Characterisation and identification of viable products from nitrogen rich agricultural wastewater

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I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of Doctor of Philosophy (PhD) is entirely my own work, that I have exercised reasonable care to ensure that the work is original and does not to the best of my knowledge breach any law of copyright and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

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Abbreviations

Air gap membrane distillation	AGMD
Ammonia	NH_3
Ammonia hydroxide	NH_3OH
Ammonium	NH_4
Ammonium sulfate	$(NH_4)_2SO_4$
Analysis of variance	ANOVA
Atomic force microscope	AFM
Biochemical oxygen demand	BOD
Boron trifluoride	BF_3
Carbon dioxide	CO_2
Chemical oxygen demand	COD
Controlled uptake long term ammonium nutrition	CULTAN
Deionised water	DI water
Dissolved air flotation	DAF
Dissolved oxygen	DO
Environmental protection agency	EPA
Fat, oil & grease	FOG
Fatty acid	FA
Fatty acid methyl ester	FAME
Free fatty acid	FFA
Gas chromatography-flame ionisation detection	GC-FID
Gas chromatography-mass spectrometer	GC-MS
Heavy metals	HM
Hydrochloric acid	HCl
Isopropyl alcohol	IPA
Liquid entry pressure	LEP
Microwave	MW
Monosaturated fatty acid	MUFA
Nitrate	NO_3^-
Nitrite	NO_2^-
Operating expense	OPEX
Organic matter	OM
Orthophosphate	PO_4^{3-}
Oxidation-reduction potential	ORP
Polyethersulfone	PES
Polypropylene	PP
Polytetrafluoroethylene	PTFE
Polyunsaturated fatty acid	PUFA
Polyvinylidene fluoride	PVDF

Potassium hydroxide	KOH
Rendering condensate wastewater	RCWW
Rendering wastewater	RWW
Saturated fatty acid	SFA
Scanning electron microscope	SEM
Sodium bicarbonate	Na_2CO_3
Sodium chloride	NaCl
Sodium hydroxide	NaOH
Sulfuric acid	H_2SO_4
Super critical-carbon dioxide	SC-CO_2
Supercritical fluid extraction	SFE
Total dissolved solids	TDS
Total Kjeldahl nitrogen	TKN
Total organic carbon	TOC
Total suspended solids	TSS
Ultra-violet	UV
Ultrafiltration	UF
Ultrasound assisted extraction	UAE
Volatile fatty acid	VFA
Wastewater	WW
Wastewater treatment plant	WWTP

Thesis abstract

Characterisation and identification of viable products from nitrogen rich agricultural wastewater - Brian Brennan 08/09/2021

Nitrogen levels in agricultural wastewater pose to be one of the greatest threats on the environment, requiring rigorous treatment before being released to receiving waters. In recent years, the dairy and beef sectors have increased capacity resulting in increased volumes of wastewater, leading to greater operation costs for wastewater treatment. Scientists and engineers have been investigating novel treatment methods to attempt reducing costs of treatment. One such method to reduce costs, which has received little research interest is utilising the wastewater to produce viable products. This thesis reviews how nitrogen in slaughterhouse related wastewater and dairy related wastewater can be utilised to produce viable products. *Nannochloropsis* sp. microalgae is cultivated on dairy wastewater and the produced fatty acids are extracted under optimum conditions to identify what viable products can be produced such as biodiesel or nutraceuticals. Hydrophobic membrane contactors are investigated and used to recover ammonia from rendering condensate wastewater to produce an ammonium sulfate fertilizer. The results demonstrated that *Nannochloropsis* sp. allowed for up to 90 % nitrogen removal while producing 0.36 ng of $C_{20:5\omega3}$ in 2 mg of *Nannochloropsis* sp. which is a characteristic fatty acid of omega-3 supplements which can help prevent against cardiovascular disease, high blood pressure and arthritis. The incorporation of polytetrafluoroethylene hydrophobic membrane contactors allowed for the recovery of ammonia from rendering condensate wastewater. The membrane contactors removed up to 65 % of the nitrogen from the wastewater and the recovered product contained a 30 % ammonium sulfate product which can be used as a competitive agriculture fertilizer compared to products already on the market. The presented thesis demonstrates agricultural wastewater has great potential to be utilised to produce viable products from its nitrogen content, but further research is required to increase the efficiency of its recovery methods.

Dissemination history

1. Oral presentations

Conference: Faraday discussions on challenging matrices; Edinburgh, Scotland 2019
Title: Comparison of extraction and derivatisation methods for sample preparation of algal oils prior to FAME analysis
Authors: *Brian Brennan*, Fiona Regan, Raquel Fernandez, Jose I. Amor and Matthew R. Jacobs.

Conference: DCU Chemistry research day; Dublin, Ireland 2019
Title: Potential of hydrophobic membranes for ammonia recovery from slaughterhouse wastewater
Authors: *Brian Brennan*, Ciprian Briciu-Burghina, Fiona Regan and Jenny Lawler.

2. Poster presentations

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Title: Comparison of extraction and derivatisation methods for sample preparation of algal oils prior to FAME analysis
Authors: *Brian Brennan*, Fiona Regan, Raquel Fernandez, Jose I. Amor and Matthew R. Jacobs.

Conference: DCU Chemistry symposium day; Dublin, Ireland 2019
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Authors: *Brian Brennan*, Matthew R. Jacobs and Fiona Regan.

Conference: IWA membrane technologies for wastewater treatment; Toulouse, France 2019
Title: First demonstration of hydrophobic membrane contactors for removal of ammonia from condensate wastewater
Author: *Brian Brennan*, Ciprian Briciu-Burghina, Sean Hickey, Thomas Abadie, Sultan M. al Ma Awali, Yan Delaure, John Durkan, Linda Holland, Brid Quilty, Mohammad Tajparast, Casper Pulit, Lorna Fitzsimons, Kieran Nolan, Fiona Regan and Jenny Lawler.

3. Publications

Title: Highlighting extraction and derivatization method comparisons for optimal sample preparation of Nannochloropsis sp. algal oils prior to FAME determination
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1. Introduction

Chapter foreword

The agriculture sector has experienced increased demands in recent years, especially the dairy and beef sector. This increased demand has resulted in higher productions and volumes of wastewater which must undergo treatment before being released into the environment. The utilisation of the nitrogen content in these wastewaters is an understudied area which promises to offer viable products. This chapter focuses on the compositions of dairy and beef related wastewater and how the nitrogen content can be recovered and utilised to produce viable products. Conventional treatment techniques are reviewed and compared to newly explored technologies such as membrane contactors and the use of microalgae to reduce nitrogen levels in the wastewater. The literature suggests that both methods allow for maximum nitrogen removal and can produce products such as biodiesel, omega-3 supplements, ammonium-based fertilizers and other ammonium-based products. However, it can be concluded that further research is required to optimise these treatment and recovery methods to successfully valorise these products.

1.1 Introduction to Ireland's agricultural sector

The agricultural sector is one of the largest contributors to environmental pollutants leading to climate change, deforestation, increased methane gas levels and discharge of pollutants to receiving waters (Shoushtarian & Negahban-Azar, 2020). The wastewater (WW) produced in the agricultural sector is of great interest as failure to appropriately treat it results in the release of high levels of organic matter (OM), nutrients (nitrogen and phosphorous) and solids into receiving waters (Farzadkia et al., 2016). Failure to adequately remove these pollutants results in decreased dissolved oxygen (DO) levels which causes killing of fish and biota in the waterways (Islam et al., 2019). Nitrogen poses to be the greatest threat to receiving waters as increased levels (>15 mg/L) consume the available DO converting any available nitrogen to nitrite (Cassidy & Belia, 2005). Agricultural WW has high levels of nitrogen due to proteins, fertilisers, manure and blood (Zoungrana et al., 2020). The agricultural sector is one of the largest sectors in the Irish economy and it is important that appropriate treatment of the high nitrogen levels is achieved. The agriculture sector is vital to the Irish economy bringing in €13.9 billion in 2019 according to the central statistics office (Griffin & Cronin, 2020). The agriculture sector comprises of, but not limited to, dairy, beef, pork, sheep, fertiliser and grain produce. Each of their contributions to the Irish exchequer can be seen in Figure 1. The data in Figure 1 is presented as a percentage of the revenue generated by each division in 2016 as reported by Teagasc (the agricultural and food development authority for Ireland) (Teagasc, 2018). The dairy sector generates the most products at 7.8 billion kg in 2019 generating €809 million. Additionally, the beef sector was shown to produce 502 million kg in 2019 generating €72 million (Griffin & Cronin, 2020). This data highlights that the dairy and beef sectors are the most important of the agriculture

industry allowing for the most generated revenue. However, in order to ensure efficient production, the industry must ensure that WW effluents are treated according to Environmental Protection Agency (EPA) regulations to prevent waterways being contaminated (Environmental Protection Agency, 2017). Conventional methods to treat nitrogen levels in these challenging agricultural WWs can be inefficient in terms of retention times and treatment costs, so new approaches to reduce nitrogen must be investigated. This thesis focuses on the utilisation of nitrogen levels in WW to produce viable products while simultaneously reducing nitrogen levels in dairy saline WW and rendering condensate WW (RCWW).

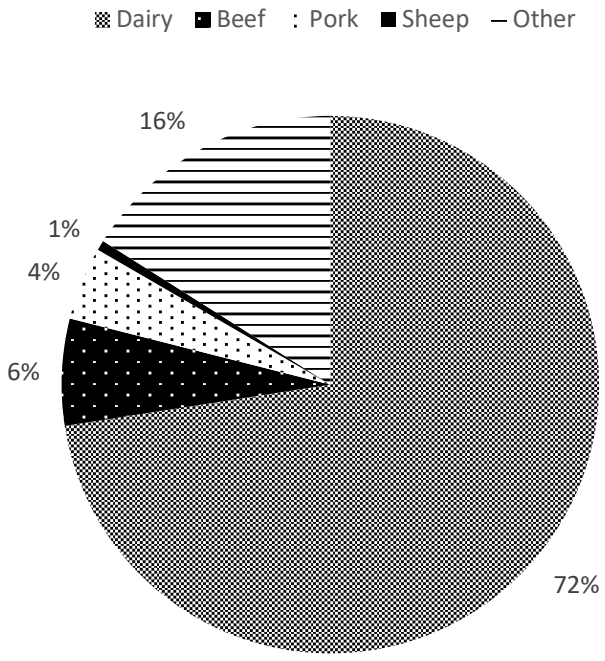

Figure 1: Contribution of each division of the agriculture sector presented as a percentage of the total revenue generated in Ireland in 2019 (other = grains, vegetables, fruits, fertilizer and poultry) (Teagasc, 2018).

1.2 Agricultural wastewater

1.2.1 Dairy wastewater

Dairy industry related WW can come from either milk or cheese processing industries (Gavala et al., 1999). WW from the dairy industry can contain urine, manure, detergents, spilled milk and mucus which contribute to high levels of nutrients, OM and solids (Healy et al., 2007). It was reported by (Sarkar et al., 2006) that dairy WW can be distinguished by its high biochemical oxygen demand (BOD), chemical oxygen demand (COD), dissolved & total suspended solids (TDS & TSS, respectively) including fat, oil & grease (FOG) and nutrients such as NH_3. Table 1 shows the composition of contaminants in dairy WW as reported by various authors. The results indicate high levels of OM, nutrients and salinity. The high salinity levels (> 1 %) may be present due to the large volumes of salt used to preserve food (Rivas Lucero et al., 2018).

Table 1: Composition of dairy wastewater (G. Q. Chen et al., 2019; Gil-Pulido et al., 2018; Rivas Lucero et al., 2018; Sarkar et al., 2006).

Constituent	TSS	TDS	COD	BOD	Total nitrogen	Total phosphorous	Salinity	pH
Concentration (mg/L)	250-600	800-1200	1500-3000	350-600	80-122	20-52	0.5-19%	5.5-7.5

Dairy WW is most commonly discarded by spreading onto agricultural land to promote growth (Healy et al., 2007). However, if this WW is not treated efficiently, the high saline levels will impact on the soil quality which will in turn degrade the quality of the crops (Rivas Lucero et al., 2018). In addition, the high levels of nutrients and OM may run off into waterways and cause serious damage (Aitken, 2003). A survey carried out by the Irish EPA in

2001 estimated that the percentage of pollution attributed to agriculture was 32, 32 and 15 % in rivers, streams and lakes, respectively (Healy et al., 2007). A more recent report from the Irish EPA stated that 53 % of water bodies in Ireland are negatively affected due to agricultural activity. This report did not break down the waters contaminated but it did highlight that river quality has worsened and lake quality has improved (O'Boyle et al., 2019). The conventional biological nitrification and denitrification method of treating nutrients is inefficient when salinity levels are greater than 1 % as the salt dehydrates the biological material which is discussed in more detail in Chapter 2 (Shen et al., 2015).

A relatively new method which is underutilised to treat nutrients in WW with high salinity levels such as dairy WW is cultivating microalgae on the WW reservoir (Matos et al., 2015). The use of microalgae has a double advantage as it allows for the removal of nutrients and salt while the nitrogen and salt content promote microalgae growth of the biomass which can be further treated to produce products such as biofuels, nutraceuticals and food additives from the fatty acid (FA) (Brennan, Regan, et al., 2020; Hodges et al., 2017; Matos et al., 2015). *Nannochloropsis* sp. is a microalgae species which naturally forms in marine environments with high salinity levels (X.-N. Ma et al., 2016). The immunity to the high saline levels by this microalga make it a very suitable species to cultivate on dairy WW to reduce nutrient levels while simultaneously providing a viable by-product. To the authors knowledge, this species has not been investigated for this use and as such, part of this thesis will focus on the utilisation and suitability of *Nannochloropsis* sp. to reduce nitrogen levels while simultaneously producing viable by-products. In addition, different extraction and derivatization methods are optimised and compared in order to determine the best method

to recover the most FA from the microalgae species cultivated from the saline WW to ensure full value is being exploited.

1.2.2 Beef sector wastewater

Animal slaughtering includes the processes of pre-slaughter handling, stunning and slaughtering to produce food for human consumption (Omole & Ogbiye, 2013). In cattle slaughterhouses, the beef is recovered as a valuable product while the carcasses, blood, organs and hides are treated separately for disposal or to produce by-products (Metzner & Temper, 1990). The blood and organs are most commonly treated and disposed of off-site (via incineration) while the hides are processed to produce leather for shoes, wallets, clothing and furniture (Valta et al., 2015). The carcasses are most commonly processed in off-site rendering plants (required by EU law (Harris & McCabe, 2015)) to produce tallow fat and bone meal (Bureau, 2006). There are various stages of the slaughtering plant which produce large volumes of WW including the slaughtering floor, rendering plant, holding yards and the offal & paunch released from the animal (Johns et al., 1995). The rendering plant poses the greatest risk to the environment due to the high protein, nitrogen and fat content released from processing of the carcasses which, if left untreated would cause serious pollution in receiving waters (Auterská & Novák, 2006). The rendering plant boils the carcasses to treat any pathogens present and to prepare the carcass to be processed. During the boiling stage, it releases a very harmful steam and fumes which are too toxic to release into the environment. As such, the steam is condensed and collected as condensate in order to treat it as RCWW (Brennan, Briciu-Burghina, et al., 2020). This RCWW has very high nitrogen and FOG levels which require extensive treatment and is conventionally carried out by biological

nitrification and denitrification which is discussed in detail below. However, this method presents challenges such as prolonged retention times, high treatment costs and may trigger the presence of acid rain due to the nitric oxides being released into the air (Wosiack et al., 2015).

A nitrogen treatment method which has gained increased interest is the use of membrane contactors which allow for the recovery of nitrogen in the form of ammonia (NH_3)/ammonium (NH_4) (Sengupta et al., 2015). The principle of membrane contactors will be discussed below in Section 1.4.4.2. The use of membrane contactors has been demonstrated on WW types including manure waste, radioactive waste and coking plant WW but has not been incorporated with a challenging matrix such as RCWW (Garcia-González & Vanotti, 2015; P. H. Lin et al., 2018; Z. Liu et al., 2016). RCWW poses to be such a challenging WW type as it has high levels of FOG and OM which can result in membrane fouling and wetting which impacts membrane performance (Brennan, Briciu-Burghina, et al., 2020). The use of membrane contactors allows for the recovery of NH_4-based products which can then be used to produce viable products such as fertilisers, flame retardant products, used as food regulators and used to promote protein purification (Amir Masoud Samani Majd et al., 2012; Hshieh & Beeson, 1995; Mostashari & Mostashari, 2008). This thesis carries out a full characterisation of RCWW and identifies the potential viable products which can be recovered from the matrix. Additionally, the use of membrane contactors is assessed at a lab- and pilot-scale in order to determine its efficiency at reducing and recovering NH_3 levels from RCWW and how it compares to conventional treatment methods.

1.2.3 Contaminants of emerging concern in agricultural wastewater

In recent years, increased contaminants which are of emerging concern have been introduced into the agricultural sector and end up in the WW streams. These contaminants include but are not limited to pharmaceuticals, hormones, pesticides and microplastics (Chollom et al., 2020; Malankowska et al., 2020; Richardson & Kimura, 2017; Snow et al., 2012; F. Wang et al., 2020). These contaminants are of great concern as they have the ability to easily enter the environment and cause known or suspected adverse ecological or human health effects (Snow et al., 2012). A brief description of the contaminants mentioned is outlined in Table 2.

Table 2: Overview of emerging contaminants present in agricultural wastewaters.

Contaminant	Source to agriculture	Danger	Explored treatment methods	Reference
Microplastics	Packaging, pharmaceuticals and feeds	Harmful to aquatic life	Biofilter, membrane bioreactors, activated sludge, solid flocs and DAF	(Iyare et al., 2020; Fan Liu et al., 2020)
Pharmaceuticals	Antibiotics and medicines for livestock	Released to environment and cause antibiotic resistance	Biodegradation, adsorption, volatilisation or hydrolysis	(Chollom et al., 2020; Richardson & Kimura, 2017)
Hormones	Livestock supplement to increase growth and improve health	Impact fish reproduction systems	Ultrasound, ozonation, advanced oxidation, membrane filtration and adsorption	(Grassi et al., 2013; Richardson & Kimura, 2017)
Pesticides	Used to remove unwanted growth and improve crop growth	Cause increased growth in aqua environments and impede on animal health	Advanced oxidation, adsorption, activated sludge, membrane bioreactors and membrane technologies	(Chollom et al., 2020)

Of the mentioned analytes, microplastics and pharmaceuticals are of greatest concern due to their existence at a molecular level and ability to be present in water matrices which

are released to rivers and streams and also adsorb to sludge matrices which is conventionally spread on to agricultural land (Richardson & Kimura, 2017). Medicines and pharmaceuticals consumed in the agricultural sector comprise of >70 % of antibiotics for both therapeutic and non-therapeutic reasons. Of these antibiotics, only 30-90 % are metabolised by animals resulting in the remainder being discharged into soils as fertilizers, or ending up in the slaughtering process and needing to be treated as SHWW (Chollom et al., 2020). A review by (Snow et al., 2012) on emerging contaminants in agricultural wastewaters reported that up to 21 different variations of antibiotics originated from a slaughterhouse - which is concerning as this can impair the growth of aquatic life and also cause antibiotic resistance if these contaminants end up in the drinking water network. Various treatment methods have been reported by (Richardson & Kimura, 2017) which allow up to 90 % removal of these veterinary medicines such as biodegradation, adsorption, volatilisation or hydrolysis. Microplastics (plastics with particle size smaller than 5 mm) have become a highly discussed topic in recent years due to their ability to adsorb and desorb toxicants like polycyclic aromatic hydrocarbons, polychlorinated biphenyls. An increased volume of these microplastics has shown to result in an increased level of the mentioned toxins (Z. Zhang & Chen, 2020). Studies by (Iyare et al., 2020; Fan Liu et al., 2020) have shown that various methods such as biofilters, membrane bioreactors, activated sludge, solid flocs and DAF can be used to remove microplastics from WW streams – however, these studies do not discuss how the recovered microplastics can be further treated and disposed after removal from the WW.

The composition of agricultural WW is one of the biggest dangers to our natural environment due its high nutrient and OM concentrations. It is critical that nitrogen levels are efficiently treated in order to prevent eutrophication development on receiving waters which leads to fish and biota killings. Conventional treatment of this challenging WW can be problematic due to high salinity levels, long retention time and high costs. Utilisation of the highly rich nitrogen content in these WW to produce viable products is a research area which needs more attention as it simultaneously allows for the reduction of nitrogen levels while producing a viable product which would allow for the generation of revenue. Microalgae cultivation thrives on dairy WW as the microalgae consume the saline levels and nitrogen content to increase microalgal biomass while reducing the nitrogen levels in the WW pond. These microalgae species can then be further treated to recover valuable FA to produce biofuels and nutraceuticals. Membrane contactors are another option which can be incorporated into the treatment process to recover the nitrogen content and produce an NH_4-based fertiliser whilst reducing nitrogen levels in the WW.

1.3 Nitrogen in agricultural wastewater

Nitrogen exists in multiple forms in agricultural WW including nitrite (NO_2^-), nitrate (NO_3^-), NH_3, organic nitrogen and Total Kjeldahl Nitrogen (TKN) (Ryu & Lee, 2016). NO_2^- and NO_3^- levels are reported to be low in SHWW whereas NH_3 levels are high (Ziara et al., 2018). Nitrogen generally takes the form of NH_3 in WW in the early processing stages as it is easily soluble and up to 50 % of nitrogen is soluble and in the form of NH_3 (P. D. Jensen et al., 2014).

Ammoniacal-nitrogen can exist in two forms, NH_3 and the NH_4 (Eykens et al., 2016). The composition of the type of NH_3 depends on pH, temperature and oxidation-reduction potential (ORP). When the nitrogen containing solution has a pH of 8 and under, it is present as NH_4^+ (Tan et al., 2006). The majority of WW streams are in the pH range 6-8, and thus alkali addition is required to change it to NH_3 (Norddahl et al., 2006). In order for nitrogen to be in the NH_3 form, it must have an ORP value of 100-350 mV which will allow nitrifying bacteria convert it to NO_3^- (B. Li & Irvin, 2007).

The sources of nitrogen can derive from various parts of plants and the concentrations of different nitrogen forms also vary depending on the plant area as seen in Table 3 (Johns et al., 1995). Very limited literature describes the nitrogen composition throughout the slaughterhouse plant. It was reported by (P. D. Jensen et al., 2014) and (Johns et al., 1995), that different sources of nitrogen in the slaughtering plant come from the slaughter floor, boning room, paunch process, offal processing and rendering plant. To the authors knowledge, there are no other studies which quantify the different nitrogen species in various parts of a slaughtering plant. Table 3 shows that the distribution of nitrogen loads is site specific. The rendering area has the highest concentrations of nitrogen waste which includes the processing of heads, hooves and bones which may have high concentrations of protein. The offal & paunch also contains high levels of inorganic nitrogen which is due to the intestinal tracts of the animals being opened to remove stomach contents. The holding yard has higher levels of NO_2^- and NO_3^- which may be due to the oxidisers degrading the NH_3 in the waste due to biological nitrification and denitrification. This suggests that NO_2^- and NO_3^- are not produced at the processing stage of the plant but instead at the collection and treatment

stages (Ziara et al., 2018). Additionally, (Ziara et al., 2018) stated that most nitrogen found in the slaughtering plant is in the NH_3 and TKN form. Low levels of NO_2^- or NO_3^- are present in the slaughtering process which suggests that they are produced during the WW collection and treatment processes. Other sources of nitrogen in slaughterhouse plants may be due to urine and faeces from both humans and animals which are introduced into the sewage system (Rajab et al., 2017). Due to the different nitrogen forms at different stages of the plant, it is suggested that the WW from all stages are collectively stored in order to more efficiently recover nitrogen to produce viable by-products (P. D. Jensen et al., 2014).

Table 3: Percentage of nutrient load from each plant section (Johns et al. 1995).

Nutrient form	Slaughter floor (%)	Rendering (%)	Holding yards (%)	Offal & paunch (%)
NO_3^-	0.2	80.5	15.8	3.5
NO_2^-	0	0	0	0
NH_3	1.5	70.9	10.7	12.3
TKN	5	48.1	4.1	19.4
Organic N	6.3	39.9	1.8	22
Total N	4.8	49.4	4.6	18.8

1.4 Treatment of nitrogen

1.4.1 Conventional treatment method

WW is commonly treated for various constituents including solids, nutrients and OM before being released into receiving waters (Masse & Masse, 2000). Figure 2 shows a typical WW treatment plant (WWTP) which suggests that floatables, oils, solids and organics are treated first using systems such as skimmers, sedimentation and dissolved air flotation (DAF) (Musa et al., 2019; Ziara et al., 2018). After de-watering the waste, the WW is treated for nutrients such as nitrogen and phosphorus using biological treatment methods such as sequencing batch reactors or more commonly used aeration and anoxic ponds (biological nitrification and denitrification) (Amenu, 2014; C. Bustillo-Lecompte et al., 2016). Extensive research has focused on the use of these ponds and they have been shown to be very effective in removing nitrogen (Atkins & Scherger, 2013; Sancho et al., 2017; Tan et al., 2006). Anaerobic digestion allows for the reduction of emissions and energy costs whilst producing biogas (Harris & McCabe, 2015). However, these methods are only used for capturing methane and they do not allow for the complete removal of nitrogen – only that in the NO_3^- form. Although these conventional methods which have been used for years have been shown to be effective in removing nitrogen, the process is restricted by slow conversion and unfavourable environmental factors in addition to having retention times taking up to 30 days to treat the total nitrogen (Pressley et al., 1972; Tan et al., 2006). Additionally, these methods have a large environmental footprint, poor capture rates and poor odour control (P. D. D. Jensen et al., 2015). (Valta et al., 2015) stated that high concentrations of NH_3 can impact the development of biogas negatively and as such, due to the high levels of NH_3 in SHWW, it

makes the study of $(NH_4)_2SO_4$ production a lot more interesting and feasible. Other methods capable of removing nitrogen and possible recovery from WW include air stripping, NH_3 volatilization, absorption, membrane systems, microalgae adsorption and sedimentation (Maroneze et al., 2014).

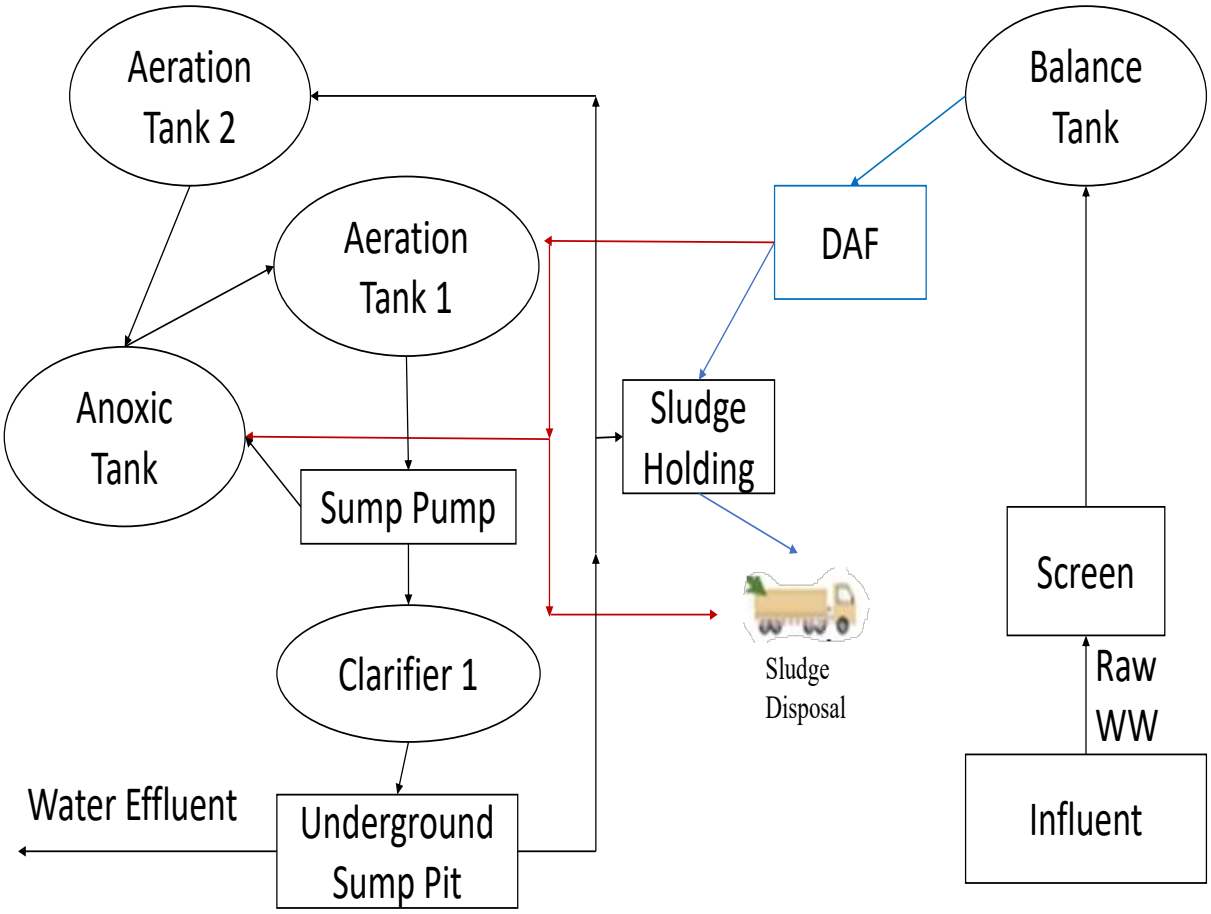

Figure 2: Schematic of a typical WWTP.

1.4.1.2 Role of nitrogen cycle in WWTP

The use of aeration and anoxic nitrogen treatment is based on the principle of the nitrogen cycle (nitrification/denitrification) as shown in Figure 3 (Batstone et al., 2015). The steps of the nitrogen cycle can be split into 1) nitrogen fixation; 2) nitrification; 3) assimilation; 4) ammonification; and 5) denitrification (Norddahl et al., 2006; Qiao et al., 2015). Biological nitrification and denitrification can occur in separate reactors or in the same reactors in sequence to allow for aerobic conditions (for nitrification) and anaerobic conditions (for denitrification) (Wosiack et al., 2015). Briefly, nitrification occurs when WW rich in nitrogen in the ammoniacal-N form is converted between NH_3 and NH_4 depending on its pH (higher and lower than 9.5, respectively) (Alcaraz Segura, 2012). In its NH_3 form, the NH_3 and oxygen (usually supplied by aeration) feed NH_3 oxidisers to produce an acid, water, NO_2^- and energy. The water is reintroduced to the system, the energy is used to grow and multiply the NH_3 oxidisers and the NO_2^- moves to the next step. NO_2^- oxidisers feed on the NO_2^- and the DO to produce NO_3^- and energy (Stein & Klotz, 2016). When NH_3 levels are <2mg/L or NO_2^- are 0.5mg/L; nitrification is regarded as being complete (Daims et al., 2015). The NO_3^- is then used in the denitrification step as an oxygen source for the heterotrophic bacteria along with COD for energy. Under anoxic conditions, the heterotrophic bacteria break down the NO_3^- and use the oxygen for synthesis while the nitrogen is released as N_2 gas in small bubbles to the surface of the waterbody and escape into the atmosphere (Letelier-Gordo et al., 2020). The combination of nitrification and denitrification can result in levels as low as 1-3 mg/L of NO_3^- in the effluent stream (Stein & Klotz, 2016).

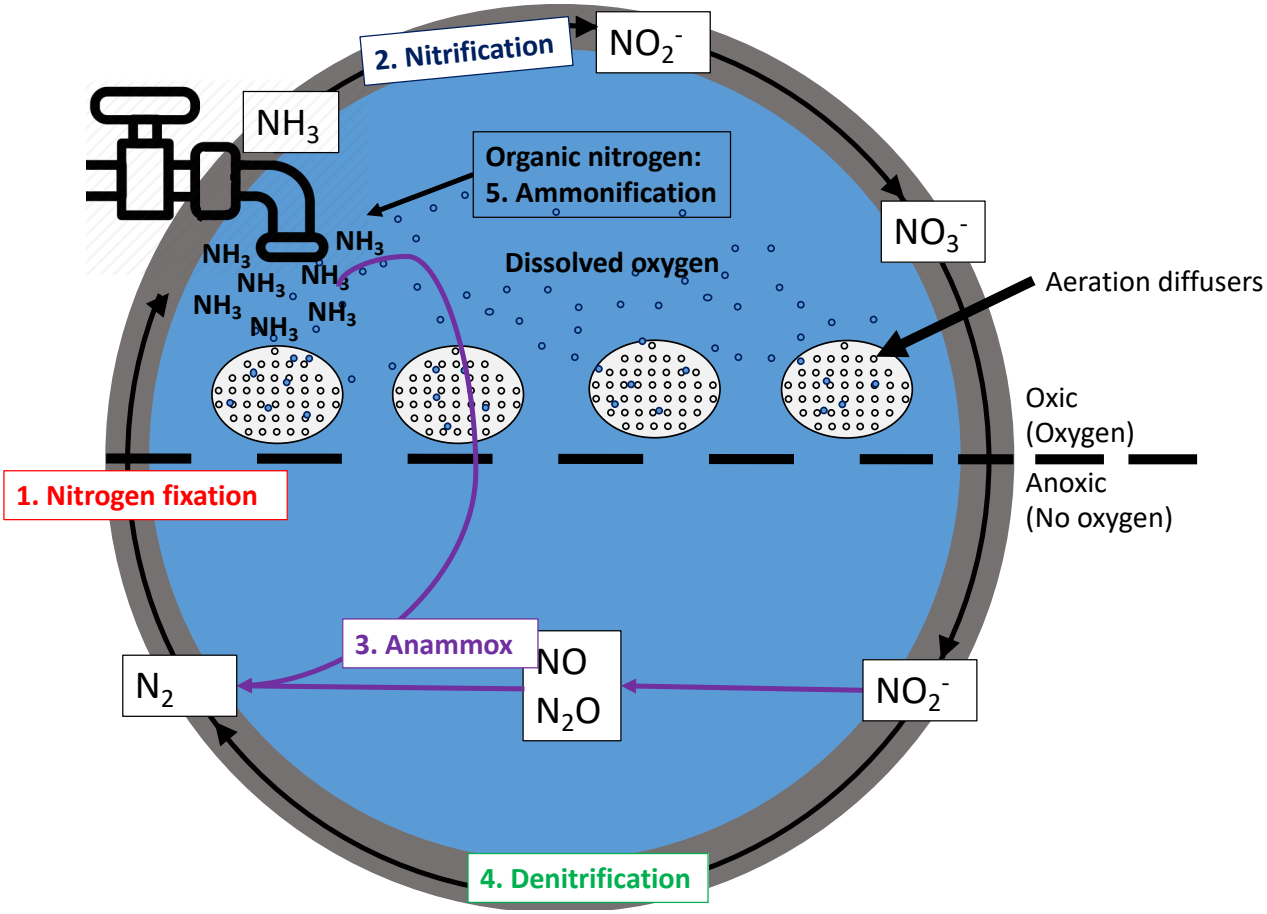

Figure 3: Overview of the nitrogen cycle showing nitrification in the top half and denitrification in the bottom half.

1.4.1.3 Comparison of SHWW practices worldwide

Slaughterhouses differ worldwide in terms of their operations, treatment methods, WW compositions and discharge limits. The most common method of treating nitrogen in WW worldwide is by the biological nitrification/denitrification method (Amenu, 2014). However, it has been reported that anaerobic lagoons are commonly used in tropical and equatorial countries while engineered reactor systems are commonly used in tropical zones (P. D. D. Jensen et al., 2015). It was reported by (Johns et al., 1995) and more recently by

(Harris & McCabe, 2015) that slaughterhouse and rendering facilities in Australia are incorporated into the one site whereas in Europe, the two processes must be carried out in separate facilities. Because of this, European researchers interested in recovering nitrogen may be more interested in the WW from rendering plants as it contains higher levels. Europe have stricter laws compared to other countries for reusing the treated effluent from rendering waste as it is not permitted to be used for human consumption. In the United States, water is reused after treatment which means 3000 L of fresh water is used per 1000 kg of livestock, whereas in Europe 40,000 L of fresh water is used for every 1000 kg of livestock (Ziara et al., 2018). Table 4 outlines the discharge limits for nutrients and OM in treated effluent from WWTP set by the European Directives (Environmental Protection Agency, 1998), World Health Organization (WHO) (C. F. Bustillo-Lecompte & Mehrvar, 2015) and other countries worldwide before they are released into the environment after treatment (Abrha & Chen, 2017; C. F. Bustillo-Lecompte & Mehrvar, 2015; EPA, 2018; M, 2015; Pin Lee et al., n.d.; Sperling, 2016; Y. Zhou et al., 2018). Table 4 shows that Australia and Canada have stricter regulations on their effluent quality compared to other countries and require it to be less harmful before releasing it back to the environment. However, the United States and some Asian countries have very relaxed regulations which result in highly polluted waterways in these countries. Regulations set out by the Ethiopian environmental authorities should specifically be pointed out due to their very relaxed regulations which contributes to the poor river quality except TSS which is considerably low (Weldesilassie et al., 2009).

Table 4: International legislative guidelines for contaminants in treated WW effluents.

Country	Total nitrogen (mg/L)	Total phosphorous (mg/L)	BOD (mg/L)	COD (mg/L)	TSS (mg/L)	Reference
Ireland	15	2	25	125	35	(O'Boyle et al., 2019)
European Union	15	2	25	125	35	(Commission, 2000)
Australia	1.5	-	20	60	15	(C. F. Bustillo-Lecompte & Mehrvar, 2015)
Canada	1.25	0.1	25	125	25	(EPA, 2018)
United States	-	-	30	-	>85 % removed	(EPA, 2018)
Brazil	2	0.1	10	180	60	(Sperling, 2016)
Ethiopia	20	3	60	250	15	(Abrha & Chen, 2017)
Tunisia	1	-	30	90	30	(M, 2015)
China	15	0.5	20	60	70	(Y. Zhou et al., 2018)
Malaysia	5	5	20	50	50	(Roslan MY et al., 2019)
Iran	10	-	30	60	40	(Farzadkia et al., 2016)
WHO	45	5	20	-	20	(Quinn & Fabiansson, 2001)

1.4.2 Novel methods to treat nitrogen

A number of different methods to recover and remove nitrogen (in the form of NH_3) from agricultural WW have been investigated including breakpoint chlorination, air stripping, selective ion exchange, MW radiation, microalgae adsorption and membrane technology (Alcaraz Segura, 2012; Alshameri et al., 2014; Ata et al., 2017; Boehler et al., 2015; Bonmatí & Flotats, 2003; Brennan, Briciu-Burghina, et al., 2020; Church et al., 2017; Hasanoğlu et al., 2013; H. C. Kim et al., 2016; Y. Kim & Logan, 2013; La et al., 2014; P. H. Liao et al., 1995; L. Lin et al., 2009; Z. Liu et al., 2016; Shen et al., 2015). The characteristics of the methods mentioned are outlined in Table 4. Nitrogen recovery is much more complicated since there is no ready precipitate (Batstone et al., 2015). A lack of investment in these technologies has prevented them being installed into agricultural WW treatment sites which prevents the

creation of viable by-products such as NH_4-based fertiliser and FA-based products (Keller et al., 2002). However, the use of microalgae has shown to completely remove nitrogen levels while producing viable products and membrane contactors have shown in a number of investigations to allow for efficient recovery of NH_3 (up to 99 %) whilst rejecting other containments using environmentally friendly approaches and producing a viable fertiliser by-product (W. Liu et al., 2018).

Table 5: Possible methods of ammonia recovery from agriculture WW.

Method	Principle	NH_3 removal	By-product	Disadvantages	References
Air stripping	NH_3 stripped from water droplets to atmosphere	71-99%	-	Can only facilitate up to 100 ppm NH_3	(Bonmatí & Flotats, 2003; P. H. Liao et al., 1995)
Ion exchange	NH_3 attaches to zeolites	84-94%	NH_3 supplement	Concentrated NH_3 waste	(Alshameri et al., 2014; Y. Kim & Logan, 2013)
MW radiation	Radiation inhibits escape of NH_3 molecule to atmosphere	87-94.2%	-	Requires high energy	(Ata et al., 2017; La et al., 2014; L. Lin et al., 2009)
Membrane technology	Driven by partial pressure difference	64-99.8%	NH_4-salt fertiliser	Membrane material subject to wetting	(Alcaraz Segura, 2012; Boehler et al., 2015; Brennan, Briciu-Burghina, et al., 2020; Hasanoğlu et al., 2010; Z. Liu et al., 2016)
Microalgae-assisted removal	Microalgae consumed nitrogen for protein and nucleic acid development	40-100%	FA products	Higher nitrogen levels can decrease FA concentration in microalgae	(Church et al., 2017; H. C. Kim et al., 2016; Shen et al., 2015)

1.4.3 Microalgae facilitated nitrogen recovery

1.4.3.2 Mechanism

As stated above, the use of microalgae has been proposed for the removal of nitrogen from saline WW as an alternative method which allows for microalgae to be utilised to produce viable products (Aravantinou et al., 2013). The use of microalgae to treat saline WW is an understudied area and *Chlorella Vulgaris, Spirulina Platensis, Picochlorum* sp. and *Acutodesmus Obliquus* are the only strands which have been studied as shown in Table 6. It was reported by (Church et al., 2017) and (Shen et al., 2015) that marine microalgae have proven efficient at removing nitrogen and phosphorous from saline WW. However,

(Aravantinou et al., 2013) reported that while marine microalgae have higher growth rates, they don't have efficient nutrient removal when compared with freshwater microalgae. It has been reported that high saline concentrations impact the efficiency in nutrient removal using conventional methods such as biological nitrification and denitrification as salt concentrations exceeding 1 % can be difficult to treat (Church et al., 2017). This is due to the salt content inhibiting the nitrifying bacteria which are responsible for removing nitrogen from the WW and the nitrifying bacteria bursting due to the build-up of water (Babatsouli et al., 2015). Table 6 shows that the strands of *Chlorella vulgaris* which were exposed to varying saline concentrations are influenced by salinity levels, but these studies are carried out by different authors who used different cultivation and experimental conditions and as such, it's not an equal comparison. The microalgae reduces nitrogen and phosphorous levels by consuming them in order to use the nitrogen to build nucleic acid and proteins while it uses phosphorous to generate phospholipids for cell membranes and generate adenosine triphosphate for cellular energy (Aravantinou et al., 2013). Table 6 shows that microalgae is capable of removing high concentrations of detectable nitrogen and phosphorous using *Chlorella Vulgaris*, *Picochlorum* sp. and *Spirulina Platensis*. It can be seen in Table 6 that not many studies have reported lipid recovery obtained from microalgae strands cultivated from saline WW. The studies which have reported the lipid recovery have a large variation (16-40 %) which could potentially be due to lipid content not being directly related to removal of nutrients as stated by (Aravantinou et al., 2013). Additionally, this variation could have been achieved due to the wide variation of experimental conditions used in different studies. However, some studies have stated that nitrogen starvation can result in higher

concentrations of lipid production (Babatsouli et al., 2015). High saline levels have been reported to allow for the microalgae to adjust their biochemical identity which was demonstrated by (Church et al., 2017) who showed that lipid content was 15.4 and 25.6 % at saline concentrations of 0 and 5 % NaCl, respectively. This suggests that higher saline concentrations promote lipid growth. A characterisation of FA in *Chlorella Vulgaris* cultivated on saline WW showed that the most dominant FA were $C_{16:0}$, $C_{18:1\omega9}$, $C_{18:2\omega6}$ and $C_{18:3\omega3}$. It was also stated that PUFA levels decrease with higher saline concentrations which is optimal for biodiesel production (Church et al., 2017).

Table 6: Nutrient removal efficiencies from saline WW of different microalgae species and lipid recovery (NR=not reported).

Microalgae species	Salinity concentration (%)	Nitrogen removal (%)	Phosphorous removal (%)	Lipid recovery (%)	Reference
Chlorella vulgaris	86	99	99	40	(Shen et al., 2015)
Picochlorum sp.	0.01	95	96	NR	(Babatsouli et al., 2015)
Chlorella vulgaris	0.1	95	30	NR	(Vo et al., 2019)
Chlorella vulgaris	1	95	30	NR	(Church et al., 2017)
Chlorella vulgaris	2.5	99	99	16	(Church et al., 2017)
Spirulina platensis	0.93	92	93	30	(W. Zhou et al., 2017)
Acutodesmus obliquus	5.2	40	43	NR	(H. C. Kim et al., 2016)

1.4.3.3 Potential of *Nannochloropsis* sp.

Nannochloropsis sp. has been reported to be a favoured microalgae species for a number of reasons. It is capable of accumulating in large amounts, it can grow on saline and other WW types under adverse conditions and accumulate in high volumes of polar lipids (up to 50 % of total biomass being lipids) (Qiu et al., 2019; Zuorro, Miglietta, et al., 2016). The lipids present in *Nannochloropsis* sp. are made up of mainly polar (phospholipids, glycolipids and betaine) and non-polar (acylglycerols, sterols and free fatty acid (FFA)) lipids (Natarajan et al., 2014). Studies looking at *Nannochloropsis* sp. have reported high concentrations of C_{16}, $C_{16.1}$, C_{18} and $C_{18.1}$ which are suitable for biodiesel production. *Nannochloropsis* sp. also contain high concentrations of $C_{20:5\omega3}$ and $C_{22:6\omega3}$ which contribute to the production of omega-3 products (Adam et al., 2012; Qiu et al., 2019). Recovering such FA from the microalgae would allow for the production of viable products. However, it is a difficult process due to the complex structure of the organism. The cell structure (composed of cellulose, polysaccharides, proteins and lipids) has a strong resistance to mechanical and chemical treatments (Chua & Schenk, 2017; Zuorro, Maffei, et al., 2016). The use of microalgae to treat saline WW has received increased interest in recent years. However, to the authors knowledge no study has focused on the use of *Nannochloropsis* sp. microalgae for this process and as such part of this thesis will determine how effective *Nannochloropsis* sp. is at reducing nitrogen levels in saline WW and determine the viable products which can be extracted.

1.4.3.4 *In-situ* lipid and fatty acid extraction methods from *Nannochloropsis* sp.

1.4.3.4.1 Microwave-assisted extraction

The use of microwave (MW)-assisted extraction offers benefits such as reduced retention time, less solvents and high product recovery (Castillo López et al., 2015; Liang et al., 2012). Briefly, the process depends on the MW energy interaction with polar materials and solvents. When the polar molecules interact with the MW energy, two processes occur: ionic conduction and dipole rotation, which in most cases occur simultaneously (Pedersen et al., 1969). The resistance offered by the solution to the ionic conduction of ions generates friction, which eventually heats up the sample. The heat generated causes the moisture inside the microalgae to heat up which starts to evaporate causing increased pressure on the cell wall to disrupt (Mandal & Hemalatha, 2007). A number of studies were carried out to determine the efficiency of MW for extracting lipids and FA from *Nannochloropsis* sp. The results initially suggest that there is a huge variation in lipid recovery and FA classification based on the solvents being used (Figure 4). These studies also showed the recovery of similar FA compositions with high SFA and MUFA, and low PUFA levels which suggests a suitable method for biodiesel recovery. A study by (Brennan, Regan, et al., 2020) showed that PUFA have a greater affinity to acetic acid and the high PUFA levels are suitable for omega-3 supplementary products (Brennan, Regan, et al., 2020; Gadipelly et al., 2014). The use of multiple solvents which was suggested by (Qadariyah L et al., 2012) aids in greater lipid recovery as the different solvents would allow for the extraction of different FAs. This ideology was demonstrated by (Brennan, Regan, et al., 2020; Martinez-Guerra et al., 2018; C. L. Teo & Idris, 2014) to show lipid recovery of 48.2, 26.4 and 51.8 % for SFA, MUFA and PUFA,

respectively. Figure 4 shows the FA composition obtained for *Nannochloropsis* sp. from different solvent(s); the average SFA is 32 %, 17 % MUFA and 12.2 % PUFA from all of the methods investigated. According to (Martinez-Guerra et al., 2018), these compositions make MW-assisted extraction the ideal method to produce biodiesel from *Nannochloropsis* sp. as it obtains the ideal cetane number, viscosity, oxidation potential and heating capacity which are all discussed further in Section 1.5.1.1.1.

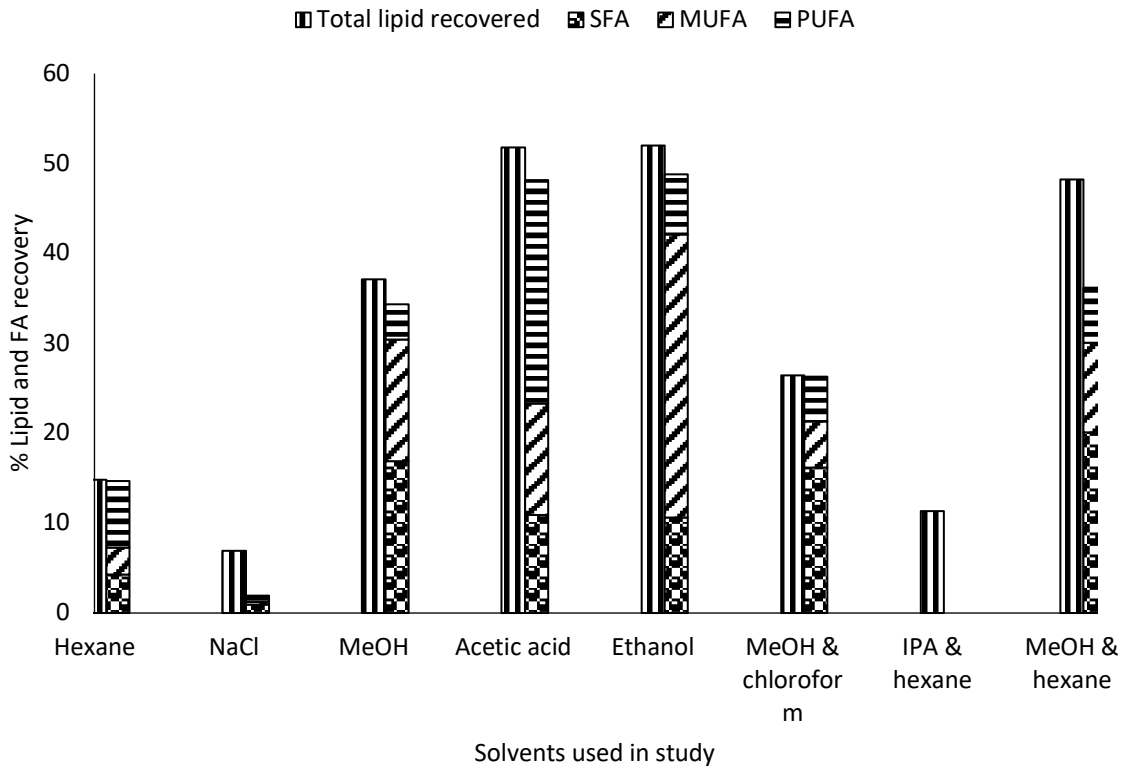

Figure 4: MW-assisted extraction of lipid and FA from *Nannochloropsis* sp. using different solvent(s). (Hexane (Bermúdez Menéndez et al., 2014); NaCl (Abugrara et al., 2019); MeOH (McKennedy et al., 2016); Acetic acid (Brennan, Regan, et al., 2020); Ethanol (Ali & Watson, 2015); MeOH&chloroform and IPA&hexane (C. L. Teo & Idris, 2014); MeOH&hexane (Martinez-Guerra et al., 2018)).

1.4.3.4.2 Supercritical CO_2-assisted extraction

Supercritical fluid extraction (SFE) is the process of using CO_2 or other solvents under high pressure to extract oils/lipids from the matrix (Pushpangadan & George, 2012). Results obtained from various SC-CO_2 studies for FAME recovery (Table 7) shows that the compositional content of the lipid is dependent on SC-CO_2 conditions. This is caused by the inability of SC-CO_2 to recover polar lipids and thus show higher concentrations of less polar lipids which could be used for omega-3 and omega-6 medicinal production (Andrich et al., 2005; Solana et al., 2014). Studies by (Mouahid et al., 2013) and (Taher et al., 2020) whom didn't include any co-solvents and only used conventional SC-CO_2 using *Nannochloropsis oculata* and *gaditana,* respectively, showed that more PUFA, less polar FA were recovered in comparison with polar SFA (Table 7). (McKennedy et al., 2016) stated that using different co-solvents demonstrates the tunability of SC-CO_2 to produce the product desired. By incorporating solvents such as hexane and ethanol it allows for the more oxidative FA which are suitable for biodiesel production. Table 7 shows the FAME composition obtained for different solvents and it shows that a hexane-ethanol mix produce high levels of SFA and MUFA which are suitable for biodiesel production (Aliev & Abdulagatov, 2017; Andrich et al., 2005; M. Chen et al., 2012; Mouahid et al., 2013; P. D. Patil et al., 2018; Taher et al., 2020).

Table 7: FA composition for Nannochloropsis sp. lipids extracted by SC-CO_2 as reported by multiple authors.

SC-CO_2 condition	**Conventional SC-CO_2**	**Conventional SC-CO_2**	**Co-solvent (hexane and ethanol)**	**Co-solvent (n-hexane)**	**Co-solvent (chloroform)**
Lipid recovery (% total biomass)	33	21.9	36	29.4	3.92
SFA	25.3	21	42.37	23.44	23.35
MUFA	14	32	52.8	46.19	46.31
PUFA	43.2	43	0.62	11.9	12.22
$C_{12:0}$	0	0	0	0.39	0.27
$C_{14:0}$	5.7	3	2.88	3.2	3.06
$C_{16:0}$	17.8	12	35.67	18.62	18.91
$C_{16:1}$	11.4	12.5	25.96	26.61	27.11
$C_{18:0}$	1.8	6	3.82	1.23	1.11
$C_{18:1\omega9}$	2.6	19.5	26.84	19.58	19.2
$C_{18:2}$	5.2	8	0	5.92	6.34
$C_{20:4\omega6}$	5	5	0	5.98	5.88
$C_{20:5\omega3}$	33	30	0.62	0	0
Reference	(Mouahid et al., 2013)	(Taher et al., 2020)	(P. D. Patil et al., 2018)	(Andrich et al., 2005)	(M. Chen et al., 2012)

Overall, the use of SC-CO_2 as a method for lipid and FAME recovery has shown to be successful and delivers lipid recoveries which are comparable and as efficient as conventional methods. The SC-CO_2 method has also demonstrated that it can be easily manipulated in order to produce FA to produce desired by-products.

1.4.3.4.3 Ultrasound-assisted extraction

Ultrasound-assisted extraction (UAE) refers to an extraction process for lipids from solid samples using ultrasound waves to disrupt the cell walls (Bendicho & Lavilla, 2018). The FAME composition obtained for different UAE studies and their conventional methods is presented in Table 8. The results represent what is expected in the *Nannochloropsis* sp. and they don't show much variation between studies (in correlation with their % lipid recovery). There is a high SFA and MUFA content which suggests that the method may be suitable for biodiesel production. However, comparisons against conventional methods suggest that there is no difference in the composition of the FA prepared by different methods. Overall, *Nannochloropsis* sp. has received little attention with the use of UAE for lipid recovery. UAE presents a quick method which successfully produces FA which are of industrial use and it can be easily scaled up. However, it does not allow for variation in FA composition by changing operational conditions like SC-CO_2. Further studies should be carried out to determine the optimum UAE conditions in order to recover the greatest lipid volume.

Table 8: Comparison of FAME composition for Nannochloropsis sp. investigated by UAE and conventional soxhlet methods (NR = Not recorded) (Lipid recovery reported as % of total biomass and FA reported as % of the lipid).

Fatty acid Composition (%)	**UAE**	**UAE**	**UAE**	**Conventional**	**UAE**
Lipid recovery	14.76	NR	55	21	6.8
SFA	28.8	34.0	23.4	24.6	29.2
MUFA	20.0	10.0	37.6	31.6	43.5
PUFA	43.7	39.5	38.1	41.7	5.9
C14:0	4.0	9	7.4	7.4	11.1
C16:0	24.7	18	14.5	16.8	12.5
C16:1	15.8	-	33.6	27.1	-
C16:2	6.3	-	1.7	1.2	-
C18:0	-	7	1.5	0.4	5.6
C18:1ω9	4.2	10	4	4.5	43.5
C18:2	15.3	10.5	1.5	2.6	5.9
C18:3	12.2	20	-	0.7	-
C20:4ω6	1.9	-	5.3	3.4	-
C20:5ω3	8.0	9	29.6	33.8	-
Reference	(Bermúdez Menéndez et al., 2014)	(Natarajan et al., 2014)	(Adam et al., 2012)	(Adam et al., 2012)	(Wiyarno et al., 2011)

1.4.3.4.4 Enzyme-assisted extraction

The use of enzymes for FAME recovery is based on the degradation of the microalgae cell wall which then facilitates the release of lipids components (Maffei et al., 2018; Zuorro, Miglietta, et al., 2016). The use of enzymes for FAME recovery offers a reduced energy intense method, due to decreased temperatures required (Y. Wang et al., 2017). The process of enzyme recovery is based on the selected enzyme(s) breaking the cell wall of the microalgae, isolating the lipid and derivatizing the lipid to FAMEs. The most common enzymes which have been tested include novozym 435, cellulose, hemicellulose and lysozyme (Onumaegbu et al.,

2018; Y. Zhang et al., 2018). Figure 5 shows the lipid recovery for various enzyme(s) all with a concentration of 10 mg/L which was determined to recover the greatest volume of lipids by various studies by (Zuorro, Maffei, et al., 2016; Zuorro, Miglietta, et al., 2016). An investigation by (Qiu et al., 2019) showed that *Nannochloropsis* sp. lipids contain up to 60 % polar lipids which can be converted to FAMEs using enzymes (lipids were used for food applications in this study). Cellulose which was the most common enzyme to be used alone, presented lipid recovery of 53.2, 48.49, 11.73 and 24 % by different studies (He et al., 2020; Liang et al., 2012; Maffei et al., 2018; Qiu et al., 2019), respectively. Cellulose is commonly used in the degradation of microalgal cell walls which contain high concentrations of cellulose (75 % of cell wall) (Qiu et al., 2019; Zuorro, Miglietta, et al., 2016). The composition of the cellulose lipid was investigated by multiple authors and it was shown to have an average of 48.9 % SFA, 35.4 % MUFA and 15.5 % PUFA as seen in Figure 5 (He et al., 2020; Qiu et al., 2019). This composition of FA in the microalgae represents an ideal biodiesel raw material as it has very small percentage of PUFA (Martinez-Guerra et al., 2018). Novozym 435 is the most widely used commercially available enzyme for assisting in the production of biodiesel as it allows for high lipid recovery (Castillo López et al., 2015). The use of Novozym 435 also offers a robust enzyme which can be used multiple times (up to 110 times as reported by (Y. Wang et al., 2017)). As seen in Figure 5, the lipids recovered by Novozym 435 were shown to be 30.7, 29.7 and 39.8 % SFA, MUFA and PUFA, respectively (average of 3 studies by (Castillo López et al., 2015; Lopez et al., 2015; Y. Wang et al., 2017)). However, the compositions of these FA appear to have equal amounts of SFA, PUFA and MUFA which would mean they are not suitable for biodiesel production (Y. Wang et al., 2017; Wu et al., 2017; Zuorro, Maffei, et

al., 2016). Additionally, hemicellulose and lypozyme were also investigated and showed to recover favourable levels of lipids at 41 and 24.5 %, respectively (Ali & Watson, 2015; Castillo López et al., 2015; Maffei et al., 2018; Y. Wang et al., 2017; Zuorro, Maffei, et al., 2016; Zuorro, Miglietta, et al., 2016).

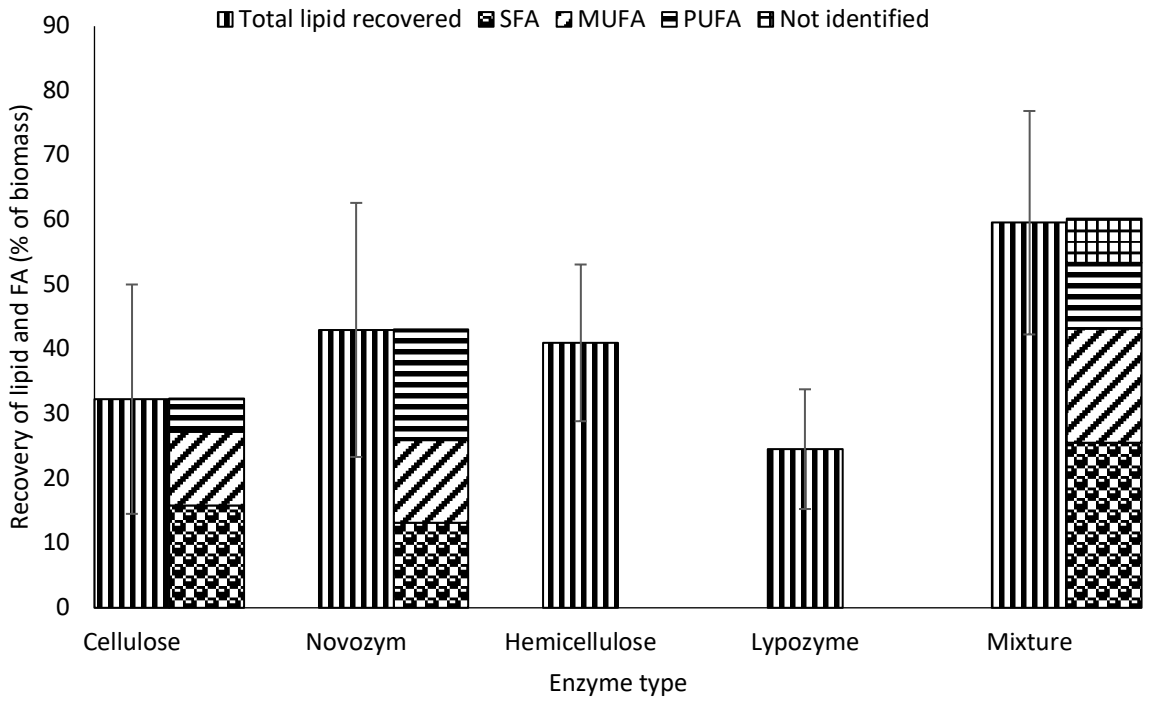

Figure 5: Summary of data gathered on the average of % lipid recovery and FA composition from *Nannochloropsis* sp. using different enzymes based on a number of studies (Cellulose: Zuorro et al., 2016a, 2016b, Qiu et al., 2019, Liang et al., 2012 and Castillo Lòpez er al., 2015; Novozym 435: Zuorro et al., 2016a, Wang et al., 2017 and Ali and Watson, 2015; Hemicellulose: Zuorro et al., 2016a, 2016b and Maffei et al., 2018; Lypozyme: Zuorro et al., 2016a and Lopez et al., 2015; Mixture: Zuorro et al., 2016a, 2016b, Wang et al., 2017 and Maffei et al., 2018.).

1.4.4 Hydrophobic membrane facilitated nitrogen recovery

1.4.4.2 Mechanism of membranes

Membrane contactors and hollow fibre membrane filtration have been used to treat WW for COD, BOD, total phosphorous and total nitrogen with removal at rates up to 85.8, 50, 97.5 and 99.8%, respectively (Aziz et al., 2019; X. Ma et al., 2019; Oyanedel et al., 2005). Whilst the use of membranes allows for a reduction in costs in treating WW due to its low operating costs, it also allows for viable by-products by recovering the nitrogen in the form of NH_3 to produce NH_4-based fertiliser (C. F. Bustillo-Lecompte & Mehrvar, 2015; Norddahl et al., 2006). Membrane contactors offer a hydrophobic porous membrane which acts as a partition between two phases to promote separation of contaminants from WW (Challinor, 2014). Membrane contactors are becoming a technology of interest due to their simple operation, high selectivity and low energy consumption (Kirsch et al., 2016). In theory, all free NH_3 can be removed using membrane contactors as long as there is enough H^+ ions in the stripping solution and sufficient contact time for the chemisorption process to occur (Wetting Phenomenon in Membrane Contactors - Causes and Prevention, 2014). Membrane contactors provide a large and stable interfacial area (Ulbricht et al., 2013). The mechanism of separation in this kind of membrane contactors is based on the mass transfer between two phases (Mandowara & Bhattacharya, 2011). Due to the hydrophobic nature of the membrane, liquid water streams will be kept outside the membrane while vapours will penetrate from the feed side with a higher partial pressure to the permeate side with a lower partial pressure (Rezakazemi et al., 2012). The driving force for liquid-liquid NH_3 removal by contact membranes is the difference in NH_3 partial pressure between the feed and the

absorbing liquid (Bonyadi & Chung, 2007). The hydrophobic microporous polymeric membrane provides the transfer area and restricts the permeation of water. The transfer takes place at the pore opening, inside the pore, or at the pore exit (Norddahl et al., 2006). As outlined by (Darestani et al., 2017), the transfer of gaseous species such as NH_3 from aqueous solutions across a hydrophobic membrane is assumed to occur in 5 stages: (1) transfer of NH_3 from the bulk solution to the boundary later at a membrane pore; (2) equilibrium of the NH_3 solution with gas (air) present in the membrane pores; (3) transfer of the NH_3 gas across the air filled pore; (4) reaction of the NH_3 gas with the receiving component (usually acid); (5) transfer of the NH_4^+ (due to pH shift $NH_3 \rightarrow NH_4^+$) salt through the boundary layer into the bulk strip solution (Mandowara & Bhattacharya, 2011). This process is usually set up in direct contact mode, i.e the feed and permeate streams in direct contact with the membrane which allows diffusion of the volatile components (EL-Bourawi et al., 2007). Figure 6 is a schematic showing a typical membrane contactor set-up (Brennan, Briciu-Burghina, et al., 2020).

