Development and application of a mechanical pretreatment to increase the biogas produced from Irish macroalgal biomass

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DECLARATION

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Author's Publications

Published Journal Paper

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List of Abbreviations

Symbol	Description
AD	Anaerobic digestion
ANOVA	Analysis of variance
BMP	Biochemical methane potential
BM	Ball milling pretreatment
BT	Beating pretreatment
CCD	Centred composite design
CNG	Compressed natural gas
CHP	Compressed heat and power
COD	Chemical oxygen demand
DOE	Design of experiment
d.b.	Dry base
d.w.	Dry weight
EC	European commission
EU	European union
FCCD	Face-centred composite design
GHG	Greenhouse gas
HPWS	High pressure water scrubbing
IE	Ireland
ILUC	Indirect land-use change
LSD	Least significance difference
MaREI	Marine renewable energy Ireland
MC	Moisture content
MW	Microwave pretreatment
OFAT	One factor at a time
PSA	Pressure swing adsorption
RES-E	Renewable energy supply-electricity
RES-H	Renewable energy supply-heat
RES-T	Renewable energy supply-transport
RSM	Response surface method
sCOD	Soluble chemical oxygen demand
SEAI	Sustainable energy authority Ireland
SOFC	Solide oxide fuel cell
tCOD	Total chemical oxygen demand
toe,ktoe,Mtoe	Tonnes of oil equivalent, thousand toe, million toe
VDI	Verein Deutscher Ingenieure (Association of German Engineers)
VFA	Volatile fatty acids
w.b.	Wet base

List of Nomenclature

Symbol	Description
A	Weight of ash, [gr]
Adeq. Precision	Adequate Precision
Adj. R^2	Adjusted R^2
Bp	Biogas produced per gram of VS [$m^3g^{-1}VS$]
Bs	Energy content of biogas from seaweed, [kWh m^{-3}]
C	Weight of container [g]
c	Concentration of the organic matter [%]
d	Desirability function
Cor. Total	Total sum of the squares corrected for the mean
D	Weight of dry matter [g]
Dc	Weight of dry matter and container [g]
df	Degree of freedom
Ec	Energy consumed by the pretreatment per gram of VS [Wh $g^{-1}VS$]
Ep	Energy of the biogas produced for gram of VS [Wh $g^{-1}VS$]
Ept	Energy consumed by the pretreatment [Wh]
HRT	Hydraulic retention time
k	Number of treatments
m	Mass of substrate fed per time unit [kg $days^{-1}$]
n	Number of replications
n_C	Number of centre points
n_F	Factorial runs
OLR	Organic load rate
Pred. R^2	Predicted R^2
$Q_{a,k,df\varepsilon}$	Critical value of the Studentised range distribution
r	Radial distance in the factorial design
s_ε	Standard error
TS	Total solids
V	Volume of substrate fed per time unit [m^3 $days^{-1}$]
VR	Digester volume [m^3]
VS	Volatile solids
w_a	Tukey's critical value
W_c	Weight of fresh sample and container [g]
x	Independent variable
y	Response variable

List of chemical symbols

Symbol	*Description*
Br	Bromine
$Ca(OH)_2$	Calcium hydroxide
CH_4	Methane
Cl	Chlorine
CO_2	Carbon dioxide
C: N	Carbon: Nitrogen ratio
C: N: P: S	Carbon: Nitrogen: Phosphor: Sulphur
F	Fluorine
H_2	Hydrogen
H_2O	Water
H_2S	Hydrogen sulphide
N_2	Nitrogen
NaOH	Sodium hydroxide
NH_3	Ammonia
O_2	Oxygen
S	Sulphur

Greek notations

Symbol	*Description*
α	Significance level
α^*	Rotatability
β	Regression coefficients
ε	Random error

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Development and application of a mechanical pretreatment to increase the biogas produced from Irish macroalgal biomass

Maria Montingelli

Abstract

Algal biomass is attracting more and more interest due to new potentials for overcoming the drawbacks relating to first and second generations of biomass for biofuel generation. Macroalgae, commonly known as seaweeds, are particularly suitable as substrate for biogas (~ 60% methane) production through anaerobic digestion (AD). However, seaweeds are not yet fully exploited as a feedstock for biogas since some obstacles need to be overcome. In general, prior AD a pretreatment step is necessary; this has to be efficient both in terms of methane yields and energy consumption. An optimal organic substrate concentration has to be selected in order to maximise the yields and avoid overloading phenomena. In Ireland, the influence of algal chemical composition variation according to genera, season and environment is still unexplored.

The aim of this research was to investigate and optimise the biogas production from Irish macroalgae when applying a mechanical pretreatment at different harvesting periods. The "Design of Experiment" (DOE) technique was employed as investigation method, since the response of interest ("methane yield") was affected by several variables which can interact with each other. The advantage with respect to the traditional method of studying the effect of "One Factor at a Time" (OFAT) is that the DOE can detect and quantify the interactions between variables.

New knowledge was developed with regards to three different mechanical pretreatments, namely beating, milling and microwave when applied to the Irish macroalgae *Laminaria sp.*, commonly known as kelps, showing that beating achieved a more optimal performance over other methods. New data were generated regarding biogas production from the Irish macroalgae *Laminaria sp.* and *Ascophyllum nodosum*, with *Laminaria sp.* exhibiting 40% more methane. During the seasonal investigation, it emerged that, in terms of the Irish climate, the summer and autumn were the best harvesting periods of *Laminaria sp.* for methane production. The organic substrate concentration influenced the most the process, with general higher yields at lower concentrations (below 2.5% of Volatile Solids). The impact of this beating pretreatment influenced positively the methane production in autumn (between 50-60%) and at the lowest organic concentration (1% of VS). In terms of pretreatment energy consumption, more energy was generated with respect to the untreated scenario for the material harvested in autumn and winter.

Chapter 1: Introduction

According to the 2012 revision of the official United Nations population estimates and projections [1], the world population of 7.2 billion in mid-2013 is projected to increase by almost one billion people within the next twelve years, reaching 8.1 billion in 2025, and to further increase to 9.6 billion in 2050 and 10.9 billion by 2100. Along with this, the International Energy Agency [2] estimates that the global energy demand is set to grow by 37% by 2040, even though the development path for a growing world population and economy is less energy-intensive than it used to be. It is also estimated that the demand of natural gas will grow by more than half, the fastest rate amongst fossil fuels, while electricity is the fastest-growing form of energy which will contribute more than any other to the reduction in the share of fossil fuels within the global energy mix. Therefore, when one considers this fast growing rate of the world population as well as the simultaneous global energy demand, it is obvious that current non-renewable energy resources (fossil fuels) will not be able to sustain such rates. Nevertheless, there is widespread the concern regarding the negative energy-related environmental impacts due to the use of non-renewable resources such as acid precipitation, stratospheric ozone depletion and global climate change [3]. During the G7 summit (June 10th 2015) [4] on the future of renewable energy, it was underlined that the use of fossil fuels has to be reduced by 50% until 2035 in order to keep the global average temperature within 2°C of pre-industrial levels. In this scenario, it is crucial a long term development is undertaken to ensure that sustainable supply of energy resource is readily available at reasonable cost and can be utilized without causing negative societal and environmental impacts [3].

Supplies of such energy resources like fossil fuels (coal, oil, and natural gas) and uranium are generally acknowledged to be finite; other energy sources such as biomass, sunlight, wind and falling water are generally considered renewable and therefore sustainable over the relatively long term [3].

For this reason, in a global context, renewable energy technologies are rapidly gaining ground, helped by global subsidies amounting to $120 billion in 2013 [2]. Projections reveal that with rapid cost reductions and continued support, renewables will account for almost half of the increase in total electricity generation up to 2040, while the use of biofuels more than triples and the use of renewables for heat more than double this increase [2]. Generally speaking, biofuels are referred to solid, liquid or gaseous fuels derived from organic matter. These are classified as first, second and third generation based on the raw material (substrate) and technology used for their production (**Table 1**).

Table 1: Classification of biofuels (modified from [5])

Biofuel classification	***Biofuel and conversion technology***	***Substrate***
1st generation	- Bioethanol by fermentation - Biodiesel by transesterification	Seeds, grains, sugars and vegetable oils
2nd generation	- Bioethanol by enzymatic hydrolysis - Methanol, Fischer-Tropsch gasoline and diesel, mixed alcohol, dimethyl ether and green diesel by thermochemical processes - Biomethane by anaerobic digestion	Lignocellulosic biomass, agricultural wastes, municipal and industrial wastes
3rd generation	- Biodiesel by transesterification - Bioethanol by fermentation - Biohydrogen by fermentation - Biomethane by anaerobic digestion	Microalgae (unicellular microorganisms), macroalgae (seaweeds)

Any large-scale increase in demand for biofuels is facilitated by the wide variety of environmental and socio-economic benefits with respect to it versus fossil fuels, nevertheless some issues have been raised [6] (**Table 2**).

Table 2: Potential benefits and challenges of biofuels, adopted from [6]

Benefits	***Challenge***
- *Energy Security*	- *Feedstock*
Domestic energy source	Collection network
Locally distributed	Storage facilities
Well connected supply-demand chain	Food-fuel competition
High reliability	
	- *Technology*
- *Economic stability*	Pretreatment
Price stability	Enzyme production
Employment generation	Efficiency improvement
Rural development	Technology cost
Reduce inter-fuels competition	Production of value added co-products
Reduce demand-supply gap	
Open new industrial dimensions	- *Policy*
Control on monopoly of fossil rich states	Land use change
	Fund for research and development
- *Environmental gains*	Pilot scale demonstration
Better waste utilization	Commercial scale deployment
Reduce local pollution	Policy for biofuels
Reduce GHGs emission from energy consumption	Procurement of subsidies on biofuels production
Reduction in landfill sites	Tax credits on production and utilization of biofuels

The greenhouse gas savings associated with first generation biofuel systems could be negated by indirect land-use change (ILUC) impacts. These impacts occur when grassland and forest are converted to crop land somewhere on the globe to meet the demand for commodities displaced by the production of biofuel feedstocks [6]. In other words, if fertile land generally used for food crops is used to produce bioenergy, this may lead, elsewhere in the world, to farmers clearing wild lands in order to meet displaced demand for crops [7]. The growth of terrestrial crops for biomass requires the use of significant amounts of land and

water and can have implications for biodiversity, food production and landscape [6].

Second generation biofuels can mitigate the issues related to the first generation of biofuels. However, it has been highlighted [5] that the production of second generation biofuel requires most sophisticated processing production equipment, thus requiring more investment per unit of production and larger scale facilities to confine capital cost scale economies. Consequently, it is evident the need in searching for more sustainable alternatives.

Third generation biofuels derived from algal biomass (micro- and macroalgae) appears to be a valuable alternative to overcome the obstacles related to the first and second generation biofuels. This kind of biomass ensures high growth yields without requiring arable land [8-10], high capacity of carbon capture during photosynthesis [11] and a negligible or low amount of lignin makes them less resistant to degradation than lignocellulosic feedstock, avoiding the need for energy-intensive pretreatments [12]. In particular, macroalgae, commonly known as seaweeds, can be converted to biofuels by various processes including thermal processes and fermentation. However, it has been claimed [11] that the most direct route to obtaining biofuel from macroalgae is via anaerobic digestion (AD) to biogas which is mainly composed of methane (CH_4). The production of biogas through AD offers significant advantages over other forms of bioenergy production, such as heat, synthesis gases and ethanol, since it is considered more competitive in efficiencies and costs [13, 14]. Compared to natural gas, algal biogas through AD has the potential to decrease greenhouse gas (GHG) emissions over 50% and fossil depletion of almost 70% [15]. The concept of exploiting macroalgae as biomass to produce biogas via AD is not completely new. In the 1970's, the US Marine Biomass program studied the optimisation of seaweeds growth biology, the engineering design of an offshore seaweeds growth facility, evaluation and optimisation of conversion by AD, and systems analysis. Up to the late 1970's, conversion of seaweeds to methane was successfully demonstrated, but several attempts to sustain seaweed growth on artificial farms were unsuccessful. Despite several breakthroughs and successes, in 1986 the program was cancelled due to the high-perceived cost of biomass energy [16]. The most recent industrial attempt of exploitation of biogas production from seaweed

biomass was represented by a pilot scale in Japan, operated by Tokyo Gas [17]. The plant was able to produce approximately 20 kL methane per ton seaweed per seaweed per day generating up to 10 kWh^{-1} of electricity. In the UK and Ireland, new interesting research is discovered in this topic. In particular, a consortium, known as the Sustainable Fuels from Marine Biomass project [18] (BioMara) led by the Scottish Marine Association, is actively investigating the feasibility of culturing seaweeds for 3rd generation biofuels. Also the Marine Renewable Energy Ireland Institute (MaREI) is collaborating with the University College Cork (UCC) [19] in order to investigate the feasibility of exploiting Irish indigenous seaweeds for the production of gaseous biofuels.

1.1 Research justification

Considering the global situation and the European Union (EU) long-term goal of reducing GHG emissions by 80-95% compared to 1990 levels by 2050 [20], the first important goals to be achieved by 2020 are to:

1. Reduce GHG emissions by 20% (compared to 1990),
2. Increase to 20% the share of renewables in energy consumption,
3. Increase energy efficiency by 20%.

Beyond 2020, the European strategy is to increase up to 40% reduction in GHG emissions by 2030 relative to 1990, and to bind on EU-wide target for renewable energy of at least 27% by 2030 [19].

In particular, according to the EU Directive 2009/28/EC [21] each Member State has had to commit to reach legally binding national targets by 2020. Ireland has to achieve a target of at least 16% of its total energy consumption and at least 10% of energy consumed in road and rail transport from renewable sources. The Irish National Renewable Energy Action Plan sets out to fulfil the 16% overall target through its use of:

1. 10% renewable energy supply in transport (RES-T),
2. 12% renewable energy supply in the heat sector (RES-H),
3. 40% renewable energy supply in the electricity sector (RES-E).

Between 2003 and 2013, the Sustainable Energy Authority of Ireland (SEAI) reported an increase of more than 300% in the renewable energy consumption. Biomass, liquid biofuels, biogas and landfill gas are generally grouped together under the term bioenergy. On this basis the majority of renewable energy gross final consumption in 2013 came from wind (47%) and bioenergy (42%), with the remainder coming from hydro, solar and geothermal (11%). **Table 3** shows the progress made towards the individual modal targets and to the overall target of 16% for the period 1990 to 2013, while **Figure 1** provides an overview of how Ireland (IE) compares with other EU Member States by 2013. At the moment, looking at the contributions of renewables in 2013, it can be seen that Ireland is half way towards meeting the 2020 targets with seven years remaining [22].

Table 3: Ireland Renewable Energy Progress to Targets 1990-2013 [22]

% of each target	*1990*	*2000*	*2005*	*2010*	*2011*	*2012*	*2013*	*2020*
RES-E	5.3	4.8	7.2	14.5	17.3	19.5	20.9	40
RES-T	0.0	0.0	0.0	2.5	3.8	4.0	4.9	10
RES-H	2.6	2.4	3.5	4.5	5.0	5.5	5.7	12
Overall	2.3	1.9	2.8	5.6	6.5	7.3	7.8	16

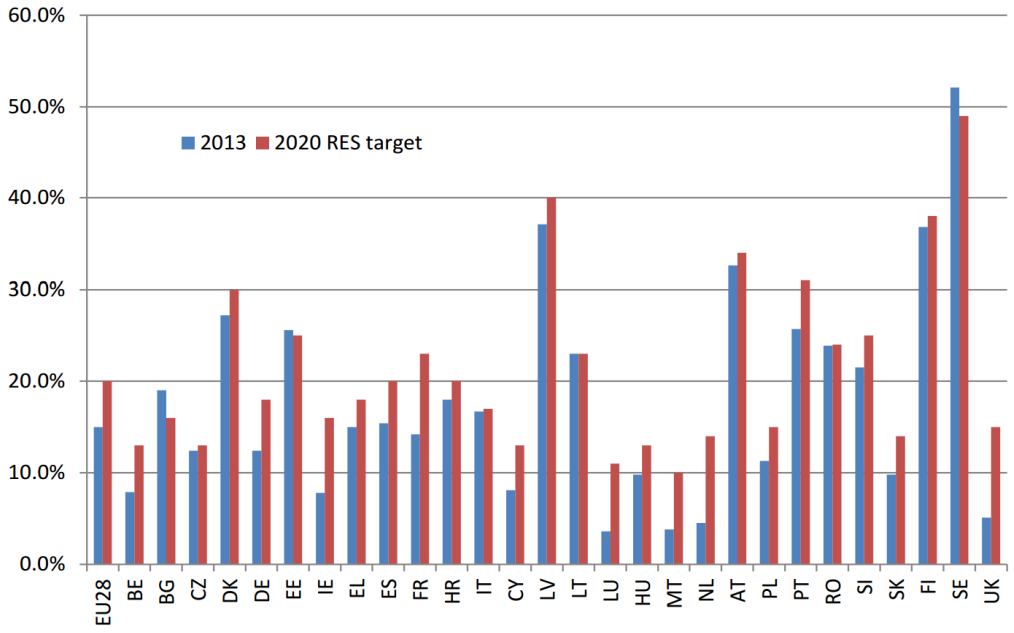


Figure 1: Progress achieved and efforts to be made by Member States towards achieving 2020 targets for renewable energy sources [23]

Biogas has the potential to contribute towards each target. Once biogas is upgraded to biomethane, it can be used for electricity generation, water heating, space heating, cooking as well as to fuel vehicle. Biogas is also used to power Combined Heat and Power (CHP) systems for heat and electricity production. On this matter, the European tendency is directed at generating electricity rather than heat or transport use, especially in the Nordic countries. According to EurObserv'ER [24], about 13.4 million tonnes oil equivalent (Mtoe) of biogas primary energy were produced during 2013 in Europe. The main producers are Germany (6717 ktoe), the UK (1824 ktoe) and Italy (1815 ktoe) thanks to their lucrative feed-in tariffs and favourable regulations for biogas.

The Irish biogas production is one of the lowest (56 ktoe) in Europe [24]. The main barrier is the lack of a consistent legislation regarding biogas/biomethane use [25, 26]. Renewable electricity and thermal energy from biogas is mainly generated in waste-water treatment plants and solid biomass CHP, where the processed biomass is sewage, animal slurries and wastes from abattoirs, breweries and other agri-food industries. Regarding the transport sector, there is currently no use of biomethane as a fuel vehicle in Ireland [22]. It is broadly known that transportation is the most difficult energy consuming sector to decarbonise and highly subjected to volatile oil price. In Ireland, the 2020 projected demand from road and rail transport has been estimated at 4.499 ktoe [27]. It has been suggested that a contribution to the projected demand of 33 ktoe will come from electric vehicles, while the balance of renewable contribution (406 ktoe) from biofuels [27]. It is noteworthy that if the sources of biofuels are wastes, non-food cellulosic material, lignocellulosic material or algae, a double weighting in the RES-T calculation can be applied [22]. For this reason, in Ireland, several studies [14, 25, 28-31] assessed the use of grass as resource for biomethane production through AD. These studies are relevant considering that grass is the main Irish biomass primarily used for ruminant production. Even though, grass was proven to contribute significantly to biogas production [32], the main concern is its competition with the Irish agricultural system [33]. Unlike grass, seaweeds do not compete for land and water with any Irish agricultural system and this biomass offers higher gross energy yields of biofuel (365 GJ ha^{-1} yr^{-1}) compared to grass biomethane (122-163 GJ ha^{-1} yr^{-1}) [34]. It represents an indigenous resource

which also can help the development of rural costal economy [11, 35] as well as an opportunity for the Irish marine sector [36]. Ireland has a long maritime tradition and significant potential for exploitation of marine resource. Most of the Irish seaweeds production is for hydrocolloid production and a significant quantity is sold as raw material for further industrial processing [37]. The estimated standing kelp stock is three million tonnes; although this estimation is highly uncertain [37]. However some obstacles need to be overcome [6, 11, 35]. Although AD from seaweeds is technically proven, the optimisation of the process is still under research [11, 38]. Efficient cultivation and harvesting are prerogatives in order to exploit the full potential of macroalgae especially on a larger scale for biofuel production [6, 11, 35]. One of the major concerns is the harvesting of wild seaweed biomass. A very careful management is required to prevent serious ecosystem damage [11].

1.2 Research objective

Considering that the algal biomass is recognised as a sustainable energy source with a big potential for Ireland, the principal aim of this research was:

- Investigate and optimise the use of macroalgal biomass for biogas production through AD when a mechanical pretreatment was applied at different harvesting times, in Ireland.

The investigation process was carried out by studying and testing:

1. Different mechanical pretreatments such as beating, milling and microwave techniques on the seaweed *Laminaria sp.*, widely available in Ireland,
2. Two seaweed species, namely *Laminaria sp.* and *Ascophyllum nodosum*, amongst the most available in Ireland,
3. Influence of seaweed harvesting period/season on the performance of the process in terms of methane yields.

The optimisation process was carried out, throughout the experimental research, by applying the “Design of Experiment” (DOE) technique to:

1. Parameters involved in the mechanical pretreatment step (time of pretreatment),
2. Parameters involved in the AD process (organic substrate concentration).

The aim of the optimisation phase was:

- Determine the optimal combination of biochemical and mechanical variables that maximise the methane production from algal biomass.

It was acknowledged that one of the major issues related to the use of mechanical pretreatment was its high energy consumption. Therefore, this research aimed to evaluate the energy consumption related to the use of the mechanical pretreatment by comparing the scenario of when the seaweed biomass was not subjected to a mechanical pretreatment.

1.3 Thesis outline

Chapter 1 has just introduced to the environmental and societal benefits derived by the use of biofuels while providing an overview of the Irish policy and strategy in order to achieve the 2020 European targets. The research objectives were also presented.

Chapter 2 aims to provide the reader with the most important concepts regarding the mechanical and biochemical aspects related to methane production. A detailed discussion is provided on the main literature results and obstacles related to the exploitation of algal biomass (both micro- and macroalgae) for methane production with a focus on the pretreatment phase.

Chapter 3 reports the planning and justification of the choices regarding the experimental part according to the DOE technique. This section describes the methodology and equipment employed all over the research activity.

Chapter 4 reports the experimental work and statistical analysis of the results observed while exploring the significance in the literature of the results achieved.

Chapter 5 presents the main conclusions and contribution to the field of the study. Also suggestions for future work are reported.

Chapter 2: Literature Review

2.1 Introduction

The aim of this chapter is to provide a general description of the biogas production process through anaerobic digestion (AD) and a critical review of the literature on the use of algal biomass for biogas purpose.

The chapter is divided in two main sections. The first section *"Background Theory"* describes in general, the AD biochemical mechanisms, feedstocks, operational parameters, pretreatment methods and end-uses of biogas. The second section *"Biogas production from algal biomass"* reviews the AD process when algal biomass is used as feedstock with a particular focus on the pretreatment phase.

2.2 Background Theory

Methane (CH_4) is the primary fuel present in natural gas. It is also produced through the biodegradation of biomass in anoxic environment, such as swamps, wetlands, sediments, and in the rumen of ruminant animals. Methane production in engineered AD systems has been employed for more than a century mainly to treat municipal sludge generated by municipal wastewater treatment plants, where the main objectives are pollution control and to kill or eliminate pathogens present in the sludge [39]. This spontaneous natural process involves the degradation and stabilization of organic materials under oxygen-free conditions by the use of particular microorganisms. The main products of AD are biogas (~ 50-75% CH_4) and microbial biomass (digestate) [40]. However, the complex balance amongst the mechanisms involved, determines a poor operation stability

which prevents AD from being widely commercialized and exploited when used with other kind of organic materials.

2.2.1 AD Biochemical Reactions

The AD process involves diverse community of bacteria that act as an integrated metabolic unit to produce methane and carbon dioxide (CO_2) through a series of sequential and concurrent reactions. The end-products of one group's metabolism are generally used as a substrate by the next group. The biological process involves four main phases, namely; hydrolysis, fermentation (acidogenesis), acetogenesis, and methanogenesis (**Figure 2**).

