Biomass and Bioenergy 144 (2021) 105902

Contents lists available at ScienceDirect

Biomass and Bioenergy

journal homepage: http://www.elsevier.com/locate/biombioe

Optimisation of anaerobic digestion of pot ale after thermochemical pre-treatment through Response Surface Methodology

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ARTICLE INFO

Keywords:
Pot ale
Anaerobic digestion
Thermochemical pre-treatment
Response surface methodology
Optimisation

ABSTRACT

Valorisation of organic wastes in renewable energy production has attracted a global attention in order to achieve a sustainable industrial growth. Anaerobic digestion (AD) is considered to be one of the most environmentally friendly waste management technology and also fulfils the necessity of a sustainable fuel generation. It has much less energy demand than other technologies like gasification or pyrolysis due to its low operation temperature. Whiskey distillery wastewater, pot ale, is classified as a high strength waste due its high organic content hence rendering it a suitable substrate for anaerobic digestion. Despite its waste-to-energy conversion potential, pot ale contains high lignin fraction which makes it resistant to biodegradation. Therefore introducing a pre-treatment step is required to enhance the biogas production and organic matter degradation. In this study anaerobic digestion of pot ale was assessed at lab scale batch mode reactor after implementation of 1 M NaOH and microwave pre-treatments at varying power settings. Response Surface Methodology was adopted for process modelling and optimisation in which inoculum substrate ratio, initial digestion pH and microwave power were investigated at three different levels. In addition, the mineral quality of the pot ale digestate has been analysed for its agricultural use.

1. Introduction

Increased need for expanding the renewable energy use in a sustainable manner as boost the second generation biofuels research as the Renewable Energy Directive II has set the overall EU renewable energy target to 32% with a 7% cap on the first generation biofuels by 2030 [1]. The major drawback of the first generation biofuels is known to be their indirect land use and its potential negative impact on the food industry due to increased demand on animal based diet [2–4]. Furthermore, sustainability issues such as water usage and greenhouse gas emission risk in land preparation step for crop growth arose from first generation biofuel production [3]. Therefore, second generation biofuel production technologies are attracting more and more interest. Among the second generation biofuels, biogas (produced by anaerobic digestion) is considered as one of the most environmental friendly fuel due to its non-toxic and easy and varying use [5].

Whiskey is one the most consumed alcoholic beverage globally correspondingly distilling industry has recently grown in countries as such Ireland and the UK [6,7]. Due to the high organic content of the liquid waste stream, known as pot ale originating from the bottom of the copper distilling stills, as well as large discharge volumes (approximately 8–15 L aqueous waste per litre of malt whiskey [8]), alcoholic beverage industry is highly polluting [9]. Uncontrolled pot ale release to the water bodies severely threatens the aquatic life in different ways i.e. decreasing the solubilised oxygen due to its high chemical and biological oxygen demand, potentially causing eutrophication and restricting photosynthesis by blocking the sunlight due to its dark colour. Excessive land and landfill applications on the other hand, are commonly associated with inhibition of seed germination and agricultural crops as a result of its acidic nature [7].

Not only the high organic content but also the yearlong supply of pot ale with no seasonal effects renders it a promising feedstock for second generation biofuel in particular biogas production through anaerobic digestion (AD). Volatilizing pot ale as feedstock of AD is also considered to be a sustainable waste management method for the distilling industry where produced biogas can be used in the manufacturing. Furthermore,

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https://doi.org/10.1016/j.biombioe.2020.105902
Received 20 July 2020; Received in revised form 5 November 2020; Accepted 22 November 2020

the co-product of the anaerobic digestion, called digestate, is considered as a safe, eco-friendly biofertiliser due its rich inorganic content – a potential superior replacement to conventional chemical fertilisers [10]. In the process of AD biochemical reactions take place at parallel steps knowns as hydrolysis, acidogenesis, acetogenesis and methanogenesis [11]. For a well-balanced reactor operation, identical reaction rates are desired to prevent accumulation of intermediate products such as volatile fatty acids (VFAs) [12,13]. Accumulation of VFAs might be inhibitory for methane production due to the pH sensitivity of the methanogenic bacteria [14–16]. Hydrolysis is the rate limiting step for AD of lignocellulosic material due to rigid, impermeable nature of lignin as well as its resistance to microbial attack and oxidative stress [17]. A pre-treatment step is therefore commonly required prior to AD of pot ale in order to eliminate structural resistance arising from the presence of lignin. Furthermore, presence of sulphate in the substrate results in formation of H_2S as a result of thermodynamically more favourable side reaction known as sulphidogenesis than methanogenesis [18]. The current state of art technology for AD of whiskey distillery waste streams has recently been reported in detail along with applied pre-treatments [7].

Chemical pre-treatments are well accepted and commonly applied on various lignocellulosic substrates prior to AD. For AD of pot ale alkaline pre-treatments are considered to be more suitable due to the necessity of neutralisation of acidic nature of pot ale [8,19]. Application of the alkaline pre-treatment reduces degree of polymerisation and crystalline structure of cellulose in addition to providing partial hydrolysis and solubilisation of hemicellulose and lignin [7,20–22].

Thermal pre-treatments are also widely applied on lignocellulosic materials. Microwave pre-treatment in particular, increases intracellular compounds by means of cell wall lysis and gel structure degradation [23,24]. It also creates continuous alignment and realignment of macromolecules in polar liquid and result in breakage of hydrogen bonds, generation of frictional heat, modification of the hydration zone, increased solubilisation of sludge, improvements of volatile solids destruction and enhancement of biogas production [23–25]. The success of microwave pre-treatment depends on parameters such as the microwave power and intensity (penetration depth), the contact time, and the temperature [23,26].

In this study, combined alkaline and microwave pre-treatment on pot ale is introduced as a hybrid thermochemical pre-treatment method prior to AD of pot ale. Moreover, a detailed pot ale characterisation along with the lignocellulose fractions as well as the impacts of applied thermochemical pre-treatment on the lignocellulosic fractions has been assessed. To the best of authors' knowledge, these approaches have never been performed on pot ale prior to anaerobic digestion In addition, micro and macro nutrient content of pot ale based digestate is introduce to literature for evaluation of its potential agricultural use. Finally, AD of thermochemically pre-treated pot ale is modelled and optimised to achieve maximum CH_4 yield and minimum CO_2 generation at the same time by using Design Expert Software.

2. Materials and methods

2.1. Feedstock and inoculum

Pot ale was obtained from a small-scale whiskey distillery in Dublin, Ireland. Activated sludge with a 6% dry matter was used as inoculum source and it was collected from a full scale biogas plant processing industrial food waste operating within mesophilic range, along with an industrial digestate sample which is used as biofertiliser based in Ireland.