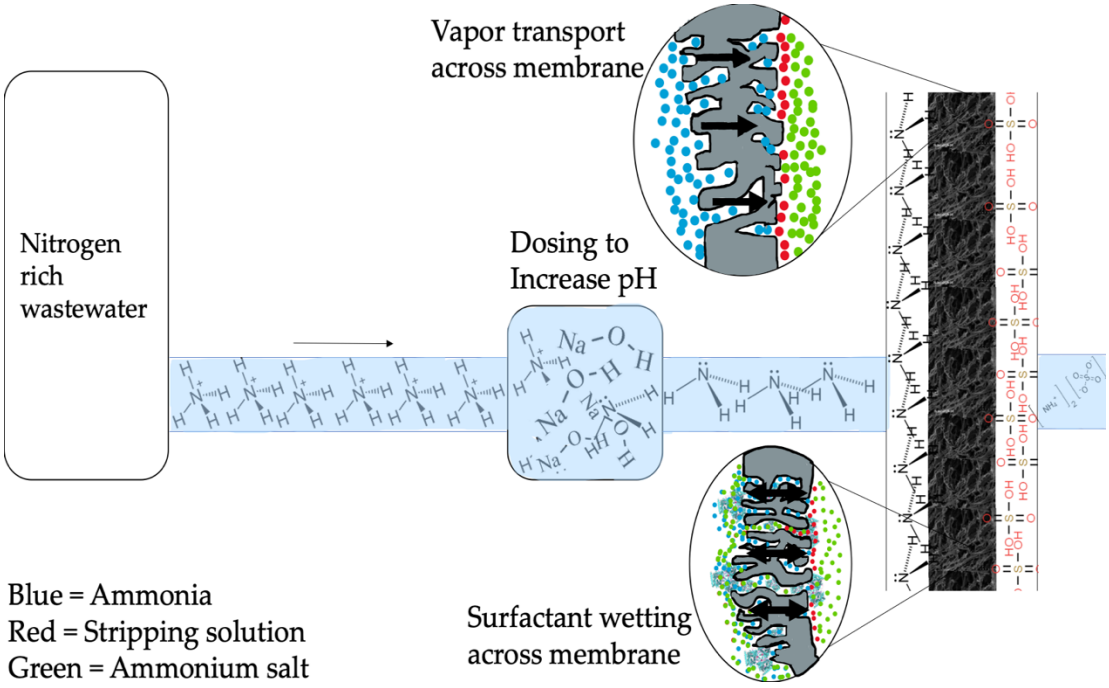

Figure 6: Schematic of typical membrane contactor set up (Brennan, Briciu-Burghina, et al., 2020).

The effect of feed temperature and stripping solution temperature should also be important with respect to NH_3 transfer across the hydrophobic membrane. A study carried out by (Licon Bernal et al., 2016) investigated the uses of nitric and phosphoric acid as the stripping solution and found that it resulted in an NH_3 recovery capacity of 95-98 % when free acid is present in the stripping solution. Additionally, a study was carried out by (Damtie et al., 2020) to compare different acids as the stripping solution (H_2SO_4, nitric acid, phosphoric acid, acid mix and water) and it was determined that H_2SO_4 recovered the greatest volume of NH_3.

1.4.4.3 Problems associated with membrane treatment

Problems associated with the use of membranes include the cost to initially set up the technology, blockage of pumps and membrane fouling (Kocherginsky et al., 2007). One of the major problems associated with the use of membranes for SHWW treatment is how susceptible they are to fouling and subsequently wetting (Vecino et al., 2019). The accumulation of particulates such as FOG, protein and OM can cause cake build up on the membrane resulting in the membrane fouling (Kirsch et al., 2016). Membrane failure is a huge economic influence on the use of membranes as they account for 72 % of the capital investment (P. D. D. Jensen et al., 2015). The types of foulants which may interfere with membrane performance include chemical foulants such as scaling, physical foulants such as deposition of particles, biological foulants such as microbes and organic fouling which interact with the membrane (Zularisam et al., 2010). Additionally, SHWW contains high levels of volatile fatty acids (VFA) which exert partial vapour pressure and are exported across the membrane with water vapor which cause contamination of the permeate and jeopardize the quality of the recovered NH_4-based fertiliser (Xie et al., 2016).

1.4.4.3.1 Membrane wetting and fouling

Normally the more hydrophobic a membrane is, the more repellent it is to water molecules which helps prevent wetting (Z. Liu et al., 2016). Membrane wetting is the process in which membranes lose their hydrophobicity and allow liquids to penetrate the membrane pores (as shown in Figure 6) (Guillen-Burrieza et al., 2016). Membrane pore wetting will result

in a direct liquid flow from feed through the wetted pores, substantially deteriorating permeate quality (Q. Liu et al., 2013). Wettability is controlled by chemical composition of the membrane and geometry of the pores (Xie et al., 2016). Components in SHWW such as fats, oils and protein can lower the liquid surface tension of the feed solution and cause wetting of the membrane pores (Darestani et al., 2017; Xie et al., 2016). Wetting on rough surfaces of membranes may be either be caused by the liquid fully penetrating the roughness grooves or by air being trapped underneath the liquid inside the rough grooves (Xie et al., 2016). Dissolved OM and colloids present in the nutrient rich waste streams can lead to membrane fouling (S. Wang et al., 2011). Liquid entry pressure (LEP) is the pressure which can be exerted on the membrane before the liquid penetrates the membranes pores. It is important for membrane materials to have a high LEP values to increase the lifetime of membrane material and reduce the chances of reduced hydrophobicity (Vecino et al., 2019).

SHWW contains high levels of oil which contribute to the wetting of membranes as the hydrophobic part of the oil adheres to the membrane surface. This results in the hydrophilic part being exposed and essentially giving the membrane a hydrophilic surface. A hydrophilic surface prevents vapours crossing the membrane, increases the pressure and results in membrane leaking due to increased pressure (Y. Chen et al., 2017; Z. Wang & Lin, 2017). The standard water quality generally required for hydrophobic membranes is 5-10 µm pre-filtration and a low fouling index (Xie et al., 2016). The wettability of a surface is directly related with the surface energy and hence, materials with a low surface energy have a high contact angle (CA) which is more immune to surface wetting (Darestani et al., 2017). It is expected that some alcohols such as ethanol can result in wetting of the membrane and more

superhydrophobic membranes are suitable to treat volatile bio-alcohols (X.-M. Li et al., 2007). The higher hydrophobicity of nanofibers is attributed to the increased surface roughness due to overlapping nanofiber layers, which results in less contact area for the solid fibre and water leading to higher CA (Q. Liu et al., 2013). The fouling and wetting of membranes impairs the membrane performance and shortens membrane lifetime, thereby reducing NH_3 recovery from SHWW which suggests that more research is required to fabricate membranes suitable for matrices with such high concentrations of oil (Xie et al., 2016).

1.4.4.3.2 Preventing membrane wetting and fouling

One method which could help overcome membrane wetting is incorporating superhydrophobic membranes which are more resistant to membrane wetting (W. Liu et al., 2018). Several authors including (Fu Liu et al., 2011), (X.-M. Li et al., 2007), (Tijing et al., 2014), (Y. Chen et al., 2017) and (Xie et al., 2016) showed that superhydrophobic membranes can be used against challenging waters as shown in Table 9. Superhydrophobic membranes are increasingly becoming the membrane of choice due to their anti-sticking, anti-contamination, anti-wettability and self-cleaning properties (Tijing et al., 2014). The results summarized that the incorporation of nanoparticles and alternative outer layers showed superhydrophobic characteristics (CA greater than 150 ˚ (X.-M. Li et al., 2007)) and were suitable for challenging matrices similar to SHWW. The incorporation of nanoparticles such as $AgNO_3$ and TiO_2 showed promising alternatives as they increase the surface roughness. (Xie et al., 2016) incorporated a hydrophilic layer on top of a polytetrafluoroethylene (PTFE) membrane and

achieved a CA of 150 ˚. This membrane is interesting as the hydrophilic part of the oil surfactants in the SHWW may adhere to the membrane (rather than hydrophobic parts like conventional membranes) which will result in a hydrophobic part of the oil being exposed to the water acting as a barrier to prevent wetting.

Table 9: Characteristics of superhydrophobic membranes fabricated to facilitate matrices similar to slaughterhouse WW (PVDF = polyvinylidene fluoride).

Membrane material	Superhydrophobic component	Fabrication method	CA (˚)	Reference
PTFE	Hydrophilic coating	Electrospinning	150	(Fu Liu et al., 2011)
PTFE	Omni phobic surface	Electrospinning	151	(X.-M. Li et al., 2007)
PTFE	$AgNO_3$	Electrospinning	158	(Tijing et al., 2014)
PVDF	Perfluoropolyether on hydrophilic substrate	Electrospinning	150	(Y. Chen et al., 2017)
PTFE	TiO_2	Electrospinning	150	(Xie et al., 2016)

An additional way of increasing the lifetime of membranes used for SHWW treatment is incorporating a cleaning mechanism to remove any surfactants. (Y. Chen et al., 2017) demonstrated that cleaning membranes with water and hexane to remove foulants allowed for the membranes to operate with increased fluxes. Various methods of cleaning contaminated membranes are discussed further in Chapter 4.

1.4.4.4 Case studies using membrane materials

A number of investigations have been carried out in order to determine the efficiency of membrane systems for removing NH_3 from challenging WW streams (summarised in

Table ***10***). Lab-scale studies by (Guo et al., 2019) and (J. Zhang et al., 2020) demonstrated that NH_3 could be removed from municipal WW and synthetic urea. (Guo et al., 2019) used a PVDF membrane with a mixture of nafion isomer and multiwall carbon nanotubes with a honeycomb surface to remove NH_3 from municipal WW from Hong Kong. The results suggested that the hollow fibre nafion isomer and multiwell carbon nanotubes support layer allowed for a greater NH_3 removal of up to 3 times greater than conventional PVDF membranes. Although this study showed high NH_3 recovery (69 %), it did not produce a product from the recovered permeate. Additionally, it was observed that higher fluxes were experienced by the conventional PVDF membrane after 12 hours due to membrane fouling and blocking but the nafion coated PVDF membrane could last up to 24 hours as nafion is more resistant to fouling. (J. Zhang et al., 2020) made a mixture of synthetic human urine and showed that the 80 % of the high NH_3 levels could be captured using polypropylene (PP) membranes. This high recovery of NH_3 allowed for the production of $(NH_4)_2SO_4$ if there is a sufficient volume of ions in the permeate side. This study also demonstrated the rejection of other compounds including PO_4^{3-} and K^+ allowing for the permeate to produce a purer product. The membrane stability was affected in terms of their hydrophobicity, mechanical and pore properties by the synthetic urine samples. However, this study used synthetic urine and more complications may occur when using real samples. (Damtie et al., 2020) also carried out a study using synthetic urine to recover NH_4-based fertilisers. This study produced a more complex synthetic urine matrix (compared to the study by Zhang et al.) and it compared different permeate solutions (H_2SO_4, nitric acid, phosphoric acid, blend of all acids and water). The results showed that any low pH acidic solution can be used on the permeate side and

their selection depends merely on the purpose for which the feed ammonium-based product is to be utilised for. H_2SO_4 showed to recover the greatest quantity of NH_3 by removing 82 % of NH_3 from the feed solution – however, this study does not report the purity of the product which is critical to determine product efficiency compared to other products on the market. Additional lab-scale studies were carried out by (Ratman et al., 2020) who investigated the use of polyethersulfone (PES) membranes to remove NH_3 from petroleum refinery WW which has a high oil content. The results from this study showed an increase in NH_3 when the membrane was treated with ultra-violet (UV) irradiation. Additionally, treatment of the membrane showed to increase the lifetime of the membrane as this matrix tends to degrade the membrane material. Disadvantages of using PES material include the fact it is a hydrophilic material which results in it requiring membrane surface treatment before being used with water samples.

Various pilot-scale studies have been carried out on challenging WW types. (Garcia-González & Vanotti, 2015) used PTFE membranes to treat manure waste and achieved 94 % NH_3 removal as seen in

Table ***10***. The authors stated that due to membranes being so selective, they remove the NH_3 which allows for greater quality methane gas production which leads to the production of biogas. (Wäeger-Baumann & Fuchs, 2012) used hollow fibre membranes on anaerobic digester effluent and recovered 70 % of the NH_3. Whilst this study achieved high removal rates, it does not meet effluent regulations and as such can't be the sole method of NH_3 removal. This study investigated the importance of the feed pH and temperature and it was determined that the pH must be greater than 10 to have a positive influence while the temperature was investigated between 20 and 40 ˚C and showed to have moderate impact. However, if lower temperatures allow for satisfactory NH_3 removal, they should be used in order to reduce energy costs. Additional studies by (Z. Liu et al., 2016) showed that 90.4 % NH_3 could be removed from radioactive waste and that the pH of the feed impacted the rate of NH_3 removal. This study demonstrated high removal rates, but membrane fouling persisted to be a problem which highlights the need for more robust membranes to be produced for challenging WW. (P. H. Lin et al., 2018) showed that PTFE membranes performed better than PP membranes to remove NH_3 from coking plant WW. The membranes allowed for 50 % reduction (from 628 mg/L to 300 mg/L) which would require further biological treatment. However, the costs of the biological treatment would be significantly reduced. Additionally, (Brennan, Briciu-Burghina, et al., 2020) also showed that PTFE membranes performed better than PP membranes. This study looked at the feasibility of membrane contactors to remove NH_3 from RCWW while producing a viable fertiliser which aided in reducing the costs of conventional treatment. Whilst this study demonstrated that a fertiliser product could be generated which reduced the costs of WW treatment, it did not efficiently reduce NH_3 levels

which would require further treatment. These two studies demonstrate that PP membranes are not robust, and fouling is easily caused by challenging WW.

Table 10: Overview of studies which used membrane contactors for recovery of ammonia from different WW sources.

Matrix	Scale	Membrane material	NH_3 recovery (%)	Product generated	Problems	Reference
Municipal WW	Lab-scale	PVDF	69	-	Membrane fouling	(Guo et al., 2019)
Synthetic urine	Lab-scale	PP	80	$(NH_4)_2SO_4$	-	(R. Zhang et al., 2020)
Synthetic urine	Lab-scale	PVDF	82	Ammonium-based fertiliser	-	(Damtie et al., 2020)
Petroleum refinery WW	Lab-scale	PES	93	-	-	(Ratman et al., 2020)
Swine manure	Pilot-scale	PTFE	94	No direct product	-	(Garcia-González & Vanotti, 2015)
Anaerobic digester effluent	Pilot-scale	-	70	-	-	(Wäeger-Baumann & Fuchs, 2012)
Radioactive plant	Pilot-scale	PTFE	90		Membrane fouling	(Z. Liu et al., 2016)
Coking plant	Pilot-scale	PTFE	50	Ammonium-based fertiliser	Membrane fouling	(P. H. Lin et al., 2018)
RCWW	Pilot-scale	PTFE	64	Ammonium-based fertiliser	Membrane fouling	(Brennan, Briciu-Burghina, et al., 2020)

1.5 Viable products from agricultural wastewater

1.5.1 Products generated from microalgae-assisted recovery

1.5.1.1 Biodiesel

Nannochloropsis sp. microalgae have received increased interest as sources of biodiesel due to the advantages offered such as carbon neutrality, reduced emissions and continues availability of biomass feedstock with the ability of fast growth (Cehn et al., 2018; Lee et al., 2014). Biodiesel can be either used in pure form or in combination with diesel for engines which do not need to be modified to facilitate them (Mubarak et al., 2015). Biodiesel

is synthesised by transesterification in which the triglyceride in the matrix reacts with a catalyst to yield esters of FA and glycerol (Ranjan et al., 2010). The need for biodiesel was introduced due to the increasing concern of climate change and the depletion of fossil fuel resources (Castillo López et al., 2015).

FA compositions of lipids extracted from *Nannochloropsis* sp. determines if the species is suitable for biodiesel production. Studies by (Ali & Watson, 2015) and (Y. Ma et al., 2014) showed that $C_{16:0}$, $C_{16.1}$, $C_{18:0}$ and $C_{18.1}$ were found in *Nannochloropsis* sp. The presence of SFA such as $C_{16:0}$ and $C_{18:0}$ have an influence on the cetane number which improves the ignition quality and increases the heat of combustion of the fuel. MUFA such as $C_{16.1}$ and $C_{18.1}$ are oxidatively unstable due to the double bonds which are susceptible to react with oxygen (Ali & Watson, 2015). It has been suggested that MUFA act as a balance between oxidative stability and low temperature properties (X.-N. Ma et al., 2016). The recommended FA ratio for optimal biodiesel is 5:4:1 for $C_{16.1}$:$C_{18.1}$:C_{14} (Huerlimann et al., 2010).

1.5.1.1.1 Biodiesel properties

The equations used to calculate the most important properties of biodiesel (including cetane number, saponification value, iodine number, viscosity, density and higher heating value) were reported by (Martinez-Guerra et al., 2018). Of these properties, the most important include the cetane number which indicates the fuel ignition quality, oxidation stability which should be high to avoid oxidation and to avoid longer storage times, viscosity values which are higher to alter injection spray characteristics damaging the chamber and

more viscous fuel can damage the fuel pump (Ali & Watson, 2015; X.-N. Ma et al., 2016). The required properties set out by the American and European fuel agencies is outlined in Table 11 (Martinez-Guerra et al., 2018). A study by (Y. Ma et al., 2014) showed that biodiesel could be successfully produced to consist of the suitable properties cetane number 54.61, iodine number 104.85 and low cloud point of 3.45 ˚C which are all in agreement with Table 11 and suggest a suitable product for engines could be produced using *Nannochloropsis* sp.

Table 11: Regulation set out by US and EU authorities for biodiesel properties for to be used in engines (Milano et al. 2018).

Property	Unit	ASTM 6751-02	EN 14214
Cetane number	-	≥47	≥51
Saponification value	(mg KOH/g)	-	-
Iodine value	(g I_2 100/g)	-	≤120
Degree of unsaturation	-	-	-
Cold filter plugging point	(˚C)	NA	≤5/≤-20
Viscosity	(mm^2/s)	1.9-6.0	3.5-5.0
Density	(g/cm^3)	NA	0.96-90
Oxidative stability	(h)	-	≥6

1.5.1.1.2 Biodiesel case studies

A study by (S. H. Teo et al., 2016) showed that the retention time to produce biodiesel from *Nannochloropsis* sp. can be performed in 20-30 min using a MW noncatalytic method. However, there is a number of problems still associated with the production of biodiesel from microalgae, most notably the cost of production due to cultivation and extraction (Goh et al., 2019; Lee et al., 2014; Onumaegbu et al., 2018). (Martinez-Guerra et al., 2018) reported that

the cost of biodiesel production is $1.32 per a litre which is not competitive with current fossil fuel oil prices, especially currently since the collapse of oil prices for the consumer due to Covid-19 (cost of production $1.39 and selling for $0.32 per L at a loss according to Shell oil website on March 20th, 2020).

Nannochloropsis sp. for biodiesel production is an attractive alternative to fossil fuels as it is compatible with current engines, has less environmental impact and satisfies all the required property characteristics (Carrero et al., 2015). A study by (T. H. Kim et al., 2015) found that *Nannochloropsis* sp. lipids and FA were capable of producing a biodiesel with 100 % yield at lab scale analysis. However, it can't be ignored that the economies of biodiesel production from *Nannochloropsis* sp. is still below the mark and requires improvements in the cultivation and drying methods in order to do so (X.-N. Ma et al., 2016). Some authors have predicted that it will be the year 2025 before microalgae can make a viable biodiesel product (Santos-Sánchez et al., 2016).

1.5.1.2 Omega-3

Omega-3 can be produced from $C_{20:5\omega3}$ and $C_{22:6\omega3}$ FA which are PUFA. They can be used to produce food, nutraceuticals and pharmaceuticals. Omega-3 provide health benefits to humans such as preventing cardio-vascular diseases, cancer, asthma, arthritis and high blood pressure (Aliev & Abdulagatov, 2017; Craggs et al., 2011). Furthermore, the intake of omega-3 containing FAs are known to play a role in controlling depression and promote animal growth (X. Li et al., 2019; X.-N. Ma et al., 2016). However it was reported by (Figueiredo et al.,

2019) that omega-3 consumption by humans worldwide is below the recommended level and thus the use of microalgae has become of interest in order to extract the FA to enrich foods or produce nutraceutical supplement tablets. The consumption of excessive amounts of omega-6 can result in the above health conditions but a low ω6:ω3 ratio (>2) counteracts this greatly (Huerlimann et al., 2010; Solana et al., 2014). PUFA such as $C_{20:5\omega3}$ and DHA have shown to successfully treat the above health conditions and the FA have been reported to be present in high concentration within *Nannochloropsis* sp. lipids (containing up to 30 % of the biomass) (Aliev & Abdulagatov, 2017; McKennedy et al., 2016; Santos-Sánchez et al., 2016). (X.-N. Ma et al., 2016) reported that the recommended daily intake of $C_{20:5\omega3}$ and DHA is 250 mg to 2 g in order to have a healthy lifestyle. A study by (Brennan, Regan, et al., 2020) reported that there was 0.36 mg $C_{20:5\omega3}$ in a 2 mg *Nannochloropsis* sp. sample meaning that in order to meet the recommended level, 694 mg of Nannochloropsis sp. would be required. Nannochloropsis sp. are a suitable species to supply these FAs as they have been reported to contain up to 50 % lipids in their biomass which is capable of doubling daily and producing high levels of $C_{20:5\omega3}$ when compared to other microalgae species (McKennedy et al., 2016; Ranjan et al., 2010).

Fish have traditionally been used to obtain these essential nutrients but the contribution of bad smells, taste and stability problems makes them an unsuitable source (V. Patil et al., 2005). In addition, the demand for fish is increasing while the fish stock worldwide is decreasing, so the use of microalgae is a suitable alternative (X.-N. Ma et al., 2016). Microalgae have been shown to be easily engineered in order to contain high concentrations of desired FA compositions. (Figueiredo et al., 2019) reported that the efficiency of $C_{20:5\omega3}$ and

DHA recovery is strongly dependent on the extraction method used which is supported by the literature. (Sá et al., 2020) amongst other authors showed that FA compositions can be altered during extraction processes by increasing the pressure using SC-CO_2 or using more polar solvents to increase $C_{20:5\omega3}$ levels from 11.8 to 581 mg/g (Aliev & Abdulagatov, 2017; X. Li et al., 2019).

1.5.2 Products generated using membrane-assisted recovery

The high levels of nitrogen present in SHWW represent a matrix which can be utilized to produce viable by-products. (Catarino et al., 2007) outlined a business eco-efficiency model which involves the act of reusing waste produced in a company in order to generate a viable product while using fewer natural resources and producing less waste. The recovery of nitrogen from SHWW would contribute to plant earnings whilst also reducing the cost of SHWW treatment (Johns et al., 1995). The use of nitrogen from SHWW to produce viable by-products has, however, received limited attention due to regulations set out by governing bodies. EU legislation does not allow for by-products to be produced from SHWW unless it is treated at 70 ˚C for 60 minutes (European Parliament and Council, 2009; Ortner et al., 2014). However, after the processing of animal parts in the rendering process the WW has undergone the required treatment and could be used to produce by-products such as fertilisers or NH_3 supplements (Deppe et al., 2016; P. H. Lin et al., 2018; Wosiack et al., 2015). Additionally, various studies have shown that $(NH_4)_2SO_4$ recovered using hydrophobic membranes can be used to produce many viable by-products including; agricultural fertiliser,

flame resistant products, food additives, wood preservatives, aid in protein purification and ammunition (Hshieh & Beeson, 1995; Vaneeckhaute et al., 2006; Wingfield, 1998; Younes et al., 2019).

1.5.2.1 $(NH_4)_2SO_4$ based fertiliser product

There is a high demand for nitrogen based fertilisers in the agriculture sector which is evident as of the total NH_3 produced worldwide each year, 85 % is consumed as fertiliser (Boehler et al., 2015; Vecino et al., 2019). Artificial fertilisers can be produced by recovering nutrients from WW which result in economic, environmental and energy consumption benefits (Darestani et al., 2017). As SHWW has high levels of NH_3, it has been identified as a suitable source of fertiliser production (Table 3). Conventionally, the Haber-Bosch method is used to produce NH_3-based fertilisers but developments in research allow for the production of a more efficient NH_3-based fertiliser. Recently, a study by (Lazouski et al., 2020) showed that gas diffusion electrodes could be used to split the hydrogen molecules from water to react with nitrogen to form NH_3 fertiliser which has great potential to be applied to SHWW. Additionally, the use of membrane contactor processes have shown recovery of NH_3 to produce $(NH_4)_2SO_4$ fertilisers (Brennan, Briciu-Burghina, et al., 2020; Malik, 2019). Hydrophobic membrane contactors with acidic solution on the permeate side (to provide the driving force for mass transfer across the membrane) allow up to 96 % recovery of NH_3, using sulfuric acid to produce a high value commercial $(NH_4)_2SO_4$ fertiliser (Sancho et al., 2017; Xie et al., 2016). The use of artificial fertilisers showed 70 % higher yield in crop growth in terms

of plant height and also showed that soil was much more nutrient dense after being dosed with the artificial fertiliser (Matheyarasu et al., 2016). The use of this fertiliser can be applied to promote the growth of leaves, develop strong roots, cures plant and vegetables of chlorosis and cures leaf yellowing (Weber et al., 2008). $(NH_4)_2SO_4$ fertilisers can be applied to land as either solid or liquid products and the summary of each method is outlined in Table 12. A study by (Brennan, Briciu-Burghina, et al., 2020) (also outlined in Chapter 5) produced a 30 % $(NH_4)_2SO_4$ liquid product from rendering condensate wastewater which has a similar composition to SHWW. The produced fertiliser was cheap to produce (€2.48/kg of nitrogen) and after pH adjusting it was suitable to be applied to large areas of land to promote growth. The methods of applying the liquid fertiliser can be carried out by a method called, controlled uptake long term ammonium nutrition (CULTAN) and contactless injection. CULTAN fertilization is a method of directly injecting the recovered nutrients into the ground in desired spots. The method allows for the distribution of the fertiliser to be even and increase the contact between fertiliser and plant roots (Deppe et al., 2016). The use of this direct contact fertilization method showed higher crop yield when compared with other fertiliser deployment types with the same concentration of nitrogen (Deppe et al., 2016). However, disadvantages include possible soil contamination of excess nitrogen or sulfate and the CULTAN fertilization method still has to be optimised to allow for longer operation times due to soils clogging injection ports (C. F. Bustillo-Lecompte & Mehrvar, 2015). The use of contactless injection was trialled by (Niemoeller et al., 2011) and the results showed injection depths of 70-90 mm were reached which meets the requirements to ensure optimal

fertilisation and crop growth. This method also had the benefit of injection pores not getting clogged.

Table 12: Comparison of solid and liquid $(NH_4)_2SO_4$ fertiliser (Niemoeller et al., 2011; Weber et al., 2008).

	Solid product	**Liquid product**
Purity	99.90%	40%
Application methods	Mixing granules with compost; dissolving the product and applying the soluble product to the surface of the plant; applying the crystal form to soil and watering it into the soil	Injecting into the soil by manual injecting fertilization (CULTAN); contactless high-pressure jet injection.
Cost of product	€7.50 per kg	€0.20 per kg

1.5.2.2 Other $(NH_4)_2SO_4$ based products

$(NH_4)_2SO_4$ can be added to flame retardant products in order to increase their combustion temperature of the material, decrease the weight loss rate and cause an increase in the production of residue or char (George, 1971). The use of $(NH_4)_2SO_4$ allows for an increased fire resistance as it loses its constitute elements which can burn to NH_3, SO_2, N_2 and H_2O (Mostashari & Mostashari, 2008). A study by (Hshieh & Beeson, 1995) showed that applying $(NH_4)_2SO_4$ to cotton fabrics increased the ignition time, residue yield and also decreased the amount of lost product. Additionally, (Mostashari & Mostashari, 2008) showed that applying $(NH_4)_2SO_4$ to materials increased the fire resistance as demonstrated in fabrics and wood allowing for the manufacturing of suitable fire blankets, fire doors *etc.* (Levan, 1984). The use of $(NH_4)_2SO_4$ has been a common additive to regulate the acidity of foods in the United States and has only become acceptable in the European Union since a report published in 2019

deemed concentrations under 300 mg/day safe (Younes et al., 2019). The addition of $(NH_4)_2SO_4$ to foods allows for an increased shelf life and strengthens the dough of the bread which allows the manufacturer to produce breads with longer lives. However, because this is being used for food, the product recovered from slaughterhouses may not be suitable under the EU law which states that WW products cannot be used for human food production (*EU Wastewater Directive 2010/ 75/0*, 2016; Fontes et al., 2013). Lastly, the use of $(NH_4)_2SO_4$ can be used to purify proteins through $(NH_4)_2SO_4$ precipitation. The method can allow for purification of proteins, folding and stabilizing protein structures, and concentrating proteins together in a solution (Wingfield, 1998).

1.5.2.3 Other NH_4-salt based products

Although H_2SO_4 is the most commonly studied acid to recover NH_3 using hydrophobic membranes - the use of nitric, hydrochloric and phosphoric acid have also been investigated in order to produce NH_4-nitrate, NH_4-chloride and NH_4-phosphate, respectively (Damtie et al., 2020). NH_4-nitrate has been used extensively as a highly rich nitrogen fertilizer and also as a component of explosives (Zapp et al., 2012). NH_4-nitrate fertilizer contains 33 % nitrogen allowing it to be a nitrogen rich fertilizer but it was reported by (Hecnar, 1995) that plants fertilized using NH_4-nitrate resulted in toxic conditions and were of harm to humans. Additionally, the storage of NH_4-nitrate is dangerous as large volumes can be easily ignited resulting in severe damages as reported by (Oxiey et al., 1992). Recently, 200-300 tons of NH_4-nitrate fertilizer were stored inadequately in a warehouse in Biuret, Lebanon which resulted

in an explosion which devastated the city in a 3 km radius and killed 190 people (Babraukas, 2020). NH_4-chloride is also used as a fertilizer and is most commonly used for rice crops in Asia (Zapp et al., 2012). In addition, NH_4-chloride has uses in food production as an acidifier, cough medicine and as a flux to coat metals for preservation (An, 2011). Lastly, NH_4-phosphate can be used as a stripping solution but due to the instability of the product, no viable product of commercial value can be produced (Schrödter et al., 2008).

2. Characteristics of challenging agricultural wastewaters

Chapter foreword

A thorough understanding of wastewater matrices is required in order to determine the most efficient treatment method and identify the most abundant components. Agriculture wastewater has shown to be a challenging matrix with high nitrogen levels requiring extensive treatment before being released into the environment. This chapter aims to identify the efficiency of novel treatment methods while determining viable products which could be produced while simultaneously reducing levels of potential contaminants. The potential of using a microalgae species, *Nannochloropsis* sp. to reduce nitrogen levels from dairy wastewater and produce viable products from its FA content. Additionally, this chapter fully characterises rendering condensate wastewater in terms of its physio-chemical characteristics and identify what by-products could be theoretically produced from its composition. The results demonstrates that *Nannochloropsis* sp. is capable of reducing nitrogen levels in dairy wastewater by up to 90 % and produce x g of biomass which could be used to produce viable products such as biodiesel or nutraceuticals as outlined in previous literature. Rendering condensate wastewater was characterised and the results demonstrate it is a very challenging matrix with high concentrations of chemical oxygen demand (10,813±427 mg/L), total Kjeldahl nitrogen (1745±90 mg/L), ammonia (887±21 mg/L), crude protein (10,911±563 mg/L), total phosphorous (51±1 mg/L), fat & oil (11,363±934 mg/L), total suspended solids (336±73 mg/L) and total dissolved solids (4397±405 mg/L) in the sample tested. Theoretical studies determined that this matrix could be utilised to simultaneously reduce contaminant levels and produce viable products (such as ammonium-based fertilizers, fire retardant products, protein supplements for animal feeds, recovery of acetic acid to

promote biological nitrification in wastewater treatment and the recovery of calcium hydroxyapatite to treat heavy metals in wastewater and soils). This chapter contributes the knowledge of identifying *Nannochloropsis* sp. a suitable matrix to treat nitrogen while also characterising RCWW for the first time.

2.1 Introduction

Agricultural WW has become of increased concern due to its challenging composition which causes damage to receiving waters. As discussed in the introduction chapter, dairy and slaughterhouse wastewater is of the greatest interest due to the size of the industries, the compositions of the WW and the potential for viable products. This chapter focuses on outlining the characteristics of dairy wastewater and how it can be used to cultivate microalgae which has the potential of producing viable products while simultaneously reducing nitrogen levels in the WW matrix. Additionally, a full physio-chemical characterisation of rendering condensate wastewater is carried out and potential products are identified which could allow for a reduction in those constituents.

The dairy industry processes high levels of salt for both nutritional and food conservation purposes and as such high levels of salt end up in the effluent (Omil et al., 1995). Similar to any agricultural WW effluent type, dairy saline WW contains high levels of nutrients and OM (J. Li et al., 2018). It has been reported that nitrogen levels up to 300 mg/L are present in dairy WW effluents, however, it is possible that there could be higher concentrations (Campos et al., 2002). Nitrogen from saline WW is conventionally treated by nitrification and denitrification using bacteria. However, the use of nitrifying bacteria can be greatly impacted by saline levels of <1 % as it causes osmotic stress on bacteria cells and result in substrate degradation (J. Li et al., 2018). Additional methods to treat saline WW and simultaneously produce viable by-products is cultivating microalgae on the saline WW pond (Church et al., 2017; Shen et al., 2015). High nitrogen removal was achieved as the *chlorella* microalgae

species consume the nitrogen to produce nucleic acid and proteins (Aravantinou et al., 2013). Microalgae are suited for saline WW treatment as they can easily adapt to saline WW through changes in their biochemical identity, biomass yield, pigment & FA formation and efficiency of removing pollutants (Vo et al., 2020). Salinity is required for microalgae to grow and some species can withstand up to 1000 ASW (Hellebust, 1985). Microalgae have the potential of producing high levels of FA which are of high value as seen in

Table *7*. These FA can be categorised as SFA, MUFA or PUFA which all have different functions (Brennan, Regan, et al., 2020). SFA and MUFA, as long as the right concentration of $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$ is present can produce a valuable biodiesel as discussed in Section 1.5.1.1. PUFA allow for the production of nutraceuticals such as omega-3 supplements from $C_{20:5\omega3}$ as discussed in Section 1.5.1.1.

Rendering plants process the unused materials of slaughterhouses such as carcasses or parts of animals, including products of animal origin not intended for direct human consumption (Arvanitoyannis & Ladas, 2008). The processing of these materials allows for the production of products such as animal-, bone- or bristle-meal as well as separating the fat from the materials to produce tallow (Metzner & Temper, 1990). In conventional rendering facilities, the WW from rendering plants is mainly generated during sterilization of the raw material and during the drying process of the waste meat/fat mixture (Metzner & Temper, 1990). The cooking vapours produced from this process are cooled and the vapour condensates are discharged into WW to be treated (Sindt, 2003). Metzner *et al.* (1990) reported that condensates are formed at a rate of 0.65 m^3 per ton of waste processed. WW from meat abattoirs have also been shown to have high levels of nutrients (nitrogen and phosphorous), FOG, crude proteins, COD, and solids (C. Bustillo-Lecompte et al., 2016). The European legislation for management of the treatment of RCWW, Animal By-Products Regulation (European Parliament and Council, 2009), is in place in order to prevent the outbreak and spread of diseases such as Bovine Spongiform Encephalopathy and Creutzfeldt-Jacob disease (Ware & Power, 2016). It has been reported that because of legal restrictions, rising treatment costs and environmentally conscious consumers, the treatment of RCWW

has become a major concern of the meat processing industry (Arvanitoyannis & Ladas, 2008). NH_3 discharge has a strong correlation with the amount of protein that has been degraded.

This chapter presents a full physiochemical characterisation of RCWW and outlines the characteristics of dairy WW which was used to cultivate *Nannochloropsis* sp. microalgae. No study has been carried out prior to this study characterising RCWW which allows for the identification of viable products. However, although the composition of dairy WW has been covered extensively, no study has identified it as being a suitable environment to cultivate *Nannochloropsis* sp. microalgae while reducing nitrogen levels.

2.2 Materials and methods

2.2.1 Materials

0.45 µm PTFE filter paper was procured from Radionics, Ireland. Solvents including acetone, hexane, *n*-hexane and ammonia hydroxide (NH_3OH) were procured from Fischer Scientific, Ireland. Standards including 25 % meta-phosphoric acid with 60 mM crotanoic acid, acetic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, propionic acid, pentadecanoic acid, a thirty-seven-component FAME mixture were obtained from Sigma Aldrich, Ireland. Additionally, NaOH, anhydrous sodium sulfate, hydrochloric acid and methyl orange reagents were obtained from Sigma Aldrich, Ireland. Reagent HACH kits were obtained to measure COD (high range 8000), TOC (high range 10173), total phosphorous (mid-range 10127) and orthophosphate (mid-range 8114) which were procured from Hach, Ireland. All chemicals and reagents used in this investigation were of analytical grade or better.

2.2.2 Sample collection and preparation

Microalgae, *Nannochloropsis* sp. were cultivated on dairy WW in a project partner site, Archimede Ricerche S.L. (Imperia, Italy). Saline and nitrogen levels was determined by the partners and the data was shared with DCU. RCWW samples were collected from a meat-slaughtering rendering plant in the south of Ireland. Samples were collected in sterile 2 L plastic bottles and conductivity, temperature, pH, DO and NH_3 were determined immediately after sampling.

2.2.3 RCWW characterisation

RCWW samples were characterised without filtration to determine a number of physicochemical properties, nutrients and micro-nutrients. Analytes were selected based on other studies focusing on wastewater contamination from rendering and meat slaughtering processes. Samples were tested for total phosphorous, total nitrogen, COD, TOC, FOG, TSS, TDS, HM, micronutrients (phosphorous, nickel, cobalt, potassium, magnesium, calcium, sodium, manganese, sulfate, sulfur, chloride) and VFAs. Samples were adjusted to pH 2 using concentrated hydrochloric acid for FOG, TOC and TKN analysis. Samples were adjusted to pH 11 for NH_3 quantitation using 1 mol/L NaOH.

Conductivity and pH were measured using a WTW Multi 320 multimeter; conductivity meter and pH electrode SenTix 4l, respectively. Temperature was measured using a Proplus® handheld multi-parameter instrument (YSI, UK), and NH_3 using an Orion NH_3 gas sensing ISE electrode. The COD, TOC, total phosphorous and ortho-phosphate were determined using spectrophotometer Hach model DR900 colorimeter according to Hach procedures 8000, 10173, 10127 and 8114, respectively. The TKN, FOG, TSS and TDS was carried out according to the APHA standard methods (APHA, 1990). Protein concentration was calculated by multiplying the difference between TKN and NH_3 by 6.25 (Jiang et al., 2011). Metal and nutrient analysis was carried out using inductively coupled plasma-emission spectroscopy (ICP-OES) according to AOAC method 43.293 (1980) at a commercial laboratory (ALS Scientific, Ireland).

2.2.4 Volatile fatty acid analysis

VFA analysis in the RCWW required extraction and derivatisation prior to analysis for determination by gas chromatography-flame ionisation detection (GC-FID) as carried out by (Erwin et al., 1961). This method was originally used to investigate VFA in blood and rumen samples but was most recently used by (Gunes et al., 2021) to investigate VFA in distillery WW. VFA extraction from the RCWW was performed by centrifuging 1.5 mL of the sample at 3000 × g for 10 min at 4 °C. 1 mL of the supernatant was transferred to a new centrifuge tube containing 200 µL of the internal standard (25 % metha-phosphoric acid with 60 mM crotanoic acid). The tubes were vortexed for 2 min and placed in a freezer for 3-4 h. The sample was then thawed and centrifuged at 12,000 × g for 15 min at 4 °C. 1 mL of the supernatant was then analysed using an Agilent 7890 GC connected to an Agilent 7693A. The GC-FID was equipped with an Agilent CP-FFAP column (25 m, 0.15 mm I.D., 0.25 µm film thickness). A 0.5 µL volume of the sample was injected into a split-less injector set to a temperature of 260 °C. Helium was used as the carrier gas at a constant flow rate of 1.5 mL/min. The initial column temperature was 115 °C and it was then increased to 175 °C at a rate of 15 °C/min; once it reached 175 °C, it was increased to 240 °C at a rate of 80 °C/min and held at this temperature for 3 min (total run time 9 min). The VFAs were detected by FID which was operated at 280 °C with a 30 mL/min hydrogen flow, 300 mL/min air flow and a make-up flow of 30 mL/min of helium. Blank injections of deionised water were performed every 5th run to ensure there were no contaminants retained by the column. The identification of unknown VFAs was achieved by comparing their retention times with known standards of acetic acid, propionic

acid, isobutyric acid, butyric acid, isovaleric acid and valeric acid. The quantification of VFAs was performed based on a 4-point calibration (25-100 % w/w).

2.2.5 Statistical analysis

Statistical analysis was carried out to determine if differences in the concentrations of NH_3, COD, DO and total nitrogen throughout the year during different seasons were significant. Analysis was also carried out on the RCWW flow rate to determine if there were higher volumes of WW at different times of the year. ANOVA was performed and followed up with *post hoc* Tukey tests where appropriate using Origin software (version 9.0) and p-values <0.05 were significantly different.

2.3 Results and discussion

2.3.1 Characteristics of dairy wastewater

The removal of excess nitrogen from WW persists to be a global urgency in order to protect receiving waters and the environment. In this study, microalgae *Nannochloropsis* sp. is used to treat nitrogen from saline WW from a dairy manufacturer. The nitrogen in the WW is consumed by the microalgae to produce nucleic acids and proteins while it uses the salt content to influence FA production within the microalgae cell which is of most interest in this chapter. Studies by (Church et al., 2017), (Babatsouli et al., 2015) and (Shen et al., 2015) have shown that the use of microalgae to treat saline WW can remove >95 % of nitrogen levels as

seen in Table 6. However, these studies mostly focus on *Chlorella Vulgaris* and not *Nannochloropsis* sp. Preliminary nitrogen analysis in the saline WW showed that initial concentration before *Nannochloropsis* sp. was added to the WW pond was 114 mg/L and 6 days after the nitrogen levels were almost depleted to <10 mg/L (corresponding to a 90 % N removal) at salinity levels of 0.8 %. No comparison study was carried out in this investigation but authors including (Church et al., 2017) and (Vo et al., 2019) showed that greater nitrogen removal could be seen when microalgae was present in the WW. Additional to the nitrogen removal, 15.6 g/m^2/day of microalgae biomass was grown while 28 kg dry solid algae were harvested/day which suggests that *Nannochloropsis* sp. allows for nitrogen removal and supplies microalgae biomass which could allow for by-products which will be discussed in detail in Section 3.3.5.

2.3.2 Characterisation of RCWW

It has been reported that rendering plants produce significant amounts of WW which contains contaminants that are relatively low in long-term environmental risks, but cannot be released directly to rivers, streams, or lakes without proper treatment (Sindt, 2003). The composition of raw RCWW is presented in

Table ***13***, indicating high levels of nutrients, OM, FOG and VFAs (

Table ***14***). Failing to appropriately treat the nutrients, solids and OM in RCWW can result in reduced DO levels which promotes eutrophication in the receiving waters (Thayalakumaran

et al., 2003). DO levels must not drop below 5 mg/L in order to sustain safe conditions for aquatic life (Roslan MY et al., 2019).

The pH is an important parameter determining the quality of the WW effluents because most chemical reactions in the aquatic environment are controlled by its value (Roslan MY et al., 2019; Sindt, 2003). It was found that the mean pH value of raw RCWW was 8.3, which was within the typical tolerance limits of 6-9 for the discharge of WW from abattoir industries (Sengupta et al., 2015).

Table 13: Physio-chemical characterisation of raw RCWW (n=3).

Nutrients		**Organic matter**		**Solids**	
Parameter	**Concentration (mg/L)**	**Parameter**	**Concentration (mg/L)**	**Parameter**	**Concentration (mg/L)**
Total phosphorous	51±1	**DO**	3.1±0.4	**TSS**	336±73
Orthophosphate	21±0.5	**COD**	10,813±427	**TDS**	4397±405
Total nitrogen	2720±82	**TOC**	2513±240		
TKN	1630±90	**FOG**	11,363±1942		
NH_3	887±21	**pH**	8.34±0.4		
Crude protein	10,911±563				

Heavy metals and micronutrients (mg/L)					
Phosphate	**Copper**	**Zinc**	**Lead**	**Chromium**	**Iron**
2.7±0.1	0.01±0	0.04±0	<0.01	<0.002	0.1±0
Potassium	**Cobalt**	**Nickel**	**Calcium**	**Magnesium**	**Sodium**
4.1±0	<0.002	<0.005	10.9±0.1	0.8±0.1	36.6±1.9
	Sulfate	**Sulfur**	**Chloride**	**Manganese**	
	10±0.8	68.8±2.5	4374±41	0.002±0	

2.3.2.1 Nutrients

Nitrogen and phosphorous are some of the most important parameters to be tested in effluent WW to determine its quality. NH_3 is produced from the biological degradation of proteins. TKN is the sum of organic nitrogen and NH_3. NH_3 is toxic to aquatic life at levels as low as 0.5 g/L (Kartohardjono et al., 2015). Total nitrogen in the raw RCWW was determined to be 2720±82 mg/L, TKN was 1630±9 mg/L, NH_3 levels were 887±2 mg/L and crude protein calculated to be 10,911±5 mg/L. (Sindt, 2003) reported that raw rendering plant WW typically has TKN values of 500 – 1000 mg/L. Nitrification and denitrification treatment may be carried out to remove up to 99 % of the total nitrogen present in the RCWW. The use of hydrophobic membranes has gained attention recently for the removal of NH_3 from WW as it can produce a viable product to generate revenue. This is discussed further in Section 4.3.6. The ratio of COD to TKN was determined to be >8, which is suitable for nitrogen removal by nitrification and denitrification (Thayalakumaran et al., 2003). Phosphorous levels may be introduced in the WW stream from meat or blood residues from the animal carcasses (Baker et al., 2020). Other sources of phosphorous may be synthetic detergents with high levels of phosphorous components which may be used during the rendering process (Ruzhitskaya & Gogina, 2017). Total phosphorous was measured at 51±1.1 mg/L. Orthophosphate was measured at 21±0.5 mg/L which is considered high, as orthophosphate is readily available for algae and aquatic plant growth. Various studies looking at phosphorous in WW from meat processing activities detected high levels of total phosphorous which must be treated. The most common method of removing phosphorous from WW involves the incorporation of phosphorous molecules into the TSS using the biological method as described by (Ruzhitskaya & Gogina, 2017). The ratio of COD and total phosphorous obtained was greater than 50 which means biological

treatment can be used to successfully treat total phosphorous before being released to meet local regulations (Thayalakumaran et al., 2003).

2.3.2.2 Organic matter

Reducing high levels of OM being released into water streams can help protect aquatic life from low DO levels. RCWW was measured to have DO levels of 3±0.4 mg/L. This low DO level is caused by the high COD, TSS and FOG levels present in the RCWW. OM present in influent and effluent is measured by COD and TSS (Vecino et al., 2019). High COD levels at 10,813±427 mg/L were measured in the RCWW. High levels of COD may be due to both biodegradable and non-biodegradable OM materials such as animal matter, FOG, nutrients and proteins (Islam et al., 2019). TOC levels were determined to be 2513±340 mg/L. TSS levels in the RCWW are 336±73 mg/L which is almost 10 times the discharge limit set out by the EPA of 35 mg/L (Ryan et al., 2016). TSS levels may be influenced by animal tissue, fats and soils from hides & hooves of animals (Verma et al., 2013). The RCWW had a high level of FOG at 11,363±934 mg/L, which is due to the high levels of unwanted tissue in the rendering process. These results indicate that raw RCWW is highly polluted and must undergo sufficient treatment before being released into receiving waters. All HM species that were measured in the raw RCWW samples (

Table ***13***) were below the EPA discharge limits. Nickel, cobalt, potassium, magnesium and manganese were at low levels in the RCWW. Calcium is found at a high level at 10±0.1 mg/L which is most likely due to the CaHA which makes up 57 % of the cattle bone composition (Field et al., 1974). The high levels of sodium (35.6±1.9 mg/L) may be due to tissue and blood

from animal waste (Ferawati et al., 2017). The RCWW has high levels of sulfur (68.8±3.5 mg/L) which could be attributed to the by-products of animals of a protein nature in the rendering process since sulfur is a constituent of some proteins.

2.3.2.3 Volatile fatty acids

VFAs are fatty acids with carbon chains with fewer than 6 carbons ($C_1 – C_5$). Various studies have looked at VFAs in WW produced from meat processing activities and have identified that acetic, propionic and butyric acid as the most abundant VFAs; whilst also identifying isobutyric and isovaleric acid in the WW composition (Metzner & Temper, 1990). VFAs are a carbon and energy source for microorganisms in the nitrification and denitrification processes which makes them important during WW treatment (Maroneze et al., 2014).

Table ***14*** shows the quantitative results obtained from the analysis of VFAs in RCWW using GC-FID while Figure 7 presents the chromatogram obtained for VFA sample analysis. The chromatogram shows low peak resolution and selectivity which may be due to the method used was optimised for a different matrix. One method of improving separation efficiency is selecting the optimal mobile phase or carrier gas. Helium is most commonly used with capillary columns as it allows for high flowrates while maintaining strong efficiency. Helium has also been reported to allow for reproducible retention times allowing for validation using standards (Hage, 2018). Future work should focus on the optimisation of the separation method to allow for optimal resolution. The results indicate that acetic, propionic and butyric acid are the most abundant VFA species present in the sample at 1519.67±36.34, 821.15±17.38 and 738.15±93.38 mg/L, respectively. These results support a large number of

studies showing these VFAs to be the most abundant with acetic acid at the highest concentration (% abundance in rendering WW according to (Auterská & Novák, 2006); acetic acid 51 %; propionic acid 26 % and butyric acid 9 %) (Auterská & Novák, 2006; Longo et al., 2015; Metzner & Temper, 1990; Mkhize et al., 2014).

Table 14: VFA analysis in RCWW using GC-FID (n=3).

Parameter	Concentration (mg/L)
Acetic acid	23,087±3208
Propionic acid	19±3.0
Isobutyric acid	4082±1349
Butyric acid	20,352±6061
Isovaleric acid	3172±738
Valeric acid	3507±0

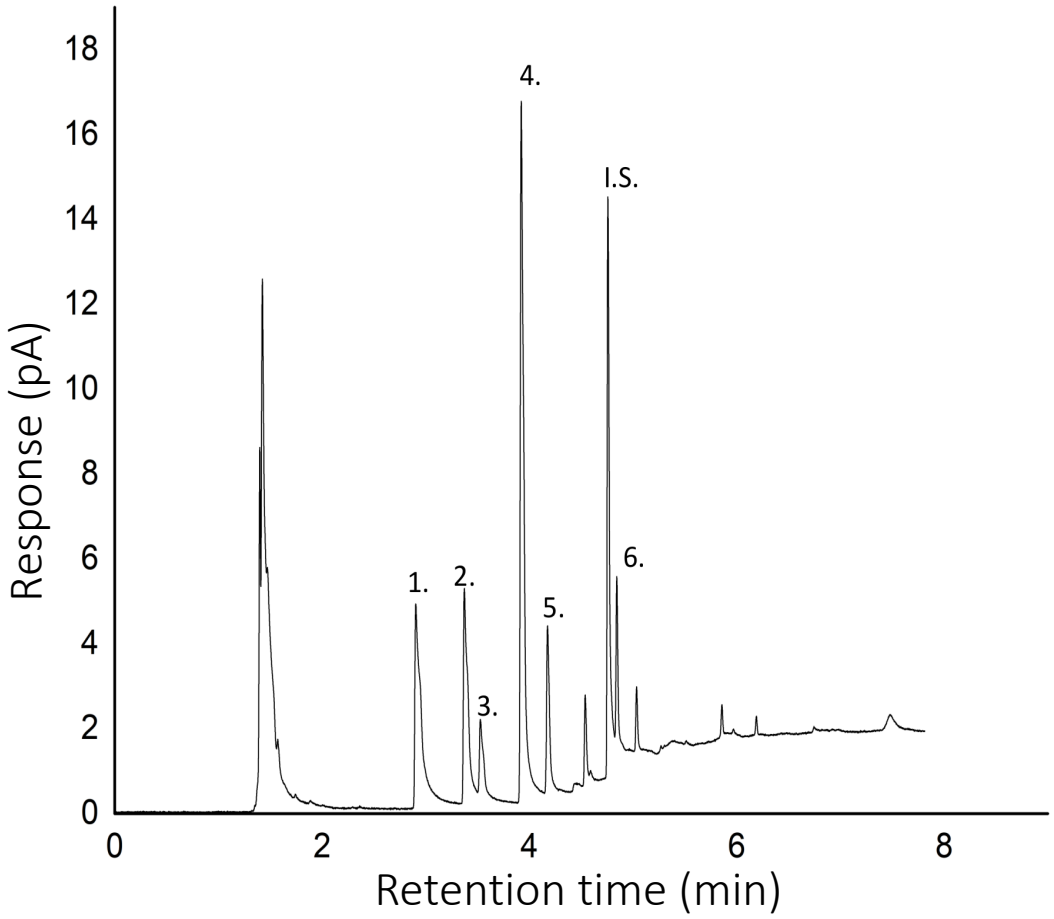

Figure 7: Chromatogram obtained from analysis of VFA in RCWW sample by GC-FID (1=acetic acid; 2=propionic acid; 3=isobutyric acid; 4=butyric acid; 5=isovaleric acid; 6=valeric acid).

2.3.3 Identification of viable products from agricultural wastewater

2.3.3.1 Viable products from dairy WW

The purpose of this thesis is to identify the products which could be generated from dairy wastewater effluent. The high saline and nitrogen levels in dairy WW were utilised to promote the growth of *Nannochloropsis* sp. which can be used to extract FAs to produce viable products. A literature review of the possible products which could be generated from the typical FA found in *Nannochloropsis* sp. is outlined in Section 1.5.1. However, no study has previously characterised FA from *Nannochloropsis* sp. cultivated from saline WW which is carried out in this thesis and discussed later in Chapter 3. However, the results presented in this chapter suggest that dairy WW is a suitable matrix to cultivate *Nannochloropsis* sp. due to its high nitrogen and saline levels which promote the species growth.

2.3.3.2 Viable products from RCWW

RCWW presents to be a very challenging matrix which must undergo rigorous treatment prior to being released which is an expensive process in order to meet local effluent regulations. A way of off-setting the cost associated with environmental compliance is to incorporate these species into a production process to yield viable by-products which would result in a cleaner effluent (Arvanitoyannis & Ladas, 2008). This would allow for nutrients and other contaminants to be reused which could help reduce the formation of bio-sludges and wastes for disposal (Ferawati et al., 2017). Table 15 shows an overview of the possible products which could be produced from the constituents present in RCWW.

Table 15: Possible products from RCWW.

Constituent	Product
Nitrogen	Ammonium-based fertiliser; flame retardant chemicals
Volatile fatty acid	Carbon source for denitrification; cosmetics
Protein	Animal feed
Calcium hydroxyapatite	Ion-absorber for heavy metals

Utilisation of nitrogen content: (Lazouski et al., 2020) demonstrated that the use of conventional gas diffusion electrodes can be used to produce NH_3 fertiliser from nitrogen and water-splitting-derived hydrogen which could potentially be derived from WW (Lazouski et al., 2020). However, this method is only capable of producing a NH_3 fertiliser whereas the use of membrane distillation allows for the production of $(NH_4)_2SO_4$. Hydrophobic membranes have become increasingly popular for extracting NH_3 from WW streams (Boehler et al., 2015; Brennan, Briciu-Burghina, et al., 2020). One of the major benefits of using membranes to treat NH_3 in WW is the production of an NH_4 salt (most commonly $(NH_4)_2SO_4$) as a by-product (Darestani et al., 2017). $(NH_4)_2SO_4$ is a fertiliser which can be applied to land to help promote the growth of crops in alkaline soils. $(NH_4)_2SO_4$ contains high levels of nitrogen and sulfur (21 and 24 % composition, respectively). The NH_4 ion is released, and it undergoes deprotonation which produces NH_3 and results in lowering the pH of the soil. It also contributes nitrogen which is essential for plant growth. The sulfur promotes the metabolism of nitrogen and chlorophyll formation and forms amino acids which are the building blocks for proteins (Darestani et al., 2017). There is no iron present in $(NH_4)_2SO_4$ but reducing the soil pH allows for iron to be absorbed more effectively by plants (Darestani et al., 2017). The use of $(NH_4)_2SO_4$ has also been used in flame retardant chemicals as it increases the combustion

temperature of the material, decreases maximum weight loss rates, and causes an increase in the production of residue or char (Darestani et al., 2017). The use of hydrophobic membranes has been shown to remove up to 99 % of NH_3 from WW in 5 h while producing a 30 % pure $(NH_4)_2SO_4$ product (Rezakazemi et al., 2012). The high levels of NH_3 present in RCWW makes it an ideal matrix to produce $(NH_4)_2SO_4$ fertiliser. It was calculated that the present RCWW produces an average of 4.7 million kg of NH_3 a year which would allow for the production of up to 711,866 kg of 30 % $(NH_4)_2SO_4$ equating to 1.1 million euro in revenue. This revenue would substantially reduce the cost of NH_3 removal. The disadvantages of using the liquid fertiliser include possible surface and ground water contamination, odour problems, greenhouse gas emission, and soil pore clogging from excessive fat loads (Mulu & Ayenew, 2015).

Utilisation of other constituents: The demand for meat and other protein rich products has increased in recent years which has resulted in calls for alternative raw materials to produce protein rich animal feeds to be produced. Crude protein was measured to be 10,911±563.7 mg/L in the RCWW which suggested that there is great potential for protein to be extracted from the RCWW for animal feeds. UF has been widely used to simultaneously purify, separate and concentrate protein materials from WW sludge. It should also be noted that the removal of protein from WW reduces the COD by 75 % (Arvanitoyannis & Ladas, 2008).

Denitrification is the loss of nitrogen molecules from NO_2^- or NO_3^- resulting in the release of N_2 gas. The denitrification process can be enhanced by adding natural carbon

sources to the WW (De Lucas et al., 2005). RCWW produces large volumes of VFA at reasonably high concentration which could be recovered and spiked into the WW system to promote denitrification (De Lucas et al., 2005). There is a total of over 3700 mg/L VFA in the RCWW sample (

Table ***14***). The most abundant VFA is acetic acid (1519±26 mg/L), which is known to be an efficient agent for enhancing biological nutrient treatment and has shown to reduce total nitrogen and total phosphorous in WW up to 95 %, over a retention time of 8 hours (Lim et al., 2000).

HM in RCWW is present at low concentrations and suggest that they do not pose a threat to the environment, however this is not the case for many industrial wastewater streams. CaHA which makes up 60 % of cattle bone composition, has been shown to be effective in the removal of HM from WW by means of absorption and ion exchange (Arvanitoyannis & Ladas, 2008; Cheung et al., 2001). The use of CaHA also allows for a 45 % cost reduction for removing HM compared to conventional methods (Deydier et al., 2003). The Ca in RCWW which is a component of CaHA has a concentration of 10±0.1 mg/L, and purification and use for HM removal has been shown to be feasible in other studies which could be applied to this matrix (Cheung et al., 2002).

2.4 Conclusion

The recovery of nitrogen from agricultural WW would allow for a simultaneous benefit of treating nitrogen concentrations and producing revenue for a company. This chapter investigates the characteristics of dairy WW and RCWW to identify viable products which could be recovered and if they are compatible with novel treatment and recovery methods. Dairy WW was reported to have levels of nitrogen at 114 mg/L and the cultivation of *Nannochloropsis* sp. on dairy WW pond allowed for 28 kg/day of microalgae biomass while reducing nitrogen levels by 90 %. Previous research has identified *Nannochloropsis* sp. to have characteristic FA for nutraceuticals and biodiesel, but these studies were cultivated on different matrices. Chapter 3 of this thesis will characterise FA from *Nannochloropsis* sp. cultivated from dairy WW to identify any viable products. A full physio-chemical characterisation was carried out on RCWW and constituents measured included COD, TN, NH3, crude protein, TP, FOG, TSS and TDS at 10,813 ± 427, 1745 ± 90, 887 ± 21, 10,911 ± 563, 51 ± 1, 11, 363 ± 934, 336 ± 73 and 4397 ± 405 mg/L, respectively. These constituents, in their current form, are higher than the discharge limits outlined by the Irish EPA (which follows EU regulatory limits) for water discharge into fresh and marine water bodies, and thus the raw RCWW must undergo treatment accordingly. a preliminary study was carried out to determine if viable products could be produced from RCWW based on the RCWW composition and other studies. It is suggested that, based on other studies and the concentration of NH_3 in this study, the NH_3 could be recovered by using membrane distillation to produce a viable ammonium sulphate fertilizer. Proteins could be recovered by using UF, to produce a protein supplement for animal feed. VFA could be recovered by using membrane filtration, to recover acetic acid, which could be used to aid in biological microbial treatment

of WW, and CaHA could potentially be recovered for use as an ion-absorbent, for removal of HM. Valorisation of these challenging WW matrices show great potential to produce viable nitrogen-based products while simultaneously reducing nitrogen levels.

3. Optimisation of fatty acid recovery methods for viable products from *Nannochloropsis* sp. cultivated on dairy wastewater

Chapter foreword

MW assisted extraction derivatization was investigated for preparing FAME derivatives to analyse the fatty acid composition of *Nannochloropsis* sp. microalgae. This method was compared against conventional derivatization and extraction methods and a direct derivatization without prior extraction. The derivatized FAMEs were analysed using GC-MS to identify and quantify the FAs present. Conventional methods have been carried out for years, but they are time consuming, require high temperatures and harmful chemicals. The developed MW assisted method is 5 times quicker (15 min) compared to the conventional and direct derivatization methods (75 min). The MW assisted method recovered significantly more FAs (51 wt %) from microalgae samples compared to the conventional (42 wt %) and direct derivatization (34 wt %). Additionaly it was shown that a sample extraction step is critical to improving the derivatization yield of algal samples for both oven assisted and MW techniques. Fourteen FA components were identified in *Nannochloropsis* sp. microalgae samples which was evaluated for its potential use for biodiesel and biopharmaceutical products. The developed MW method allows for a rapid, robust and accurate preparation of algae samples in order to determine their FA composition.

3.1 Introduction

Nannochloropsis sp. microalgae are complex organisms which have become of interest in recent years due to their composition, especially the lipids present. FA are the largest component of lipids and the physical, chemical and physiological properties of a lipid class depend on its FA class (Ichihara & Fukubayashi, 2010). The lipids and FA present in algae have gained increasing interest due to their potential to contribute to the production of biodiesel and pharmaceuticals (Laurens et al., 2012). Biodiesel made from FA and lipids present in bio-products such as algae can offer a more sustainable and green method to provide energy (Maghraby & Fakhry, 2015). The benefits of using algae for biodiesel production include its ability to grow in fresh water or marine environments without the need for arable land (L. Chen et al., 2012). Microalgae have also been utilized for treating sewage waste and saline WW (Aliev & Abdulagatov, 2016) along with providing a means for the production of polyunsaturated FAs (PUFA), pigments, antioxidants, pharmaceuticals; and biomass for animal feed, fertilizer and energy crops (Sahu et al., 2013).

In order for FA composition to be identified and quantified by GC-MS, they must be converted to FAME (Indarti et al., 2005). Conversion of FAs to FAMEs increases the volatility and thermal stability of FAs by preventing decarboxylation of the acid group during injection, additionally elimination of the free acid improves GC peak shapes and detection of FAs by GC (Brunton et al., 2015; Indarti et al., 2005; Kinsella et al., 1977). Sample preparation prior to GC-MS, requires a series of drying, hydrolysis, extraction, purification, transesterification and analysis steps (Indarti et al., 2005), and the most common procedure used FA sampler

preparation and analysis was developed by (Bligh & Dyer, 1959). Extraction is the first step in the analysis of FAs present in algae as it allows isolation of the FA and triglyceride components present in the sample (Mandal & Hemalatha, 2007). The extraction step eliminates undesired compounds such as proteins, carbohydrates and salts that could interfere with derivatization (Mandal & Hemalatha, 2007). After extraction, the TGA and FAs are converted into FAMEs by derivatization. This is most commonly carried out by hydrolysing the TGA in the presence of a strong acid or base (such as NaOH or KOH (R. L. Liu et al., 2012)) in order to break up the TGA. The sample then undergoes transesterification in the presence of an acid or base catalyst, such as boron trifluoride (BF_3), with an alcohol (typically methanol or ethanol) to form the desired FAME derivatives (Ostermann et al., 2014)-(Dahiya, 2015). The FAMEs are then extracted into an organic solvent to allow for analysis and quantification by GC-MS (Brunton et al., 2015). FA recovery efficiency using the Bligh Dyer method was shown to be up to 98 % (Tammekivi et al., 2019). The quality of the derivatization can also be assessed by using thin layer chromatography as demonstrated by (Gehrke & Goerlitz, 1963) but this does not quantify the amounts of FAMEs present. Conventional derivatization methods have proven to be effective, however, they have a number of disadvantages. Typically, these methods require high temperatures, are time consuming and the multiple steps required for sample preparation increase the probability of errors (Khoomrung et al., 2012). As the interest in bio-products derived from algae has increased in recent years, it has become important to develop a quick and reliable method for FAME analysis.

There have been a number of investigations evaluating simplified extraction and derivatization procedures for FAME preparation on a range of different sample types

(Abdulkadir & Tsuchiya, 2007). MW irradiation is a well-developed method that has been used extensively in organic synthesis, derivatizations and extractions (R. L. Liu et al., 2012). There are a number of advantages to MW assisted extraction and derivatization as a method to prepare FAMEs from lipid containing samples; such as ease of operation, lower energy consumption, shorter reaction times and faster heating provided by the apparatus (R. L. Liu et al., 2012). Another benefit to MW-assisted recovery is that it allows for a more uniform thermal transfer and ensure that all the samples is heated up equally unlike a heating block which heats sample vials from the walls/bottom (R. L. Liu et al., 2012). (R. L. Liu et al., 2012) used MW-assisted recovery to investigate a one-step method for FAME analysis of herbal medicines. Their results showed that MW-assisted recovery was more energy efficient, reduced sample preparation time and improved extraction and derivatization efficiency compared with the conventional methods. Similarly, (Brunton et al., 2015) employed MW-assisted recovery for determination of FA in food and found it to be rapid, simple and it provided superior recoveries compared to conventional methods. A study by (Aliev & Abdulagatov, 2016) looked at the use of supercritical fluid extraction for preparing FAMEs from algae. This method had the advantage of being a high speed, low temperature and an oxygen free sample preparation procedure. However, the apparatus and process required is complex compared to conventional or MW-assisted method.

There has been an increase in the use of MW-assisted recovery for preparing FAMEs in recent years and with that, an efficient method should be developed for the analysis of algae. An ideal method should be quick, simple, precise and accurate (Khoomrung et al., 2012). This study focuses on developing a quick, simple and efficient MW-assisted recovery method for

Nannochloropsis sp. and comparing it with conventional derivatization methods, such as the method reported by Bligh and Dyer (Ostermann et al., 2014). This study also investigates the optimum extraction and compares the conventional method to direct derivatization techniques without prior extraction prior to improve derivatization speed and workflow.

3.2 Materials and methods

3.2.1 Materials

Nannochloropsis sp. microalgae was provided by Archimede Ricerche S.L. (Imperia, Italy). The algae samples were stored at 4 °C in glass vials. Solvents including isopropanol (IPA), ethanol, hexane, cyclohexane, acetic acid and toluene were procured from Sigma Aldrich, Ireland. Acetone and methanol (MeOH) were obtained from Honeywell, Ireland. Chloroform was procured from Fisher Scientific, Ireland. The internal standard, pentadecanoic acid ($C_{15:0}$), a 37 component FAME standard and derivatization reagent Boron trifluoride (BF_3) (10 % in methanol) were procured from Sigma Aldrich, Ireland. All other chemicals and reagents including sodium hydroxide (NaOH), potassium hydroxide (KOH), sulfuric acid (H_2SO_4), hydrochloric acid (HCl), sodium chloride (NaCl) and sodium carbonate (Na_2CO_3) were obtained from Sigma Aldrich, Ireland. All chemicals used in this investigation were of analytical grade or better. Deionized (DI) water was obtained from an Elga, Purelab Ultra system.