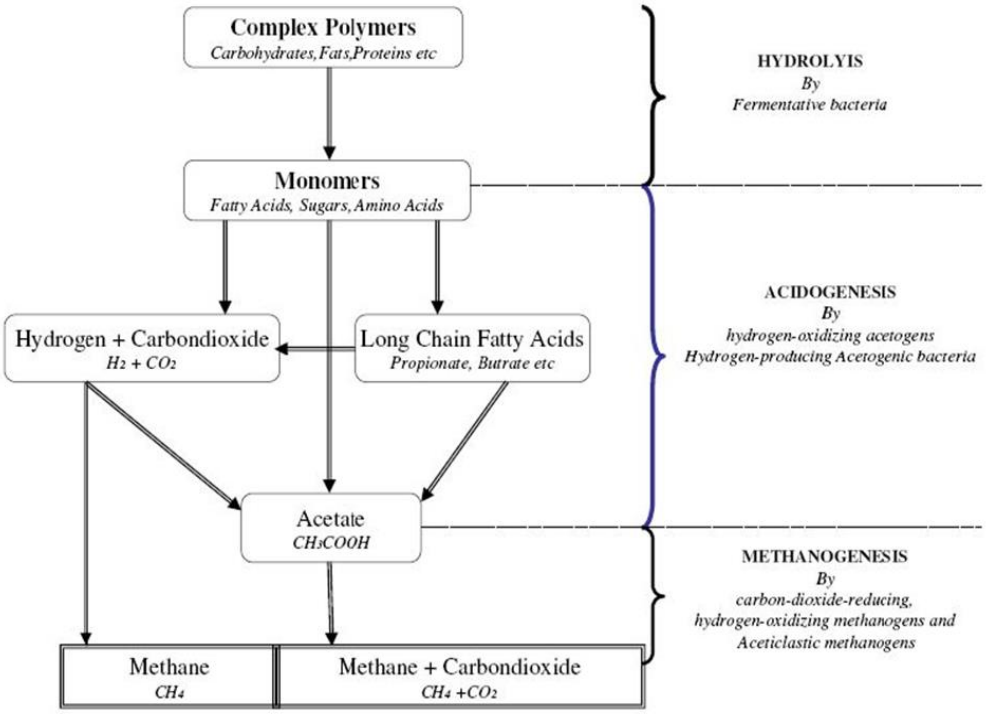


Figure 2: AD phases [41]

Hydrolysis is theoretically the first step of AD during which the complex organic matter (polymers) are decomposed into smaller units (mono- and oligomers). During hydrolysis, polymers like carbohydrates, lipids, nucleic acids and proteins are converted into glucose, glycerol, purines and pyridines. Hydrolytic microorganisms excrete hydrolytic enzymes, converting biopolymers into simpler and soluble compounds [42]. Different polymers require several different types of enzymes as it is shown in the following **Figure 3**.

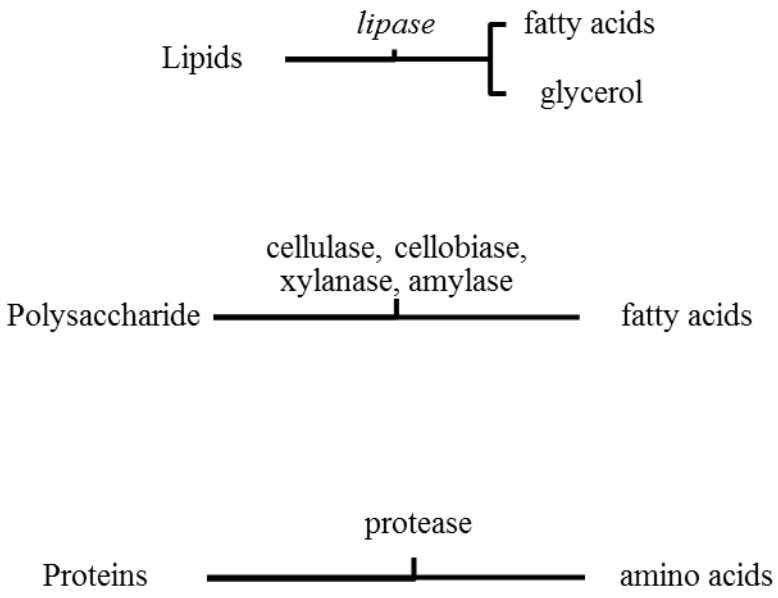


Figure 3: AD biochemical reactions

The rate of hydrolysis depends on several factors, such as pH, substrate composition, and particle size [43]. In the case of vegetable substrates containing cellulose, hemi-cellulose and lignin, hydrolysis is indeed the speed determining process of the overall AD, since those compounds are not easily accessible by the hydrolytic enzymes. A pretreatment phase has generally been introduced as the main solution [44].

The second phase of the overall process is fermentation where the products of hydrolysis are converted by fermentative bacteria into methanogenic substrates. Simple sugars, amino acids and fatty acids are degraded into acetate, CO_2 and hydrogen (70% H_2) as well as into volatile fatty acids (VFA) and alcohols (30%) [42]. As the fermentation process results in the formation of various short-chain organic acids, this stage of AD is also referred to as the acid-forming stage or acidogenesis [43].

During acetogenesis, VFA and alcohols are oxidised into methanogenic substrates like acetate, H_2 and CO_2. VFA, with carbon chains longer than two units and alcohols, with carbon chains longer than one unit, are oxidized into acetate and hydrogen. The production of hydrogen increases the hydrogen partial pressure. This can be regarded as a "waste product" of acetogenesis and inhibits the metabolism of the acetogenic bacteria. During methanogenesis, hydrogen is

converted into methane. Acetogenesis and methanogenesis usually run parallel, as symbiosis of two groups of organisms [42].

In the methanogenesis phase, methane is produced through two distinct routes by two different microbial groups. Approximately two-thirds of the methane produced, derives from the fermentation of acetic acid by acetoclastic methanogens. The remaining methane is produced from the conversion of hydrogen and carbon dioxide by hydrogen-oxidizing methanogens, as shown in the following **Figure 4**.

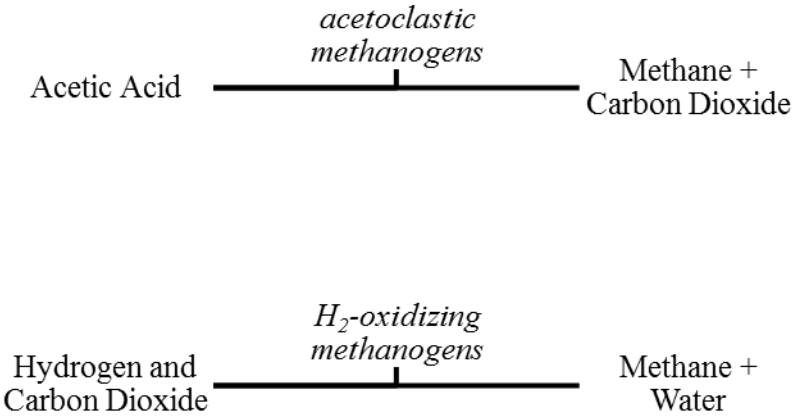


Figure 4: Methanogenesis biochemical reactions

Methanogenesis is a critical step in the entire AD process, as it is severely influenced by operation conditions. Composition of feedstock, feeding rate, temperature, and pH are examples of factors influencing the methanogenesis process. Digester overloading, temperature changes or large amounts of oxygen can result in termination of methane production [42]. Besides, the microorganisms involved during methanogenesis and acidogenesis differ widely in terms of physiology, nutritional needs, growth kinetics, and sensitivity to environmental conditions. Thus, failure to maintain the balance between these two groups of microorganisms is the primary cause of AD instability [40].

2.2.2 AD feedstocks

Biomass wastes are the most suitable feedstocks for AD; reciprocally, AD is the most suitable technology to recover the bioenergy from large amounts of biomass

wastes. The main characteristics of different feedstocks pertinent to AD are summarized in **Table 4**.

Table 4: Biochemical methane potential (BMP) of common feedstocks used in biogas production [39]

Feedstock	*Characteristics*	*BMP (m^3 CH_4 dry ton^{-1})*
Livestock manure		
Beef and dairy cattle manure	High-nitrogen, low-readily fermentable carbohydrates, high microbial biomass, high water content, may have inert material	148-250
Piggery manure	High-nitrogen, relatively low-fermentable carbohydrates, high microbial biomass, high water content	275-356, 450
Poultry manure	High-nitrogen and phosphorus, relatively low-fermentable carbohydrates, high microbial biomass, high water content	460
Food-processing wastes		
Brewery residues/wastes	Low-nitrogen, low-readily fermentable carbohydrates, high water content	147
Fresh fruit wastes	Low-nitrogen, high-readily fermentable carbohydrates, high water content	254-495
Slaughterhouse wastewater	High-nitrogen, high water content	297
Municipal sludge	High microbial biomass, low-readily fermentable carbohydrates, high water content.	85-110, 390
Municipal wastes	Low-nitrogen, low readily-fermentable carbohydrates, low water content	300-550
Crop Residues		
Corn stover	Low-nitrogen, low readily-fermentable carbohydrates, low water content.	250
Wheat straw	Low-nitrogen, low readily-fermentable carbohydrates, low water content.	161-241
Energy crops		
Grass silage	Low-nitrogen, high readily-fermentable carbohydrates, low water content.	390
Willow	Low-nitrogen, low readily-fermentable carbohydrates, low water content.	160

2.2.2.1 Lignocellulosic Biomass

Present-day operating biogas stations are based on the treatment of lignocellulosic biomass which includes agriculture and forestry wastes, municipal solid waste, waste paper, wood and herbaceous energy crops. Lignocellulosic materials are composed mainly of cellulose, hemicellulose and lignin. Both the cellulosic and hemicellulosic fractions of biomass are converted to monosaccharides that can be subsequently fermented to biogas. Some properties such as lignin content, cellulose accessibility to cellulase, and cellulose crystallinity result in a very slow biodegradation of native untreated lignocelluloses and a low extent of degradation which does not normally exceed 20% [45]. Thus, this type of biomass requires aggressive pretreatments in order to yield an optimal amount of biogas. The pretreatment step is a very expensive operation which makes lignocellulosic feedstocks not uneconomically viable at a large scale. The pretreatment step will be discussed further in this chapter.

2.2.2.2 Algal Biomass

As previously introduced, algal biomass and in particular macroalgae is the renewable resource investigated in this work. Marine macroalgae or seaweeds are plants adapted to the marine environment, generally in coastal areas. There are a very large number of species around the world. Broadly, three types of seaweeds are defined according to their pigments; brown seaweeds (*e.g. Laminaria, Fucus, Sargassum*), red seaweeds (*e.g. Gelidium, Palmaria, Porphyra*) and green seaweeds (*e.g. Ulva, Codium)* [46]. In comparison to terrestrial biomass crops, macroalgae contain little cellulose and no lignin and therefore undergo a more complete hydrolysis. Gas yields are related both to ash content (and its inverse relationship with volatile solids content) and the level of storage sugars; and, as seaweed biochemical composition varies with season, gas yield therefore varies. The Carbon: Nitrogen (C:N) ratio is also an important part of optimising a digesters diet and strengthens the argument for the co-digestion of seaweeds with other more N rich substrates, for example waste food or agricultural slurries [11]. Due to their biochemical properties, brown seaweeds have been used for industrial applications since the early 20th century, and now attention is turning in many regions towards brown seaweed resources as source of energy. Also

green seaweeds, in particular *Ulva sp.* are being researched as potential renewable AD feedstocks [46].

Amongst the variety of macroalgae species that exist around Ireland (**Appendix A**); this work investigated the production of biogas from brown macroalgae *Laminaria sp.* and *Ascophyllum nodosum.* In the case of *Laminaria sp.*, five different species are present in Ireland such as *L. digitata, L. hyperborea, Saccharina latissima, Sacchoriza polyschides and Alaria esculenta. Laminaria sp.* and *Ascophyllum nodosum* are commonly known as kelps and wracks respectively. On the East coast of Ireland (Counties Louth, Dublin, Wicklow and North County Wexford) kelp occur to depths of up to 8 m, whereas in the clearer waters of the North-Western, Western and South-Western Irish coasts, they may occur up to 25 m and, exceptionally, up to 32 m [47]. *Ascophyllum nodosum* is a North Atlantic endemic species, which grows in the intertidal zone and can be found in abundance in the South-West, Mid-West and North-West coast of Ireland [47]. The brown colour of these algae, results from the dominance of the xanthophyll pigment fucoxanthin, which masks other pigments, chlorophyll *a* and *c* (there is no chlorophyll *b*), beta-carotene and other xanthophylls. Food reserves are typically complex polysaccharides, sugars and higher alcohols. The principal carbohydrate reserve is laminaran (also called laminarin) [47]. The walls are made of cellulose and alginic acid, a long-chained heteropolysaccharide. *Ascophyllum nodosum* and *Laminaria hyperborea* are widely used for alginate extraction. Alginates, derivatives of alginic acids, are used commercially for toothpastes, soaps, ice cream, tinned meats, fabric printing, and a host of other applications. It forms a stable viscous gel in water, and its primary function in the above applications is as a binder, stabilizer, emulsifier or moulding agent [47]. A simple biochemical profile of brown macroalgae (*Laminaria sp.*) is provided in the **Table 5**.

Table 5: Representative *Laminaria sp.* biochemical profile [46]

Variable	*Unit*	*Value*
Moisture content	% w/w wet base	88
Ash content	% w/w dry base	26
Volatile solids	% w/w d.b.	74
C	% w/w d.b.	34.6
H	% w/w d.b.	4.7
O	% w/w d.b.	31.2
N	% w/w d.b.	2.4
S	% w/w d.b.	1
Cl	% w/w d.b.	-
F	% w/w d.b.	-
Br	% w/w d.b.	-
Higher Heating Value	MJ/Kg d.b.	13.2
Lower Heating Value	MJ/Kg d.b.	12.2
Cellulose	% w/w d.b.	6
Hemicellulose	% w/w d.b.	0
Lignin	% w/w d.b.	0
Lipids	% w/w d.b.	2
Proteins	% w/w d.b.	12
Starch	% w/w d.b.	0
Alginates	% w/w d.b.	23
Laminaran	% w/w d.b.	14
Fucoidan	% w/w d.b.	5
Mannitol	% w/w d.b.	12
Total Fermentable Sugars	% w/w d.b.	60

2.2.3 AD Stability parameters

The stability of AD is influenced by some critical parameters, thus it is crucial that appropriate conditions for anaerobic microorganisms are provided. The growth and activity of anaerobic microorganisms is significantly influenced by conditions such as exclusion of oxygen, temperature, pH, nutrients supply, stirring intensity and presence of inhibitors [42]. This section aims to provide a general outlook of the most important stability and operational parameters used in AD.

2.2.3.1 Temperature

Temperature plays an important role in the AD process as different species of bacteria are active at different temperatures. The digestion can occur under psychrophilic (10 to 20°C), mesophilic (20 to 40°C), and thermophilic (40 to 60°C) conditions [43]. **Figure 5** describes the influence of temperature on the rate of AD. Psychrophilic temperatures are known to decrease microbial growth, substrate utilization, rates, and biogas production [48, 49]. It may also result in an exhaustion of cell energy, a leakage of intracellular substances or complete lysis [50]. Thermophilic temperatures provide many advantages compared to mesophilic and psychrophilic processes, such as faster degradation rates, higher gas production, less effluent viscosity and higher pathogen destruction [51]. At the same time some drawbacks must be considered such as high temperatures that result in a larger degree of imbalance. During the digestion process it is important to maintain a constant temperature, as temperature changes or fluctuations will affect the biogas production negatively. Thermophilic bacteria are more sensitive to temperature fluctuation of ± 1°C and require longer time to adapt to a new temperature. On the other hand, mesophilic bacteria are less sensitive, since temperature fluctuations of ± 3°C are tolerated without significant reductions in methane production [42]. It has been observed that thermophilic temperatures can cause lower biogas yield due to the production of volatile gases such as ammonia which suppresses methanogenic activities [52]. Even though higher biogas and methane production can be achieved at thermophilic conditions, it is recommended to verify if the higher demand for energy is necessary and justified by maintaining these high temperatures. Thus, most biogas plants generally operate at mesophilic conditions (35-37 °C) [53] as the process is more stable and requires lower energy expense [54, 55].

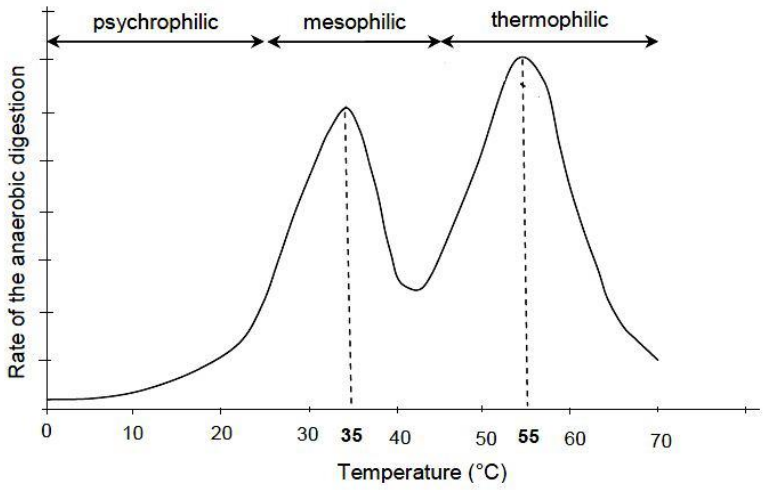


Figure 5: Influence of temperature on the rate of AD [56]

2.2.3.2 Values of pH

The bacterial community responsible for methanogenesis, *i.e. Methanospirillum hungatei*, *Methanosarcina barkeri* and *Methanobacterium formicicum*,requires a neutral pH, of around 7. Below pH 6 and above pH 8.3, methane falls off rapidly [57]. Methanogenesis occurs efficiently at pH 6.5-8.2 [58], while hydrolysis and acidogenesis occurs at pH 5.5 and 6.5, respectively [48]. Since the ideal pH range for hydrolysis and methanogenesis varies, thus in order to obtain a more efficient process, the application of two-phase reactors with a separate hydrolysis stage has been proven to be advantageous [59].

2.2.3.3 Volatile fatty acids (VFA)

The stability of the AD process is reflected by the concentration of intermediate products like the VFA. These acids are intermediate compounds (acetate, propionate, butyrate, lactate), produced during acidogenesis, with a carbon chain of up to six atoms [42]. It has been observed that under conditions of overloading, methanogenic activity does not remove hydrogen and volatile organic acids as quickly as they are produced. The result can be an excessive accumulation of VFA inside the digester, which can lead to a drop of pH-value with a consequent reduction of methanogenic activity and in some cases also an inhibition of the hydrolysis/acidogenesis phase [60]. However, the accumulation of VFA will not always be expressed by a drop of pH value, due to the buffer capacity of the digester, which depends on the biomass types contained in it. For instance, animal

manure has a surplus of alkalinity, which means that the VFA accumulation should exceed a very high level, before this can be detected due to significant decrease of pH value [42].

2.2.3.4 Ammonia

Ammonia is produced by the biological degradation of the nitrogenous matter, mostly in the form of proteins and urea. Since nitrogen is an important nutrient for anaerobic microorganisms, it is believed that ammonia concentrations below 200 mg L^{-1} are beneficial for the digestion process. At the same time, high ammonia concentration inside the digester, especially free ammonia (the unionised form of ammonia, NH_3), is considered to be responsible for process inhibition. A value of 80 mg L^{-1} of NH_3 has been found to be the minimum inhibitory level [61]. Amongst the four types of anaerobic microorganisms, the methanogens are the least tolerant and the most likely to cease to grow due to ammonia inhibition, with a consequent reduction of methane yields [42]. The concentration of free ammonia is therefore direct proportional to temperature and pH. This means that increasing pH and temperature will lead to increased inhibition, as these factors increase the fraction of free ammonia. The process instability, due to high levels of ammonia, results in VFAs accumulation, which leads to a decrease in pH and a consequent declining concentration of NH_3. At this point, the process is in the so-called "inhibited steady state", where it would run stably but at a lower methane yield [40].

2.2.3.5 Hydrogen sulphide (H_2S)

The Hydrogen sulphide production during AD may reduce the methane yield by competition between methanogens and sulphate-reducing bacteria [62, 63]. The inhibitory sulphide level ranges from 100 to 800 mg L^{-1} for dissolved sulphide or approximately 50-400 mg L^{-1} for undissociated H_2S [62]. High concentrations of H_2S are problematic for use in the generation of biogas, due to its corrosive properties, within pipes and cogeneration engines. The maximum concentration of H_2S specified by co-generator manufacturers is around 100 mg L^{-1}.

2.2.3.6 AD nutrients

Macronutrients such as carbon (C), nitrogen (N), phosphor (P), and sulphur (S) are crucial for the growth and survival of AD microorganisms. The unbalanced

nutrients are regarded as an important factor limiting AD, thus a nutrient ratio of the elements C: N: P: S at 600:15:5:3 is considered sufficient for methanization [64]. The insufficient provision of nutrients, as well as unbalanced supply ratios can cause inhibition and disturbances in the AD process. In particular, as the C: N ratio plays a crucial role, the optimum C: N value for the AD has been found between 20: 1 and 30:1 [65]. Low ratios can result in high ammonia released and excessive accumulation of VFAs in the digester [65] resulting in a possible failure of the AD. One method used to avoid excessive ammonia accumulation is to adjust low feedstock C: N ratios by adding high carbon content materials, thereby improving the digestion performance [60].

2.2.3.7 Characterisation of Substrate/Carbon Source

Substrate of AD should always be characterised in terms of total solids (TS) and volatile solids (VS), chemical oxygen demand (COD), nitrogen and phosphorus content [66]. The total solids are defined as the dry matter of a sample heated at 105°C until constant weight is achieved, while VS are defined as the dry matter, which is evaporated by combustion at 550°C. The analysis of VS determines the total amount of organic matter in a substrate while COD is defined as the amount of oxygen that is consumed during oxidation of the organic substance. High ratios of COD: VS indicate substrates with an organic matter that requires more oxygen to decompose than that with lower ratios. In general, substrates containing organic matter that is high in energy (high COD: VS), and are highly digestible also have high specific methane yields.

2.2.3.8 Chemical oxygen demand (COD)

The test used to detect the COD measures the oxygen equivalent of the organic material that can be oxidized chemically using dichromate in an acid solution [67]. It is a simple test to run, and it measures the organic matter in the absence of oxygen. On the other hand, it must be noted that a digestion substrate will have a COD even if the chemicals in the sample are toxic or indigestible to microorganisms. In the case of solid substrates such as seaweeds, it is important to ensure that the sample analysed is homogenous [68]. The total COD (tCOD) can then be fractionated into the principal fractions particulate and soluble COD (sCOD). Particulate and soluble COD can be fractioned further into:

- Readily biodegradable sCOD,
- Slowly biodegradable colloidal and particulate (enmeshed) COD,
- Non-biodegradable sCOD,
- Non-biodegradable colloidal and particulate COD.

Unfortunately, there is little standardization on the definition of soluble COD versus particulate COD. Where filtration is used as technique to fractionate a sample, the relative distribution between soluble and particulate COD will vary greatly depending on the pore size of the filter [69].

2.2.3.9 Moisture content

The digestion process is classified as ‘‘wet’’ or ‘‘dry’’ according to the amount of TS in the feedstock. Wet bioreactors have TS of 16% or less, while dry bioreactors contain 22-40% TS, with the intermediate rating termed ‘semi dry’ [55]. High moisture content generally facilitates AD. It has been proven that the highest methane production rates occur at 60-80% humidity [70]. Some results showed that specific methanogenic activity at low moisture content was remarkably lower than that at high moisture content. In particular, specific methanogenic activity increased linearly when the moisture content of the substrate increased from 65% to 82% [71].

2.2.3.10 Mixing

The effects of mixing the content within a digester are still understood. Mixing is not necessary for methanogenic biology to take place. It does not greatly modify the biogas productivities, namely the production rate and yield, or biogas composition. The main aim of mixing is to achieve a uniform temperature inside the methane digester and reduce scum formation and settlement inside the digestion tank [57].