2.2. Analytical methods

Solid content of pot ale sample as received was determined in terms of total solids (TS), volatile solids (VS) and moisture content (MC) according to Ref. [27]. The concentrations of nitrogen in forms of nitrite (Ferrous Sulphate Hach Method 8153) and nitrate (Cadmium Reduction Hach Method 8039) were determined using Hach kits for DR 2000 Spectrometer, whereas ammonia (Salicylate Test N Tube Hach Method 10031) was measured using a Hach DR 900 Spectrometer.

Chemical oxygen demand (COD), biological oxygen demand (BOD) and sulphate concentrations were determined before to and after AD to assess the efficiency of organic matter degradation though methanogenesis and sulfidogenesis pathways, as well as for characterising pot ale as received. The concentrations of COD and sulphate were measured according to Hach Method 8000 by using standard Hach high range plus vials (200–15000 mg L^{-1}) and USEPA Sulfa-Ver 4 Hach Method 8051 provided by Hach Lange Ltd., using a DR 2000 spectrometer. Lovibon BD600 unit provided by Lennox Ltd., Ireland was used to determine BOD concentrations. The supernatant of all samples were analysed (before and after anaerobic digestion) in the form of a mix of sludge after centrifuging at 10000 rpm for 30 min using a Sorvall RC 5B Plus centrifuge in order to prevent the risk of masking high solid content sludge on organic content [28,29]. Pot ale samples were dried at 35 °C, grounded and sieved to obtain 0.5–1 μm radius for determination of hemicellulose, cellulose and lignin fractions in accordance with the detergent method developed by Ref. [30,31]. Lignocellulosic fractions of pot ale were analysed before and after the application of alkaline pre-treatment stands alone as well as in combination with microwave pre-treatment for assessing the amendments in the lignocellulose fractions with regard to the applied pre-treatment.

Pot ale was also characterised in terms of common short chain volatile fatty acids (VFAs), such as acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid and valeric acid were measured by using gas chromatography (Agilent 7890) with an Agilent CP 7686 column. In addition to the characterisation purpose, VFAs were determined in inoculum substrate mix prior to and after AD to assess potential VFA inhibition.

Finally, the mineral quality of untreated pot ale, inoculum and selected digestate sample were determined in terms of phosphorous (P), potassium (K), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn) and zinc (Zn) using ICP-OES (Inductively Coupled Plasma - Optical Emission Spectrometry, Agilent 5100). Furthermore, an industrial digestate (currently being used as biofertiliser) sample was collected, from an anaerobic digestion plant processing food waste, and analysed and used as reference material to assess the quality of pot ale digestate and its potential for agricultural use.

2.3. Pre-treatment methods

Alkaline pre-treatment was conducted by using 1 M NaOH solution prior to microwave pre-treatment. 1 M NaOH solution (approximately 30 ml) was added dropwise into 400 ml pot ale until pH 10 was reached, it was then stirred for 6 min at 600 rpm according to significant results previously published [32]. Microwave pre-treatment was performed by using a Sharp Compact microwave oven with an 800 W maximum power. The time of microwave pre-treatment was decided as 11 min based on the temperature profile (given in Table 1) created with 10, 30 and 50% of overall power, as a longer period of time at lower power setting is advised in the literature in order to avoid potential inhibition arising from Maillard reactions [22] as well as production of phenolic compounds and furfurals as a result of high temperature (>150 °C) thermal pre-treatment [33–35]. The temperature of the pot ale sample treated at 30% power (240 W) reached the boiling point after 11 min of treatment, whereas the samples treated with 50% power (400 W) reached the boiling point after 7 min and kept boiling for 4 min. In the case of the 10% power (80 W), the sample could only have reached 66 °C after 11 min. In other words, following chemical pre-treatment, pot ale was subjected to low temperature thermal pre-treatment by using microwave in order to benefit from both thermal and athermal impacts [36] while avoiding the formation of the inhibitory compounds as well

Table 1
Temperature profile of microwave pre-treatment on alkali pre-treated pot ale.

Time of Pre-treatment (min)	Temperature Profile (°C)		
	10% (80 W)	30% (240 W)	50% (400 W)
1	20	30	36
2	25	38	50
3	29	46	62
4	33	54	74
5	38	61	84
6	42	69	95
7	47	77	100
8	51	84	103
9	55	90	105
10	60	95	107
11	66	99	110

as minimising the energy demand for the pre-treatment.

2.4. *Anaerobic digestibility*

A lab scale batch reactor was operated for anaerobic digestion. Each reactor consisted of 500 ml glass flasks with an active volume of 400 ml and the biogas sampling bags. The bioreactors were filled with 1 M NaOH and microware (10, 30 and 50%) pre-treated pot ale sample with inoculum substrate ratios (I/S) of 1, 3 and 5 on VS basis. No media was used to dilute pot ale. After mixing inoculum and substrate the pH for each sample was measured by using a Hanna precision pH meter (accuracy ± 0.01), model pH 213. The pH levels were then adjusted to 7, 8 and 9 by using buffer solutions to assess the impacts of initial pH values on AD [37]. Controls were prepared with I/S of 1, 3 and 5 on VS basis then the pH levels were adjusted to 7, 8 and 9. All reactors were then sealed with glass adapters with controlled valves and each system was purged with nitrogen gas for 5 min to ensure anaerobic conditions. All reactions were carried out in triplicates and the incubation time was set at 21 days. The produced biogas during digestion was stored in airtight Sigma foil gas sampling bags and measured after 2, 6 days and at the end of the digestion by using Geotech Biogas 5000 gas analyser. Memmert water baths were employed to ensure operation mesophilic temperature 35 ± 1 °C.

A kinetic study was conducted in order to evaluate the effects of pre-treatments on reaction rates by means of comparing the hydrolysis rate constants [38]. The first order kinetic model (Eq. (1)) was fitted to the CH_4 yield to evaluate the influences of the pre-treatment by comparing the hydrolysis constant. A paired *t*-test was used for statistical analysis.

$$B_t = B_0\left[1 - e^{(-k_h t)}\right] \tag{1}$$

where; B_t (ml CH_4 gVS^{-1}) is the cumulative CH_4 yield at time t.

B_0 (ml CH_4 gVS^{-1}) is the ultimate CH_4 yield,
k_h (d^{-1}) is the first-order hydrolysis constant.
t (d) is the time of digestion.

2.5. *Response Surface Methodology (RSM)*

RSM provides combination of statistical and mathematical techniques which are used for process modelling, interpreting and predicting the impact of several input variables on the response of interest "y" which is methane yield in this case. A Box Behnken Design (BBD) assessing three numerical factors (A: I/S, B: Initial pH and C: Microwave power) at three levels was adopted as RSM in Design Expert software version 10 due to its ability to model with the least number of experiments. The levels of each design factors are given in Table 2 along with the coding format. Levels of the design factors were decided based on preliminary significant results as well as previously published research by Ref. [38,39].

Table 2
Summary of the design factors and the responses.