3.2.2 Optimisation of Methods

Three methods were investigated to find the optimum procedure to extract and derivatize FAs present in algae. The FA analysis methods investigated include a conventional extraction and derivatization procedure, a direct derivatization procedure which received no extraction prior to derivatization (Applegate, 2007); and a MW assisted extraction derivatization procedure. All optimization experiments were carried out in triplicate (n=3). Investigations were performed as per the flow chart Figure 8. The peak area responses and number of FAME peaks detected were used to determine which conditions were optimal. Each procedure parameter (*e.g.,* extraction solvent, fractionation solvent, hydrolysis reagent, hydrolysis reagent volume, transesterification reagent volume, hydrolysis reaction time and transesterification reaction time for the conventional method) was sequentially optimised and the best condition from each step was then carried forward to subsequent optimization experiments (Figure 8). Throughout the methods section, optimum conditions have been bolded.

3.2.2.1 Optimisation of conventional extraction

The conventional method of extraction was modified from that outlined by (Ostermann et al., 2014). The extracting solvent (acetic acid, acetone, ethanol, IPA, methanol) and the fractionation solvent (chloroform, cyclohexane, hexane and toluene) were varied (Figure 8). The sample was extracted by weighing out 2 mg of the algae sample and dissolving it in 30 µL of extraction solvent and then spiking it with 1 mL of 3.1 mg/L internal standard in MeOH. 15

µL of the fractionation solvent was added and the sample was then shaken for 4 min (Clifton 62551). A 500 µL volume of DI water and 500 µL fractionation solvent was added to separate the organic and inorganic layers which was aided by shaking the sample for 2 min and centrifugation (Eppendorf centrifuge, model 5804) for 5 min at 3500 × g. The aqueous layer was collected and extracted again using the same procedure. The organic layers were then combined and evaporated with nitrogen gas to dryness. This dried extract was then derivatized as follows.

3.2.2.2 Optimisation of conventional derivatization procedure

The dried algae sample extract was mixed with a volume (0.5, 1.0, 1.5 or 2 mL) of 0.5 mol/L hydrolysis reagent (NaOH, KOH, Na_2CO_3, HCl or H_2SO_4), and hydrolysed in an oven at 80 °C (*Technico TEC-240-010S)* for a range of reaction times (15, 30, 45, 60, 75 and 90 min). Transesterification was performed at 100 °C using 10 % BF_3 in MeOH, and the amount of BF_3 was optimized (1, 2 and 3 mL) in addition to the reaction time (10, 20, 30 or 40 min). Once transesterified, 2 mL of DI water and 1 mL hexane was added to the sample and it was shaken for 4 min and centrifuged at 3500 × g for 5 min. The hexane layer was then collected and analysed by GC-MS.

3.2.2.3 Optimisation of direct derivatization

The optimisation of the direct derivatization method was carried out as outlined in Section 3.2.2.2 without performing the initial extraction step (Section 3.2.2.1). Briefly, 2 mg of the

algae sample was spiked with 1 mL of 3.1 mg/L internal standard and then derivatized as per section 3.2.2.2. The order in which this investigation was carried out is outlined in Figure 8.

3.2.2.4 Optimisation of MW method

A 2 mg quantity of microalgae sample was added to a glass vial (Borosilicate glass with a Teflon cap). 10 µL of extraction solvent (acetic acid, acetone, ethanol, IPA, methanol), 30 µL of the fractionation solvent (chloroform, cyclohexane, hexane and toluene) and 1 mL of 3.1 mg/L internal standard was added to the vial. 1 mg of solid NaOH was added into the solution. The samples were placed in a MW reactor (Anton Parr, Monowave 300 Ireland) operated at a power of 435 W, at a temperature of 45 °C, for three reaction times (10, 15 and 20 min). Following the reaction, different volumes (10, 20 and 30 µL) of 1 mol/L NaCl were added and the sample was left to cool for 1 min. A volume of 1 mL of fractionation solvent and 1 mL of water was added. The organic layers were then collected and analysed by GC-MS.

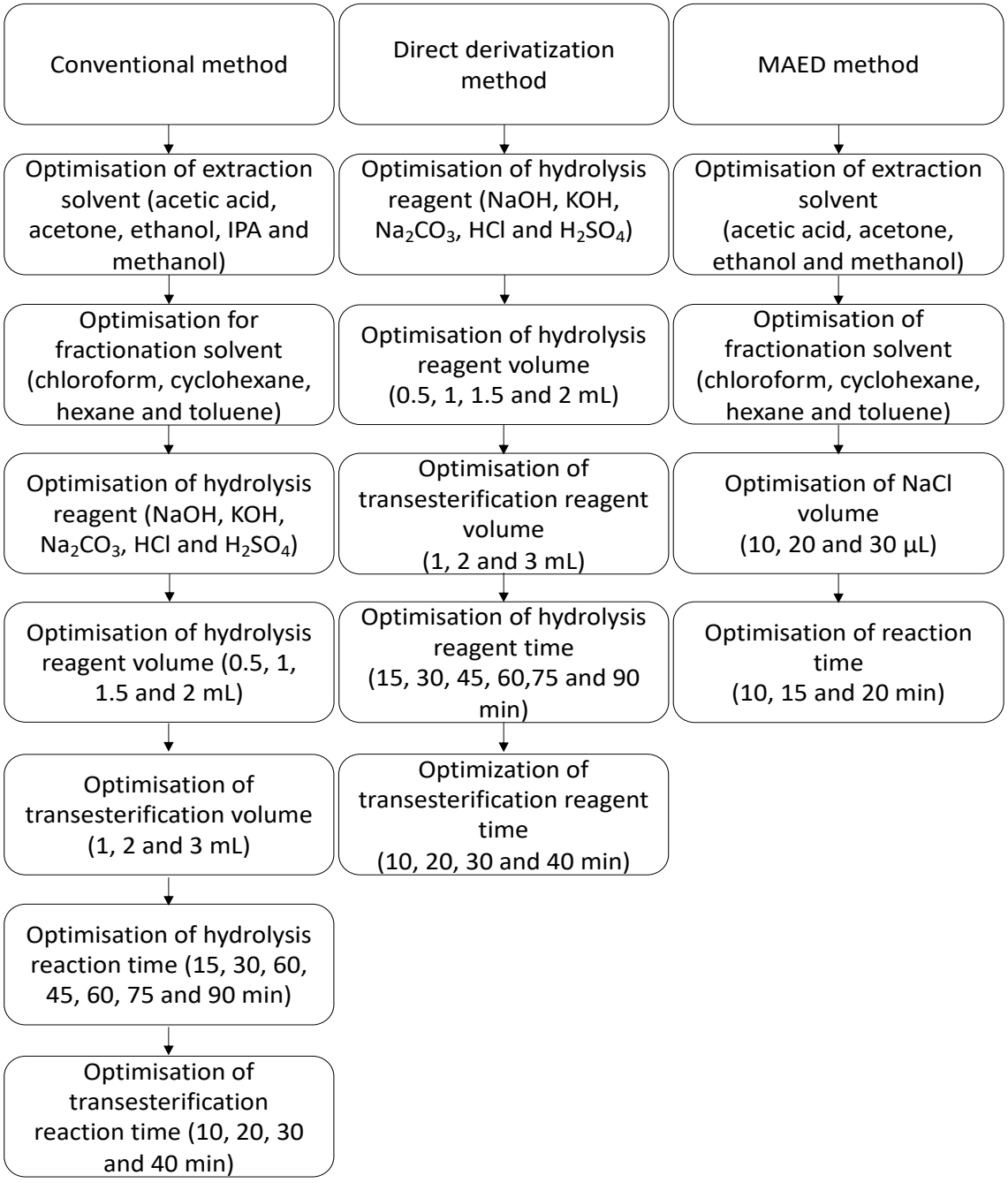

Figure 8: Order of experiments to determine the optimum parameters for the 3 different methods of extraction and derivatization investigated.

3.2.3 GC-MS analysis

The FAMEs were separated and analysed using an Agilent 6890 GC connected to a single quadrupole mass spectrometer Agilent 5973 network MSD. The GC-MS was equipped with a highly polar Agilent VF-23ms GC column (30 m, 0.25 mm I.D., 0.25 µm film thickness). The GC-MS separation was based on the work of (Hu Liu & Liu, 2017) who characterised the FA concentration and distribution in microalgae plants in north-eastern Tibetan Plateau. The method was varied in terms of increasing the split injection to increase the selectivity. A 1 µL volume of sample was injected with a 20:1 split ratio to an injector set to a temperature of 310 °C. Helium was used as the carrier gas at a constant flow rate of 1.2 mL/min. The initial column temperature was 40 °C for 1 min, it was then increased to 150 °C at a rate of 10 °C/min, once reaching 150 °C, it increased to 315 °C (6 °C/min) and held at this temperature for 20 min. The FAMEs were detected with electron ionization (70 eV) in scan mode with *m/z* of 50 to 500 AMU. The identification of unknown FAMEs was achieved by comparing their retention times and mass spectrum profiles with known standards and NIST97 mass spectral library. The quantification of FAMEs was performed based on calibration with a 37 component FAME standard mixture.

3.2.4 Statistical analysis

Statistical analysis was carried out to test whether differences between peak number and peak responses were significantly different between the evaluated conditions. One-way analysis of variation (ANOVA) performed ($p < 0.05$) and followed up with *post hoc* Tukey Tests

where appropriate using Origin software (version 9.0). Statistical analysis was applied to each optimisation step to evaluate which conditions provided the optimal recovery of FAs from algae samples.

3.3 Results and Discussion

3.3.1 Standard identification and quantification

A 37 component FAME standard mix was used to determine the optimum GC-MS instrument conditions and to identify and quantify peaks in the microalgae sample. The method used showed that FAME peaks started eluting at a retention time of 4.73 min ($C_{5:0}$ butyrate acid methyl ester) and the last FAME present in the standard mix eluted at 54.87 min ($C_{24:1\omega9}$, nervonic acid methyl ester). Various dilution factors and split injections were investigated and GC conditions were varied from the method used as outlined by (Hu Liu & Liu, 2017). Figure 9 shows the chromatogram for the respective varying conditions including (A) injection without a sample dilution or split; (B) injection with 20:1 split; (C) injection with 1 in 100 dilution and 20:1 split and (D) injection with 1 in 20 dilution & 20:1 split. It is suggested that (D) is the optimum method as it allowed for the separation for all 37 FAME peaks with the best resolution as shown in Figure 9D while other methods showed very poor resolution, separation and too diluted samples. Peak separation may have been further improved by optimising the split injection or optimising the heating regime. Additionally, different gases could be used as the carrier gases but helium was selected as it was already installed in the instrument being use but also allows for high flowrates while maintain good separation and

reproducibility. Although a cyanopropyl column was used for this separation, good quality separation of cis-trans composition is difficult as they have the same molecular weight and separation is based on the cis isomer having a stronger interaction with the cyano-dipoles. However, the polarity of the stationary phase is not sufficient to fully separate the complex cis-trans mixtures. Figure 9 shows the retention time for each of the FAMEs in the standard mix. Initial peak identification was carried out using chromatogram of other studies and NIST library on Table 16. These times and the corresponding mass spectrums were then used for the identification of FAMEs obtained from the microalgae samples as explained later in Section 3.3.5.

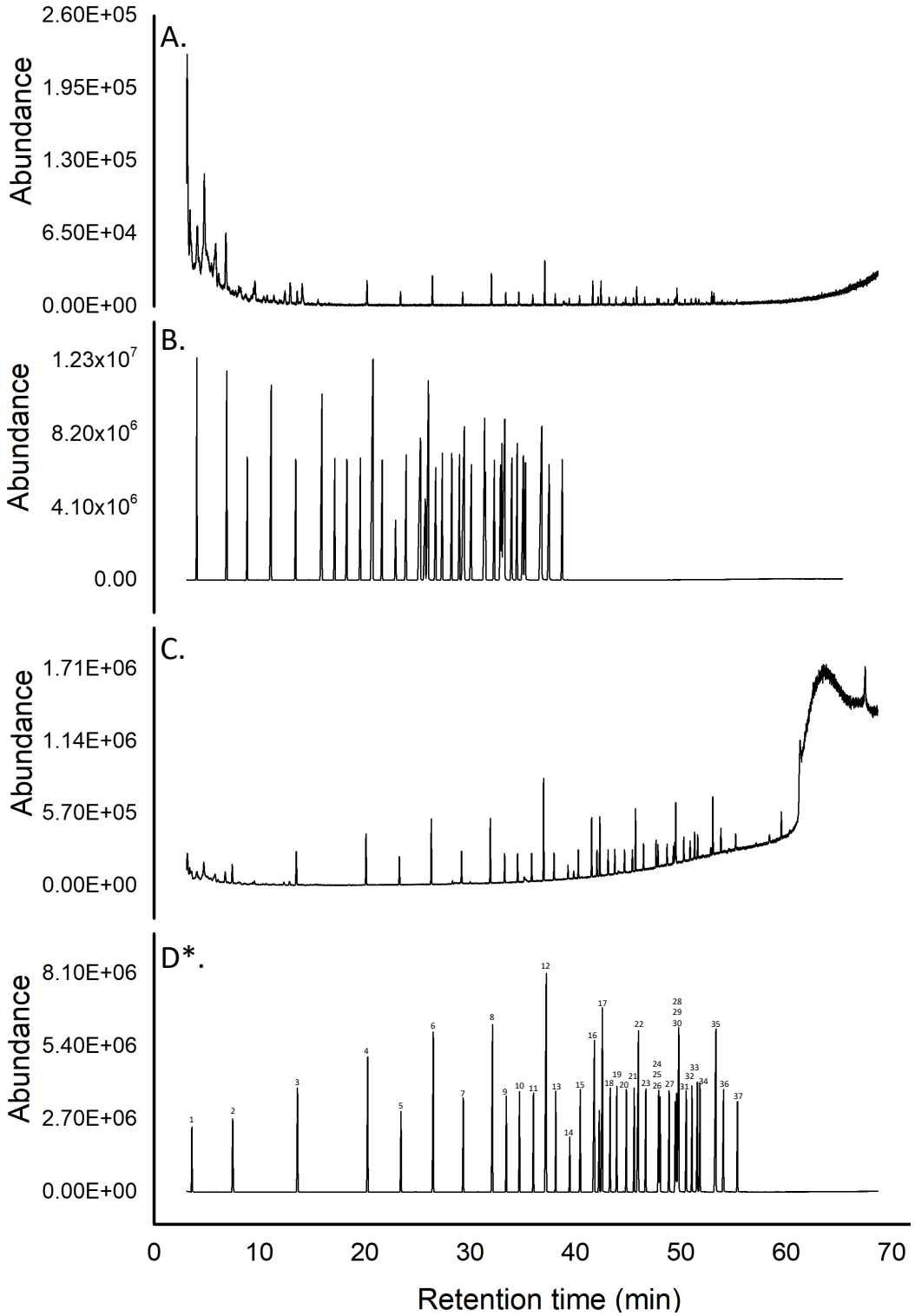

Figure 9: Chromatograms obtained from various instrumental conditions and sample dilutions for the analysis of the 37 FAME mix standard with (A) no split injection or dilution (B) 20:1 split injection (C) 20:1 split injection and 1 in 100 sample dilution and (D) 20:1 split injection and 1 in 20 sample dilution. Optimal conditions and dilution marked with * (peak number identification in chromatogram D defined in Table 16).

The identification of peaks was confirmed from the mass spectra identifying the characteristic fragmentation patterns. The mass spectra and fragmentation patterns for the analytes of interest ($C_{16:0}$, $C_{16:1}$, $C_{18:1}$ & $C_{20:5\omega3}$) are shown in Figure 10. According to (Chakraborty & Raj, 2007), mass spectrometry with EI provides limited information about the structure of FA. An ion at M-31 represents the loss of the -OCH_3 group which is the structure for methyl esters. The base peak for FAMEs is *m/z*=74 which is present in all FA compositions obtained from the McLafferty rearrangement. The characteristic ions for SFA ($C_{14:0}$) are *m/z*=71 according to the McLafferty rearrangement. The abundance of MUFA ions was higher than that of PUFA ions. $C_{16:1}$ showed to have a characteristic ion at *m/z*=96 due to the double bond according to (Mjøs, 2004). $C_{18:1}$ showed to have a characteristic ion at *m/z*=55 as the double bond is located at the start of the chain. PUFA $C_{20:5\omega3}$ showed to have a characteristic fragment ion at *m/*z=117 which defines the n3 terminal group. Additionally, LC-PUFA with double bonds (n≥4) as in $C_{20:5\omega3}$ also have a characteristic ion *m/z*=91 (Wasta & Mjøs, 2013). The fragmentation pattern of the remaining analytes detected in the 37 FAME mix are not discussed in detail as they do not produce viable product of as much interest as those discussed. However, their most abundant ions present, and characteristics are shown in Table 16 in addition to the probability (%) according to the NIST library.

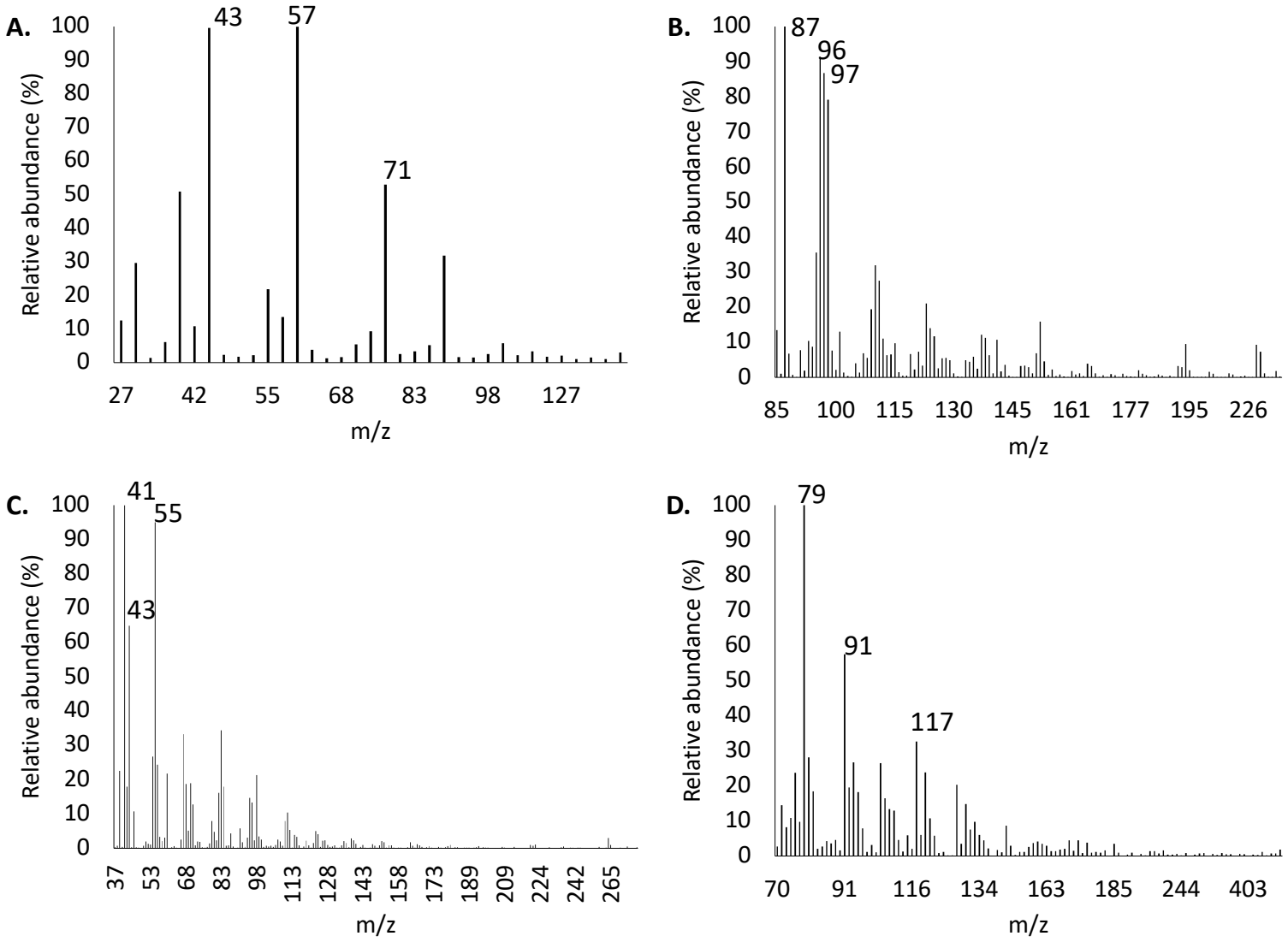

Figure 10: Mass spectra's showing fragmentation pattern for analytes of interest to produce viable products (Biodiesel (A, B and C) & nutraceuticals (D)) from GCMS analysis for (A) $C_{14:0}$ (B) $C_{16:1}$ (C) $C_{18:1}$ and (D) $C_{20:5\omega3}$.

Table 16: Mass spectral data with most abundant peaks, retention time and percentage match according to NIST library.

Peak number	Fatty acid	Molecular weight of FA (g/mol)	Retention time (Min)	Total peak	m/z Top peak	m/z Second peak	m/z Third peak	% Match (NIST library)
1	$C_{5:0}$	102.13	4.7	29	74	43	71	93
2	$C_{7:0}$	130.18	7.4	22	74	43	87	94
3	$C_{8:0}$	158.24	13.5	34	87	101	115	91
4	$C_{10:0}$	186.29	20.1	46	87	143	101	97
5	$C_{11:0}$	200.32	23.3	37	74	87	41	96
6	$C_{12:0}$	214.34	26.3	56	87	143	101	98
7	$C_{13:0}$	228.37	29.2	39	74	87	43	98
8	$C_{14:0}$	242.4	31.9	35	57	43	71	98
9	$C_{14:1\omega5}$	240.38	33.3	96	74	55	83	68
10	$C_{15:0}$	256.42	34.5	10	257	258	255	97
11	$C_{15:1}$	254.41	35.8	78	55	74	69	76
12	$C_{16:0}$	270.45	37.0	67	87	143	101	98
13	$C_{16:1\omega7}$	268.43	37.9	129	87	96	97	97
14	$C_{17:0}$	284.48	39.3	129	87	97	96	98
15	$C_{18:1\omega7}$	282.43	40.3	284	55	69	74	83
16	$C_{18:0}$	298.5	41.5	75	87	143	101	98
17	$C_{18:1\omega9t}$	296.49	42.0	129	264	97	96	98
18	$C_{18:1\omega9}$	296.49	42.3	84	74	55	69	99
19	$C_{18:2\omega6t}$	294.47	43.1	97	67	81	55	96
20	$C_{18:2\omega6}$	294.47	43.7	296	95	96	109	49
21	$C_{18:3\omega6}$	292.46	44.6	89	80	67	79	55
22	$C_{18:3\omega3}$	292.46	45.4	84	87	143	97	99
23	$C_{20:0}$	326.56	45.7	222	55	69	41	58
24	$C_{20:1\omega9}$	324.54	46.5	70	67	41	55	95
25	$C_{20:2\omega6}$	322.53	47.6	284	43	41	42	70
26	$C_{20:3\omega6}$	320.51	47.8	103	261	287	262	43
27	$C_{20:3\omega3}$	320.51	48.7	37	105	107	69	45
28	$C_{20:4\omega6}$	318.5	49.3	102	87	69	43	59
29	$C_{20:5\omega3}$	316.48	49.4	224	79	91	117	99
30	$C_{21:0}$	340.58	49.5	309	74	28	87	58
31	$C_{22:0}$	354.61	50.3	92	87	143	97	58
32	$C_{22:1\omega9}$	352.59	50.9	276	55	41	43	94
33	$C_{22:2\omega6}$	350.58	51.3	178	67	81	95	43
34	$C_{22:6\omega3}$	342.52	51.6	152	79	91	67	99
35	$C_{23:0}$	368.64	53.0	180	74	87	43	47
36	$C_{24:0}$	382.66	53.8	58	74	75	199	41
37	$C_{24:1\omega9}$	380.65	55.2	232	55	348	69	89

3.3.2 Optimisation of conventional method

As outlined in the methods section (Section 3.2.2), there are two steps to the conventional method: the extraction step and the derivatisation step. Both steps were optimised for a range of conditions including the solvents, reaction times and catalysts used.

3.3.2.1 Optimisation of conventional extraction method

An investigation on solvents used to extract and fractionate the FA from the algae samples was carried out using acetic acid, acetone, ethanol, IPA and MeOH. Chloroform, cyclohexane, hexane and toluene were used for fractionation. Acetone was found to recover the highest number of FAMEs in terms of both the greatest number of FAME peaks and highest total peak area at 14±0 and $7.6 \times 10^8 \pm 1.5 \times 10^5$, compared to the other conditions evaluated (Table 17). A study by (Ren et al., 2017) found that the greatest FA recovery was achieved by using acetone as the extraction solvent with a yield of 72.3 % compared to methanol (35.8 %) and dichloromethane (60.3 %). The other solvents (acetic acid, ethanol, IPA and methanol) investigated produced 12, 13, 13 and 13 FAME peaks, respectively. The Tukey test (Table 17) showed that acetone was significantly better than the other conditions. The optimum fractionation solvent according to the FAME peak number and peak area was hexane which produced 14±0 FAME peaks and total FAME peak area of $1.1 \times 10^9 \pm 4.3 \times 10^6$. Chloroform, cyclohexane and toluene all recovered lower levels of FAME with 12, 7 and 0 FA peaks, respectively. Other studies have shown hexane to be the optimal solvent for fractionation and it has been used to maintain the solubility of lipids and FAs whilst extracting FAMEs

following derivatization, which was supported by the present results (Khoomrung et al., 2012).

3.3.2.2 Optimisation of conventional derivatization method

3.3.2.2.1 Selection of alkali catalyst for hydrolysis and volume

The catalyst used for hydrolysis is a key factor in derivatization performance as it promotes the breakdown of the sample from triglycerides to FAs (R. L. Liu et al., 2012). NaOH, KOH, Na_2CO_3, HCl and H_2SO_4 were evaluated under the same conditions to determine the optimal catalyst. NaOH had the greatest yield of FAs when it was used as the hydrolysis reagent as it produced the largest number of peaks and peak area at 14±0 and $1.7 \times 10^9 \pm 5.3 \times 10^6$, respectively. Statistical analysis indicated that this condition was significantly better than the other conditions (Table 18). Homogeneous alkaline catalysts have been shown to be superior to acidic and metallic oxides so it was expected that NaOH or KOH would be the superior catalyst (R. L. Liu et al., 2012). A 0.5 mL volume of 1 mol/L NaOH provided the best recovery of FA and it was found that as the volume of NaOH increased, excess hydroxide caused reduced derivatization efficiency. A peak area of 5.5×10^8 and 14 peaks was achieved and this recovery was superior to the other catalysts evaluated at the 95 % confidence level (Table 18).

3.3.2.2.2 Selection of BF_3 volume

Optimization of the volume of BF_3 used for transesterification of FAs in algae oil samples was carried out with 1, 2 and 3 mL increments. Transesterification converts FAs and triglycerides to methyl ester derivatives (Otera, 1993). The results show that 2 mL of BF_3 was optimal to obtain the greatest recovery of FAs with 14 peaks produced and an average peak area of $1.6 \times 10^9 \pm 2.5 \times 10^7$ compared to the other conditions (Table 18). Increasing the volume of BF_3 did not yield better FAME recoveries and using lower volumes decreases the cost of FA derivatization. Similar scaled studies by (Khoomrung et al., 2012) also found that that 2 mL of BF_3 is the optimal volume for the transesterification of FA containing samples.

Table 17: Number of peaks and peak area obtained for optimisation of parameters for conventional extraction method.

Parameter	Setting	Peak area (1)	Peak area (2)	Peak area (3)	Peak number (1)	Peak number (2)	Peak number (3)	Average peak area	Standard deviation peak area	RSD % peak area	Average peak number	Standard deviation peak number	RSD % peak number
Extracting solvent	Acetic acid	2.8E+08	2.8E+08	2.8E+08	1.2E+01	1.2E+01	1.2E+01	2.8E+08	1.8E+06	6.5E-03	1.2E+01	0.0E+00	0.0E+00
	Acetone	7.6E+08	7.6E+08	7.6E+08	1.4E+01	1.4E+01	1.4E+01	7.6E+08	1.8E+05	2.4E-04	1.4E+01	0.0E+00	0.0E+00
	Ethanol	6.8E+08	6.9E+08	6.8E+08	1.3E+01	1.3E+01	1.3E+01	6.8E+08	4.9E+06	7.2E-03	1.3E+01	0.0E+00	0.0E+00
	IPA	5.5E+08	5.5E+08	5.4E+08	1.3E+01	1.3E+01	1.3E+01	5.4E+08	1.2E+06	2.2E-03	1.3E+01	0.0E+00	0.0E+00
	Methanol	3.2E+07	3.2E+07	3.2E+07	1.3E+01	1.3E+01	1.3E+01	3.2E+07	4.7E+03	1.5E-04	1.3E+01	0.0E+00	0.0E+00
Separating solvent	Chloroform	1.7E+07	1.7E+07	1.7E+07	1.0E+01	1.0E+01	1.0E+01	1.7E+07	1.2E+04	6.8E-04	1.0E+01	0.0E+00	0.0E+00
	Cyclohexane	1.5E+07	1.9E+07	1.4E+07	7.0E+00	8.0E+00	7.0E+00	1.6E+07	2.6E+06	1.6E-01	7.3E+00	5.8E-01	7.9E-02
	Hexane	1.1E+09	1.1E+09	1.1E+09	1.4E+01	1.4E+01	1.4E+01	1.1E+09	4.3E+06	4.0E-03	1.4E+01	0.0E+00	0.0E+00
	Toluene	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00

Table 18: Number of peaks and peak area obtained for optimisation of parameters for conventional derivatisation method.

Parameter	Setting	Peak area (1)	Peak area (2)	Peak area (3)	Peak number (1)	Peak number (2)	Peak number (3)	Average peak area	Standard deviation peak area	RSD % peak area	Average peak number	Standard deviation peak number	RSD % peak number
Hydrolysis reagent	H_2SO_4	3.3E+08	3.1E+08	3.1E+08	1.2E+01	1.2E+01	1.2E+01	3.2E+08	1.5E+07	4.6E-02	1.2E+01	0.0E+00	0.0E+00
	HCl	1.5E+09	1.5E+09	1.5E+09	1.0E+01	1.0E+01	1.0E+01	1.5E+09	2.1E+05	1.4E-04	1.0E+01	0.0E+00	0.0E+00
	KOH	1.1E+08	1.1E+08	1.1E+08	1.4E+01	1.4E+01	1.4E+01	1.1E+08	1.5E+05	1.4E-03	1.4E+01	0.0E+00	0.0E+00
	Na_2CO_3	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	#DIV/0!	0.0E+00	0.0E+00	#DIV/0!
	NaOH	1.7E+09	1.7E+09	1.7E+09	1.4E+01	1.4E+01	1.4E+01	1.7E+09	5.3E+06	3.2E-03	1.4E+01	0.0E+00	0.0E+00
Hydrolysis reagent volume (NaOH)	0.5 mL	5.5E+08	5.5E+08	5.6E+08	1.4E+01	1.4E+01	1.4E+01	5.5E+08	3.9E+05	7.1E-04	1.4E+01	0.0E+00	0.0E+00
	1 mL	5.0E+08	5.0E+08	5.0E+08	1.4E+01	1.4E+01	1.4E+01	5.0E+08	7.4E+05	1.5E-03	1.4E+01	0.0E+00	0.0E+00
	1.5 mL	1.4E+08	1.4E+08	1.5E+08	1.4E+01	1.4E+01	1.4E+01	1.4E+08	1.2E+06	8.0E-03	1.4E+01	0.0E+00	0.0E+00
	2 mL	1.2E+08	1.2E+08	1.2E+08	1.4E+01	1.4E+01	1.4E+01	1.2E+08	3.4E+06	2.8E-02	1.4E+01	0.0E+00	0.0E+00
Transesterification volume	1 mL	1.0E+09	1.0E+09	1.0E+09	1.3E+01	1.3E+01	1.3E+01	1.0E+09	4.3E+04	4.1E-05	1.3E+01	0.0E+00	0.0E+00
	2 mL	1.6E+09	1.6E+09	1.6E+09	1.4E+01	1.4E+01	1.4E+01	1.6E+09	2.5E+07	1.5E-02	1.4E+01	0.0E+00	0.0E+00
	3 mL	3.3E+08	0.0E+00	3.0E+08	9.0E+00	0.0E+00	9.0E+00	2.1E+08	1.8E+08	8.7E-01	6.0E+00	5.2E+00	8.7E-01
Hydrolysis reaction time	15 min	8.1E+07	8.1E+07	8.2E+07	1.2E+01	1.2E+01	1.2E+01	8.1E+07	1.0E+05	1.2E-03	1.2E+01	0.0E+00	0.0E+00
	30 min	2.3E+08	2.1E+08	2.1E+08	1.4E+01	1.4E+01	1.4E+01	2.1E+08	1.1E+07	5.2E-02	1.4E+01	0.0E+00	0.0E+00
	45 min	2.3E+08	2.3E+08	2.3E+08	1.4E+01	1.4E+01	1.4E+01	2.3E+08	2.0E+06	8.5E-03	1.4E+01	0.0E+00	0.0E+00
	60 min	1.8E+08	1.8E+08	1.8E+08	1.4E+01	1.4E+01	1.4E+01	1.8E+08	1.0E+06	5.6E-03	1.4E+01	0.0E+00	0.0E+00
	75 min	1.6E+08	1.6E+08	1.6E+08	1.4E+01	1.4E+01	1.4E+01	1.6E+08	1.5E+05	9.4E-04	1.4E+01	0.0E+00	0.0E+00

Table 18 (continued): Number of peaks and peak area obtained for optimisation of parameters for conventional derivatisation method.

Parameter	Setting	Peak area (1)	Peak area (2)	Peak area (3)	Peak number (1)	Peak number (2)	Peak number (3)	Average peak area	Standard deviation peak area	RSD % peak area	Average peak number	Standard deviation peak number	RSD % peak number
Hydrolysis reaction time	90 min	5.7E+07	5.6E+07	5.9E+07	1.2E+01	1.2E+01	1.2E+01	5.8E+07	1.6E+06	2.8E-02	1.2E+01	0.0E+00	0.0E+00
Transesterification reaction time	10 min	2.9E+08	2.9E+08	2.9E+08	1.4E+01	1.4E+01	1.4E+01	2.9E+08	2.4E+05	8.2E-04	1.4E+01	0.0E+00	0.0E+00
	20 min	2.1E+08	2.1E+08	2.1E+08	1.4E+01	1.4E+01	1.4E+01	2.1E+08	1.9E+05	8.6E-04	1.4E+01	0.0E+00	0.0E+00
	30 min	2.4E+08	2.4E+08	2.4E+08	1.4E+01	1.4E+01	1.4E+01	2.4E+08	5.2E+05	2.1E-03	1.4E+01	0.0E+00	0.0E+00
	40 min	1.8E+08	1.8E+08	1.8E+08	1.4E+01	1.4E+01	1.4E+01	1.8E+08	5.2E+05	2.9E-03	1.4E+01	0.0E+00	0.0E+00

3.3.2.2.3 Selection of reaction times for transesterification and hydrolysis

The efficiency of hydrolysis and transesterification depends on the extraction of FAs from the microalgae into the solvents and reaction with the catalysts used (NaOH for hydrolysis & BF_3 for transesterification). The conversion of lipids to FAMES is determined by the reaction time and temperature, with longer times and higher temperatures typically increasing recoveries, although excessive heat and reaction durations can result in sample deterioration (R. L. Liu et al., 2012). The optimal oven-based hydrolysis time was 45 min with 14 FA peaks produced and FA peak area of $2.3 \times 10^8 \pm 2.0 \times 10^6$ (Table 19). This 45 min condition provided significantly better recovery that the other durations evaluated (Table 18) .The optimum transesterification time was 30 min with 14 FAME peaks produced and a total peak area of $2.4 \times 10^8 \pm 5.2 \times 10^5$, which was significantly better than the 10, 20, and 40 min durations also tested (Table 18).

Table 19: Summary of optimised methods for the extraction and derivatization of algal samples.

Parameter	Conventional method	Direct derivatization method	MW
Extraction solvent volume	Acetone	-	Acetic acid
Fractionation solvent volume	Hexane	-	Chloroform
Hydrolysis reagent	NaOH	-	-
Hydrolysis solvent volume (mL)	0.5	0.5	-
Transesterification volume (mL)	2	2	-
Transesterification reaction time (min)	45	45	15
Hydrolysis reaction time (min)	30	30	-
NaCl volume (µL)	-	-	20

3.3.3 Optimisation of MW-assisted extraction

3.3.3.1 Selection of best solvents and solvent volumes for MW-assisted extraction

The same solvents were investigated for the MW-assisted recovery method as were for the conventional and direct synthesis. Acetic acid extracted the most FAMEs from the algae oil using MW-assisted recovery as it produced 14±0 FAME peaks and yielded a total peak area of $3.8 \times 10^8 \pm 1.3 \times 10^6$, compared to the other conditions, while acetone, which was the optimal solvent in the conventional method, performed significantly worse (10 times reduction in total peak area) when used with MW-assisted recovery (Table 20). No studies have been carried out on the fatty acids content of *Nannochloropsis* sp. microalgae plants using MW-assisted recovery, however, there are a variety of studies using MW-assisted recovery for FA derivatization on other samples that have found the best extraction solvents

to be methanol and acetone for yeasts and herbal medicines (Khoomrung et al., 2012; Ren et al., 2017). Further analysis of the acetic acid solvent system was carried out to optimise the volume of solvent and it was found that 10 µL extracted significantly more FAs from the algae sample compared to the other conditions evaluated (Table 20). A number of studies report that hexane was the optimum fractionation solvent when combined with MW-assisted recovery (Brunton et al., 2015; Khoomrung et al., 2012; R. L. Liu et al., 2012). The present investigation indicated that chloroform was superior to hexane and the other solvents, yielding 12 FAMEs and a total peak area of $2.6 \times 10^8 \pm 4.8 \times 10^5$, respectively.

3.3.3.2 Optimisation of reaction time for MW and volume of NaCl to quench derivatization

The optimum reaction time depends on the type and size of the sample. MW-assisted recovery can be up to 4 times faster than conventional oven based heating methods due to the ability of MW to efficiently heat samples and enhance derivatization reactions (Khoomrung et al., 2012). At 15 min reaction time yielded 14 peaks and a total FAME peak area of $3.1 \times 10^7 \pm 2.4 \times 10^4$ which was significantly more than the 10 and 20 min durations also evaluated (Table 20). The volume of NaCl used for quenching the derivatization was optimised and it was found that 20 µL NaCl was effective compared . The largest total peak area and 14 peaks were produced when using 20 µL NaCl, compared to only 12 peaks and diminished total peak areas delivered by 10 and 30 µL of NaCl (Table 20).

Table 20: Number of peaks and peak area obtained for optimisation of parameters for MW-assisted method.

Parameter	Setting	Peak area (1)	Peak area (2)	Peak area (3)	Peak number (1)	Peak number (2)	Peak number (3)	Average peak area	Standard deviation peak area	RSD % peak area	Average peak number	Standard deviation peak number	RSD % peak number
Fractionation solvent	Chloroform	2.6E+08	2.6E+08	2.6E+08	1.2E+01	1.2E+01	1.2E+01	2.6E+08	4.8E+05	1.8E-03	1.2E+01	0.0E+00	0.0E+00
	Cyclohexane	1.5E+07	1.5E+07	1.5E+07	7.0E+00	7.0E+00	7.0E+00	1.5E+07	1.3E+04	8.6E-04	7.0E+00	0.0E+00	0.0E+00
	Hexane	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
	Toluene	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Extraction solvent	Acetone	1.8E+08	1.8E+08	1.8E+08	1.4E+01	1.4E+01	1.4E+01	1.8E+08	6.1E+05	3.4E-03	1.4E+01	0.0E+00	0.0E+00
	Methanol	1.5E+08	1.5E+08	1.5E+08	1.4E+01	1.4E+01	1.4E+01	1.5E+08	5.8E+05	3.9E-03	1.4E+01	0.0E+00	0.0E+00
	Acetic acid	3.8E+08	3.8E+08	3.8E+08	1.4E+01	1.4E+01	1.4E+01	3.8E+08	1.3E+06	3.6E-03	1.4E+01	0.0E+00	0.0E+00
	Ethanol	1.8E+07	1.9E+07	1.8E+07	6.0E+00	6.0E+00	6.0E+00	1.8E+07	8.0E+04	4.3E-03	6.0E+00	0.0E+00	0.0E+00
Reaction time	10 min	2.4E+07	2.4E+07	2.4E+07	1.0E+01	1.0E+01	1.0E+01	2.4E+07	5.5E+04	2.3E-03	1.0E+01	0.0E+00	0.0E+00
	15 min	3.1E+07	3.1E+07	3.1E+07	1.4E+01	1.4E+01	1.4E+01	3.1E+07	3.4E+04	1.1E-03	1.4E+01	0.0E+00	0.0E+00
	20 min	2.1E+07	2.1E+07	2.0E+07	1.0E+01	1.0E+01	1.0E+01	2.0E+07	7.3E+04	3.6E-03	1.0E+01	0.0E+00	0.0E+00
Volume of NaCl	10 µL	1.7E+07	1.7E+07	1.7E+07	1.3E+01	1.2E+01	1.2E+01	1.2E+01	1.4E+04	8.5E-04	1.2E+01	5.8E-01	4.7E-02
	20 µL	2.1E+07	2.1E+07	2.1E+07	1.4E+01	1.4E+01	1.4E+01	2.1E+07	4.4E+04	2.1E-03	1.4E+01	0.0E+00	0.0E+00
	30 µL	1.6E+07	1.6E+07	1.6E+07	1.2E+01	1.2E+01	1.2E+01	1.6E+07	1.9E+03	1.1E-04	1.2E+01	0.0E+00	0.0E+00

3.3.4 Statistical analysis comparing recovery methods

The FA composition of *Nannochloropsis* sp. microalgae samples were reported as a percentage mass of the total sample. 14 FAs were identified in the algal plant extract using each of the three preparation methods. The MW-assisted recovery method was the most effective at extracting and derivatizing FAs out of the three optimized methods, since it yielded the highest total peak area of FAMEs (Figure 11), which was significantly higher than the number of FAMEs obtained using conventional and direct derivatization (Table 20).

Table ***21*** summarises the FA composition determined by each of the methods. Table 20 shows that MW-assisted recovery was significantly different to the other two methods for all FAs detected except $C_{8:0,}$ $C_{16:1w7t}$ and $C_{20:4w6}$. Of the FA which MW-assisted recovery showed no significant difference, both the peak area and FA weight composition were smaller for MW-assisted recovery method. The MW-assisted recovery method yielded a higher TFA (51.8±0.5 %) and SFA content (13.2±1.3 %) than that of the direct derivatization method (TFA 34.1±0.4 % and SFA 10.2±1.1 %) and the conventional method (TFA 42.6±0.8 % and SFA 12.8±1.3 %). It is also seen that for TFA and SFA the conventional method showed greater derivatization efficiencies than the direct derivatisation method which confirms that the extraction and purification step is required to improve recovery of these FAs. The MW-assisted recovery had the best repeatability with an RSD of 8.5 % which was superior to conventional method (11.2 % RSD) and the direct derivatization method (10.7 % RSD), (Table 20). Since the MW-assisted recovery method had the greatest average recovery for TFA it was selected as the optimal method for the analysis of FA in the microalgae samples.

Both MUFA and PUFA recoveries were investigated by the 3 different methods. It was found that PUFA had the highest yield of all FA types and MW-assisted recovery extracted and derivatized the sample for PUFA the most efficiently with a yield of 35.0±2.8 % whilst the method with no extraction had quite a low yield in comparison at 18.7±1.6 % and the conventional method with 24.8±3.1 % (

Table ***21***). Statistical analysis confirmed that the differences in recoveries between techniques were significant (Table 20). The better recovery of PUFA when utilizing the MW-assisted recovery method may be due to the milder temperature used during derivatization which could minimise oxidation and of PUFA compared with the other methods 12. The MW-assisted recovery method also had the best precision 8 % while it was 8.4 % for the direct derivatization and 12.4 % for the conventional method. The differences in recoveries between the MW-assisted recovery, conventional and direct derivatisation methods highlight the importance of using low reaction temperatures and performing an initial extraction prior to derivatization. Interestingly, the MW-assisted recovery had lower yields of MUFA (3.5±0.2 %) compared to the direct derivatization (5.6±0.7 %) and conventional method (4.9±0.6 %). ANOVA analysis indicated that the direct derivatization method may be superior for FA recovery of MUFA compared to the MW-assisted recovery and conventional methods (Table 20) in applications where targeted analysis of these species is the goal. Overall, the total unsaturated FA (TUFA) obtained from MW-assisted recovery (38.5±2.9 %) was significantly better (Table 20) than the direct derivatization (23.9±2.6 %) and conventional methods (29.7±3.6 %). The proportions of FA determined in the present microalgae samples match other studies evaluating FA composition in algae, for example, (Sukenik & Carmeli, 1990) analysed FAs in similar *Nannochloropsis* sp. microalgae and detected FAs ranging in sizes from myristoleic acid ($C_{14:0}$) to arachidic acid ($C_{20:0}$) along with unsaturated and polyunsaturated variants of these fatty acids.

The MW-assisted recovery method was found to be a superior method due to its high efficiencies in extracting and derivatizing the FA within algae samples. Furthermore, the MW-

assisted recovery method is almost 4 times faster than the conventional and direct derivatization method similar to other MW-assisted recovery studies. Additionally, incorporating an extraction step prior to derivatization recovered 8 % more FA than direct derivatization methods, highlighting the importance of for maximising FA recoveries despite the additional time required to perform this step.

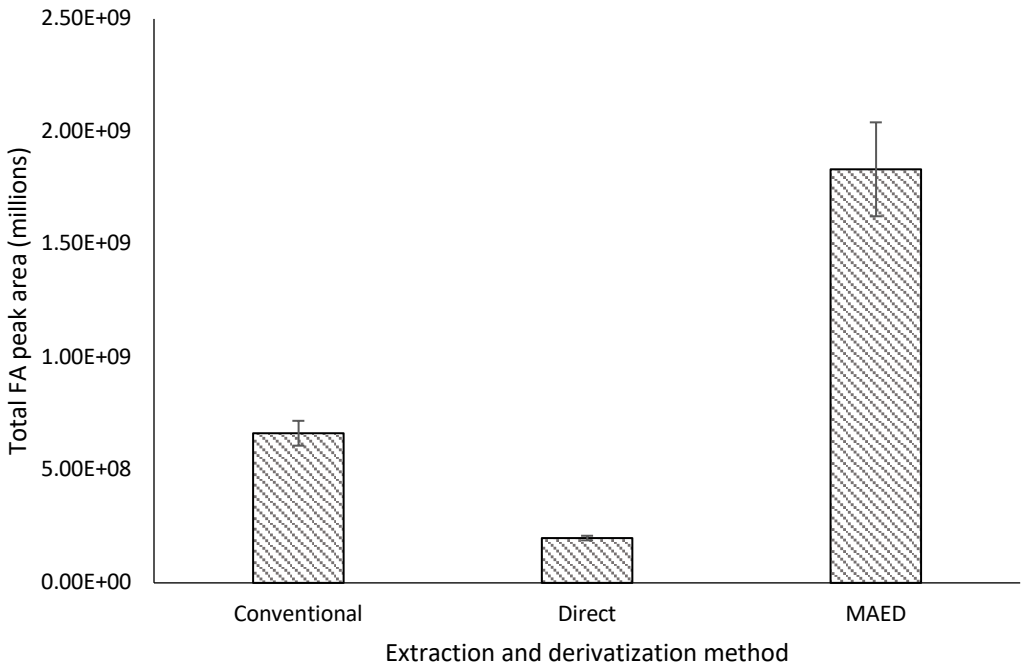

Figure 11: Comparison of total FA peak area produced by conventional, direct derivatization and MW methods (n=3).

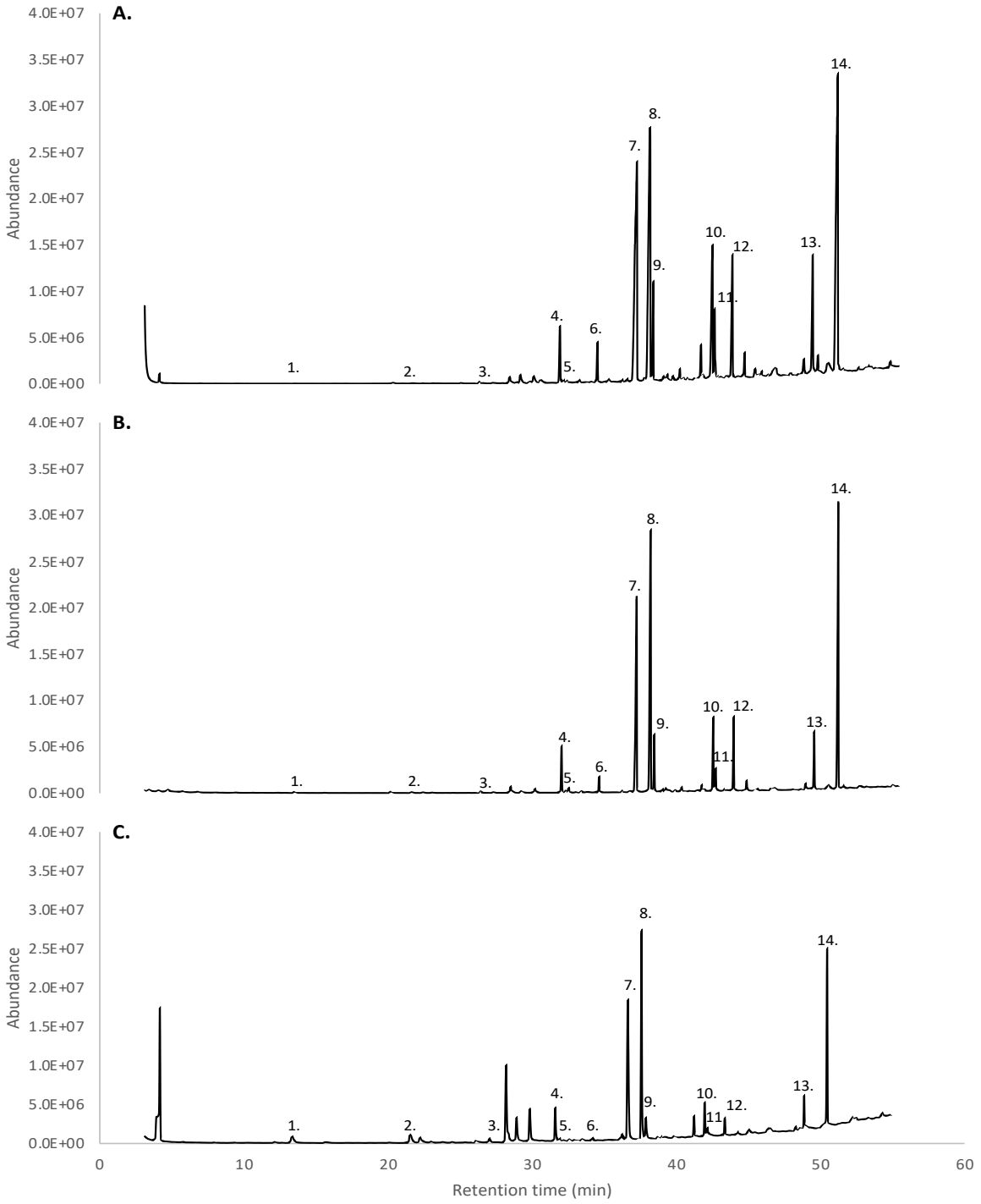

Figure 12: GC-MS chromatogram of FAMEs for 3 different methods of sample preparation; A= MW-assisted method, B = conventional method and C = direct derivatization method (1=$C_{8:0}$, 2=$C_{10:0}$, 3=$C_{12:0}$, 4=$C_{13:0}$, 5=$C_{14:0}$, 6=$C_{16:0}$, 7=$C_{16:1\omega7t}$, 8=$C_{16:1\omega7}$, 9=$C_{18:0}$, 10=$C_{18:1\omega9}$, 11=$C_{18:1\omega7}$, 12=$C_{18:2\omega6}$, 13=$C_{20:4\omega6}$ and 14=$C_{20:5\omega3}$).

3.3.5 Sample composition and potential by-products

FAME composition in *Nannochloropsis* sp. was characterised according to the 37 FAME mix standard outlined in Section 3.3.1. The retention times for the *Nannochloropsis* sp. samples (as shown from the chromatograms in Figure 12) were compared to that of the 37-standard mix (Table 16 to confirm which FA were present.

3.3.5.1 FA quantification

FA composition of the *Nannochloropsis* sp. sample was quantified against a 37 FAME standard mix and the internal standard, $C_{15:0}$. The concentration of each FAME in the 37 FAME mix was outlined in the manufacture's product specifications. The FAME concentration correlates with the peak area produced on the chromatogram and the peak areas were then used to quantify the FA in the *Nannochloropsis* sp. samples with the aid of the internal standard. By determining the signal ratio between the internal standard and the analytes in the 37 FAME mix and spiking the *Nannochloropsis* sp. samples with the internal standard allowed for the quantification of FA in the sample. An example of the calculations to determine the signal ratio and thus the concentrations of each FA present in the sample is presented below using $C_{20:5\omega3}$ recovered using the MW method. Results obtained from similar calculations for the remaining FA using different recovery methods are presented in

Table ***21*** with the signal ratio for each analyte and the corresponding concentration (expressed in µg) and % FA content.

Calculation of signal ratio between analyte and internal standard (Example calculation based on $C_{20:5\omega3}$)

Step 1: Calculate mass on column

$$Mass\ on\ column = \frac{[C_{20:5\omega3}\ in\ 37\ FAME\ mix]}{1000} \times \frac{Dilution\ factor}{(Volume\ injected)/(Split\ Ratio)} \times 1000$$

$$Mass\ on\ column = \frac{[200\ \mu g/mL]}{1000} \times \frac{20}{(1\ \mu L)/(20)} \times 1000$$

$$Mass\ on\ column = 0.5\ ng$$

Step 2: Calculate the area of peak per ng

$$Area/ng = \frac{Area}{Mass\ on\ column}$$

$$Area/ng = \frac{476{,}339}{0.5\ ng}$$

$$\frac{Area}{ng} = 952{,}678\ ng$$

Step 3: Signal ratio

$$Signal\ ratio = \frac{C_{20:5\omega3}\ area/ng}{Internal\ standard\ area/ng}$$

$$Signal\ ratio = \frac{952{,}678\ ng}{1{,}876{,}730\ ng}$$

$$Signal\ ratio = 0.5076$$

Calculation of concentration of analyte in *Nannochloropsis* sp. sample (Example calculation based on $C_{20:5\omega3}$)

Step 1: Calculate normalised signal between sample and internal standard

$$Normalised\ signal = \frac{\mathrm{C20{:}\ 5\omega3}\ peak\ area}{Internal\ standard\ peak\ area}$$

$$Normalised\ signal = \frac{97{,}572{,}945}{1{,}662{,}795}$$

$$Normalised\ signal = 58.68$$

Step 2: Calculate the ion count relative to the internal standard

$$Ion\ count = Signal\ ratio \times normalised\ signal$$

$$Ion\ count = 0.51 \times 58.68$$

$$Ion\ count = 29.79$$

Step 3: FA mass in original sample

$$Mass\ in\ original\ sample = mass\ on\ column \times sample\ size \times dilution\ factor$$

$$Mass\ in\ original\ sample = 18.62\ ng \times 1000\ \mu L \times 20\ \mu L$$

$$Mass\ in\ original\ sample = 372{,}344\ ng$$

Step 4: FA mass expressed in µg

$$Mass\ in\ \mu g = 372{,}344\ ng \times 1000$$

$$Mass\ in\ \mu g = 372.34\ \mu g$$

Step 5: FA content as % of total sample weight

$$\%\ FA\ content = \frac{FA\ mass\ (\mu g)}{Sample\ mass\ (\mu g)} \times 100$$

$$\%\ FA\ content = \frac{372.34\ \mu g}{2000\ \mu g} \times 100$$

$$\%\ FA\ content = 18.62\ \%$$

3.3.5.2 Biodiesel potential

Biodiesel consists of FAMEs which are synthesised from TGA and FAs that are present in the microalgae plants (Maghraby & Fakhry, 2015). The FAs that yield the greatest biofuel potential are the C_{16} to C_{18} chain length FAs, in particularly palmitic ($C_{16:0}$), palmitoleic ($C_{16:1}$), heptadecanoic ($C_{17:0}$), stearic ($C_{18:0}$), oleic acid ($C_{18:1}$) and α-Linolenic acid ($C_{18:3}$) (Arif et al., 2019). Fuel potential is defined as the fraction of lipids that compose of the maximum amount of FAs which are capable of undergoing transesterification (Arif et al., 2019). The FAMEs which were detected to be of the highest concentration in the microalgae sample according to their

weight and were $C_{16:0}$, $C_{16:1\omega7t}$, $C_{18:1\omega9}$ and $C_{18:2\omega6}$ at 9.2, 10.2, 1.3 and 4.8 wt %, respectively. These FAs are considered to be the excellent FAs for biodiesel production (Arif et al., 2019; Maghraby & Fakhry, 2015). The ideal molar ratio of $C_{16:1}$, $C_{18:1}$ and $C_{14:0}$ is 5:4:1 for the most efficient biodiesel according to (Maghraby & Fakhry, 2015). However, of these FAs, the ratio in the *Nannochloropsis* sp. microalgae sample analysed is 58:11:1 (with FA content of 11.5, 2.2 and 0.2 %, respectively). This ratio suggests that the present microalgae sample analysed may not be ideal for biodiesel production and require blending with a myristic acid methyl ester source. A higher SFA content improves oxidative and thermal stability along and ignition quality leading to biodiesel products with superior performance (Maghraby & Fakhry, 2015).

3.3.5.3 Nutraceutical potential

The FA with the highest content is $C_{20:5\omega3}$ at 18.7 % (

Table ***21***). While this FA does not have a use in the production of biodiesel, it has an important role in the treatment and prevention of diseases such as cardio vascular, diabetes, eye disorder, arthritis and to control cholesterol and blood pressure (Aliev & Abdulagatov, 2017). Studies have shown that $C_{20:5\omega3}$ is found to be present at high concentrations in marine species and high saline levels also promote the growth of PUFA (V. Patil et al., 2007). This indicates that *Nannochloropsis* sp. may have the potential for pharmaceutical and personal uses. Additionally, $C_{20:5\omega3}$ provides a great source for omega-3 nutraceuticals which help to lower blood pressure, reduce triglycerides, help heart related diseases and induce brain development (Chua & Schenk, 2017). Conventionally omega-3 is obtained from fish stock but fish levels are decreasing which means a new source for omega-3 is required making *Nannochloropsis* sp. cultivated from saline WW more valuable (Fithriani & Ambarwaty, 2020). Arachidonate acid ($C_{20:4\omega6}$) is also present in the sample in low levels at 1.4 %. $C_{20:4\omega6}$ is also an important FA as it allows for the development of the brain and for pre and post-natal brain and retina development (V. Patil et al., 2007).

3.3.5.4 Other potential products

Other SFA present in the algae sample at low levels include $C_{8:0}$, $C_{10:0}$, $C_{12:0}$ and $C_{13:0}$ at 0.7, 0.1, 0.20 and 2.1 %, respectively. It is uncommon to find these FAs in algae plants as algae usually contains FAs with a chain length between $C_{14:0}$ and $C_{24:0}$ (Hu Liu & Liu, 2017). However, if these microalgae species were gathered in large volumes they could allow for the production of different materials. $C_{8:0}$ has been used for the production of medicine for

patients suffering from pancreatic insufficiency and fat malabsorption (Lemarié et al., 2016). $C_{10:0}$ has been used for the production of fruit flavours and perfumes (Beare-Rogers et al., 2001). $C_{12:0}$ is naturally found in coconut oil and milk is used for the production of soaps and cosmetics (Anneken et al., 2012). It is unusual to find $C_{13:0}$ in microalgae as according to (Breuer et al., 2013), microalgae only contain carbon chains with even numbers. However, some studies have shown low levels of $C_{13:0}$ to be present in different microalgae species (Koichi & Tsuchiya, 1978).

Table 21: FA mass (µg) and FA composition (%) of total sample in *Nannochloropsis* sp. microalgae plants obtained using 3 different extraction & derivatisation methods (n=3).

	Signal ratio	MW method		Conventional method		Direct synthesis	
		Mass (µg)	FA Content (%)	Mass (µg)	FA Content (%)	Mass (µg)	FA Content (%)
$C_{8:0}$	0.7	13.3	0.7±1.0	0.8	0.04±0.5	0.0	0.1±0.01
$C_{10:0}$	0.9	1.0	0.1±0.3	1.2	0.1±0.1	0.1	0.04±0.01
$C_{12:0}$	1.0	3.9	0.2±0.02	3.3	0.2±0.02	0.1	0.1±0.03
$C_{13:0}$	1.2	41.6	2.1±0.02	41.4	2.1±0.1	2.4	1.2±0.1
$C_{14:0}$	1.2	4.8	0.2±0.1	7.6	0.4±0.2	0.2	0.1±1.4
$C_{16:0}$	1.0	183.2	9.2±0.8	196.6	9.8±2.5	16.8	8.3±2.0
$C_{16:1\omega7t}$	1.1	203.9	10.2±1.0	295.2	14.8±1.9	19.5	9.8±0.05
$C_{16:1\omega7}$	1.0	26.8	1.3±1.3	39.5	1.9±0.3	3.0	1.5±1.2
$C_{18:0}$	0.9	17.0	0.9±0.1	5.6	0.3±0.1	1.0	0.5±0.03
$C_{18:1\omega9}$	0.8	25.7	1.3±0.1	43.4	2.2±0.5	4.9	2.5±0.2
$C_{18:1\omega7}$	0.9	17.3	0.9±0.7	16.4	0.8±0.1	2.3	1.2±0.1
$C_{18:2\omega6}$	1.1	95.3	4.8±0.3	54.9	2.8±0.3	5.4	2.7±0.3
$C_{20:4\omega6}$	0.5	27.7	1.4±0.1	21.8	1.1±1.2	2.4	1.2±0.02
$C_{20:5\omega3}$	0.5	373.6	18.7±1.3	123.4	6.2±1.0	10.1	5.1±0.3
∑TFA		1035.2	51.8±0.5	851.2	42.6±0.6	68.3	34.1±0.4
∑SFA		250.5	13.2±1.3	256.6	12.8±1.3	20.6	10.3±1.1
∑MUFA		69.8	3.5±0.2	99.3	4.9±0.6	11.3	5.6±0.7
∑PUFA		700.6	35.0±2.8	495.3	24.8±3.1	37.4	18.7±1.6
∑TUFA		770.4	38.5±2.9	594.6	29.7±3.6	47.7	23.9±2.6

3.4 Conclusion

Three methods were optimized and evaluated for the preparing FAMEs from algae samples, including a conventional oven-based derivatization, a direct derivatization without lipid pre-extraction and a MW-assisted recovery method. The choice of reaction temperature, reaction time and extraction/fraction solvents were critical to method performance as they influenced the amount of FA extracted and derivatized. The MW-assisted recovery method combined with acetic acid as an extractant and chloroform as a fraction solvent provided the most efficient method for FA

derivatization to FAMEs. FAs in microalgae samples were rapidly derivatized in 15 min compared to the conventional and direct derivatization methods which required reaction times 3 times longer. Furthermore, the MW-assisted recovery method yielded highest conversion of FAs to FAMEs compared to the conventional and direct derivatization approaches. The developed method is robust and will be invaluable for future assessments of FA content in algae that may be utilised for biodiesel or pharmaceutical applications.

4. Assessment of commercially available membrane materials for ammonia recovery from rendering condensate wastewater: lab- and pilot-scale study

Chapter Foreword

The use of hydrophobic membranes for the removal of NH_3 from water streams has become an increasingly popular method due to the potential product generation and reduced costs of treatment. There is a race to find a suitable membrane material which can withstand challenging WW and be up scaled for industrial scale treatment. In this study, nine commercially available hydrophobic membranes were used to treat ammonia in a challenging agricultural matrix, slaughterhouse RCWW. The materials investigated (PP, PTFE, PVDF and PES) varied in terms of material, pore size, surface roughness, fabrication methods and membrane thickness. Membrane suitability was determined based on its NH_3 removal efficiency and how susceptible it was to membrane wetting. Membrane wetting was determined using various characteristic methods such as surface roughness, LEP, CA, porosity and membrane morphologies. The results demonstrated that PTFE materials were the most suitable at lab-scale as it allowed for no visible signs of membrane wetting and the greatest NH_3 removal (63 %). Further analysis of this membrane material at pilot-scale showed removal rates of up to 68 % before membrane wetting occurred after 6 days of operation. Additionally, this chapter demonstrates that a lab-scale cleaning protocol allows for increased membrane lifetime which could incorporated at full-scale to show similar increased lifetimes. Product characterisation also showed that a viable liquid $(NH_4)_2SO_4$ product could be generated with a 30 % purity which allows for a reduction in the treatment method. Overall, this study showed that certain commercially available hydrophobic membranes are suitable to treat challenging matrices such as RCWW at lab- and pilot-scale while producing a viable product to decrease WW treatment costs.