2.2.4 AD operational parameters

2.2.4.1 Organic Loading Rate and Hydraulic Retention Time

Amongst the AD operational parameters, the Organic Load Rate (*OLR*) and the Hydraulic Retention Time (*HRT*) are considered to be the most important, since they influence the biogas yields as well as the plant economy. The *OLR* indicates

how much organic dry matter can be fed into the digester, per volume and time unit, according to the following equation (1):

$$OLR = \frac{m * c}{VR} \quad (1)$$

In the above equation (1) *OLR* represents the organic load [kg $days^{-1}$ m^{-3}], *m* is the mass of substrate fed per time unit [kg $days^{-1}$], *c* is the concentration of organic matter [%], and *VR* is the digester volume [m^3].

The *HRT* is the average retention time of the overall fluid elements in the reactor. The hydraulic retention time is correlated to the digester volume and the volume of substrate fed per time unit, according to the following equation:

$$HRT = \frac{VR}{V} = \frac{m * c}{V * OLR} \quad (2)$$

In the above equation (2) *HRT* represents the hydraulic retention time [days] and *V* is the volume of substrate fed per time unit [m^3 $days^{-1}$].

Therefore, according to the equation *HRT* (2), increasing the *OLR* reduces the *HRT* which leads to a reduction of the digester's size and consequently, its capital cost. However, sufficient time should be allowed for the microorganisms to break down the organic material and convert it into gas. Generally, the methane yield is constant and maximised when the process is operated at low *OLR* and high *HRT*. When *HRT* is reduced, an increase in *OLR* could result in imbalances in the bacterial population, leading to VS accumulation and digester failure [72]. Optimal *HRT* and *OLR* should then be selected based on a compromise between getting the highest possible biogas yield and having a justifiable plant economy [42].

2.2.4.2 Feeding systems

According to the continuity of feeding, AD is classified as batch or continuous system. Batch system consists of a tank of substrate that is inoculated with bacteria and kept at a suitable temperature. The bacteria begin to grow, and under suitable conditions they continue to grow at a regular rate until the substrate is used up or they alter the conditions in the tank (acid formation) to those adverse to growth. The bacteria then enter a phase of slower and, finally, no growth until

the culture has then reached its end. The residual substrate and bacterial cells must be cleared out and the tank refilled with fresh substrate and inoculum bacteria; or the inoculum may be provided by leaving a little of the previous culture residues in the tank. Anaerobic batch reactors are useful because they can perform quick digestion with simple and inexpensive equipment, and also are helpful in assessing the rate of digestion [73]. On the other hand, have some limitations are known such as high fluctuations in gas production as well as gas quality, biogas losses during emptying the bioreactors and restricted bioreactor heights [70].

If towards the end of the growth phase in a batch culture, fresh medium is added, and an equal volume of the spent medium and bacterial cells are taken out, then the remaining bacteria can continue to grow on the new medium [74]. If this addition and removal of medium and removal of bacteria is repeated every few minutes or continuously then the bacteria can settle down to grow at a rate equal to the rate of medium addition; that is, they double in numbers in the time taken for one fermentor volume of medium to pass through the fermentor. This is the case of a continuous system; where the number of bacteria in the fermentor remains constant with time and so the rates of dissimilation of substrates and production of biogas [74]. Continuous bioreactors can be classified as “one-stage” and “two-stage” or “multi-stage” continuously fed system. In the “one-stage” system all of the biochemical reactors take place in one bioreactor. On the other hand, in the “two-stage” or “multi-stage” system various biochemical processes such as hydrolysis, acidification, acetogenesis and methanogenesis take place separately [55]. This type of system increases the stability of the process by controlling the acidification phase through optimisation of the hydraulic retention time in order to prevent overloading and the accumulation of toxic material. The biomass concentration and other conditions can also be optimised independently for each stage [55].

2.2.5 Pretreatment

In AD, the hydrolysis phase can be identified as the rate-limiting step [75]. A pretreatment is generally necessary in order to reduce the impact of the rate-limiting step. A large number of pretreatments have been tested; these methods can be roughly classified into physical, chemical, biological processes and

combination of them. **Table 6** provides an overview of the most studied pretreatments.

Table 6: Pretreatment processes [76]

Pretreatment methods	*Processes*	*Possible changes in biomass*	*Remarks*
Physical pretreatments	*Milling:* Ball milling Two-roll milling Hammer milling Colloid milling Vibro-energy milling *Irradiation:* Gamma-ray Electron-beam Microwave *Others:* Hydrothermal High pressure steaming Expansion Extrusion Pyrolysis	Increase in accessible surface area and pore size Decrease in cellulose crystallinity Decrease in degrees of polymerization	Highly energy-demanding No removal of lignin No chemicals required Not yet feasible large-scale industrial application
Chemical and physicochemical pretreatments	*Explosion:* Steam; Ammonia fiber CO_2 SO_2 *Alkali:* Sodium hydroxide Ammonia Ammonium Sulfite *Acid:* Sulphuric Acid Hydrochloric Acid Phosphoric Acid *Gas:* Chlorine dioxide Nitrogen dioxide Sulphur dioxide *Oxidizing agents:* Hydrogen peroxide Wet oxidation Ozone *Solvent extraction of lignin:* Ethanol-water Benzene-water Ethylene glycol Butanol water Swelling agents	Increase in accessible surface area Partial or nearly complete delignification Decrease in degrees of polymerization Partial or complete hydrolysis of hemicelluloses	Most effective and promising for industrial applications Usually rapid treatment rate Harsh conditions Chemical requirements
Biological pretreatments	*Fungi and actinomycetes*	Delignification Reduction in degree of polymerization of cellulose Partial hydrolysis of hemicellulose	Low energy requirement No chemical requirement Mild environmental conditions Very low treatment rate

An ideal biomass pretreatment process should be simple, and be able to enhance the solubilisation of polymer to monomer sugars without formation of degradation products. The process should be also inexpensive, less energy-demanding, and not cause any pollution [45]. During the selection of a pretreatment process, the kind of biomass to be treated should also be considered, since a pretreatment method may be a good choice for one type of biomass but may not be suitable for another [43]. In the next sub-paragraphs the main types of pretreatment are discussed.

2.2.5.1 Physical pretreatment

The main effect of physical pretreatment is to increase the accessible surface area and size of pores available for the hydrolytic enzymes. Different types of physical processes can be identified based on the type of force applied. Mechanical processes involve a comminution step using milling or grinding methods, while irradiation processes use gamma rays, electron beam and microwaves in order to improve the enzymatic hydrolysis.

Mechanical pretreatments have been mainly applied to lignocellulosic feedstock. They have been found effective at altering the inherent structure of lignocelluloses and decreasing the particle size and the degree of cellulose crystallinity. The result is an increase of the total hydrolysis yield by 5-25 % and a digestion time reduction by 23-59% [45]. Biomass can be comminuted by a combination of chopping, milling and grinding. In general, after chopping a particle size of 10-30 mm can be achieved, while grinding or milling permit finer sizes in the range of 0.2-2 mm. Many varying machines are commercially available; however the choice of the right grinding or milling machine depends particularly on the moisture content in the biomass. Colloid mills and extruders are suitable for wet materials with moisture contents of more than 15-20%, whereas two-roll, attrition, hammer or knife mills are only suitable for dry biomass with moisture contents of up to 10-15% [45]. The ball mills can be used for either dry or wet biomass. As it is well known, the main drawback of mechanical pretreatments is their high energy use requirement. This parameter depends on many factors such as the type of the mill, initial and final particle sizes, and biomass characteristics (processing amount, composition and moisture content). It has been estimated that the size reduction step can consume up to

33% of the total electrical demand, thus a thorough evaluation of the machines employed as well as of the biomass characteristics would improve the whole process economics [45], something of particular interest in this research.

Also irradiation processes can improve enzymatic hydrolysis of lignocellulosic biomass. Microwave and ultrasound methods are the most studied processes. Microwave can be used as an alternative method for conventional heating and can give better results than classical thermal pretreatment. However during the process, the production of inhibitory compounds can take place. This is highly dependent on the characteristics of biomass processed. Ultrasound can be used especially for disintegration of waste-activated sludge and effluents. The application of ultrasounds causes cavitation into the medium which causes the disruption of cell walls, thus more matter becomes available for hydrolytic enzymes. Even though the irradiation methods can improve the hydrolysis performance, they are expensive, pose difficulties in industrial application and can produce toxic compounds [76].

2.2.5.2 Chemical pretreatment

Chemical pretreatments involve the use of chemical agents which promote hydrolysis. This can be achieved through the use of strong acids, alkalis and oxidants [77]. Alkali pretreatment refers to the application of alkaline solutions such as sodium hydroxide (NaOH), slaked lime $Ca(OH)_2$ or ammonia (NH_3) to remove recalcitrant substances such as lignin and hemicellulose. Acidic pretreatments generally employ the use of solutions of dilute sulfuric acid, hydrochloric acid, and phosphoric acid to hydrolyse the biomass. Amongst the chemical pretreatments for lignocellulosic biomass, these are mostly applied. They can achieve high reaction rates, improve cellulose hydrolysis and remove up to 100% of hemicellulose. The pretreatment is not effective in dissolving lignin, but it can disrupt it and increases the cellulose's availability to enzymatic hydrolysis. However, they are characterised by high reaction temperature and formation of different types of inhibitors [76]. A lower operating temperature can be used when performing high concentrated-acid pretreatments. These methods are not preferred because they are corrosive and must be recovered to make the pretreatment economically feasible [44]. Another chemical pretreatment method is ozonation, this pretreatment employs ozone which is a strong oxidant which

does not cause an increase of the salt concentration and no chemical residues remain [77]. Since it is also disinfects from pathogens, ozonation has gained great interest especially for sludge pretreatment [77].

2.2.5.3 Biological pretreatment

Biological pretreatments employ microorganisms, mostly fungi, which can produce enzymes capable of degrading the recalcitrant compounds such as lignin and hemicellulose, since cellulose is more resistance to the biological attack. The main advantages of this type of process are its low energy requirement, the elimination of any chemical requirements, and mild environmental conditions [76]. On the other hand, residence times of days, the requirement of careful growth conditions, and the large amount of space to perform biological pretreatments are the disadvantages that make this method pretreatment less attractive on an industrial scale [44].

2.2.6 Biogas uses

Biogas has many energy utilisations; it can be used for heat production by direct combustion, electricity production by fuel cells or micro-turbines, Combined Heat and Power (CHP) generation or as vehicle fuel. Biogas is a mixture of methane and carbon dioxide with generally small amounts of sulfuric components (H_2S), as shown in **Table 7**.

Table 7: Biogas composition [42]

Component	*Chemical symbol*	*Concentration*
Methane	CH_4	50 – 75 volume-%
Carbon dioxide	CO_2	25 – 45 volume-%
Water vapor	H_2O	2 – 7 volume-%
Oxygen	O_2	< 2 volume-%
Nitrogen	N_2	<2 volume-%
Ammonia	NH_3	< 1 volume-%
Hydrogen	H_2	< 1 volume-%
Hydrogen sulphide	H_2S	20 – 20.000 ppm
Nitrogen	N_2	<2 volume-%

[ppm: parts per million; Volume. -%: volumetric percentage]

The composition and properties of biogas depend in some degree on feedstock types, digestion systems, temperature and retention time. The calorific value of typical biogas (60 % CH_4 and 40 % CO_2) ranges from 5.5 to 6.5 kWh m^{-3}, this is comparable to natural gas, which has an energy value of 5.8-7.8 kWh m^{-3} [39]. Hence, biogas can potentially be used as a substitute for natural gas. This section provides an overview of the main applications of biogas.

2.2.6.1 Biogas in CHP generation

Biogas produced from nearly all large-scale AD reactors is used to power CHP systems to generate heat and electricity, which are used to operate AD reactors and associated facilities (*e.g.*, office buildings). It has been estimated that approximately 30% of the energy present in biogas can be converted into electricity, while approximately 55% can be recovered as heat, leaving only 15% being wasted. Hence, CHP systems are very efficient in utilizing biogas. However, it should be noted that the H_2S present in biogas and some of its combustion products are acidic and can present a corrosion risk to biogas-handling and CHP systems. The corrosion posed by H_2S can be reduced by removing it through either the use of iron hydroxide into the digester or a H_2S bio-scrubber. Other solutions can be the use of alkaline lubricant oil as well as new CHP systems such as the recently developed externally fired gas-turbines [39].

2.1.6.2 Upgrading biogas to biomethane

The major difference between biogas and natural gas is in relation to its CO_2 content. Biogas contains 30-40% CO_2 and 55-70% CH_4, while natural gas consists primarily of methane (75-82%). Biogas also contains small quantities of water vapour, hydrogen sulphide, nitrogen, oxygen, ammonia, siloxanes and particles. For efficient operation, protection of mechanical equipment from corrosion, and to maximise the volumetric energy density, contaminants and gases with no energy value need to be removed. The final result is biomethane which is chemically identical to natural gas [78].

For most upgrading systems removal of H_2S prior to upgrading is necessary. This is usually achieved by the addition of iron hydroxide to the digester; if large quantities of hydrogen sulphide are present in the biogas (*i.e.* greater than 2000

ppm) the use of a H_2S bio-scrubber may be necessary before CO_2 removal. There are various techniques and methods for CO_2 removal. The three most commercially available upgrading techniques are high pressure water scrubbing (HPWS), pressure swing adsorption (PSA) and chemical (amine) scrubbing [79, 80]. The first two systems are currently the dominant upgrading systems in the biomethane industry. However, HPWS systems were identified as being the least complex in operation and therefore are currently the most attractive and employed systems [14].

Once the biogas has been upgraded to biomethane, it can be injected into the gas grid or used as a transport fuel in compressed natural gas (CNG) vehicles. The end product is practically identical to natural gas and it can be blended as bio-natural gas or sold separately [14].

2.1.6.3 Biogas and Fuel Cells

Electricity production from biogas using fuel cells is an attractive alternative because of improved efficiency and the reduced production of pollutants. Fuel cells are devices that electrochemically convert the chemical energy contained in the fuel into direct current electricity and oxidation products of the fuel [81]. The process is without emissions, quiet and also effective when compared to conventional combustion engines [82]. However, conventional fuel cells can only use pure H_2 - rich gas as fuel, thus the biogas from AD must first be reformed. A possible alternative can be the use of a new type of fuel cell, the solid oxide fuel cell (SOFC), which can use biogas directly without prior reformation. Nevertheless, this technology is still in its infancy, and no commercial application has yet been reported [39].

2.3 Biogas production from algal biomass

This section was the subject of a published review paper entitled *"Biogas production from algal biomass"* [38]. This paper provides a comprehensive and critical literature review of the major obstacles related to the exploitation of both macroalgae and microalgae biomass in order to demonstrate a more comprehensive understanding of the topic. A summary of the main factors influencing both macro- and microalgae biomass is described in [38], while in the next section are reported the key findings from the literature research which this research aimed to address.

2.4 Summary of the key findings

From the literature investigation, several issues related to the exploitation of algal biomass for biogas production were identified. In particular, this research will focus on the following key findings which were considered important for the exploitation of macroalgal biomass in Ireland.

Depending on the type of pretreatment and algal species, it is evident that an enhancement in methane yield can be achieved. Mechanical pretreatments up to date have been preferred due to their simplicity and effectiveness. However, from the available literature it was not possible to identify the most suitable pretreatment strategy for a specific algal species. The application of different AD parameters does not permit to compare pretreatments according to methane yields. There was also a lack of data regarding the energy evaluation of algal biomass pretreatments, to identify the current status of the economic feasibility of this pretreatment step.

A large variation in methane yields was observed according to the species employed. Brown seaweeds exhibited the highest methane yields with respect to green and red seaweeds. Several studies investigated the brown *Laminariales*, while very little literature was available regarding other Irish brown seaweeds species such as *Ascophyllum nodosum*, *Alaria esculenta* and *Pelvetia canaliculata.*

Amongst the AD parameters that influence the methane production from algal biomass, the organic substrate concentration was reported as one of the most critical.

Methane production is highly influenced by the chemical composition of seaweeds. In general, high sugar content determines high methane production. In seaweeds the sugar levels are mainly influenced by seasonal and environmental conditions. The seasonality effect on the biogas production from seaweed biomass has not been explored in Ireland to date; this is a focus of this research.

A more detailed analysis of such issues is reported in the next chapter. The objectives, methods, materials and execution of each experiment are also described.

Chapter 3: Experimental Design, Materials and Methods

3.1 Introduction

Following the main findings of the literature review, this chapter presents the objectives and methodologies used throughout the experimental part of this research. The "Design of Experiments" (DOE) technique was selected to statistically analyse the experimentation. This technique was first introduced by Fisher [83] through the development of the factorial experimental design methods. It is a formal structured technique for studying any situation that involves a response which varies as a function of one or more independent variables (also called factors) and which may interact with each other [84]. A more traditional method is the "One Factor at a Time" (OFAT). This method consists of varying only one variable at a time while keeping other variables fixed. There is a general acceptance [84-87] that the DOE is more efficient in order to determine the impact of two or more factors and their interactions on a response, than the OFAT approach. The main reasons are that [85]:

1. The estimates of the effects of each factor are more precise,
2. The interaction between factors can be estimated systematically,
3. Experimental information is collected over a larger region of the factor space,
4. It requires less resource for the amount of information obtained.

Others techniques such as the "trial and error" method [88, 89] and the "best guess" approach [86] were found to have some disadvantages. Compared to DOE, these techniques do not explore the entire experimental space and do not

estimate factors' impacts and interactions. In general, they are time consuming and there is no evidence that an optimised solution is attained.

Therefore, the DOE technique was selected as it permitted to:

1. Estimate the impacts of more variables on the response,
2. Estimate the interactions between variables,
3. Generalise and optimise the solutions,
4. Minimise resources.

3.2 Procedure for experimentation

In this research, the following DOE procedure recommended by Montgomery *et al.* [86] has been applied in order to plan, execute, analyse and report an experiment [66]:

1. Recognition and detailed statement of a problem,
2. Cause-and-effect analysis of the main process inputs (variables) and outputs (responses),
3. Selection of the response variable and choice of factors, levels, and ranges,
4. Choice of experimental design,
5. Experiment execution,
6. Statistical analysis of the data,
7. Discussion of the statistical analysis,
8. Conclusions and recommendations,

For each experiment, the first four steps of the procedure have been developed by considering as the main support, the literature review and the guidelines reported in the VDI 4630 [90]. In particular, the VDI 4630 provides rules for assessing the fermentability of organic materials and indications regarding the necessary equipment and apparatus required for the corresponding test set-ups.

The first four steps are described in the following sections while the other steps are presented in the following chapters.

3.3 Preliminary analysis

A schematic representation of the AD process is illustrated in **Figure 6**. The main input materials of the process are the algal biomass, inoculum and water; after digestion, the main output of the process that this research is interested in, is biogas (~50-60% CH_4). The goal was then to investigate and optimise the response in terms of biogas/methane production.

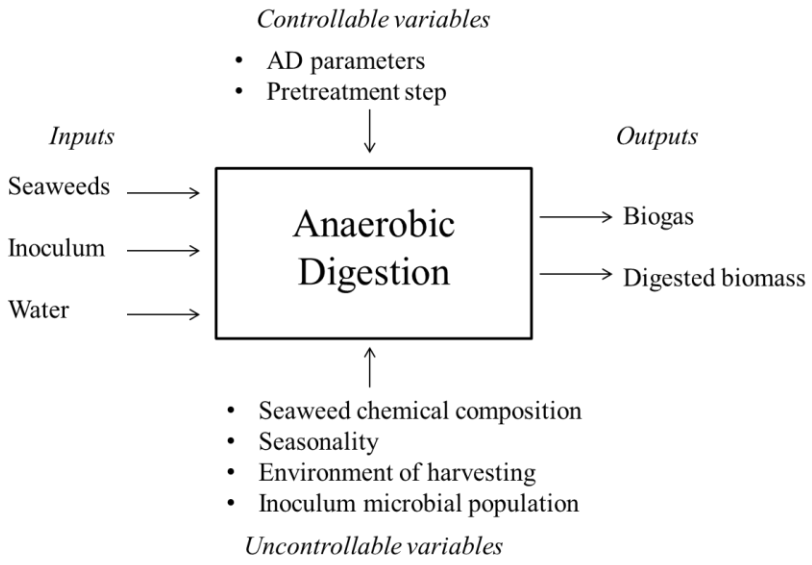


Figure 6: General model of the process under investigation

The variables that can influence the response are represented in **Figure 6**. Some of these variables are *controllable* such as the parameters used during the AD and the use of a pretreatment step, while other variables are *uncontrollable*. Among the *uncontrollable* variables, some of them can be considered as *nuisance* factors that may influence the experimental response but in which we are not directly interested [86]. Examples of *nuisance* variables are the inoculum microbial composition and the environment of harvesting. A general cause-and-effect diagram was carried out in order to identify in detail all of the possible variables that affected the process (**Figure 7**).

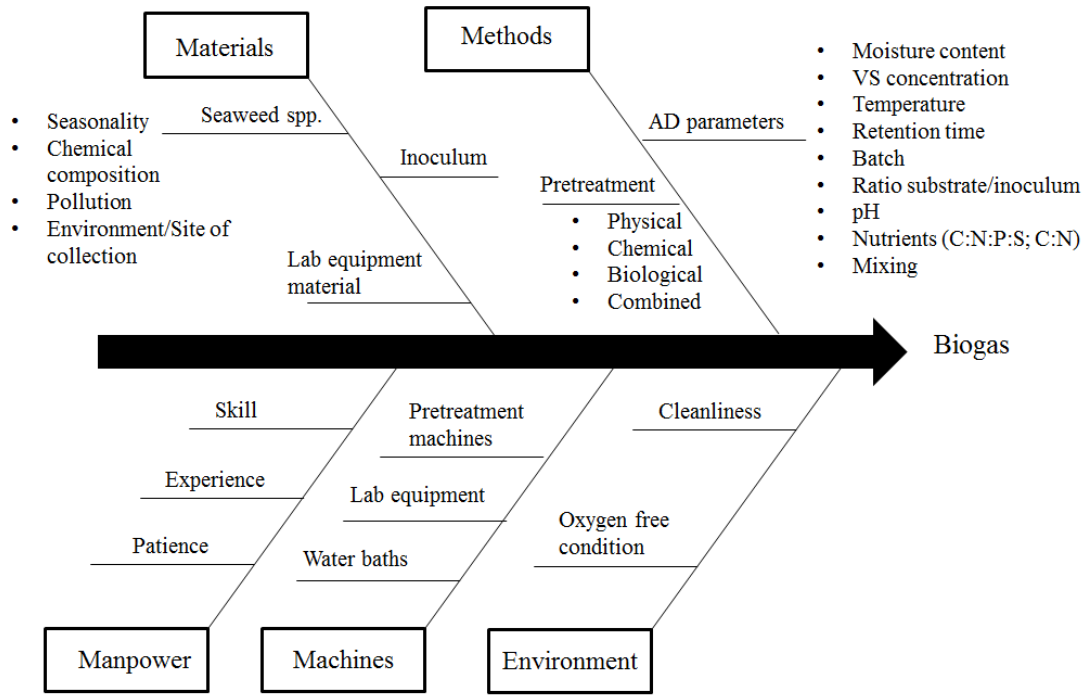


Figure 7: General cause-and-effect diagram for biogas production

All of the identified variables were classified into main categories: materials, methods, machine, manpower and environment. The aim of this preliminary analysis was to explain how these variables affect the output of the system. Therefore, the description of the materials, methods and laboratory equipment employed in the experimental work is provided in the *Materials and Methods Section*. Among all the variables, the following factors were thought to require special attention.

Considering the materials category:

1. The seaweed species used as substrate influences the biogas and methane production. In general, the chemical composition varies according to the species, season and site of collection, as extensively discussed in the *Literature Review Chapter 2*. Thus, it was worthwhile to investigate what kind of seaweed species amongst the indigenous ones could better suit the process in terms of methane production. This point was developed and addressed in experiment 2.
2. The inoculum was a very high source of variation for the entire process. The main function of the inoculum was to provide the necessary "broad trophic"

microbial composition in order to ensure that different substrates would not face any limitations [66]. If the digester was not fed with a suitable microbial population, then the substrates would not be efficiently digested with a consequent of failure of the process. It was suggested to use as inoculum untreated digested sludge from a municipal sewage treatment works and one which is not obviously subject to inhibition [90]. Although this variable influenced the output of the system, it was chosen not to investigate this kind of influence. In this case, the inoculum represented a *nuisance* variable. By holding constant a *nuisance* variable, it was possible to minimise such influence on the final output of the system. Thus, the same kind of inoculum as well as the same liquid volume in the AD reactor was used throughout the experimental study.