Parameters under investigation (Design factors)	Actual Levels	Coded Levels	Responses
I/S	1	−1	Cumulative CH_4 Yield (ml gVS^{-1})
	3	0	
	5	1	Cumulative CO_2 Yield (ml gVS^{-1})
Initial pH	7	−1	
	8	0	Cumulative H_2S (mg L^{-1})
	9	1	
Microwave Power (%)	10	−1	
	30	0	
	50	1	

BBD combines two level factorial design and adds several centre points [40]. In this study, investigation of three factors, the total number of the design points was equal to 12 and then 5 centre points were added, making 17 design points. Recorded experimental data was examined by stepwise regression and second order polynomial model (given in Eq (2)) has shown the best fit in order to identify the impact relevant mathematical model terms. The mathematical model was then used for data interpretation and prediction.

$$Y = b_0 + \sum b_i X_i + \sum b_{ii} X^2{}_{ii} + \sum b_{ij} X_i X_j \tag{2}$$

The adequacy of the developed model was tested with sequential lack of fit test and regression analysis (such as R^2, Adjusted R^2 and Adequacy Precision ratio) as well as the analysis of variance (ANOVA). Only when *p* value of the model and of each term in the model was smaller than significance level, which was $\alpha = 0.05$, is considered to be adequate within the confidence interval (1-α) [41–43]. The developed models were further challenged by independent validation experiments and paired *t*-test was conducted for statistical analysis of the differences between predicted results and validation experiment results. The models passed all significance tests subsequently, it was optimised by in accordance with combination of numerical and graphical optimisation by employing the desirability function explained in detail by Pandian et al., 2011 offered by DOE software.

3. Results and discussion

3.1. *Pot ale characterisation and modifications in lignocellulose fractionations*

Characteristics of non-treated pot ale sample are given in Table 3.

Table 3
Characteristics of pot ale as received.

Pot Ale Characteristic	Value
TS (g $gsample^{-1}$)	0.089 ± 0.0004
VS (g $gsample^{-1}$)	0.077 ± 0.008
Moisture %	91.13 ± 0.042
pH	4.5 ± 0.3
COD (mg L^{-1})	38867 ± 115
BOD (mg L^{-1})	30965 ± 666
SO_4^{2-} (mg L^{-1})	190 ± 31
VFAs (mg L^{-1})	$8.05 \times 10^9 \pm 8.57$
Hemicellulose (%)	11.5 ± 0.3
Cellulose (%)	10.6 ± 1.8
Lignin (%)	26.9 ± 1.6
N–NO_3^- (mg L^{-1})	111 ± 20
N–NH_3 (mg L^{-1})	45 ± 7
N–NO_2^- (mg L^{-1})	33 ± 4
P (mg L^{-1})	476.5 ± 11.8
K (mg L^{-1})	954.2 ± 11.3
Cu (mg L^{-1})	53.04 ± 3.21
Fe (mg L^{-1})	2.55 ± 0.21
Mg (mg L^{-1})	107.28 ± 0.44
Mn (mg L^{-1})	0.30 ± 0.01
Zn (mg L^{-1})	0.49 ± 0.01

The lignocellulosic composition of non-treated, alkaline (1 M NaOH) and combined alkaline and microwave (at 10, 30 and 50% power) pre-treated pot ale are given in Fig. 1. Pre-treated samples had a significant increase ($p < 0.05$) in the hemicellulose fraction as well as a significant delignification ($p < 0.05$) indicating an enhanced biodegradability in comparison to the non-treated pot ale (Control). The maximum lignin removal was achieved when 1 M NaOH pre-treatment was followed by 30% microwave pre-treatment reducing the lignin composition to 13.2 ± 1.3% from 26.9 ± 1.6% (Control in Fig. 1). Moreover, combining alkaline pre-treatment with 10 and 30% microwave pre-treatments has further enhanced the hemicellulose fraction to 27.0 ± 4.5% and 32.9 ± 0.5% in comparison with 1 M NaOH pre-treatment with the p values of 0.0140 and 0.0005 respectively. However, combining 1 M NaOH pre-treatment with 50% microwave pre-treatment caused a decrease in the hemicellulose fraction. Hemicellulose is the most thermo-chemically sensitive component of the lignocellulosic structure [21]. Therefore the decrease was attributed to hemicellulose hydrolysis as a result of high microwave power treatment [33]. Alkaline pre-treatment followed by 30% microwave power has shown a significant reduction in cellulose fraction (5.3 ± 0.2%) with regard to the alkaline pre-treatment alone 10.7 ± 0.2 with a p value of 0.0053.

3.2. Methane yield and organic matter degradation

BBD matrix for modelling combined alkaline and microwave pre-treatments and recorded responses (cumulative CH_4, CO_2 and H_2S production) are given in Table 4. In addition to the design points, biogas results of the non-treated pot ale as control group (with 3 different inoculum ratios) are given in Table 4 along with corresponding responses. The initial pH value was found to be an insignificant parameter for the methane yields based on the *t*-test carried out on methane yields achieved by experiments at different initial pH values, therefore only pH 8 adjusted controls were included to Table 4. Standard numbers (std) were assigned to different reaction conditions presented in Table 4 in order to simply the referencing within the text. Significant enhancements in CH_4 yields were seen due to the applied thermochemical pre-treatment in comparison with the control for all inoculum substrate ratios (I/S). The highest methane yield of 1614 ± 168 ml CH_4 gVS^{-1} was achieved when alkaline pre-treatment followed with 30% (240 W) microwave pre-treatment on pot ale with an I/S of 5 on dry basis (std no 4 in Table 4). The methane yield of std 4 has shown a more than 3-fold increase over the control (Control 3 in Table 4). The significant enhancement on the methane yield was explained by the increased hydrolysis kinetics due to the amendments on lignocellulosic structure of pot ale as a result of thermochemical pre-treatment. The hydrolysis constant of std 4 was found to be 0.3041 ± 0.041 day^{-1} whereas it was only 0.2617 ± 0.003 day^{-1} for the corresponding control (Control 3) after the first 6 days of AD ($p < 0.05$). On contrary, no significant difference was seen in the hydrolysis constants after the first 2 days of AD.

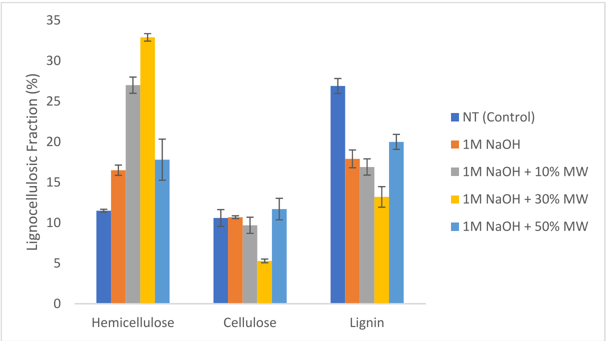

Fig. 1. Lignocellulosic structure of pot ale before and after alkaline, combined alkali-microwave pre-treatments.