4.1 Introduction

Recovering NH_3 from WW allows for the simultaneous reduction of nitrogen levels while offering the potential to produce a nitrogen-based products (Ding et al., 2006). RCWW which is produced from the condensation of vapours released after heat processing of animal carcasses, has high nitrogen concentrations. The high nitrogen content in RCWW allows it to be a suitable matrix to produce NH_4-based fertilisers (Brennan, Briciu-Burghina, et al., 2020). The use of hydrophobic membrane technologies has been shown to be an effective method to recover NH_3 from WW streams (Hasanoğlu et al., 2010; Qu et al., 2013; Rezakazemi et al., 2012). The use of hydrophobic membranes offers the benefits of being selective to NH_3 removal, low energy consumption, faster retention times compared with conventional methods and the capability of producing a viable ammonium salt fertiliser which can generate revenue to offset against the cost of operation (Brennan, Briciu-Burghina, et al., 2020; Ding et al., 2006; Zarebska et al., 2014). However, membranes are highly susceptible to wetting from surfactants and fouling due to organic loading on the feed side of the membrane (Lauterböck et al., 2013). RCWW was reported in Chapter 2 to be a challenging matrix and have high concentrations of surfactants and components such as TSS and FOG. The high levels of surfactants have the potential to build up on the membrane surface and/or membrane pores which may result in the deterioration of the flux, increased power consumption, change in membrane hydrophobicity and decreased membrane life span (El-Bourawi et al., 2006; Licon Bernal et al., 2016; Zarebska et al., 2014). Wetting of the membrane jeopardizes the performance dramatically (Yazgan-Birgi et al., 2018). Various authors have introduced cleaning steps in order to remove foulants such as proteins, fats and OM from the membrane

material to improve the life span and reduce wetting (C. Liu et al., 2001; Nguyen & Roddick, 2011; Rudolph et al., 2018). In order to ensure the most efficient cleaning method is being used, it is important to recognise the source of the fouling which can be categorized into 4 groups; inorganic fouling, particle and colloidal fouling, microbial fouling or organic fouling (C. Liu et al., 2001; Naim et al., 2012). Membrane fouling from RCWW may be caused from organic foulants (proteins, fats and OM) which are present in high concentrations. Caustic cleaning can be introduced to clean membranes which have been fouled by organic foulants (Nguyen & Roddick, 2011).

The most common membrane materials to remove NH_3 from WW include polypropylene PP, PVDF and PTFE (Darestani et al., 2017; Tan et al., 2006). Other authors have also suggested using PES with a modified surface (omni phobic layer (OEM)) due to its hydrophilic properties which would facilitate high concentrations of oils in the RCWW (Q. Chen et al., 2017; Eykens et al., 2016). Support layers on membrane materials are believed to improve the membrane stability and for that reason, various membranes with support layers are used in this study (W. Liu et al., 2018). A number of factors are considered in order to determine the efficiency of a membrane material including; how much NH_3 it removes, minimum heat loss of the feed sample through the membrane material and its resistance to wetting (Eykens et al., 2016; Huaqing Liu et al., 2018). The parameters which influence membrane efficiency include LEP, membrane thickness, porosity, pore size, roughness and membrane morphologies (Lauterböck et al., 2013). An increase in the use of superhydrophobic membranes has occurred in recent years due to their resistance to highly polluted WW (Z. Liu et al., 2016).

Superhydrophobic surfaces have a CA>150˚ which allow for increased resistance to wettability and self-cleaning properties (Razmjou et al., 2012).

Various studies have been carried out most recently by Malik (Malik, 2019), (Zoungrana et al., 2020) and (Bae & Kim, 2020) to demonstrate the use of hydrophobic membrane contactors to remove NH_3 from challenging water samples. Additionally, (Eykens et al., 2016) compared different commercially available membranes to recover salt from saline WW. However, a critical comparison of commercially available membrane materials to remove NH_3 from challenging matrices such as RCWW has not been carried out. As such, the following chapter investigates the performance of different commercially available hydrophobic membranes for NH_3 removal from RCWW at lab-scale. Optimal membrane materials are then used in a pilot-scale study on-site in a meat-processing plant. This chapter presents a critical study to compare commercially available hydrophobic membranes and their suitability for removing NH_3 from a challenging matrix such as RCWW at lab- and pilot-scale. The recovery of an $(NH_4)_2SO_4$ product is investigated to identify the potential of using membrane technologies to recover nitrogen to produce viable nitrogen-based products.

4.2 Materials and methods

4.2.1 Materials

RCWW used in this study was obtained from a rendering facility located in the south of Ireland. The lab- and pilot- scale membrane contactor set-up was procured from Blue-tec bv,

Industriewag 16, 6871 KA, Renkum, The Netherlands. 9 different membrane materials were investigated in this study as outlined in

Table 22, specifying their material type, support layer, pore size, membrane thickness, fabrication method and manufacturer. Membrane materials used vary in terms of their material type, pore size, support layer and hydrophobicity allowing for a large variation of membrane types to be investigated. 1 L high-density polyethylene bottles were used to collect the RCWW sample and were procured from Radionics, Ireland. Whatman 0.45 µm PTFE filter paper was procured from Fisher Scientific, Ireland. Solvents including 35 % NH_3OH, 99 % n-hexane and 98 % H_2SO_4 were procured from Merck, Ireland. Pure NaOH granules were procured from Merck, Ireland. Bulk NaOH (30 %) (w/w) and H_2SO_4 (96 %) (w/w) which were of analytical grade were procured from Brenntag Chemicals Distribution LTD, Ireland. $(NH_4)_2SO_4$ (99 %) (w/w %) was procured from Fischer, Ireland. All chemicals and reagents used were of analytical grade.

Table 22: Specification of membrane materials investigated in this study.

	Material	Support layer	Pore size (µm)	Membrane thickness (mm)	Fabrication method	Manufacturer	Country	Membrane code
1	PP	-	0.45	0.05	Stretching	Blue-Tec	The Netherlands	PP1
2	PP	-	0.22	0.2	Fibre pressing	Tisch	United States	PP2
3	PP	-	0.45	0.2	Fibre pressing	Tisch	United States	PP3
4	PTFE	-	0.45	0.1	ePTFE Stretching	Blue-Tec	The Netherlands	PTFE 1
5	PTFE	PP	0.45	0.2	ePTFE Stretching	Tisch	United States	PTFE 2
6	PVDF	PET	0.45	0.12	Phase inversion	Tisch	United States	PVDF 1
7	PVDF	-	0.45	0.12	Phase inversion	Pall	Ireland	PVDF 2
8	PES	Omni phobic	0.22	0.13	Phase inversion	Sartorius	Germany	PESU
9	PES	Fluor	0.22	0.6	Stretching	Blue-Tec	The Netherlands	SHP

4.2.2 Sampling

RCWW samples were collected for lab-scale analysis in sterile 1 L plastic bottles and frozen until they were used. A previous study (Chapter 2) characterised the RCWW sample and identified it to contain high levels of FOG and proteins (Section 2.2.3). To prevent the RCWW sample causing immediate wetting on the membranes and clogging of the system, the sample underwent pre-treatment before being exposed to the system (for lab-scale investigations). TSS was removed using 0.45 µm PTFE filter paper according to the APHA standard method (APHA, 1990). FOG was removed from the RCWW according to the APHA standard method (APHA, 1990). Samples were further diluted by 10-fold using DI water to have NH_3 concentrations of approximately 100 mg/L (Elga Purelab Ultra system, Ultra System,

Switzerland). The pH of the diluted RCWW was adjusted to 10.5 using 10 mL of 1 M NaOH and was measured using Jenway 3510 pH Meter procured from Jenway, UK.

Pilot-scale studies were carried out on-site in ABP Food Group WWTP. NH_3 rich RCWW was obtained from the feed from the rendering plant to the aeration tank as outline in Figure 13. Large tanks were filled with RCWW to ensure continues operation of the membrane contactors.

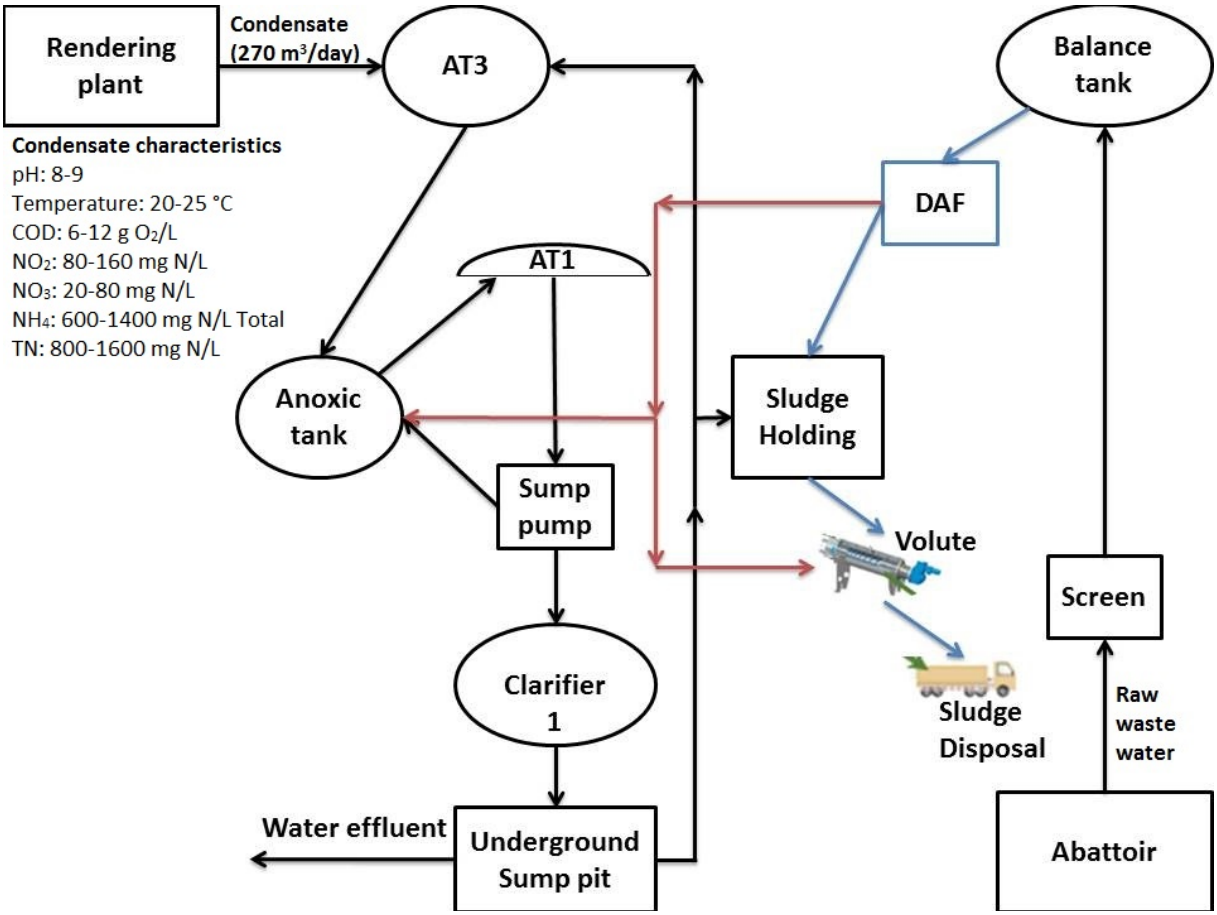

Figure 13: Graphical representation showing the configuration of the current, activated sludge WWTP and characterization of RCWW. AT1 = Aeration Tank 1, AT3 = Aeration Tank 3, DAF = Dissolved Air Flotation.

4.2.3 Membrane contactor operation

4.2.3.1 Lab-scale operation

Membrane contactor efficiency was investigated on all 9 membranes over a 3-hour period to determine the NH_3 removal rate from RCWW and the energy efficiency of the membranes. Direct contact membrane distillation was also carried out with the same membrane materials and a similar concentration of NH_3OH solution to determine any difference between the matrices. **Error! Reference source not found.** 14 shows the schematic diagram of the

membrane contactor system used in this investigation. The membrane contactor system consists of a feed side and stripping solution side which operates a re-circulation loop which allows for the feed to be repeatedly treated allowing for maximum NH_3 removal. A summary of the membrane system can be described as follows; the diluted feed solution is decanted into the feed reservoir and adjusted to pH 10.5 in order to ensure all the NH_3 is in its volatile gaseous form and maintained throughout the process. The feed pump carries it to the membrane housing where liquid remains on the feed side of the membrane under investigation and the volatile gaseous NH_3 crosses the membrane pores as explained by (Brennan, Briciu-Burghina, et al., 2020) to react with the stripping solution (0.1 M H_2SO_4) to produce $(NH_4)_2SO_4$. The parameters of the lab-scale system used in this study are presented in Table 23.

Table 23: Parameters used for operation of lab-scale membrane system.

Parameter	Feed flow rate (L/h)	Feed temperature (˚C)	Feed pH	Pressure (Bar)	Permeate flow rate (L/h)	Permeate temperature (˚C)
Level	120	35	10.5	0.2	120	35

The NH_3 removal efficiency was determined by measuring the concentration of NH_3 in the feed solution periodically over 3 hours (every 30 min) using a NH_3 electrode (Orion High Performance NH_3 Electrode, Thermo Fischer Scientific, Ireland). The energy efficiency of the membranes was investigated by measuring the temperature of the feed and retentate to determine if there was any heat loss to the stripping side through the membrane. The energy

used to power the whole system (pumps and heaters) throughout the 3 hours was also determined using a Nevsetpo Power Meter (Amazon, UK) which gave an automatic kWh reading. The above process was carried out using 1) NH_3OH solution and 2) RCWW. The two separate matrices were investigated in order to determine the effect of RCWW on the membranes compared to the NH_3OH solution.

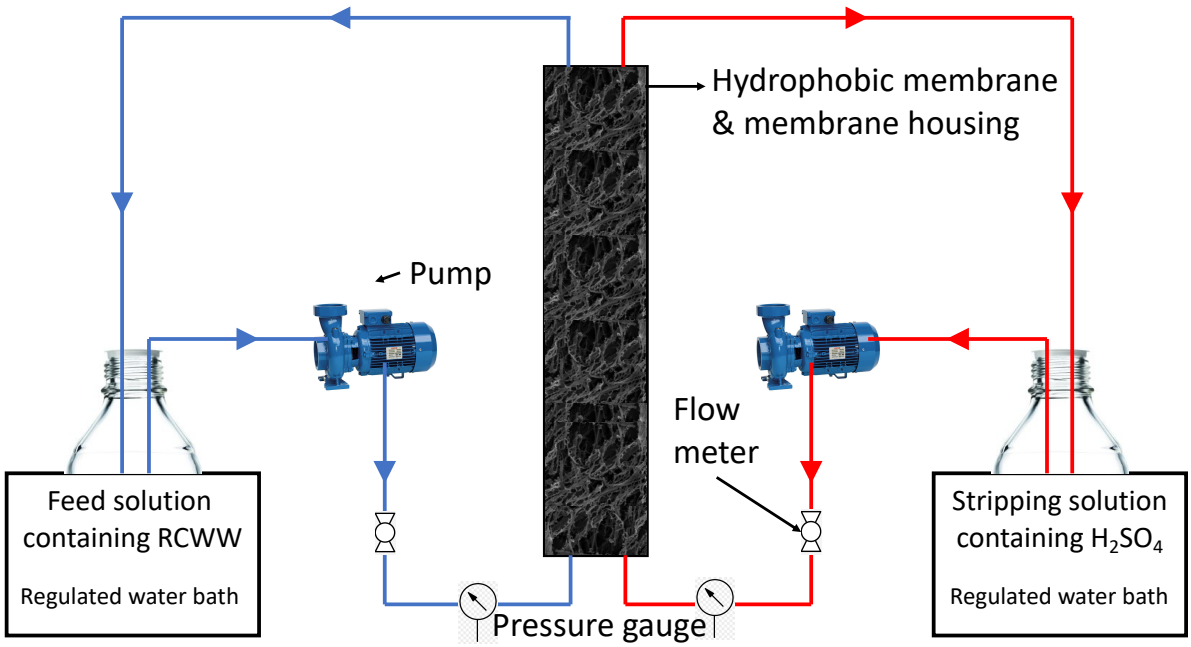

Figure 14: Schematic diagram of lab-scale membrane distillation set-up used in this study.

4.2.3.2 Pilot-scale operation

The NH_3 stripping unit was designed according to requirements and to feature automated, unattended operation, data logging, and remote access. The specifications for two modules evaluated in the investigation are summarized in

Table 24. The membrane materials which were used were PP1 and PTFE1 and were the membranes of choice based on results from lab-scale analysis.

Table 24: Summary of the specifications for the pilot-scale membrane modules tested in this study.

	Membrane 1	**Membrane 2**
Membrane material	PTFE1	PP1
Configuration (type)	Spiral wound	Spiral wound
Surface area (m^2)	6.7	3.7
Flow feed side (m^3/h)	0.05–0.7	0.05–1
Flow acid side (m^3/h)	0.05–0.7	0.05–0.5
Pressure max (bar)	0.6	0.5
Temperature max (°C)	45	50

All experiments were conducted in a pilot scale system module as described briefly in the schematic diagram in Figure 15 and in detail in the schematic diagram in Figure 16. The NH_3 rich RCWW sample was obtained from the rendering plant waste which is being fed into the nitrification tank (AT3 tank in Figure 13) in the ABP WW treatment facility. The RCWW samples pass through a series of heaters and NaOH dosing pumps to ensure that the temperature and pH are at the optimum conditions to ensure the NH_3 is in its volatile gaseous form. The RCWW sample then passes through a settler tank and 50 µL filters to ensure the RCWW sample does not have particles present which may clog the membrane pores. Once the sample is in its volatile gaseous form the RCWW stream is pumped into the feed side of the hollow fibre membrane whilst the stripping solution, H_2SO_4, flows along the permeate side of the hydrophobic membrane. The gaseous NH_3 diffuses through the hydrophobic membrane and reacts with the H_2SO_4 on the permeate side. The reaction that occurs between NH_3 and H_2SO_4 can be seen in Equation 1 which produces $(NH_4)_2SO_4$. Both solutions on either side are then recycled to their respective reservoirs which can be seen in Figure 15.

$$2NH_3 + H_2SO_4 \rightarrow (NH_4)_2SO_4$$ (Equation 1)

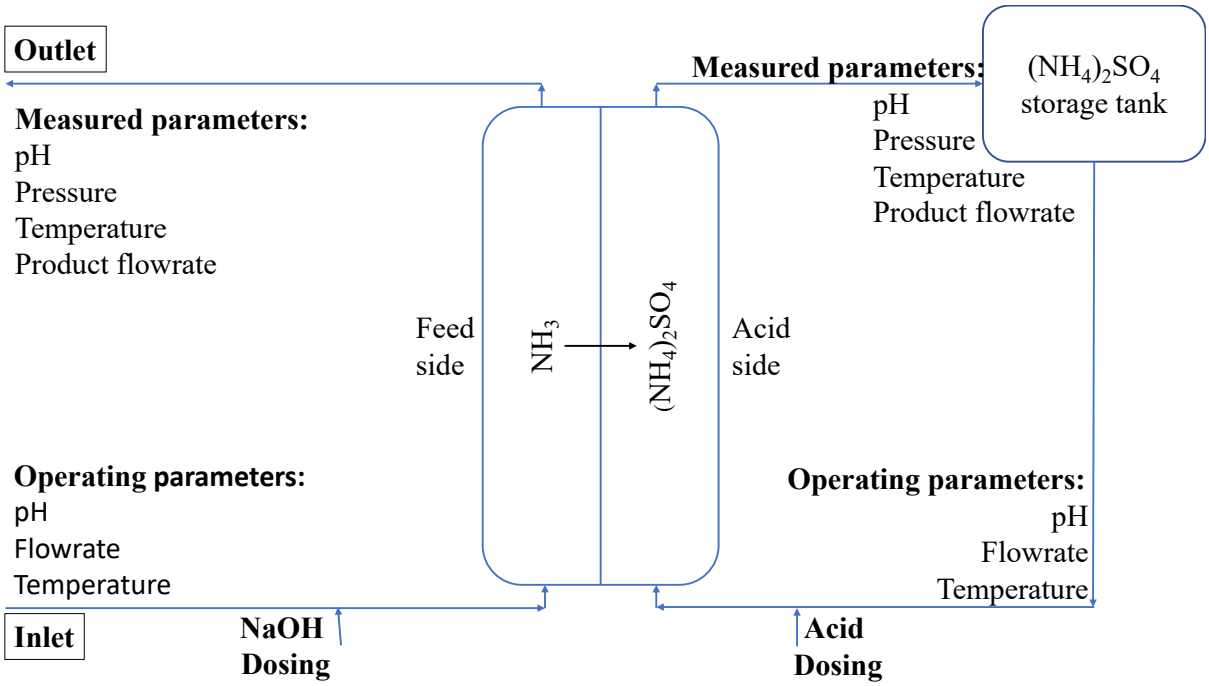

Figure 15: Simplified schematic of process overview of pilot-scale (not to scale).

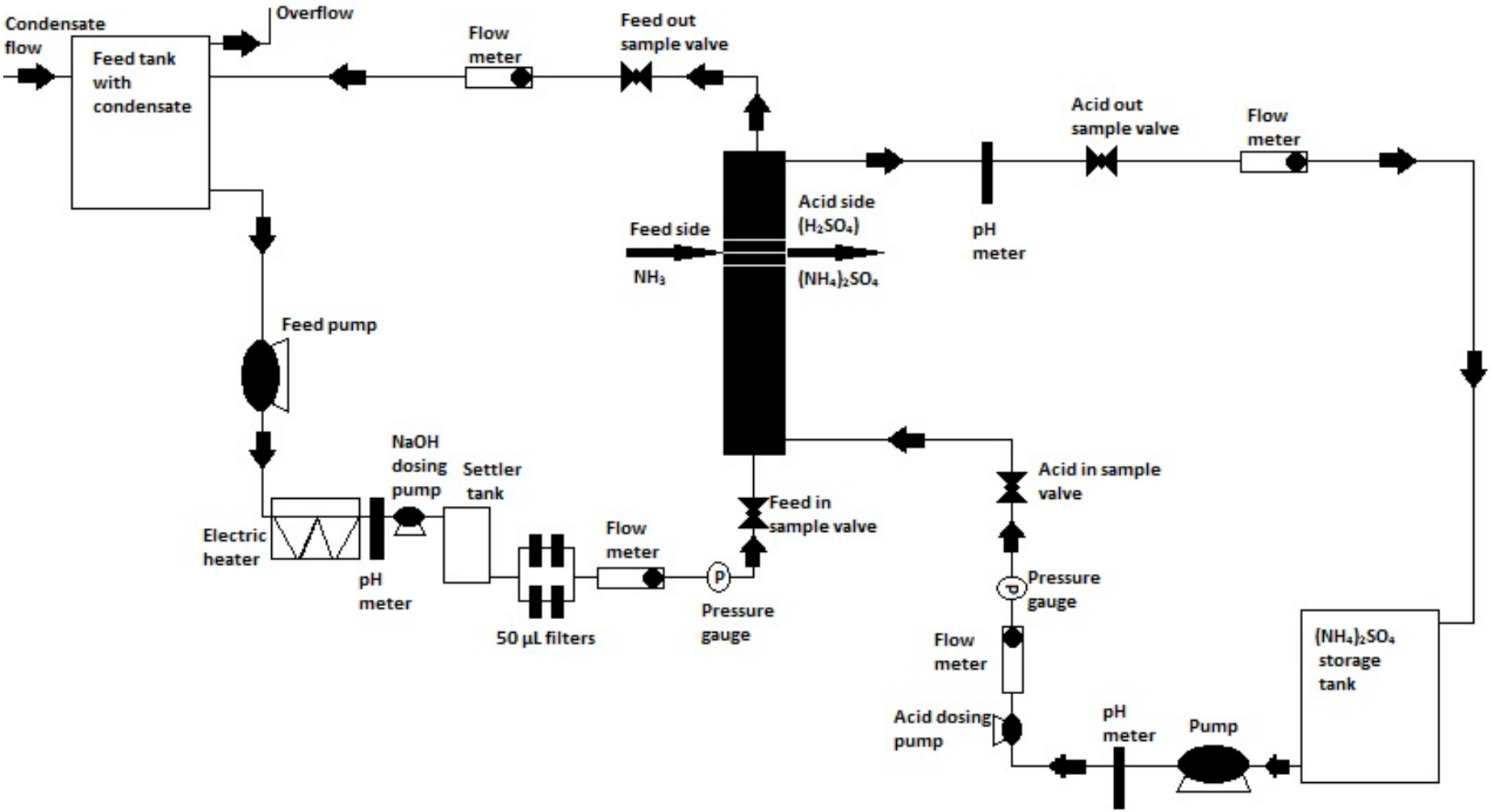

Figure 16: Detailed schematic diagram of pilot-scale membrane system used in this investigation (not to scale)

4.2.4 Physiochemical characterisation

The RCWW samples were analysed to characterize them according to their physicochemical properties including pH, temperature and turbidity. By investigating these properties, pre-treatment steps of the RCWW samples were then formulated depending on the results for both lab- and pilot-scale analysis. The temperature was measured using a YSI Proplus® handheld multi-parameter instrument (YSI, Xylem, Hertfordshire, UK) and the pH was tested using a WTW Multi 320 multimeter, pH electrode SenTix 4l. The turbidity of the sample was analysed using a portable turbidity meter Turb® 430 IR (VWR, Dublin, Ireland). Particle size distribution was carried out using a Malvern Mastersizer 3000E using a Hydro EV wet dispersion unit procured from Malvern Panalytical with a stir speed of 8001200 RPM and sonification of 50 %. An investigation was carried out in order to determine the relationship between the temperature and pH of the RCWW samples. Titration experiments were carried out at different temperatures to determine the required volumes of NaOH to raise the pH to optimum levels for NH_3 removal. Particle size distribution and turbidity in the RCWW samples was investigated to determine if any pre-treatment is required to protect the membranes from clogging.

4.2.5 Effect of matrix on membrane materials

Membrane materials were characterised in their pristine condition and after being exposed to treated RCWW (as outlined in Section 4.2.2). This was carried out in order to determine the effect RCWW had on membrane materials and how it could affect the

performance of membrane materials. All membrane materials were investigated in triplicate (n=3) before and after being exposed to treated RCWW. Additional membranes were exposed to a NH_3OH solution for 3 hours to further examine the effect of RCWW.

The surface and cross-sectional morphologies including the fibre and pore size of all investigated membranes were obtained using scanning electron microscope (SEM) (Hitachi S3400n SEM, Tungsten system) (samples were investigated in triplicate and 20 pore sizes and fibre diameters were measured in each sample). Samples were gold coated using a 750T sputter coater (Quorum Technologies, Lewes, UK) and carried out with 20-25 kV acceleration. Surface roughness was quantitatively determined using atomic force microscope (AFM) (BRUKER ICON DIMESNION AFM) using Silicon AFM probes in non-contact/tapping mode (13 kHz resonant frequency and 0.2 N/m force constant), procured from Nano and More, UK. Membrane porosity was performed as outlined by (Woo et al., 2017). The surface energy of all membrane materials was determined by measuring the CA (FTA200 Dynamic Contact Angle Analyzer) using DI water (Elga Purelab Ultra system) in order to determine the hydrophobicity of the membrane surface. LEP is the minimum pressure applied to a pristine membrane which results in liquid penetrating inside the membrane pores. LEP was investigated using a LEP set-up with Amicon Test Cells and carried out as outlined by Smolders and Franken (Smolders & Franken, 1989).

4.2.6 Membrane wetting determination

In order to determine if RCWW resulted in membrane wetting after being exposed to RCWW at pilot-scale, a leakage test was carried out. This is carried out by flushing clean water on the feed side of the membrane module in a recirculation mode and H_2SO_4 on the permeate side. Conductivity is measured in the feed tank over time. Considering a high conductivity gradient is present between the feed and permeate side of the membrane, if the membrane is leaking, then mass transfer is reversed and an increase in conductivity should be noticed on the feed side and ultimately in the CIP tank. The membrane material was exposed to RCWW at different time intervals (0 min, 10 min and 2 h) and it then underwent operation with clean water and H_2SO_4 mixture. If leakage occurred after RCWW it would result in ions from the H_2SO_4 passing to the feed side by electrolyte diffusion and increasing the conductivity of the CIP tank.

4.2.7 Membrane cleaning

A membrane cleaning study was carried out to determine if a cleaning protocol could increase the lifetime of the membrane material and return the flux to original conditions after the membrane exposed to RCWW was cleaned. The membrane cleaning protocol was developed based on work carried out by various authors using NaOH as the cleaning solution (C. Liu et al., 2001; Nguyen & Roddick, 2011). Briefly, a clean membrane was used to remove NH_3 from a 100 mg/L NH_3OH solution. Thereafter, the same membrane material was exposed

to RCWW and used to remove NH_3 from RCWW (RCWW was treated as outlined in Section 4.2.2). After exposure to RCWW, 10 L of DI water was pumped around the membrane system to initially clean the membrane material (not returned to feed tank). 2 L of 0.1 % wt. NaOH was then recirculated through the feed side of the membrane for 1 h to hydrolyse and solubilise any fats and proteins from the membrane material as outlined by (C. Liu et al., 2001). After the NaOH solution was recirculated, 10 L of DI water was used to rinse the membrane again without recirculating. The clean membrane was used again to treat a 100 mg/L NH_3OH solution, and the removal rate was compared to the initial NH_3OH solution removal rate. A comparison experiment was carried out with the same protocol without the NaOH cleaning step in order to determine the importance of the cleaning step.

4.2.8 Statistical analysis

Statistical analysis was carried out to determine if there was any difference in the results obtained for membrane characterisation between pristine membranes and membranes exposed to NH_3OH or RCWW. Statistical analysis was carried out using a 2-sample t-test and if the p-value obtained was <0.05 for the two membrane materials, it was deemed statistically different (Nahm, 2017).

4.3 Results and discussion

RCWW is a waste product from the processing of animal waste which possesses a high NH_3 concentration. The volume of animal waste processed daily varies which results in a varied NH_3 concentration in the RCWW. Operation of the rendering plant starts on a Monday and it begins to produce RCWW on Monday evening which is then fed into an aeration tank where it is conventionally treated for NH_3. From this feed, samples were taken to test on the membrane system. On Fridays, RCWW flow ceases due to the plant being shut down for the weekend. This results in no NH_3 being produced over the weekend. Figure 17 shows the concentrations of NH_3 over the course of a month which shows the variation which is experienced. It can be seen that the highest levels of NH_3 are experienced mid-week or on Tuesday just after operations have started up again. Levels indicated by zero identify when operations were not running in the plant.

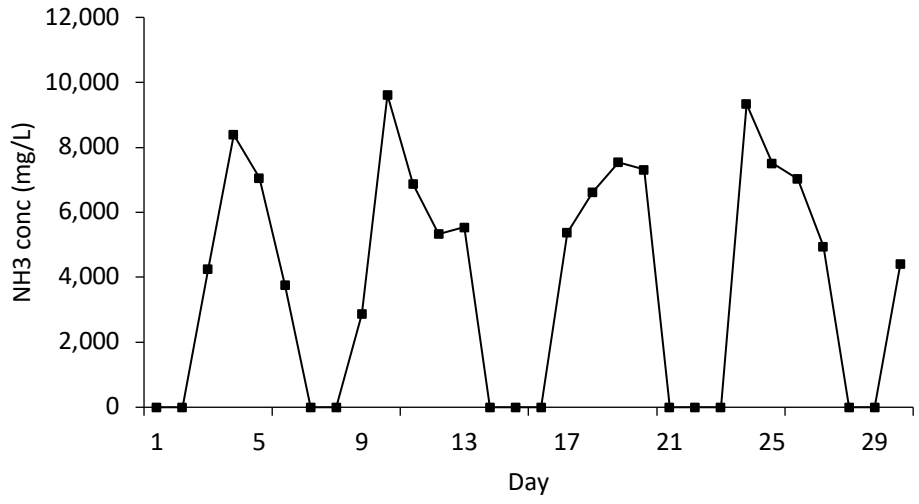

Figure 17: Variation in NH_3 levels in RCWW over the course of one month (April 2018).

A number of tests were carried out in order to characterise the RCWW according to its physicochemical properties including its pH, temperature and turbidity, and how they exert influence on each other. By doing so, it ensures the RCWW is pre-treated efficiently to be in its volatile form so that it is capable of crossing over the hydrophobic membrane in gaseous form. Investigating the turbidity, it allows for the RCWW to be filtered appropriately in order to prevent it from clogging the membrane pores. The second investigation carried out was completed to determine the suitability of various membrane materials for removing NH_3 from RCWW. The rate of NH_3 removal was determined by measuring the NH_3 levels in the feed inlet and feed outlet (retentate). Failure of membranes was further investigated for membrane wetting using CA and leakage using conductivity analysis.

4.3.1 Physiochemical characterisation of matrix

An investigation was carried out to determine the relationship between the pH and temperature of the RCWW. Two RCWW samples, one with an ambient temperature of 20 °C and the other sample at 75 °C were investigated. As seen in Figure 18, it was noted that the initial pH drops as the temperature increases. This is caused by equilibrium shifting due to increase ionization of solute molecules according to Le Chatelier's principle. If the temperature of a dynamic system is increased, the equilibrium will move to lower the temperature by absorbing the extra heat. That means that the forward reaction will be favoured, and more hydrogen ions and hydroxide ions will be formed increasing the equilibrium constant (Kw). Further tests were carried out with RCWW and DI water to

determine the temperature dependence of the pH. Due to this dependence of the pH, an increased volume of NaOH is required in order to adjust the pH of the RCWW. Figure 18 shows how much NaOH is needed in order to adjust the pH of the RCWW to its optimum conditions (pH of 10.5). This suggests that using a higher temperature (75 °C) will increase the cost efficiency of NH_3 removal as less NaOH will be used to increase the pH to 10.5. However, more energy will be used as the RCWW with an initial temperature of 20 °C will need to be heated. However, by increasing the temperature it decreases the potential of membrane clogging which may be more influential in the NH_3 removal process as discussed below.

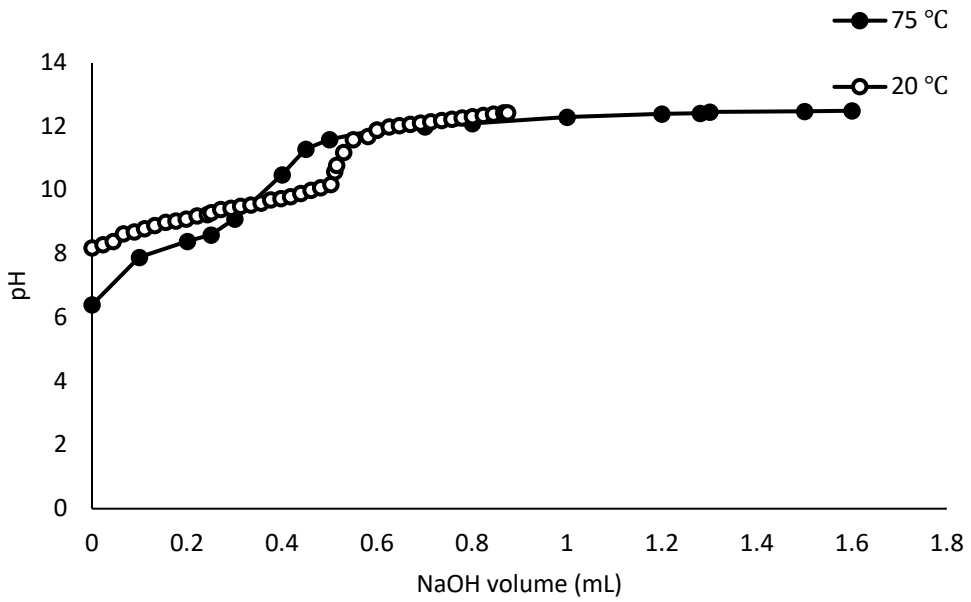

Figure 18: Titration of the same RCWW sample (60 mL) with 30% (w/v) NaOH at 20 and 75 °C.

Sample turbidity in RCWW samples was investigated to determine if any pre-treatment is required to protect the membranes from clogging. A decrease in turbidity was noted with

an increase in temperature and pH. The RCWW sample heated up to 75 °C and pH adjusted to 12 had a turbidity of 40 NTU whilst the RCWW sample at an ambient temperature and its natural pH (approximately pH 7-8) had a turbidity of 180 NTU. The results show that there is a strong correlation between an increase in temperature & pH and a lower turbidity and particle size distribution. The RCWW contains protein agglomerates and FOG. Increased temperature and ionization (due to NaOH) has a solubilizing effect on these molecules causing larger particles/agglomerates to break down into smaller constituents which is required to help prevent membrane fouling. It was concluded that operation at high pH and temperatures above 10.5 and 45 °C, respectively, avoid the need for a pre-filter finer than 50 µm to be implemented to protect the membrane contactor, as protein agglomerates and FOG are solubilized under these conditions. As such the optimum pH and temperature of the feed solution should be 10.5 and 45 °C, respectively in order to make the feed solution suitable for the membrane material. In addition, the high pH and temperature reduce the likelihood of biofouling of the membrane.

4.3.2 Effect of RCWW on membrane materials

4.3.2.1 Characterisation methodologies

The structural and physical characteristics of a membranes material are critical to predict the performance and efficiency of a membrane material (Bae & Kim, 2020). Various authors including (Rehman et al., 2019), (Guillen-Burrieza et al., 2016) and (Rezaei et al., 2018) have reported that membrane morphologies (pore size and fibre diameter), surface roughness,

surface energy, LEP and porosity to be of the most important characteristic parameters. Additionally, (Lauterböck et al., 2013) has reported that surface area and surface porous structure can influence the removal rate of NH_3 in membranes but there a very limited studies which have investigated the surface area of membrane materials for this purpose. Membrane morphologies such as pore size and fibre diameter are important parameters as optimal pore size (0.1 – 1 µm) has been linked with reduced membrane wettability while the number of fibres and fibre diameter can influence the removal rate and wetting efficiency (Eykens et al., 2016). In this study, the Hitachi S3400n SEM was used. This instrument allows for a resolution of 4 nm when using a low vacuum and acceleration voltage of 30 kV. As this study used a lower acceleration voltage (20-25 kV), a lower resolution is expected (Bayazid et al., 2020). Due to the ongoing Covid-19 pandemic, this was the best instrument available at the time of testing. The Hitachi S5500 may be more suitable allowing for resolution of 0.4 nm and therefore higher quality images. Due to large variation between samples, 30 measurements were recorded for the pore size and fibre diameter to gain a strong overview of the sample. Surface roughness is investigated using AFM and is an important parameter as roughness indicates how susceptible a material is to having particles bind to the surface. The more rough a surface is, the less likely a sample will bind to the surface which is ideal for RCWW samples which have high volumes of components which will cause wetting if bound to the surface (Shirazi et al., 2013). AFM is carried out on all membrane materials to determine which membrane surface has the roughest surface. Additionally, it is carried out after RCWW exposure to determine if RCWW negatively impacts the membranes surface. Samples were carried out in triplicate which is agreement with many other previous studies which looked at

similar samples and matrices. Surface energy (contact angle) studies were carried out to investigate how hydrophobic different membrane materials were, how RCWW effected the hydrophobicity overtime and the surface energy difference between DI water and RCWW samples. The results obtained from these experiments would provide beneficial information regarding how susceptible the membranes are to wetting. LEP and porosity were carried out in accordance with other published studies and provide beneficial information regarding the mechanical strength of the membrane and how porous a membrane material is, respectively. Surface porous structures of membrane materials can be investigated using Brunauer Emmett Teller (BET) analysis. BET analysis has the advantage of indicating the physical adsorption of gas molecules which is critical in this study due the molecular size of the NH_3 molecules and the microporous structures. Unfortunately, due to the ongoing Covid-19 pandemic, access to a BET instrument was unavailable and this analysis could not be carried out. Porosity tests were carried out using ethanol which gave an indication of the volume of pores divided by the total volume of the membrane (Woo et al., 2017).

4.3.2.2 Membrane wetting

It has been reported that more hydrophobic materials are more suitable for changing matrices. Optimum hydrophobic materials should allow for minimal contact between the liquid and surface to prevent surfactants penetrating the membrane pores and thus wetting of the membrane occurring (Rezaei et al., 2017). Surface energy CA results for the 9 membrane materials used in this investigation are presented in **Error! Reference source not found.** 25. The results presented show the CA obtained for clean membranes, membranes which were subject to NH_3OH solution exposure for 3 hours and membrane exposed to RCWW for 3 hours under similar conditions.

Table 25: CA for each investigated membrane using deionised water for clean membranes, membranes exposed to NH_3OH for 3 hours and membranes exposed to RCWW for 3 hours (n=3).

Membrane material	Clean membrane	NH_3OH exposure	RCWW exposure
PP1	111.3±2.7	101.7±2.3	96.7±2.8
PP2	120.1±0.9	120.1±3.0	110.4±1.1
PP3	130.7±1.2	127.2±8.4	120.2±2.5
PTFE1	119.3±2.9	121.2±7.2	118.2±6.4
PTFE2	123.0±4.1	127.3±1.5	123.8±3.4
PVDF1	98.5±2.0	89.0±3.9	81.9±1.9
PVDF2	126.4±1.1	117.3±4.7	100.5±6.7
PES	107.3±2.4	109.4±1.4	108.8±1.7
SHP	128.6±1.5	127.0±1.4	132.1±4.5

Supplier specifications outlined that SHP was a superhydrophobic membrane (CA>150˚) but initial analysis (128±1.5˚) determined that the membrane is not superhydrophobic. CA values obtained after exposure to NH_3OH and RCWW are consistent

suggesting that these matrices have no effect on the surface energy. Statistical analysis suggests that the CA values for PP and PVDF membranes were the only materials affected after being exposed to either NH_3OH solution or RCWW. PP1, which was fabricated by stretching of polymers, shows to have an initial CA of 111.3±2.7˚ which reduced by almost 15˚ after being exposed to RCWW suggesting that the OM present in the RCWW effects the hydrophobicity of the material. PP2 and PP3 were both fabricated using fibre pressing and have pore sizes of 0.22 and 0.45 µm, respectively. PP3 which has the bigger pore size shows to have higher CA values suggesting that increased pore sizes increase hydrophobicity. It should be noted that the pore sizes of PP1 and PP3 were similar and PP3 showed to have significantly higher CA which suggests that the fabrication method also impacts the hydrophobicity of the material which is very important when designing fabrication methods. Both PVDF membranes investigated (PVDF1 and PVDF2) were of similar pore size and fabricated the same way – however, PVDF1 had a polyethylene terephthalate (PET) substrate layer. A study by (Jasmee et al., 2018) showed that the hydrophobicity of PET was 75.3˚ which may be the reason why PVDF1 has a lower CA compared to PVDF2 (98.5 and 126.4˚, respectively). After exposure to RCWW, PVDF1 loses its hydrophobic nature while PVDF2 remains hydrophobic but is impacted significantly. PTFE and PES membrane materials demonstrated a stable resistance to the RCWW, and their hydrophobicity did not decrease after being exposed to either NH_3OH or RCWW. The SHP membrane which was made of PES with a support layers of perfluorooctanoic acid nanoparticles showed greater hydrophobicity compared to omni phobic membranes (used on the PES membrane). However, it was later reported by the manufacturer, after investigations were completed, that the

perfluorooctanoic acid support layer, was discontinued due to their toxic nature. The PTFE membranes differed in terms of their support layer, which PTFE2 had a PP support layer. The results show that this support layer allowed for a slightly higher CA value (123˚ vs 119˚). It has been reported by multiple studies that PTFE offers strong and hydrophobic membrane to treat NH_3 from WW which is resistant to wetting as supported by the results achieved in the study presented in this chapter (Ahn et al., 2011). One of the most important requirements for membrane contactors is their ability to resist membrane wetting, which is evident in PTFE and PES membranes and as such, these two membrane materials appear to be the most suitable material to treat RCWW in terms of membrane wetting resistance.

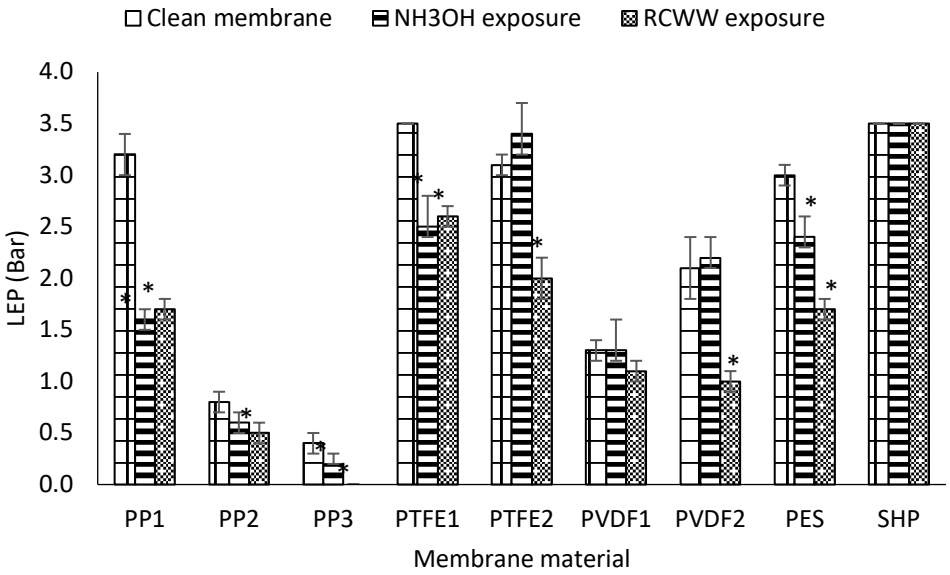

Figure 19: LEP values obtained for membrane materials with and without exposure to NH_3OH and RCWW. Statistical analysis showing significance between different membrane conditions expressed as p-value (p-value > 0.05 suggests the conditions are not significantly different; n=3).

The LEP results obtained for the various membranes in this study are presented in **Error! Reference source not found.** 19. According to (Rezaei et al., 2017), OM and surfactants in the feed solution can result in decreased LEP values due to a build-up on the surface which is expected in the results of this study. The results show that clean PP1, PTFE1, PTFE2, PES and SHP have LEP values > 2.5 bar which suggest they are suitable for membrane operation. However, after being exposed to RCWW for 3 hours, only PTFE1 and SHP maintained LEP values >2.5 bar. Interestingly, PP1 decreased to 1.6 bar after being exposed to NH_3OH resulting in the membranes wetting with exposure to a liquid without any surfactants which suggests this is a very weak membrane material. Similar results were obtained for PP1 after being exposed to RCWW for 3 hours (1.7 bar). Seven of the membranes which were investigated (PP1, PP2, PP3, PTFE1, PTFE2, PVDF2 and PES) showed significant decrease in LEP after being exposed to RCWW (not impacted by NH_3OH solution) which supports claims that OM and surfactants influence the LEP. The decrease in LEP may be due to a build-up of surfactants on the membrane material which causes decreased pore sizes as shown in Figure 20. This build-up of surfactants may prevent the vapour phase NH_3 from passing through the membrane which builds up pressure in the feed side and eventually resulting in the membrane failing. The SHP membrane material showed to consistently stay greater than 3.5 bar suggesting a strong structure and that this membrane is capable of RCWW treatment. However, as stated above the support layer of the SHP membrane could be an unsuitable material choice, and support layers with similar hydrophobic characteristics should be explored. PTFE1 also showed potential but its LEP values decreased after exposure to both

NH_3OH & RCWW, and a cleaning protocol may need to be introduced in order to keep the integrity of membrane.

4.3.2.3 Membrane morphology

Porosity has been described as one of the most important membrane characteristics in terms of flux and energy efficiency (Eykens et al., 2016). Porosity is defined as the ratio of open space (in terms of the pores) over the total area of the membrane (Dong et al., 2014). A membrane with a higher porosity had been reported by (Okajima & Kurihara, 1966) to prevent wetting. However, (Lauterböck et al., 2013) challenged this, and also the results presented in **Error! Reference source not found.** 20 show that this statement may not be true. The results show that the most porous membranes were in the following order PP1>PES>PTFE2>PTFE1>PVDF1>PVDF2>PP3>SHP>PP2. PP1 shows to have the highest porosity at approximately 86 % and the material was not impacted after being exposed to NH_3OH or RCWW. This result is quite interesting as PP1 showed to be impacted the most in terms of CA and LEP as discussed in Section 4.3.2.2.1. A study by (Eykens et al., 2016) also showed PP with no support layer to have high porosity values at 83 %. SHP which showed to have the most promising anti-wetting characteristics in Section 4.3.2.2.1 also had one of the lowest porosities at 43 %. PP3 was the only membrane which had significantly changed porosity (**Error! Reference source not found.** 20) after exposure to RCWW and it actually increased which suggests that the constituents within the RCWW may actually alter the pores of the membranes in order to make them more porous and thus result in wetting of the membrane.

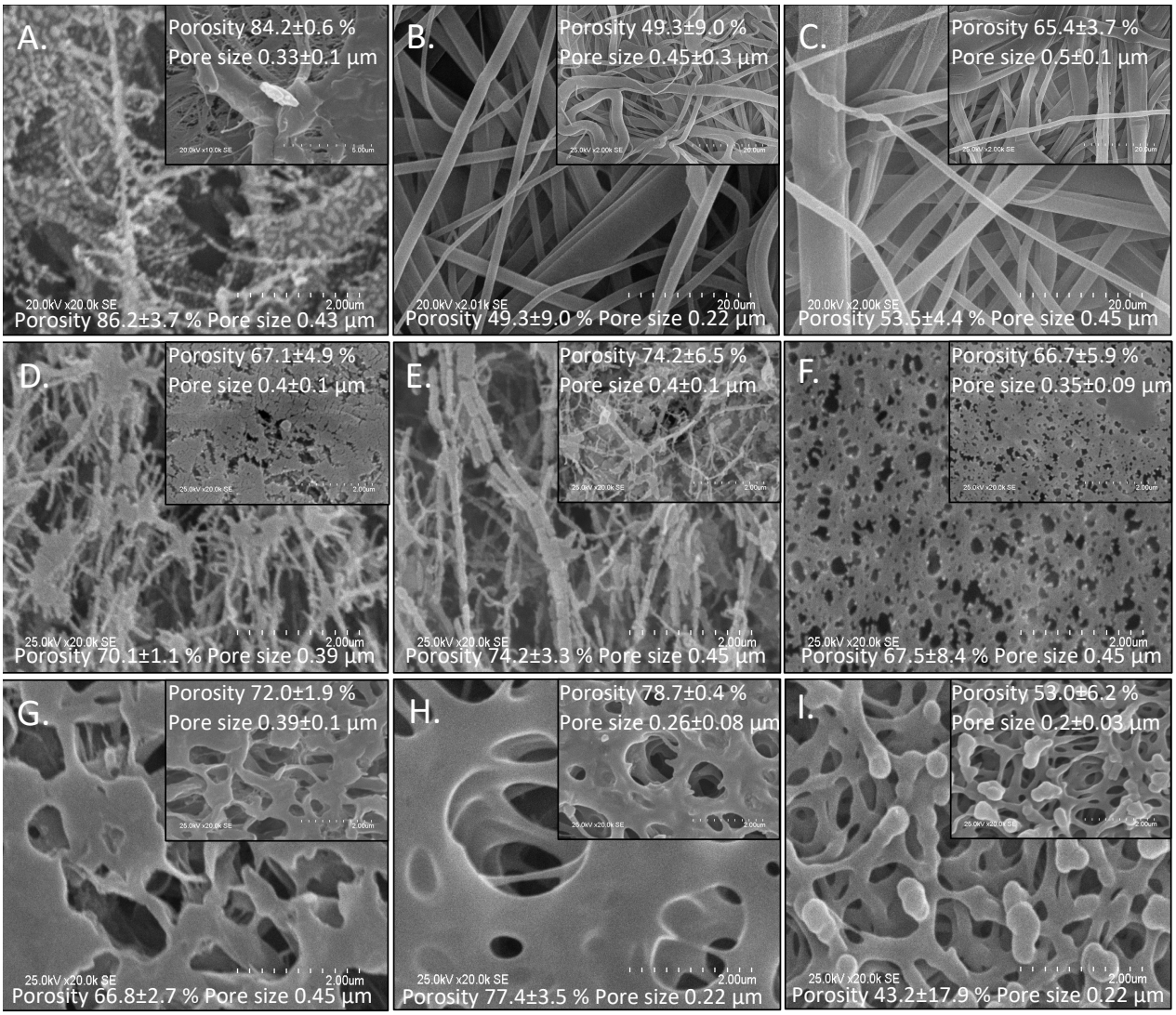

Figure 20: SEM images obtained for clean membranes (primary larger picture) and membranes after 3-hour exposure to RCWW (secondary smaller picture) for (A) PP1, (B) PP2, (C) PP3, (D) PTFE1, (E) PTFE2, (F) PVDF1, (G) PVDF2, (H) PES & (I) SHP. Both images also contain pore size and porosity obtained for respective materials (pore size and fibre diameter measured in triplicate samples from 20 points; porosity measured in triplicate).

The pore size for the 9 membrane materials was determined in their pristine condition, after exposure to NH_3OH & after exposure to RCWW. The quantitative results can be found in **Error! Reference source not found.** 20. The average pore diameter obtained experimentally were all in accordance with the specifications provided by the manufacturer. The results suggest that the pore size of PP2, PP3, PTFE2, PVDF1, PVDF2 and PES either

increase or decrease after being exposed to RCWW. PP2 had a pristine pore size of 0.22 µm and after exposure of RCWW, the maximum pore size found was 1.04 µm suggesting that a rupture in the membrane occurred increasing the pore size. This increase in pore size is in accordance with the decreased LEP and CA results obtained for PP2 after exposure to RCWW suggesting there is membrane wetting occurring. PTFE2 and PES showed to have significantly larger pore sizes after being exposed to RCWW which may be due to membrane wetting. Both PVDF membranes (PVDF1 & 2) showed to have smaller pore sizes which may have occurred due to a build-up of surfactants on the membrane material. The results suggest that the morphological structures of PTFE1 and SHP may be the most suitable for RCWW use as they're not affected by RCWW exposure.

Error! Reference source not found. 20 A-I shows the flat-surface SEM image for the membrane materials. The RCWW exposed images for PP1 shows to have white particles attached to membrane surface which may be foulants and also shows fewer open pores. The cross-sectional image shows the fibres to be broken after exposure to RCWW. PP2 & PP3 exposed to RCWW is very clean with little evidence of fouling on the flat image despite the results presented for CA, LEP and surface roughness. PTFE2 (**Error! Reference source not found.** 20E) has a similar composition to PTFE1 which is expected as they were both produced using ePTFE stretching. A study by (Huang et al., 2004) also fabricated PTFE membranes using ePTFE and presented similar compositions. The SEM images suggest that after the membrane is exposed to RCWW, the pore size distribution is decreased as a result of foulant build up. Additionally, the cross-sectional image shows to have a flat surface with a rough coating which may offer the rough surface experienced by this membrane. After exposure to RCWW, the

surface roughness can also be seen to reduce (quantitively in **Error! Reference source not found.** 20) and not be visible in the SEM image. Both PVDF membranes were fabricated using phase inversion which is a very common method for PVDF membranes and was also demonstrated by (Tao et al., 2013) who presented similar membrane morphological structures. The PVDF membranes appear to be smooth flat surfaces with distinct pores and no multiple layers. SEM images obtained after RCWW exposure suggest strong foulant as the number of pores appears to reduce significantly. The images obtained for the PES membrane shows a very interesting structure with pores which appear large. It is also visible that the membrane has multiple layers with the pores over lapping. Lastly the SHP membrane which is PES is presented in **Error! Reference source not found.** 20I which shows to have a branching structure similar to PTFE1 and PTFE2 which were also fabricated using the stretching method. Additionally, this membrane shows to have nanoparticles incorporated into its structure which is attributed to the perfluorooctanoic acid nanoparticles which give it its increased surface roughness and hydrophobicity. The RCWW exposed image suggests very little impact caused by the RCWW as the image appears unchanged suggesting that the SHP membrane is resistant to the RCWW.

Overall, the images of the various membranes show that they are mostly resistant to RCWW as the images after RCWW exposure appear to be mostly clean and don't show any strong fouling. Some images (PP2, PP3, PVDF1 and PVDF2) show the most impacts of pore size disruption and PES showed to have its structure impacted after RCWW exposure. PTFE1 and SHP membranes showed to have strong resistance – PTFE1 showed to have a build-up of foulants but SHP showed to have no variation at all after exposure to RCWW.

4.3.2.2.3 Membrane roughness

AFM is a method used to quantitively determine the roughness of a membranes topography by analysing the peaks and valleys present in the surface (Y. Liao et al., 2013). All nine membranes used in this study were investigated using AFM and the mean roughness is presented in **Error! Reference source not found.** 21 and 22. The surface height map is shown in **Error! Reference source not found.** 21 for PP2, PTFE1, PES and SHP which present interesting interactions between the membrane material and RCWW. The most notable thing about the results presented is the large deviation which suggests that the membranes surface is not consistent. The area of the sample examined was 5 μm^2 and samples were investigated in triplicate. The PP membrane (PP1, PP2 and PP3) investigated which had varying pore sizes and fabrication methods were shown to exhibit variation in surface roughness. PP1 showed to have a considerably high surface roughness compared to the other two which may have been due to the fabrication method. A study by (Komaladewi et al., 2018) indicated that using stretching as a fabrication method resulted with increased surface roughness. However, this study did not use fibre pressing as a comparison which was used for PP2 and PP3. Additionally, an increased surface roughness was presented in PP3 (compared to PP2) which supports existing literature than there is a correlation between increased pore size (0.22 & 0.45 µm) and increased surface roughness (Tsuyuhara et al., 2010). PES showed to have a high surface roughness which is unusual for a hydrophilic material, but the hydrophobic nature and roughness could be due to the omni phobic layer (Zheng et al., 2018). Lastly, CA results suggest that the SHP membrane is not superhydrophobic – however, the perfluorooctanoic acid nanoparticles which give it its superhydrophobic surface coating provide strong surface

roughness. (X. Chen et al., 2020) demonstrated surface roughness values of >400 nm for PTFE membranes with a perfluorooctanoic acid coating. Additionally, a study by (Rezaei et al., 2018) showed that while superhydrophobic coating can influence greater hydrophobicity and rougher surfaces, certain foulants can result in complex interactions and decrease the surface roughness.

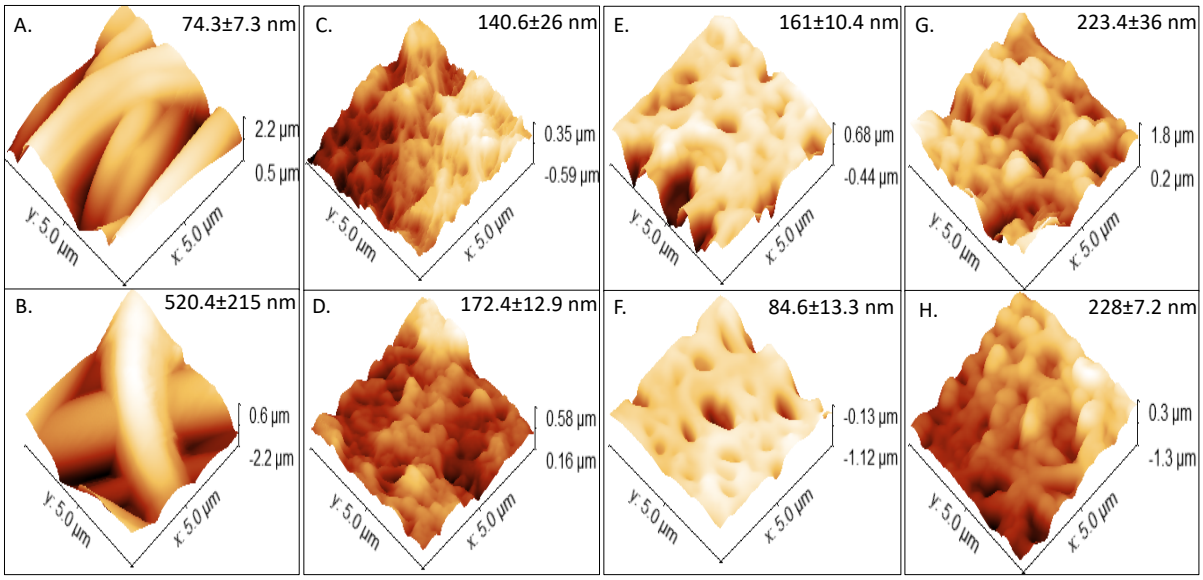

Figure 21: Surface roughness AFM images obtained for clean membranes (Top) and membranes (bottom) exposed to RCWW for 3 h for (A&B) PP2, (C&D) PTFE1, (E&F) PES and (G&H) SHP (n=3). Surface roughness (R_a) expressed as nm on top right of each sample image.

PP1, PTFE1, PTFE2, PVDF2 and SHP did not show any significant difference in their surface roughness values after being exposed to either a NH_3OH or RCWW matrix for 3 hours. This suggests membrane durability in these membranes. However, the CA for PP1 and PVDF2 were impeded after exposure to RCWW which suggests that the surface roughness experienced

here may be just foulants built up on the membrane presenting as rough surface. As such this leaves PTFE1, PTFE2 and SHP as the only membranes which were not affected after exposure to RCWW. The brightest regions of the SEM images represent peaks in the surface while the dark regions represent pores or valleys (Brennan, Briciu-Burghina, et al., 2020). The membranes which experienced a decrease in surface roughness can be seen to have strong peaks and valleys in their clean membrane images and after exposure to RCWW, the contrast of the image becomes one colour to present a coating of foulants and oil from the RCWW to give a more-smooth surface. However, the PTFE membranes can be seen to have consistent images across the different membrane conditions. It can be concluded that membrane exposure to RCWW results in the roughness to decline due to the high content of oil and OM. The results suggest that PTFE membranes offer the greatest resistance to RCWW in terms of surface roughness. In addition, the SHP membrane contains a coating which results in superior resistance to the RCWW.

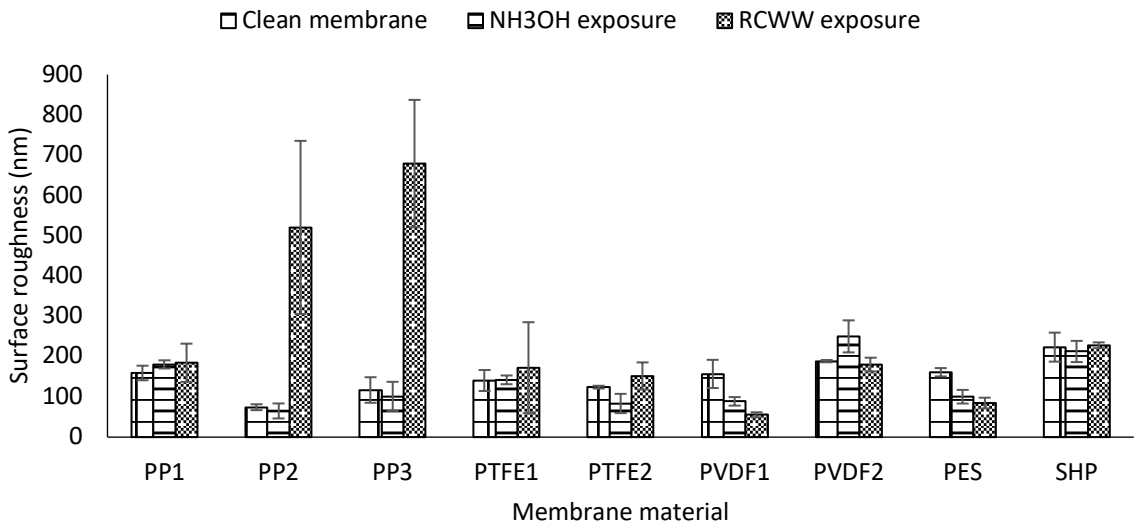

Figure 22: Quantitative results for mean surface roughness (R_a, nm) of the membrane materials at different conditions (n=3).

4.3.3 Membrane integrity

Another, much quicker test to determine membrane integrity is the leaking test. This is carried out by flushing clean water on the feed side of the membrane module in a recirculation mode and H_2SO_4 on the permeate side. Conductivity is measured in the feed tank over time. Considering a high conductivity gradient is present between the feed and permeate side of the membrane, if the membrane is leaking, then mass transfer is reversed and an increase in conductivity should be noticed on the feed side and ultimately in the CIP tank. The membrane material was exposed to RCWW at different time intervals (0 min, 10 min and 2 h) and it then underwent operation with clean water and H_2SO_4 mixture. If leakage occurred after RCWW it would result in ions from the H_2SO_4 passing to the feed side by electrolyte diffusion and increasing the conductivity of the CIP tank. The results after different RCWW exposure times are presented in Figure 23. The results suggest that the much higher

conductivity rate after 2 h of exposure as oppose to 10 min exposure which suggests that 2 h exposure is wetting the membrane surface which in turn causes a mixing of the two liquids.

Results collected from the wetting and leakage tests for PP1 membranes suggest that PP1 is not as efficient as PTFE1 membranes for application to RCWW over long periods of time due to surface wetting. Differences observed between the surface wettability of DI water and RCWW determined that although such PP1 membrane could be used with other WW types, they are not suitable for RCWW without frequent cleaning.

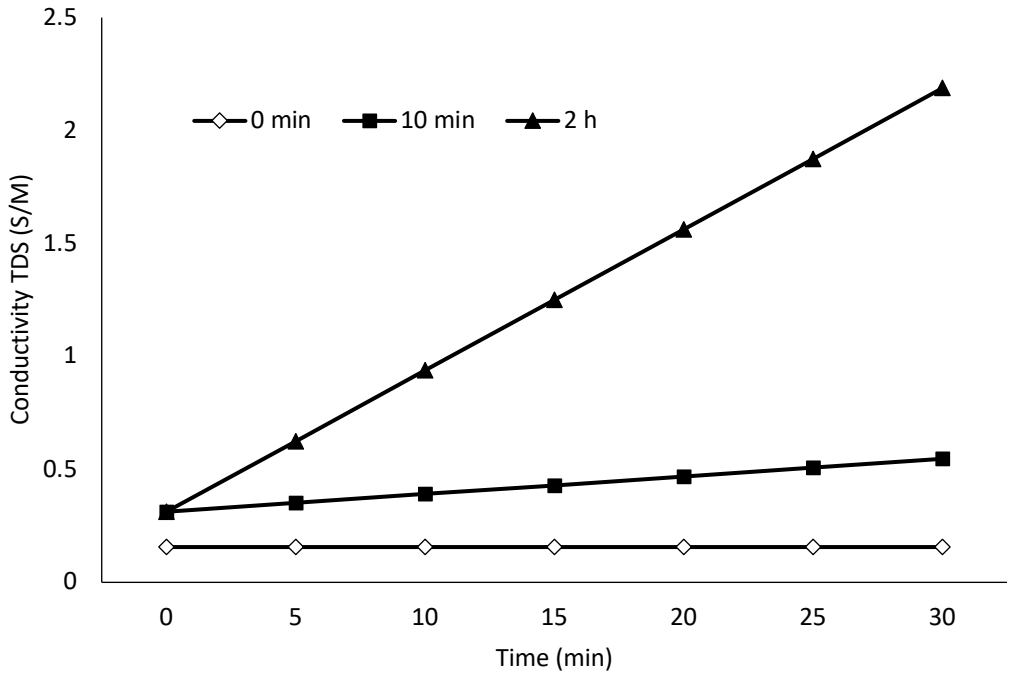

Figure 23: Rate of conductivity increase in the CIP tank after different RCWW exposure times using the PP1 membrane.