3. Most of the lab equipment (reactors, stoppers, cylinders) were made of glass as preferred material especially for all those parts which are in contact with the biogas, as suggested by the procedure [90]. Tubes and valves were made of plastic and kept as short as possible.

Considering the methods category:

1. The influence of pretreatment on biogas production from seaweed has been extensively discussed in the *Literature Review Chapter 2*. It emerged that depending on type of pretreatment and seaweed species, an evident enhancement in methane yield can be achieved. Nevertheless, few studies investigated the effect of different pretreatments on seaweed considering similar AD conditions [38]. For this reason, a further investigation was carried out in experiment 1. The pretreatment variable was intentionally varied by holding constant the AD parameters for each pretreatment method under investigation.
2. The AD parameters influenced highly the final response. Some parameters were held constant throughout all the experiments. In **Table 8**, an overview of the AD settings used for all the experiments is presented. Amongst all the AD parameters, only the VS concentration was under investigation. It is known [72] that an excessive substrate concentration leads to imbalances in the bacterial population, VS accumulation and digester failure. On the other hand, excessively low substrate concentration can result in starving conditions within

the digester and a consequent reduced methane generation [69]. To date, only few studies have addressed the influence of substrate concentration on the AD of seaweed [91-93]. In general, suitable substrate concentration must be investigated according to the nature and composition of the algal substrate [38]. For this reason, the VS concentration was intentionally varied in experiments 2-3-4, after a proper selection of both pretreatment and seaweed species.

Table 8: AD parameters

Factor	***Value***	***Reason***
Temperature	Mesophilic range (38°C)	Better AD stability in the presence of inhibitory compounds [40]
Experimental set-up	Batch	Easier to perform at lab scale [66, 90], Fundamental evaluation of the possible biogas yield and of the anaerobic biological degradability of a material or mixture of materials, Qualitative appraisal of the speed of anaerobic degradation of the material under investigation, Qualitative evaluation of the inhibitory effect of the material under investigation in the range of concentrations in the test
Mixing	Once a day	Encouraging degassing of the biogas and preventing the formation of dry and inactive layers [90]
Ratio Substrate/Inoculum	50:50 % in volume	Ensuring the controllability of the inoculum variability and to provide sufficient microbial activity
Nutrients (C:N:P:S; C:N)	No adjustment	The algal compounds represent the main nutrients' source. The C: N ratio was not considered as parameter under investigation
pH	No adjustment	Adding digested sewage sludge as inoculum brought the pH value in the optimal range for AD
Moisture content/Dry matter	Adjusted according to the VS % under investigation	As the VS concentration was a variable under investigation, the dry matter of the substrate was adjusted through addition of water in order to obtain the desired VS concentration. The factor of dilution was related to the amount of VS (%TS) measured in fresh seaweed

Considering the machines category:

1. Water baths were used as the heating unit. On a daily basis, it was ensured that the level of water in the bath was always higher than the fill in the reactor vessels by the operator. The reason was to avoid temperature fluctuations within the entire volume of the incubator since these are known to affect negatively the microbial activity and consequently the biogas production [90].
2. Both the lab equipment and the pretreatment machines were inspected prior every experiment in order to ensure no external contamination was observed as well as proper functioning.

Finally, **Table 9** reports a classification of the variables identified. In particular, the seaweeds seasonality was classified as a non-controllable variable. Nevertheless, it was highly important for bioenergy purpose, not only to identify suitable species but also to choose and note the best harvesting times [94]. For this reason, it was appropriate to investigate the changes of the system's output according to the time of harvesting as well.

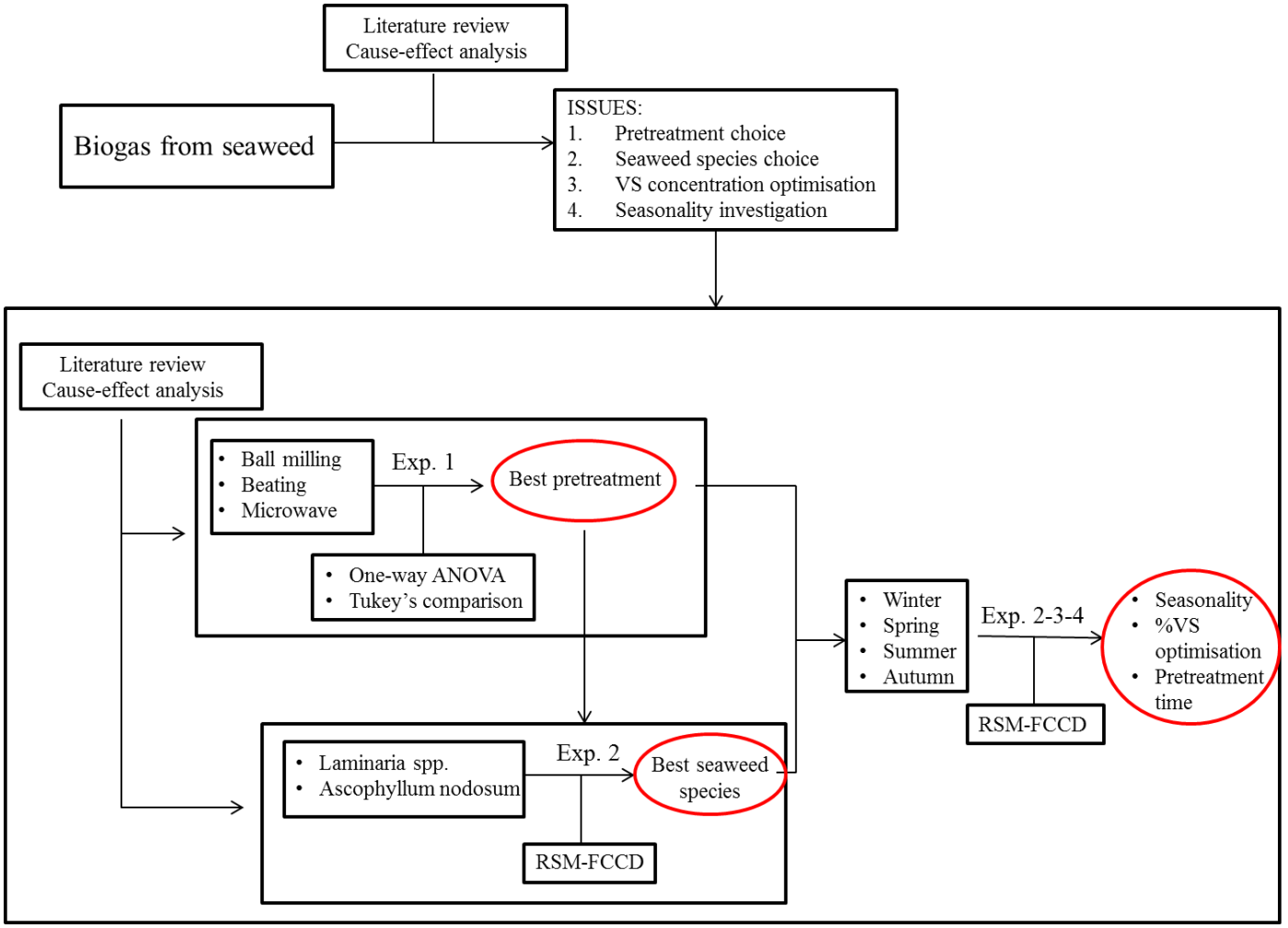
Table 9: Variables' classification for biogas production

Variables under study	***Variables under control***	***Variables cannot be controlled***
Seaweed species	Inoculum	Seaweeds seasonality
VS concentration	AD parameters	Pollution of the environment of harvesting
Pretreatment	Lab equipment/set-up	
	Manpower	
	Site of collection	

Thus, the preliminary analysis revealed some major issues related to the exploitation of seaweeds for biogas production:

1. Choice of a suitable pretreatment,
2. Choice of a suitable seaweed species,
3. VS concentration optimisation,
4. Seasonality investigation.

These issues were addressed through a series of four experiments. **Figure 8** shows the developed strategy of experimentation. Each experiment was supported by a detailed literature review and a preliminary cause-and-effect analysis in order to reveal further variables which can influence the process. The output of each experiment was used as input for the next set of experiments. Suitable statistics were also chosen according to the objectives of each experiment.



RSM = Response Surface Method
FCCD = Face-centred central composite design

Figure 8: Proposed strategy of experimentation

3.3.1 Experiment 1: pretreatment choice

It is worth noting that a pretreatment method must be maintained as simple as possible and the products must be highly fermentable [77, 95], therefore most of the studies investigated were based on the use of physical pretreatments due to their simplicity. Nevertheless, few studies investigated the effect of different pretreatments on macroalgae considering similar AD conditions [38], thus a rigorous comparison between pretreatments results may be difficult. Among the macroalgae, the *Laminaria sp.* and *Ulva sp.* are most recommended for the production of biogas. In Ireland, it is possible to find five kelp species such as *Laminaria digitata*, *L. hyperborea*, *Saccharina latissima*, *Sacchorhiza polyschides* and *Alaria esculenta*. In particular, this experiment dealt with the use of a mixture of cast *Laminaria sp.* The objective of this experiment was to evaluate the effect of three physical pretreatments; beating (BT), ball milling (BM) and microwave (MW) on the methane yields of the Irish macroalgae *Laminaria sp.* at pre-selected digestion parameters. **Figure 9** displays the cause-and-effect analysis which identified the main variables that influenced the use of these pretreatments when macroalgae *Laminaria sp.* was used as a feedstock.

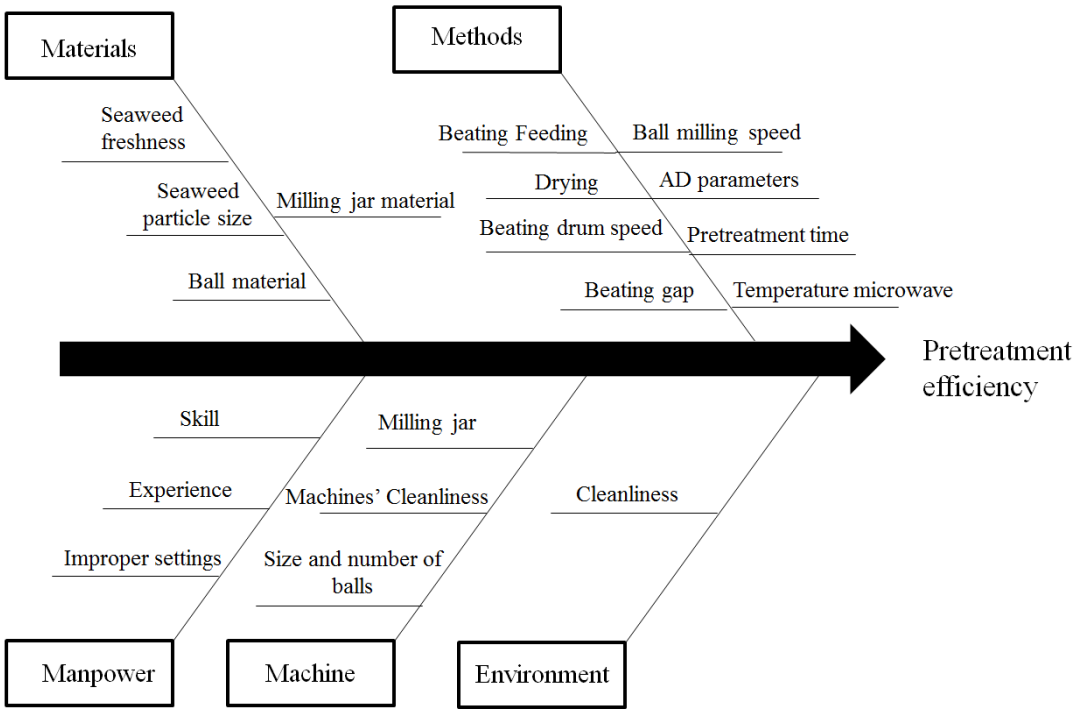


Figure 9: Cause-and-effect diagram pretreatment (experiment 1)

Amongst all the variables identified in the cause-and-effect analysis, some of them were thought to require further discussion:

In particular, regarding the materials:

1. The seaweed freshness had to be considered. While the beating and microwave pretreatment did not require a prior drying step of the feedstock, the ball milling required drying the seaweed due to its consistency.
2. After beating and ball milling a reduction of particle size was achieved. The particle size of the substrate influenced the biogas production. In general, the bigger is the specific surface of the biomass, the higher is the biogas yield, but the relationship is not linear [96]. The comminution of fine particles contributes less than the comminution of big particles [96].
3. The milling jar and balls material was chosen in order to avoid any kind of contamination of the substrate.

Regarding the methods:

1. The feeding of the beater machine in terms of water and seaweed ratio was adjusted according to the amount of TS necessary for the digestion process. In this experiment, the machine was fed in order to obtain a final solution at 5% TS concentration.
2. The beating machine did not allow an adjustment of the drum speed, which was kept constant at 580 rpm.
3. In the literature, the effect of microwave on *Laminaria sp.* for biogas production was not explored. It is known that this kind of pretreatment is influenced by microwave frequency, radiation time and biomass concentration while, the main effect is an enhanced solubilisation of the substrate [97].
4. The pretreatment time was set according to the kind of pretreatment. Previous work by Tedesco *et al.* [98] studied the influence of the beating time as well as the machine gap on the biogas production from *Laminaria sp.* According to this study a beating time of 10 min in conjunction with a machine gap of 76 µm was the best combination in order to obtain the highest biogas yield from *Laminaria sp.* In the case of ball milling, a pretreatment time of 18 h was used [95]. For microwave pretreatment the retention time was set at 30 sec, after the boiling of the liquid phase.

Regarding the machine category, a description of each pretreatment machine is reported in the *Material and Methods Section 3.6.2*. This experiment involved a single variable (pretreatment) at several different levels (**Table 10**), while the pretreatment efficiency was measured in terms of biogas/methane production.

3.3.1.2 One-way ANOVA and Tukey's multiple comparisons test

Since the aim of the experiment was to test for differences between the biogas/methane averages produced after a particular treatment, this was the case of a one-way classification of the data. The statistical analysis of a one-way classification data is generally done by using a one-way ANOVA. Specifically, through ANOVA it was possible to reveal if there were differences between one or more pairs of treatment averages, but it did not indicate which pairs were different. For this reason, the one-way ANOVA was followed by post-ANOVA methods of analysis to identify the different pairs. Amongst all the formal methods [84, 99, 100], the Tukey's multiple comparisons test was chosen in order to identify significant differences between treatments. The aim of the statistical analysis was to assess whether the $k = 5$ group means, differ from one another. The ANOVA *F* test is a popular statistical procedure for assessing group differences; however, when $k > 2$, a significant *F*-test would have to be probed further, in order to locate specific differences amongst the group means. Tukey's multiple comparison procedure, is a commonly cited method when the researcher's multiple comparison hypotheses are for pairwise differences and the rate of Type I error has to be controlled for the set of all possible pairwise contrasts [99, 101, 102] .

The critical value of Tukey's test is:

$$w_a = \frac{s_\varepsilon Q_{a,k,df_\varepsilon}}{\sqrt{n}} \quad (3)$$

In the above equation, Q_{a,k,df_ε} is the critical value of the *Studentised range distribution* for $\alpha = 0.05$, s_ε represents the standard error of the ANOVA and *n* is the number of observation. The critical value (Q_{a,k,df_ε}) depends on the significance level α, the number of treatments k, and the number of error degrees of freedom for the ANOVA df_ε. If the difference between any pair of averages exceeds w_a,

then it is possible to conclude that those averages are significantly different from each other [84]. Although the Tukey's test is less powerful (less sensitive to small differences between treatment averages) than other similar tests, for example the Duncan's test [103], it is widely used, since it involves fewer calculations and it is easier to report [84].

Table 10: Variables matrix: experiment 1

Variable under investigation-Factor	***Levels***	***Response***
Pretreatment (categoric)	Untreated	Biogas production
	Beating	[ml g^{-1} VS]
	Microwave	Methane production
	Ball milling (1 mm)	[ml g^{-1} VS]
	Ball milling (2 mm)	

3.3.2 Experiment 2: seaweed choice

After testing each of the different pretreatments, a selection of a suitable species in terms of biogas/methane performance was carried out. As output from experiment 1, the beating was selected as optimised pretreatment to be used.

Amongst all of the seaweed species, the literature review showed that the brown seaweed species were the most promising in terms of biogas yields. In particular, brown seaweeds such as *Laminaria sp.* and *Ascophyllum nodosum* are the most commercially important Irish seaweed species [47]. About 16,000 tonnes of *Ascophyllum nodosum* are harvested each year in Ireland, dried and milled in factories at Arramara Teoranta, Cill Chiaráin (Kilkerrin), Co. Galway; and some 3,000 tonnes of the resulting seaweed meal is exported and processed in Scotland for the production of alginic acid. *Laminaria hyperborea* stipes are collected in drift in Scotland and Ireland and the rods are used for the manufacture of high-grade alginates. Thus, *Laminaria sp.* and *Ascophyllum nodosum* were ideal candidates as species for further investigation. The cause-and-effect analysis (**Figure 10**) considered both species as the main subjects for biogas production.

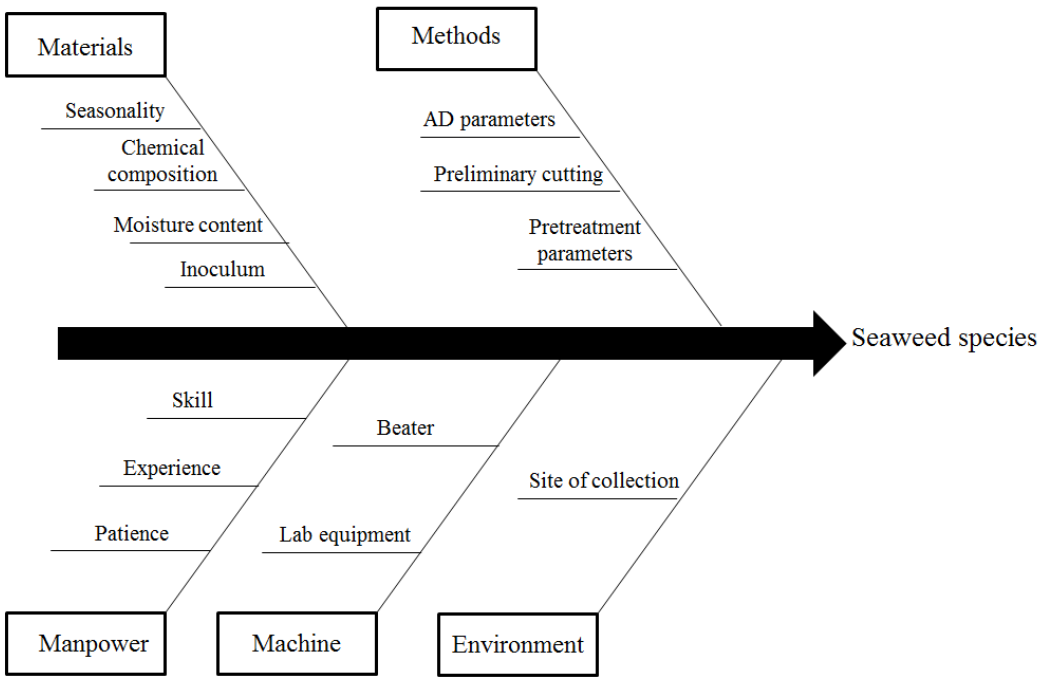


Figure 10: Cause-and-effect diagram seaweed species (experiment 2)

Most of the variables reported in **Figure 10** were already discussed. Nevertheless, some observations about materials and environment must be considered.

By looking at the materials category, the chemical composition of seaweeds was the main factor influencing the biogas/methane yield. The carbohydrates levels in *Laminaria sp.* were related to the season [94, 104] while in the case of *Ascophyllum nodosum* no real seasonal variation has been reported in the literature [92]. One of the main differences between the chemical compositions of these two macroalgal species is the presence of polyphenols. In comparison to carbohydrates, the polyphenol content of the *Laminariales* make up proportionally less of the seaweed tissue compared to that found in *Ascophyllum nodosum* [94]. This compound is extremely important for the AD process as it is known that polyphenols have an inhibitory action on methane production [105].
The environmental conditions are considered as one of the factors which influence the chemical composition of seaweeds and therefore the AD process [106, 107]. As this was not the subject of this research, the site of collection represented a *nuisance* variable. Therefore, the harvesting of both species was conducted from the same site.

3.3.2.1 Response surface method: face-centred central composite design

The aim of this experiment was to determine the best species in terms of biogas/methane yields between *Ascophyllum nodosum* and *Laminaria sp.* by evaluating the influence of beating pretreatment and substrate concentration. The response surface methodology (RSM) was selected in order to evaluate the influence of these two variables on the response (the biogas/methane production) and the interaction between them according to the seaweed species. The methodology was initially developed and described by Box *et al.* [108] and widely applied for chemical and biochemical processes [109]. In particular, it is recently used as an optimisation technique for biofuels production [98, 110-112]. This is a collection of mathematical and statistical techniques which allows the designing of an experiment in order to optimise a final response (y) which is influenced by several variables ($x_1, x_2, \ldots, x_k$) [86, 113]. The main advantage of this method over others DOE designs such as Taguchi and 2-level factorial design methods [149, 150] was to reveal interactions and even quadratic effects of influencing parameters on AD by limiting the number of planned experiments [86]. In most RSM problems, the form of the relationship between the response and the independent variables are unknown. Thus, the first step in RSM was to find a suitable approximation for the true functional relationship between y and the set of independent variables. In particular, when the experimenter is relatively close to the optimum, a model that incorporates curvature is usually required to approximate the response. In most cases, a second-order model (4) is adequate [86].

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum \sum_{i<j} \beta_{ij} x_i x_j + \varepsilon \qquad (4)$$

Where β are the regression coefficients of the model and ε is the random error. Amongst the response surface designs, the central composite designs or CCD are the most popular for fitting a second-order model. Generally, the CCD consists of a $2k$ factorial (where k is the number of study variables) with n_F factorial runs, $2k$ axial or star runs, and n_c centre runs. In general, there are two parameters in the CCD that have to be specified: the distance α^* of the axial runs from the design centre and the number of centre points n_c [86].

The choice of α^* determines the *rotatability* of the design. It is important for the second-order model to provide good predictions throughout the region of interest. Thus, the model should have a reasonably consistent and stable variance of the predicted response at points of interest [86]. In particular, *rotatability* refers to the uniformity of prediction error. In rotatable designs, all points at the same radial distance (r) from the centre point have the same magnitude of prediction error [116]. The value of α^* for *rotatability* depends on the number of points in the factorial portion of the design; in fact, $\alpha^* = (n_F)^{1/4}$ yields a rotatable CCD where n_F is the number of points used in the factorial portion of the design [86]. *Rotatability* is extremely important when the location of the optimum point within the region of interest is not clear before the experiment is conducted; therefore it is desirable that all points of the design at a given distance (r) from the centre point in any direction have the same magnitude of prediction error.

An α^* value equal to 1 renders a CCD not rotatable, this is the case of a face-centred CCD (FCCD). This kind of design is chosen whenever the region of interest is cuboidal rather than spherical (the rotatability is indeed a spherical property) [86]. In these cases, the FCCD represents a useful variation of CCD as it requires less centre points and levels of each factor and thus it is a simpler design to carry out. For example, in order to make rotatable a 2^2 CCD, α^* must be chosen equal to 1.4, which means operating the process at five level settings of each variable, while the FCCD, in contrast, requires operating the process at only three level settings of each variable [86].

When deciding between a FCCD and a rotatable CCD, it must be considered whether the rotatable design benefits of uniform prediction error and the extension of the design region adequately offset the added complexity of operating the process at two additional level settings of each variable. This leads to a much greater opportunity for sources of experimental error associated with setup and operations. Compared to FCCDs, rotatable CCDs offer reduced prediction error for, and improved estimation of, quadratic effects. However, given a reasonable magnitude of overall experimental error, these benefits do not outweigh the added complexity of requiring each variable to be run at five levels [116].