The delay was attributed to slow microbial grow rate as a result of bacterial acclimation to the alkalinity arising from the chemical pre-treatment applied [45]. Pot ale samples pre-treated under the same conditions resulted in higher CH_4 yields when the I/S was higher. For instance, std 2, 4, 6, 8 seeded with an I/S of 5 (Table 4) had the same pre-treatment and initial pH level with std 1, 3, 5, 7 seeded with an I/S of 1 respectively and significantly higher CH_4 yields were achieved by std 2, 4, 6, 8. Among the samples containing an I/S of 5 (Std 2, 4, 6, 8), the highest methane yields were observed as 1430 ± 50 (std 2) and 1614 ± 168 ml gVS^{-1} (std 4) when 1 M NaOH pre-treatment was combined with 30% microwave power as a result of significantly higher delignification due to the applied pre-treatment (Fig. 1). Despite lower lignin degradation capacity of 1 M NaOH and microwave pre-treatment at 50% power, std 8 had a considerably high methane yield (1019 ± 51 ml gVS^{-1}). This was attributed to achieving a greater reduction in particle size and correspondingly greater increase in surface area as a result of higher microwave power delivered in comparison to 30% [36].

On the other hand, the highest H_2S concentrations (>1000 mg L^{-1}) were seen in the samples (std no 1, 3, 5, 7 and Control 1) which had the lowest I/S indicating a bacterial completion between methanogens and sulphate reducing bacteria as such higher H_2S generation was observed as a result of sulfidogenesis [7,38,46]. The starting pH value of the digestion has no significant effect on the methane yield and the pH remained within the neutral range (6.8–7.4) for the entire digestion period for all samples.

The efficiency of AD was measured with the organic matter removal percentages in terms of COD, BOD and SO_4 [47–49] and the analysis results are given in Table 5. SO_4 removal occurs as result of side reaction where sulphate is converted to sulphur by sulphate reducing bacteria to produce H_2S [50]. In anaerobic digestion process methane forming and sulphate reducing bacteria competes for H_2 at parallel steps in which the activity of the sulphate reducing bacteria is thermodynamically more favourable [46]. Therefore, SO_4 removals were measured to monitor potential inhibitions on methanogenic activity. The highest COD (69 ± 1.4%) and BOD (66 ± 1.7%) removal percentages were achieved with the std no 4 and the std no 1 respectively where the pre-treatment conditions were 1 M NaOH in combination with 30% (240 W) microwave. It was attributed to achieving an increased surface area and soluble organic matter in the reaction environment [51] as well as lignocellulosic structure amendments [52] due to the applied low temperature thermochemical pre-treatment. Furthermore, the SO_4 removal of std no 4 was found to be the lowest, 40 ± 8.3%, among the samples seeded with an I/S of 5. Std no 4 has also shown the highest CH_4 yield (Table 4) indicating that bacterial competition between methanogenesis and sulfidogenesis stages was in favour of methanogenesis [53–55] therefore, the organic matter was broken down through methanogenesis pathway predominantly. As the inoculum substrate ratio directly linked to the process kinetics, higher inoculum ratios results in greater organic matter degradation by methanogens [56]. On the other hand, higher COD and BOD removals were seen when microwave pre-treatment applied at higher power for the I/S of 1. For example, COD and BOD degradations of std no 7 were found to be significantly higher than std no 5 according to the applied *t*-test with the p values of 0.001 and 0.010 respectively. On contrary, std 6 has shown a significantly greater COD and BOD removals than the std 8 with according to the p values of 0.023 and 0.037 respectively indicating that lower microwave power achieved higher biodegradation when the I/S was 5. These results are explained by the necessity of the high microwave power pre-treatment to achieve a greater substrate solubilisation when the reaction rate is slow as a result of low inoculum substate ratio [56,57]. Various levels of SO_4 removals ranging from 40 ± 8.3 to 78 ± 0.9% were seen. No VFA was detected in the inoculum substrate samples after anaerobic digestion indicating that potential VFA inhibition in anaerobic digestion of lignocellulosic matter has been eliminated [58].

Table 4
Design matrix for combined alkaline and microwave pre-treatments.

Std	Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
		A: I/S	B: pH	C: Microwave Power	CH_4 Yield	CO_2 Yield	H_2S Production
		–	–	%	ml gVS^{-1}	ml gVS^{-1}	mg L^{-1}
Control 1		1	8	N/A	227 ± 6	191 ± 2	1049 ± 31
Control 2		3	8	N/A	391 ± 23	235 ± 13	734 ± 143
Control 3		5	8	N/A	518 ± 1	286 ± 10	701 ± 210
1	2	1	7	30	728 ± 30	554 ± 22	1117 ± 231
2	8	5	7	30	1430 ± 50	948 ± 12	680 ± 100
3	12	1	9	30	525 ± 115	307 ± 47	1028 ± 409
4	16	5	9	30	1614 ± 168	867 ± 75	727 ± 517
5	10	1	8	10	557 ± 7	480 ± 2	1300 ± 81
6	15	5	8	10	764 ± 11	677 ± 15	570 ± 66
7	13	1	8	50	685 ± 13	509 ± 15	1207 ± 138
8	17	5	8	50	1019 ± 51	632 ± 39	576 ± 20
9	9	3	7	10	547 ± 178	445 ± 90	624 ± 98
10	4	3	9	10	640 ± 11	459 ± 17	645 ± 88
11	6	3	7	50	879 ± 111	630 ± 66	839 ± 92
12	11	3	9	50	861 ± 18	462 ± 26	948 ± 36
13	14	3	8	30	918 ± 84	712 ± 60	795 ± 202
14	3	3	8	30	1058 ± 115	612 ± 37	444 ± 102
15	1	3	8	30	1121 ± 72	637 ± 52	455 ± 35
16	7	3	8	30	1108 ± 106	747 ± 54	766 ± 99
17	5	3	8	30	916 ± 85	621 ± 23	958 ± 67

Table 5
Organic matter removal percentages of each sample.

Std no	[a]Sample Abbreviation	[b]COD_0 (mg L^{-1})	COD removal (%)	[b]BOD_0 (mg L^{-1})	BOD removal (%)	[b]$(SO_4)_0$ (mg L^{-1})	SO_4 removal (%)
I/S = 1							
1	I/S1 7 30%MW	17093	62 ± 1.8	13760	66 ± 1.7	497	59 ± 8.2
3	I/S1 9 30%MW	27520	51 ± 6.0	20960	50 ± 4.1	475	57 ± 4.7
5	I/S1 8 10%MW	19840	34 ± 1.7	14027	34 ± 6.0	517	51 ± 1.6
7	I/S1 8 50%MW	30667	65 ± 0.2	22507	65 ± 1.3	539	61 ± 2.9
I/S = 3							
9	I/S3 7 10%MW	19627	55 ± 1.2	13280	51 ± 3.8	853	78 ± 0.9
10	I/S3 9 10%MW	16000	37 ± 6.4	13493	36 ± 5.1	704	52 ± 2.4
11	I/S3 7 50%MW	21547	48 ± 1.1	15147	48 ± 5.0	891	60 ± 2.8
12	I/S3 9 50%MW	20053	44 ± 4.5	14293	44 ± 6.3	944	60 ± 2.1
13–17	I/S3 8 30%MW	19488	38 ± 5.0	14144	33 ± 5.0	880	69 ± 2.6
I/S = 5							
2	I/S5 7 30%MW	20133	50 ± 4.5	14240	53 ± 3.9	565	62 ± 3.9
4	I/S5 9 30%MW	24747	69 ± 1.4	18293	58 ± 5.0	853	40 ± 8.3
6	I/S5 8 10%MW	20213	46 ± 2.8	16053	52 ± 4.3	843	61 ± 0.5
8	I/S5 8 50%MW	18293	37 ± 3.5	13483	40 ± 2.6	800	58 ± 2.6

[a] All samples were exposed to 1 M NaOH pre-treatment prior to varying microwave power.
[b] Average of triplicate run.