4.3.4 Membrane efficiency

4.3.4.1 Lab scale efficiency

4.3.4.1.1 NH_3 removal efficiency

Lab-scale studies on all 9 membranes were carried out to determine the rate of NH_3 removal from a 100 mg/L NH_3OH solution and a RCWW solution with a similar NH_3 concentration. The results obtained for each of the analysis is presented in Figure 24 and

Table *26* including the p-value for statistical analysis determining if there is a significant difference in the volume of NH_3 removed between to two matrices using the same membrane materials. The membrane material which allowed for the highest NH_3 removal from the NH_3OH solution was PP3 (70.3 %) which has a 0.45 µm pore size and contains no support layer. PP2 which has the same chemical composition as PP3 and varies in terms of its pore size (0.22 µm) showed to have one of the weakest NH_3 removals with the same matrix at 48.4 %. This suggests that the pore size of the membrane affects the rate of removal. Both PVDF membranes have similar pore sizes and were fabricated using the same methods. PVDF1 contains a PET support layer which showed to impact the rate of removal and resulted in a decreased NH_3 removal (33.7 % vs. 53.2 %). However, the PTFE membranes showed to react differently to a support layer as PTFE2 had a PP support layer which allowed for almost 11 % greater removal (56 vs 67 %). Figure 24 shows the rate of removal for each of the membranes from both matrices (NH_3OH solution and RCWW). The graph shows that for the majority of the materials, the greatest volume of NH_3 is removed in the first 30 mins suggesting that the driving force for mass transfer decreases as the concentration of $(NH_4)_2SO_4$ on the stripping side increases. However, the removal rate of NH_3 from the 100 mg/L NH_3OH solution using the SHP membrane showed to have a continuous removal rate and then spiked after 150 min. Further analysis was carried out to determine what was the maximum level of NH_3 could be removed after 360 min and it was determined that soon after 180 min, the rate of removal started to plateau as seen in Figure 25. After 360 min operation, it allowed for 54 % removal of NH_3.

Table 26: Comparison of NH_3 removal efficiency achieved in different matrices by membrane materials (n=3).

Membrane material	NH_3OH (% removal)	RCWW (% removal)	p-value	Significant difference between matrices
PP1	60.8±1.7	49.4±2.7	0.043	Yes
PP2	48.4±3.6	60.7±4.2	0.09	No
PP3	70.3±1.1	39.5±3.9	0.008	Yes
PTFE1	59.0±3.5	62.2±0.7	0.05	Yes
PTFE2	67.5±2.0	54.1±1.3	0.011	Yes
PVDF1	33.7±5.4	39.6±4.3	0.1	No
PVDF2	53.2±1.9	47.2±0.9	0.01	Yes
PES	56.0±2.5	53.6±3.5	0.52	No
SHP	50.1±2.9	47.6±2.8	0.418	No

However, when the membranes were deployed to treat RCWW some of the membrane materials (PP1, PP3, PTFE2 and PVDF2) showed to be significantly affected by the composition of RCWW. PP3 which had the greatest NH_3 removal when using NH_3OH showed to reduce significantly (70 to 39 %) when RCWW was the matrix. Results in Figure 19 & Figure 20 and Table 25 show that the CA & LEP decreased while the porosity increased suggesting that there may be membrane wetting and that the permeate side is crossing over to the feed side and preventing NH_3 removal. PP1 and PTFE1 showed to also be significantly impacted by the exposure of RCWW which is most likely due to the clogging of pores which was presented by SEM analysis. The use of PP membranes was used by (J. Zhang et al. 2020) to remove NH_3 from synthetic urine and a removal rate of 80 % was achieved. However, this matrix was not as complex as RCWW and did not contain such high concentrations of OM, proteins and fats which may have allowed for greater removal. PTFE2 and PVDF2 membranes both showed to decrease after RCWW exposure which may also be due to the membrane materials being susceptible to wetting. A study looking at municipal WW using PVDF membranes removed up

to 69 % of NH_3 and a study using PTFE membranes to remove NH_3 from coking plant WW removed 50 % NH_3 which suggests that both of these membrane materials remove adequate levels of NH_3 from complex matrices (Guo et al. 2019; P. H. Lin et al. 2018). The remainder of the membrane materials (PP2, PTFE1, PVDF1, PES and SHP) showed to remove the same levels of NH_3 in both matrices suggesting that the composition of RCWW didn't impact their ability to allow NH_3 through the pores. However, the characterisation of the membranes showed that the membranes were structurally affected by the RCWW so these membranes may be impacted more over time and as such, a membrane with a high removal rate and low wetting capacity by RCWW should be used which theoretically makes PTFE1 and SHP a suitable candidate for future studies (although SHP is not suitable due to the toxic nature of the membrane as discussed previously).

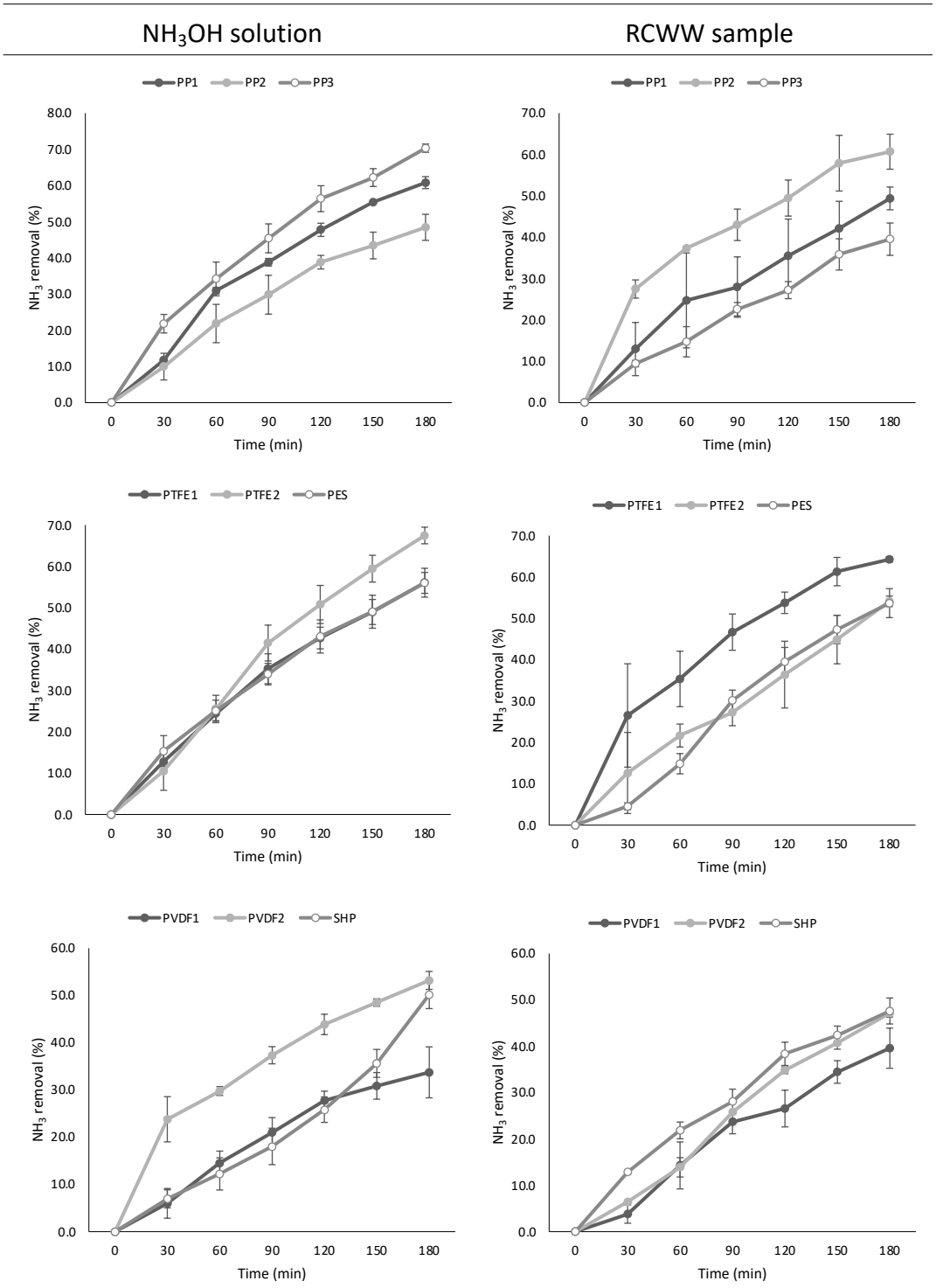

Figure 24: NH_3 removal efficiency expressed as a percentage achieved by each membrane material from NH_3OH solution and RCWW matrix (n=3).

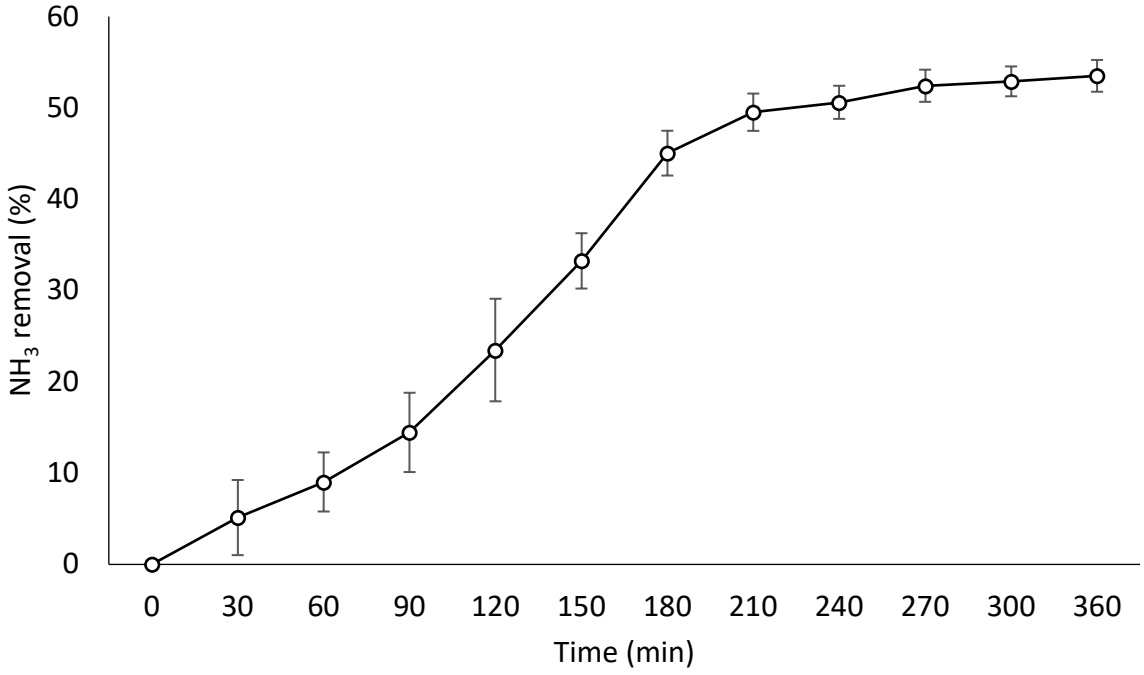

Figure 25: Analysis of NH_3 removal (expressed as %) from NH_3OH solution using SHP with a prolonged time period (360 min) (n=3).

4.3.4.1.2 Energy efficiency

Energy efficiency studies were carried out to determine if different membrane materials required different energy usages. The energy efficiency was determined by measuring the kilowatt-hour used to heat the water and power the pumps. Additionally, the temperature of the feed solution was measured before and after treatment using the membranes to determine the rate of temperature loss through the membrane. The results shown in Table 27 suggest that the same energy is required to operate the system for 3 hours (0.853 kWh) regardless of the membrane material being used. However, there are different NH_3 removal rates achieved after 3 hours using different membrane materials (Figure 24) which suggests that the membranes which remove the highest % of NH_3 may be more efficient in terms of

removing a certain volume of nitrogen over a time period. PTFE1 would be the most efficient membrane in this case as it removes the highest volume of NH_3 after 3 hours from the RCWW (64 %). The heat loss by the membrane was also recorded by measuring the temperature of the feed solution before and after it was passed through the membrane material for treatment. Each of the membranes investigated showed that the feed solution temperature dropped by 1.3 ˚C between the feed inlet and retentate.

Table **22** shows that the membranes used in this study had different thicknesses, but this did not impact on the heat loss efficiency as there was no significant difference shown between membranes. The results suggest that energy usage is not influenced by different membrane materials and the most efficient membrane is one which removes the highest % of NH_3.

Table 27: Energy efficiency data obtained for all membranes investigated in this study showing the energy usage for 3-hour analysis and temperature loss through the membrane (n=3).

Membrane material	Energy used (kWh)	Temperature loss (˚C)
PP1	0.862±0.02	1.1±0.2
PP2	0.891±0.12	1.1±0.5
PP3	0.799±0.20	2±1
PTFE1	0.823±0.01	1.8±0.3
PTFE2	0.854±0.12	0.8±0.09
PVDF1	0.879±0.21	1.1±0.2
PVDF2	0.863±0.08	1.3±0.2
PES	0.899±0.05	0.9±0.2
SHP	0.843±0.08	1.3±0.4

4.3.4.2 Pilot scale efficiency

A series of tests were carried out on the PTFE1 membrane to investigate the efficiency of NH_3 removal from RCWW. RCWW was introduced into the system for testing and reproducibility was achieved by testing 2 modules of the same structure. As seen in Figure 17 the composition of the RCWW is different every day with different concentrations of different components (including NH_3) and any possible wetting agents present. As seen in Figure 26, up to 64 % NH_3 was removed from the RCWW by the 1st module and up to 65 % removal was achieved using the 2nd module. These levels of removal show great potential for the use of membrane systems for RCWW treatment as they can be used to reduce NH_3 levels and reduce

them further by installing a series of membranes for sequential NH_3 reduction. Additionally, this process has a shorter retention time when compared with conventional methods and it produces a viable fertiliser product which can be sold. Module 1 shows to treat the RCWW efficiently for 10 days without cleaning the membrane and after the 10 days its removal efficiency shows to decrease. Module 2 was seen to be efficient for 6 days before its NH_3 removal rate started to decrease. The differences in the efficiency of the 2 modules may have been due to the composition of the RCWW sample and an increase in wetting agents present for the 2nd module. However, the membranes present a promising method for treating NH_3 in RCWW and could be further optimised by implementing a cleaning procedure for the membranes to prevent them being affected by wetting agents. The occurrence of wetting on the membrane materials can be combatted by introducing a cleaning procedure as described in Section 4.27 which uses a 0.1 % wt. NaOH solution in order to clean emulsified membranes at risk of fouling and wetting and showed the flux to return to normal.

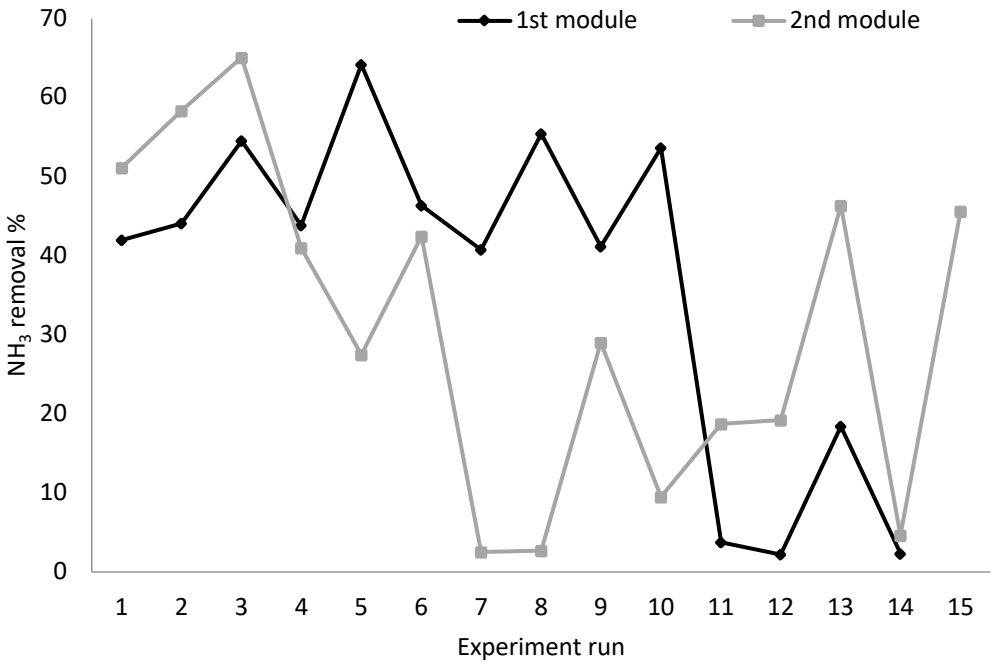

Figure 26: Performance of 1st and 2nd PTFE generation module (Flow feed side 0.05–1 L/h, permeate flow rate 0.05–0.5 L/h, pressure 0.5 Bar and temperature 50 °C).

The 2nd generation module used in this study was made from PP1 which failed initial clean water tests due to RCWW being present in the pipework of the system from existing tests. A cleaning method could not be incorporated due to the high level of wetting and time did not permit for a suitable cleaning protocol to be introduced. This suggested that PP1 was not compatible with RCWW and instigated further tests to be carried out to confirm this hypothesis.

4.3.5 Effect of membrane cleaning

Membrane cleaning has been reported to be an integral part of the membrane process in order to ensure maximum separation and improved economics of the membrane process (C. Liu et al., 2001). The use of 0.1 % wt. NaOH caustic solution was used in order to dissolve the foulants which are deposited on the membrane surface and remove them. PP1 membrane material was used to investigate the effect of the cleaning protocol as this membrane showed to foul easily as seen in Section 4.3.3.1 which would allow the effect of membrane cleaning to be identified easily. The cleaning protocol is outlined in Section 4.2.7 and the comparison of membranes which underwent the cleaning protocol and membrane which did not receive any cleaning are outlined in Figure 27. The membrane materials were characterised with and without the cleaning procedure. The results presented in Figure 27 immediately suggest that the cleaning protocol has a positive impact on membrane wettability. The CA without cleaning the membrane with 0.1 % wt. NaOH was 92.1˚ but after cleaning the membrane it increased to 105.6˚ which is similar to the CA obtained by a pristine membrane material at 111˚ (Table 25). A study by (Rudolph et al., 2018) also experienced an increase in hydrophobicity back to pristine conditions after the use of NaOH to clean the membrane. Additionally, the cleaning procedure can be seen to increase the LEP from 1.7 to 2.7 bar (without and with cleaning, respectively). The cleaning protocol did not increase the LEP back to its original pristine condition, but it did increase it enough in order to be deemed suitable for membrane separation use (minimum LEP at 2.5 bar for membrane material) (Eykens et al., 2016). Organic foulant occurs on the membrane surface which may reduce the hydrophobicity over time and also block pores which decreases the LEP (Naim et al., 2012). By introducing an alkaline caustic

solution, the cleaning surfactants diffuse into the fouling layer and act via dissolution and solubilization to detach the fouling from the membrane material allowing for the wetting characteristics return to original values as seen in the results presented here.

The pore size, fibre diameter and porosity showed to have no significant difference between a cleaning protocol being introduced and no cleaning protocol. However, examining the SEM images shown in Figure 27, it seems there is a fouling layer on the membrane which did not receive any cleaning which may either block the membrane pores or result in an increased pressure which results in wetting of the membrane and greater pore sizes. Surface roughness analysis was carried out using AFM and the results showed that the surface roughness was reduced with no cleaning protocol in place (Figure 27). It was proposed by (Hou et al., 2014) that there is a direct correlation between CA and surface roughness which is suggested in the results presented in this study. The decrease in the surface roughness may be due to a build-up of foulants within the pores and grooves which is suggested by Figure 27 as the first image (C) which is the membrane which underwent cleaning clearly shows visible fibres whereas the second image (D) with no cleaning protocol does not show the fibres as visibly and shows a clump of particles in one corner. Additionally, it should be noted that the effect of membrane cleaning using NaOH allowed for the surface roughness to return to its original pristine conditions as shown in Figure 21.

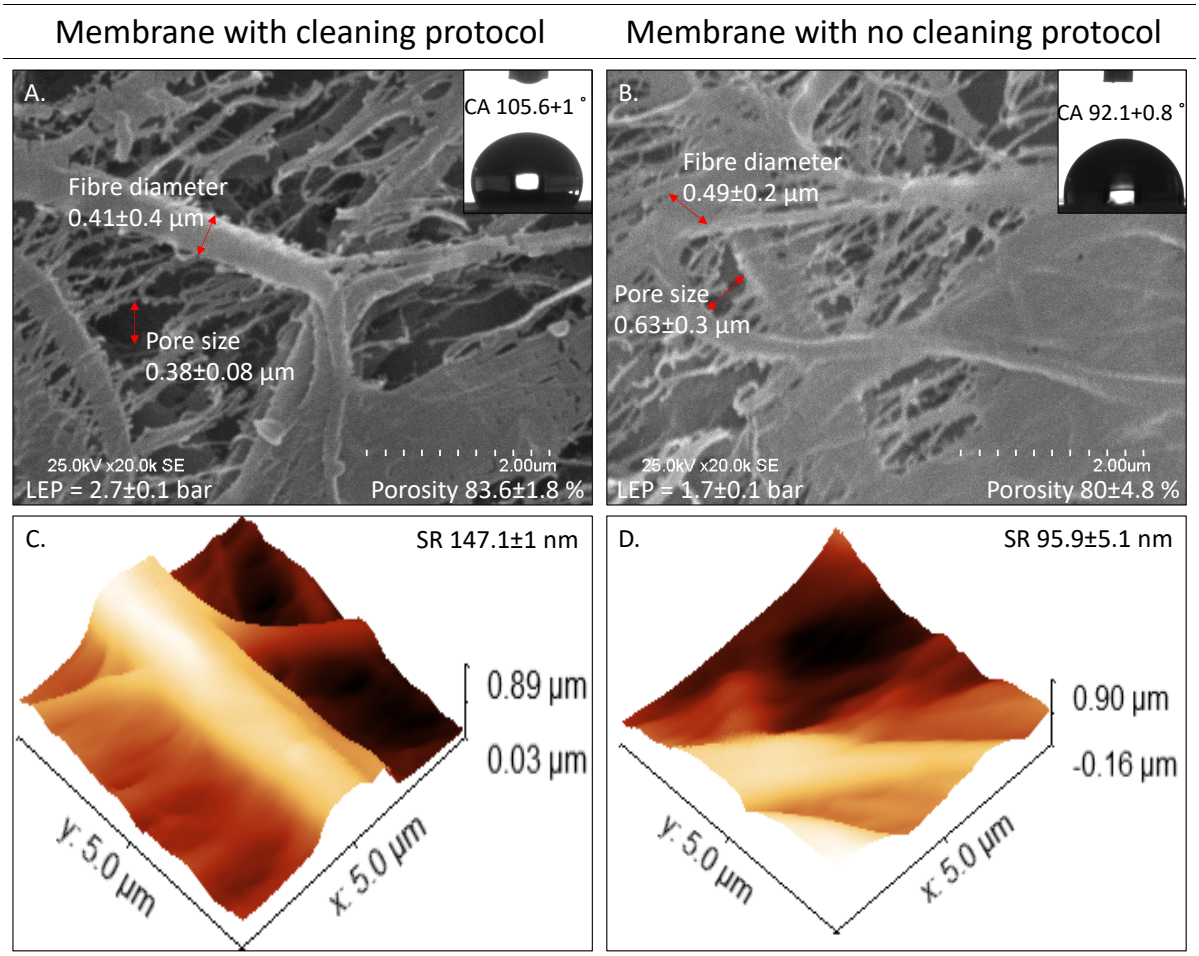

Figure 27: SEM (top) and AFM (bottom) images for (A & C) PP1 membranes which underwent 0.1 % wt. NaOH cleaning procedure and (B & D) PP1 membranes which did not undergo the cleaning protocol (n=3). Membrane characteristics also displayed in images (SR=Surface roughness).

NH_3 removal efficiency from a 100 mg/L NH_3OH solution can be seen in Figure 28 which presents the NH_3 removal achieved using (1) a clean PP1 membrane with no RCWW exposure, (2) a PP1 membrane which underwent NaOH cleaning after RCWW exposure and (3) a PP1 membrane which did not undergo the NaOH cleaning protocol after RCWW exposure. The results show that a clean PP1 membrane is capable of removing up to 52 % of NH_3 without any exposure to RCWW. The aim of the cleaning protocol is to achieve this removal efficiency

again after PP1 is exposed to RCWW. After exposure to RCWW and PP1 was cleaned using NaOH, the removal efficiency was found to be 43 % while PP1 that was exposed to RCWW with no cleaning protocol implemented, the NH_3 efficiency declined to 29 %. Although full removal efficiency was not returned, it can be concluded that the use of a cleaning protocol helps improve the removal quality after the membrane is exposed to RCWW. The use of 0.1 % wt. NaOH shows to be a sufficient caustic solution to clean the membrane but higher concentrations may allow for greater recovery. Also, the results shown in Figure 28 show that in the first 30 minutes, similar levels of NH_3 are being removed by all membranes. After this, the rate of removal in the membrane which had no cleaning protocol introduced declines in comparison to the other two membrane materials. This may be due to foulants present on the membrane being displaced and starting to block pores preventing the gaseous NH_3 to pass through the membrane.

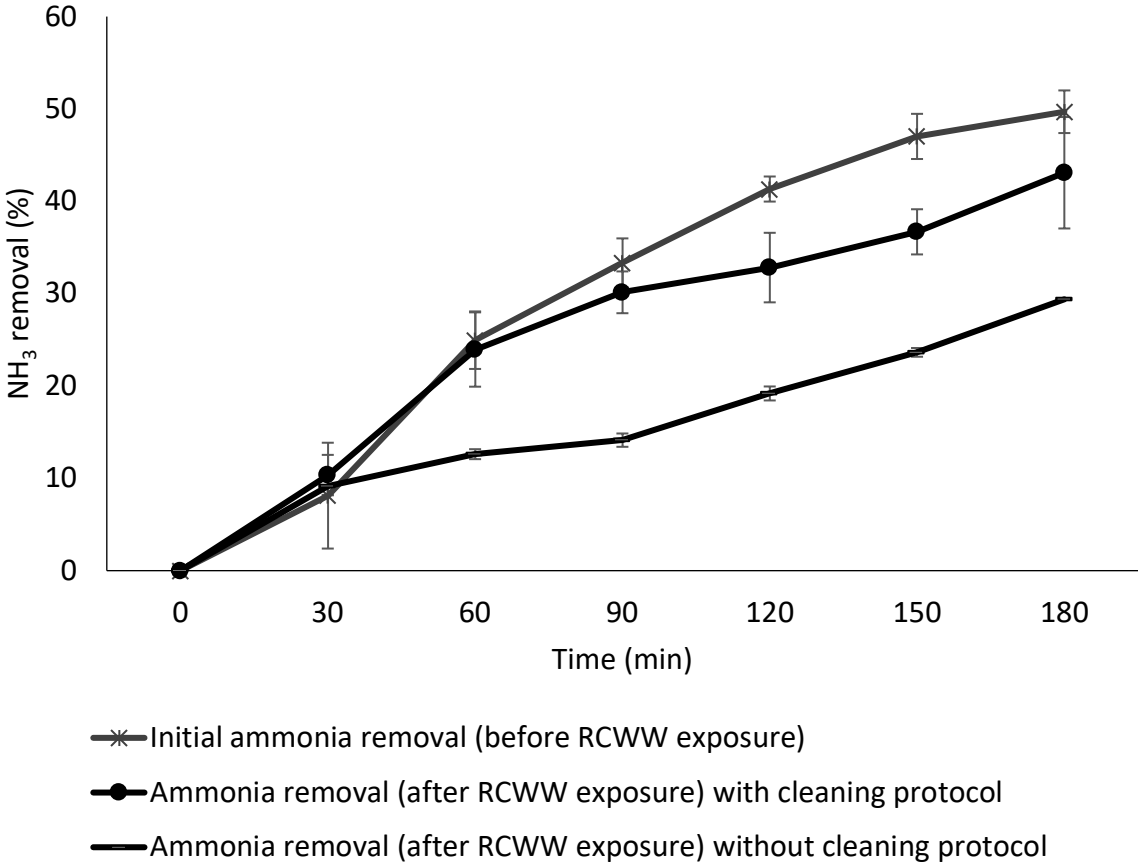

Figure 28: Effect of 0.1 % wt. NaOH cleaning procedure on PP1 membrane after 3-hour exposure to RCWW for removing NH_3 (n=3).

The results presented characterising the membranes with and without NaOH cleaning along with the NH_3 removal efficiency show the importance of the cleaning protocol. By incorporating a cleaning protocol, it allows for greater volumes of NH_3 to be removed and a longer lasting membrane material. Failing to introduce a suitable membrane cleaning protocol may result in higher operation costs due to more membrane material being required and down time.

4.3.6 Product characterisation

After membrane diffusion, NH_3 reacts with H_2SO_4 to form $(NH_4)_2SO_4$ as seen in Equation 1 (GASSER, 1964). The primary function of $(NH_4)_2SO_4$ is an agricultural fertiliser in alkaline soils to help promote growth. The NH_4 ion is released, and it undergoes deprotonation which produces NH_3 and results in lowering the pH of the soil. It also contributes nitrogen which is essential for plant growth. The sulfur promotes the metabolism of nitrogen, chlorophyll formation and forms amino acids which is the building blocks for proteins (Darestani et al., 2017). The product formed in this study was found to have a pH of 2–2.5 and a purity of 30 %. Liquid products on the market were found to have a purity of 40 % making our product a viable and green product, with economic benefit of valorisation of waste produced from the rendering plant (International Commission of Agricultural Engineering (CIGR) et al., 1999). In addition, the maximum purity which is possible in a liquid form is 40 % and $(NH_4)_2SO_4$ with purities higher than 40 % result in crystallisation (Boehler et al., 2015). The liquid $(NH_4)_2SO_4$ may be introduced to the soil by injecting it into the soil has been shown to be carried out by manual injection fertilizing (CULTAN) or by contactless high pressure jet injection (Deppe et al., 2016). Both methods work perfectly in normal conditions but the CULTAN method can result in soil blocking the injectors.

The $(NH_4)_2SO_4$ solution produced here was found to be very acidic with a pH of 2–2.5. For a product to be of value for application to soils, the pH needs to be increased. A series of titrations were carried out to determine which reagent most efficiently increased the pH of the product (40 mL) based on the costs and quality. The reagents which were used include NaOH, $NaHCO_3$, $CaCO_3$, RCWW and sludge waste from the aeration tank. Table 28 shows how

much of each reagent was required in order to increase the pH from ~ 2 to 7. It was found that RCWW had no effect on changing the pH. The aeration tank sample only changed the pH in one test. NaOH proved to change the pH using the least volume and it also had the smallest standard deviation. $NaHCO_3$ and $CaCO_3$ required larger volumes and thus NaOH was chosen as the most efficient reagent to adjust the pH of the product in order for it to be applied as land fertiliser.

Table 28: Titrations to investigate most efficient reagent to increase pH of product (titrand = 40 mL).

Reagent	Run 1 (mL)	Run 2 (mL)	Run 3 (mL)	Average (mL)	St. Dev (mL)
111 g/L NaOH	280.00	220.00	200.00	233.33	41.63
15 g/L $NaHCO_3$	3520.00	6300.00	4860.00	4893.33	1390.30
9.6 g/L $CaCO_3$	690.00	865.00	No change	777.50	123.74
RCWW	No change	No change	No change	-	-
Sludge	540.00	No change	No change	540.00	0.00

4.3.7 Cost analysis

A preliminary cost analysis on the membrane system to remove (NH_3) was carried out and compared with the current method being used to treat RCWW. The operational costs (including chemicals, energy consumption from heaters & pumps and filters) were calculated based on the assumption that the (NH_3) membrane system is in operation 7 days a week. The costs for the chemicals were based on the volume used in experiments throughout the project (titrations, volume of H_2SO_4 etc.) and the energy was estimated by measuring the energy usage with an energy meter. The results in

Table *29*, show that the capital cost of the current method is €500,000 and the operation costs (energy for aeration) equate to €1.71 per Kg of NH_3 removed. No viable product is produced from the current treatment, so no potential revenue is generated on it. The capital cost of the (NH_3) membrane removal system is €360,000 for the pilot and supply of membranes needed. The operational costs to remove one kg of NH_3 using the stripping pilot is a total of €2.48. However, the (NH_3) stripping unit produces $(NH_4)_2SO_4$ which can then be sold as a fertilizer at a price of €1.54 for $(NH_4)_2SO_4$ (30%) (*w*/*w*). This would suggest that the cost of treatment by (NH_3) stripping unit is €0.94 which is cheaper than the current treatment method. However, it should be noted that the current method is a continuous system which has less costs associated with ceasing and starting operation while the tested membrane system was only a batch system. Due to the two systems being different (continues vs batch) it is difficult to make a conclusive comparison. Considering the capital costs and operation costs of the two treatment methods, it can be suggested that preliminary results suggest that (NH_3) removal using membrane technology is a cheaper method as both capital and operation costs are cheaper.

Table 29: Summary of cost benefit analysis ([a] 0.009 € kWh^{-1} – was used to estimate the cost. The energy usage (€/kg N) was calculated from the total ammonia removed/year and total blower energy use/year (data obtained from 2016 plant site)).

	(NH_3) Removal Using Membrane Technology (70% Efficiency)			**Current Treatment**	
CAPEX	€300,000 (full scale) + €36,000 (membranes)			€500,000 (Aeration tank + diffusers, blowers)	
	(€/kg N)			(€/kg N)	
	NaOH	H_2SO_4	Energy [a]	Energy	Bio- augmentation
OPEX	1.54	0.71	0.26	1.065	0.645
	Maintenance costs (pre-filters)			Maintenance cost	
	0.0012			ND	
OPEX total	2.48			1.71	
OPEX—PC	0.94			1.71	
Product capitalization (PC)	$(NH_4)_2SO_4$ (30%) (*w*/*w*) 1.54			NA	

4.4 Conclusion

This chapter aims to identify if membrane materials are a suitable method to recover NH_3 from a challenging matrix, RCWW and produce a viable $(NH_4)_2SO_4$ product. 9 different commercially available hydrophobic membranes are assessed at lab- and pilot-scale to determine if they can efficiently remove NH_3 and provide strong wettability resistance to the high levels of surfactants and OM present in RCWW. Lab-scale assessment showed that all 9 membrane materials allowed for sufficient NH_3 removal from both NH_3OH and RCWW solutions (up to 64 % removal after 3 hours). However, the most membrane materials experienced a deterioration in their performance after being in contact with RCWW and the structure of the material started to breakdown. However, PTFE1 membrane showed to have strong resistance to RCWW and allowed for high NH_3 removal efficiency (up to 62 %). The combination of strong resistance to RCWW and high NH_3 removal resulted in PTFE1 being selected as the optimal membrane material and used in pilot-scale studies. A membrane

cleaning protocol was introduced for PP1 and showed that initial membrane performance and NH_3 removal could be retrieved after a caustic cleaning which would allow for longer membrane lifetime. For this reason, PTFE1 and PP1 were used in pilot scale studies. However, initial tests showed that PP1 was incompatible with RCWW at a larger scale and the membrane failed almost immediately. PTFE1 showed stronger resistance and allowed for 64 % NH_3 removal for up to 7 days. In addition, the process produced an $(NH_4)_2SO_4$ product with a 30 % purity which would allow for the reduction in treatment costs. Overall, the use of membrane contactors allowed for the reduction of nitrogen levels in RCWW while producing a viable NH_4-based product.

5. Conclusions and future work

The work presented in this thesis demonstrates the potential for WW from agricultural sources to be utilised to produce viable products from the rich nitrogen content - although, further work is required before it can be made feasible. The challenging WW types which were investigated include nitrogen rich saline WW from dairy industries and nitrogen rich rendering condensate wastewater from a beef-slaughtering industry. Nitrogen content was utilised in this study as the literature suggests it has the most opportunities to produce a product which would generate revenue.

Saline levels were found to have a salinity level of 0.8 % in dairy WW which interferes with bacteria nitrifiers used in the conventional methods to treat nitrogen in WW. The nitrogen concentration in the investigated dairy WW matrix was 114 mg/L which required treatment to allow for discharge into receiving waters. This thesis successfully demonstrated that the use of microalgae *Nannochloropsis* sp. could be deployed on the WW in order to reduce nitrogen levels to <10 mg/L. The results showed a satisfactory nitrogen removal (90 %) and also an increase in *Nannochloropsis* sp. biomass growth as the microalgae consumes the nitrogen for nucleic acid and protein development. Various studies have been carried out to utilise nitrogen in saline WW to promote microalgae growth, however, these studies have mostly focused on *Chlorella Vulgaris* which are a freshwater microalgae species. The use of *Nannochloropsis* sp. which is a naturally occurring marine species has not been utilised for this purpose and as such it was used in this study. As *Nannochloropsis* sp. is a natural marine species, it was expected it would thrive in a saline environment in addition to its reputation of accumulating large volumes of FA. As stated, the microalgae species performed as expected and reduced the nitrogen levels efficiently and produced large volumes of microalgae biomass

(15.6 g/m^2/day). The *Nannochloropsis* sp. was treated further in order to extract the FA to produce a viable by-product. The MW-assisted *in*-situ extraction and derivatisation method was optimised in order to recover the highest quantity of FAME possible. The MW was then compared with the conventional method to determine the efficiency. The results demonstrated that there were high quantities of FA present in the microalgae cell. However, due to the low dielectric loss of the salt due to the saline WW, it resulted in low levels of SFA and MUFA which are the optimal FA for high quality biodiesel recovery. However, there was very high quantities of PUFA such as $C_{20:5\omega3}$ which can be used for omega-3 supplements making the microalgae a viable source to produce nutraceuticals. The use of other extraction methods such as SC-CO_2 may allow for selective FA extraction which could reduce the PUFA recovered and allow for a more stable biodiesel product to be generated.

Overall, the use of *Nannochloropsis* sp. has demonstrated to be an efficient microalgae species to reduce nitrogen levels in saline WW and capable of producing FA which can generate a valuable by-product such as omega-3 supplements. The findings presented in this thesis have identified a number of opportunities for future work which was not previously identified. The harvesting of microalgae on wastewater ponds with varying concentrations of salt and nitrogen could identify why saturated fatty acids are so low and how it could be improved to identify more suitable FA for biodiesel production. Additionally, various extraction method such as supercritical-CO_2 could be investigated to determine if it allows for the recovery of more polar fatty acids as suggested by other studies. Additionally, the produced biodiesel and nutraceuticals should be further purified and characterised to

determine the efficiency and scale up the operation in order to efficiently treat large volumes of WW and make it a more common economical business practice.

The composition of RCWW was extensively investigated and it was determined it contained high levels of nutrients and organic matter; COD (10,813±427 mg/L), TKN (1745±90 mg/L), NH_3 (887±21 mg/L), crude protein (10,911±563 mg/L), total phosphorous (51±1 mg/L), FOG (11,363±934 mg/L), TSS (336±73 mg/L) and TDS (4397±405 mg/L). The challenging composition of this WW requires extensive treatment before being released back to the environment which incurs high costs. However, it was investigated how feasible it would be to recover the NH_3 present in the RCWW to produce a viable by-product using hydrophobic membranes. Various studies have investigated the use of membrane filtration to recover NH_3 and produce $(NH_4)_2SO_4$ fertiliser which can be applied to agricultural land to promote crop growth. This thesis focused on the suitability of hydrophobic membranes (in a direct contact membrane configuration) to recover NH_3 in a matrix with high volumes of surfactants such as RCWW. While there have been many studies carried out using hydrophobic membranes to recover NH_3, no studies have contained as many surfactants as RCWW. Initial lab-scale assessments were carried out to investigate the suitability of various commercially available membranes on RCWW. The membranes investigated varied in terms of their membrane material, pore size, hydrophobicity and support layers. Characterisation of the membrane materials before and after RCWW exposure demonstrated that PP and PVDF membranes were susceptible to wetting but PTFE membranes had high resistance to the RCWW. Membrane distillation studies were carried out with the RCWW and removal efficiencies of up to 62 % were determined using PTFE1 membrane material. Other membrane materials

such as PTFE2 and SHP also showed promising results. The energy efficiency for each of the membranes was calculated and the energy requirement did not vary between membranes. A cleaning protocol was introduced using 0.1 % wt. NaOH and it was determined the hydrophobic characteristics of PP1 were almost fully recovered after cleaning suggesting that wetted membranes could undergo a cleaning procedure to prolong operation. Overall, it was determined that PTFE1 membrane is the most suitable which will be developed on for future studies.

A pilot-scale investigation was carried out in order to determine the suitability of hydrophobic membranes for removing NH_3 from RCWW. PP1 and PTFE1 membranes were used in this study as they showed promising results in lab-scale assessment. However, PP membrane failed immediately after coming in contact with RCWW which suggest that it is not suitable for this process. It was demonstrated that the LEP and surface roughness decreased immediately which may have caused the RCWW to penetrate the membrane material and leak to the other side. PTFE membranes showed to successfully reduce NH_3 by 64 % which suggests this is a promising method of NH_3 to produce $(NH_4)_2SO_4$ fertiliser. However, the PTFE membrane showed to deteriorate over time. It was suggested that to overcome this deterioration, the 0.1 % wt. NaOH cleaning method should be incorporated to allow for a longer lifetime. The cleaning protocol study at pilot-scale could not be investigated due to time constraints. Although the PTFE membrane showed high NH_3 removal, it is not high enough to allow the WW to be returned to receiving waters. To combat this, it is suggested that multiple membrane systems could be set up in series in order to deplete the NH_3 concentrations. Or alternatively, the PTFE membranes could be incorporated into the existing

method and treat RCWW before it's treated conventionally to help reduce costs. A full characterisation was carried out on the produced by-product ($(NH_4)_2SO_4$) and results showed that a 30 % purity product was formed which could be sold as a competitive liquid fertiliser product. The revenue that could be generated from the liquid fertiliser could reduce the cost of treatment by 90 %. Whilst there has been extensive research into membrane science in recent years, there is still a wealth of information to be identified in order to make this a viable method to recover products and treat the WW. The fabrication of more complex, superhydrophobic membrane materials should be identified in order to prolong membrane lifetime which will allow for longer treatment times, product more $(NH_4)_2SO_4$ product and reduce costs. Additional research should also focus on the cleaning protocols which could be implemented to extend the lifetime of the membrane materials. Once a suitable membrane material is identified, the operating parameters at full-scale should be identified to maximise NH_3 removal and allow for the optimal integration.

This thesis has demonstrated that nitrogen levels in challenging WW can be used to produce viable by-products to help reduce WW treatment costs or generate revenue for companies. The saline WW can be used to produce microalgae with FA to produce nutraceuticals and further work could be carried out to optimise the process and generate a high-quality biodiesel product. RCWW showed to have high NH_3 concentrations which could be used to produce a viable $(NH_4)_2SO_4$ fertiliser which can reduce the costs of WW treatment whilst promoting healthy growth in agricultural lands. This research has made some developments in the area of WW treatment that will help make agricultural WW a more valuable resource to produce viable products. The fatty acid in *Nannochloropsis* sp. cultivated

in saline dairy wastewater has not been characterised before and this research demonstrated that it can simultaneously reduce nitrogen levels while producing fatty acids suitable for omega-3 nutraceuticals. Additionally, the composition of RCWW has been characterised which has not been done in the last 30 years since before the meat industry has exploded. This thesis identifies that RCWW and SHWW can be heavily utilised to produce an array of viable products and most notably ammonium-based fertilizers using membrane contactors. This research has identified that PTFE membranes are the most suitable for a challenging matrix such as RCWW and that PES membranes have potential if treated appropriately. Overall this thesis has identified potential possibilities for agricultural WW to be utilised to produce viable products on condition of some method optimisations.

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Review

In-situ lipid and fatty acid extraction methods to recover viable products from *Nannochloropsis* sp.

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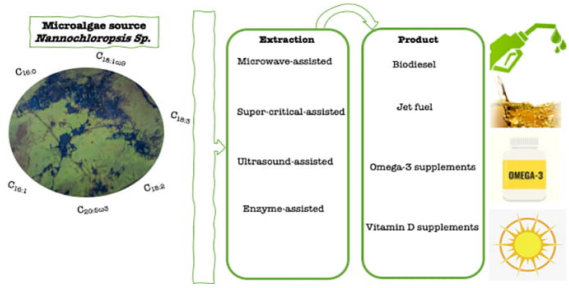
HIGHLIGHTS

- A critical review of FA and lipid extraction methods for application to microalgae.
- Combined extraction methods (*e.g.* microwave and ultrasound) show improved extraction performance
- Enzyme-assisted extraction offers green production, but scale up is a challenge.
- A summary of the FA composition obtained using extraction methods is provided.
- Viable products from *Nannochloropsis* Sp. are given, highlighting biodiesel and Omega-based products.

GRAPHICAL ABSTRACT



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ABSTRACT

Nannochloropsis sp. has received increased attention by researchers in recent years due to its complexity and abundance of lipid structures. The lipids of this microalgae species have been identified to contain large quantities of neutral lipids which are capable of producing raw materials for nutraceuticals, food additives and biofuels. The production of biodiesel has received the greatest attention as there is an increase in global demand for both more fuel and more environmentally sustainable methods to produce such resources. The greatest challenges facing industries to mass produce viable products from microalgae involve the degradation of the cell wall and extracting the fatty acid of interest due to high costs. Various studies have shown that the extraction lipids from the microalgae can greatly influence the overall fatty acid composition. Different extraction methods can result in recovering higher quantities of either saturated fatty acids, monounsaturated fatty acids or polyunsaturated fatty acids. Biodiesel production requires higher quantities of saturated fatty acids and monosaturated fatty acids as increased quantities of polyunsaturated fatty acids result in oxidation which decreases the performance of the biodiesel. Whereas, polyunsaturated fatty acids are required in order to produce pharmaceuticals and food additives such as omega 3.

This review will focus on how different *in-situ* extraction methods for lipid and fatty acid recovery, influence the fatty acid composition of various *Nannochloropsis* species (*oculate, gaditana, salina* and *oceanica*). The mechanical methods (microwave, ultrasonic and supercritical-carbon dioxide) of extraction for *Nannochloropsis* sp. will be critically evaluated. The use of enzymes will also be addressed, for their ability to extract fatty acids in a more environmentally friendly manner. This paper will report on the viable by-products which can be produced using different extraction methods.

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Contents

1. Introduction

Microalgae are naturally occurring green unicellular organisms which can be found in either freshwater or marine systems which produce high levels of lipids (Wu et al., 2017). The environmental impact of microalgae on fresh and marine waterways has received much interest. They are often preferred to other crops for biomass production due to their ability to grow on water and not compete for arable land for food supplies (Bermúdez Menéndez et al., 2014). Their high lipid content can be 200 times greater than other plants producing lipid volumes of 32,374.9 L/ha $year^{-1}$ (Ali and Watson, 2015; Qadariyah et al., 2018). Microalgae is capable of adsorbing CO_2 making it an attractive green process with reports showing 100 tons of microalgal biomass fixing 183 tons of CO_2 (Ali and Watson, 2015; Ranjan et al., 2010). It has been reported by various authors that microalgae can be used to deplete nitrogen levels up to 100% removal. However, no studies have focused on the use of *Nannochloropsis* sp. to reduce nitrogen levels (Babatsouli et al., 2015; Church et al., 2017; Shen et al., 2015). The use of microalgae to produce viable products such as biodiesel is favoured. (Huerlimann et al., 2010; Zuorro et al., 2016a). This is due to reduced environmental impacts compared with conventional fossil oil recovery. Additionally, recovering biodiesel from microalgae over plants which can be used for human consumption is favoured in order to prevent a depletion of food sources and competing for arable land.

Nannochloropsis sp. has been reported to be a favoured microalgae species for a number of reasons (Ljubic et al., 2020). It is capable of accumulating in large amounts, it can grow on brackish and other wastewaters under adverse conditions and accumulate large amounts of polar lipids (up to 50% of total biomass being lipid) (Qiu et al., 2019; Zuorro et al., 2016b). The lipids present in microalgae are made up of mainly polar (phospholipids, glycolipids and betaine) and non-polar (acylglycerols, sterols and free fatty acid (FFA)) lipids (Natarajan et al., 2014). Studies looking at *Nannochloropsis* sp. have reported high concentrations of C_{16}, $C_{16.1}$, C_{18} and $C_{18.1}$ which are suitable for biodiesel production. *Nannochloropsis* sp. *also* contain high concentrations of $C_{20:5\omega3}$ which contribute to the production of omega-3 products (Adam et al., 2012; Qiu et al., 2019). Recovering these FA from the microalgae species would allow for the production of viable products. However, it is a difficult process due to the complex structure of the organism. The cell structure (composed of cellulose, polysaccharides, proteins and lipids) has a strong resistance to mechanical and chemical treatments (Chua and Schenk, 2017; Zuorro et al., 2016a). Species of *Nannochloropsis* used in fatty acid recovery, include *oculata, gaditana, salina and oceanica.*

For years scientists have attempted to disrupt the strong cell structures of *Nannochloropsis* sp. using conventional methods such as soxhlet extraction and chemical degradation (Zghaibi et al., 2019). After the lipids are extracted, they undergo a transesterification into fatty acid methyl esters (FAME) using an alkali or acid catalyst at high temperatures in the presence of methanol (Santana et al., 2012). However, this conventional two step method requires high temperatures which may deteriorate the polyunsaturated fatty acids (PUFA) (Aliev and Abdulagatov, 2017; Taher et al., 2020). These disadvantages have called for *in-situ* extraction methods which use less harmful chemicals and can disrupt the microalgae cell efficiently (Goh et al., 2019).

Due to negative aspects of the conventional methods, novel *in-situ* methods for extraction of lipids and FA from *Nannochloropsis* sp. are needed to produce viable products. Many methods have gained attention in the last 5 years. (Ali and Watson, 2015; Qiu et al., 2019; Wiyarno et al., 2011). From this period, microwave-, ultrasound-, supercritical CO_2 (SC-CO_2) and enzyme-assisted extractions are the most studied methods on *Nannochloropsis* sp. and therefore they are the focus of this paper. The data presented in this review also suggests that these are the most promising methods in achieving lipid and FA recovery.

The energy requirement to dry, extract and hydrolyse the FA, accounts for 10.7, 14.5 and 14.8% of the total energy consumption, respectively (Wang et al., 2017). In order to reduce these energy inputs, the use of *in-situ* methods must be integrated (Qadariyah et al., 2012). Wet biomass extraction can be difficult and result in the recovery of fewer lipids. This is due to the algal cells gathering a hydrated shell which acts as a barrier to energy and mass transfer (Martinez-Guerra et al., 2018). The choice of a particular technique for lipid extraction depends on algal species, initial lipid content and desired FA (Ranjan et al., 2010). SC-CO_2 have been reported as a suitable method for PUFA recovery but high pressures result in high energy costs (Zghaibi et al., 2019).

In-situ extraction and derivatisation methods have received increasing interest in recent years for the recovery of lipids from *Nannochloropsis* sp.

Many papers have assessed the methods used in terms of the lipids and proteins recovered. However, to the authors knowledge there has been no review on the most common methods to critically review their performance in terms of FA composition and the possible viable products. A large number of studies have focused on the lipid recovery from *Nannochloropsis* sp. using various methods. This paper reviews the most common *in-situ* lipid and FA extraction methods for *Nannochloropsis*. The review covers 10 years of studies involving *Nannochloropsis* sp. as a source of lipids and FAs for bio-based products. Search terms such as *Nannochloropsis* sp. lipid extraction, microwave-, ultrasound-, supercritical- or enzyme-assisted extraction of lipids from *Nannochloropsis* sp. and recovery of viable products from *Nannochloropsis* sp. were used. Over 140 references were gathered and subsequently >70 references have been included in this review. It addresses how the methods influence the FA composition of the end product. The viable products from the microalgae are also discussed.

2. Extraction-assisted methods

2.1. Microwave-assisted extraction

The use of microwave (MW) to aid in FAME recovery from microalgae has increased recently. MW allows for a controlled and accurate heating with uniform thermal transfer to ensure all the samples are heated up equally (Martinez-Guerra et al., 2018; Mubarak et al., 2015). Microwave heating leads to localized high temperature and pressure gradient which results in cellular wall degradation and enhanced mass transfer (Martinez-Guerra et al., 2018). This unique heating mechanism leads to a reduced extraction time (15 min) compared to conventional heating (>60 min) (Brennan et al., 2020).

2.1.1. Principles of MW assisted extraction

The principle of MW assisted lipid extraction is described in detail by (Mandal and Hemalatha, 2007) who used the method for medicinal plants. However, the same principle is used for microalgae.

2.1.2. Factors which effect lipid recovery using MW

The use of MW for lipid and FA recovery extracts up to 51.8% of the dry biomass as lipids with 13.2% SFA, 3.5% MUFA and 35% PUFA as reported by (Brennan et al., 2020). The drying and extraction processes are the most energy demanding steps in the recovery of viable products from *Nannochloropsis sp.* (Mubarak et al., 2015). However, some authors have described the process of having much lower retention times. Therefore this decreases the cost, also making it a greener process (Zghaibi et al., 2019). A study by (Ali and Watson, 2015) investigating *Nannochloropsis oculata* showed that the energy and cost from the MW contribution of the process for lipid recovery was very low at 0.160 mJ/kg of lipid recovered. The same study showed that the use of ethanol contributed more to the energy consumption at 90.9 mJ/kg of lipid recovered, suggesting that the solvent choice is important. A study by (McKennedy et al., 2016) using *Nannochloropsis oculate,* extracted lipids with methanol leading to a lower yield. However, as seen in Fig. 1 and Table 1, ethanol is reported to achieve the optimal extraction of lipids using MW radiation (Mandal and Hemalatha, 2007).

Factors which effect MW lipid extraction include temperature, time, matrix characteristics, solvent type and dielectric properties (Mandal and Hemalatha, 2007; Zghaibi et al., 2019). Solvents which have a lower dielectric loss, allow for less microwave adsorption and thus greater heating efficiency. Polar solvents such as methanol and ethanol achieve better extraction than hexane and chloroform. It was demonstrated by (Bermúdez Menéndez et al., 2014) that using a non-polar solvent such as hexane with *Nannochloropsis gaditana* recovered only 14.8% lipids from the dry biomass. (Zghaibi et al., 2019) used NaCl on *Nannochloropsis oceanica* to recover lipids (Table 1). This represents a greener process however, the recovery was low due to its low dielectric constant (Hoekstra and Cappillino, 1971). NaCl is reported to recover less lipid and FA volume, but it is more cost efficient. The cost to extract 1 kg of lipid with the Bligh Dyer method and Soxhlet extraction is $1380 and $10,683, respectively. The costs of the MW method with NaCl was reported to be in the region of $774 to recover the same volume of FA (Zghaibi et al., 2019).

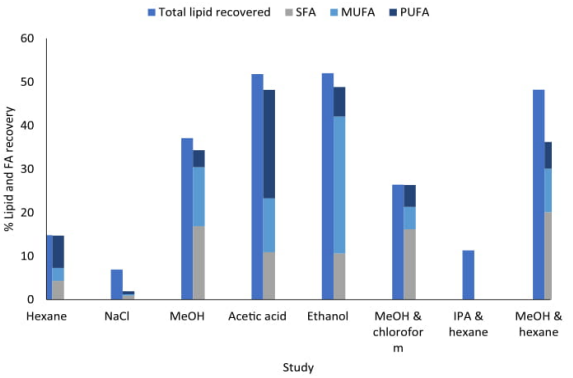

Fig. 1. MW-assisted extraction of lipids and FA from Nannochloropsis sp. using different solvent(s). (Hexane (Bermúdez Menéndez et al., 2014); NaCl (Abugrara et al., 2019); MeOH (McKennedy et al., 2016); Acetic acid (Brennan et al., 2020); Ethanol (Ali and Watson, 2015); MeOH&chloroform and IPA&hexane (Teo and Idris, 2014); MeOH&hexane (Martinez-Guerra et al., 2018)).

2.1.3. MW in-situ methods

The mechanical effect of ultrasonication (US) can allow for the release of soluble compounds from the microalgae (by disrupting the cell wall by cavitation and bubble collapse). This enhances mass transfer and facilitates solvent access to the cell components (Bermúdez Menéndez et al., 2014). By combining MW and US enhances mass transfer by MW with US improving rapid heating (Martinez-Guerra et al., 2018). A study by (Martinez-Guerra et al., 2018) showed that using MW with methanol and hexane, obtained a lipid recovery of 22.8%. By adding US in the process, more than doubled the recovery to 48.2% and lowered the energy required. The results showed that the US method did not affect the FA composition. The recovered fraction was composed of $C_{16:0}$ and other SFA with very low PUFA, allowing for good quality biodiesel production (Martinez-Guerra et al., 2018).

2.1.4. Efficiency of lipid and FA extraction using MW

Studies to determine the efficiency of MW for extracting lipids and FA from *Nannochloropsis* sp. indicate a huge variation in lipid recovery based on the solvents (Fig. 1 and Table 2). Studies have shown recovery of similar FA compositions (Fig. 1), high SFA and MUFA, with low PUFA

Table 1
Lipid and FA recovery from Nannochloropsis Sp. using different solvents with the aid of MW-assisted extraction (Ali and Watson, 2015; Bermúdez Menéndez et al., 2014; Brennan et al., 2020; Martinez-Guerra et al., 2018; McKennedy et al., 2016; Teo and Idris, 2014; Zghaibi et al., 2019).

Solvent composition	Lipid recovery (%)	SFA (%)	MUFA	PUFA
Hexane	14.8	29.1	20.1	50.2
Sodium chloride	6.9	13.5	4.4	9.9
Methanol	37.1	45.5	36.5	10.5
Acetic acid	51.8	21.0	23.9	48.1
Ethanol	52.0	20.4	60.5	13.0
Methanol and chloroform	26.4	61.2	19.5	19.0
Propanol and hexane	11.3	NR	NR	NR
Methanol and hexane	48.2	41.7	20.7	12.7

Table 2
Lipid and FA composition of Nannochloropsis Sp. investigated using MW with different solvent(s) ((Abugrara et al., 2019; Ali and Watson, 2015; Bermúdez Menéndez et al., 2014; Brennan et al., 2020; Martinez-Guerra et al., 2018; McKennedy et al., 2016; Teo and Idris, 2014).

Fatty acid composition (%)	Hexane	Sodium chloride	Methanol	Acetic acid	Ethanol	Methanol and chloroform	Methanol and hexane
$C_{14:0}$	4.1	4.0	6.1	0.4	–	11.9	10.8
$C_{16:0}$	24.9	9.2	38.4	17.8	18.0	16.5	11.3
$C_{16:1}$	15.8	0.5	27.7	19.7	4.5	–	6.2
$C_{18:0}$	–	0.3	1.0	2.5	2.4	32.9	8.7
$C_{18:1\omega9}$	4.3	1.1	8.8	2.5	56.0	10.6	4.7
$C_{18:1\omega9t}$	–	1.6	–	1.7	–	9.0	–
$C_{18:2}$	14.6	0.1	1.2	9.3	–	19.0	6.1
$C_{18:3}$	12.0	0.3	0.7	–	13.0	–	6.6
$C_{20:0}$	–	0.1	–	–	–	–	10.9
$C_{20:4\omega6}$	1.9	1.2	–	2.7	–	–	–
$C_{20:5\omega3}$	7.4	8.2	6.5	36.1	–	–	–
$C_{22:1}$	–	0.7	–	–	–	–	9.9

levels suggesting suitability for biodiesel recovery. A study by (Brennan et al., 2020) showed that PUFA have a greater affinity to acetic acid. The high PUFA levels are suitable for omega 3 supplementary products. However, this study was based on microalgae cultivated from brine wastewater, unsuitable for pharmaceutical (Brennan et al., 2020; Gadipelly et al., 2014). The use of multiple solvents, (Qadariyah et al., 2012) is reported to aid lipid recovery, with different solvents allowing for the extraction of different FAs (Brennan et al., 2020; Martinez-Guerra et al., 2018; Teo and Idris, 2014). Table 2 and Fig. 1 show the FA composition obtained for *Nannochloropsis* sp. from different solvent (s); the average SFA is 32%, 17% MUFA and 12.2% PUFA from all of the methods investigated. According to (Martinez-Guerra et al., 2018), these compositions make MW the ideal method to produce biodiesel from *Nannochloropsis*. The ideal cetane number, viscosity, oxidation potential and heating capacity discussed further in Section 3.1, are achieved.

2.2. Supercritical fluid-assisted extraction

Supercritical fluid extraction (SFE) is the process of using CO_2 or other solvents under high pressure to extract oils/lipids from the matrix (Pushpangadan and George, 2012; Obeid et al., 2018). SFE has been recognised as an efficient alternative to organic solvents and conventional methods (Mouahid et al., 2013). Supercritical CO_2 (SC-CO_2) has a lipid solubility similar to organic solvents with higher diffusivity and lower viscosity (Taher et al., 2020). The use of SC-CO_2 over conventional methods has been reported to extract up to 2.5 times more lipid and use less energy (Aliev and Abdulagatov, 2017). In addition, the SC-CO_2 method is efficient at recovering non-polar lipids. Polar solvents can be incorporated in order to influence the recovery of polar lipids (Patil et al., 2018). However, SC-CO_2 is a complex process with costly apparatus compared to conventional methods (Brennan et al., 2020). The SC-CO_2 method is affected by water content in the sample therefore impacting the biomass recovery and reducing the lipid recovery (Taher et al., 2020).

2.2.1. SC-CO_2 in-situ methods

Nannochloropsis salina (Patil et al., 2018) was used with a MW step to rupture the cell walls, prior to extraction by SC-CO_2. The results showed that a lipid recovery of 12% of the total biomass, could be obtained. This was double that obtained using the conventional method. (Taher et al., 2020) who investigated *Nannochloropsis gaditana* incorporated an enzymatic process prior to the SC-CO_2 extraction to further derivatize the lipids into FAMEs. Fig. 2 shows that lipase had increases of yield up to 80%. However, the cost of enzymes can greatly increase the cost of biodiesel production. A study by (McKennedy et al., 2016) (using *Nannochloropsis* oculata) showed that by incorporating co-solvents can allow for reduced temperature and pressure. Therefore operation costs were reduced, while producing FAME suitable for biodiesel. Ethanol has a low solubility to lipids however, when under supercritical conditions it can dissolve more lipids (Chen et al., 2012). By mixing ethanol with hexane as a co-solvent for SC-CO_2, improved lipid recovery is achieved (in comparison with conventional SC-CO_2) and FAME production. (Patil et al., 2018). Fig. 2 shows the lipid recovery obtained as a % of the total biomass for SC-CO_2 and SC-CO_2 assisted methods (Aliev and Abdulagatov, 2017; Andrich et al., 2005; Chen et al., 2012; Patil et al., 2018; Taher et al., 2020).

2.2.2. FA composition obtained by SC-CO_2

Results obtained from SC-CO_2 studies for FAME recovery (Table 3) show that the composition of the lipid is dependent on the extraction technique. SC-CO_2 does not recover polar lipids but shows higher concentrations of less polar lipids useful for omega 3 and omega 6 medicinal production (Andrich et al., 2005; Solana et al., 2014). Studies by (Mouahid et al., 2013) and (Taher et al., 2020) using conventional SC-CO_2 on *Nannochloropsis oculata* and *gaditana*, respectively showed that more PUFA FA were recovered rather than the more polar SFA (Table 3). (McKennedy et al., 2016) stated that using different co-solvents demonstrates the tunability of SC-CO_2 to produce a desired product. Solvents such as hexane and ethanol allow for the more oxidative FA which are suitable for biodiesel production. Table 3 shows the

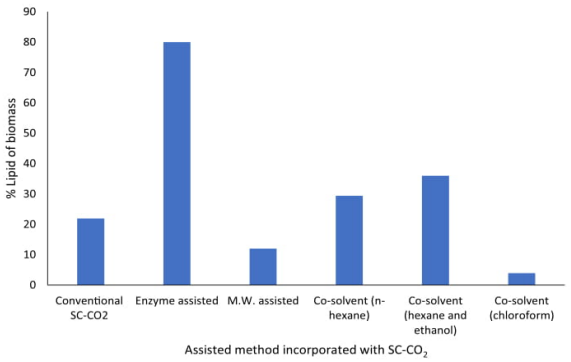

Fig. 2. Lipid recovery with SC-CO_2 using different assisting methods (enzyme- (Taher et al., 2020), MW- (Patil et al., 2018) and co-solvent- (n-hexane with *Nannochloropsis* sp. *salina*) (Aliev and Abdulagatov, 2017); (hexane & ethanol) (Chen et al., 2012); (chloroform with *Nannochloropsis* sp. *salina*) (Aliev and Abdulagatov, 2017) assisted methods) compared with conventional SC-CO_2 (Andrich et al., 2005).

Table 3
FA composition for Nannochloropsis Sp. lipids extracted by SC-CO_2.

SC-CO_2 condition	Conventional SC-CO_2	Conventional SC-CO_2	Co-solvent (hexane and ethanol)	Co-solvent (n-hexane)	Co-solvent (chloroform)
Lipid recovery (% total biomass)	33	21.9	36	29.4	3.92
SFA	25.3	21	42.37	23.44	23.35
MUFA	14	32	52.8	46.19	46.31
PUFA	43.2	43	0.62	11.9	12.22
$C_{12:0}$	0	0	0	0.39	0.27
$C_{14:0}$	5.7	3	2.88	3.2	3.06
$C_{16:0}$	17.8	12	35.67	18.62	18.91
$C_{16:1}$	11.4	12.5	25.96	26.61	27.11
$C_{18:0}$	1.8	6	3.82	1.23	1.11
$C_{18:1\omega9}$	2.6	19.5	26.84	19.58	19.2
$C_{18:2}$	5.2	8	0	5.92	6.34
$C_{20:4\omega6}$	5	5	0	5.98	5.88
$C_{20:5\omega3}$	33	30	0.62	0	0

FAME composition obtained for different solvents and it shows that hexane-ethanol produce high levels of SFA and MUFA which are suitable for biodiesel production (Aliev and Abdulagatov, 2017; Andrich et al., 2005; Chen et al., 2012; Mouahid et al., 2013; Patil et al., 2018; Taher et al., 2020).

2.3. Ultrasound-assisted extraction

Ultrasound-assisted extraction (UAE) refers to an extraction process of lipids from solid samples using ultrasound waves to disrupt the cell walls (Bendicho and Lavilla, 2018). This allows for a greater solvent penetration into the microalgae, increasing contact between the sample and solvent thereby improving mass transfer (Duarte et al., 2014; Mubarak et al., 2015). An improved extraction yield, reduction in temperature, solvent consumption and extraction time have been reported (Parniakov et al., 2015). UAE has been reported to give a higher purity product (Wiyarno et al., 2011) where optimisation of temperature (Adam et al., 2012) and retention time making it a greener process (Adam et al., 2012). A drawback of UAE is the difficulty of recovering solvents to be reused (Mandal et al., 2015). (Duarte et al., 2014) reported that it is difficult to incorporate UAE with other methods – although (Martinez-Guerra et al., 2018) and (Bermúdez Menéndez et al., 2014) showed that UAE and MW assisted extraction can be incorporated and increase the lipid yield more efficiently. The UAE method is outlined extensively by (Ferreira et al., 2016; Mubarak et al., 2015; Natarajan et al., 2014).