In the case of this experiment, a FCCD design was selected. The optimum region was not completely unknown and the experimental error associated with operating the process at two more additional levels was very high. **Figure 11** represents the FCCD employed. The selected design involved two study variables (beating time and VS concentration) and it consisted of a centre point, four factorial points (the intersection points of the coded variable bounds) and four axial points (points parallel to each variable axis on a circle of radius equal to 1.0 and origin at the centre point). The dots in **Figure 11** identify the variable level setting combinations that constituted the nine design points (experiment runs). Since the goal of this experiment was to select the best seaweed species between *Laminaria sp.* and *Ascophyllum nodosum*, a categorical factor 'seaweed species' was considered as well. The levels values (**Table 11**) were chosen, by considering previous studies on the subject. Tedesco *et al.* [98] investigated a beating pretreatment on *Laminaria sp.* by testing a range between 5 and 15 min as time of pretreatment. The best result in terms of methane production was observed after 10 min of pretreatment. Regarding the organic matter concentration, Hanssen *et al.* [92] found out that the optimum methane production from *Laminaria sp.* and *Ascophyllum nodosum* was achieved with a VS concentration below 6 %. According to these results, a centre point at 10 min and 2.5 % of VS concentration was designed. The use of centre points was useful in order to provide good variance of prediction throughout the experimental region [86].

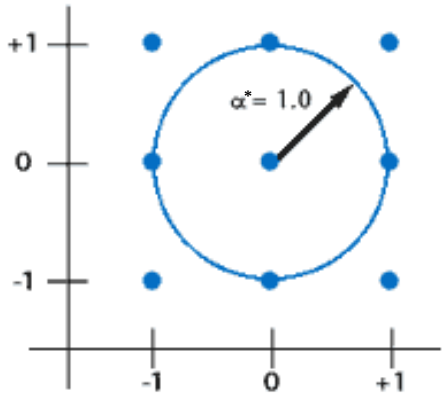


Figure 11: FCCD of two variables, one centre point, α* = 1

Table 11: Variables matrix: experiment 2

Variable under investigation-Factor		*Levels*	*Response*
Seaweed species (categoric)	1.	*Laminaria sp.*	Biogas production [ml g^{-1} VS]
	2.	*A. nodosum*	Methane production [ml g^{-1} VS]
VS concentration (numeric)	1.	1%	
	2.	2.5%	
	3.	4%	
Beating time (numeric)	1.	5 min	
	2.	10 min	
	3.	15 min	

3.3.3 Experiments 2-3-4: seasonality investigation

The variation of seaweeds chemical composition according to the season was one of the drawbacks in the exploitation of this feedstock for bioenergy purpose. Previous studies [94, 104, 117] showed that the chemical composition of seaweeds changes according to the season and how the conversion of this kind of biomass into biofuels was affected. Nevertheless, the literature lacks of studies that investigate an optimisation of the AD when the algal biomass is harvested at different times of the year.

Experiment 2 revealed the selection of *Laminaria sp.* as the most suitable seaweed species for biogas conversion when beating pretreatment was applied. At this stage, the other two issues that this research aimed to address were: the optimisation of the VS concentration along with the pretreatment time, and the influence of the harvesting period of *Laminaria sp.* For this reason a series of experiments were performed throughout a year. The data from experiment 2 were used as representative of May, while other two experiments, one in November and another in March were performed respectively. May was selected as representative of the end of spring and start of summer, November as representative of the end of autumn and start of winter, finally March as representative of the end of winter and start of spring.

For each experiment, the interaction between VS concentration and time of pretreatment and the effect on the response were evaluated through the RSM technique (**Table 12**).

Table 12: Variables matrix: experiment 2-3-4

Variable under investigation-Factor	*Levels*	*Response*
VS concentration (numeric)	1. 1% 2. 2.5% 3. 4%	Biogas production [ml g^{-1} VS] Methane production [ml g^{-1} VS]
Beating time (numeric)	1. 5 min 2. 10 min 3. 15 min	

3.4 Analytical methods

3.4.1 Total solids (TS) and volatile solids (VS) analysis

The amount of *TS* was determined by drying the seaweeds at 105°C to a constant weight. The *TS* fraction was then calculated by using the formula:

$$TS\,[\%] = 100 - MC\,[\%] \tag{5}$$

The moisture content (*MC*) of each sample was calculated according to the following formula:

$$MC\,[\%] = \left[1 - \left(\frac{D_C - C}{W_C - C}\right)\right] * 100 \tag{6}$$

In the above equation, D_C [gr] = weight of dry matter and container, C [gr] = weight of container, and W_C [gr] = weight of fresh sample and container.

The *VS* amount was determined by combusting a known weight of dried sample at 575 ± 25°C overnight, according to standard methods (NREL/MRI LAP 1994, 2008) [118, 119].

The *VS* fraction was calculated as percentage of *TS* according to the formula:

$$VS\,[\%TS] = \left(\frac{D - A}{D}\right) * 100 \quad (7)$$

In the above equation, *D* [gr] = weight of dry matter at 105°C, *A* [gr] = weight of ash. The ash is represented by the weight of dry matter after ignition at 575°C. Both *TS* and *VS* analysis were carried out in triplicate.

3.4.2 Total and soluble COD

Total (tCOD) and soluble COD (sCOD) were determined through the colorimetric method. For COD analysis the procedure followed is reported as Method 8000 for water, wastewater and seawater by Hach Lange Company. The measurements were carried out using Hach standard kit (range 0-1500 mg L^{-1}, Hach Lange, Düsseldorf, Germany) and a Hach Lange DR2000 spectrometer to read the samples. Prior to sCOD determination, a vacuum filtration through a glass microfiber filter (1.5 µm of pore size) at first and then through a membrane filter (0.1 µm of pore size) was performed. Both tCOD and sCOD were determined by diluting the samples at a dilution factor of 1:100.

3.5 Materials

3.5.1 Experiment 1: pretreatment choice

Laminaria sp. was manually collected on shore in Howth (Dublin, Ireland) in early November 2013. There was no selection of a particular *Laminaria* species, in order to reproduce the case of harvesting readily biomass available on the beach. In the mixture harvested from the beach, three main species were identified, namely *L. digitata* (**Figure 12-a**), *L. hyperborea* (**Figure 12-b**), and *Saccharina l.* (**Figure 12-c**). The TS content was found at 18 ± 2 % Wt on wet basis, while the VS content was at 85 ± 1 % Wt on dry basis. Before pretreatment, the fresh seaweed was roughly cut and immediately used without washing. On the same day, the treated and untreated seaweeds were inoculated and subject to AD.

3.5.2 Experiment 2: seaweed choice

A mixture of *Laminaria sp.* seaweed (*L. digitata*, *Saccharina l.*, and *L. hyperborea*) was manually collected on shore in Howth in early May 2014. From

the same site *Ascophyllum nodosum* (**Figure 12-d**) was manually collected in August 2014. Before pretreatment, fresh seaweeds were roughly cut and immediately treated without washing. **Table 13** reports the TS and VS contents for the two species.

Table 13: TS and VS analysis based on experiment 2

Species	***TS [% Wt on wet basis]***	***VS [% Wt on dry basis]***
Laminaria sp.	14 ± 1	66 ± 8
Ascophyllum nodosum	30 ± 3	73 ± 5

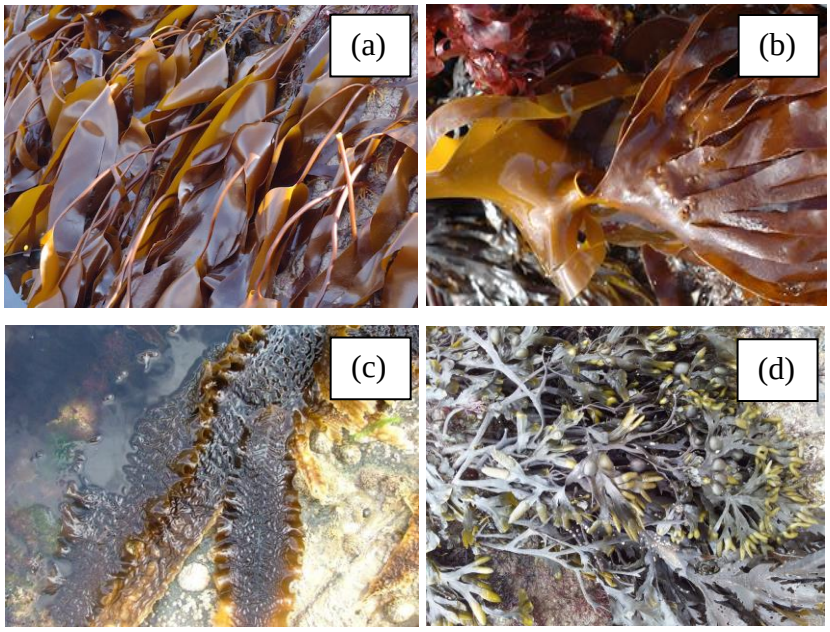


Figure 12: ***Laminaria digitata (a), L. hyperborea (b), Saccharina l. (c)*** **and** ***Ascophyllum nodosum (d)***

3.5.3 Experiments 2-3-4: seasonality investigation

Samples of *Laminaria sp.* (*L. digitata*, *Saccharina l.*, and *L. hyperborea*) were collected from the beach in Howth in May 2014, November 2014 and early March 2015. For each month, before beating pretreatment, fresh seaweeds were roughly cut without washing. **Table 14** reports the TS and VS analysis.

Table 14: TS and VS analysis, experiments 2-3-4

Period	*TS [% Wt on wet basis]*	*VS [% Wt of TS]*
May 2014	14 ± 1	66 ± 8
November 2014	19 ± 2	84 ± 1
March 2015	13 ± 2	74 ± 1

3.5.4 Inoculum

The inoculum used for all the experiments was digested sewage sludge from a wastewater treatment plant (Celtic Anglian Water Ltd., Ringsend, Dublin) operating at mesophilic temperature. **Figure 13** reports the stage of the wastewater treatment process when the sludge was extracted.

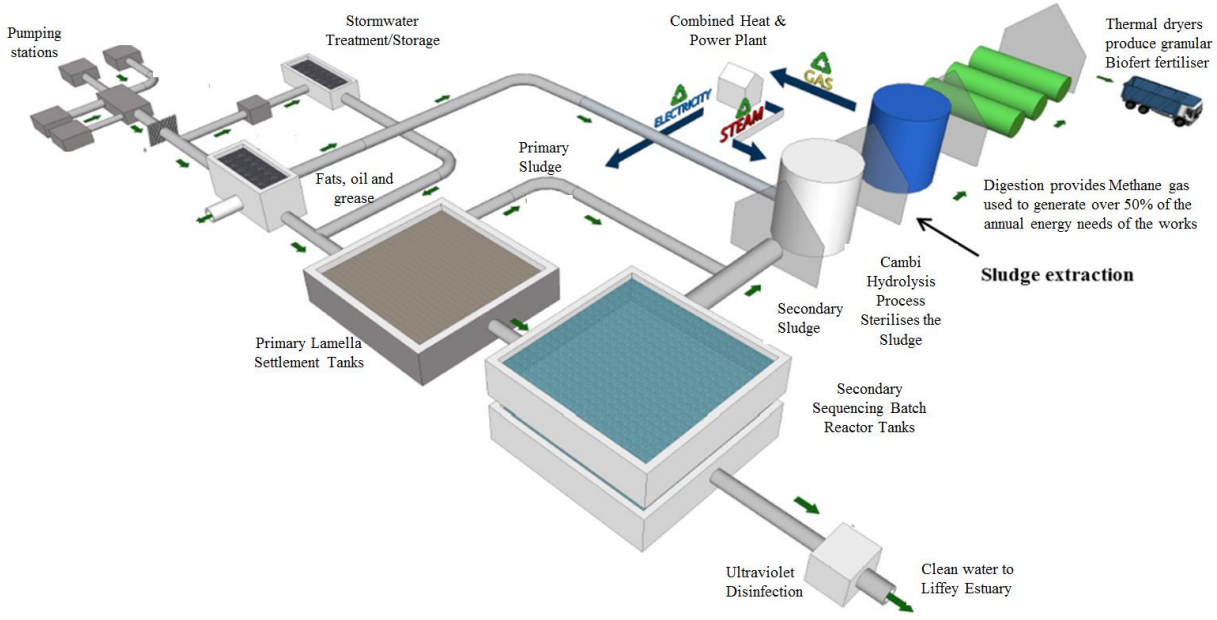


Figure 13: Ringsend Waste Water Treatment Works, Dublin City

The TS content of the inoculum was found equal to 3.6 ± 0.5 % Wt on its wet basis, while the VS content was equal to 79.5 ± 4 % Wt on its dry basis. The pH was measured equal to 8 ± 0.1. The tCOD and sCOD were found equal to 60.15 ± 6.8 g O_2 L^{-1} and 5.8 ± 0.42 g O_2 L^{-1} respectively. Once collected from the plant, the inoculum was immediately used and prevented from degasify in order to reproduce the operating conditions of a co-digestion system for further studies.

Hence, sludge only reactors were incubated in order to estimate the biogas production of the inoculum, which was then subtracted from the seaweed-sludge yields.

3.6 Methods

3.6.1 AD experiment set-up

A batch system was used as the AD experiment set-up. The bioreactors consisted of borosilicate glass flasks of 500 ml in capacity. After inoculum addition, the pH for each sample was measured by using a Hanna precision pH meter (accuracy ± 0.01), model pH 213. All reactors were sealed with borosilicate glass adapters equipped with controlled gas opening valves. Each reactor was connected to an airtight Linde plastic-gas bag, where the biogas produced during all the incubation time was collected. The whole system was purged with nitrogen flow for 5 minutes in order to achieve anaerobic conditions. For all experiments, water baths were used to incubate the reactors at an operating mesophilic temperature of 38 ± 1°C. During incubation, the bioreactors were shaken manually once a day. The biogas volume was measured by using gas sampling tubes which were installed in a gas jar with confining liquid according to procedure VDI 4630 [90]. The entire experiment set-up is represented in **Figure 14-15**. A biogas analyser, model Drager X-am 7000, was used to verify that the system was anaerobically isolated, and to measure the percentage of CH_4 in the biogas.

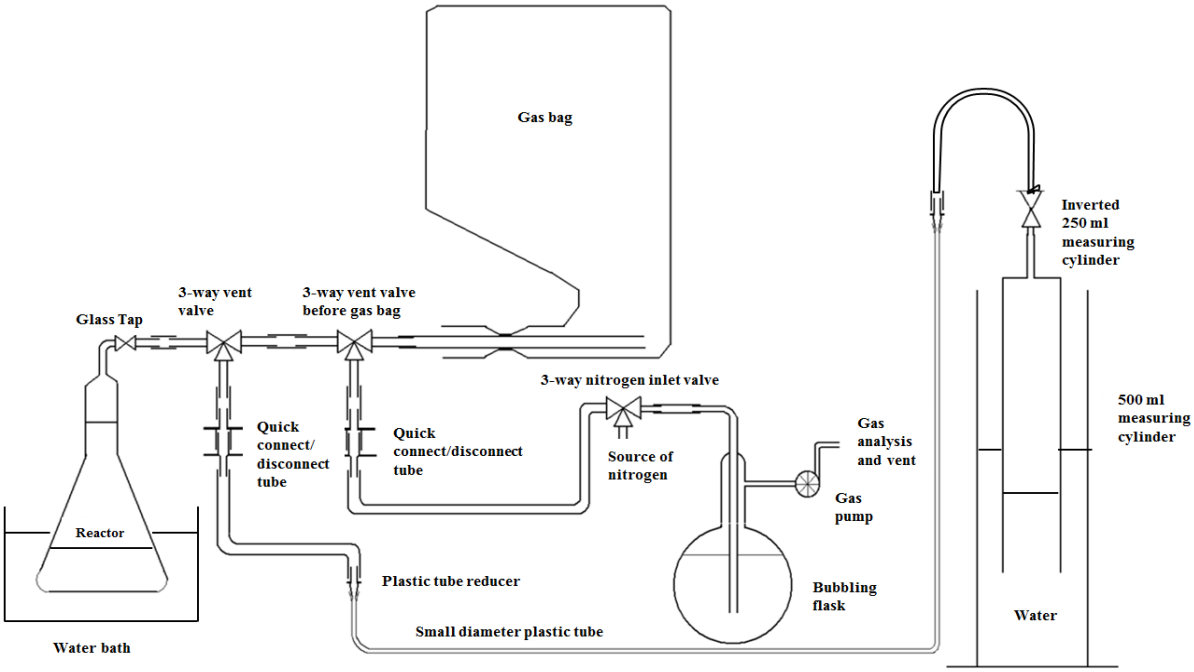


Figure 14: AD experiment set-up

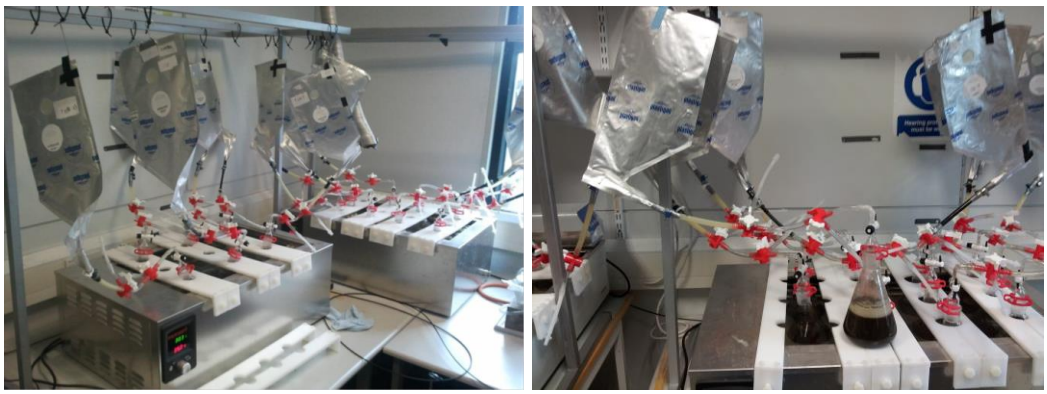


Figure 15: Lab AD set-up, water baths and reactors

3.6.1.1 Experiment 1: pretreatment choice

Each bioreactor was filled with a solution of 200 ml of tap water at a constant 5% TS concentration of treated seaweed for each type of pretreatment. Then 200 ml of inoculum was added for a total working volume of 400 ml. These were performed in duplicate. The untreated seaweed sample was composed of a solution of 200 ml of tap water at 5% TS concentration of untreated seaweed and 200 ml of inoculum. These were performed in duplicate. For this experiment, the incubation time was set at 25 days. Biogas collections for analysis were performed at 3, 13, and 25 days after the start of incubation. Sludge only reactors were prepared with 200 ml of sludge and 200 ml of tap water in order to obtain

the same working volume of 400 ml as for inoculated seaweed bioreactors. Sludge only reactors were prepared in duplicate.

3.6.1.2 Experiment 2: seaweed choice

Batch AD tests were carried out in duplicate both for *Laminaria sp.* and *Ascophyllum nodosum*, after the experimental conditions were applied. For each VS concentration tested (1, 2.5 and 4%), each bioreactor was filled with 200 ml of treated seaweed at different beating times (5, 10 and 15 min) and 200 ml of inoculum for a total liquid volume of 400 ml. Tests of untreated seaweed and inoculum for each different seaweed VS concentrations were also included. Samples of sludge-only were prepared as in experiment 1. The incubation time was set at 14 days. The biogas produced during the reaction was collected in airtight Linde plastic-gas bags and collected after 6 days and at the end of digestion.

3.6.1.3 Experiments 2-3-4: seasonality investigation

For this investigation the same AD set up as for the seaweed choice experiment was applied. The same experiment was reproduced three times along a year; in May, November and following March by using *Laminaria sp.* as only seaweed.

3.6.2 Pretreatment methods: beating (BT), ball milling (BM) and microwave (MW)

The equipment used for the beating pretreatment was a Hollander beater; model Reina (**Figure 16**, **Table 15**). This kind of machine was originally built for the pulp and paper industry. It was equipped with a crank handle which allows adjustment of the gap between the drum's blades and the bed-plate. The minimum gap achievable was 76 µm, which corresponded to one single turn of the crank handle. The machine performs two main actions; (a) - cutting action caused by the grooves located on the bed-plate, and (b) - high pressure beating action of the feedstock against an inclined plate placed at the exit-out of the drum. The drum of the machine permitted a constant rotational speed of 580 rpm. Even though, the machine was capable of operating both wet and dry biomass, it was necessary to add water in order to cause the recirculation of the feedstock. The result was a pulp of different consistencies according to the gap and the processing time applied. The machine was operated at the minimum gap of 76

µm and pretreatment time of 10 minutes, according to a previous optimisation [98].

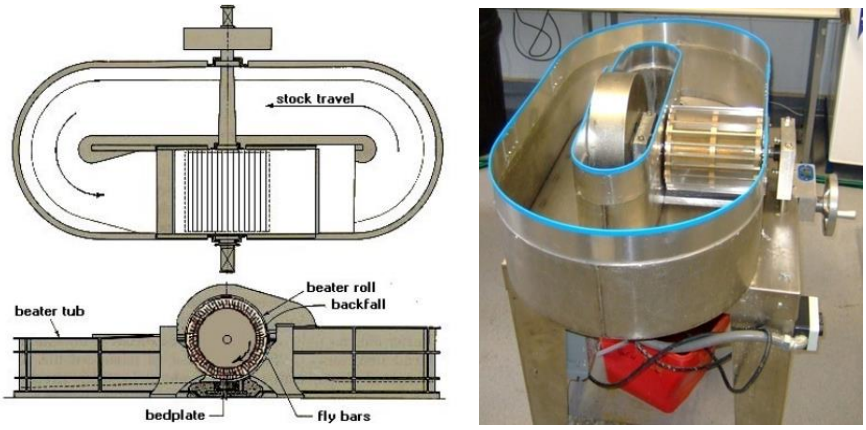


Figure 16: Hollander beater [120]

Table 15: Hollander beater specifications

Motor	1hp (746 watts)
	220 v
	6.9 Amps
	1 Phase
	1450 rpm
V-Belt drive	2.5: 1 Reduction
Drum Speed	580 rpm
Tub Volume	Maximum Capacity = 90 Litres
	Working Capacity = 40 litres
Drum Diameter	200mm
Drum Paddles	24 paddles

Due to the seaweed consistency, a ball milling of fresh seaweed was not possible to perform. Thus, the seaweed was previously dried for 24 h at 80°C and then milled in a conventional ball milling (**Figure 17**) for a period of 18 hours by using a porcelain milling jar with 20 alumina balls (15 mm diameter). The resulting powder was sieved in order to obtain two different particle sizes of 1 mm and 2 mm respectively.

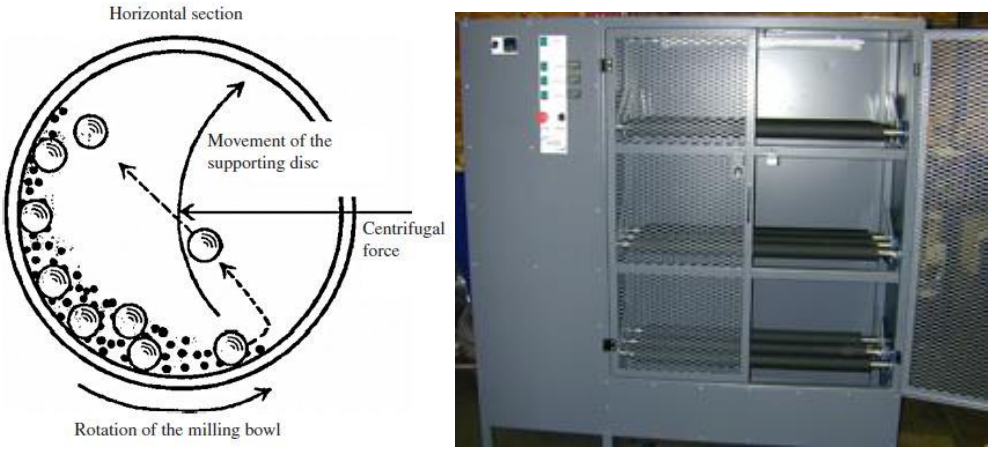


Figure 17: Ball milling [121]

Roughly cut fresh seaweed together with tap water was subject to microwave pretreatment. The samples were exposed to microwave at 560 W, until a temperature of 100°C was achieved through the boiling of the liquid phase. At this point, the samples were left boiling for a retention time of 30 seconds. The pretreatment was performed with a conventional microwave oven (Sharp Compact R230A, 50 Hz).