3.3. Mathematical model estimation and validation

The ANOVA (Table 6 - Table 7) for each response of interest estimated that the models adopted were significant according to *p* values (<0.05) of the models as well as insignificant *p* values (>0.05) of the lack of fit test. Insignificant model terms were eliminated from the model by stepwise regression in order to achieve a high accuracy in data prediction with the developed model. All adequacy measures (R^2 values) were found to be close to 1 indicating a sufficient regression for model development. Moreover, adequate precision was found to be greater than 4 which defines adequate model discrimination.

The ANOVA tables indicate that I/S (A), first and second order effect

Table 6
ANOVA table for CH_4 yield.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1.18E+06	3	3.92E+05	17.45	<0.0001	significant
A- I/S	6.80E+05	1	6.80E+05	30.24	0.0001	
C- Microwave Power (%)	1.10E+05	1	1.10E+05	4.87	0.0459	
C^2	3.87E+05	1	3.87E+05	17.23	0.0011	
Residual	2.92E+05	13	22481.86			
Lack of Fit	2.52E+05	9	27971.49	2.76	0.1704	not significant
Pure Error	40520.8	4	10130.2	17.45	<0.0001	
Cor Total	1.47E+06	16				

R^2: 0.8010, Adj R^2: 0.7551, Adeq Precision: 13.783.

Table 7
ANOVA table for CO_2 yield.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	303782.4	4	75945.59	12.62	0.0002	significant
A-I/S	202884.5	1	202884.5	33.71	8.39E-05	
B-Initial pH	58140.5	1	58140.5	9.66	0.0090	
C-Microwave Power (%)	98	1	98	0.02	0.9005	
C^2	42659.38	1	42659.38	7.09	0.0207	
Residual	72213.39	12	6017.782			
Lack of Fit	60312.19	8	7539.024	2.53387	0.1926	not significant
Pure Error	11901.2	4	2975.3			
Cor Total	375995.8	16				

R^2: 0.8079, Adj R^2: 0.7439, Adeq Precision: 12.0657.

of microwave power (C, C^2) were the significant model terms for CH_4 yield (Table 6) while I/S (A), initial pH (B) and the second order effect of microwave power (C^2) were the significant model terms for CO_2 yield (Table 7) in a 95% confidence interval for AD of pot ale. Initial reaction pH did not appear to be an important parameter for biomethane yield. It was attributed to sufficient buffering capacity of the inoculum as the pH value stabilised and remained within the range of 6.8–7.4 for all samples after 2 days. A small decrease in the pH level was seen as a result hydrolysis and acidogenesis steps (acidification phase) in the first 2 days of AD [50,59].

The magnitude of the design factors (in different units) were reduced to −1 to 1 scale allowing the comparison and the presentation of their impact on the interest of response in a unitless scale. The coding format of the design factors is given in Table 2.

Mathematical models of CH_4 and CO_2 yields were given as function of the independent variables (A: I/S, B: Initial pH, C: Microwave power) in terms of coded Eqs (3) and (4) and actual factors Eqs (5) and (6) respectively.

Final Equation in terms of coded factors:

$$CH_4\left(\frac{ml}{gVS}\right) = 1046.44 + 291.5\,A + 117\,C - 302.44\,C^2 \tag{3}$$

$$CO_2\left(\frac{ml}{g\ VS}\right) = 662.11 + 159.25\,A - 85.25\,B - 3.5\,C - 100.36\,C^2 \tag{4}$$

By considering the coefficient of the mathematical models with coded factors the extent of influence of each parameter was varying based on the response of interest. The quadratic effect of microwave power (C^2) had the most powerful influence (negative) on CH_4 yield. It was then followed by ascending effects of I/S (A) and microwave power (C). The influence strength of design parameters for CO_2 yield was identified as A > -C^2 > -B > –C.

Final Equation in terms of actual factors:

$$CH_4\left(\frac{ml}{gVS}\right) = -246.8 + 145.6\frac{I}{S} + 51.22 \times MW\ Power - 0.76 \times MW\ Power^2 \tag{5}$$

$$CO_2\left(\frac{ml}{gVS}\right) = 884.7 + 78.6\ \frac{I}{S} = -85\ pH_0 + 14.9 \times MW\ Power - 0.3MW\ Power^2 \tag{6}$$

The mathematical models in terms of actual factors for CH_4 and CO_2 yields were used for data prediction for further model challenge and validation.

3.4. Validation of developed models and model graphs

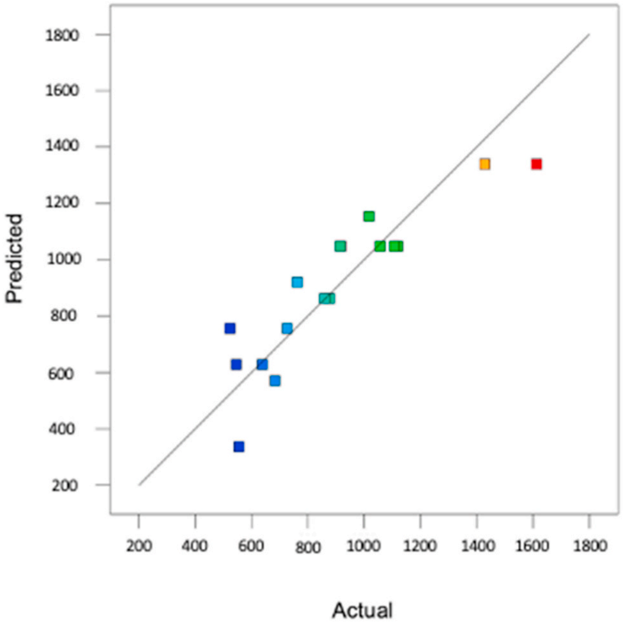

Fig. 2. Scatter diagram for CH_4 yield (ml gVS^{-1}).

Scatter diagram of predicted vs actual methane yields is given in Fig. 2. In the scatter diagram a homogeneous distribution of the design points close to the diagonal line was seen indicating that model is adequate. In addition, normal distribution of residuals (the difference between actual and predicted data) were seen in methane generation. The normal distribution plot proves the assumption of normal data distribution; therefore the ANOVA can be applied for statistical analysis of the estimated models. A similar trend was seen in CO_2 yield.