The FAME composition for *Nannochloropsis* sp. obtained for different UAE studies and their conventional methods is presented in Table 4. A high SFA and MUFA content is reported which suggests that the method may be suitable for biodiesel production. However, comparisons against conventional methods suggest that there is no significance in the composition of the FA prepared by different methods. Overall, *Nannochloropsis* sp. has received little attention with the use of UAE for lipid recovery. UAE represents a quick method which successfully produces FA of industrial use and it can be easily scaled up. However, it does not allow for variation in FA composition.

Table 4
FAME composition for Nannochloropsis Sp. investigated by UAE and conventional Soxhlet method (Adam et al., 2012; Bermúdez Menéndez et al., 2014; Natarajan et al., 2014; Wiyarno et al., 2011) (NR = Not recorded) (Lipid recovery reported as % of total biomass and FA reported as % of the lipid).

Fatty acid composition (%)	UAE	UAE	UAE	Conventional	UAE
Lipid recovery	14.76	NR	55	21	6.8
SFA	28.8	34.0	23.4	24.6	29.2
MUFA	20.0	10.0	37.6	31.6	43.5
PUFA	43.7	39.5	38.1	41.7	5.9
$C_{14:0}$	4.0	9	7.4	7.4	11.1
$C_{16:0}$	24.7	18	14.5	16.8	12.5
$C_{16:1}$	15.8	–	33.6	27.1	–
$C_{16:2}$	6.3	–	1.7	1.2	–
$C_{18:0}$	–	7	1.5	0.4	5.6
$C_{18:1\omega9}$	4.2	10	4	4.5	43.5
$C_{18:2}$	15.3	10.5	1.5	2.6	5.9
$C_{18:3}$	12.2	20	–	0.7	–
$C_{20:4\omega6}$	1.9	–	5.3	3.4	–
$C_{20:5\omega3}$	8.0	9	29.6	33.8	–

2.4. Enzyme-assisted extraction

2.4.1. Principles of enzyme-assisted extraction

The process of enzyme recovery is based on the selected enzyme (s) breaking the cell wall of the microalgae, isolating the lipid and derivatizing the lipid to FAME (Maffei et al., 2018; Zuorro et al., 2016b). The use of enzymes for FAME recovery offers a reduction in energy, due to decreased temperatures required. (Wang et al., 2017) who studied *Nannochloropsis oceanica* demonstrated that enzymatic recovery of FAME could be carried out at room temperature allowing for FAME recovery of up to 99%. Disadvantages of enzymatic-assisted extraction include the inability to upscale due to the high cost of enzymes (Lopez et al., 2015; Wu et al., 2017). However, various studies by (Castillo López et al., 2015) on *Nannochloropsis gaditana* showed that novozym has a high reuse rate of up to 6 times. A study by (Wang et al., 2017) also using novozym enzymes, showed that they could be reused up to 110 times. The increased life time may be due to the addition of t-butanol and methanol when compared to the other study, which used methanol. When using enzymes to recover FAMEs, the cost of the enzymes represent 22% of the total costs which can be reduced in half by recycling the enzymes as long as the enzymes working efficiency remained at 90% (Wang et al., 2017).

2.4.2. FA composition obtained by enzyme-assisted extraction

The most common enzymes which have been tested include novozym, cellulose, hemicellulose and lysozyme (Onumaegbu et al., 2018; Zhang et al., 2018). Fig. 3 shows the lipid recovery for various enzyme(s) all with a concentration of 10 mg/g which was determined to recover the greatest volume of lipids by various studies by (Zuorro et al., 2016b, 2016a). An investigation by (Qiu et al., 2019) showed that *Nannochloropsis* sp. lipids contain up to 60% polar lipids which can be converted to FAMEs using enzyme (lipids were used for food applications in this study). Cellulose which was the most common enzyme to be used alone, gave lipid recovery of between 11.73 and 53.2% in different studies ((He et al., 2020; Liang et al., 2012; Maffei et al., 2018; Qiu et al., 2019). The composition of the cellulose lipid reported by multiple authors showed an average of 48.9% SFA, 35.4% MUFA and 15.5% PUFA as seen in Fig. 3 (He et al., 2020; Qiu et al., 2019). This composition of FA in the microalgae represents an ideal biodiesel raw material, with a very small percentage of PUFA (Martinez-Guerra et al., 2018). The FAMEs which are at highest

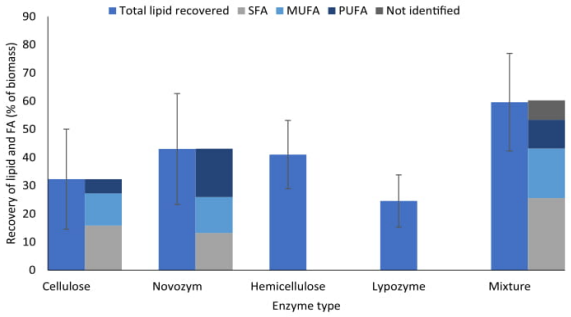

Fig. 3. Summary of data gathered on the average of % lipid recovery and FA composition from *Nannochloropsis* sp. using different enzymesbased on a number of studies (Cellulose: Zuorro et al., 2016a, 2016b, Qiu et al., 2019, Liang et al., 2012 and Castillo López et al., 2015; Novozym: Zuorro et al., 2016a, Wang et al., 2017 and Ali and Watson, 2015; Hemicellulose: Zuorro et al., 2016a, 2016b and Maffei et al., 2018; Lypozyme: Zuorro et al., 2016a and Lopez et al., 2015; Mixture: Zuorro et al., 2016a, 2016b, Wang et al., 2017 and Maffei et al., 2018.).

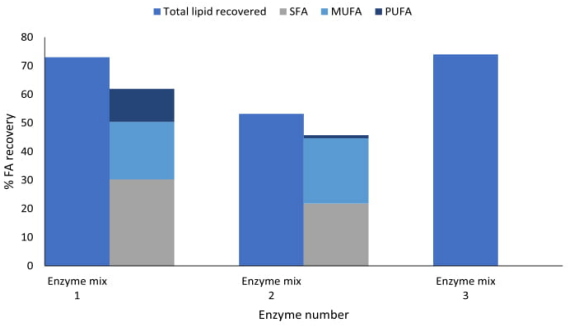

Fig. 4. Summary lipid recovery and FA composition from Nannochloropsis sp. using enzyme mixtures from 3 different studies; enzyme mix 1 = mixture of cellulose/protease/lysozyme/pectinose (Wu et al., 2017), enzyme mix 2 = cellulose/hemicellulose by (Zuorro et al., 2016a) and enzyme mix 3 = cellulose/hemicellulose by (Maffei et al., 2018).

concentrations include $C_{16:0}$ and $C_{16:1}$ which are characteristic biodiesel carbons outlined in Table 5 (Teo et al., 2014).

Novozym is the most widely used commercially available enzyme for assisting in the production of biodiesel as it allows for high lipid recovery (Castillo López et al., 2015). As seen in Fig. 3, the lipids recovered (average of 3 studies by (Castillo López et al., 2015; Lopez et al., 2015; Wang et al., 2017)) by novozym showed to have 30.7, 29.7 and 39.8% for SFA, MUFA and PUFA respectively. The compositions of these FA appear to have equal amounts of SFA, PUFA and MUFA which would mean they are not suitable for biodiesel production (Wang et al., 2017; Wu et al., 2017; Zuorro et al., 2016b). Hemicellulose and lypozyme were also investigated demonstrating recovery of favourable levels of lipids at 41 and 24.5%, respectively (Ali and Watson, 2015; Lopez et al., 2015; Maffei et al., 2018; Wang et al., 2017; Zuorro et al., 2016b, 2016a).

2.4.3. Factors effecting enzyme-assisted recovery

Fig. 3 shows that a mixture of enzymes can recover the highest volume of lipids (Maffei et al., 2018; Wu et al., 2017; Zuorro et al., 2016b). Fig. 4 shows the lipid and FA composition obtained by different enzyme mixtures (enzyme mix 1; mixture of cellulose/protease/lysozyme/pectinose (Wu et al., 2017), enzyme mix 2; cellulose/hemicellulose by (Zuorro et al., 2016a) and enzyme mix 3; cellulose/hemicellulose by (Maffei et al., 2018). A study by (Zuorro et al., 2016b) showed that lipid recovery with no enzymes gave 14% lipids from biomass. By introducing enzymes to assist with lipid extraction showed improvements with hemicellulose, cellulose and lysozyme at 34, 24 and 18%, respectively. Hemicellulose is reported to have optimal impact due to its composition of mannanase, galactosidase and gluconate. Zuorro's results showed (Zuorro et al., 2016b) that a combination of enzymes (cellulose and hemicellulose) led to greater lipid recovery at 53.2%. These lipids were further characterized to contain 41% SFA and 43% PUFA, which is an ideal ratio for the production of biodiesel. Another enzyme mixture consisting of cellulose and hemicellulose (Maffei et al., 2018) found that the mixture allowed for a recovery of 74%. A study carried out by (Wu et al., 2017) showed that an enzyme cocktail of cellulose, protease, lysozyme and pectinase extracted 73.1% of the lipid from microalgae. The results in Table 5 indicate that enzyme-assisted extraction enhances the recovery of SFA and MUFA.

Table 5
FA composition obtained by enzymatic-assisted extraction.

Fatty acid composition (%)	Enzyme mixture	Cellulose	Nonozyme
SFA	42.91	48.99	30.6
MUFA	28.63	35.44	28.7
PUFA	16.87	15	40.4
$C_{14:0}$	6.5	7.48	7.3
$C_{16:0}$	35.4	35.26	23.3
$C_{16:1}$	28.46	28.15	24.8
$C_{18:0}$	0.32	6.25	–
$C_{18:1\omega9}$	–	7.29	3.9
$C_{18:2}$	2.95	1.61	3.8
$C_{18:3}$	0.26	0.53	–
$C_{20:4\omega6}$	3.61	1.13	6.8
$C_{20:5\omega3}$	10.31	12.26	29.8

3. Viable products from *Nannochloropsis* sp.

3.1. Biodiesel

Nannochloropsis sp. microalgae has received increased interest as sources of biodiesel. This is due to advantages such as carbon neutrality, reduced emissions and availability of biomass feedstock due to fast growth (Cehn et al., 2018; Lee et al., 2014; Bwapwa et al., 2020). Biodiesel can be either used in pure form or in combination with diesel for engines which do not need to be modified to facilitate them (Mubarak et al., 2015). Biodiesel is synthesised by transesterification in which the triglyceride in the matrix reacts with a catalyst to yield esters of FA and glycerol (Ranjan et al., 2010). There is a growing need for biodiesel due to the impact of fossil fuel resources on climate change (Castillo López et al., 2015).

3.1.1. Biodiesel FA composition

FA compositions of lipid extracted from *Nannochloropsis* sp. determines if the species is suitable for biodiesel production. Studies by (Ali and Watson, 2015) and (Ma et al., 2014) showed that $C_{16:0}$, $C_{16.1}$, C_{18} and $C_{18.1}$ were found in *Nannochloropsis* sp. The presence of SFA such as $C_{16:0}$ and $C_{18:0}$ determines the cetane number, which improves the ignition quality and the heat of combustion of the fuel. UFA such as $C_{16.1}$ and $C_{18.1}$ are oxidatively unstable due to the double bonds which are susceptible to reaction with oxygen (Ali and Watson, 2015). It has been suggested that MUFA act as a balance between oxidative stability and low temperature properties (Ma et al., 2016). The recommended FA ratio for optimal biodiesel is 5:4:1 for $C_{16.1}$:$C_{18.1}$:C_{14} (Huerlimann et al., 2010). The equations used to calculate the most important properties of biodiesel (including cetane number, saponification value, iodine number, viscosity, density and higher heating value) were reported by (Martinez-Guerra et al., 2018). Of these properties, the most important include the cetane number which indicates the fuel

ignition quality, oxidation stability which should be high to avoid oxidation and to avoid longer storage times, viscosity values which are higher to alter injection spray characteristics damaging the chamber and more viscous fuel can damage the fuel pump (Ali and Watson, 2015; Ma et al., 2016). The required properties set out by the American and European fuel agencies is outlined in Table 6 (Martinez-Guerra et al., 2018). A study by (Ma et al., 2014) showed that biodiesel could be successfully produced to consist of the suitable properties cetane number 54.61, iodine number 104.85 and low cloud point of 3.45 °C. This suggests a suitable product (Table 6) for engines can be produced using *Nannochloropsis* sp.

3.1.2. Biodiesel case studies

Nannochloropsis sp. for biodiesel production is reported to be an attractive alternative to fossil fuels as it is compatible with current engines. (Carrero et al., 2015). A study by (Teo et al., 2016) showed that the retention time to produce biodiesel from *Nannochloropsis* sp. can be performed in 20–30 min using a MW noncatalytic method. However, problems associated with the production of biodiesel from microalgae include the cost of production. This is due mostly to cultivation and extraction (Goh et al., 2019; Lee et al., 2014; Onumaegbu et al., 2018). A study by (Kim et al., 2015) found that *Nannochloropsis* sp. lipids and FA were capable of producing a biodiesel with 100% yield at lab scale analysis. However, biodiesel production from *Nannochloropsis* sp. requires improvement in the cultivation and drying methods in order to achieve the scale required (Ma et al., 2016). Some authors have predicted microalgae can make a viable biodiesel product (Santos-Sánchez et al., 2016) in five years from 2016.

3.2. Omega-3

Omega-3 FA are recovered from $C_{20:5\omega3}$ and $C_{22:6\omega3}$ which are PUFA. They can be used to produce food, nutraceuticals and pharmaceuticals. Omega-3 is reported to provide health benefits such as preventing cardio-vascular diseases, cancer, asthma, arthritis and high blood pressure (Aliev and Abdulagatov, 2016; Craggs et al., 2011). Furthermore, the intake of omega-3 containing FAs are known to play a role in controlling depression and promote animal growth (Li et al., 2019; Ma et al., 2016). It was reported (Figueiredo et al., 2019) that omega-3 consumption by humans worldwide is below the recommended. Therefore, the use of microalgae is of interest to produce FA for enriching foods or production of nutraceutical supplements. PUFA such as EPA and DHA have been reported to provide benefits for health conditions with high concentrations from *Nannochloropsis* sp. lipids (containing up to 30% of the biomass) (Aliev and Abdulagatov, 2016; McKennedy et al., 2016; Santos-Sánchez et al., 2016). (Ma et al., 2016) reported that the recommended daily intake of EPA and DHA is 250 mg to 2 g in order to have a healthy lifestyle. A study by (Brennan et al., 2020) reported 0.36 mg EPA in a 2 mg *Nannochloropsis* sp. sample therefore in order to meet the recommended levels, >690 mg of Nannochloropsis sp. would be required. Nannochloropsis sp. are a suitable species to supply these FAs as they've been reported to contain up to 50% lipids in their biomass which is capable of doubling daily and producing high levels of $C_{20:5\omega3}$ when compared to other microalgae species (McKennedy et al., 2016; Ranjan et al., 2010).

Table 6
Regulation set out by US and EU authorities for biodiesel properties for to be used in engines.

Property	Unit	ASTM 6751–02	EN 14214
Cetane number	–	≥47	≥51
Saponification value	(mg KOH/g)	–	–
Iodine value	(g I_2 100/g)	–	≤120
Degree of unsaturation	–	–	–
Cold filter plugging point	(°C)	NA	≤5/≤ − 20
Cloud point		Report	Report
Viscosity	(mm^2/s)	1.9–6.0	3.5–5.0
Density	(g/cm^3)	NA	0.96–90
Oxidative stability	(h)	–	≥6

4. Conclusion

Extensive research has previously been carried out characterising FA from *Nannochloropsis* sp. using different extraction methods. However, of the studies investigated, no study has looked at how different methods impact the FA composition of the recovered lipid. This is a comprehensive review of the *in-situ* mechanical and enzymatic methods of recovering FAMEs from *Nannochloropsis* sp. The methods discussed include microwave-, ultrasound-, SC-CO_2- and enzyme assisted extraction. The viable products which can be produced from the FA recovered are discussed. The comparison of methods suggest that the MW method produces high levels of SFA and MUFA which makes it suitable for biodiesel production. However, MW is a difficult and costly method to scale up which presents challenges to using it at industrial scale. SC-CO_2-assisted extraction shows some merits which allows for the conditions to be altered in order to produce desired FAs. The UAE method showed to have little research focusing on *Nannochloropsis* sp. However, from the reviewed literature it could be concluded that the simple and low costing method produces highest levels of SFA and MUFA. Enzyme-assisted extraction was reported to offer a green alternative with lower temperatures and chemical consumption. A mixture of enzymes allowed for the greatest recovery. Using a mixture resulted in a maximum disruption of the cell wall. For the recovery of nutraceuticals, SC-CO_2-assisted extraction appears to be the most efficient as the system parameters can be modified to obtain the highest concentrations of EPA and DHA. In terms of biodiesel, enzymatic-assisted extraction shows great potential with its high lipid recovery, ability to reuse enzyme substrates and green approach to help lower CO_2 emissions. Published literature has focused on the recovery of biodiesel and omega-3 lipids extensively. Biodiesel recovery shows great potential but there are still further developments to make it a viable product. EPA and DHA have become very popular for producing omega-3 containing nutraceuticals.

The review shows that different extraction methods can impact the FA composition recovered from the microalgae species. The MW method shows high recovery of biodiesel suitable FA while SC-CO_2 shows the greatest recovery for nutraceutical suitable FA. The review also shows strong potential to produce viable products from the FA recovered from *Nannochloropsis* sp. Further research is required to address the challenge of scaling up these operations.

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Declaration of competing interest

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Recovery of viable ammonia–nitrogen products from agricultural slaughterhouse wastewater by membrane contactors: a review

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Efficient removal of nitrogen from wastewater is vital to ensure the safekeeping of waterways and their biota. Increased demand for meat supplies worldwide has led to increased wastewater production consisting of higher nitrogen levels. In order to reduce nitrogen treatment costs using biological denitrification, slaughterhouses need to start looking at the potential of producing by-products from wastewater. The production of viable products from industry-based wastewater has been shown to reduce treatment costs and also generate a source of revenue for the company. The sources of nitrogen in slaughterhouse wastewater are discussed, and the risk it poses to the environment and the different treatment methods are reviewed. Additionally, the need for new methods of ammonia treatment is outlined, including the potential of recovering nitrogen to produce viable products. The use of hydrophobic membranes to recover ammonia from challenging wastewaters is critically analysed and the possible implications it may encounter with slaughterhouse wastewater. The viable products which can be derived from the nitrogen in slaughterhouse wastewater are identified and studies by multiple authors show that the production of ammonium-salts can be used to aid in agricultural fertiliser production, flame retardant compositions, food additives and protein purification precipitation. A comprehensive review of studies evaluating the composition of slaughterhouse wastewater is presented, including the impact of the challenging matrix on membrane materials, which has not being reviewed to date. Additionally, a detailed discussion on how the nitrogen content is recovered using hydrophobic membranes in order to produce viable products is also presented, which has not been discussed before in relation to slaughterhouse wastewater.

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Water impact

Efficient removal of nitrogen from wastewater is critical to ensure good water quality. Increased demand for meat supplies worldwide has led to increased wastewater production consisting of higher nitrogen levels. In order to reduce nitrogen treatment costs using biological denitrification, slaughterhouses need to start looking at the potential of producing by-products from wastewater. Membrane technologies are used to remove nitrogen species from wastewaters, and are generating viable end-products.

1. Introduction

Slaughterhouses consume a considerable volume of freshwater yearly which results in a large volume of wastewater (WW) rich in organic contaminants and nutrients.[1] It has been reported that the slaughterhouse industry uses 29% of the fresh water consumed by the agriculture sector every year.[2] The global processing of cattle has increased in the last 50 years due to the increasing demand for red meat with 27.6 Mt being processed in 1961 and 63.9 Mt in 2013.[3] Additionally, more than 30 million cattle heads were processed in 2016 which produced substantial volumes of WW which require rigorous and costly treatment.[4] Nitrogen is one of the most problematic constituents in slaughterhouse WW (SHWW) due to its effects on the environment.[5] Nitrogen is usually in the form of ammonia (NH_3) or organic nitrogen in SHWW.[6] It has been reported that 53% of rivers in the United States have medium to high levels of NH_3 due to inadequate WW treatment.[7] Failing to effectively treat NH_3 in SHWW results in poor water quality arising from excessive algae growth, turbidity, bad odour, depletion of dissolved oxygen (DO) and mortality of fish & other aerobic organisms in receiving

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waters.[8,9] The highest concentration of NH_3 in SHWW is from the rendering plant, due to the processing of the animal carcasses which breaks down the protein bonds which are made up of nitrogen.[10] The most common method of treating NH_3 from SHWW is through biological nitrification–denitrification which has a combined retention time of 20 days.[11] However, in order for WW treatment plants (WWTP) to be capable of treating the increasing levels of nitrogen, they must adapt to alternative methods of treatment or even utilise the SHWW to recover viable by-product which are capable of reducing the costs of treatment or generate revenue for the company.[12] The recovery of nitrogen from WW has a double benefit as it reduces the nitrogen levels before it's released to receiving waters and allows for the production of viable by-products.[13] Nitrogen can be recovered using ion exchange, air stripping, microwave radiation or hydrophobic membrane contactors.[14] Membrane contactors use a stripping solution to capture the nitrogen (in the form in NH_3) to produce ammonium (NH_4)-salt.[15] Various studies have shown that NH_4-based salts can be recovered to produce a liquid fertiliser which would be of interest to famers who can apply the fertiliser to promote crop growth.[16]

Overall, there is a critical need to reduce and recover NH_3 in a more energy efficient way ensuring it is adequately treated. A report by the Irish EPA[17] which investigated the efficiency of urban and industry treatment methods indicated that 34% of WWTP were releasing inadequately treated effluent. This inadequacy may be due to overloading of the plant, poorly performing facilities or insufficient treatment residence time. This has led to a call for new improved technologies to treat WW. Production of viable nitrogen-based by-products from the WW is an option for reducing costs associated with ammonia removal, and here we comprehensively review the characterisation of SHWW and potential for valorization *via* nitrogen-based by-products, with a focus on membrane contactors. Studies by Bustillo-Lecompte and Mehrvar,[18] Mittal *et al.*[19] and Fuchs *et al.*[20] review the composition of SHWW and critically analyse how they are treated to recover nitrogen but they do not review the use of membrane contactors. Beckinghausen *et al.*[21] and Rezaei *et al.*[22] discuss the advancements of membrane technologies to treat nitrogen in wastewater but do not discuss a matrix as challenging as SHWW which can compromise the membranes structure and integrity. Darestani *et al.*[23] reviews the use of hollow fibre membranes to recover nitrogen from agricultural WW but does not review other membrane configurations and additionally, Jensen *et al.*[24] reviews the use of membrane contactors to treat agricultural wastewater for saline levels (NaCl) but not nitrogen. No review paper successfully discusses the advances in nitrogen recovery from SHWW while discussing the effects of the matrix and the efficiency compared to conventional methods. Additionally, an in-depth identification of viable nitrogen based products from slaughterhouse using membranes has not been performed. This review paper aims to compile all of these factors by characterising slaughterhouse wastewater in order to identify the efficiency of nitrogen removal using membrane technology compared with conventional methods and identifying the viable nitrogen-based products which can be recovered.

2. Nitrogen in animal slaughterhouses

2.1 Sources of nitrogen

Nitrogen exists in multiple forms throughout slaughterhouse sites including nitrite (NO_2^-), nitrate (NO_3^-), NH_3, organic nitrogen and total Kjeldahl nitrogen (TKN).[25] NO_2^- and NO_3^- levels are reported to be low (<0.5 mg L^{-1} N) in raw SHWW whereas NH_3 levels are high (<2 mg L^{-1}).[4] Nitrogen generally takes the form of NH_3 in WW in early processing stages as it is easily soluble and up to 50% of nitrogen is soluble and in the form of NH_3.[11] NH_3 can exist in two forms, either as NH_3 or NH_4^+ ion.[26] The composition of the type of NH_3 depends on pH, temperature and oxidation–reduction potential (ORP). When an NH_3 solution has a pH of 8 and under, the ammonium ion is present as NH_4^+.[27] However, at 9.5 and above it will be present as NH_3.[28] In order for nitrogen to be in the NH_3 form, it must have an ORP value of 100–350 mV which will allow nitrifying bacteria convert it to NO_3^-.[29]

The sources of nitrogen can derive from various parts of the plant and the concentrations of different nitrogen forms also vary depending on the plant area as seen in Fig. 1.[30] Very limited literature describes the nitrogen composition throughout the slaughterhouse plant. It was reported by Jensen *et al.*[11] and Johns *et al.*,[30] that different sources of nitrogen in the plant come from the slaughter floor, boning room, paunch process, offal processing and rendering plant. To the authors' knowledge, there are no other studies which quantify the different nitrogen species in various parts of a slaughtering plant. Fig. 1 shows that the distribution of nitrogen loads is site specific. The rendering area has the highest concentrations of nitrogen waste which includes the processing of heads, hooves and bones which may have high concentrations of protein (>5 mg L^{-1}). The offal & paunch also contains high levels of inorganic nitrogen (>0.5 mg L^{-1}) which is due to the intestinal tracts of the animals being opened to remove stomach contents. The holding yard has higher levels of NO_2^- and NO_3^- which may be due to the oxidisers degrading the NH_3 in the waste due to nitrification, while low levels of NO_2^- or NO_3^- are present in the slaughtering process level. This suggests that NO_2^- and NO_3^- are not produced at the processing stage of the plant but instead at the collection and treatment stages.[4] Additionally, Ziara *et al.*[4] stated that most nitrogen found in the slaughtering plant is in the NH_3 and TKN form. Other sources of nitrogen in slaughterhouse plants may be due to urine and faeces from both humans and animals which are introduced into the sewage system.[31] Due to the different nitrogen forms at different stages of the plant, it is suggested that the WW from all stages are collectively stored in order to more efficiently recover nitrogen to produce viable by-products.[11]

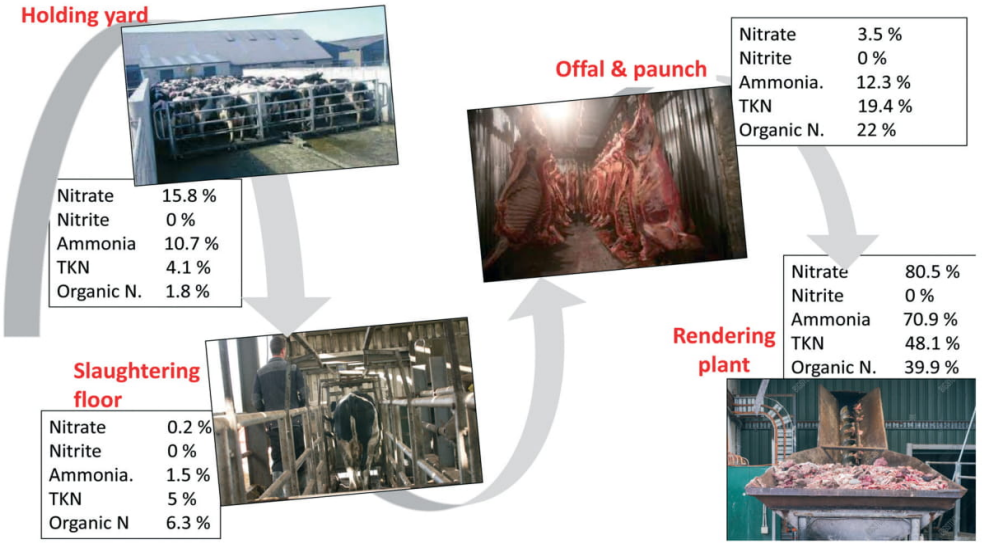

Fig. 1 Percentage of nutrient load at each stage of slaughtering process.

2.2 Conventional nitrogen treatment methods

SHWW is usually treated on site for various contaminants including solids, nutrients and organics before being released into receiving waters.[32] Fig. 2 shows a typical WWTP for SHWW which suggests that floatables, oils, solids and organics are treated first using systems such as skimmers, sedimentation and dissolved air flotation (DAF).[1,4] After de-watering the waste, the WW is treated for nutrients such as nitrogen and phosphorus using biological treatment methods such as sequencing batch reactors or more common, biological nitrification–denitrification.[18,33] Extensive research has focused on the use of these ponds and they have been shown to be very effective in removing nitrogen.[15,27,34] Denitrification allows for the reduction of emissions and energy costs whilst producing biogas.[3] However, these methods are only used for capturing methane and they do not allow for the complete removal of nitrogen – only that in

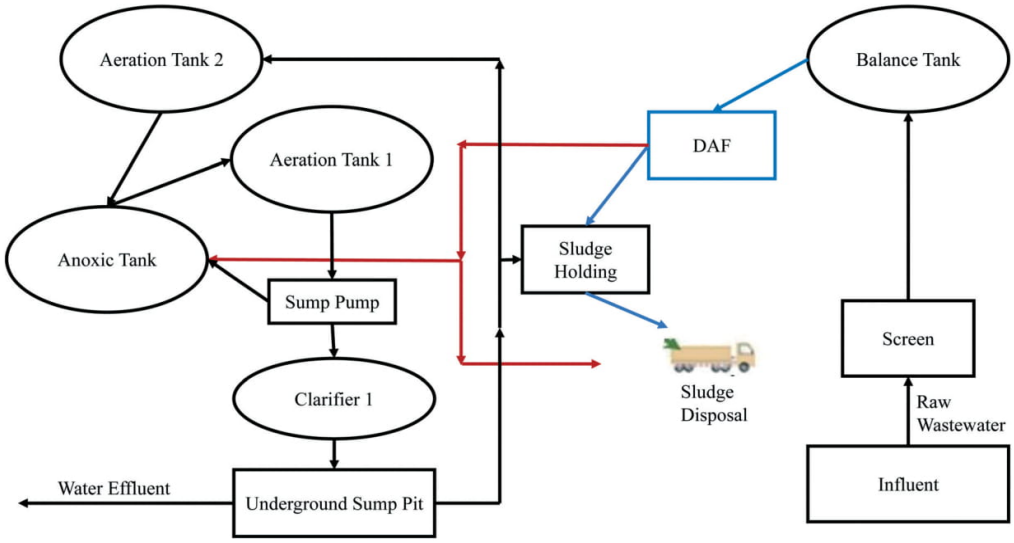

Fig. 2 Schematic of a typical WWTP to treat SHWW.

the NO_3^- form. Although these conventional methods which have been used for years have been shown to be effective in removing nitrogen, the process is restricted by slow conversion and unfavourable environmental factors in addition to having retention times taking up to 30 days to treat the total nitrogen.[27,35] Additionally, these methods have a large environmental footprint, poor capture rates and poor odour control.[24] Valta *et al.*[36] stated that high concentrations of NH_3 can impact the development of biogas negatively and as such, due to the high levels of NH_3 in SHWW, it makes the study of NH_4-based salt production a lot more interesting and feasible. Other methods capable of removing nitrogen and possible recovery from SHWW include air stripping, NH_3 volatilization, absorption, struvite precipitation, membrane systems and sedimentation.[37]

2.3 Role of nitrogen cycle in slaughterhouse wastewater treatment plant

The use of the biological nitrification–denitrification treatment in SHWW is based on the principle of the nitrogen cycle as shown in Fig. 3.[38] The steps of the nitrogen cycle can be split into 1) nitrogen fixation; 2) nitrification; 3) assimilation; 4) ammonification; and 5) denitrification.[39,40] In order to ensure the process is efficient, the steps of nitrification and denitrification occur in separate reactors as nitrification requires aerobic conditions while denitrification requires anaerobic conditions.[8] Briefly, nitrification occurs when SHWW rich in nitrogen in the ammoniacal-N form is converted between NH_3 and NH_4 depending on its pH (basic and acidic, respectively).[41] In its NH_3 form, the NH_3 and oxygen (usually supplied by aeration) feed NH_3 oxidisers to produce an acid, water, NO_2^- and energy. The water is reintroduced to the system, the energy is used to grow and multiply the NH_3 oxidisers and the NO_2^- moves to the next step. NO_2^- oxidisers feed on the NO_2^- and the DO to produce NO_3^- and energy.[42] When NH_3 levels are <2 mg L^{-1} or NO_2^- is 0.5 mg L^{-1}; nitrification is regarded as being complete.[43] The NO_3^- is then used in the denitrification step as an oxygen source for the heterotrophic bacteria along with chemical oxygen demand (COD) for energy. Under anoxic conditions, the heterotrophic bacteria break down the NO_3^- and use the oxygen for synthesis while the nitrogen is released as N_2 gas in small bubbles to the surface of the waterbody and escape into the atmosphere.[44] The combination of biological nitrification and denitrification can result in nitrogen levels as low as 1–3 mg L^{-1} of NO_3^- in the effluent stream.[42]

3. Recovery of nitrogen from slaughterhouse wastewater

A number of different methods to recover nitrogen (in the form of NH_3) from SHWW have been investigated including breakpoint chlorination, air stripping, selective ion exchange, microwave radiation, struvite precipitation and membrane technology.[5,13,52,53,41,45–51] The characteristics of the methods mentioned are outlined in Table 1. Nitrogen recovery is much more complicated since there is no ready precipitate.[38] A lack of investment in these technologies has prevented them

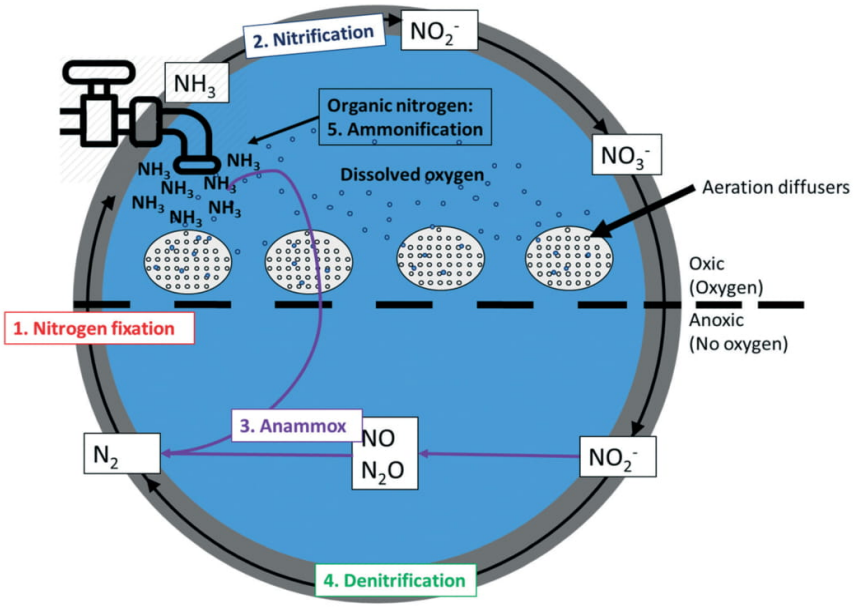

Fig. 3 Overview of the nitrogen cycle showing nitrification in the region above the dashed line and denitrification in the lower section.

Table 1 Possible methods of ammonia recovery from SHWW

Method	Principle	Ammonia removal	By-product	Disadvantages	Ref.
Air stripping	NH_3 stripped from water droplets to atmosphere	71–99%	—	Can only facilitate up to 100 ppm NH_3	(45, 46)
Ion exchange	NH_3 attaches to zeolites	84–94%	NH_3 supplement	Concentrated NH_3 waste	(47, 48)
Microwave radiation	Radiation inhibits escape of NH_3 molecule to atmosphere	87–94.2%	—	Requires high energy	(49–51)
Struvite precipitation	Ammonia, magnesium and phosphorous react together to form a crystal which can be isolated from WW	45–70%	Fertilizer	May require high volumes of magnesium phosphate and long treatment time	(56–58)
Membrane technology	Driven by partial pressure difference	64–99.8%	Ammonium-salt fertiliser	Membrane material subject to wetting	(5, 13, 41, 52, 59)

being installed into SHWW treatment sites which prevents the creation of viable by-products such as NH_4^+-based fertiliser.[54] However, the use of membranes in a number of investigations has shown to allow for efficient recovery of NH_3 (up to 99%) whilst rejecting other contaminants and producing a viable fertiliser by-product.[55]

3.1 Recovery of nitrogen using membranes

Membrane contactors have been used to treat SHWW for COD, BOD, total phosphorous and total nitrogen with removal at rates up to 85.8, 50, 97.5 and 99.8%, respectivel.[60–62] Whilst the use of membrane contactors allows for a reduction in WW treatment costs due to its low operating costs, it also allows for viable by-products by recovering the nitrogen in the form of NH_3 to produce NH_4^+-salt fertiliser.[2,40] Membrane contactors offer a hydrophobic porous membrane which acts as a partition between two phases to promote separation of contaminants from WW,[63] and can be installed using flat sheet, spiral wound or hollow fibre configurations. Membrane contactors are becoming a technology of interest due to their simple operation, high selectivity and low energy consumption.[64] Membrane contactors have a feed side and a permeate side which usually contains a strong acid. The feed side containing an NH_3/NH_4 solution is deprotonated to ensure all of the NH_3 is in a volatile form. As the feed solution is circulated into the membrane contactor, the partial pressure of NH_3 on the permeate side causes the NH_3 enter the membrane pores, cross to the permeate side and react with the stripping solution. Because the NH_3 immediately reacts with the stripping solution to produce an NH_4-salt, the concentration of NH_3 is very low on the permeate side allowing for continues transfer due to the partial pressure which acts as the driving force.[13] The mechanism of separation in this kind of membrane contactors is based on the mass transfer between two phases.[28] In theory, all free NH_3 can be removed using membrane contactors as long as there is enough H^+ ions in the stripping solution and sufficient contact time for the chemisorption process to occur.[65] Due to the hydrophobic nature of the membrane materials, liquid water streams will be kept outside the membrane while vapors will penetrate from the feed side with a higher partial pressure to the permeate side with a lower partial pressure.[66] The hydrophobic microporous polymeric membrane provides the transfer area and restricts the permeation of water. The transfer takes place at the pore opening, inside the pore, or at the pore exit.[40] As outlined by Darestani *et al.*,[23] the transfer of gaseous species such as NH_3 from aqueous solutions across a hydrophobic membrane is assumed to occur in 5 stages: (1) transfer of NH_3 from the bulk solution to the boundary later at a membrane pore; (2) equilibrium of the NH_3 solution with gas (air) present in the membrane pores; (3) transfer of the NH_3 gas across the air filled pore; (4) reaction of the NH_3 gas with the receiving component (usually acid); (5) transfer of the NH_4^+ (due to pH shift $NH_3 \rightarrow NH_4^+$) salt through the boundary layer into the bulk strip solution.[28] This process is usually set up in direct contact mode, *i.e.* the feed and permeate streams are in direct contact with the membrane which allows diffusion of the volatile components.[67] Fig. 4 is a schematic showing a typical membrane contactor set-up. The figure outlines show the NH_3 molecules move from the feed side through the membrane pores to react with the stripping solution and form a NH_4-salt.[13] The image also shows (on the bottom right) how role of surfactants and how they can result in membrane wetting. As discussed further below, fats and proteins can result in membrane wetting which causes liquids to penetrate the membrane at the surface and eventually liquid to cross the barrier.[13] Surfactants present in challenging wastewaters can result in clogged pores, surface coating or surface modifications which lead to increased pressure which damages the membrane material.

The effect of feed temperature and stripping solution temperature should also be important with respect to NH_3 transfer across the hydrophobic membrane. A study carried out by Bernal *et al.*[68] investigated the uses of nitric acid and phosphoric acid as the stripping solution and found that it resulted in an NH_3 recovery capacity higher than 95–98% when free acid is present in the stripping solution. Additionally, a study was carried out by Damtie *et al.*[69] to compare different acids as the stripping solution (H_2SO_4,

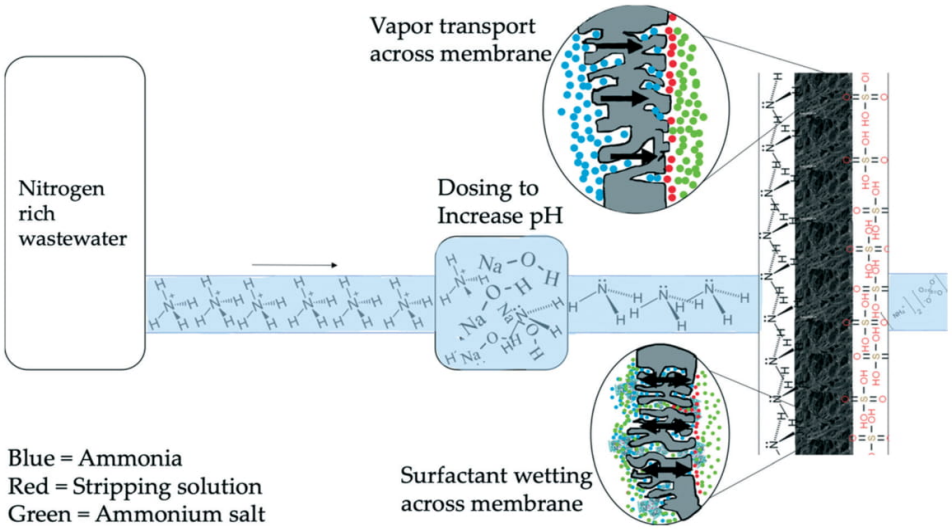

Fig. 4 Schematic outlining the operation of a typical direct contact membrane set up and an example of potential membrane wetting.[13]

nitric acid, phosphoric acid, acid mix and water) and it was determined that H_2SO_4 recovered the greatest volume of NH_3.

3.2 Membrane failures associated with slaughterhouse wastewater

Problems associated with the use of membrane contactors include the cost to initially set up the technology, blockage of pumps and membrane fouling.[70] One of the major problems associated with the use of membrane contactors for SHWW treatment is how susceptible they are to fouling and subsequently wetting.[71] The accumulation of particulates such as fats, grease, protein and organic matter can cause build up on the membrane material resulting in the membrane fouling.[64] Membrane failure is a huge economic influence on the use of membrane contactors as they account for 72% of the capital investment.[24] The types of foulants which may interfere with membrane performance include chemical foulants such as scaling, physical foulants such as deposition of particles, biological foulants such as microbes and organic fouling which interact with the membrane material.[72] SHWW has high concentrations of TSS, FOG and OM which cause wetting of membranes.[2] It was outlined by Brennan *et al.*[13] that high concentrations of TSS and FOG impacted the performance of membrane materials and reduced the efficiency of NH_3 after 6 days. An additional study by Zarebska *et al.*[73] investigated the efficiency of membrane distillation for removing NH_3 from swine WW. The results indicated that the highly challenging composition of the WW was a limiting factor in the performance of the removal efficiency. Additionally, SHWW contains high levels of volatile fatty acids which exert partial vapour pressure and are exported across the membrane with water vapor which can cause contamination of the permeate and jeopardize the quality of the recovered NH_4^+-based fertiliser.[74] A recent study carried out by Lee *et al.*[75] demonstrated that an alkaline feed solution can reduce the risk of wetting by OM which could eliminate the requirement for a cleaning protocol. The presented data suggests the critical need for a membrane material which can withstand high levels of pollutants or a suitable cleaning protocol be introduced in order to revive membranes.

3.2.1 Membrane wetting and fouling. Normally more hydrophobic membrane materials are more repellant to water molecules which helps prevent wetting.[52] Membrane wetting is the process in which membrane materials lose their hydrophobicity and allow for liquids to penetrate the membrane pores.[76] Membrane pore wetting will result in a direct liquid flow from feed through the wetted pores, substantially deteriorating permeate quality.[77] Wettability is controlled by chemical composition of the membrane and geometry of the pores.[74] Components in SHWW such as fats, oils and protein can lower the liquid surface tension of the feed solution and cause wetting of the membrane pores.[23,74] Wetting on rough surfaces of membrane materials may be either be caused by the liquid fully penetrating the roughness grooves or by air being trapped underneath the liquid inside the rough grooves.[74] Dissolved organic matter and colloids present in the nutrient rich waste streams can lead to membrane fouling.[78] Liquid entry pressure (LEP) is the pressure which can be exerted on the membrane contactors before the liquid penetrates the membranes pores. It is important for membrane materials to have a high LEP values to increase the lifetime of membrane material and limit the likelihood of reduced hydrophobicity.[71]

SHWW contains high levels of oil which contribute to the wetting of membranes as the hydrophobic part of the oil

adheres to the membrane surface. This results in the hydrophilic part being exposed to the essentially giving the membrane material a hydrophilic surface. A hydrophilic surface prevents vapors crossing the membrane contactor, increases the pressure and results in membrane leaking due to increased pressure.[79,80] The standard water quality generally required for hydrophobic membranes is 5–10 μm pre-filtration and a low fouling index.[74] The wettability of a surface is directly related with the surface energy and hence, materials with a low surface energy have a high contact angle which is more immune to surface wetting.[23] It is expected that some alcohols such as ethanol can result in wetting of the membrane materials and more superhydrophobic membranes are suitable to treat volatile bio alcohols.[81] The higher hydrophobicity of nanofibers is attributed to the increased surface roughness due to overlapping nanofiber layers, which results in less contact area for the solid fiber and water leading to higher CA.[77] The fouling and wetting of membrane materials impairs the membrane performance and shortens membrane lifetime, thereby reducing NH_3 recovery from SHWW which suggests that more research is required to fabricate membranes suitable for matrices with such high concentrations of oil.[74]

3.2.2 Preventing membrane wetting and fouling. One method which could help to overcome membrane wetting is incorporating superhydrophobic membrane materials which are more resistant to membrane wetting.[55] Several authors including Liu *et al.*,[82] Li *et al.*,[81] Tijing *et al.*,[83] Chen *et al.*[79] and Xie *et al.*[74] showed that superhydrophobic membrane materials can be used against challenging waters as shown in Table 2. Superhydrophobic membrane materials are increasingly becoming the membrane material of choice due to their anti-sticking, anti-contamination, anti-wettability and self-cleaning properties.[83] The results summarized that the incorporation of nanoparticles and alternative outer layers showed superhydrophobic characteristics (contact angle greater than 150°)[81] and were suitable for challenging matrices similar to SHWW. The incorporation of nanoparticles such as $AgNO_3$ and TiO_2 showed promising alternatives as they increase the surface roughness. Xie *et al.*[74] incorporated a hydrophilic layer on top of a PTFE membrane and achieved a contact angle of 150°. This membrane material is interesting as the hydrophilic part of the oil surfactants in the SHWW may adhere to the membrane surface (rather than hydrophobic parts like conventional membranes) which will result in a hydrophobic part of the oil being exposed to the water acting as a barrier to prevent wetting.

An additional way of increasing the lifetime of membrane contactors used for SHWW treatment is incorporating a cleaning mechanism to remove any surfactants. Chen *et al.*[79] demonstrated that cleaning membranes with water and hexane to remove foulants allowed for the membrane contactors to operate with increased fluxes. A caustic cleaning can be introduced to clean membrane materials which have been fouled by organic foulants.[84] A caustic cleaning using an alkaline solution (such as NaOH) has been readily adopted as it allows for hydrolysis and solubilization reactions between the NaOH surfactant and foulants which allow the foulants to be easily washed away.[85] A study by Rudolph *et al.*[86] suggested the alternative use of enzymes to treat fouled membrane materials as caustic NaOH is carried out under harsh conditions which causes membrane aging and impacts on the membrane flux. However, the results showed that the use of enzymes did not positively impact on the flux recovery and SEM images showed that the use of enzymes did not clean the membrane as efficiently as NaOH.

3.3 Membrane studies

A number of investigations have been carried out in order to determine the efficiency of membrane systems for removing NH_3 from challenging WW streams (summarised in Table 3). Lab-scale studies by Guo *et al.*[87] and Zhang *et al.*[88] demonstrated that NH_3 could be removed from municipal WW and synthetic urea using direct contact membrane distillation. Guo *et al.*[87] used a PVDF membrane with a mixture of Nafion isomer and multiwall carbon nanotubes with a honey comb surface to remove NH_3 from municipal WW from Hong Kong. The results suggested that the hollow fibre Nafion isomer and multiwell carbon nanotubes support layer allowed for a greater NH_3 removal of up to 3 times greater than conventional PVDF membranes. Although this study showed high NH_3 recovery (69%) it did not produce a product from the recovered permeate. Additionally, it was observed that higher fluxes were experienced by the conventional PVDF membrane after 12 hours due to membrane fouling and blocking but the Nafion coated PVDF membrane could last up to 24 hours as Nafion is more resistant to fouling. Zhang *et al.*[88] made a mixture of synthetic human urine and showed that the 80% of the high NH_3 levels could be captured using PP membrane contactors.

Table 2 Characteristics of superhydrophobic membranes fabricated to facilitate challenging matrices similar to SHWW[74,79,81-83]

Membrane material	Superhydrophobic component	Fabrication method	Contact angle
PTFE	Hydrophilic coating	Electrospinning	150°
PTFE	Omni phobic surface	Electrospinning	151°
PTFE	$AgNO_3$	Electrospinning	158°
PVDF	Perfluoropolyether on hydrophilic substrate	Electrospinning	150°
PTFE	TiO_2	Electrospinning	150°

Table 3 Overview of studies which used membrane contactors for recovery of NH_3 from different WW sources (MD = membrane distillation)

Matrix	Scale	Membrane material	Membrane configuration	NH_3 recovery (%)	Product generated	Problems	Ref.
Municipal WW	Lab-scale	PVDF	Direct contact MD	69	—	Membrane fouling	(87)
Synthetic urine	Lab-scale	PP	Direct contact MD	80	$(NH_4)_2SO_4$	—	(88)
Synthetic urine	Lab-scale	PVDF	Direct contact MD	82	Ammonium-based fertiliser	—	(69)
Petroleum refinery WW	Lab-scale	PES	Sweep gas MD	93	—	—	(89)
Swine manure	Pilot-scale	PTFE	Hollow-fibre MD (lumen = acid)	94	No direct product	—	(90)
Anaerobic digester effluent	Pilot-scale	—	Hollow-fibre MD (lumen = acid)	70	—	—	(91)
Radioactive waste	Pilot-scale	PTFE	Hollow-fibre MD	90		Membrane fouling	(52)
Coking plant WW	Pilot-scale	PTFE	Hollow-fibre MD (lumen = acid)	50	Ammonium-based fertiliser	Membrane fouling	(93)
Rendering condensate WW	Pilot-scale	PTFE	Direct contact MD	64	Ammonium-based fertiliser	Membrane fouling	(13)

This high recovery of NH_3 allows for the production of $(NH_4)_2SO_4$ if there is a sufficient volume of ions in the permeate side. This study also demonstrated the rejection of other compounds including PO_4^{3-} and K^+ allowing for the permeate to produce a more pure product. The membrane material stability was affected in terms of their hydrophobicity, mechanical and pore properties by the synthetic urine samples. However, this study used synthetic urine and more complications may occur when using real samples. Damtie *et al.*[69] also carried out a study using synthetic urine to recover NH_4-based fertilisers using direct contact membrane distillation. This study produced a more complex synthetic urine matrix (compared to the study by Zhang *et al.*[88]) and it compared different permeate solutions (H_2SO_4, nitric acid, phosphoric acid, blend of all acids and water). The results showed that any low pH acidic solution can be used on the permeate side and their selection depends merely on the purpose for which the feed ammonium based product is to be utilised for. H_2SO_4 showed to recover the greatest quantity of NH_3 by removing 82% of NH_3 from the feed solution – however, this study does not report the purity of the product which is critical to determine product efficiency compared to other products on the market. Additional lab-scale studies were carried out by Ratman *et al.*[89] who investigated the use of PES membranes to remove NH_3 from petroleum refinery WW which has a high oil content. The results from this study showed an increase in NH_3 when the membrane materials was treated with UV irradiation. Additionally, treatment of the membrane material showed to increase the lifetime of the membrane as this matrix tends to degrade the membrane material. Disadvantages of using PES material include the fact it is a hydrophilic material which requires pre-treatment before being used with water samples. This study was carried out using sweep gas membrane distillation which showed efficient removal rates but did not allow for the recovery of a viable product.

Various pilot-scale studies using direct contact membrane distillation and hollow fibre treatment have been carried out on challenging WW types. Garcia-González and Vanotti[90] used PTFE hollow fibre membranes (acid on the lumen side) to treat manure waste and achieved 94% NH_3 removal as seen in Table 3. The authors stated that due to membrane materials being so selective, they remove the NH_3 which allows for greater quality methane gas production which leads to the production of biogas. Wäeger-Baumann and Fuchs[91] used hollow fiber membranes (acid on the lumen side) on anaerobic digester effluent and recovered 70% of the NH_3. Whilst this study achieves high removal rates, it does not meet effluent regulations and as such can't be the sole method of NH_3 removal. This study investigated the importance of the feed pH and temperature and it was determined that the pH must be greater than 10 to have a positive influence while the temperature was investigated between 20 and 40 °C and showed to have moderate impact. However, if lower temperatures allow for satisfactory NH_3 removal, they should be used in order to reduce energy costs. A more recent study by Vecino *et al.*[92] showed that liquid–liquid membranes could be incorporated to treat WW from Barcelona and recover the high ammonia concentrations (up to 4000 mg L^{-1}) and reduce them by 85% using nitric acid. However, the quality of this product was quantified at 5–10% (w/w) after liquid–liquid membrane recovery but was increased to 15.6% once the recovered product was concentrated using electrodialysis with the optimum conditions allowing for a high concentrated nitrogen fertiliser for soils. Additional studies by Liu *et al.*[52] showed that 90.4% NH_3 could be removed from radioactive waste using hollow fibre membranes and that the pH of the feed increased the rate of NH_3 removal. This study demonstrated high removal rates, but membrane fouling persisted to be a problem which highlights the need for more robust membranes to be produced for challenging WW. Lin *et al.*[93] showed that PTFE membrane performed better than PP

membranes to remove NH_3 from coking plant wastewater. The membranes allowed for 50% reduction (from 628 mg L^{-1} to 300 mg L^{-1}) using hollow fibre membranes (acid on lumen side) which would allow require further biological treatment. However, the costs of the biological treatment would be significantly reduced. Additionally, Brennan *et al.*[13] also showed that PTFE membrane contactors performed better than PP membranes. However, this study was in a direct contact membrane distillation suggesting that this membrane configuration allowed for higher efficiency for PTFE membranes. This study looked at the feasibility of membrane contactors to remove NH_3 from RCWW while producing a viable fertiliser which aided in reducing the costs of conventional treatment. Whilst this study demonstrated that a fertilizer product could be generated which reduced the costs of WW treatment, it did not efficiently reduce NH_3 levels which would require further treatment. These two studies demonstrate that PP membranes are not robust and fouling is easily caused by challenging WW.

4. Nitrogen based by-products from slaughterhouse wastewater

The high levels of nitrogen present in SHWW represent a matrix which can be highly utilized to produce viable by-products. Catarino *et al.*[94] outlined a business eco-efficiency model which involves the act of reusing waste produced in a company in order to generate a viable product using less natural resources and producing less waste. The recovery of nitrogen from SHWW would contribute to plant earnings whilst also reducing the cost of SHWW treatment.[30] The use of nitrogen from SHWW to produce viable by-products has, however, received limited attention due to regulations set out by governing bodies. EU legislation does not allow for by-products to be produced from SHWW unless it is treated at 70 °C for 60 minutes.[95,96] However, after the processing of animal parts in the rendering process, WW has undergone the required treatment and could be used to produce by-products such as fertilisers or NH_3 supplements.[8,93,97] Additionally, various studies have shown that NH_4-salts recovered using hydrophobic membranes can be used to produce many viable by products including; agricultural fertiliser, flame resistant products, food additives, wood preservatives, aid in protein purification and ammunition.[98–101]

4.1 $(NH_4)_2SO_4$ based fertiliser products

There is a high demand for nitrogen based fertilisers in the agriculture sector which is evident as the total NH_3 produced worldwide each year, 85% is consumed as fertiliser.[5,71] Artificial fertilisers can be produced by recovering nutrients from WW which result in economic, environmental and energy consumption benefits.[23] As SHWW has high levels of NH_3, it has been identified as a suitable source of fertiliser production (Fig. 1). Conventionally, the Haber–Bosch method is used to produce ammonia-based fertilisers but developments in research allow for the production of a more efficient NH_3-based fertiliser. Recently, a study by Lazouski *et al.*[102] showed that gas diffusion electrodes could be used to split the hydrogen molecules from water to react with nitrogen to form NH_3 fertiliser which has great potential to be applied to SHWW. Additionally, the use of membrane contactor processes have shown recovery of NH_3 to produce $(NH_4)_2SO_4$ fertilisers.[13,103] Hydrophobic membrane contactors with chemical reaction on the permeate side (to provide the driving force for mass transfer across the membrane) allow up to 96% recovery of NH_3, using sulfuric acid to produce a high value commercial $(NH_4)_2SO_4$ fertiliser.[15,74] The use of artificial fertilisers produced from slaughterhouse WW showed 70% higher yield in crop growth in terms of plant height and also showed that soil was much more nutrient dense after being dosed with the artificial fertiliser.[12] The use of this fertiliser can be applied to promote the growth of leaves, develop strong roots, cures plant and vegetables of chlorosis and cures leaf yellowing.[16] $(NH_4)_2SO_4$ fertilisers can be applied to land as either solid or liquid products and the summary of each method is outlined in Table 4. A study by Brennan *et al.* produced a 30% $(NH_4)_2SO_4$ liquid product from rendering condensate wastewater which has a similar composition to SHWW. The produced fertiliser was cheap to produce (€2.48 per kg of nitrogen) and after pH adjusting, it was suitable to be applied to large areas of land to promote growth. The methods of applying the liquid fertiliser can be carried out by controlled uptake long term ammonium nutrition (CULTAN) and contactless injection. CULTAN fertilization is a method of directly injecting the recovered nutrients into the ground in desired spots. The method allows for the distribution of the fertiliser to be even and increase the contact between fertiliser and plant roots.[97] The use of this direct contact fertilization method showed higher crop yield when compared with other fertiliser deployment

Table 4 Comparison of solid and liquid $(NH_4)_2SO_4$ fertiliser

	Solid product	Liquid product
Purity	99.90%	40%
Application methods	Mixing granules with compost; dissolving the product and applying the soluble product to the surface of the plant; applying the crystal form to soil and watering it into the soil	Injecting into the soil by manual injecting fertilization (CULTAN); contactless high pressure jet injection
Cost of product	€7.50 per kg	€0.20 per kg

types with the same concentration of nitrogen.[97] However, disadvantages include possible soil contamination of excess nitrogen or sulfate and the CULTAN fertilization method still has to be optimised to allow for longer operation times due to soils clogging injection ports.[2] The use of contactless injection was trialled by Niemoeller *et al.*[104] and the results showed injection depths of 70–90 mm were reached which meets the requirements to ensure optimal fertilisation and crop growth. This method also had the benefit of injection pores not getting clogged.

4.2 Other $(NH_4)_2SO_4$ based products

$(NH_4)_2SO_4$ can be added to flame retardant products in order to increase their combustion temperature of the material, decrease the weight loss rate and cause an increase in the production of residue or char.[105] The use of $(NH_4)_2SO_4$ allows for an increased fire resistance as it loses its constitute elements which can burn to NH_3, SO_2, N_2 and H_2O.[106] A study by Hshieh and Beeson[99] showed that applying ammonium sulfate to cotton fabrics increased the ignition time, residue yield and also decreased the amount of lost product. Additionally, Mostashari and Mostashari[106] showed that applying $(NH_4)_2SO_4$ to the material increased the fire resistance and was ranked as the best additive in reducing flame spread in fabrics and also in wood meaning it could be used as a suitable fire blanket, fire door *etc.*[107] The use of $(NH_4)_2SO_4$ has been a common additive to regulate the acidity of foods in the United States and has only become acceptable in the European Union since a report published in 2019 deemed concentrations under 300 mg per day safe.[98] The addition of $(NH_4)_2SO_4$ allows for an increased shelf life and strengthens the dough of the bread which allows the manufacturer to produce breads with longer lives. However, because this is being used for food, the product recovered from slaughterhouses may not be suitable under the EU law which states that WW products cannot be used for human food production.[108,109] Lastly, the use of $(NH_4)_2SO_4$ can be used to purify proteins through ammonium sulfate precipitation. The method can allow for purification of proteins, folding and stabilizing protein structures, and concentrating proteins together in a solution.[101]

4.3 Other NH_4-salt based products

Although H_2SO_4 is the most commonly studied acid to recover NH_3 using hydrophobic membranes – the use of nitric, hydrochloric and phosphoric acid have also been investigated in order to produce NH_4-nitrate, NH_4-chloride and NH_4-phosphate, respectively.[69] NH_4-Nitrate has been used extensively as a highly rich nitrogen fertilizer and also as a component of explosives.[110] NH_4-Nitrate fertilizer contains 33% nitrogen allowing it to be a nitrogen rich fertiliser, but it was reported by Hecnar *et al.*[111] that plants fertilized using NH_4-nitrate resulted in toxic conditions and were of harm to humans. Additionally, the storage of NH_4-nitrate is dangerous as large volumes can be easily ignited resulting in severe damages as reported by Oxiey *et al.*[112] Recently, 200–300 tons of NH_4-nitrate fertilizer were stored inadequately in a warehouse in Biuret, Lebanon which resulted in an explosion which devastated the city in a 3 km radius and killed 190 people.[113] NH_4-Chloride is also used as a fertilizer and is most commonly used for rice crops in Asia.[110] In addition, NH_4-chloride has uses in food production as an acidifier, cough medicine and to coat metals as a preservation.[114] Lastly, NH_4-phosphate can be used as a stripping solution but due to the instability of the product, no viable product of commercial value has so far been produced.[115]

5. Future perspectives

Slaughterhouses worldwide are processing more animals which is leading to higher volumes of wastewater rich in nitrogen which will damage the environment if not treated efficiently. The use of membrane contactor has shown to successfully recover nitrogen in the form of NH_3 from different matrices and reduce the nitrogen content in effluents. Although membrane contactors have shown to be successful in treating a variety of wastewaters, matrices as challenging as SHWW remain difficult to treat. As such, more research and advancements are required in order to make this an efficient treatment method which will allow for the recovery of NH_4-based products allowing for revenue generation which will reduce capital and operation costs. It is recommended that further research should focus on pre-treatment methods and membrane cleaning protocols. SHWW shows to have high concentrations of OM and fats which cause wetting of membranes. If such surfactants are removed before the SHWW is exposed to membranes, it may allow for the successful implementation of the technology. Additionally, the incorporation of a caustic cleaning has shown to be effective in cleaning membranes and reviving them to treat different matrices. The development of an effective caustic cleaning protocol and how it can be incorporated into the membrane contactor experimental plan to allow for periodic cleaning may increase the lifetime of membrane materials.

6. Conclusion

A comprehensive review on the potential of producing a viable product from slaughterhouse wastewater using a membrane contactor type operation is presented. The sources of nitrogen throughout the slaughterhouse were reviewed and it was determined that rendering of the animal parts produces the highest levels of nitrogen (levels in excess of 2000 mg L^{-1}) whilst reports also suggesting levels at the slaughtering floor, holding yards and from the offal & paunch. Ammonium-sulfate was identified to aid in agricultural fertiliser production, food additives, flame retardant materials and protein purification. The recovery of nitrogen in the form of ammonium sulfate has been

identified to produce viable products which reduce the cost of water treatment and possibly generate revenue for the company. The use of membrane contactor process with acid sweep was discussed and the effects of slaughterhouse wastewater on the membrane material was examined by comparing to by other authors who used membranes to treat other challenging waters. The use of membrane contactors provides great potential for nitrogen recovery, but further research is needed in order to fabricate membranes suitable for treatment of slaughterhouse wastewater which is a challenging matrix due to its high oil and protein content. This review critically analyses the composition of slaughterhouse wastewater from around the world and shows the high concentrations of contaminants including nitrogen which require extensive treatment before being released into receiving waters. However, this review highlights the strong potential to recover nitrogen and simultaneously reduce nitrogen levels in the wastewater and produce a viable product. Various products were identified including fertilizers, flame retardant products and food additives. The data and information presented in this review suggest there is great potential for viable products to be produced from the nitrogen content in SHWW using hydrophobic membranes. However, it can't be ignored that the potential of membrane wetting due to SHWW surfactants is a limiting factor which must be addressed in future studies.

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Conflicts of interest

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Highlighting extraction and derivatization method comparisons for optimal sample preparation of *Nannochloropsis* sp. algal oils prior to FAME determination†

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Microwave assisted extraction derivatization (MAED) was investigated for preparing fatty acid methyl ester (FAME) derivatives to analyse the fatty acid (FA) composition of *Nannochloropsis* sp. algae. This method was compared against the conventional methods of oven assisted derivatization and direct derivatization without prior extraction. The derivatized FAMEs were analysed using GC-MS to identify and quantify the FAs present. Conventional methods have been carried out for years, but they are time consuming and require high temperatures and harmful chemicals. The MAED approach developed is 5 times (15 min) faster compared to the conventional and direct derivatization methods (75 min) optimised for FA analysis of *Nannochloropsis* sp. algae. The MAED method recovered significantly more FAs (51 wt%) from algal samples compared to conventional (42 wt%) and direct derivatization (34 wt%). Additionally it was shown that a sample extraction step is critical to improving the derivatization yield of algal samples for both oven assisted and MAED techniques. Fourteen FA components were identified in *Nannochloropsis* sp. algal samples and were evaluated for their potential use for biodiesel and biopharmaceutical products. The developed MAED method allows for a rapid, robust and accurate preparation of algal samples in order to determine their FA composition.

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1 Introduction

Nannochloropsis sp. algae are complex organisms which have become of interest in recent years due to their composition, especially the lipids present. Fatty acids (FA) are the largest component of lipids and the physical, chemical and physiological properties of a lipid class depend on its FA class.[1] The lipids and FAs present in algae have gained increasing interest due to their potential to contribute to the production of biodiesel and pharmaceuticals.[2] Biodiesel made from FAs and lipids present in bio-products such as algae can offer a more sustainable and green method to provide energy.[3] The benefits of using algae for biodiesel production include their ability to grow in fresh water or marine environments without the need for arable land.[4] Algae have also been utilized for treating sewage waste[5] along with providing a means for the production of polyunsaturated FAs (PUFAs), pigments, antioxidants, pharmaceuticals, biomass for animal feed, fertilizers and energy crops.[6]

In order for FA composition to be identified and quantified by GC-MS, they must be converted to fatty acid methyl esters (FAMEs).[7] Conversion of FAs to FAMEs increases the volatility and thermal stability of FAs by preventing decarboxylation of the acid group during injection; additionally, the elimination of the free acid improves GC peak shapes and detection of FAs by GC.[7–9] Sample preparation prior to GC-MS requires a series of drying, hydrolysis, extraction, purification, transesterification and analysis steps,[7] and the most common procedure used for FA sample preparation and analysis was developed by Bligh and Dyer.[10] Extraction is the first step in the analysis of FAs present in algae as it allows isolation of the FA and triglyceride components present in the sample.[11] The extraction step eliminates undesired compounds such as proteins, carbohydrates and salts which could interfere with derivatization.[11] After extraction, the triglycerides and FAs are converted into FAMEs by derivatization. This is most commonly carried out by hydrolysing the triglycerides in the presence of a strong acid or base (such as sodium or potassium hydroxide[12]) in order to break up the triglycerides. The sample then undergoes transesterification in the presence of an acid or base catalyst, such as boron trifluoride (BF_3), with an alcohol (typically methanol or ethanol) to form the desired FAME

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derivatives.[13,14] The FAMEs are then extracted into an organic solvent to allow for analysis and quantification by GC-MS.[8] FA recovery efficiency using the Bligh–Dyer method was shown to be up to 98%.[15] The quality of the derivatization can also be assessed by using thin layer chromatography as demonstrated by Gehrke *et al.* but this does not quantify the amounts of FAMEs present.[16] Conventional derivatization methods have proven to be effective; however, they have a number of disadvantages. Typically, these methods require high temperatures, are time consuming and the multiple steps required for sample preparation increase the probability of errors.[17] As the interest in bio-products derived from algae has increased in recent years, it has become important to develop a quick and reliable method for FAME analysis.