3.6.3 Experiment 1: pretreatment choice, statistical analysis

The data were analysed by a one-way ANOVA. The one-way ANOVA was used as this was the case of a quantitative outcome ("methane yield") with a categorical variable ("pretreatment") that had more levels of treatment. In this work five levels have been considered for the "pretreatment" variable such as untreated, beating (BT), ball milling at 1 mm (BM 1mm), ball milling at 2 mm (BM 2 mm) and microwave (MW). The ANOVA was based on *F*-testing and this analysis was followed by Tukey's pairwise comparisons between averages. A one-way ANOVA at each collection (3, 13, 25 days) was considered in order to verify if there were differences among the methane yields of the treatments that could not be explained by random variation. The statistical significance of the data was evaluated through the P-value approach [86], with 95% confidence level ($\alpha = 0.05$). Thus, if the P-value was found less than 0.05, the methane yields at a particular collection could be considered statistically significant and a pairwise Tukey's test could be run. Also in the case of the Tukey's test, the P-value approach was used as a statistic to determine the significance of the terms. In particular, for values < 0.05, the difference between pretreatments methane

averages was estimated statistically significant while for a P-value > 0.05 the difference between pretreatments methane averages was not statistically significant.

3.6.4 Experiment 2: seaweed choice, statistical analysis

The RSM used in the present study was a FCCD involving two different factors, namely the beating time and the VS concentration for each seaweeds species. Each factor was set at three levels; 5, 10 and 15 min as time of beating and 1, 2.5 and 4% as VS concentration. A total of 13 experiments were conducted for each species, with the first 9 experiments organized in a 3^2 full factorial design with two operating variables and the remaining 4 involving the replications of the centre point. The values of the centre point were selected as beating time 10 min and VS concentration 2.5%. Since the aim of the study was to select the best seaweed species between *Laminaria sp.* and *Ascophyllum nodosum*, a categorical variable 'seaweed species' was considered, with a total of 26 runs as reported in **Table 16**. The ANOVA was used in order to check the adequacy of the model developed and to obtain the interaction between the process variables. The quality of the fit polynomial model was expressed by the coefficient of determination R^2, and its statistical significance was checked by the Fisher's *F*-test. Model terms were evaluated by the P-value with 95% confidence level ($\alpha = 0.05$). The statistical design (**Table 16**) was generated, evaluated for the quality of fit of the model and the constant and regression coefficients by using the Design-Expert® software (version 9.0.3.1). Such software was selected among others such as Minitab, JMP and Statit, because it is DOE dedicated, easy to use and offers an interactive graphics, diagnostics and optimisation tools.

Table 16: Design matrix for experiment 2

Exp. No.	*Factors*		
	X_1: VS concentration [%]	**X_2: beating time [min]**	**X_3: Seaweed species**
1	1	15	*Laminaria*
2	2.5	10	*Ascophyllum*
3	2.5	10	*Laminaria*
4	4	15	*Ascophyllum*
5	2.5	10	*Laminaria*
6	2.5	10	*Ascophyllum*
7	4	10	*Ascophyllum*
8	4	15	*Laminaria*
9	2.5	10	*Ascophyllum*
10	1	15	*Ascophyllum*
11	2.5	15	*Laminaria*
12	4	5	*Laminaria*
13	2.5	10	*Laminaria*
14	2.5	10	*Laminaria*
15	2.5	10	*Ascophyllum*
16	2.5	10	*Ascophyllum*
17	2.5	5	*Laminaria*
18	4	10	*Laminaria*
19	1	10	*Ascophyllum*
20	1	5	*Ascophyllum*
21	1	5	*Laminaria*
22	2.5	5	*Ascophyllum*
23	2.5	15	*Ascophyllum*
24	4	5	*Ascophyllum*
25	2.5	10	*Laminaria*
26	1	10	*Laminaria*

3.6.5 Experiment 2-3-4: seasonality investigation, statistical analysis

The same FCCD used for experiment 2 was developed for experiments 3 and 4. In this case, only one species was tested (*Laminaria sp.*) and the FCCD considered two variables (beating time, VS concentration) at three levels and one centre point (10 min; 2.5 %). This design was replicated three times over a year, according to different harvesting month. The ANOVA was used to evaluate the adequacy of the model for each experiment as already explained for experiment 2.

3.6.6 Energy calculation method

The following formulas were employed in each experiment in order to calculate the energy balance related to the use of the mechanical pretreatment.

$$B_S = CH_4\ \% * \frac{9.67}{97} \quad (8)$$

Where B_s [kWh m^{-3}] is the energy content of the biogas produced by seaweed, CH_4 % is the average content of methane of the seaweed biogas, 9.67 kWh is the energy content of 1 Nm^3 (Normal cubic meter) of biogas at 97% content of methane [122].

$$E_p = B_p * B_s \quad (9)$$

In the above equation, E_p [Wh g^{-1}VS] is the energy related to the biogas produced from 1 g of VS of seaweed and B_p [m^3 g^{-1}VS] is the quantity of biogas produced for each gram of VS of seaweed.

$$E_C = \frac{E_{pt}}{VS_m} \quad (10)$$

In the above equation, E_C [Wh g^{-1}VS] is the energy consumed by the pretreatment in order to process 1 g of VS of seaweed, E_{pt} [Wh] is the energy consumed during the pretreatment measured by a kilowatt hour meter, VS_m [g] is the total amount of VS into the machine.

$$Net\ E_P = E_P - E_C \quad (11)$$

The *Net* E_P [Wh g^{-1}VS] is the energy produced by 1 g of VS of seaweed treated. In the case of untreated seaweed the E_C term was equal to zero since no mechanical pretreatment was applied.

$$Energy\ Gain = \frac{(Net\ E_P)_{pretreatment} - (Net\ E_P)_{untreated}}{(Net\ E_P)_{untreated}} * 100 \quad (12)$$

The *Energy Gain* [%] is the difference in percentage between the energy provided by the biogas produced from treated seaweed $(Net\ E_P)_{pretreatment}$ and the energy from the biogas provided by the untreated seaweed $(Net\ E_P)_{untreated}$. The *Energy Gain* was negative when the $(Net\ E_P)_{untreated}$ term is > than the $(Net\ E_P)_{pretreatment}$ term, which meant that the use of the pretreatment caused a loss of energy compared to the case of untreated seaweed.

3.7 Chapter summary

In this chapter the “Design of Experiment” technique was identified as experimental methodology. The preliminary analysis of the AD process when using seaweeds as feedstock was carried out. As result of such analysis, the objectives, materials and methods were described for each experiment. The execution of each experiment was also reported.

The following chapter reports the results obtained for each planned experiment along with a discussion of the main findings.

Chapter 4: Results and Discussion

4.1 Introduction

This chapter reports on the experimental work, statistical analysis and results discussion. In this research work, a total of four experiments were carried out. The first experiment (experiment 1) investigated the best pretreatment method, among three different pretreatments (beating, ball milling and microwave), that suited macroalgal biomass for biogas production. Experiment 2 dealt with the comparison of the methane yields from two seaweed species such as *Laminaria sp.* and *Ascophyllum nodosum (A. nodosum),* while optimising the substrate concentration as well as the pretreatment phase. According to experiment 1 and 2 results, the last two experiments (experiments 3 and 4) investigated the use of *Laminaria sp.* as feedstock for biogas production at different periods over a year (seasonal effect). At the same time, an optimisation in terms of substrate concentration and beating pretreatment for each harvesting period was carried out.

4.2 Experiment 1: pretreatment comparison

The objective of this investigation was to evaluate the effect of three physical pretreatments; beating (BT), ball milling (BM) and microwave (MW) on the methane yields of the Irish macroalgae *Laminaria sp.* at pre-selected digestion parameters. Also, an energy balance study was carried out based on the energy consumption of the best pretreatment in terms of methane production. To the author's knowledge, no prior study on the comparison of methane yields from

Laminaria sp. has been reported on previously, when BT, BM and MW were used as pretreatment.

4.2.1 Methane production and statistical analysis

The methane yields at each collection and the final cumulative methane production after 25 days of digestion for each treatment is reported in **Table 17**. Also the trends of cumulative biogas and methane production are reported in **Figure 18**.

Table 17: Data on cumulative methane production after 3, 13 and 25 days of digestion

	Pretreatment				
	Untreated	**BT**	**BM 1mm**	**BM 2mm**	**MW**
Methane at 1st collection[a] [ml/g VS]	93±4	127±3	71±2	64±5	99±7
Methane content at 3 days (%)	41±2	44±1	43±2	43±0	46±1
Methane at 2nd collection[b] [ml/g VS]	212±2	178±4	147±1	148±9	68±2
Methane content at 13 days (%)	67±4	65±2	58±1	60±2	61±4
Methane at 3rd collection[c] [ml/g VS]	23±3	30±1	23±1	48±2	77±2
Methane content at 25 days (%)	60±1	50±2	41±4	51±1	55±2
Cumulative methane[d] [ml/g VS]	328±5	335±8	241±3	260±15	244±6

[a] *Single-factor ANOVA analysis of the data set showed that P=0.0011*
[b] *Single-factor ANOVA analysis of the data set showed that P<0.0001*
[c] *Single-factor ANOVA analysis of the data set showed that P<0.0001*
[d] *Single-factor ANOVA analysis of the data set showed that P=0.0011*

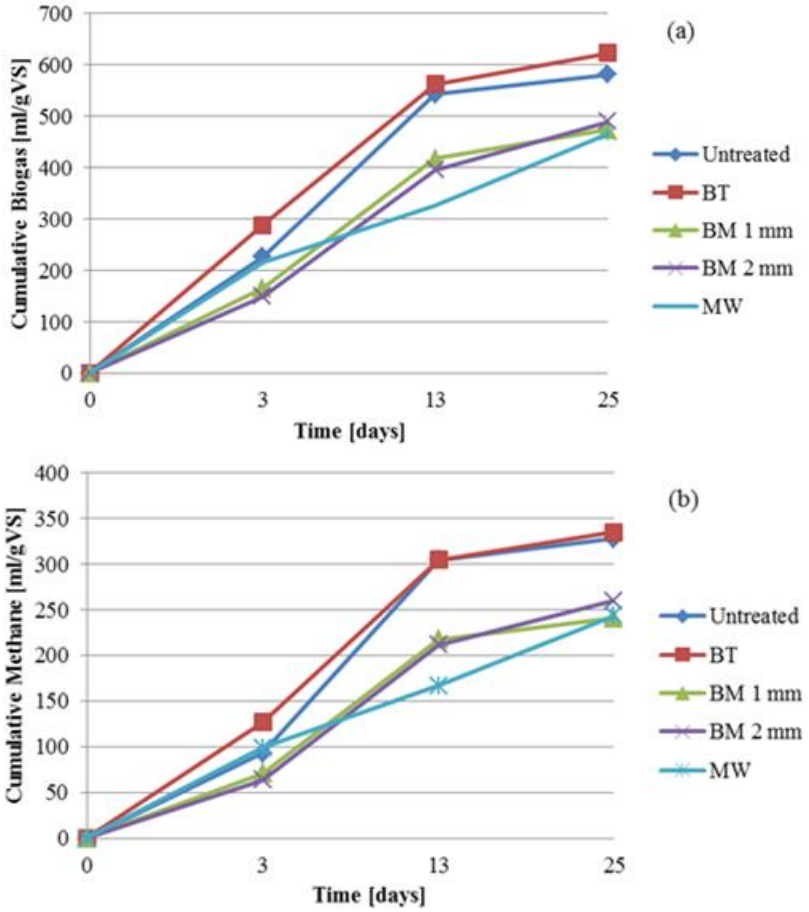


Figure 18: Cumulative (a): biogas and (b): methane at 3, 13, and 25 days of digestion.

All of the P-values were found to be less than 0.05 (**Table 17**), which means that for each collection the differences observed in the methane yields were likely due to the different pretreatments applied. Thus, the methane means at each collection and the overall cumulative means for each pretreatment were subject to Tukey's pairwise testing in order to identify significant differences between pretreatments. The results of the Tukey's pairwise tests are reported in **Table 18**.

Table 18: Tukey's pairwise comparisons after 3, 13, 25 days of digestion and on the cumulative methane production

Treatment	*P-values 1st collection*	*P-values 2nd collection*	*P-values 3rd collection*	*P-values cumulative data*
Untreated vs BT	0.0162	0.0141	0.0893	0.9543
Untreated vs BM 1 mm	0.0864	0.0007	0.9999	0.004
Untreated vs BM 2 mm	0.0356	0.0007	0.0006	0.0127
Untreated vs MW	0.8382	0.0001	0.0001	0.0048
BT vs BM1	0.0017	0.0171	0.0893	0.0027
BT vs BM2	0.0011	0.0195	0.0037	0.0078
BT vs MW	0.0382	0.0001	0.0001	0.0032
BM1 vs BM2	0.8706	0.9998	0.0006	0.5143
BM1 vs MW	0.0332	0.0003	0.0001	0.9978
BM2 vs MW	0.0152	0.0003	0.0003	0.659

After 3 days of digestion, amongst all the pretreatments applied, only the BT and MW yielded more methane with respect to the untreated seaweed. BT and MW performed 37 % and 7 % respectively more methane than the untreated. From the pairwise comparison, only the methane produced by the BT sample was found to be significantly higher than the untreated sample. This would indicate that the increase in methane was a result of the BT pretreatment. In the case of the MW pretreatment, the difference of 7 % in methane observed between the treated and untreated sample was found to be "not significant". Both BM at 1mm and at 2 mm samples produced less methane than raw seaweed. By comparing the methane produced after BM at 2 mm with the untreated sample, the difference was found significant, which indicates that the BM may have negatively affected the methane production, by hampering the start of digestion. In the case of BM at 1 mm sample, the difference with the untreated was found to be "not significant",

even though a P-value between 0.05 and 0.1 (P=0.0864) indicated a marginal significance. The methane content for all samples was found ranging from 40 to 47% (**Table 17**).

During the second collection (13 days) the highest methane production was registered for the untreated sample. For all pretreated samples, a significant lower methane production was recorded when compared to the untreated sample. This suggests that the major effect of the BT pretreatment was a boost of the initial phase of the AD, while the other pretreatments might have affected negatively the process. In terms of methane content, during this stage the highest methane percentages were registered with a peak of 70% for the untreated sample (**Table 17**).

During the last collection (25 days), MW, BT and BM at 2 mm registered more methane with respect to the untreated, with MW and BM being significantly higher, while the differences observed in the BT and BM at 1 mm with respect to the untreated sample were found to be "not significant". At the end of the incubation period, the cumulative methane for the untreated seaweed was found equal to 328 ml g^{-1}VS.

Measurements of pH were carried out in order to investigate the occurrence of some kind of strong inhibition. As **Table 19** indicates, the pH values at the end of digestion were found to be rather stable between 7.4 and 7.6. This did not suggest the occurrence of any strong inhibition during the incubation period. Nevertheless, it must be noticed that the measurement of pH was not significant enough in order to diagnose the occurrence of an inhibition. An 'inhibited steady state' can still be the case [40]. A further investigation regarding ammonia and VFAs may be useful in order to diagnose possible inhibition states, that might be occurred after BM and MW pretreatments are applied.

Table 19: pH values before and after digestion

Sample	*pH (before digestion)*	*pH (after digestion)*
Untreated	7.56±0.05	7.48±0.06
BT	7.47±0.01	7.43±0.02
BM 1 mm	7.18±0.09	7.60±0.09
BM 2 mm	7.22±0.1	7.55±0.07
MW	7.48±0.02	7.48±0.02

4.2.2 Energy Evaluation

Amongst the pretreatment methods tested in this experiment, the only method which exhibited a higher methane production with respect to the untreated sample was the BT pretreatment.

By comparing the methane yields obtained through BM and MW with the raw seaweed, the current research data showed that these pretreatments affected negatively the process. Thus, only the BT pretreatment was subject to an energy balance. In general, the pretreatment step needs to be both effective and economically advantageous in terms of the overall process efficiency [123-128]. High pretreatment costs have been identified as one of the key barriers for commercialization of other kinds of biomass (*i.e.* lignocellulosic biomass) [129]. The energy balance was carried out by comparing the energy content of the biogas produced by the raw seaweed biomass with the energy content of the biogas produced by using pretreated biomass. When considering the energy content of the biogas produced through the use of BT pretreatment, the data was corrected by subtracting the electric energy used during the pretreatment step in order to count the influence of the pretreatment as shown in *Paragraph 3.6.6.* **Table 20** shows the energy balance calculations after 3, 13, and 25 days of digestion based on cumulative biogas. The energy content of the biogas was based on a value of 9.67 KWh/Nm^3 at 97% of methane [122]. The energy consumption of the BT pretreatment was measured during the experiment and found to be equal to 0.083 Wh g^{-1}VS, *i.e.* 14 kWh ton^{-1} of raw material. As

expected, a positive energy gain of 28% was achieved after 3 days of digestion. At the end of digestion, the energy balance showed that the biogas produced after the use of pretreatment reached a break-even point. This suggested that the BT pretreatment was beneficial in order to boost the AD of *Laminaria sp.*, but did not permit an increase in the production of energy when compared to untreated seaweed.

Table 20: Energy balance for the BT pretreatment

Digestion Time	*B_S: Biogas Energy Content [KWh/Nm^3]*	*Net E_P: Energy Produced [Wh g^{-1} VS]*	*Energy Gain [%]*
Day 3			
Untreated	4.09	0.93	
BT	4.39	1.18	+28
Day 13			
Untreated	6.68	3.04	
BT	6.48	2.96	-3
Day 25			
Untreated	5.98	3.27	
BT	4.98	3.26	0

4.2.3 Discussion of the key findings

The results obtained in the present work agree with most of the literature, where a general methane yield from *Laminaria sp.* ranging between 200 and 350 ml g^{-1}VS was found [92, 104]. In this study, when a pretreatment was applied, only the BT sample achieved a higher cumulative methane yield of 335 ± 8 ml g^{-1}VS with respect to the raw seaweed. However, this increase was found to be not statistically significant. On the other hand, BM at 1 mm and 2 mm lowered the methane yield by 27 and 21% respectively, compared to the untreated sample. The Tukey's pairwise comparison tests revealed that those reductions were statistically significant. There was a very high probability that the BM pretreatment hampered the AD of seaweed. Thus a particle size decrease up to 1 mm of dried macroalgae negatively affected the methane production. Unlike lignocellulosic biomass, which must be reduced to 1-2 mm in order to decrease heat and mass transfer limitations during the hydrolysis step [45], this work showed that for macroalgae a reduction of particle size in the mentioned range

did not exhibit any improvement in AD performance. This finding was in accordance with Chynoweth *et al.* [130], who reported that in the case of macroalgae, a reduction of particle size from 2-4 mm to finer sizes did not increase gas yields. The main effect of a particle size reduction was to increase the surface area available to the anaerobic microorganisms, resulting in an increase of gas production [131, 132]. However, an excessive particle size reduction can speed up the hydrolysis and acidogenesis phases of AD and increase the production rates of soluble organic matter such as VFA. The main consequence of this phenomenon is an organic overloading that leads to an accumulation of VFAs and decreased pH [133]. When the pH drops to within the acidic range, the methanogenic activity is hampered with a consequent inhibition of the biogas production rate [40, 133]. As the size reduction step in the case of lignocellulosic feedstock consumes about 33% of the total electrical demand [45], this finding would reduce the pretreatment cost and thus improve the overall process efficiency as a harsh treatment was avoided in the case of macroalgae. Generally, the drying of algal biomass is necessary when milling or grinding pretreatments are used. A drying step would allow a more efficient storage of seaweed as well as higher organic loading rate in a continuous system without lowering the hydraulic retention time. However, this has the effect of increasing energy consumption and increasing costs. Few studies have investigated the influence of the drying pretreatment on methane production by using *Laminaria sp.* as substrate. Vanegas *et al.* [134] investigated the AD of the Irish seaweed *Laminaria digitata* after drying and milling up to a particle size less than 1 mm. A methane yield of 184 ml g^{-1}VS was registered, whereas in this study a higher yield of 241 ± 3 ml g^{-1}VS was found. Unfortunately, the mentioned study does not report the methane yields in the case of fresh and untreated seaweed so a rigorous comparison is not possible. This research showed that the combination of a drying and milling step did not improve the overall methane yield. On the contrary, it is very likely that it negatively affected the entire process.

In the case of BT pretreatment, the data showed that the major effect was to promote the start of the digestion. Only during the first days of digestion was found a statistically significant increase in methane whereas during the following days almost no improvement was registered with respect to the raw substrate.

This result confirmed the hypothesis that the pretreatment can speed up the start of the AD [76, 135], even though only resulting in a marginal improvement of the overall methane production. A previous study by Tedesco *et al.* [136] investigated the particle size reduction achieved through the use of BT pretreatment when *Laminaria sp.* was used as substrate for AD. The mentioned study showed an improvement in methane of up to 53% was achieved, when the BT pretreatment was carried out at the same machine's settings as in this study. Tedesco *et al.* [136] reported that after such pretreatment, almost 80% of the particles were sized below 1.6 mm^2, in terms of frontal surface area. This seems to be in disagreement with the finding that particle sizes below 1-2 mm do not lead to an improvement in methane production from seaweed. However, it must be noted that the BT pretreatment was carried out without drying the seaweed and adding water during the pretreatment of the biomass. On the other hand, in order to carry out a BM of seaweed, it was necessary to dry the biomass prior to pretreatment. Hence, the drying step may have influenced the AD of seaweed. Consequently, during the BT pretreatment, the use of fresh seaweed as well as the release of more readily digestible compounds may have promoted the digestion process, when a particle surface area below 1.6 mm^2 was achieved. For BT pretreatment an energy consumption of 14 kWh ton^{-1} of raw material was calculated. This is comparable with other mechanical pretreatments [45, 137]. It was calculated that the energy balance was positive during the first days of digestion, while it settled around the break-even point after 13 days up to the end of digestion. In the case of ley crop silage [137], the energy balance resulted negative for the first 25 days and positive for longer retention times. This meant that, in the case of seaweeds, better performance can be achieved by lowering the digestion time.

The MW pretreatment registered an overall 27% less methane with respect to the untreated seaweed, this reduction was estimated statistically significant by the pairwise comparison. In general, a microwave pretreatment increases the biomass solubilisation which should accelerate and/or increase the anaerobic biodegradability [97]. On the contrary, our data showed that the use of microwave pretreatment at 100°C impacted negatively on the methane production from *Laminaria sp.* Thus, a harsh pretreatment at high temperatures did not improve

the methane production from *Laminaria sp.* seaweed, but it hampered the AD process. To date, there are no studies in the literature dealing with the use of microwave pretreatment for AD of *Laminaria sp.* However, Jard *et al.* [138] studied the use of thermal pretreatment on AD of the seaweed *Palmaria palmata*. The study demonstrated that an increase of temperature from 20°C to 120°C caused an increase in the biomass solubilisation but did not have any significant impact on *P. palmata*'s methane potential. This would suggest that an increase of the biomass solubilisation does not necessary lead to an increase of methane production, as this depends on the type of compounds released. The Jard *et al.*'s study [138] is not directly comparable with this study as a different substrate as well as pretreatment means were used, but it confirmed that the methane production from seaweed biomass did not benefit by using pretreatments which involve the use of high temperatures. Vivekanand *et al.* [139] registered a marginal improvement in the methane yield when a steam explosion pretreatment at 130°C was applied to the brown seaweed *Saccharina latissima*. In this case, the authors concluded that although the thermal pretreatment increased the yield of biogas from seaweed such a harsh pretreatment was more relevant for the more recalcitrant lignocellulosic substrates.