In addition to the post analysis provided by the software, three validation points (which were not used for model development) were chosen and the results of the experiments are given in Table 8 along with the predicted values by the models for CH_4 and CO_2 generations. These validation points are selected to challenge the models with different I/S and microwave powers which are significant model terms for methane yield.

The difference between the predicted and the experimental results are then statistically analysed by 2 tailed *t*-test. There was no significant difference between the experimental data and the predicted data in any

Table 8
Results of validation experiments.

Validation Points	CH_4 Yield (ml gVS^{-1})		CO_2 Yield (ml gVS^{-1})	
	Experimental	Predicted	Experimental	Predicted
I/S2 8 30% MW	825 ± 14 *p:* 0.0878	897	557 ± 61 *p:* 0.1498	537
I/S4 8 30% MW	1067 ± 22 *p:* 0.0816	1189	525 ± 36 *p:* 0.0605	694
I/S4 8 50% MW	980 ± 16 *p:* 0.3633	997	453 ± 26 *p:* 0.0735	512

case and the developed models were validated within the 95% level of confidence.

The perturbation plots, which were created with coded factors, for CH_4 and CO_2 yields are given in Fig. 3 a and b respectively. The significant design parameters for CH_4 yield A (I/S) and C (microwave power) were plotted by keeping the other factor constant at a time. I/S (factor A) has shown a linear relationship with methane yield. However, microwave power (factor C) has a polynomial relationship with methane generation with a peak around centre. Factor A and C have shown similar effects on CO_2 yield as CH_4. In addition, initial pH of the reaction (factor B) was a significant parameter which resulted in lower CO_2 generation at its lower levels.

The contour graphs of CH_4 and CO_2 yields are given in Fig. 4 a and b respectively. Mainly polynomial curves were seen in both graphs. The higher CH_4 yields were achieved with the conditions of higher I/S ratios and approximately mid-range microwave power.

3.5. Process optimisation

A combined numerical and graphical optimisation methods provided by Design Expert Software were applied on mathematical models developed for CH_4 (Eq (5)) and CO_2 (Eq (6)) yields. The optimum factor levels to achieve the highest CH_4 production as well as the lowest CO_2 and H_2S generation at the same time were identified to minimise the cost intensive biogas upgrading requirements prior to its utilisation for electricity and/or heat generation in combined heat power units (CHP) as explained by Refs. [16,60–62]. In addition, minimising the CO_2 and H_2S content of biogas increases its calorific value. Therefore, achieving minimum possible H_2S concentration is crucial part of optimisation. The design factors (A:I/S, B: initial pH and C: microwave power) were left in range. CH_4 yield was aimed to maximise with an importance level of 5. CO_2 and H_2S generations on the other hand was minimised with the importance levels of 3 and 5 respectively. A higher importance was assigned to minimisation of H_2S due its highly corrosive nature even in small concentration (~1–3% by volume) [62]. A numerical optimisation study was initially conducted on the RSM design matrix (Table 4) based on this criteria [44], subsequently a graphical optimisation was performed on the suggested numerical solutions to identify the target yields which are associated to the factor levels [43,63,64]. The numerical solutions were computed by Design Expert with a highest CH_4 yield of 1274 ml gVS^{-1} at an I/S of 4.6, initial pH 8.9 and 39% microwave power. The CO_2 yield and H_2S generation was predicted to be 684 ml gVS^{-1} and 630 mg L^{-1}under the same conditions respectively.

The graphical optimisation result is given in Fig. 5. The target area marked in yellow is identified by overlapped limits of each interest of respond corresponding to the criteria set by the authors in accordance with the numerical optimisation solutions. As such the lower and upper limits as follows (1211–1274 ml gVS^{-1} for methane, 653–694 ml gVS^{-1} for CO_2 and 630–634 mg L^{-1} for H_2S). Fig. 5 provides a brief approach to design operational parameters for anaerobic co-digestion of pot ale with sludge.

3.6. Digestate quality

A pot ale digestate sample was taken from the first validation experiment (Table 8) in form of sludge and pot ale mixture after 21 days of digestion, and analysed for the mineral (P, K, Mg, Zn, Mn, Cu, Fe) and the heavy metal (As, Cd, Co and Mo) content. The results are given in Table 9 along with inoculum and industrial digestate sample. No heavy metals were detected in any of the samples.

The composition of pot ale digestate was determined to be superior to the industrial digestate. The Cu concentration of pot ale digestate was found to be approximately 5-fold higher than the industrial digestate. The difference was attributed high Cu content of pot ale due to mass transfer in copper stills during distillation [65]. In addition, significantly higher concentrations ($p < 0.05$) of micro and macro soil nutrients (P, K, Mg, Zn, Mn and Fe) of pot ale digestate was associated with the richer mineral composition of pot ale since the inorganic content of digestate is directly linked to raw material.

From environmental and public health standpoints, using pot ale digestate as biofertiliser is considered to be a promising replacement material to animal by product digestates due to their high risk of containing of parthenogenic bacteria such as *Salmonella* and *Klebsiella spp. Klebsiella* were previously reported to be implicated in human infections. *Salmonella* are known to be transmittable to human and animals though contaminated food and water [66]. The presence of this pathogen has been reported in digestate originating from cattle manure, pig slurry [67], cow dung and chicken dropping [68], food waste and human excreta [66]. Therefore pasteurisation step before applying digestate to farm land is required by the EU Regulation No 1774/2002 [69].