There have been a number of investigations evaluating simplified extraction and derivatization procedures for FAME preparation on a range of different sample types.[18] Microwave irradiation is a well-developed method that has been used extensively in organic synthesis, derivatization and extraction.[12] There are a number of advantages to microwave assisted extraction and derivatization (MAED) as a method to prepare FAMEs from lipid containing samples, such as ease of operation, lower energy consumption, shorter reaction times and faster heating provided by the apparatus.[12] Another benefit to MAED is that it allows for more uniform thermal transfer and ensures that all the samples are heated up equally unlike a heating block which heats sample vials from the walls/bottom.[12] Liu *et al.* used MAED to investigate a one-step method for FAME analysis of herbal medicines.[12] Their results showed that MAED was more energy efficient, reduced sample preparation time and improved extraction and derivatization efficiency compared with conventional methods. Similarly, Brunton *et al.* employed MAED for the determination of FAs in food and found it to be rapid and simple and it provided superior recoveries compared to conventional methods.[8] A study by Aliev *et al.* looked at the use of supercritical fluid extraction for preparing FAMEs from algae.[5] This method had the advantage of being a high speed, low temperature and oxygen free sample preparation procedure.[5] However, the apparatus and process required are complex compared to conventional or MAED methods.

There has been an increase in the use of MAED for preparing FAMEs in recent years and with that, an efficient method should be developed for the analysis of algae. An ideal method should be quick, simple, precise and accurate.[17] This study focuses on developing a quick, simple and efficient MAED method for *Nannochloropsis* sp. and compares it with conventional derivatization methods, such as the method reported by Bligh and Dyer.[10] This study also investigates the optimum extraction and compares the conventional method to direct derivatization techniques without prior extraction prior to improving derivatization speed and workflow.

2 Experimental

2.1 Materials

Nannochloropsis sp. algae were provided by Archimede Ricerche S.L. (Imperia, Italy). The algal samples were stored at 4 °C in glass vials. Solvents including isopropanol (IPA), ethanol, hexane, cyclohexane, acetic acid and toluene were procured from Sigma Aldrich, Ireland. Acetone and methanol (MeOH) were obtained from Honeywell, Ireland. Chloroform was procured from Fisher Scientific, Ireland. The internal standard, pentadecanoic acid ($C_{15:0}$), a 37 component FAME standard and derivatization reagent boron trifluoride (BF_3) (10% in methanol) were procured from Sigma Aldrich, Ireland. All other chemicals and reagents including sodium hydroxide (NaOH), potassium hydroxide (KOH), sulfuric acid (H_2SO_4), hydrochloric acid (HCl), sodium chloride (NaCl) and sodium carbonate (Na_2CO_3) were obtained from Sigma Aldrich, Ireland. All chemicals used in this investigation were of analytical grade or better. Deionized (DI) water was obtained from an Elga Purelab Ultra system.

2.2 Optimisation of methods

Three methods were investigated to find the optimum procedure to extract and derivatize FAs present in algae. The FA analysis methods investigated include a conventional extraction and derivatization procedure, a direct derivatization procedure which received no extraction prior to derivatization,[19] and a microwave assisted extraction derivatization procedure. All optimization experiments were carried out in triplicate ($n = 3$). Investigations were performed as per the flow chart in Fig. 1. The peak area responses and the number of FAME peaks detected were used to determine which conditions were optimal. Each procedure parameter (*e.g.* extraction solvent, fractionation solvent, hydrolysis reagent, hydrolysis reagent volume, transesterification reagent volume, hydrolysis reaction time and transesterification reaction time for the conventional method) was sequentially optimised and the best condition from each step was then carried forward to subsequent optimization experiments (Fig. 1). Throughout the methods section, optimum conditions have been bolded.

2.2.1 Optimisation of the conventional extraction. The conventional method of extraction was modified from that outlined by Ostermann *et al.*[13] The extraction solvent (acetic acid, acetone, ethanol, IPA, and methanol) and the fractionation solvent (chloroform, cyclohexane, hexane and toluene) were varied (Fig. 1). The sample was extracted by weighing out 2 mg of the algal sample and dissolving it in 30 μL of the extraction solvent and then spiking it with 1 mL of 3.1 mg L^{-1} internal standard in MeOH. 15 μL of the fractionation solvent was added and the sample was then shaken for 4 min (Clifton 62551). A 500 μL volume of DI water and 500 μL fractionation solvent were added to separate the organic and inorganic layers which was aided by shaking the sample for 2 min and centrifugation (Eppendorf centrifuge, model 5804) for 5 min at 3500 × *g*. The aqueous layer was collected and extracted again using the same procedure. The organic layers were then combined and evaporated with nitrogen gas to dryness. This dried extract was then derivatized as follows.

2.2.2 Optimisation of the conventional derivatization procedure. The dried algal sample extract was mixed with a volume (0.5, 1.0, 1.5 or 2 mL) of 0.5 mol L^{-1} hydrolysis reagent (NaOH, KOH, Na_2CO_3, HCl or H_2SO_4) and hydrolysed in an oven

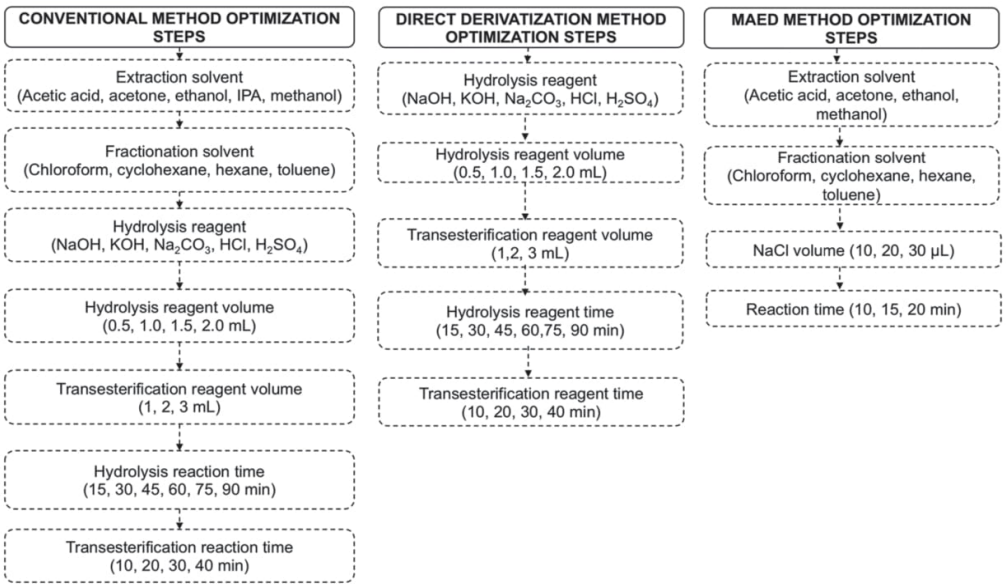

Fig. 1 Order of experiments to determine the optimum parameters for the 3 different methods of extraction and derivatization investigated.

at 80 °C (Technico TEC-240-010S) for a range of reaction times (15, 30, 45, 60, 75 and 90 min). Transesterification was performed at 100 °C using 10% BF_3 in MeOH, and the amount of BF_3 was optimized (1, 2 and 3 mL) in addition to the reaction time (10, 20, 30 or 40 min). Once transesterified, 2 mL of DI water and 1 mL hexane were added to the sample and it was shaken for 4 min and centrifuged at 3500 × *g* for 5 min. The hexane layer was then collected and analysed by GC-MS.

2.2.3 Optimisation of direct derivatization. The optimisation of the direct derivatization method was carried out as outlined in Section 2.2.2 without performing the initial extraction step (Section 2.1.1). Briefly, 2 mg of the algal sample was spiked with 1 mL of 3.1 mg L^{-1} internal standard and then derivatized as per Section 2.2.2. The order in which this investigation was carried out is outlined in Fig. 1.

2.2.4 Optimisation of the MAED method. A 2 mg quantity of algal sample was added to a glass vial (Borosilicate glass with a Teflon cap). 10 µL of the extraction solvent (acetic acid, acetone, ethanol, IPA, and methanol), 30 µL of the fractionation solvent (chloroform, cyclohexane, hexane and toluene) and 1 mL of 3.1 mg L^{-1} internal standard were added to the vial. 1 mg of solid NaOH was added to the solution. The samples were placed in a microwave reactor (Anton Parr, Monowave 300 Ireland) operated at a power of 435 W and a temperature of 45 °C, for three reaction times (10, 15 and 20 min). Following the reaction, different volumes (10, 20 and 30 µL) of 1 mol L^{-1} NaCl were added and the sample was left to cool for 1 min. A volume of 1 mL of the fractionation solvent and 1 mL of water were added. The organic layers were then collected and analysed by GC-MS.

2.3 GC-MS analysis

The FAMEs were separated and analysed using an Agilent 6890 GC connected to a single quadrupole mass spectrometer Agilent 5973 network MSD. The GC-MS was equipped with a highly polar Agilent VF-23ms GC column (30 m, 0.25 mm I.D., 0.25 µm film thickness). GC-MS separation was based on the work of Khoomrung *et al.*[17] A 1 µL volume of the sample was injected with a 20 : 1 split ratio into an injector set to a temperature of 250 °C. Helium was used as the carrier gas at a constant flow rate of 1.0 mL min^{-1}. The initial column temperature was 50 °C for 1.5 min, and it was then increased to 180 °C at a rate of 25 °C min^{-1}; once it reached 180 °C, it was increased to 220 °C (10 °C min^{-1}) and held at this temperature for 1 min. It was then increased to 250 °C (15 °C min^{-1}) and held for 3 min. The FAMEs were detected with electron ionization (70 eV) in scan mode with a *m*/*z* of 50 to 500 AMU. The identification of unknown FAMEs was achieved by comparing their retention times and mass spectrum profiles with known standards and the NIST97 mass spectral library. The quantification of FAMEs was performed based on calibration with a 37 component FAME standard mixture.

2.4 Statistical analysis

Statistical analysis was carried out to test whether differences between the peak number and peak responses were significantly different between the evaluated conditions. One-way analysis of variance (ANOVA) was performed ($p < 0.05$) and followed up with *post hoc* Tukey tests where appropriate using Origin software (version 9.0). Statistical analysis was applied to each optimisation step to evaluate which conditions provided the optimal recovery of FAs from algal samples.

3 Results and discussion

3.1 Standard identification and quantification

A 37 component FAME standard mix was used to determine the optimum GC-MS instrument conditions and to identify and quantify peaks in the algal sample. The method used showed that FAME peaks started eluting at a retention time of 4.73 min ($C_{5:0}$ butyrate acid methyl ester) and the last FAME present eluted at 54.87 min ($C_{24:1\omega9}$, nervonic acid methyl ester). Table 1 shows the retention time for each of the FAMEs in the standard mix; these times and the corresponding mass spectra were then used for the identification of FAMEs obtained from the algal samples. The chromatogram and retention times produced from the FAME mix are in agreement with studies carried out on a similar FAME standard mix by Benjamin *et al.*[15] Chromatograms of the optimized extraction conditions are shown in Fig. 2.

3.2 Optimisation of the conventional method

As outlined in the methods section, there are two steps in the conventional method: the extraction step and the derivatisation step. Both steps were optimised for a range of conditions including the solvents, reaction times and catalysts used.

3.2.1 Optimisation of the conventional extraction method. An investigation of solvents used to extract and fractionate the FA from the algal samples was carried out using acetic acid, acetone, ethanol, IPA and MeOH. Chloroform, cyclohexane, hexane and toluene were used for fractionation. Acetone was found to recover the highest amount of FAMEs in terms of both the greatest number of FAME peaks and highest total peak area of 14 ± 0 and $7.6 \times 10^8 \pm 1.5 \times 10^5$, compared to the other conditions evaluated (see the ESI† Section 3.1). A study by Ren *et al.* found that the greatest FA recovery was achieved by using acetone as the extraction solvent with a yield of 72.3% compared to methanol (35.8%) and dichloromethane (60.3%).[20]

Table 1 37 standard FAME mix with the carbon number and retention time (RT)

FAME	RT (min)	$C_{18:2\omega6t}$	43.09
$C_{5:0}$	4.73	$C_{18:2\omega6}$	43.72
$C_{7:0}$	7.42	$C_{18:3\omega6}$	44.65
$C_{8:0}$	13.50	$C_{18:3\omega3}$	45.39
$C_{10:0}$	20.11	$C_{20:0}$	45.69
$C_{11:0}$	23.27	$C_{20:1\omega9}$	46.45
$C_{12:0}$	26.30	$C_{20:2\omega6}$	47.65
$C_{13:0}$	29.17	$C_{20:3\omega6}$	47.82
$C_{14:0}$	31.90	$C_{20:3\omega3}$	48.69
$C_{14:1\omega5}$	33.26	$C_{20:4\omega6}$	49.28
$C_{15:0}$	34.48	$C_{20:5\omega3}$	49.40
$C_{15:1}$	35.81	$C_{21:0}$	49.51
$C_{16:0}$	36.95	$C_{22:0}$	50.27
$C_{16:1\omega7}$	37.95	$C_{22:1\omega9}$	50.88
$C_{17:0}$	39.29	$C_{22:2\omega6}$	51.30
$C_{18:1\omega7}$	40.27	$C_{22:6\omega3}$	51.59
$C_{18:0}$	41.53	$C_{23:0}$	53.02
$C_{18:1\omega9t}$	42.03	$C_{24:0}$	53.79
$C_{18:1\omega9}$	42.30	$C_{24:1\omega9}$	55.19

The other solvents (acetic acid, ethanol, IPA and methanol) investigated produced 12, 13, 13 and 13 FAME peaks respectively. The Tukey test (ESI 4.1†) showed that acetone was significantly better than the other conditions. The optimum fractionation solvent according to the FAME peak number and peak area was hexane which produced 14 ± 0 FAME peaks and a total FAME peak area of $1.1 \times 10^9 \pm 4.3 \times 10^6$. Chloroform, cyclohexane and toluene all recovered lower levels of FAME with 12, 7 and 0 FA peaks, respectively. Other studies have shown hexane to be the optimal solvent for fractionation and it has been used to maintain the solubility of lipids and FAs whilst extracting FAMEs following derivatization, which was supported by the present results.[17]

3.2.2 Optimisation of the conventional derivatization method

3.2.2.1 Selection of the alkali catalyst for hydrolysis and volume. The catalyst used for hydrolysis is a key factor in the derivatization performance as it promotes the breakdown of the sample from triglycerides to FAs.[12] NaOH, KOH, Na_2CO_3, HCl and H_2SO_4 were evaluated under the same conditions to determine the optimal catalyst. NaOH had the greatest yield of FAs when used as the hydrolysis reagent as it produced the largest number of peaks and peak area of 14 ± 0 and $1.7 \times 10^9 \pm 5.3 \times 10^6$, respectively. Statistical analysis indicated that this condition was significantly better than the other conditions (ESI 4.2†). Homogeneous alkaline catalysts have been shown to be superior to acidic and metallic oxides so it was expected that NaOH or KOH would be the superior catalyst.[12] A 0.5 mL volume of 1 mol L^{-1} NaOH provided the best recovery of FA and it was found that as the volume of NaOH increased, excess hydroxide caused reduced derivatization efficiency. A peak area of 5.5×10^8 and 14 peaks were achieved and this recovery was superior to that of the other catalysts evaluated at a 95% confidence level (see the ESI 4.2†).

3.2.2.2 Selection of the BF_3 volume. Optimization of the volume of BF_3 used for transesterification of FAs in algal oil samples was carried out with 1, 2 and 3 mL increases. Transesterification converts FAs and triglycerides to methyl ester derivatives.[21] The results show that 2 mL of BF_3 was optimal to obtain the greatest recovery of FAs with 14 peaks produced and an average peak area of $1.6 \times 10^9 \pm 2.5 \times 10^7$ compared to the other conditions (ESI 4.1†). Increasing the volume of BF_3 did not yield better FAME recoveries and using lower volumes decreases the cost of FA derivatization. Similar scaled studies by Khoomrung *et al.* also found that 2 mL of BF_3 is the optimal volume for the transesterification of FA containing samples.[17]

3.2.2.3 Selection of reaction times for hydrolysis and transesterification. The efficiency of hydrolysis and transesterification depends on the extraction of FAs from the algae into the solvents and reaction with the catalysts used (NaOH for hydrolysis & BF_3 for transesterification). The conversion of lipids to FAMES is determined by the reaction time and temperature, with longer times and higher temperatures typically increasing recoveries, although excessive heat and reaction durations can result in sample deterioration.[12] The optimal oven-based hydrolysis time was 45 min with 14 FA peaks produced and a FA peak area of $2.3 \times 10^8 \pm 2.0 \times 10^6$ (Table 2).

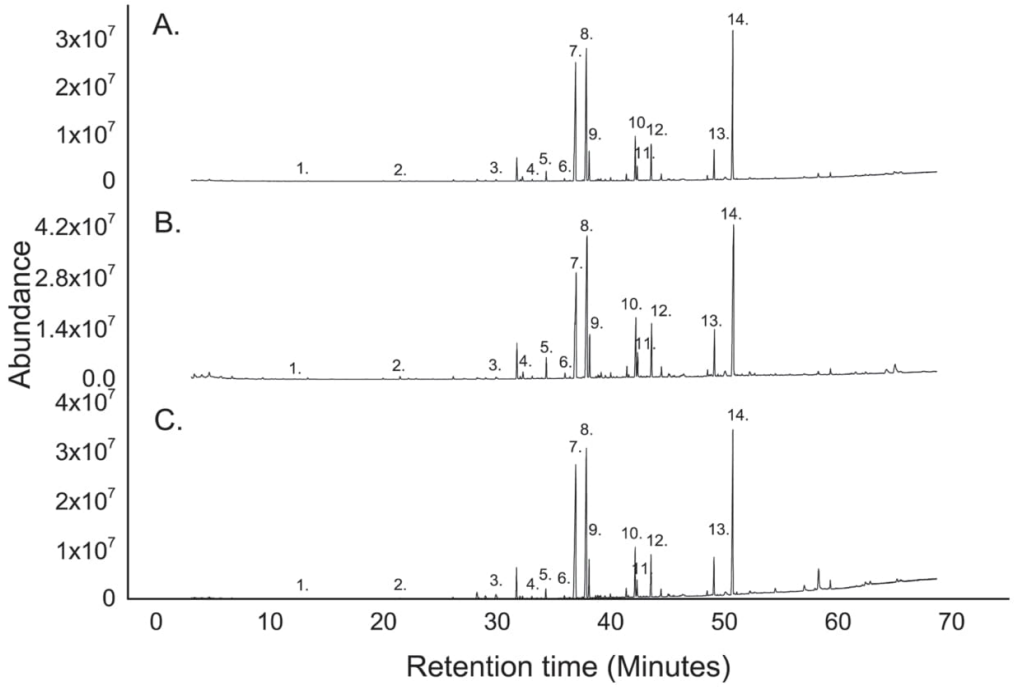

Fig. 2 GC-MS chromatogram of FAMEs for 3 different methods of sample preparation; A = direct method, B = MAED method and C = conventional derivatization method (1 = $C_{8:0}$, 2 = $C_{10:0}$, 3 = $C_{12:0}$, 4 = $C_{13:0}$, 5 = $C_{14:0}$, 6 = $C_{16:0}$, 7 = $C_{16:1\omega7t}$, 8 = $C_{16:1\omega7}$, 9 = $C_{18:0}$, 10 = $C_{18:1w9}$, 11 = $C_{18:1\omega7}$, 12 = $C_{18:2\omega6}$, 13 = $C_{20:4\omega6}$ and 14 = $C_{20:5\omega3}$).

This 45 min condition provided significantly better recovery that the other durations evaluated (see the ESI 4.1†).The optimum transesterification time was 30 min with 14 FAME peaks produced and a total peak area of $2.4 \times 10^8 \pm 5.2 \times 10^5$, which were significantly better than those achieved with the 10, 20, and 40 min durations also tested (see the ESI 4.1†).

3.3 Optimisation of MAED

3.3.1 Selection of the best solvents and solvent volumes for MAED. The same solvents as those for the conventional and direct synthesis were investigated for the MAED method. Acetic acid extracted the most FAMEs from the algal oil using MAED as it produced 14 ± 0 FAME peaks and yielded a total peak area of $3.8 \times 10^8 \pm 1.3 \times 10^6$, compared to the other conditions, while acetone, which was the optimal solvent in the conventional method, performed significantly worse (10 times reduction in the total peak area) when used with MAED (ESI 4.3†). No studies have been carried out on the fatty acid content of *Nannochloropsis* sp. algal plants using MAED; however, there are a variety of studies using MAED for FA derivatization on other samples which have found the best extraction solvents to be methanol and acetone for yeasts and herbal medicines.[17,20] Further analysis of the acetic acid solvent system was carried out to optimise the volume of the solvent and it was found that 10 μL extracted significantly more FAs from the algal sample

Table 2 Summary of optimised methods for the extraction and derivatization of algal samples

Parameter	Conventional method	Direct derivatization method	MAED
Extraction solvent volume	Acetone	—	Acetic acid
Fractionation solvent volume	Hexane	—	Chloroform
Hydrolysis reagent	NaOH		—
Hydrolysis solvent volume (mL)	0.5	0.5	—
Transesterification volume (mL)	2	2	—
Transesterification reaction time (min)	45	45	15
Hydrolysis reaction time (min)	30	30	—
NaCl volume (μL)	—	—	20

compared to the other conditions evaluated (see the ESI 4.3†). A number of studies report that hexane was the optimum fractionation solvent when combined with MAED.[8,12,17] The present investigation indicated that chloroform was superior to hexane and the other solvents, yielding 12 FAMEs and a total peak area of $2.6 \times 10^8 \pm 4.8 \times 10^5$, respectively.

3.3.2 Optimisation of reaction time for MAED and the volume of NaCl to quench derivatization. The optimum reaction time depends on the type and size of the sample. MAED can be up to 4 times faster than conventional oven based heating methods due to the ability of microwaves to efficiently heat samples and enhance derivatization reactions.[17] At 15 min reaction time, 14 peaks and a total FAME peak area of $3.1 \times 10^7 \pm 2.4 \times 10^4$ were achieved, which were significantly more than those achieved with the 10 and 20 min durations also evaluated (ESI 4.3†). The volume of NaCl used for quenching the derivatization was optimised and it was found that 20 μL NaCl was effective compared. The largest total peak area and 14 peaks were produced when using 20 μL NaCl, compared to only 12 peaks and decreased total peak areas delivered with 10 and 30 μL of NaCl (ESI 4.3†).

3.4 Statistical analysis comparing optimized methods

The FA composition of *Nannochloropsis* sp. algal samples was reported as a percentage mass of the total sample. 14 FAs were identified in the algal plant extract using each of the three preparation methods. The MAED method was the most effective at extracting and derivatizing FAs out of the three optimized methods, since it yielded the highest total peak area of FAMEs (Fig. 3), which was significantly higher than the amount of FAMEs obtained using conventional and direct derivatization (see ESI 4.4†). Table 3 summarises the FA composition determined by each of the methods. ESI 4.4† shows that MAED was significantly different from the other two methods for all FAs detected except $C_{8:0}$, $C_{16:1w7t}$ and $C_{20:4w6}$. Of the FAs for which MAED showed no significant difference, both the peak area and FA weight composition were smaller for the MAED method. The MAED method yielded a higher TFA (51.8 ± 0.5%) and saturated fatty acid (SFA) content (13.2 ± 1.3%) than those of the direct derivatization method (TFA 34.1 ± 0.4% and SFA 10.2 ± 1.1%) and the conventional method (TFA 42.6 ± 0.8% and SFA 12.8 ± 1.3%). It is also seen that for TFA and SFA, the conventional method showed greater derivatization efficiencies than the direct derivatisation method which confirms that the extraction and purification steps are required to improve the recovery of these FAs. MAED had the best repeatability with an RSD of 8.5% which was superior to that of the conventional method (11.2% RSD) and the direct derivatization method (10.7% RSD), (ESI 4.4†). Since the MAED method had the greatest average recovery for TFA, it was selected as the optimal method for the analysis of FAs in algal samples.

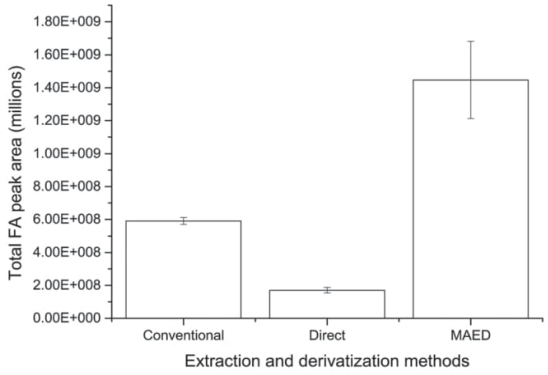

Fig. 3 Comparison of the total FA peak area produced by conventional, direct derivatization and MAED methods ($n = 3$).

Both monounsaturated FA (MUFA) and polyunsaturated FA (PUFA) recoveries were investigated by the 3 different methods. It was found that PUFAs had the highest yield of all FA types and MAED extracted and derivatized the sample for PUFAs the most efficiently with a yield of 35.0 ± 2.8% whilst the method with no extraction had quite a low yield of 18.7 ± 1.6% in comparison and the conventional method had a yield of 24.8 ± 3.1% (Table 3). Statistical analysis confirmed that the differences in recoveries between techniques were significant (ESI 4.4†). The better recovery of PUFAs when utilizing the MAED method may be due to the milder temperature used during derivatization which could minimise oxidation and of PUFAs compared with the other methods.[12] The MAED method also had the best precision of 8% while it was 8.4% for the direct derivatization and 12.4% for the conventional method. The differences in recoveries between the MAED, conventional and direct derivatisation methods highlight the importance of using low reaction temperatures and performing an initial extraction prior to derivatization. Interestingly, MAED had lower yields of MUFA (3.5 ± 0.2%) compared to the direct derivatization (5.6 ± 0.7%) and conventional method (4.9 ± 0.6%). ANOVA indicated that the direct derivatization method may be superior for FA recovery of MUFAs compared to the MAED and conventional methods (ESI 4.4†) in applications where targeted analysis of these species is the goal. Overall, the total unsaturated FA (TUFA) obtained from MAED (38.5 ± 2.9%) was significantly better (ESI 4.4†) than the direct derivatization (23.9 ± 2.6%) and conventional methods (29.7 ± 3.6%). The proportions of FA determined in the present algal samples match other studies evaluating FA composition in algae, for example, Sukenik *et al.* analysed FAs in similar *Nannochloropsis* sp. algae and detected FAs ranging in sizes from myristoleic acid ($C_{14:0}$) to arachidic acid ($C_{20:0}$) along with unsaturated and polyunsaturated variants of these fatty acids.[22]

The MAED method was found to be a superior method due to its high efficiencies in extracting and derivatizing the FA within algae samples. Furthermore, the MAED method is almost 4 times faster than the conventional and direct derivatization method similar to other MAED studies.[8] Additionally, incorporating an extraction step prior to derivatization recovered 8% more FA than direct derivatization methods, highlighting the importance of for maximising FA recoveries despite the additional time required to perform this step.

Table 3 FA composition (%) in algal plants obtained with 3 different extraction & derivatisation methods

	Direct synthesis ($n = 3$)	Conventional method ($n = 3$)	MAED method ($n = 3$)
FA	FA content (weight%, SD)	FA content (weight%, SD)	FA content (weight%, SD)
$C_{8:0}$	0.1 ± 0.01	0.04 ± 0.5	0.7 ± 1.0
$C_{10:0}$	0.04 ± 0.01	0.1 ± 0.1	0.1 ± 0.3
$C_{12:0}$	0.1 ± 0.03	0.2 ± 0.02	0.2 ± 0.02
$C_{13:0}$	1.2 ± 0.1	2.1 ± 0.1	2.1 ± 0.02
$C_{14:0}$	0.1 ± 1.4	0.4 ± 0.2	0.2 ± 0.1
$C_{16:0}$	8.3 ± 2.0	9.8 ± 2.5	9.2 ± 0.8
$C_{16:1\omega7t}$	9.8 ± 0.05	14.8 ± 1.9	10.2 ± 1.0
$C_{16:1\omega7}$	1.5 ± 1.2	1.9 ± 0.3	1.3 ± 1.3
$C_{18:0}$	0.5 ± 0.03	0.3 ± 0.1	0.9 ± 0.1
$C_{18:1\omega9}$	2.5 ± 0.2	2.2 ± 0.5	1.3 ± 0.1
$C_{18:1\omega7}$	1.2 ± 0.1	0.8 ± 0.1	0.9 ± 0.7
$C_{18:2\omega6}$	2.7 ± 0.3	2.8 ± 0.3	4.8 ± 0.3
$C_{20:4\omega6}$	1.2 ± 0.02	1.1 ± 1.2	1.4 ± 0.1
$C_{20:5\omega3}$	5.1 ± 0.3	6.2 ± 1.0	18.7 ± 1.3
∑TFA	34.1 ± 0.4	42.6 ± 0.6	51.8 ± 0.5
∑SFA	10.3 ± 1.1	12.8 ± 1.3	13.2 ± 1.3
∑MUFA	5.6 ± 0.7	4.9 ± 0.6	3.5 ± 0.2
∑PUFA	18.7 ± 1.6	24.8 ± 3.1	35.0 ± 2.8
∑TUFA	23.9 ± 2.6	29.7 ± 3.6	38.5 ± 2.9

3.5 Sample composition and potential for biodiesel applications

Biodiesel consists of FAMEs which are synthesised from triglycerides and FAs that are present in the algal plants.[3] The FAs that yield the greatest biofuel potential are C_{16} to C_{18} chain length FAs, in particular, palmitic ($C_{16:0}$), palmitoleic ($C_{16:1}$), heptadecanoic ($C_{17:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$) and α-linolenic acid ($C_{18:3}$).[23] Fuel potential is defined as the fraction of lipids that comprise the maximum amount of FAs which are capable of undergoing transesterification.[23] The FAMEs which were detected to be of the highest concentration in the algal sample according to their weight were $C_{16:0}$, $C_{16:1\omega7t}$, $C_{18:1\omega9}$ and $C_{18:2\omega6}$ at 9.2, 10.2, 1.3 and 4.8 wt%, respectively. These FAs are considered to be excellent FAs for biodiesel production.[3,23] The ideal molar ratio of $C_{16:1}$, $C_{18:1}$ and $C_{14:0}$ is 5 : 4 : 1 for the most efficient biodiesel according to Maghraby *et al.*[3] However, of these FAs, the ratio in the *Nannochloropsis* sp. algal sample analysed is 58 : 11 : 1 (with a FA content of 11.5, 2.2 and 0.2%, respectively). This ratio suggests that the present algal sample analysed may not be ideal for biodiesel production and requires blending with a myristic acid methyl ester source. A higher SFA content improves oxidative and thermal stability and ignition quality leading to biodiesel products with superior performance.[3]

The FA with the highest content is eicosapentaenoic acid ($C_{20:5\omega3}$) at 18.7 wt%. While this FA does not have a use in the production of biodiesel, it is useful in the treatment of diseases such as cardiovascular disease, diabetes, eye disorder, and arthritis and for control of cholesterol and blood pressure.[5] This indicates that this algal species may have the potential for pharmaceutical and personal applications. Arachidonate acid ($C_{20:4\omega6}$) was also present in the sample at low levels of 1.4%. Arachidonate acid is also an important FA for pre- and post-natal brain and retina development.[24] Other SFAs present in the algal sample at low levels include caprylic ($C_{8:0}$, 0.8%), capric ($C_{10:0}$, 0.1%), lauric ($C_{12:0}$, 0.2%) and tridecanoic ($C_{13:0}$, 2.1%) acid. It is uncommon to find these FAs in algal plants as algae usually contain FAs with a chain length between $C_{14:0}$ and $C_{24:0}$.[25] However, if these algal species were gathered in large volumes they could allow for the production of different materials. $C_{8:0}$ has been used for the production of medicine for patients suffering from pancreatic insufficiency and fat malabsorption.[26] $C_{10:0}$ has been used for the production of fruit flavours and perfumes.[27] $C_{12:0}$ is naturally found in coconut oil and milk and is used for the production of soaps and cosmetics.[28] It is unusual to find $C_{13:0}$ in algae as according Breuer *et al.*, algae typically produce FAs with even numbers of carbon atoms.[29] However, some studies have shown trace levels of $C_{13:0}$ to be present in different algal species.[30]

4 Conclusion

Three methods were optimized and evaluated for preparing FAMEs from algal samples, including conventional oven-based derivatization, direct derivatization without lipid pre-extraction and a microwave assisted derivatization method. The choice of reaction temperature, reaction time and extraction/fractionation solvents was critical to method performance as they influenced the amount of FA extracted and derivatized. The MAED method combined with acetic acid as an extractant and chloroform as a fractionation solvent provided the most efficient method for FA derivatization to FAMEs. FAs in algal samples were rapidly derivatized in 15 min compared to the conventional and direct derivatization methods which required

reaction times 3 times longer. Furthermore, the MAED method yielded the highest conversion of FAs to FAMEs compared to the conventional and direct derivatization approaches. The developed method is robust and will be invaluable for future assessments of FA content in algae that may be utilised for biodiesel or pharmaceutical applications.

Conflicts of interest

There are no conflicts of interest to declare.

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Potential Viable Products Identified from Characterisation of Agricultural Slaughterhouse Rendering Wastewater

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Abstract: The composition of challenging matrices must be fully understood in order to determine the impact of the matrix and to establish suitable treatment methods. Rendering condensate wastewater is a complex matrix which is understudied. It is produced when the vapour from rendering facilities (heat processing of slaughterhouse waste material) is cooled as a liquid for discharge. This study offers a full physicochemical characterisation of rendering condensate wastewater and its potential for valorisation via production of viable by-products. A study of seasonal variation of levels of dissolved oxygen, chemical oxygen demand, total nitrogen and ammonia was carried out on the wastewater. The results show that the wastewater was high strength all year-round, with a chemical oxygen demand of 10,813 ± 427 mg/L and high concentrations of total Kjeldahl nitrogen (1745 ± 90 mg/L), ammonia (887 ± 21 mg/L), crude protein (10,911 ± 563 mg/L), total phosphorous (51 ± 1 mg/L), fat and oil (11,363 ± 934 mg/L), total suspended solids (336 ± 73 mg/L) and total dissolved solids (4397 ± 405 mg/L). This characterisation demonstrates the requirement for adequate treatment of the condensate before releasing it to the environment. While there is a reasonably constant flow rate and dissolved oxygen level throughout the year, higher chemical oxygen demand, total nitrogen and ammonia levels were found in the warmer summer months. From this study, rendering condensate slaughterhouse wastewater is shown to have potential for production of marketable goods. These products may include ammonium sulphate fertilizer, protein supplements for animal feeds and recovery of acetic acid calcium hydroxyapatite, thus enhancing both the financial and environmental sustainability of slaughterhouse operations. This work demonstrates a valuable assessment of a complex wastewater, while taking advantage of on-site access to samples and process data to inform the potential for wastewater reuse.

Keywords: agriculture; by-products; nutrient recovery; rendering condensate wastewater; slaughterhouse; wastewater

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1. Introduction

As the number of slaughterhouses increases around the world due to the growing demand for meat, the volume of raw organic material (OM) being sent to rendering plants is also rising [1]. The increase in the demand for meat can be seen by the volume of meat consumed worldwide increasing by 40% in a 10-year period [2]. Rendering plants process the unused materials of slaughterhouses, such as carcasses or parts of animals, including products of animal origin not intended for direct human consumption [2]. The processing of these materials allows for the production of products such as animal-, bone- or bristle-meal, as well as separating the fat from the materials to produce tallow [3]. In conventional rendering facilities, the wastewater (WW) from rendering plants is mainly generated during sterilization of the raw material and during the drying process of the waste meat/fat mixture [3]. The cooking vapours produced from this process are cooled, and the vapour

condensates are discharged along with other WW streams from the slaughterhouse, to be treated [4]. Metzner et al. (1990) reported that condensates are formed at a rate of 0.65 m^3 per ton of waste processed. WW from meat abattoirs has also been shown to have high levels of nutrients (nitrogen (N) and phosphorous (P)); fats, oil and grease (FOG); crude proteins; chemical oxygen demand (COD); and solids [5].

The WW produced from the condensed vapours from a rendering plant is known as rendering condensate wastewater (RCWW). The volume and strength of RCWW is directly proportional to the amount of raw material processed and the amount of water used during the rendering process. The RCWW is generally hot and has high levels of condensed volatile fatty acids (VFAs) [6], COD, FOG, total organic carbon (TOC), total nitrogen (TN), total suspended solids (TSS), heavy metals (HM) and ammonia (NH_3) [5,7], which, if discharged into receiving water bodies, can cause eutrophication (Table 1). RCWW commonly contains high levels of nutrients, such as TN and total phosphorous (TP), due to the degradation of protein and animal tissue [4]. As such, it is imperative that RCWW is appropriately treated before released to the environment [7]. The most common VFAs in slaughterhouse WW (SHWW) include acetic acid, propionic acid, butyric acid and valeric acid, which have an influence on the removal of nutrients in the WW treatment plant [8] and are associated with odour problems [9].

Table 1. Characterisation of various wastewater (WW) types associated with the meat-slaughtering process (rendering wastewater (RWW); not reported (NR); slaughterhouse wastewater (SHWW); chemical oxygen demand (COD); fats, oil and grease (FOG); total phosphorous (TP); total nitrogen (TN), total suspended solids (TSS), heavy metals (HMs)) [3,5,10–13].

Type of WW	RWW	RWW	SHWW	SHWW	SHWW	Hog WW
pH	7.5	NR	6.5	7.3	7.2	6.9
COD (mg/L)	9500	6000	8575	11,546	109.8	8627
TP (mg/L)	200	<4	112.5	202	173	NR
TN (mg/L)	1100	430	445.5	103	NR	593
Crude protein (mg/L)	2187	0	980	375	2160	1104
FOG (mg/L)	525	110	121.5	1825	NR	NR
TSS (mg/L)	NR	<6	1550	3835	15.1	NR
HMs (mg/L)	NR	<2	NR	NR	NR	369

The OM concentrations in SHWW (which is similar to RCWW) is usually high and the residues are moderately solubilized [14]. SHWW typically contains fractions not found in RCWW, including blood and manure, leading to differences in the physicochemical characteristics. RCWW and SHWW are often combined for WW treatment [13]. Failing to treat the OM efficiently may lead to contamination of receiving waters [7], with excessive FOG discharge resulting in floating solids accumulation in lakes and streams [4]. Similarly, the most common WW treatment methods include anaerobic, aerobic and dissolved air flotation (DAF) treatment [15]. N and P are removed by using aerobic and anaerobic tanks, whilst most OMs and FOG can be removed by using DAF [16].

The European legislation for management of the treatment of RCWW, Animal By-Products Regulation [17], is in place in order to prevent the outbreak and spread of diseases such as Bovine Spongiform Encephalopathy and Creutzfeldt–Jacob disease [18]. It has been reported that, because of legal restrictions, rising treatment costs and environmentally conscious consumers, the treatment of RCWW has become a major concern of the meat processing industry [2]. Proteins from carcass debris are the major pollutant besides FOG in processing WW [19]. However, contaminants in RCWW represent potential opportunities for recovery as valuable commercial products. For example, FOG discharge is grease that can be recovered as finished grease in the rendering operations for tallow fat [4]. VFA can be extracted to obtain acetic acid for use as a carbon rich supplement to increase COD levels aiding in the denitrification and TP removal process [20]. The protein loss to WW can be estimated by multiplying the Total Kjeldahl Nitrogen (TKN) concentration by 6.25,

as the TKN method degrades protein bonds, which are composed of N compounds [21]. NH_3 discharge has a strong correlation with the amount of protein that has been degraded. NH_3 present in the WW can be removed by using the denitrification process or it could be recovered by using hydrophobic membranes, and when reacted with sulphuric acid (H_2SO_4), it produces ammonium sulphate ($(NH_4)_2SO_4$), which can be used as a nitrogen and sulphur-rich fertiliser [22,23]. Cattle bones are mostly composed of hydroxyproline, N and calcium hydroxyapatite (CaHA) at 3.7, 5.8 and 57.8%, respectively [24]. CaHA can be recovered by using ultrafiltration (UF) and used to treat HM by ion-exchange processes [25].

This study represents the first full physicochemical characterisation of RCWW (specifically the condensate WW produced from the rendering process). The study includes an analysis of seasonal variation, to determine how COD, NH_3, dissolved oxygen (DO) and TN concentrations change temporally in a 12-month period and with flow of RCWW. Previous studies have presented limited or incomplete datasets without temporal variation. Based on the findings of this work, it is possible to identify a suite of potential by-products which could be produced from RCWW.

2. Materials and Methods

2.1. Sample Collection and Preparation

RCWW samples were collected from a meat-slaughtering rendering plant in the south of Ireland. Samples were collected in sterile 2 L plastic bottles, and conductivity, temperature, pH, DO and NH_3 were determined immediately after sampling.

2.2. Materials and Reagents

A 0.45 µm polytetrafluoroethylene (PTFE) filter paper was procured from Radionics, Ireland. Solvents including acetone, hexane, n-hexane and ammonia hydroxide (NH_3OH) were procured from Fischer Scientific, Ireland. Standards including 25% meta-phosphoric acid with 60 mM crotanoic acid, acetic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, propionic acid and pentadecanoic acid were obtained from Sigma Aldrich, Ireland. Additionally, sodium hydroxide, anhydrous sodium sulphate, hydrochloric acid and methyl orange reagents were obtained from Sigma Aldrich, Ireland. Reagent HACH kits were obtained to measure COD (high range: 8000), TOC (high range: 10,173), TP (mid-range: 10,127) and orthophosphate (mid-range: 8114), which were procured from Hach, Ireland. All chemicals and reagents used in this investigation were of analytical grade or better.

2.3. Sample Characterisation

RCWW samples were characterised without filtration, to determine a number of physicochemical properties, nutrients and micro-nutrients. Analytes were selected based on other studies focusing on wastewater contamination from rendering and meat-slaughtering processes (Table 1). Samples were tested for TP, TN, COD, TOC, FOG, TSS, total dissolved solids (TDS), HM, micronutrients (phosphorous, nickel, cobalt, potassium, magnesium, calcium, sodium, manganese, sulphate, sulphur and chloride) and fatty acids (FAs). Samples were adjusted to pH 2, using concentrated hydrochloric acid for FOG, TOC and TKN analysis. Samples were adjusted to pH 11 for NH_3 quantitation, using 1 mol/L NaOH.

Conductivity and pH were measured by using a WTW Multi 320 multimeter, and a conductivity meter and pH electrode SenTix 4l, respectively. Temperature was measured by using a Proplus® handheld multi-parameter instrument (YSI, Hertfordshire, UK), and NH_3 using an Orion NH_3 gas-sensing ion selective electrode (ISE) electrode. The COD, TOC, TP and ortho-phosphate were determined by using spectrophotometer Hach model DR900 colorimeter according to Hach procedures 8000, 10,173, 10,127 and 8114. The TKN, FOG, TSS and TDS was carried out according to the APHA standard methods [26]. Protein concentration was calculated by multiplying the difference between TKN and NH_3 by 6.25 [27]. Metal and nutrient analysis was carried out by using inductively coupled plasma–

emission spectroscopy, according to the Association of Analytical Chemist (AOAC) method 43.293 (1980) at a commercial laboratory (ALS Scientific, Clonmel, Ireland).

2.4. Volatile Fatty Acid Analysis

VFA analysis in the RCWW required extraction and derivatization prior to analysis for determination by gas chromatography (GC). VFA analysis were performed by centrifuging 1.5 mL of the sample at 3000× *g* for 10 min at 4 °C. Then, 1 mL of the supernatant was transferred to a new centrifuge tube containing 200 µL of the internal standard (25% metha-phosphoric acid with 60 mM crotanoic acid). The tubes were vortexed for 2 min and placed in a freezer for 3–4 h. The sample was then thawed and centrifuged at 12,000× *g*, for 15 min, at 4 °C. Then, 1 mL of the supernatant was then analysed by using an Agilent 7890 GC connected to an Agilent 7693A flame ionisation detector (GC–FID). The GC–FID was equipped with an Agilent CP-FFAP column (25 m, 0.15 mm internal diameter (i.d.), 0.25 µm film thickness). A 0.5 µL volume of the sample was injected into a splitless injector set to a temperature of 260 °C. Helium was used as the carrier gas at a constant flow rate of 1.5 mL/min. The initial column temperature was 115 °C, and it was then increased to 175 °C at a rate of 15 °C/min; once it reached 175 °C, it was increased to 240 °C at a rate of 80 °C/min and held at this temperature for 3 min (total run time 9 min). The VFAs were detected by FID, which was operated at 280 °C with a 30 mL/min hydrogen flow, 300 mL/min air flow and a make-up flow of 30 mL/min of helium. Blank injections of deionised water were performed every 5th run, to ensure there were no contaminants retained by the column. The identification of unknown VFAs was achieved by comparing their retention times with known standards of acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid and valeric acid. The quantification of VFAs was performed based on a 4-point calibration (25–100% *w*/*w*).

2.5. Statistical Analysis

Statistical analysis was carried out to determine if differences in the concentrations of NH_3, COD, DO and TN throughout the year, during different seasons, were significant. Analysis was also carried out on the RCWW flow rate, to determine if there were higher volumes of WW at different times of the year. One-way analysis of variance (ANOVA) was performed ($p < 0.05$) and followed up with post hoc Tukey tests, where appropriate, using Origin software (version 9.0).

3. Results and Discussion

3.1. Characterisation of Raw RCWW

It has been reported that rendering plants produce significant amounts of WW which contains contaminants that are relatively low in long-term environmental risks, but cannot be released directly to the rivers, streams or lakes, without proper treatment [4]. The composition of raw RCWW is presented in Table 2, indicating high levels of nutrients, OM, FOG and VFAs (Table 3). Failing to appropriately treat the nutrients, solids and OM in RCWW can result in reduced DO levels, which promotes eutrophication in the receiving waters [28]. DO levels must not drop below 5 mg/L, in order to sustain safe conditions for aquatic life [1].

The pH is an important parameter determining the quality of the WW effluents because most chemical reactions in the aquatic environment are controlled by its value [1,4]. It was found that the mean pH value of raw RCWW was 8.3, which was within the typical tolerance limits of 6–9 for the discharge of WW from abattoir industries [29].

Table 2. Physiochemical characterisation of RCWW (n = 3).

Nutrients		Organic Matter		Solids	
Parameter	**Concentration (mg/L)**	**Parameter**	**Concentration (mg/L)**	**Parameter**	**Concentration (mg/L)**
TP	51 ± 1	DO	3.1 ± 0.4	TSS	336 ± 73
Orthophosphate	21 ± 0.5	COD	10,813 ± 427	TDS	4397 ± 405
TN	2720 ± 82	TOC	2513 ± 240		
TKN	1630 ± 90	FOG	11,363 ± 1942		
NH_3	887 ± 21	pH	8.34 ± 0.4		
Crude protein	10,911 ± 563				
Heavy metals and micronutrients (mg/L)					
P	Copper	Zinc	Lead	Chromium	Iron
2.7 ± 0.1	0.01 ± 0	0.04 ± 0	<0.01	<0.002	0.1 ± 0
Potassium	Cobalt	Nickel	Calcium	Magnesium	Sodium
4.1 ± 0	<0.002	<0.005	10.9 ± 0.1	0.8 ± 0.1	36.6 ± 1.9
	Sulphate	Sulphur	Chloride	Manganese	
	10 ± 0.8	68.8 ± 2.5	4374 ± 41	0.002 ± 0	

TKN, Total Kjeldahl Nitrogen.

Table 3. VFA analysis in RCWW, using GC–FID (n = 3).

Parameter	Concentration (mg/L)
Acetic acid	1519.7 ± 36.3
Propionic acid	821.2 ± 17.4
Isobutyric acid	190.9 ± 15.1
Butyric acid	738.1 ± 93.4
Isovaleric acid	233.2 ± 21.8
Valeric acid	297.4 ± 38.0

3.1.1. Nutrients

N and P are some of the most important parameters to be tested in effluent WW, to determine its quality. NH_3 is produced from the biological degradation of proteins. TKN is the sum of organic nitrogen and NH_3. NH_3 is toxic to aquatic life at levels as low as 0.5 g/L [30]. TN in the raw RCWW was determined to be 2720 ± 82 mg/L, TKN was 1630 ± 9 mg/L, NH_3 levels were 887 ± 2 mg/L and crude protein was calculated to be 10,911 ± 5 mg/L. Reference [4] reported that raw rendering plant WW typically has TKN values of 500–1000 mg/L. Local regulations in Europe and Ireland require effluent nitrogen levels to be below 15 mg/L before being released and, as such, RCWW must be treated [31]. Nitrification and denitrification treatment may be carried out in order to remove 99% of the TN present in the RCWW. The use of hydrophobic membranes has gained attention recently for the removal of NH_3 from WW, as it can produce a viable product to generate revenue. This is discussed further in Section 3.3. The ratio of COD to TKN was determined to be >8, which is suitable for nitrogen removal by nitrification and denitrification [28]. P levels may be introduced in the WW stream from meat or blood residues from the animal carcasses [15]. Other sources of P may be synthetic detergents with high levels of P components, which may be used during the rendering process [32]. TP was measured at 51 ± 1.1 mg/L. Orthophosphate was measured at 21 ± 0.5 mg/L, which is considered high, as orthophosphate is readily available for algae and aquatic plant growth. Various studies looking at P in WW from meat-processing activities detected high levels of TP, which must be treated (levels must be below 15 mg/L) [31]. The most common method of removing P from WW involves the incorporation of P molecules into the TSS, using the biological method as described by Reference [32]. The ratio of COD and TP obtained was greater than 50, which means that biological treatment can be used to successfully treat TP before being released to meet local regulations [28].

3.1.2. Organic Matter

Reducing high levels of OM being released into water streams can help protect aquatic life from low DO levels. RCWW was measured to have DO levels of 3 ± 0.4 mg/L. This low DO level is caused by the high COD, TSS and FOG levels present in the RCWW. OM present in influent and effluent is measured by COD and TSS [33]. High COD levels at 10,813 ± 427 mg/L were measured in the RCWW. High levels of COD may be due to both biodegradable and non-biodegradable OM materials, such as animal matter, FOG, nutrients and proteins [34]. COD levels must be below 125 mg/L in effluent before it is released [31]. TOC levels of 2513 ± 340 mg/L were present in RCWW. TSS levels in the RCWW are 336 ± 73 mg/L, which is almost 10 times the discharge limit set out by the EPA of 35 mg/L [35]. TSS levels may be influenced by animal tissue, fats and soils from the hides and hooves of animals [36]. The RCWW had a high level of FOG at 11,363 ± 934 mg/L, which is due to the high levels of unwanted tissue in the rendering process. These results indicate that raw RCWW is highly polluted and must undergo sufficient treatment before being released into receiving waters. All heavy metal species that were measured in the raw RCWW samples (Table 2) were below the EPA discharge limits. Nickel, cobalt, potassium, magnesium and manganese were at low levels in the RCWW. Calcium is found at a high level at 10 ± 0.1 mg/L, which is most likely due to the CaHA, which makes up 57% of the cattle bone composition [24]. The high levels of sodium (35.6 ± 1.9 mg/L) may be due to tissue and blood from animal waste [37]. The RCWW has high levels of sulphur (68.8 ± 3.5 mg/L), which could be attributed to the by-products of animals of a protein nature in the rendering process, since sulphur is a constituent of some proteins.

3.1.3. Volatile Fatty Acids

VFAs are fatty acids with carbon chains with fewer than six carbons (C_1–C_5). Various studies have looked at VFAs in WW produced from meat-processing activities and have identified that acetic, propionic and butyric acid as the most abundant VFAs, whilst also identifying isobutyric and isovaleric acid in the WW composition [3]. VFAs are a carbon and energy source for microorganisms in the nitrification and denitrification processes, which makes them important during WW treatment [38]. Table 3 shows the quantitative results obtained from the analysis of VFAs in RCWW, using GC–FID with a sample RCWW VFA chromatogram given in Figure 1. The results indicate that acetic, propionic and butyric acid are the most abundant VFA species present in the sample at 1519.67 ± 36.34, 821.15 ± 17.38 and 738.15 ± 93.38 mg/L, respectively. These results support a large number of studies showing these VFAs to be the most abundant with acetic acid at the highest concentration (% abundance in rendering WW according to Reference [6]; acetic acid 51%; propionic acid 26% and butyric acid 9%) [3,6,8,39].

3.2. Seasonal Variation Analysis

The study was carried out on the site of a rendering plant. Typically, operation of the rendering plant took place from late Monday afternoon to Friday evening. This meant that there were three days of downtime. The rendering plant released the condensed WW whilst it was in operation, and no WW was released while not in operation. This typical operating scenario leads to a high variation in concentrations of target analytes, as discussed herein, and can be seen in data plotted which shows a wide range of concentrations around a median value. Furthermore, seasonal effects were found to also impact the composition of the RCWW.

A seasonal variation study was carried out on the RCWW based on daily data supplied by the rendering plant. Parameters that were investigated include the daily flow rate, DO, COD, TN and NH_3. Figures 2 and 3 show the monthly average of the daily flow rate of RCWW and the monthly average for each of the pollutants investigated. Contaminant loadings of WW from meat-processing industries have been reported to vary seasonally, daily or even per working shift [2]. The composition of the WW can vary based on a number of parameters, such as the volume of material being processed, the type of material

being processed and the season of the year [40]. The flow of RCWW showed strong temporal monthly and annual variation. The average RCWW flow was 285.4 m^3/day, with a standard deviation of 105 m^3/day, which suggests a high variation. However, further statistical analysis showed a *p*-value of 0.25 (Supplementary Materials Tables S1 and S2), which suggests there was no significant variation in the flow rate throughout the year.

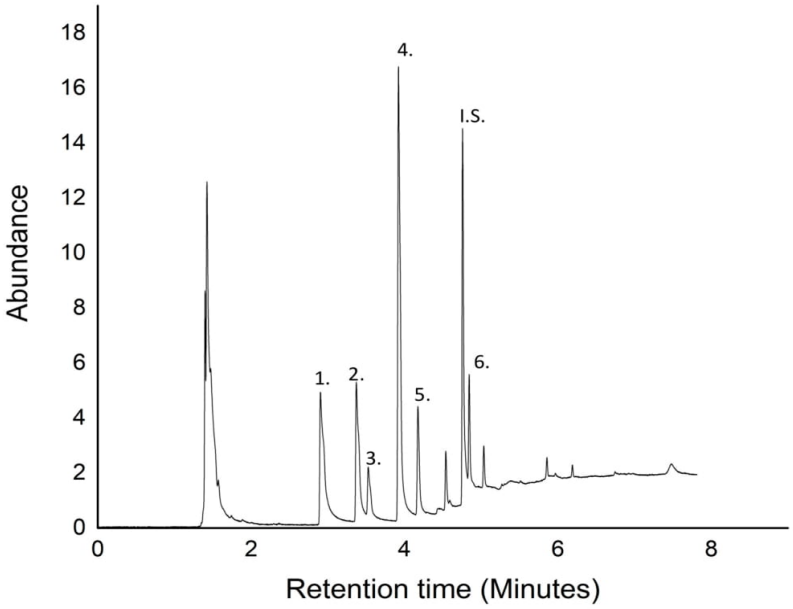

Figure 1. Chromatogram obtained from analysis of VFA in RCWW sample by GC–FID (1 = acetic acid; 2 = propionic acid; 3 = isobutyric acid; 4 = butyric acid; 5 = isovaleric acid; 6 = valeric acid) (IS = internal standard).

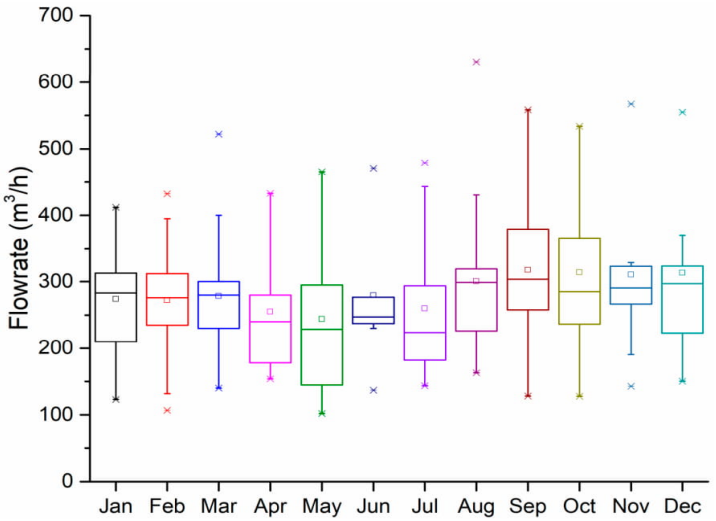

Figure 2. Average monthly rendering RCWW flow from the plant in 2017.

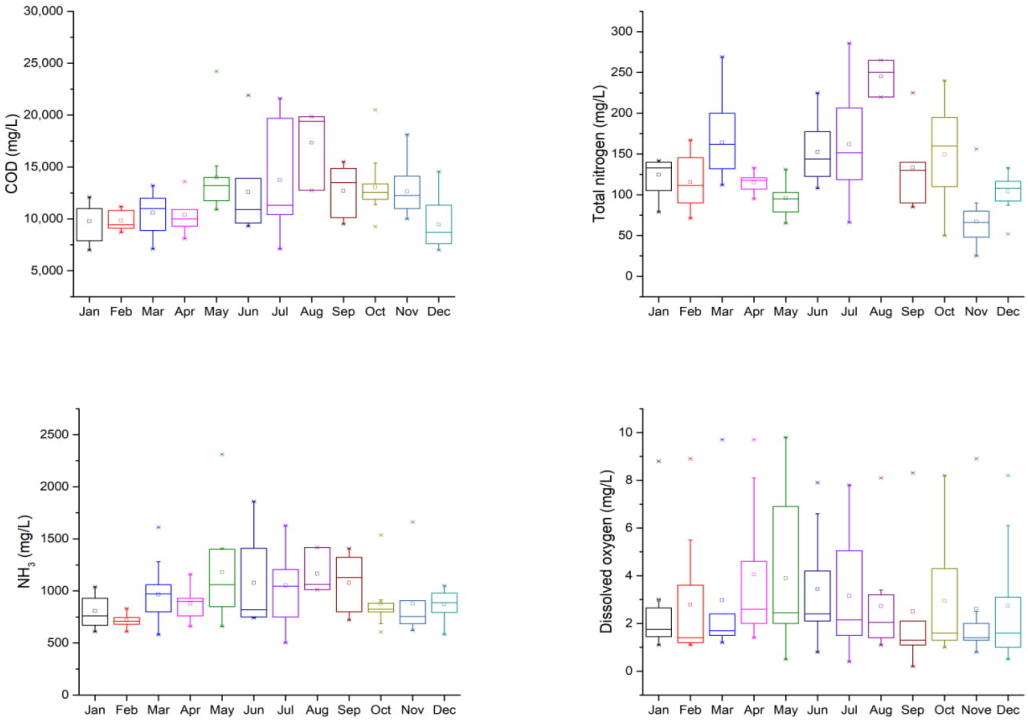

Figure 3. Monthly average concentrations for COD, NH_3, TN and DO in RCWW.

Figure 3 shows that, throughout the year, the RCWW had a DO level below the acceptable limit for discharge. The average DO level was 3.0 mg/L, with a standard deviation of 2.6 mg/L, which suggested that there was a high variation of DO throughout the year. Statistical analysis showed that there was no significant difference in the DO levels throughout the year (p = 0.75). Figure 3 also showed COD, NH_3 and TN levels throughout the year in RCWW. NH_3 had an average of 982 mg/L and standard deviation of 357 mg/L, which suggested that there was substantial temporal variation of NH_3 throughout the year. As NH_3 can exist as either NH_3 or NH_4^+ depending on its equilibrium (pH and temperature), NH_3 may be higher in the summer, due to increased temperatures, which is supported by the results [41]. Statistical analysis showed that the variation of NH_3 concentrations was significant and that August (as shown by the Tukey test presented in the Supplementary Materials) differed to other months, which supports the spike seen in Figure 3 (p-value = 8.5×10^{-7}). TN had an average of 126.4 mg/L, standard deviation of 52.0 mg/L and a p-value of 1.23×10^{-13}, suggesting a variation throughout the year, which may be due to a variation in the temperature of the RCWW. August represents the highest level of TN, which is usually the warmest part of the year. The mass of the NH_3 and TN was calculated for each month, and it was shown that higher masses of NH_3 and TN are released in the summer months (Figure 4). The calculated masses show that higher quantities were released in the summer months and the winter months, which may be due to a combination of higher temperatures, higher loading in the production plant and larger animals being processed. The average COD level is 12,296 mg/L, with a standard deviation of 3857 mg/L. While it had a relatively large standard deviation, the COD levels remained high throughout the year, which requires extensive treatment. Figure 3 suggests August had the highest levels, which is in agreement with the ANOVA and Tukey tests (p-value = 2.85×10^{-9}).

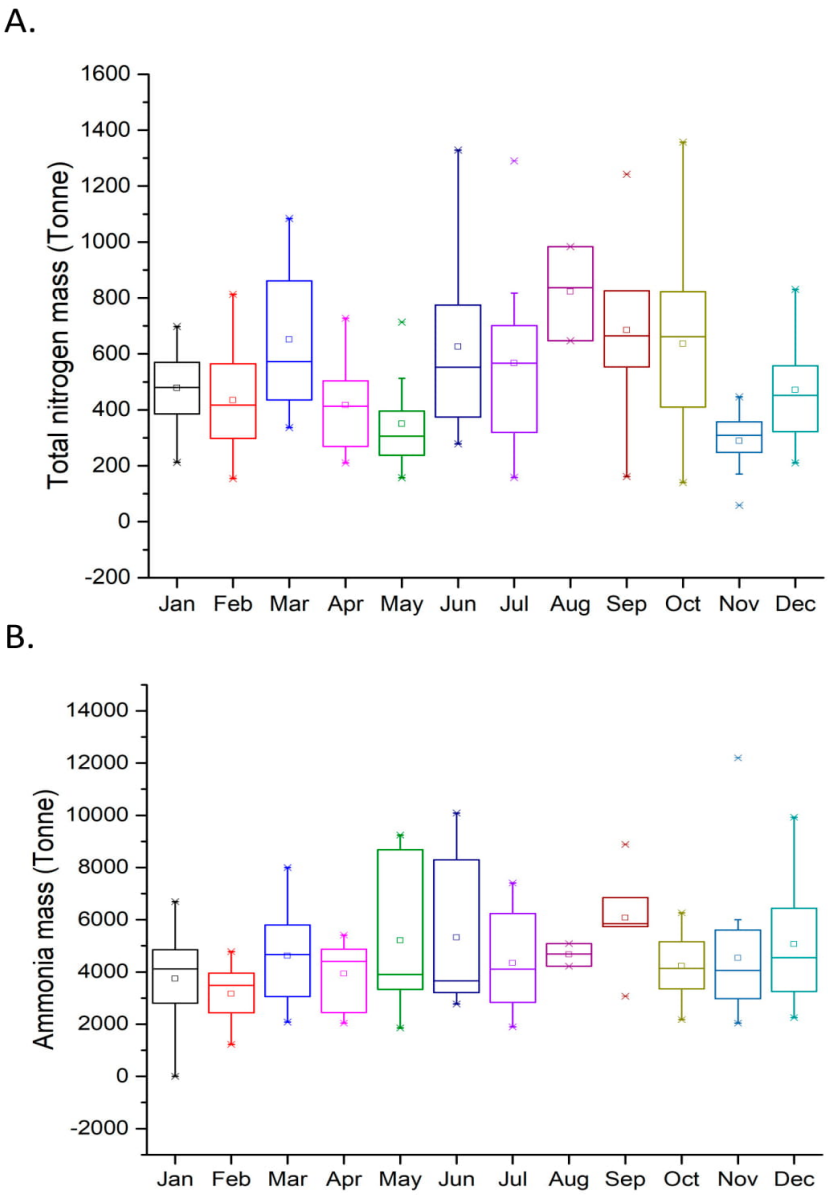

Figure 4. Total mass (tonne) of (**A**) TN and (**B**) NH_3 released per month in the rendering condensate wastewater (the middle box indicates the median).

3.3. Identification of Viable By-Products from Rendering Condensate

The treatment of WW for discharge in compliance with local environmental regulations is an expensive process. A way of off-setting the cost associated with environmental compliance is to incorporate these species into a production process to yield viable by-products which would result in a cleaner effluent [2]. This would allow for nutrients and

other contaminants to be reused which could help reduce the formation of bio-sludges and wastes for disposal [37]. Table 4 shows an overview of the possible products which could be produced from the constituents present in RCWW.

Table 4. Possible products from RCWW.