The main finding of this experiment was that the BT pretreatment showed a better performance with respect to MW and BM pretreatment. In particular, it was observed that the BT pretreatment speeded up the digestion process with an energy gain. Therefore, the BT pretreatment was selected as pretreatment for the next set of experiments and a shorter retention time of 14 days was adopted.

After the selection of the pretreatment method, two different brown seaweed species were investigated (*Laminaria sp.* and *Ascophyllum nodosum*). In the next section, the results of such investigation are presented and discussed. Also the impact of the BT pretreatment time and the organic substrate concentration were investigated with regards to these species.

4.3 Experiment 2: seaweed choice

This experimental study evaluated the influence of beating pretreatment and substrate concentration on AD applied to two common Irish seaweeds namely *A. nodosum* and *Laminaria sp.* Through experiment 1, it was possible to select the

beating as method of pretreatment. The pretreatment phase was tested in terms of beating time, while the substrate concentration was considered in terms of VS concentration. Response surface methodology (RSM) was utilised in order to evaluate the influence of BT and VS concentration on methane production and the interaction between them.

4.3.1 Methane production

Figures 19-20 and **Table 21** report the cumulative methane yields registered for both species at different experimental combination after 14 days of digestion. The experimental error was reported as standard deviation calculated between measurements. A graphical appreciation of such error is reported as bars in **Figures 19-20**.

Laminaria sp. yielded higher methane than *A. nodosum* for all experimental combinations. In terms of methane content, most of *Laminaria sp.* samples exhibited an average of 50% of CH_4, with a peak of 70% for the untreated and a minimum of 20% for the highest VS concentration of 4%. On the other hand, *A. nodosum* exhibited a constant average of 40-45% of CH_4 along all the samples. It was observed that the behaviour of the treated samples with respect to the untreated condition depended mainly on the algal species. *Ascophyllum nodosum* treated for 15 min and at 1% VS yielded up to 30% more methane than the untreated sample, while *Laminaria sp.* showed about 9% more methane than the untreated samples only at 2.5 % of VS and after 10 and 15 min of beating.

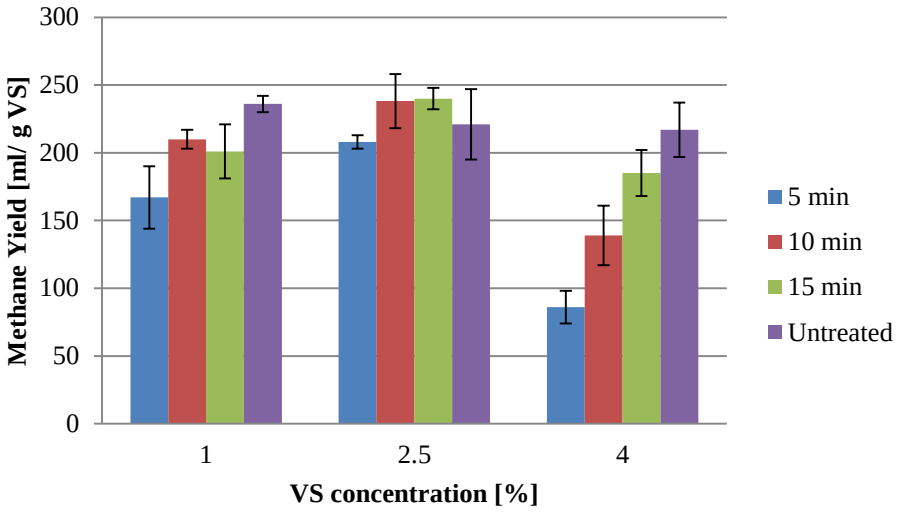


Figure 19: *Laminaria sp.* methane yields

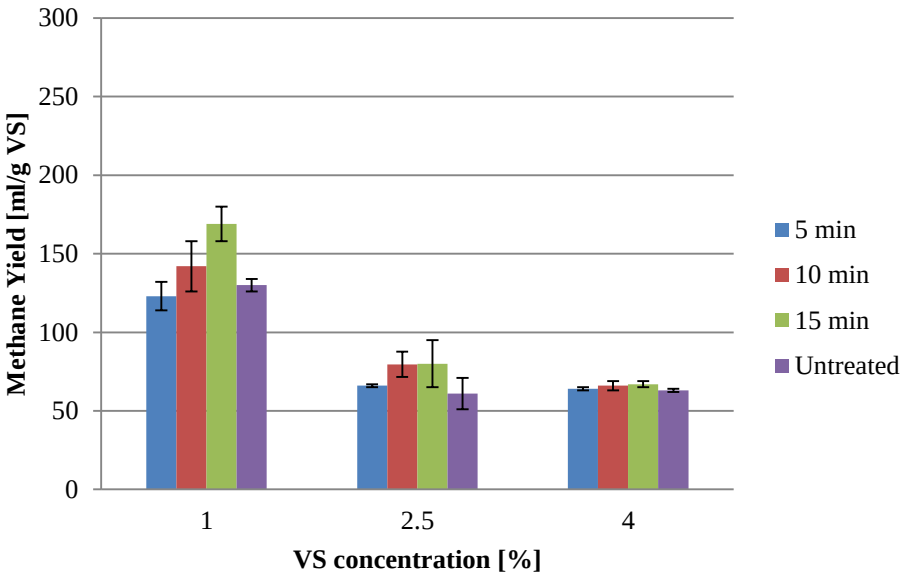


Figure 20: *A. nodosum* methane yields

Table 21: Methane yields, biogas yields, sCOD for *Laminaria sp.* and *A. nodosum*

Sample		***Laminaria sp.***			
VS [%]	**BT [min]**	**Initial sCOD [g O L^{-1}]**	**Final sCOD [g O L^{-1}]**	**CH_4 [ml g^{-1} VS]**	**Biogas [ml g^{-1} VS]**
1	0	N.A.	N.A.	236±6	482±8
1	5	5.08±0.48	2.7±0.33	167 ± 23	402±20
1	10	4.78±0.28	2.08±0.38	210 ± 7	491±10
1	15	5.03±0.36	2.68±0.27	201 ± 20	463±25
2.5	0	N.A.	N.A.	221±26	451±24
2.5	5	5.63±0.61	2.80±0.46	208 ± 5	433±1
2.5	10	6.30±0.21	2.93±0.29	238 ± 20	494±22
2.5	15	5.53±0.96	2.2±0.55	240 ± 8	615±7
4	0	N.A.	N.A.	217±20	413±18
4	5	7.60±0.39	5.8±0.26	86 ± 12	222±23
4	10	7.53±1.13	4.63±0.49	139 ± 22	317±26
4	15	7.08±0.79	4.58±0.68	185 ±17	374±25
		A. nodosum			
1	0	N.A.	N.A.	130±4	315±8
1	5	4.67±0.16	3.19±0.11	123±9	294±23
1	10	4.88±0.21	3.41±0.31	142±16	337±28
1	15	4.40±0.19	3.39±0.26	169±11	402±20
2.5	0	N.A.	N.A.	61±10	150±29
2.5	5	6.07±0.27	3.33±0.11	73 ± 1	177±4
2.5	10	6.62±0.13	3.61±0.33	80 ± 8	193±19
2.5	15	6.55±0.12	3.26±0.38	80 ± 15	189±29
4	0	N.A.	N.A.	63±1	156±2
4	5	7.83±0.05	3.43±0.22	64 ± 1	156±3
4	10	9.02±0.73	3.45±0.25	66 ± 3	161±8
4	15	10.75±0.52	4.18±0.45	67 ± 2	164±6

Both species exhibited the lowest methane yields at the highest level of VS. At 4% VS, the sCOD values measured for *Laminaria sp.* (**Table 21**), indicated that the available organic matter did not pass through the digestion process. The final sCOD was higher with respect to other experimental conditions which exhibited a final sCOD in the range of 2-3 g O L^{-1}. This suggests that the use of longer retention time can be beneficial in order to allow a more complete consumption of the degradable substrate. On the contrary, *A. nodosum* at 4% VS exhibited a final sCOD in the same range of the other samples which yielded higher methane. In this case, an inhibition occurred due to an overloading of the digester. It was likely that most of the degradable organic matter was transformed into other co-products than methane. Thus, a reduction of the sCOD was registered since most of the organic matter was used for the microbial activity. However, it must be

noted that the pH values (**Table 22**) did not suggest the occurrence of an inhibition. Nevertheless, as already highlighted in experiment 1, the pH measurement can suggest an inhibition, but in this case it was not sufficient.

Table 22: pH values for *Laminaria sp.* and *A. nodosum*

Sample		*Laminaria sp.* pH		*A. nodosum* pH	
VS [%]	**BT [min]**	**Initial**	**Final**	**Initial**	**Final**
1	0	7.44±0.03	7.41±0.02	7.92±0.03	7.44±0.02
1	5	7.27±0.04	7.52±0.03	7.96±0.03	7.45±0.04
1	10	7.25±0.01	7.37±0.02	7.93±0.01	7.41±0.01
1	15	7.28±0.02	7.36±0.04	7.99±0.03	7.44±0.02
2.5	0	7.45±0.02	7.40±0.02	7.90±0.02	7.33±0.01
2.5	5	7.07±0.01	7.59±0.01	7.71±0.01	7.34±0.05
2.5	10	7.07±0.02	7.61±0.04	7.81±0.04	7.38±0.06
2.5	15	7.04±0.04	7.60±0.05	7.84±0.02	7.31±0.01
4	0	7.47±0.01	7.45±0.01	7.82±0.03	7.31±0.01
4	5	7.03±0.02	7.40±0.03	7.48±0.03	7.46±0.01
4	10	6.98±0.02	7.69±0.03	7.46±0.01	7.39±0.02
4	15	6.93±0.05	7.58±0.01	7.47±0.01	7.41±0.04

At this point, methane yields after 6 days of digestion were observed (**Table 23**). The *Laminaria sp.* data revealed that at 4% of VS an evident hampering of the digestion was caused by the pretreatment since much higher yields were observed for the untreated samples. Besides, for all the treated samples at 4% an initial pH above 7 was measured (**Table 22**), whilst for the untreated samples the pH resulted equal to 7.47 ± 0.01 which was more suitable for AD. Thus, it was likely that the enhanced solubilisation of the organic matter caused by the beating pretreatment determined a decrease in pH with respect to the untreated samples. However, since the treated samples after 14 days of digestion exhibited a suitable pH (ranging between 7.40 and 7.58), it was probable that the buffer capacity of the system was sufficient in order to allow the anaerobic microorganisms to survive and adapt. Thus, at 4% of VS longer retention times after pretreatment would allow for a better performance of the digester.

At 2.5% of VS, there was an increase of 50% methane for all the treated samples. Such increase confirmed the results of experiment 1, where the main effect of the beating pretreatment was to accelerate the start of digestion while resulting in a marginal methane enhancement at the end of digestion.

Unlike *Laminaria sp.*, at 6 days of digestion *A. nodosum* did not exhibit an enhancement of methane after pretreatment even though a general improvement in the methane yields of treated samples with respect to the untreated was observed at the end of digestion, after 14 days.

According to these results, it was evident that the pretreatment phase impacted differently according to the seaweed species used as well as the VS concentration. The RSM analysis was carried out in order to evaluate the impact of the pretreatment and VS concentration on the methane response according to the macroalgal species.

Table 23: *Laminaria sp.* and *A. nodosum* methane yields at 6 days of digestion

Sample		***Laminaria sp.* at 6 days of digestion**		***Ascophyllum nodosum* at 6 days of digestion**	
VS [%]	**BT [min]**	**CH_4 [ml g^{-1} VS]**	**Treated vs Untreated [%]**	**CH_4 [ml g^{-1} VS]**	**Treated vs Untreated [%]**
1	0	150 ± 2		93 ± 1	
1	5	128 ± 10	-15	73 ± 7	-22
1	10	128 ± 8	-15	78 ± 8	-16
1	15	116 ± 19	-23	96 ± 8	3
2.5	0	104 ± 14		52 ± 9	
2.5	5	159 ± 21	53	54 ± 3	4
2.5	10	160 ± 19	54	59 ± 5	13
2.5	15	161 ± 9	55	40 ± 20	-23
4	0	140 ± 10		57 ± 1	
4	5	23 ± 6	-509	50 ± 4	-12
4	10	33 ± 4	-324	51 ± 4	-11
4	15	55 ± 3	-155	53 ± 1	-7

4.3.2 Model estimation

The RSM design matrix with the methane response for each combination of factors levels is shown in **Table 24**.

Table 24: Design matrix with methane response

Exp. No.	*Factors*			
	X_1: VS concentration [%]	**X_2: beating time [min]**	**X_3: Seaweed species**	**Response: Methane [ml g^{-1} VS]**
1	1	15	*Laminaria*	201
2	2.5	10	*A.nodosum*	66
3	2.5	10	*Laminaria*	270
4	4	15	*A.nodosum*	67
5	2.5	10	*Laminaria*	216
6	2.5	10	*A.nodosum*	86
7	4	10	*A.nodosum*	66
8	4	15	*Laminaria*	185
9	2.5	10	*A.nodosum*	74
10	1	15	*A.nodosum*	169
11	2.5	15	*Laminaria*	240
12	4	5	*Laminaria*	86
13	2.5	10	*Laminaria*	248
14	2.5	10	*Laminaria*	237
15	2.5	10	*A.nodosum*	89
16	2.5	10	*A.nodosum*	83
17	2.5	5	*Laminaria*	208
18	4	10	*Laminaria*	139
19	1	10	*A.nodosum*	142
20	1	5	*A.nodosum*	123
21	1	5	*Laminaria*	167
22	2.5	5	*A.nodosum*	73
23	2.5	15	*A.nodosum*	80
24	4	5	*A.nodosum*	64
25	2.5	10	*Laminaria*	220
26	1	10	*Laminaria*	210

The ANOVA table as yielded by the software (**Table 25**) showed that the estimated model was significant as well as the model terms A, B, C, BC, A^2, ABC and A^2C. At the same time, the P-value related to the 'Lack of Fit', was > 0.05, which implied that the Lack of Fit was not significant. This meant that the model developed adequately fit the data.

Table 25: ANOVA experiment 2

Source	*Sum of Squares*	*df*	*Mean Square*	*F Value*	*P-value Prob > F*	
Model	1.194E+005	11	10853.43	50.98	< 0.0001	significant
A: VS [%]	13668.75	1	13668.75	64.20	< 0.0001	
B: BT [min]	4070.08	1	4070.08	19.12	0.0006	
C: Species	73633.24	1	73633.24	345.84	< 0.0001	
AB	60.50	1	60.50	0.28	0.6024	
AC	396.75	1	396.75	1.86	0.1938	
BC	990.08	1	990.08	4.65	0.0489	
A^2	1913.50	1	1913.50	8.99	0.0096	
B^2	320.07	1	320.07	1.50	0.2404	
ABC	1458.00	1	1458.00	6.85	0.0203	
A^2C	11362.12	1	11362.12	53.37	< 0.0001	
B^2C	259.45	1	259.45	1.22	0.2883	
Residual	2980.76	14	212.91			
Lack of Fit	690.76	6	115.13	0.40	0.8589	Not significant
Pure Error	2290.00	8	286.25			
Cor Total	1.224E+005	25				

R^2 = 0.9756; Adj. R^2 = 0.9565; Pred. R^2= 0.9157; Adeq. Precision= 18.896.

The values of R^2, adjusted R^2, and predicted-R^2 were all close to 1, which indicated that the chosen model was adequate to predict the CH_4 yields from the variables within the experimental boundaries. Equally, an adequate precision greater than 4 indicated that this model could be used to navigate the design space.

Equation (13) represents the final model equation in terms of coded factor. By default, the software encoded the high levels of the factors as +1 and the low levels of the factors as -1 (**Table 26**). The equation was calculated by the software and obtained for the CH_4 yield (Y) as a function of the independent variables A (VS concentration) and B (beating time) and C (species).

Table 26: Variables coded factors

	Coded factors		
Variable	*-1*	*0*	*+1*
A: VS concentration [%]	1	2.5	4
B: Beating Time [min]	5	10	15
C: Species	*Laminaria sp.*	N.A.	*A. nodosum*

$$Y = +158.60 - 33.75\,A + 18.42\,B - 79.67\,C + 2.75\,AB - 5.75\,AC - 9.08\,BC - 18.61\,A^2 - 7.61\,B^2 - 3.5\,ABC + 45.35\,A^2C + 6.85\,B^2C \quad (13)$$

By comparing the factors' coefficients, the species selected (C) represented the highest impact on the response. When *Laminaria sp.* was selected, the impact of the relative coefficient on methane production resulted to be positive, while the impact was negative in the case of *A. nodosum*. The other two strong impacts were the interaction A^2C and the VS concentration (A) respectively. In the case of the A^2C term, the impact was dependent on the value of the C term (negative for *Laminaria sp.* and positive for *A. nodosum*), while term A has a positive impact at low VS concentrations.

The software computed the final equations (Eq. 14) and (Eq. 15) in terms of actual factors for *Laminaria sp.* and *A. nodosum* respectively:

$$Y = +48.57 + 101.81\,A + 11.66\,B + 2.17\,AB - 28.43\,A^2 + 0.58\,B^2 \quad (14)$$

$$Y = +161.51 - 71.43\,A + 6.06\,B - 1.44\,AB + 11.89\,A^2 - 0.03\,B^2 \quad (15)$$

Figure 21 shows the normal probability of residuals. Since the plotted dots resembled a straight line, it was assumed that the underlying error distribution was normal and therefore, the ANOVA procedure could be considered as an exact test of hypothesis of no difference in treatment means. A possible problem could be represented by the red point at the far right of the graph. It could be an outlier and therefore required further investigation [86]. Thus, the Design-Expert diagnostics tool was run. **Figure 22** shows that the standard deviation of such point (highlighted point) was very low, indicating that this was not the case of an outlier.

In any case, the predicted values versus the actual values (**Figure 23**) plot shows a good prediction of the model as most of the points were grouped around the diagonal line. This meant that there was a strong correlation between the model's predicted results and the actual results.

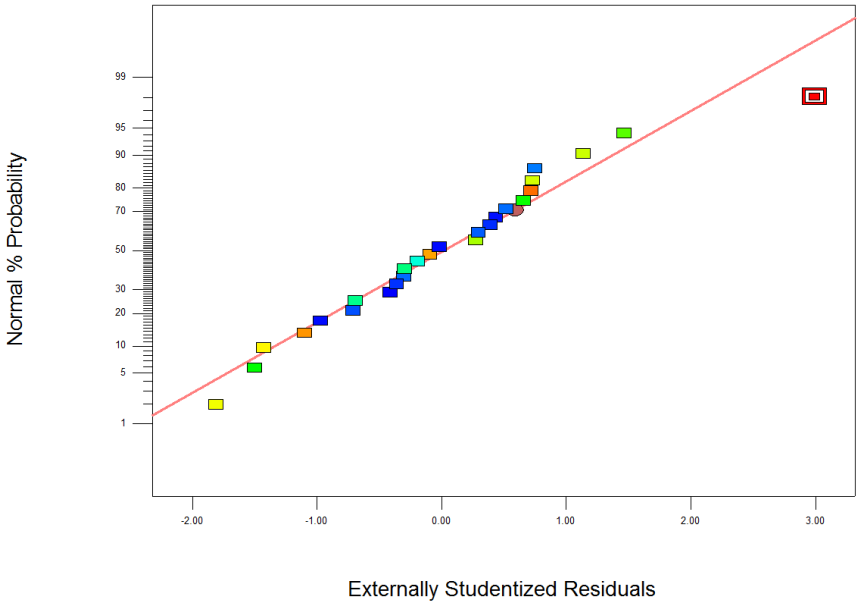


Figure 21: Normal probability plot

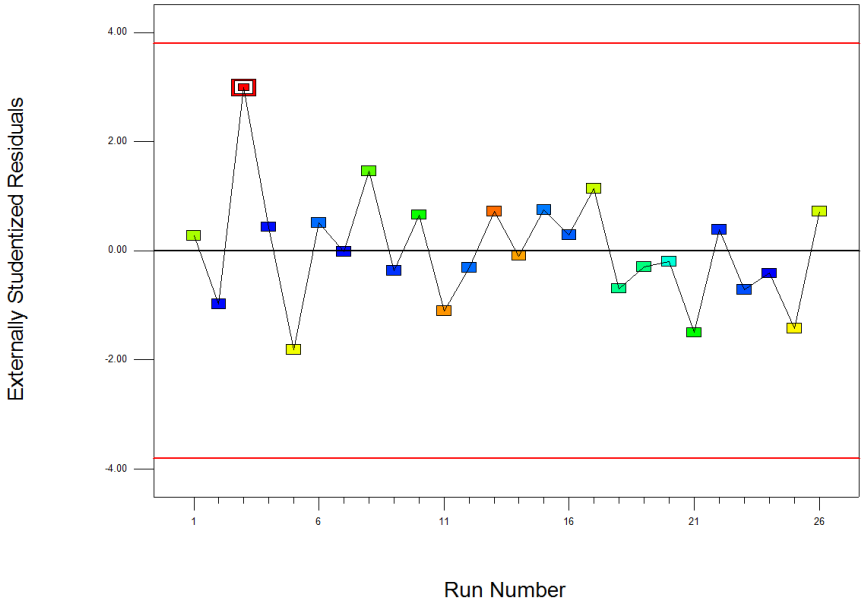


Figure 22: Residual vs run

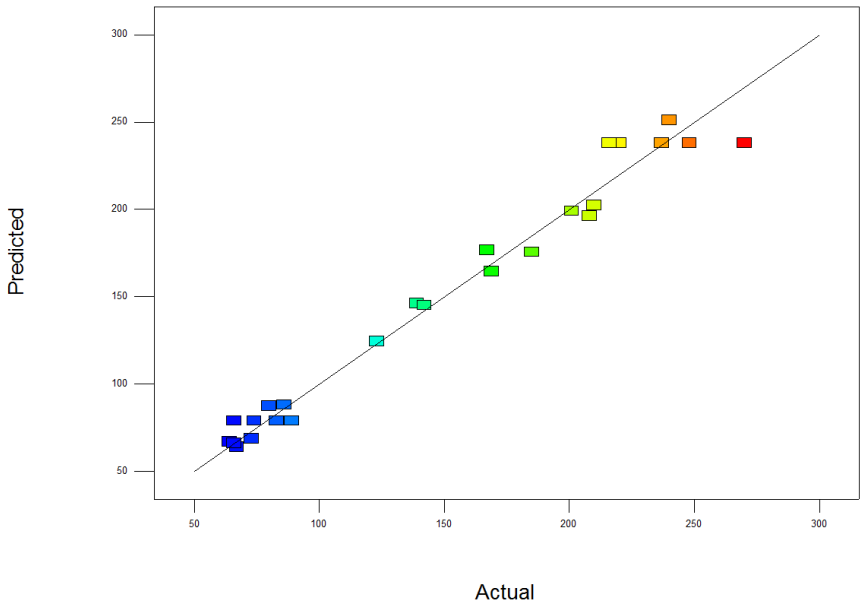


Figure 23: Predicted vs actual residuals

The resulting surfaces for each species and the correspondent contour plots are represented in **Figures 24-25**. In the case of *Laminaria sp.* (**Figure 24**) the optimum region for methane production was visible around the centre point (2.5%) of the VS concentration factor and in correspondence of the highest level of the beating time factor. Whilst for *A. nodosum* (**Figure 25**), the methane yield increased as the VS concentration reduced and the beating time increased.