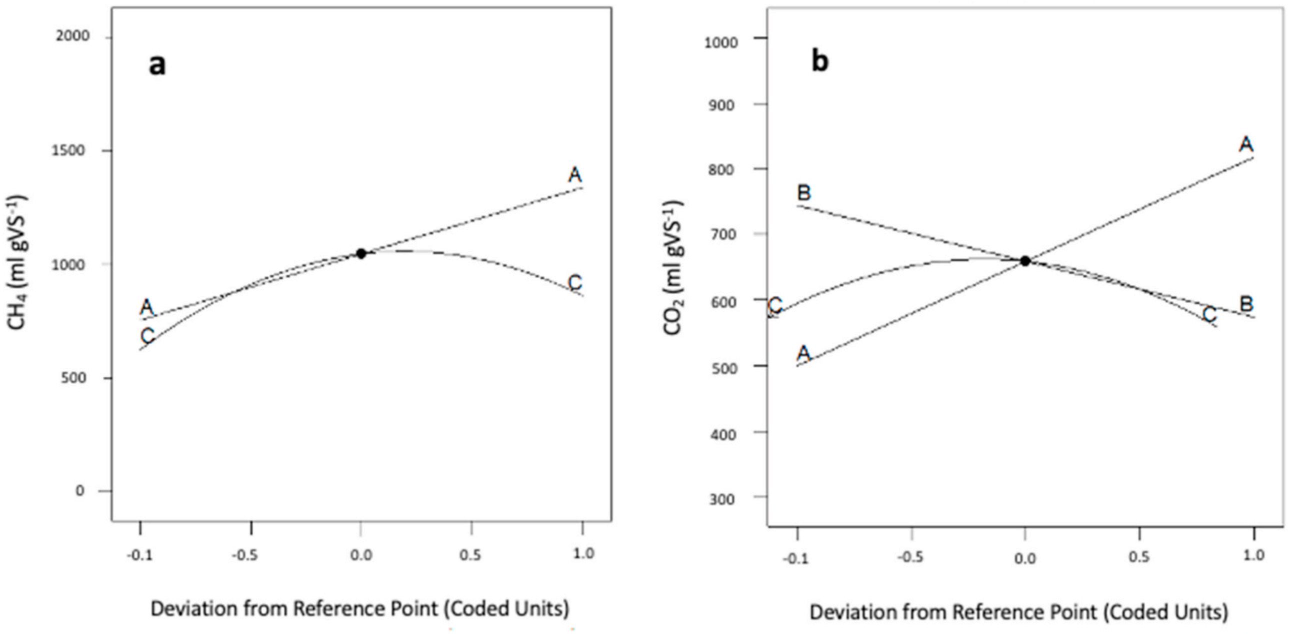

Fig. 3. Perturbation graphs of the a. CH_4 and b. CO_2 yields.

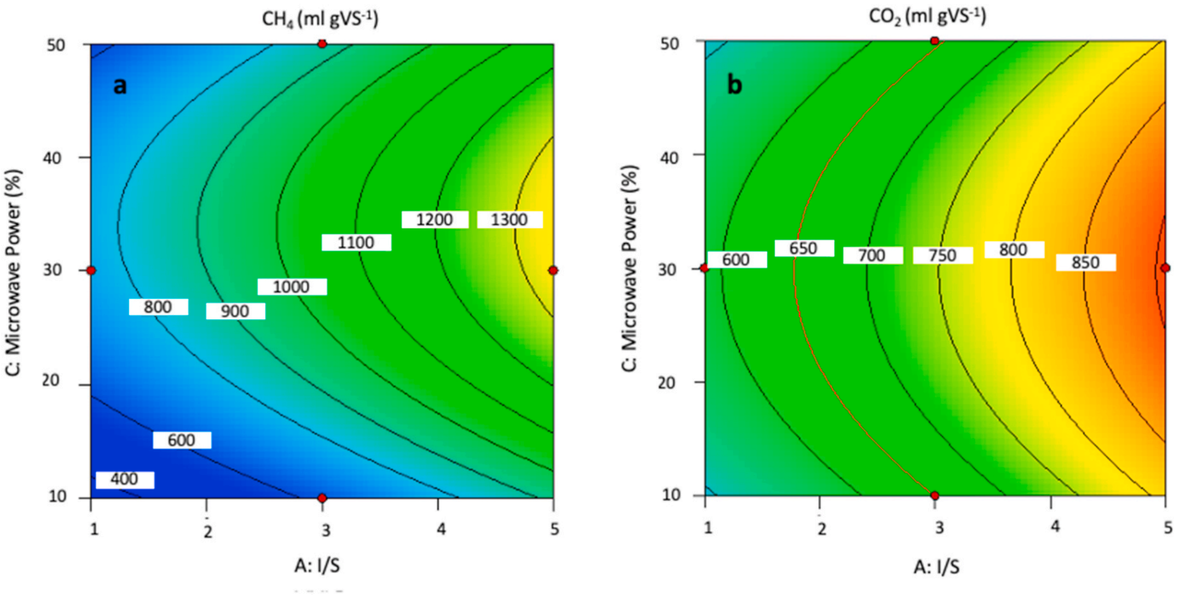

Fig. 4. Contour graph for a. CH_4 and b. CO_2.

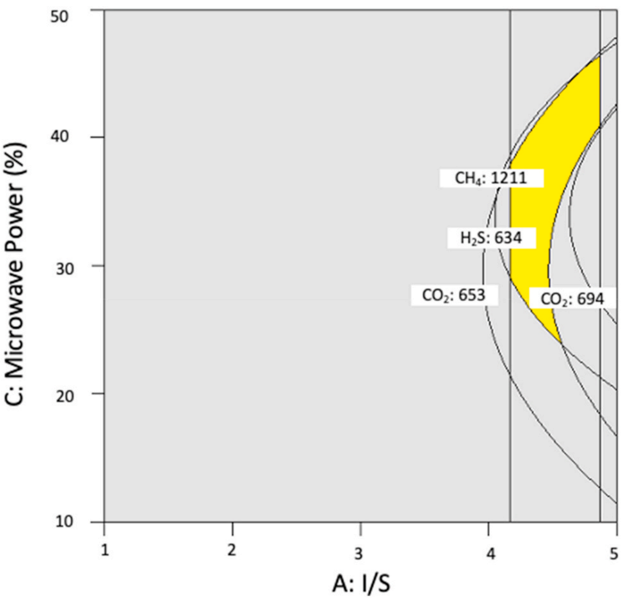

Fig. 5. Optimum zone with highest estimated CH_4 yield.

Table 9

Total mineral analyses of inoculum, industrial digestate and pot ale digestate.

Compound	Inoculum	Industrial digestate	Pot ale digestate
P	1620.5 ± 18.2	1082.6 ± 6.8	1391.6 ± 17.7
K	1225.3 ± 3.7	1090.0 ± 13.4	1174.4 ± 6.1
Mg	209.6 ± 11.4	104.8 ± 2.3	151.9 ± 13.3
Zn	16.87 ± 0.4	12.52 ± 0.3	14.00 ± 0.21
Mn	18.47 ± 0.1	12.54 ± 0.2	15.13 ± 0.13
Cu	3.40 ± 0.1	2.70 ± 0.4	13.15 ± 0.14
Fe	349.0 ± 3.8	224.3 ± 3.5	279.8 ± 3.9

All units are in mg L^{-1}.

Although presence of pathogenic bacteria is mainly associated with animal and human based digestate testing microbiological characteristics of pot ale digestate is recommended for increased public safety.

4. Industrial implementation

Annual volume of pot ale discharge is directly linked to the production capacity of whiskey distilleries. However, mass balance of whiskey manufacturing process does not vary remarkably for individual plants depending on manufacturing scale, raw material selection etc [49,70]. Therefore, a typical mass balance for malt whiskey distillery producing 1 million litres of alcohol per annum (Fig. 6) was used to estimate annual biogas production and predict the economical reliability of the technology.