Component	Product
Nitrogen	Ammonium-based fertiliser; flame-retardant chemicals
Volatile fatty acid	Carbon source for denitrification; cosmetics; biogas
Protein	Animal feed
Calcium hydroxyapatite	Ion-absorber for heavy metals
COD	Biogas

3.3.1. Ammonium-Based Fertiliser

RCWW had high levels of NH_3 throughout the year, as seen in Figure 3. Lazouski et al. demonstrated that the use of conventional gas diffusion electrodes can be used to produce NH_3 fertiliser from nitrogen and water-splitting-derived hydrogen, which could potentially be derived from wastewater [42]. However, this method is only capable of producing a NH_3 fertiliser, whereas the use of membrane distillation allows for the production of $(NH_4)_2SO_4$. Hydrophobic membranes have become increasingly popular for extracting NH_3 from WW streams [23,43]. One of the major benefits of using membranes to treat NH_3 in WW is the production of an NH_4 salt (most commonly $(NH_4)_2SO_4$) as a by-product [44]. $(NH_4)_2SO_4$ is a fertilizer which can be applied to land, to help promote the growth of crops in alkaline soils. Ammonium sulphate contains high levels of nitrogen and sulphur (21 and 24% composition, respectively). The ammonium ion is released, and it undergoes deprotonation, which produces NH_3 and results in lowering the pH of the soil. It also contributes nitrogen, which is essential for plant growth. The sulphur promotes the metabolism of nitrogen and chlorophyll formation and forms amino acids, which are the building blocks for proteins [44]. There is no iron present in $(NH_4)_2SO_4$, but reducing the soil pH allows for iron to be absorbed more effectively by plants [44]. The use of $(NH_4)_2SO_4$ has also been used in flame-retardant chemicals, as it increases the combustion temperature of the material, decreases maximum weight loss rates and causes an increase in the production of residue or char [44]. The use of hydrophobic membranes has been shown to remove up to 99% of NH_3 from WW in 5 h, while producing a 30% pure $(NH_4)_2SO_4$ product [45]. The high levels of NH_3 present in RCWW makes it an ideal matrix to produce $(NH_4)_2SO_4$ fertilizer. A study by Brennan et al. [23] stated that the operation costs to produce 1 kg of $(NH_4)_2SO_4$ was €2.48 and could be sold for €1.54. It was calculated that the present RCWW produces an average of 4.7 million kg of NH_3 a year, which would allow for the production of up to 711,866 kg of 30% $(NH_4)_2SO_4$, equating to 1.1 million euro in revenue. While the cost of producing the fertilizer is greater than the revenue generated, it would substantially reduce the cost of NH_3 removal, compared with conventional treatment methods which do not produce any revenue. The disadvantages of using the liquid fertilizer include possible surface water and groundwater contamination, odour problems, greenhouse gas emission, and soil-pore clogging from excessive fat loads [11].

3.3.2. Animal Feed

An increase in demand for protein and meat-based diets has led to an increase in the number of animals to be processed and requiring feed. Therefore, an alternative for producing animal feed is required in order to provide for this increasing market [2]. SHWW has shown to have a protein composition comparable to other meal products used in animal feed [3]. Crude protein was measured to be 10,911 $\pm$ 563.7 mg/L in the RCWW, which suggested that there is great potential for protein to be extracted from the RCWW for animal feeds. Ultrafiltration (UF) has been widely used to simultaneously purify, separate and concentrate protein materials from WW sludge. The use of UF has shown to produce a product containing 30–35% protein, which could be supplemented into animal feed once

other contaminants are treated [46]. It should also be noted that the removal of protein from WW reduces the COD by 75% [2].

3.3.3. Carbon Sources

Denitrification is the loss of nitrogen molecules from nitrites or nitrates resulting in the release of N_2 gas. The denitrification process can be enhanced by adding natural carbon sources to the WW [47]. RCWW produces large volumes of VFA at reasonably high concentration, which could be recovered and spiked into the WW system, to promote denitrification [47]. Commercially available carbon sources are used to aid biological nutrient treatment, but in recent years, the cost of these carbon sources have increased, making the use of processed carbon sources an appealing alternative. Ultrafiltration has been used in a number of studies, in order to separate, isolate, recover and utilize VFA in the permeate stream, while rejecting the suspended solids [39]. The use of membrane filtration allows for the recovery of up to 50% acetic acid, 40% propionic acid, 18% isobutyric acid, 18% butyric acid, 15% isovaleric acid and 8% valeric acid [48]. There is a total of over 3700 mg/L VFA in the RCWW sample (Table 3). The most abundant VFA is acetic acid (1519 $\pm$ 26 mg/L), which is known to be an efficient agent for enhancing biological nutrient treatment and has been shown to reduce TN and TP in WW up to 95%, over a retention time of 8 h [20]. The use of propionic acid has also shown to be effective in reducing P levels [39]. Additionally, propionic acid has been used in the cosmetic industry, as a base for perfume in combination with butyl rubber, in order to form a more stable product with a longer shelf life [22]. Other studies by Vilvert et al. [49] and Gunes et al. [50] demonstrated that biogases could be recovered from matrices of similar compositions (mostly the high COD and VFA concentrations).

3.3.4. Heavy Metal Treatment

HMs in RCWW are shown to be at low concentrations and suggest that they do not pose a threat to the environment; however, this is not the case for many industrial wastewater streams. CaHA, which makes up 60% of cattle bone composition, has been shown to be effective in the removal of HM from WW by means of absorption and ion exchange [2,51] and allows for a 45% cost reduction for removing HMs, as compared to conventional methods [52]. Various studies have investigated the removal of HMs from WW samples, using CaHA from cattle bones, and it was demonstrated that Cd, Pb and Cr could be completely removed from WW samples by using CaHA, while Cu and Fe showed removals of up to 40% [25,51,53,54]. The Ca in RCWW, which is a component of CaHA, has a concentration of 10 $\pm$ 0.1 mg/L, and purification and use for HM removal has been shown to be feasible [25].

The previous sections highlight the potential products from RCWW. In order for the successful delivery and exploitation of these products, efficient recovery and purification methods must be developed. The recovery of NH_4-based products from SHWW is discussed by Brennan et al. [55], and membrane contactors were identified as a suitable technology to recover NH_4-based products. Experimental work has shown that PTFE membranes were capable of producing a 30% $(NH_4)_2SO_4$ product. However, further work is needed to produce more robust membrane materials, to allow for longer treatment and recovery times.

Protein-rich animal feed was also identified as a viable product. Further research should focus on the optimization of ultrafiltration, to address this challenging matrix, in order to recover the protein content and purify the product. The high carbon content in RCWW shows great potential to reduce costs for anaerobic treatment. Further analysis should be carried out at both the lab- and pilot-scale, to determine if this matrix can be supplemented into anaerobic waste, to determine if it acts as an efficient carbon source. Lastly, heavy-metal treatment can be carried out from the CaHA content; however, the concentrations in the RCWW may be considered low, so further research should focus on isolating and purifying the CaHA and using it to adsorb heavy metals.

4. Conclusions

The processing of unused material from slaughterhouses to produce tallow fat and/or animal feed, using high-temperature treatments, yields condensate vapours which become RCWW. A physiochemical characterisation of raw RCWW was carried out in this study. The results demonstrated that there were high concentrations of organic and inorganic constituents present. Constituents measured in RCWW include COD, TN, NH_3, crude protein, TP, FOG, TSS and TDS at 10,813 ± 427, 1745 ± 90, 887 ± 21, 10,911 ± 563, 51 ± 1, 11, 363 ± 934, 336 ± 73 and 4397 ± 405 mg/L, respectively. These constituents, in their current form, are higher than the discharge limits outlined by the Irish EPA (which follows EU regulatory limits) for water discharge into fresh and marine water bodies, and thus the raw RCWW must undergo treatment accordingly. HMs were also characterized in the WW sample, and it was determined that all HMs were present at very low levels and would not be useful to produce viable products. A seasonal-variation study was carried out on the RCWW, and it was determined that the flow rate and DO levels remained constant throughout the year. The warmer summer months also showed higher levels of TN, NH_3 and COD, suggesting that the temperature may impact the levels of these contaminants. Lastly, a preliminary study was carried out to determine if viable products could be produced from RCWW based on the RCWW composition and other studies. It is suggested that, based on other studies and the concentration of NH_3 in this study, the NH_3 could be recovered by using membrane distillation to produce a viable ammonium sulphate fertilizer. Proteins could be recovered by using UF, to produce a protein supplement for animal feed. VFA could be recovered by using membrane filtration, to recover acetic acid, which could be used to aid in biological microbial treatment of WW, and CaHA could potentially be recovered for use as an ion-absorbent, for removal of HM. Valorisation of this potentially valuable WW or co-product stream should be considered by slaughterhouses with rendering facilities, allowing for cost savings in WW treatment, along with potential benefits of by-product production.

Supplementary Materials: The following are available online at https://www.mdpi.com/2073-4441/13/3/352/s1, Table S1: Analysis of variance on flow rate of rendering condensate wastewater throughout the year; Table S2: Analysis of variance on dissolved oxygen concentrations in rendering condensate wastewater throughout the year; Table S3: Analysis of variance of chemical oxygen demand concentrations in rendering condensate wastewater throughout the year; Table S4: Analysis of variance on total nitrogen concentrations in rendering condensate wastewater throughout the year; Table S5: Analysis of variance on ammonia concentrations in rendering condensate wastewater throughout the year; Table S6: Tukey Test of flowrate of rendering condensate wastewater throughout the year; Table S7: Tukey Test of dissolved oxygen concentrations in rendering condensate wastewater throughout year; Table S8: Tukey Test of chemical oxygen demand in rendering condensate wastewater throughout the year; Table S9: Tukey Test of total nitrogen concentrations in rendering condensate wastewater throughout the year; Table S10: Tukey Test of ammonia concentrations in rendering condensate wastewater throughout the year.

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Conflicts of Interest: The authors have no conflicts to declare.

Abbreviations

NH_3	Ammonia
NH_3OH	Ammonia hydroxide
$(NH_4)_2SO_4$	Ammonium sulfate
ANOVA	Analysis of variance
CaHA	Calcium hydroxyapatite
COD	Chemical oxygen demand
DAF	Dissolved air flotation
DO	Dissolved oxygen
FOG	Fats, oil & grease
FA	Fatty acids
GV	Gas chromatography
GC-FID	Gas chromatography flame ionisation detection
HM	Heavy metals
N	Nitrogen
NR	Not reported
OM	Organic matter
P	Phosphorous
PTFE	Polytetrafluoroethylene
RCWW	Rendering condensate wastewater
RWW	Rendering wastewater
SHWW	Slaughterhouse wastewater
H_2SO_4	Sulfuric acid
TDS	Total dissolved solids
TKN	Total Kjeldahl Nitrogen
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorous
TSS	Total suspended solids
UF	Ultrafiltration
VFA	Volatile fatty acid
WW	Wastewater

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Pilot Scale Study: First Demonstration of Hydrophobic Membranes for the Removal of Ammonia Molecules from Rendering Condensate Wastewater

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Abstract: Hydrophobic membrane contactors represent a promising solution to the problem of recycling ammoniacal nitrogen (N-NH_4) molecules from waste, water or wastewater resources. The process has been shown to work best with wastewater streams that present high N-NH_4 concentrations, low buffering capacities and low total suspended solids. The removal of N-NH_4 from rendering condensate, produced during heat treatment of waste animal tissue, was assessed in this research using a hydrophobic membrane contactor. This study investigates how the molecular composition of rendering condensate wastewater undergo changes in its chemistry in order to achieve suitability to be treated using hydrophobic membranes and form a suitable product. The main objective was to test the ammonia stripping technology using two types of hydrophobic membrane materials, polypropylene (PP) and polytetrafluoroethylene (PTFE) at pilot scale and carry out: (i) Process modification for NH_3 molecule removal and (ii) product characterization from the process. The results demonstrate that PP membranes are not compatible with the condensate waste as it caused wetting. The PTFE membranes showed potential and had a longer lifetime than the PP membranes and removed up to 64% of NH_3 molecules from the condensate waste. The product formed contained a 30% concentrated ammonium sulphate salt which has a potential application as a fertilizer. This is the first demonstration of hydrophobic membrane contactors for treatment of condensate wastewater.

Keywords: ammonia; hydrophobic; membranes; polypropylene; polytetrafluoroethylene; rendering condensate; wastewater

1. Introduction

Increasing research interest has focused on the use of hydrophobic membrane contactors in recent years as a method of recovering ammonia (NH_3) molecules from wastewater (WW) streams due to a number of advantages including: fast separation from WW due to the large surface area of the membranes, low energy input per mole of (NH_3) removed, production of a viable fertilizer as the final product which can be sold, independent control of gas and liquid flow rates, and ease of operation [1,2]. However, to date, no studies have focused on using hydrophobic membrane contactors to treat rendering facility condensate wastewater (RCWW) as the matrix.

RCWW is a stream of WW rich in (NH_3) molecules produced from the processing of meat industry waste into viable products which involves the heat processing of animal waste tissue [3]. The purpose of the rendering process is to separate water, fat and protein components and produce stable products of commercial value via steam treatment [4]. RCWW is known to have very high (NH_3) concentration and has been reported to contain between 500 and 1000 mg/L Total Kjeldahl Nitrogen (TKN) which is the sum of organic nitrogen and NH_3 molecules [5]. It is crucial that (NH_3) molecules are treated appropriately before it is released into the environment as even small concentrations such as 0.05 mg/L can result in some fish species suffering from poor growth, fertility issues and making them more susceptible to bacterial infection. Whilst other fish can withstand high concentrations and at 2 mg/L they suffer from tissue damage, extreme lethargy and death [2,6]. The conventional methods to treat (NH_3) molecules in WW include air stripping, break-point chlorination, selective ion exchange and biological nitrification/denitrification [7]. These methods all have their own benefits but are inefficient as air-stripping is not suitable for low concentrations of (NH_3) in WW, break-point chlorination requires large treatment volumes, the ion exchange method requires expensive organic resins while the biological nitrification/denitrification method are slow processes that require large treatment vessels as well as significant energy input for air supply to aerobic phases [8].

EU regulations require that (NH_3) levels being released back into the environment must be below 15 mg/L [9,10]. In order to achieve the required legislative limits, an efficient method to remove (NH_3) molecules must be installed, easily integrated to the WW treatment facility, with low operational and capital costs and short retention time. This makes the use of hydrophobic membrane contactors ideal as various studies have shown as much as 99% (NH_3) removal from waste streams [11]. The mechanism of separation by hydrophobic membrane contactors is based on the mass transfer between the gas phase and the liquid phase [7]. Ammonium (N-NH_4) in the feed phase is converted into an (NH_3) volatile gas phase by increase of pH and/or temperature [1]. The volatile (NH_3) molecules then diffuse through the membrane pores from the feed side containing the WW to the permeate side containing the stripping solution, which is typically an acid that reacts with the (NH_3) molecules to form (N-NH_4) salts (most commonly sulfuric acid (H_2SO_4)) [6]. The feed and permeate sides are separated by a hydrophobic membrane sheet which acts as a barrier [12]. Polypropylene (PP) and polytetrafluoroethylene (PTFE) are the most commonly used membranes as they provide good separation to the two phases due to their high hydrophobicity [12]. The driving force of the mass transfer is the difference in NH_3 concentrations in the 2 phases [13]. The (NH_3) molecules immediately reacts with the stripping solution on the permeate side of the membrane and forms a non-volatile compound; as such the (NH_3) molecule levels on the permeate side is essentially zero [7].

Membrane wetting has been reported to be a significant issue affecting the feasibility of the use of hydrophobic membrane contactor technology [14]. Certain surfactants in WW such as fat, oils & grease (FOG), dissolved organic matter and colloids lower the liquid surface tension of the feed solution and cause wetting of the membrane pores [15]. Wettability is controlled by the chemical composition of the membrane [2]. The membrane can be characterized under 3 wetting modes; non-wetted, fully wetted and partially wetted. The non-wetted mode is when the membrane pores are completely filled with gas and is the highly preferred mode. Partial wetting is when the liquid can partially enter the membrane pores and causes wetting. Finally, fully wetted membrane pores result in a direct liquid flow from the feed side to the permeate side deteriorating the separation quality [16].

This study is the first demonstration of membrane contactors for the treatment of (NH_3) molecules from RCWW. The study involves the measurement of the physicochemical characteristics of RCWW and the required pre-treatment steps for the most efficient removal of (NH_3) molecules from RCWW. The use of PP and PTFE membranes for the removal of (NH_3) molecules from RCWW, a matrix which has not been examined in the literature to date, was investigated. The membranes were characterized in terms of their surface energy (contact angle (CA), chemical morphologies, surface roughness, porosity and liquid entry pressure (LEP) before and after being exposed to the RCWW at different time intervals. An initial cost analysis of NH_3 molecule removal using hydrophobic membrane contactors with production of ammonium sulphate ($(NH_4)_2SO_4$) under different operating conditions is presented. The mass transfer efficiency and wettability of the membranes are determined, and the $(NH_4)_2SO_4$ product characteristics are discussed.

2. Results and Discussion

RCWW is a waste product from the processing of animal waste which possess a high (NH_3) concentration. The volume of animal waste processed daily varies which results in a varied (NH_3) concentration in the RCWW. Operation of the rendering plant starts on a Monday and it begins to produce RCWW on Monday evening which is then fed into an aeration tank where it is conventionally treated for (NH_3). From this feed, samples are taken to test on the membrane system. On Fridays, RCWW flow ceases due to the plant being shut down for the weekend. This results in no (NH_3) being produced over the weekend. Figure 1 shows the concentrations of (NH_3) over the course of a month which shows the variation which is experienced. It can be seen that the highest levels of N-NH_4 are experienced mid-week or on Tuesday just after operations have started up again. Levels indicated by zero identify when operations were not running in the plant.

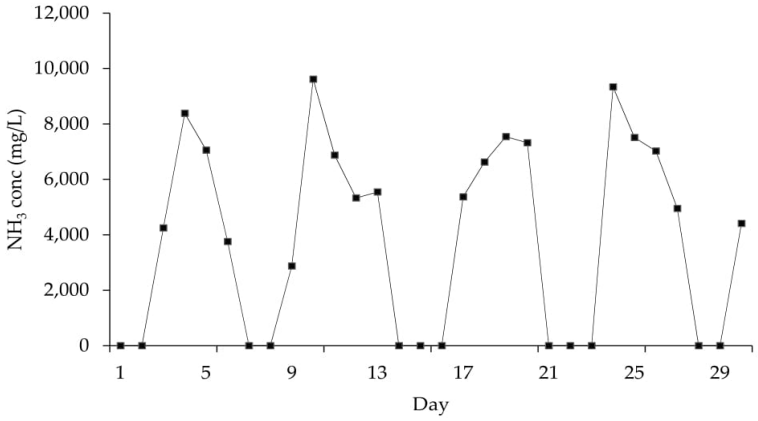

Figure 1. Variation in NH_3 levels in RCWW over the course of one month (April 2018).

A number of tests were carried out in order to characterise the RCWW according to its physicochemical properties including its pH, temperature and turbidity, and how they exert influence on each other. By doing so, it ensures the RCWW is pre-treated efficiently to be in its volatile form so that it is capable of crossing over the hydrophobic membrane in gaseous form. Investigating the turbidity, it allows for the RCWW to be filtered appropriately in order to prevent it from clogging the membrane pores. The second investigation carried out was completed to determine the suitability of polytetrafluoroethylene (PTFE) and polypropylene (PP) membranes for removing (NH_3) in RCWW. The rate of (NH_3) removal was determined by measuring the (NH_3) levels in the feed inlet and feed outlet (retentate). Failure of membranes was further investigated for membrane wetting using CA and leakage using conductivity analysis.

2.1. Physiochemical Characterisation of RCWW

An investigation was carried out in order to determine the relationship between the pH and temperature of the RCWW. Two RCWW samples, one with an ambient temperature of 20 °C and the other sample at 75 °C were investigated. As seen below in Figure 2, it was noted that the initial pH drops as the temperature increases. This is caused by equilibrium shifting due to increase ionization of solute molecules according to Le Chatelier's principle. If the temperature of a dynamic system is increased, the equilibrium will move to lower the temperature by adsorbing the extra heat. That means that the forward reaction will be favoured, and more hydrogen ions and hydroxide ions will be formed increasing the equilibrium constant (Kw). Further tests were carried out with RCWW and deionised water (DI water) to determine the temperature dependence of the pH. Due to this dependence of the pH, an increased volume of sodium hydroxide (NaOH) is required in order to adjust the pH of the RCWW. Figure 2 shows how much NaOH is needed in order to adjust the pH of the RCWW to its optimum conditions (pH of 10.5). This suggests that using a higher temperature (75 °C) will increase the cost efficiency of (NH_3) removal as less NaOH will be used to increase the pH to 10.5. However, more energy will be used as the RCWW with an initial temperature of 20 °C will need to be heated. However, by increasing the temperature it decreases the potential of membrane clogging which may be more influential in the (NH_3) removal process as discussed below.

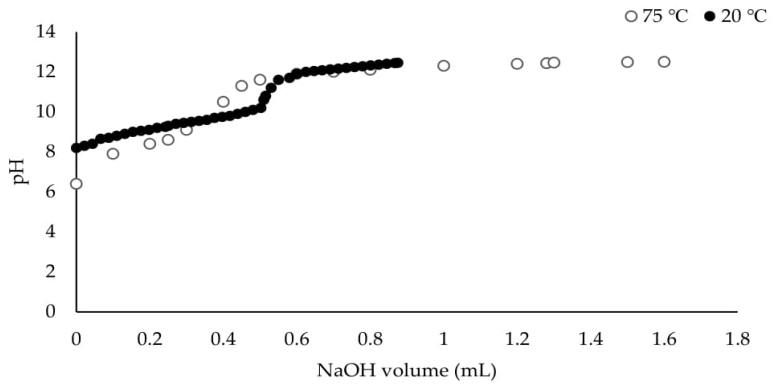

Figure 2. Titration of the same RCWW sample (60 mL) with 30% (*w*/*v*) NaOH at 20 and 75 °C.

Sample turbidity in RCWW samples was investigated to determine if any pre-treatment is required to protect the membranes from clogging. A decrease in turbidity was noted with an increase in temperature and pH. The RCWW sample heated up to 75 °C and pH adjusted to 12 had a turbidity of 40 NTU whilst the RCWW sample at an ambient temperature and its natural pH (approx. 7–8) had a turbidity of 180 NTU. The results show that there is a strong correlation between an increase in temperature & pH and a lower turbidity and particle size distribution. The RCWW contains protein agglomerates and FOG. Increased temperature and ionization (due to NaOH) has a solubilizing effect on these molecules causing larger particles/agglomerates to break down into smaller constituents which is required to help prevent membrane fouling. It was concluded that operation at high pH and temperatures above 10.5 and 45 °C, respectively, avoid the need for a pre-filter finer than 50 µm to be implemented to protect the membrane contactor, as protein agglomerates and FOG are solubilized under these conditions. As such the optimum pH and temperature of the feed solution should be 10.5 and 45 °C, respectively in order to make the feed solution suitable for the membrane material. In addition, the high pH and temperature reduce the likelihood of biofouling of the membrane.

2.2. Effect of RCWW on Membrane Materials

Two commonly used membranes were used in this study, PP and PTFE. The following section discusses the characteristics of these membranes and how they are compromised after being exposed with RCWW.

2.2.1. Membrane Wetting

Membrane wettability is the ability of a liquid to wet a surface which is controlled by interfacial forces between liquid, solid and vapors [17]. Membrane wetting may be due to inorganic & organic fouling, surfactants, transmembrane pressure or membrane degradation [18]. Three wetting patterns have been identified in the literature in hydrophobic microporous membranes: non-wetting, partial wetting and overall wetting. [16,19]. Compatibility between the membrane type and the liquid absorbent in membrane contactors is an effective parameter in wettability determination. Generally, compatibility depends on absorbent surface tension as well as membrane surface energy [19,20]. Liquids with lower surface tension have greater tendency to wet the surface, while membranes with higher surface energy are more vulnerable to wetting [16]. Organic compounds such as FOG in feed solutions result in a decreased surface tension, and in time, membrane wetting as the hydrophobic organic contaminants begin to adhere to the hydrophobic membrane surface with their hydrophilic component staying in the feed solution which results in a hydrophilic surface on the membrane which results in a decreased CA [18]. CA measurements were used in order to test the hydrophobicity of the membrane surfaces with DI water, RCWW and RCWW with FOG removed.

Table 1 presents a summary of the CA for PP and PTFE membranes with 1 μL of DI water, RCWW and RCWW with FOG removed. The results show that PTFE presents a higher hydrophobicity at 123.1° compared to 115.7° for PP. These results agree with other studies which suggest that PTFE usually poses a rougher surface and a higher CA [21]. The results also show that DI water poses a higher surface tension compared with the RCWW. This decrease in hydrophobicity may be due to a high concentration of organic contaminants present in the RCWW which decreases the surface tension. RCWW which had FOG removed also showed to have a much higher CA which suggests that the FOG present in the RCWW may be causing the membrane surface to become more hydrophilic as suggested by [18]. Figure 1 (supplementary material) represents how the different matrix solutions poses varying surface energy interactions with the membrane surfaces.

Table 1. CA values obtained for PP and PTFE membranes using different matrixes.

Matrix	PP	PTFE
DI water	115.7° ± 2.3°	123.1° ± 2.4°
RCWW	92.2° ± 3.4°	99.4° ± 3.1°
RCWW with no FOG	107.3° ± 1.3°	111° ± 2.6°

Further investigation was carried out to determine the effect of RCWW to the membrane hydrophobicity over time. PP and PTFE samples were exposed to RCWW at varying times as described in Section 3.2. Initial results (Figure 3) showed that there was no major effect to the membranes hydrophobicity after being exposed to RCWW for at least 6 h as they still remained hydrophobic (above 90°).

Exceeding the membrane liquid entry pressure (LEP) is another cause of membrane wetting. LEP is a result of the feed solution penetrating the membrane and passing through the membrane to the permeate side. PP and PTFE membranes were investigated to determine their LEP and to investigate how RCWW effected the LEP of the samples over time. Table 2 shows that the LEP of the membranes with no exposure to RCWW is greater than 2 bar. This suggests the membrane materials are suitable for membrane distillation as the LEP of membranes should be at least 1.5 bar in order to be used for MD [22]. Effect of exposure to RCWW suggests that PP membranes are greatly affected by RCWW as

their LEP reduces immediately to 0.57 bar and RCWW penetrates the membranes without any pressure thereafter which may be in part due to their large pore size. This suggests that PP membranes are not suitable for MD with RCWW. PTFE membranes performed considerably better as they allowed sufficient LEP values up to 30 min exposure to RCWW. After 30 min the membrane LEP are seen to be below the recommended level and after 120 min they failed without any pressure. However, the membrane system used in this investigation did not exceed 0.6 bar which would allow PTFE to treat RCWW for up to one hour before requiring cleaning. After 60 min of operation the membrane can start to fail due to wetting which may be due to the surface of the membrane being coated; the reduction in surface roughness is evident (Table 3).

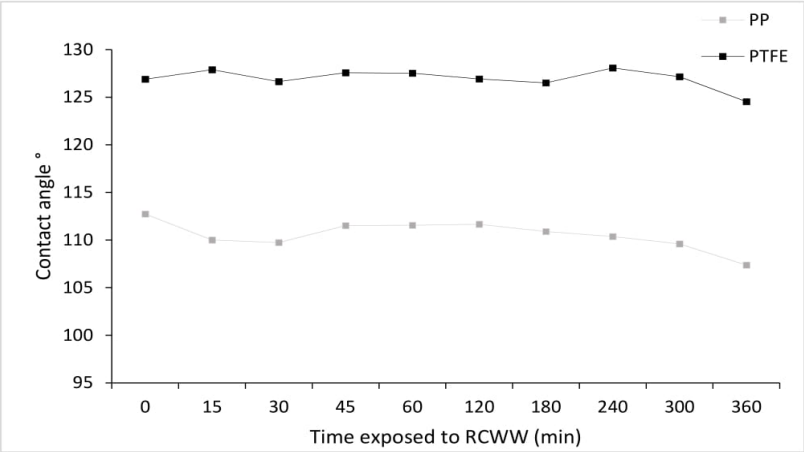

Figure 3. CA for membrane samples (PP & PTFE) exposed to RCWW at different time intervals.

Table 2. LEP values obtained for PP and PTFE samples exposed to RCWW at different time intervals.

Time (min)	0	15	30	45	60	120
PP (Bar)	> 2	0.57 ± 0.12	-	-	-	-
PTFE (Bar)	> 2	> 2	1.67 ± 0.06	1.07 ± 0.06	0.7 ± 0.1	0.07 ± 0.01

Table 3. Quantitative analysis of PP and PTFE membrane surface roughness.

Time (min)	PP RMS Roughness (nm)	PTFE RMS Roughness (nm)
0	154.64	249.23
15	134.48	164.4
30	132.86	-
45	511.33 *	131.61
60	118.92	120.42
120	962.2 *	114.87
180	113.66	109.74
240	255.98 *	94.98
300	109.49	556.12 *
360	86.71	85.57

* RCWW residue may have remained on the membrane surface and caused high surface roughness value.

2.2.2. Membrane Roughness

A quantitative measurement of PP and PTFE was carried out by characterizing the membranes in terms of their roughness (R_a), root mean square roughness (RMS), mean difference between the highest peak and lowest valley R_{max} and surface area (SA). Atomic force microscopy (AFM) is a powerful tool which allows us to fully understand a samples morphology and 3D images of its topography [21]. In AFM images, the brightest regions represent peaks in the surface and dark regions represent pores or valleys [23]. The images in Figure 4 show (A) PTFE sample with no RCWW exposure, (B) PTFE sample after 120 min RCWW exposure, (C) PP sample with no RCWW exposure and (D) PP sample after 15 min RCWW exposure. 120 and 15 min are presented for PTFE and PP, respectively as they are the points at which membrane failure was first experienced in terms of LEP (Section 2.2.1). Analysis for all other time intervals investigated can be found in the supplementary material (Supplementary Material Figures S1–S4). It should be noted that some time intervals consist of a surface roughness outlier which is assumed to be due to RCWW remaining on the surface on the membrane samples as they are much higher than the initial samples which had no exposure to RCWW (Table 3). The AFM images show variation in surface roughness to samples exposed to RCWW for different time intervals as seen in Figure 4. Both PTFE and PP membrane samples had a root mean square (RMS) roughness value of 249.23 and 154.64 nm, respectively. These results suggest that PTFE have a rougher surface which is in agreement with the values obtained for CA (Section 2.2.1). Other studies have also shown that PTFE typically has a greater surface roughness [21]. Additionally, a study by [24] shows that there is a direct relationship between membrane surface roughness and permeate flux which is in agreement with the PTFE membrane presented in this paper Section 2.3.1. The same author also showed that a membrane with greater surface roughness resulted in decreased membrane fouling. Samples which were exposed to RCWW were shown to lose surface roughness with prolonged exposure (Table 3). The interaction between the particles present in the RCWW and the composite membrane surface caused the roughness to decline. In Figure 4A, samples with no RCWW exposure can be seen to have high peaks and after 120 min exposure (Figure 4B), these peaks are seen to break up and a lower, smoother surface starting to develop. Similar results can be seen for the PP membrane surface. PTFE membranes can be seen to withstand their surface roughness for a much longer period than PP as after 120 min they both seem to have consistent roughness. Overall, the AFM analysis showed that PTFE membrane had a rougher surface than PP, which contributed to the increased flux and longer lifetime. It was also shown that RCWW has a great effect on the membranes surface topography and results in the roughness decreasing over time. Various studies by Vecino et al. [25] showed that oil has a major impact on the surface roughness due to the hydrophobic part of the oil particle adhering to the membrane surface and leaving the hydrophilic part exposed to the sample, which allows the water to enter the membrane pores. Due to the high oily content of RCWW, this may be a major contribution to the surface wetting and thus, appropriate pre-treatments are required in order to remove the oil content from RCWW which was demonstrated by Zheng et al. [26] using omni phobic membranes which showed removal of oil from the feed solution using similar direct contact membrane setup.

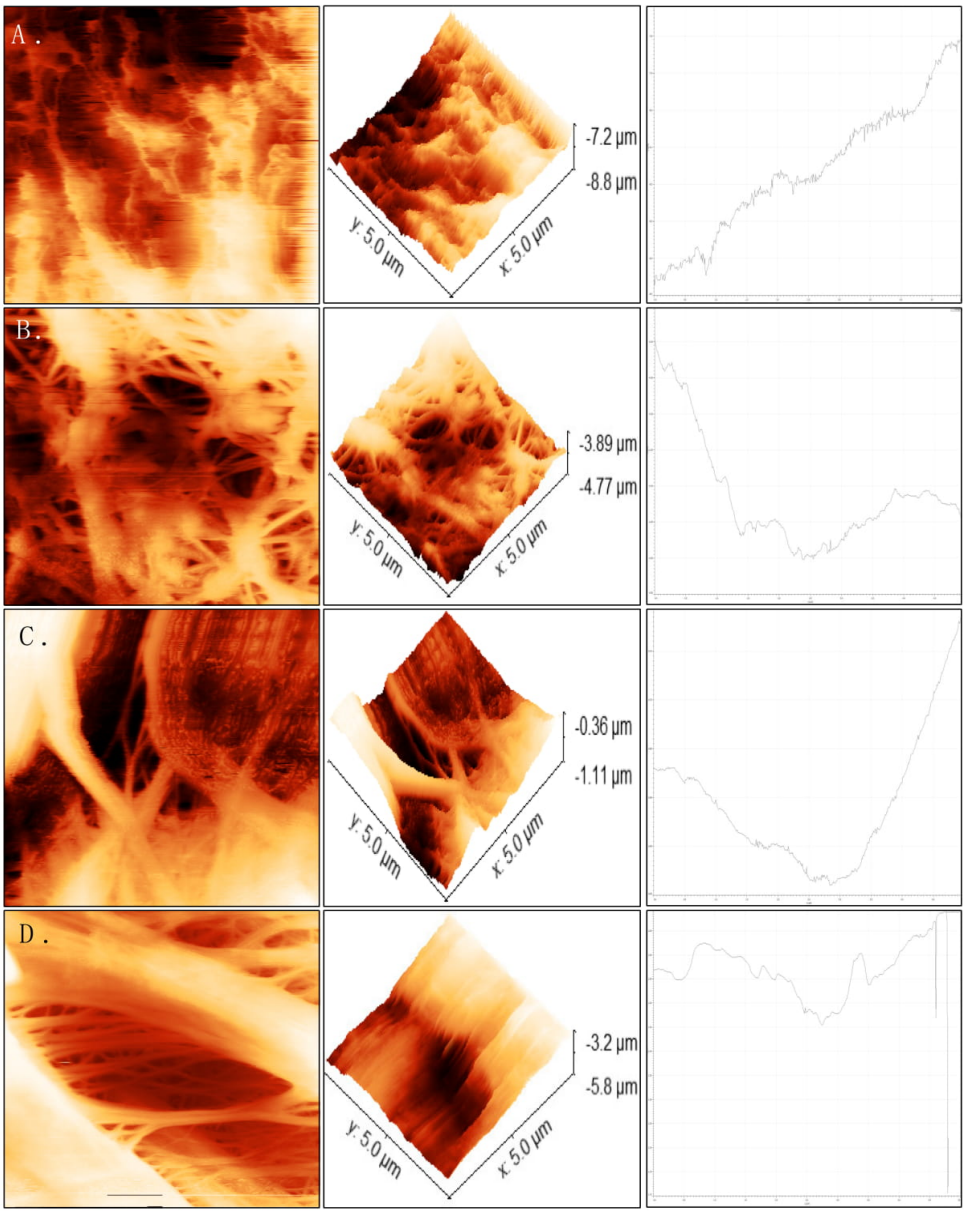

Figure 4. AFM analysis with flat maps, surface height maps and 2D profile for (**A**) PTFE with no RCWW exposure, (**B**) PTFE after 120 min RCWW exposure, (**C**) PP with no RCWW exposure and (**D**) PP after 15 min RCWW exposure.

2.2.3. Membrane Morphology

SEM images shown in Figure 5 present a flat and cross-sectional view of both PTFE and PP before and after RCWW exposure. Similarly to the results presented for AFM analysis, the images for PTFE at 120 min and PP at 15 min RCWW exposure are presented as they represent the maximum exertion according to the LEP results (Section 2.2.1) with the parameters used in the membrane system. The PTFE image with no RCWW exposure (Figure 5A) shows cell like structures with branching and sufficient even pores. The cross-sectional view shows continuous cross-linked fibres. The average pore size for PTFE with no RCWW exposure is 0.39 ± 0.1 µm (Table 4). PTFE samples after 120 min exposure time (Figure 5B) are shown to have a coating on the top as the surface structure is not as defined. Pore size in these samples have decreased (0.3 ± 0.07 µm) which is likely due to pore clogging from the RCWW. The cross-sectional view also shows that fibres are beginning to break which is most likely due to organic matter and particles entering the pores, penetrating the membrane and causing membrane wetting. PP membranes (Figure 5C,D) have a branch like structure with nanoparticles webbed to the larger fibre. Pore sizes (Table 4) appear to remain the same size with and without RCWW exposure. Fibre size can be seen to decrease after exposure and more abundance of nanoparticles webbed are present. The cross-sectional views also show fibres beginning to breakdown.

Membranes provide a barrier between two liquids and in order to achieve high vapor permeability, the membrane should poses high porosity [27]. Additionally to achieving high flux, a high porosity can also help to prevent membrane wetting [16]. The results for the porosity test are presented in Table 4 and they suggest that the PP membrane is more porous at 86% compared to PTFE at 50%. These results are in agreement with other studies by Shirazi [21]. Statistical analysis using ANOVA also suggested that exposure to RCWW does not affect the membrane porosity as the p-value for PP at 0 and 60 min is 0.27 and PTFE at 0 and 60 min is 0.44 which is greater than 0.05 which suggests there is no significant difference and that exposure to rendering RCWW has no impact on the porosity.

Table 4. PTFE and PP membrane morphology characteristics and porosity.

Time (min)	PTFE			PP		
	Porosity (%)	Pore Size (µm)	Fibre Diameter (µm)	Porosity (%)	Pore Size (µm)	Fibre Diameter (µm)
0	50 ± 1	0.39 ± 0.1	23.2 ± 2.9	86 ± 3	0.43 ± 0.1	12.1 ± 1.8
15	-	-	-	-	0.41 ± 0.17	9.1 ± 1.4
60	49 ± 1	-	-	83 ± 1	-	-
120	-	0.3 ± 0.07	17 ± 3.6	-	-	-

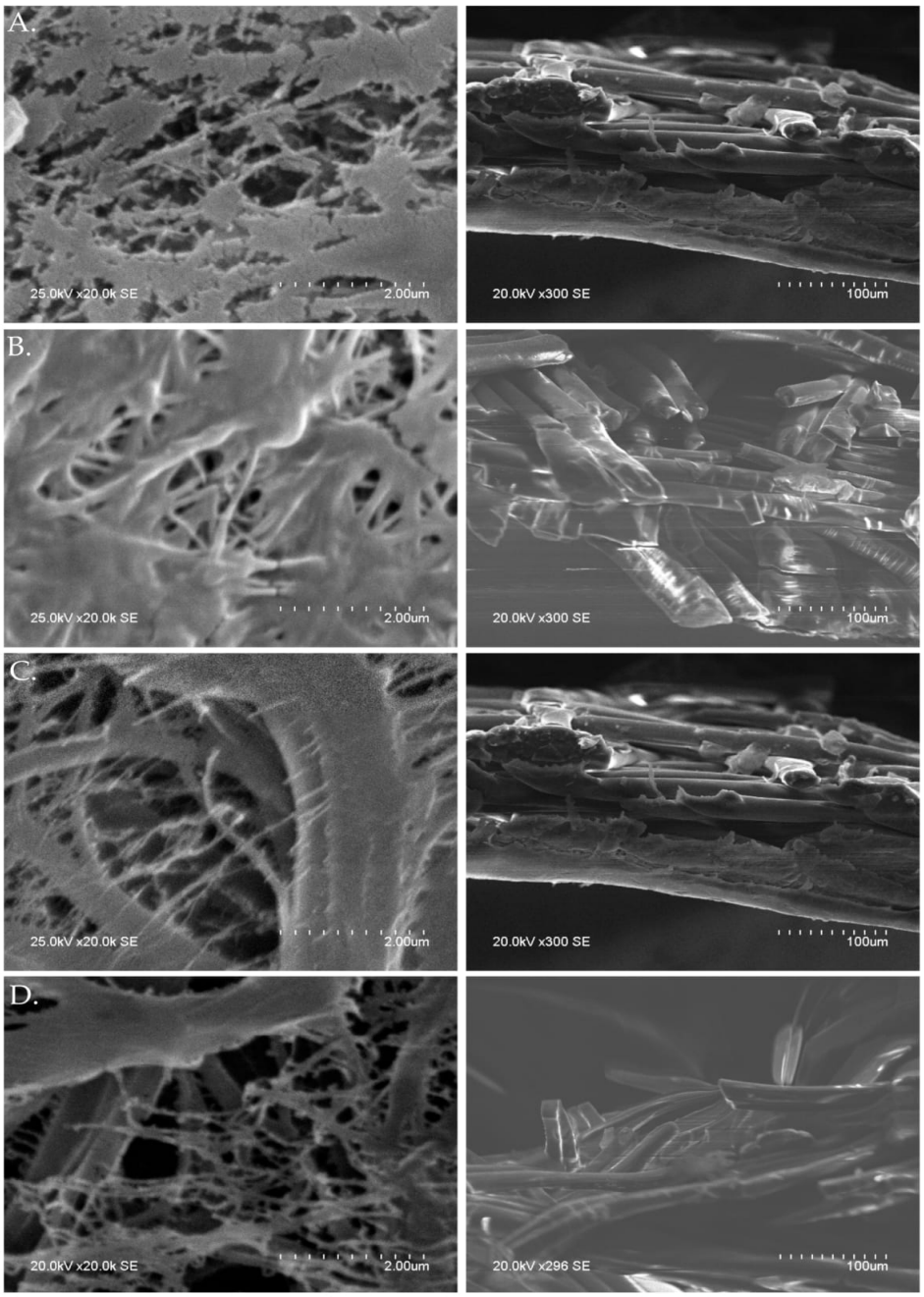

Figure 5. Surface and cross section SEM images for the morphologies of (**A**) PTFE with no RCWW exposure, (**B**) PTFE after 120 min RCWW exposure, (**C**) PP with no RCWW exposure and (**D**) PP after 15 min RCWW exposure.

2.3. Membrane Performance

2.3.1. (NH_3) Removal

A series of tests were carried out on the PTFE membrane to investigate the efficiency of (NH_3) removal from RCWW. RCWW was introduced into the system for testing and reproducibility was achieved by testing 2 modules of the same structure. As seen in Figure 1 the composition of the RCWW is different every day with different concentrations of different components (including (NH_3) and any possible wetting agents present. As seen in Figure 6, up to 64% (NH_3) was removed from the RCWW by the 1st module and up to 65% removal was achieved using the 2nd module. These levels of removal show great potential for the use of membrane systems for RCWW treatment as they can be used to reduce (NH_3) levels and reduce them further by installing a series of membranes in parallel. Additionally this process has a shorter retention time when compared with conventional methods and it produces a viable fertilizer product which can be sold. Module 1 shows to treat the RCWW efficiently for 10 days without cleaning the membrane and after the 10 days its removal efficiency shows to decrease. Module 2 was seen to be efficient for 6 days before its (NH_3) removal rate started to decrease. The differences in the efficiency of the 2 modules may have been due to the composition of the RCWW sample and an increase in wetting agents present for the 2nd module. However, the membranes present a promising method for treating (NH_3) in RCWW and could be further optimised by implementing a cleaning procedure for the membranes to prevent them being affected by wetting agents. The occurrence of wetting on the membrane materials can be combatted by introducing a cleaning procedure as described by Chen et al. [28] who used hexane and water in the cleaning in place (CIP) operation in order to clean emulsified membranes at risk of fouling and wetting and showed the flux to return to normal.

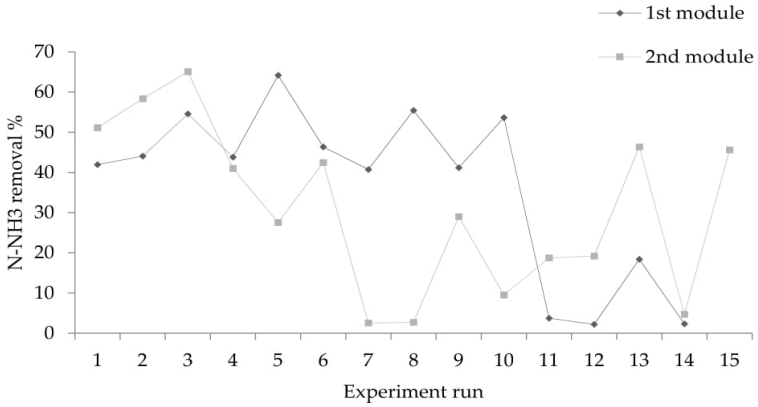

Figure 6. Performance of 1st and 2nd PTFE generation module (Flow feed side 0.05–1 L/h, permeate flow rate 0.05–0.5 L/h, pressure 0.5 Bar and temperature 50 °C).

The 2nd generation module used in this study was made from PP which failed initial clean water tests due to RCWW being present in the pipework of the system from existing tests. This suggested that PP was not compatible with RCWW and instigated further tests to be carried out to confirm this hypothesis.

2.3.2. Membrane Leakage

Another, much quicker test to determine membrane integrity is the leaking test. This is carried out by flushing clean water on the feed side of the membrane module in a recirculation mode and sulfuric acid on the permeate side. Conductivity is measured in the feed tank over time. Considering a

high conductivity gradient is present between the feed and permeate side of the membrane, if the membrane is leaking, then mass transfer is reversed and an increase in conductivity should be noticed on the feed side and ultimately in the CIP tank. The membrane material was exposed to RCWW at different time intervals (0 min, 10 min and 2 h) and it then underwent operation with clean water and sulfuric acid mixture. If leakage occurred after RCWW it would result in ions from the sulfuric acid passing to the feed side by electrolyte diffusion and increasing the conductivity of the CIP tank. The results after different RCWW exposure times are presented in Figure 7. The results suggest that the much higher conductivity rate after 2 h of exposure as oppose to 10 min exposure which suggests that 2 h exposure is wetting the membrane surface which in turn causes a mixing of the two liquids.

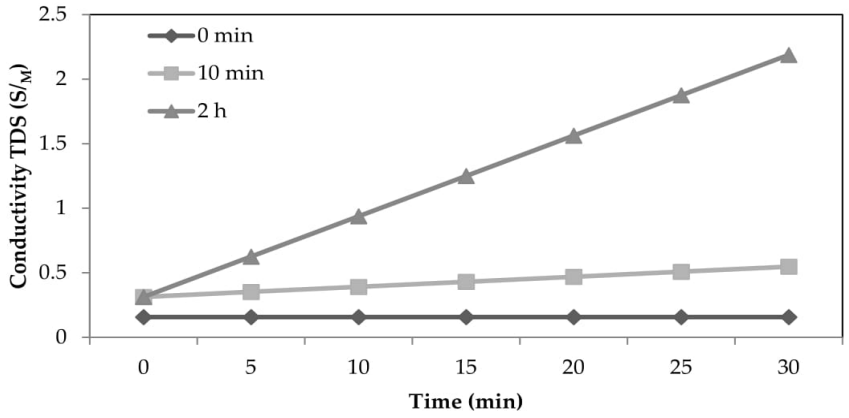

Figure 7. Rate of conductivity increase in the CIP tank after different RCWW exposure times using the PP membrane.

Results collected from the wetting and leakage tests for PP membranes suggest that PP is not as efficient as PTFE membranes for application to RCWW over long periods of time due to surface wetting. Differences observed between the surface wettability of DI water and RCWW determined that although such PP membrane could be used with other WW types they are not suitable for RCWW without frequent cleaning. In order to implement PP membranes, an appropriate automatic cleaning procedure could be implemented at set times in order to recover the membranes and allow them to be used for longer periods.

2.4. Product Characterisation

As stated above, after membrane diffusion NH_3 reacts with H_2SO_4 to form $(NH_4)_2SO_4$ as seen in Equation (1) [29]. The primary function of $(NH_4)_2SO_4$ is an agricultural fertilizer in alkaline soils to help promote growth. The ammonium ion is released and it undergoes deprotonation which produces NH_3 and results in lowering the pH of the soil. It also contributes nitrogen which is essential for plant growth. The sulfur promotes the metabolism of nitrogen, chlorophyll formation and forms amino acids which is the building blocks for proteins [2]. The product formed in this study was found to have a pH of 2–2.5 and a purity of 30%. Liquid products on the market were found to have a purity of 40% making our product a viable and green product, with economic benefit of valorisation of waste produced from the rendering plant [30]. The liquid $(NH_4)_2SO_4$ may be introduced to the soil by injecting it into the soil has been shown to be carried out by manual injection fertilizing (CULTAN) or by contactless high pressure jet injection [31]. Both methods work perfectly in normal conditions but the contactless method does not reach large depth in very dry soil.

The $(NH_4)_2SO_4$ solution produced here was found to be very acidic with a pH of 2–2.5. For a product to be of value for application to soils, the pH needs to be increased. A series of titrations were carried out to determine which reagent most efficiently increased the pH of the product (40 mL) based on the costs and quality. The reagents which were used include NaOH, sodium bicarbonate ($NaHCO_3$), calcium carbonate ($CaCO_3$), RCWW and sludge waste from the aeration tank. Table 5 shows how much of each reagent was required in order to increase the pH from ~ 2 to 7. It was found that RCWW had no effect on changing the pH. The aeration tank sample only changed the pH in one test. NaOH proved to change the pH using the least volume and it also had the smallest standard deviation. $NaHCO_3$ and $CaCO_3$ required larger volumes and thus NaOH was chosen as the most efficient reagent to adjust the pH of the product in order for it to be applied as land fertilizer.

Table 5. Titrations to investigate most efficient reagent to increase pH of product (titrand = 40 mL).

Reagent	Run 1 (mL)	Run 2 (mL)	Run 3 (mL)	Average (mL)	St. Dev (mL)
111 g/L NaOH	280.00	220.00	200.00	233.33	41.63
15 g/L $NaHCO_3$	3520.00	6300.00	4860.00	4893.33	1390.30
9.6 g/L $CaCO_3$	690.00	865.00	No change	777.50	123.74
RCWW	No change	No change	No change	-	-
Sludge	540.00	No change	No change	540.00	0.00

2.5. Molecule and Ion Movement through Membrane Material

RCWW contains high levels of NH_4 molecules which must be converted to NH_3 molecules in order to pass through the hydrophobic membrane (as shown in Figure 8). Once the RCWW solution containing NH_4 molecules is converted to NH_3 gas molecules, the NH_3 molecules are soluble within the RCWW solution and as it approaches the membrane surface it enters the membrane pores due to partial pressure between the feed and permeate side of the membranes (depicted as blue in Figure 8). The NH_3 vapor molecule passes through the membrane pores and approaches the surface of the permeate side immediately reacting with H_2SO_4 molecules (represented by red in Figure 8). $(NH_4)_2SO_4$ (represented by green in Figure 8) is produced from the reaction at the surface of membrane outside the pores which then circulates back to the permeate tank. As the reaction between these two happens immediately, it results in minimal NH_3 being present in the permeate side allowing for a high pressure difference between the feed and permeate side which would allow for the diffusion of NH_3 molecules across the membrane as long as there is $ions^+$ for the NH_3 molecules to react with. Surfactants such as fats and proteins result in wetting of the membrane which causes liquids to penetrate the membrane at the surface and eventually liquid to cross the barrier. Figure 8 shows how the surfactants can cause the membrane to leak and RCWW passes from the feed side to the permeate side and also the H_2SO_4 from the permeate to the feed side which results in the reaction to $(NH_4)_2SO_4$ in both the feed side and across the membrane material.

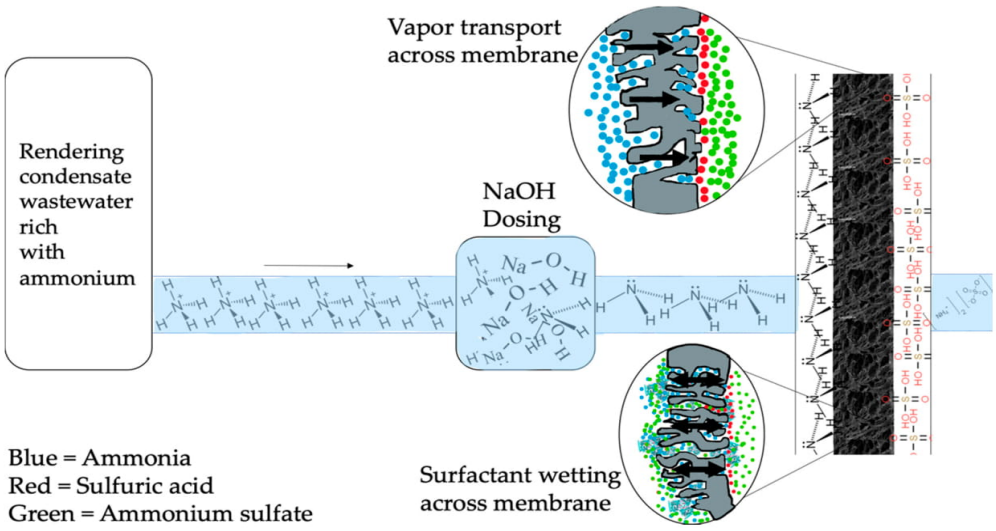

Figure 8. Molecular composition throughout membrane process.

2.6. Preliminary Life Cycle Assessment

A preliminary cost analysis on the membrane system to remove (NH_3) was carried out and compared with the current method being used to treat RCWW. The operational costs (including chemicals, energy consumption from heaters & pumps and filters) were calculated based on the assumption that the (NH_3) membrane system is in operation 7 days a week. The costs for the chemicals were based on the volume used in experiments throughout the project and the energy was estimated by measuring the energy usage with an energy meter. The results in Table 6, show that the capital cost of the current method is €500,000 and the operation costs (energy for aeration) equate to €1.71 per Kg of NH_3 removed. No viable product is produced from the current treatment, so no potential revenue is generated on it. The capital cost of the (NH_3) membrane removal system is €360,000 for the pilot and supply of membranes needed. The operational costs to remove one kg of NH_3 using the stripping pilot is a total of €2.48. However, the (NH_3) stripping unit produces $(NH_4)_2SO_4$ which can then be sold as a fertilizer at a price of €1.54 for $(NH_4)_2SO_4$ (30%) (*w*/*w*). This would suggest that the cost of treatment by (NH_3) stripping unit is €0.94 which is cheaper than the current treatment method. However, it should be noted that the current method is a continuous system which has less costs associated with ceasing and starting operation while the tested membrane system was only a batch system. Due to the two systems being different (continues vs batch) it is difficult to make a conclusive comparison. Considering the capital costs and operation costs of the two treatment methods, it can be suggested that preliminary results suggest that (NH_3) removal using membrane technology is a cheaper method as both capital and operation costs are cheaper.

Table 6. Summary of cost benefit analysis ([a] 0.009 € kWh^{-1} – was used to estimate the cost. The energy usage (€/kg N) was calculated from the total ammonia removed/year and total blower energy use/year (data obtained from 2016 plant site)).

	(NH_3) Removal Using Membrane Technology (70% Efficiency)			**Current Treatment**	
CAPEX	€300,000 (full scale) + €36,000 (membranes)			€500,000 (Aeration tank + diffusers, blowers)	
	(€/kg N)			(€/kg N)	
	NaOH	H_2SO_4	Energy [a]	Energy	Bio- augmentation
OPEX	1.54	0.71	0.26	1.065	0.645
	Maintenance costs (pre-filters)			Maintenance cost	
	0.0012			ND	
OPEX total	2.48			1.71	
OPEX—PC	0.94			1.71	
Product capitalization (PC)	$(NH_4)_2SO_4$ (30%) (*w*/*w*) 1.54			NA	

3. Materials and Methods

3.1. Materials

The pilot study was carried out on site in a meat processing plant located in ABP Food Group, Kilcommon, Co. Tipperary, Ireland. Figure 9 shows the conventional method of treatment which is being used in the plant using biological nitrification in an aerobic tank. (NH_3) rich WW was obtained from RCWW produced by a rendering plant processing animal products. The (NH_3) stripping unit and membrane materials (PP and PTFE) were designed and sourced from BLUE-tec bv, Industrieweg 16, 6871 KA, Renkum, The Netherlands. Bulk NaOH (30%) (*w*/*w*) and H_2SO_4 (96%) (*w*/*w*) which were of analytical grade were procured from Brenntag Chemicals Distribution LTD, Ireland. $(NH_4)_2SO_4$ (99%) (*w*/*w*%) was procured from Fischer, Ireland and is of an analytical grade.

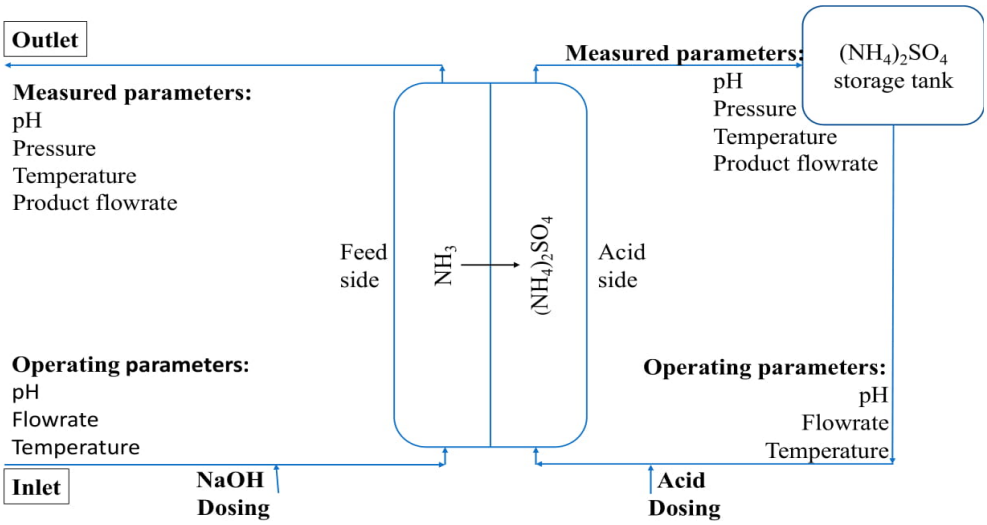

Figure 9. Simplified schematic of process overview (not to scale).

3.2. Characterisation of Membranes

Membrane samples were initially investigated in triplicate prior to being exposed to RCWW and investigated again in triplicate after being exposed to RCWW at different time intervals (0, 15, 30, 45, 60,

120, 180, 240, 300 and 360 min). After exposure to RCWW, samples were sprayed with DI water for 15 s and dried overnight at 103 °C. This investigation allows for the effect of RCWW exposure over time on the membranes to be investigated. The surface and cross-sectional morphologies including the fibre size of both PP and PTFE membranes were obtained using SEM (Hitachi S3400n SEM, Tungsten system). Samples were gold coated and carried out with 20 mV acceleration. The sample pore size and pore size distribution were also investigated using the same SEM instrument and conditions using Image J image analysis software. Surface roughness was quantitatively determined using AFM (BRUKER ICON DIMENSION AFM) using Silicon AFM probes in non-contact/tapping mode (13 kHz resonant frequency and 0.2 N/m force constant), procured from Nano and More, UK. Membrane porosity was performed as outlined by Woo et al., 2017. The surface energy of the PP and PTFE membrane were determined by measuring the CA (FTA200 Dynamic Contact Angle Analyzer) using deionized (DI) water (Elga Purelab Ultra system) in order to determine the hydrophobicity of the membrane surface. LEP is the minimum pressure applied to a dry membrane which results in liquid penetrating inside the membrane pores. LEP was investigated using a LEP set-up with Amicon Test Cells and carried out as outlined by Smolders and Franken [32].

3.3. Pilot Operation

The (NH_3) stripping unit was designed according to requirements and to feature automated, unattended operation, data logging, and remote access. The specifications for two modules evaluated in the investigation are summarized in Table 7. The membrane materials which were used were PP and PTFE.

Table 7. Summary of the specifications for the membrane modules tested.

	Membrane 1	**Membrane 2**
Membrane material	PTFE	PP
Configuration (type)	Spiral wound	Spiral wound
Surface area (m^2)	6.7	3.7
Flow feed side (m^3/h)	0.05–0.7	0.05–1
Flow acid side (m^3/h)	0.05–0.7	0.05–0.5
Pressure max (bar)	0.6	0.5
Temperature max (°C)	45	50

All experiments were conducted in a pilot scale system module as described briefly in the schematic diagram in Figure 9. The (NH_3) rich RCWW sample was obtained from the rendering plant waste which is being fed into the nitrification tank (AT3 tank in Figure 10) in the ABP WW treatment facility. The RCWW samples pass through a series of heaters and NaOH dosing pumps to ensure that the temperature and pH are at the optimum conditions to ensure the (NH_3) is in its volatile gaseous form. The RCWW sample then passes through a settler tank and 50 µL filters to ensure the RCWW sample does not have particles present which may clog the membrane pores. Once the sample is in its volatile gaseous form the RCWW stream is pumped into the feed side of the hollow fibre membrane whilst the stripping solution, H_2SO_4, flows along the permeate side of the hydrophobic membrane. The gaseous (NH_3) diffuses through the hydrophobic membrane and reacts with the H_2SO_4 on the permeate side. The reaction that occurs between (NH_3) and H_2SO_4 can be seen in Equation (1) which produces $(NH_4)_2SO_4$. Both solutions on either side are then recycled to their respective reservoirs which can be seen in Figure 9.

$$2NH_3 + H_2SO_4 \rightarrow (NH_4)_2SO_4 \quad (1)$$

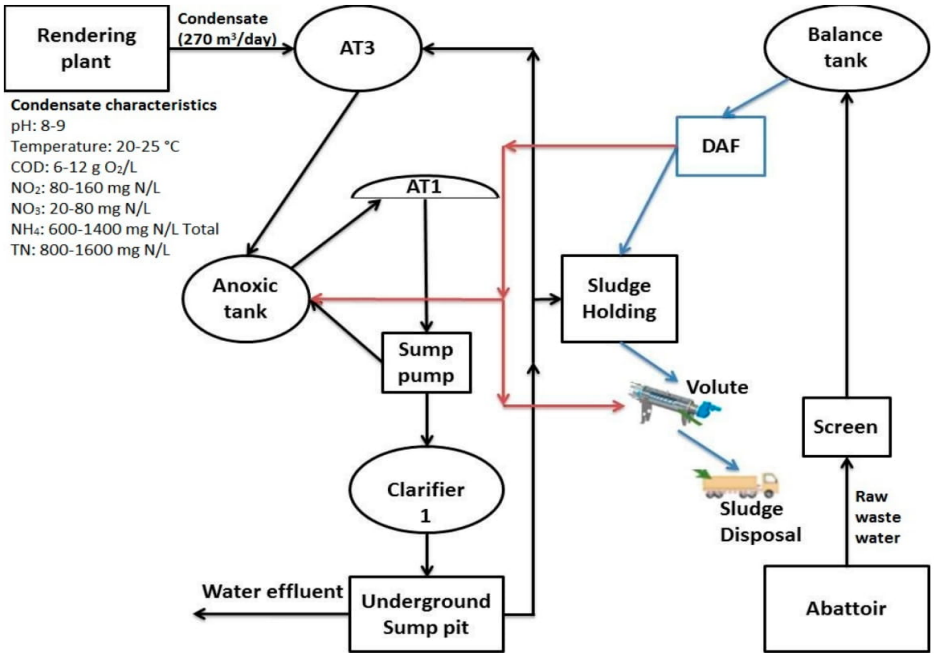

Figure 10. Graphical representation showing the configuration of the current, activated sludge WWTP and characterization of RCWW. AT1 = Aeration Tank 1, AT3 = Aeration Tank 3, DAF = Dissolved Air Flotation.

3.4. Physicochemical Characterisation of RCWW

The RCWW samples were analyzed to characterize them according to their physiochemical properties including pH, temperature and turbidity. By investigating these properties, pre-treatment steps of the RCWW samples were then formulated depending on the results. The temperature was measured using a YSI Proplus® handheld multi-parameter instrument (YSI, Xylem, Hertfordshire, UK) and the pH was tested using a WTW Multi 320 multimeter, pH electrode SenTix 4l. The turbidity of the sample was analyzed using a portable turbidity meter Turb® 430 IR (VWR, Dublin, Ireland). Particle size distribution was carried out using a Malvern Mastersizer 3000E using a Hydro EV wet dispersion unit procured from Malvern Panalytical with a stir speed of 8001200 RPM and sonification of 50%. An investigation was carried out in order to determine the relationship between the temperature and pH of the RCWW samples. Titration experiments were carried out at different temperatures to determine the required volumes of NaOH to raise the pH to optimum levels for (NH_3) removal. Particle size distribution and turbidity in the RCWW samples was investigated to determine if any pre-treatment is required to protect the membranes from clogging.

4. Conclusions

This paper describes the first pilot application of hydrophobic membranes for the removal of (NH_3) from raw condensate wastewater from the rendering operation of a meat processing plant. Hydrophobic membrane contactors are a relatively new process which have the potential to remove (NH_3) from WW. RCWW waste is produced from the processing of meat industry waste and is highly concentrated with (NH_3). To the authors knowledge no literature or studies have focused on the use of hydrophobic membranes for treating (NH_3) in RCWW waste. In this study, the physicochemical properties of RCWW are characterized, and the optimal pre-treatment steps are determined. The efficiency of PTFE and PP

membranes was investigated for NH_3 removal. The PTFE and PP membranes were characterized and the effect RCWW posed on the membranes was investigated. Additionally, the $(NH_4)_2SO_4$ product was characterized and the cost comparison between the membrane method and the conventional method was analyzed. The results showed that the pH changes depending on the temperature of the RCWW and as the temperature increases, the pH increases which results in an decreased volume of NaOH being required to achieve the optimum pH to change the NH_4 into gaseous NH_3. Characterization of the PTFE and PP membranes showed that PTFE membranes were slightly more hydrophobic with a higher CA, surface roughness and LEP. Characterization studies also showed that membrane exposure to RCWW over time affects the structure of the membranes. Analysis of PTFE membranes showed that the membrane method was efficient and that there was up to 65% removal of NH_3 removal from the RCWW. However, the PP membrane failed initial water tests and it was concluded that the RCWW contaminated the PP membrane and caused wetting. PTFE membranes may be suitable if they were set up in parallel to allow for multiple treatment steps. The PTFE membranes could also be used as a pre-treatment step to the conventional aeration method to help reduce costs. Additionally, a cost benefit analysis was carried out and showed that the cost of running the NH_3 stripping unit was more expensive than current treatment methods at €1.71 and €2.48, respectively. However, the NH_3 stripping unit produces $(NH_4)_2SO_4$ which can be sold as a fertilizer and reduces the operational product capitalization. Future work should focus on the composition of the RCWW and identify reasons for wetting and fouling of the membrane. Compatible materials which are capable of withstanding the condensate WW should also be identified for hydrophobic membrane fabrication.

Supplementary Materials: Supplementary materials can be found at http://www.mdpi.com/1422-0067/21/11/3914/s1.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

NH_3	Ammonia
WW	Wastewater
RCWW	Rendering condensate wastewater
NH_4	Ammonium
PP	Polypropylene
PTFE	Polytetrafluorethylene
FOG	Fat, oil and grease
NaOH	Sodium hydroxide
$((NH_4)_2SO_4)$	Ammonium sulphate
LEP	Liquid entry pressure
H_2SO_4	Sulfuric acid
CA	Contact angle

DI water	Deionised water
$NaHCO_3$	Sodium bicarbonate
$CaCO_3$	Calcium carbonate
CAPEX	Capital expenditure
OPEX	Operational expenditure
PC	Product capitalization
SEM	Scanning electron microscopy
AFM	Atomic force microscopy

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