The continuous stirred tank reactor (CSTR) is one of the most preferred configurations for industrial applications due to its operational simplicity in comparison with the 3rd generation high rate reactors [72] for biogas production. Subsequently, generated biogas commonly used in CHP units for heat and electricity generation due to its uncomplicated infrastructure requirement [73–75]. Therefore, the contribution of the applied pre-treatment in the annual energy recovery potential of a whiskey distillery producing 1 million litre whiskey was estimated by combining a CSTR and a CHP unit. The hydraulic retention time (HRT) was assumed to be 6 days as approximately 80% of biogas generation was completed within the first 6 days of AD. The first 6 days of biogas quality and quantity of the full scale application was assumed to be same as the lab scale experiments to estimate the methane yield. The energy density of methane was taken as 36 MJ m^{-3} for the estimation of combustion energy output potential via CHP [76]. Typical energy electrical and thermal efficiencies of a CHP unit were taken into account as 38 and 39% of the energy input respectively [75]. Energy recovery potentials of Control 3, std 4 (Table 4) and the optimised conditions (Section 3.5) are given in Table 10. The detailed calculation is provided in supplementary data.

The energy recovery potential of non-treated pot ale sample with an I/S of 5 (Control 3) at full scale was estimated to be 3891 MWh year^{-1} which corresponds to 1479 and 1517 kWh electricity and heat recovery per annum. Introducing 1 M NaOH and 30 W microwave pre-treatment (Std4) prior to AD, resulted in a 2.8 fold increase the in the estimated energy recovery potential by reaching 10 898 MWh year^{-1}. Therefore it can be concluded that thermochemical pre-treatment can significantly improve the energy recovery potential of AD technology. The energy recovery potential of the full scale AD of pot ale at the optimised conditions (I/S of 4.6, initial pH 8.9 and 39% microwave power), on the other hand, was estimated to be 9672 MWh h^{-1} which is approximately

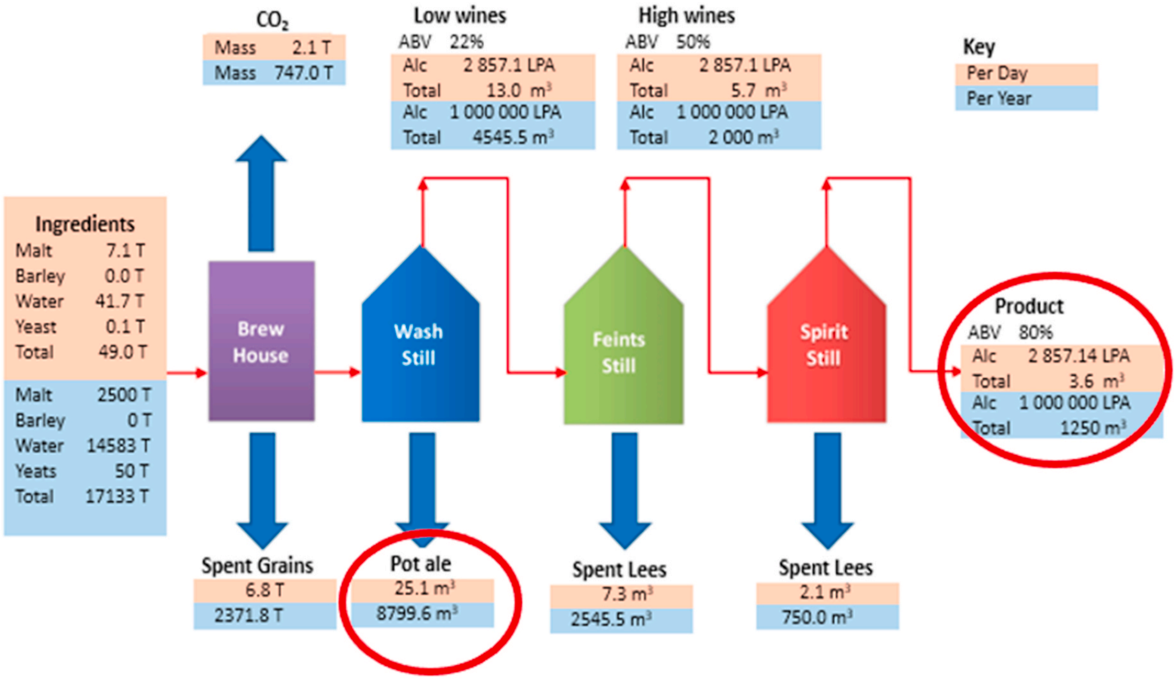

Fig. 6. Mass balance for a typical malt whiskey distillery adopted from Ref. [71].

Table 10
Estimated energy recovery potentials at full scale.

	Lab scale biogas yield (m^3 tonVS^{-1})	Full scale biogas production (m^3 year^{-1})	Full scale CH_4 production (m^3 CH_4 year^{-1})	Energy input to CHP (MWh year^{-1})	Electrical energy output of CHP (MWh year^{-1})	Electrical energy output of CHP (MWh year^{-1})
Control 3	755 ± 3	710 908	386 023	3891	1479	1517
Std 4	2208 ± 94	2 079 053	1081 107	10 898	4141	4250
Optimised condition	N/A	N/A	959 490	9672	3675	3772

11% less than the std 4. However, operating under optimised conditions reduces H_2S generation from 727 to 630 mg L^{-1} and CO_2 generation from 867 to 653 ml gVS^{-1} which corresponds to 13 and 25% reduction in H2S and CO2 emission respectively. This reduction can potentially eliminate the biogas upgrading step prior to CHP unit as discussed in Section 3.5.

5. Conclusions

Implementation of thermochemical pre-treatment has amended the lignocellulosic structure of pot ale which resulted in increased reaction kinetics and correspondingly enhanced biogas quality and quantity. Combining 1 M NaOH pre-treatment with 30% microwave pre-treatment has led a 3-fold increase in the CH_4 yield reaching a maximum of 1614 ± 168 ml gVS^{-1} (std no 4 in Table 4) in comparison to control. In addition to increased methane yields, applied pre-treatment has significantly increased the organic matter degradation in terms of COD and BOD reaching 69 ± 1.4% and 66 ± 1.7% removals respectively. The optimum conditions for AD of thermochemically pre-treated pot ale were determined as I/S of 4.6, initial pH level of 8.9 and 312 W of microwave via Design Expert software. The experiment results of the pre-treated samples suggest achieving satisfactory levels of organic matter degradation as well as increased methane yields as expected. Despite achieving promising result by implementing a pre-treatment step at lab scale, there several challenges in scaling up to industrial level. The aid of full scale simulation tools are most required for scaling up progressively since the fundamental studies of AD technology consists of empirical methodologies. The reliability of this process can be improved by linking the applicability of the pre-treatment step to micro and macro scale distilleries via well-established simulations enabling investigation of different scenarios. The state of art model fir AD is known to be ADM1 model where the substrate composition, bacterial growth rate and potential inhibitions are taken into account for scaling up studies.

Acknowledgements

The authors gratefully acknowledge Alltech Ireland Ltd. for the financial support for this research, Project ID 15-S-ST-10568. The authors are most grateful to Brain Fay and Indre Sinkunaite for their help in analysis to produce relevant data.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.biombioe.2020.105902.

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