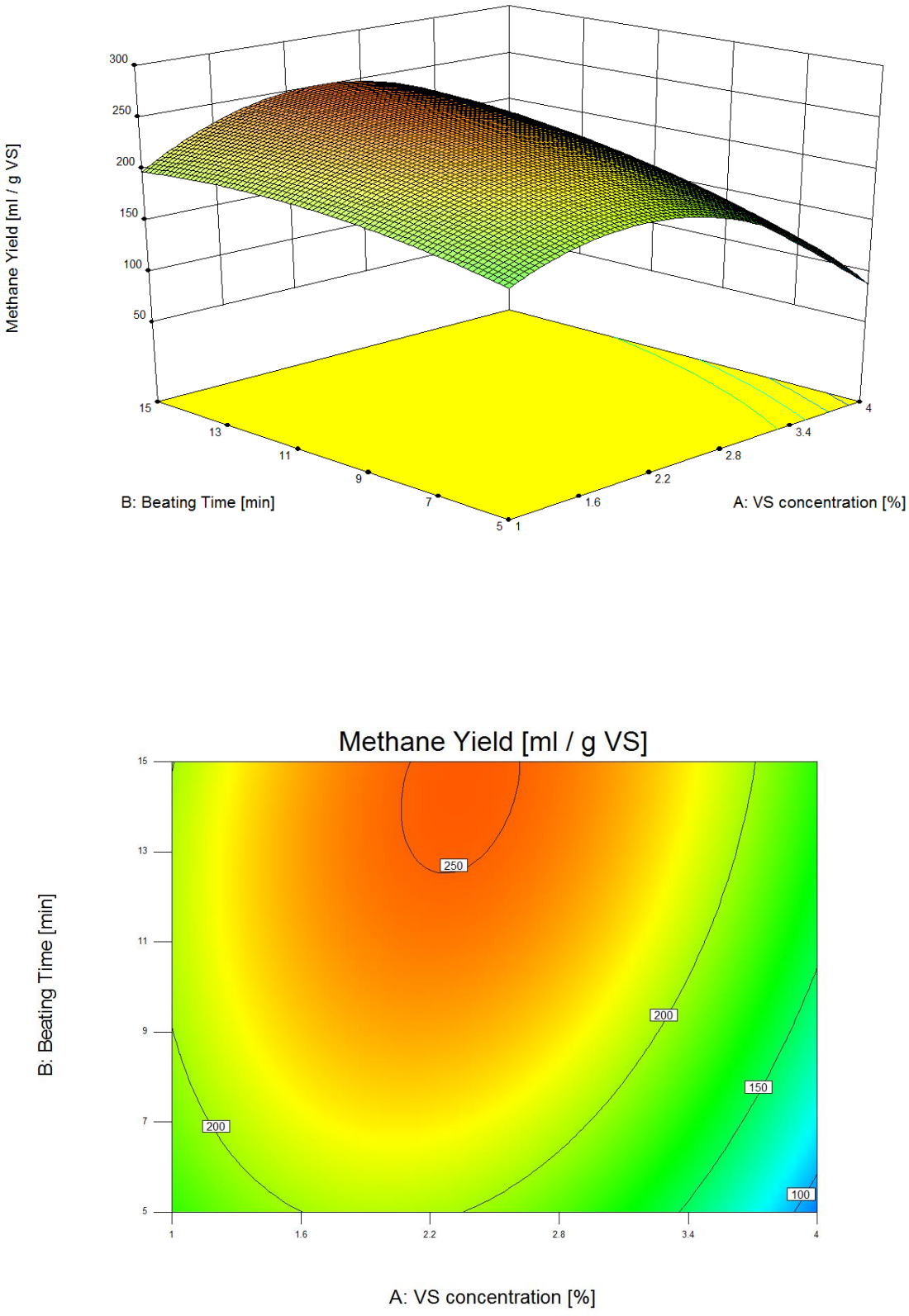


Figure 24: *Laminaria sp.* response surface and contour plot

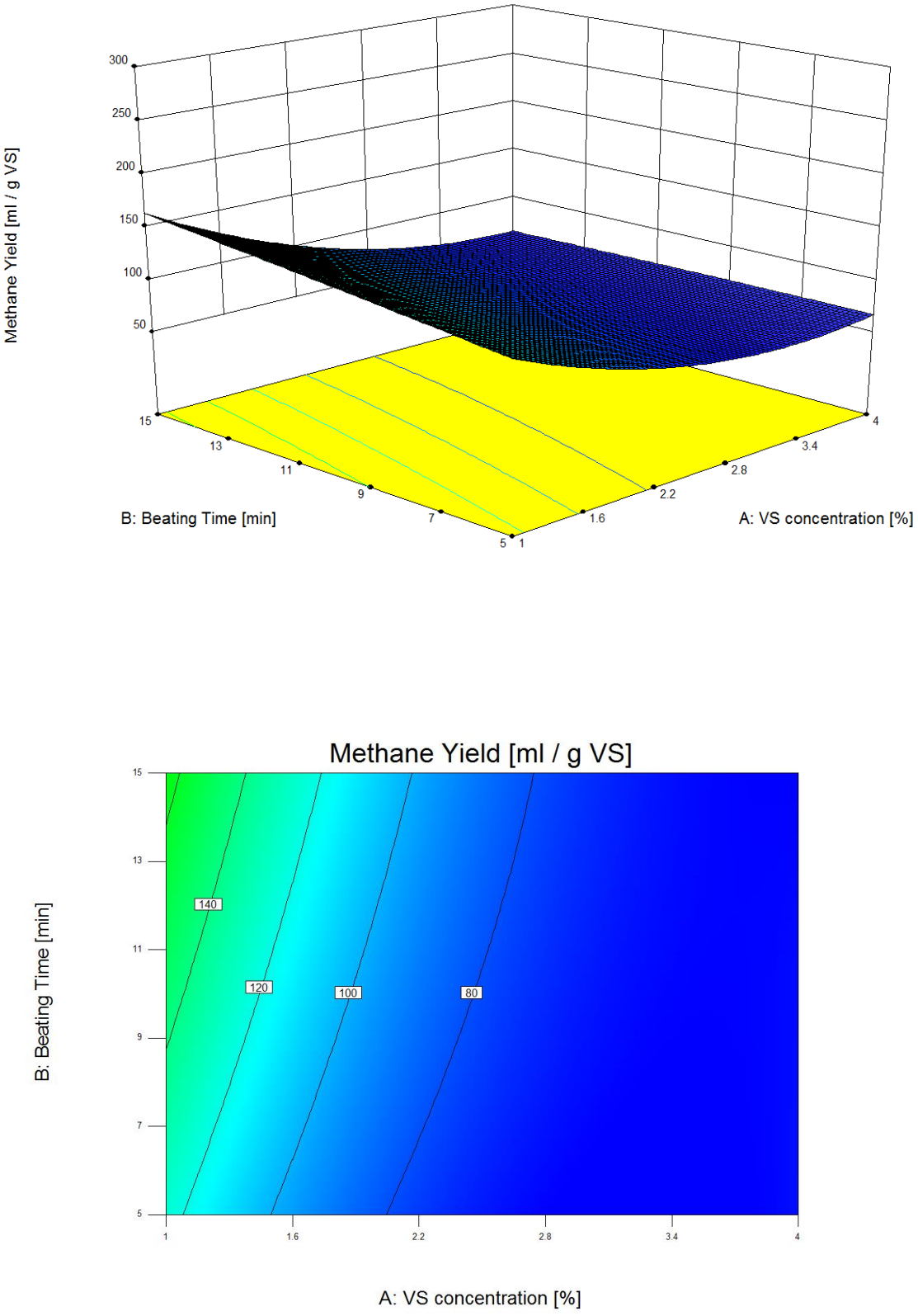


Figure 25: *A. nodosum* response surface and contour plot

An immediate investigation of such trends was possible through the perturbation plots (**Figures 26-27**). The perturbation plots displayed the effect of changing each factor while holding the other one constant. The curvature of the VS concentration (A) factor for both species suggested that this factor influenced the methane yield response more than the time of pretreatment (B). The higher impact of the VS concentration relative to the beating time factor was also confirmed by the correspondent coefficients in the general model equation (Eq. 13). In particular, for both species the methane yield decreased when the VS concentration increased from the centre point (2.5%) up to the highest level (4%). *Laminaria sp.* exhibited the best methane yields when the VS concentration ranged between 1.75% and 2.5%, whilst for *A. nodosum*, the methane yield increased dramatically from the centre point (2.5%) up to the minimum level (1%).

Increasing the beating time (B) influenced positively the methane yield for both species. The impact of such factor was more important in the case of *Laminaria sp.* as a slight curvature was observable with respect to the *A. nodosum* plot.

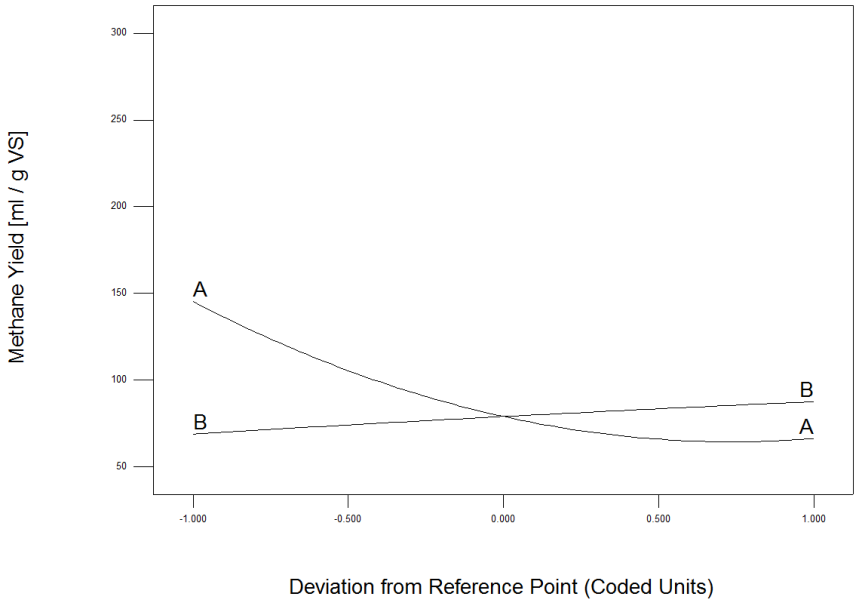


Figure 26: Perturbation plot *A. nodosum*

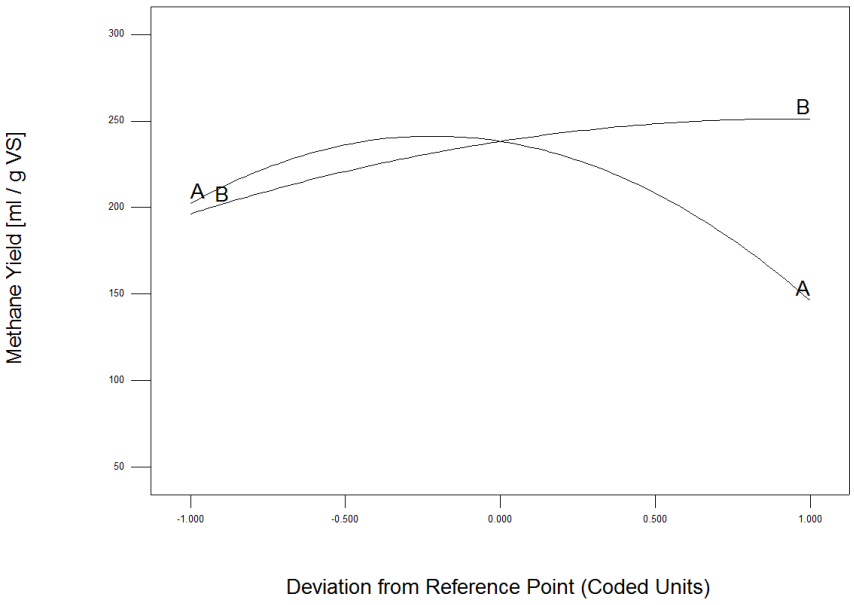


Figure 27: Perturbation plot *Laminaria sp.*

Figures 28-29 represent the AB interaction plot for *Laminaria sp.* and *A. nodosum*. It is interesting to notice that when the VS concentration was set at 4%, in the case of *A. nodosum*, the beating time had almost no effect on the response, while for *Laminaria sp.* an increase of beating time determined an increase in the methane yield. At this concentration, the pretreatment phase seemed to have the strongest impact on *Laminaria sp.*, even though resulting in lower methane yields compared to lower levels of VS.

For both species, at the lowest level of VS concentration (1%), the methane yields were higher compared to a 4% of VS. Unlike *Laminaria sp.*, *A. nodosum* interaction plot did not show any overlapping between the least significance difference (LSD) intervals at 5 and 15 min, thus the predictions at those points were significant. Thus, at 1% of VS concentration it was possible to improve the methane production from *A. nodosum* by enhancing the time of beating up to 15 min. On the other hand, when treating *Laminaria sp.* at 1% of VS, there was no statistical evidence which suggested that an enhancement of pretreatment time improved significantly the methane yield from this species.

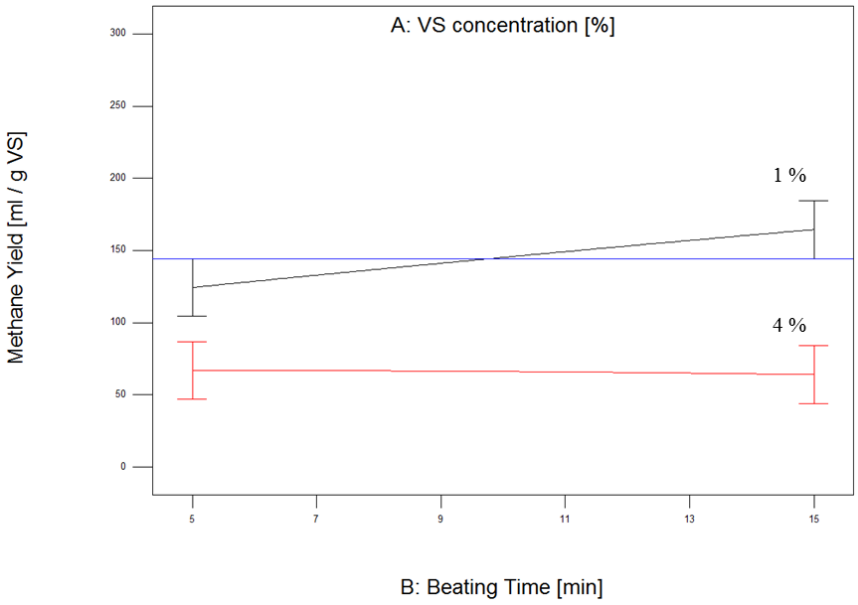


Figure 28: *A. nodosum* AB interaction

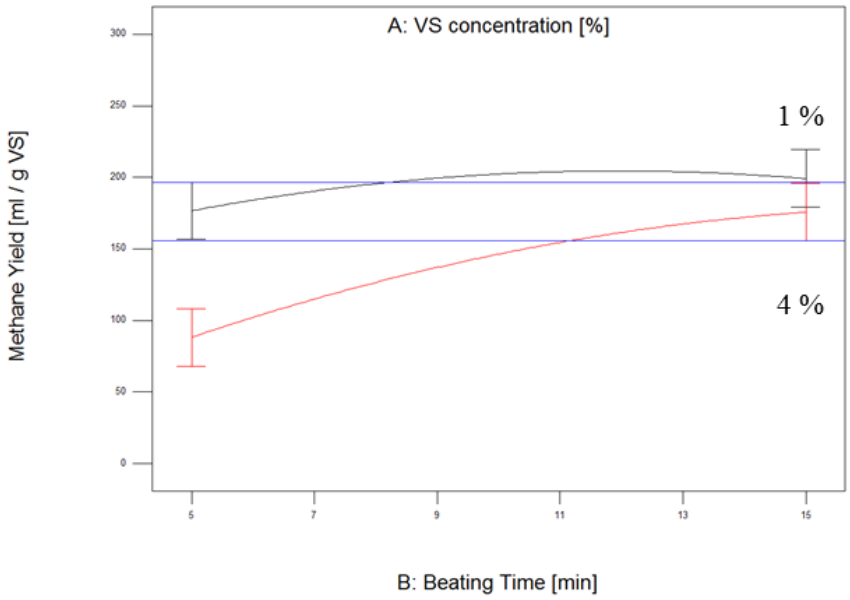


Figure 29: *Laminaria sp.* AB interaction

The pretreatment phase had the strongest impact on *Laminaria sp.* when the VS concentration was set at 4% even though resulting in lower methane yields compared to lower levels of VS. Nevertheless, it is interesting to note that, at 15 min of pretreatment there was no significant difference between the methane yields reached at 1% and 4% of VS. Thus, when increasing the beating time to 15

min, the influence of the VS concentration on the methane yields from *Laminaria sp.* did not have any effect.

Figure 30 shows the BC interaction plot when the VS concentration was set at 2.5%. Both at 5 and 15 min there was no overlapping from left to right of the LSD bars, which means that between species there was a significant difference in methane yields at those two levels of treatment time. In the case of *A. nodosum*, since there was an overlap between the LSD bars at 5 and 15 min, at 2.5 % the pretreatment phase does not have any significant effect on the methane yield of this species, unlike *Laminaria sp.* which showed a significant difference between the yields at 5 and 15 min, with a better performance at 15 min.

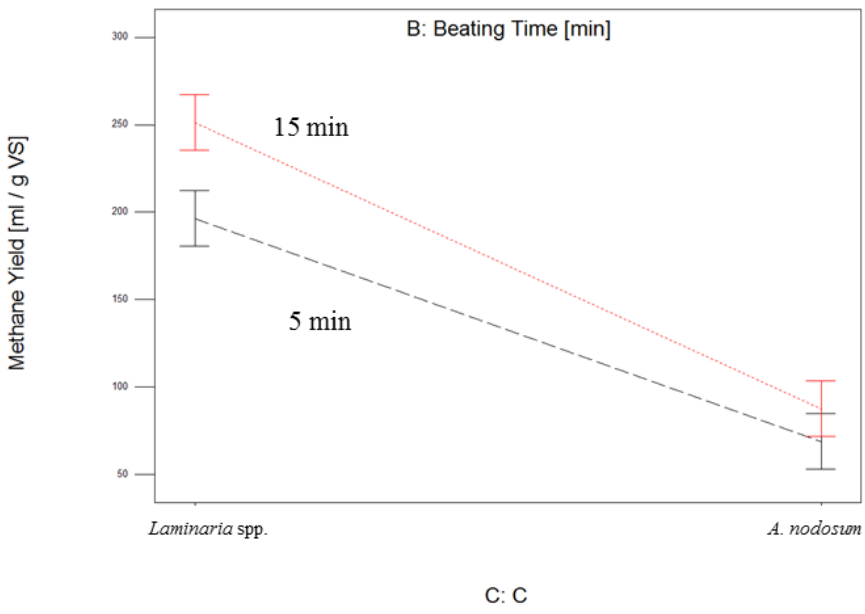


Figure 30: BC interaction when A = 2.5% for *Laminaria sp.* and *A. nodosum*

4.3.3 Optimisation

An important tool offered by Design-Expert was the possibility to optimise the response while this was subject to specific constraints of the independent variables. This approach is known as a constrained optimisation problem [86]. The Design-Expert software package solved this version of the problem using a direct search procedure.

In this experiment, the main goal was to find the optimal combination of species, VS concentration and beating time that could maximise the methane yield (**Table 27**). The strategy of the software was to employ a desirability function (*d*) which varied between 0 and 1. When the response was at its goal, then *d* was equal to 1, on the contrary, when the response was outside an acceptable region, *d* was equal to 0 [86]. **Table 28** represents the output of the software when all the variables were set in the tested ranges and the main goal was to maximise the methane yield.

Table 27: Constraints optimisation 1

Variable	***Goal***	***Lower Limit***	***Upper Limit***
A: VS [%]	in range	1	4
B: BT [min]	in range	5	15
C: Species	in range	*Laminaria sp.*	*A.nodosum*
Methane [ml g^{-1} VS]	maximise	64	270

Table 28: Solution optimisation 1

Solution	***A: VS [%]***	***B: BT [min]***	***C: Species***	***Methane [ml g^{-1} VS]***	***Desirability***
1	2.342	14.453	Laminaria	252.009	0.913
2	1.000	15.000	A. nodosum	164.497	0.488
3	1.000	14.892	A. nodosum	164.094	0.486
4	1.000	14.331	A. nodosum	162.001	0.476
5	1.000	14.250	A. nodosum	161.695	0.474
6	1.000	13.250	A. nodosum	157.906	0.456
7	1.000	9.250	A. nodosum	142.143	0.379
8	4.000	5.331	A. nodosum	66.834	0.014

The software confirmed that when the aim was to maximise the methane yield, the best solution (d = 0.913) was to use around 2% of organic matter from *Laminaria sp.* and after a beating pretreatment of almost 15 min.

A further optimisation considered minimising the beating time while maximising the methane yield (**Table 29)**. In general, this combination is beneficial for the economics of the system as less energy is necessary for pretreatment.

Table 29: Constraints optimisation 2

Variable	*Goal*	*Lower Limit*	*Upper Limit*
A: VS [%]	in range	1	4
B: BT [min]	minimise	5	15
C: Species	in range	*Laminaria sp.*	*A. nodosum*
Methane [ml g^{-1} VS]	maximise	64	270

Table 30: Solution optimisation 2

Solution	*A: VS [%]*	*B: BT [min]*	*C: Species*	*Methane [ml g^{-1} VS]*	*Desirability*
1	2.008	5.678	Laminaria	210.608	0.787
2	1.000	5.941	A. nodosum	128.370	0.466
3	1.000	5.903	A. nodosum	128.208	0.466
4	1.000	5.997	A. nodosum	128.606	0.466
5	1.000	5.822	A. nodosum	127.861	0.466
6	1.000	5.765	A. nodosum	127.619	0.466
7	1.000	5.715	A. nodosum	127.403	0.466
8	1.000	7.631	A. nodosum	135.485	0.460
9	1.000	9.240	A. nodosum	142.104	0.444
10	1.000	10.750	A. nodosum	148.168	0.415
11	1.000	11.750	A. nodosum	152.109	0.386
12	4.000	5.000	A. nodosum	66.830	0.069

In this case (**Table 30**) the highest desirability (d = 0.787) corresponded to employ *Laminaria sp.* after 5 min of pretreatment with a VS concentration of 2%.

It was noticed that in this optimisation the predicted methane response was 17 % less than the previous optimisation in favour of a reduction of 10 min of the pretreatment time. At this point, it was interesting to investigate if a reduction of 10 min in beating time could make up for a reduction of 17% of methane yield.

Table 31 reports such analysis by employing the methane yields predicted by the software at 15 and 5 min. The energy consumed [Wh g^{-1} VS] was calculated by measuring the electricity consumption of the machine at 15 min (0.12 KWh) and 5 min (0.04 KWh). The biogas energy content was calculated equal to 3.99 kWh m^{-3} [122], according to an average methane percentage of 40%. The analysis revealed that reducing the beating time of 10 min does not make up for a reduction of 17% of methane yield. The net energy at 15 min results to be 8 % more energy output than the net energy produced at 5 min, according to the methane yields estimated by the software.

Table 31: Energy evaluation of optimisation 1 and 2

	Optimisation 1: ***BT = 15 min***	***Optimisation 2:*** ***BT = 5 min***
Methane yield [ml g^{-1} VS]	252	211
Methane content [%]	40	40
VS concentration [%]	2	2
Ep: Energy produced [Wh g^{-1} VS]	2.01	1.68
Ec: Energy consumed [Wh g^{-1} VS]	0.29	0.10
Net Ep [Wh g^{-1} VS]	1.72	1.59

4.3.4 Discussion of the key findings

The results showed that *Laminaria sp.* produced up to 240 ml CH_4 g^{-1} VS, while *A. nodosum* reached up to 169 ml CH_4 g^{-1} VS, which corresponded to 40% more methane from *Laminaria sp.* The observed difference between the two species could be explained by the presence of polyphenols. Polyphenols are known for their inhibitory action towards microbial activities, mainly due to inhibition of vital enzymes [94]. Moen *et al.* [105] found out that a limiting factor for the conversion of organic matter during AD of *A. nodosum* was the inhibitory effect of the polyphenols on methane production, while *Laminaria hyperborea* stipes were easily hydrolysed, since they contained much less polyphenols. It was

reported that the content of polyphenols in *A. nodosum* ranges between a maximum of 13% of dry matter during winter and a minimum of 9% in the summer [94, 105]. While Schiener *et al.* [94] reported an average polyphenol content of only 0.15% of dry matter for both *L. digitata* and *L. hyperborea* and 0.41% for *S. latissima*, being at high levels between May and July and low levels in October. For this experiment, *A. nodosum* was harvested in August, while *Laminaria sp.* was harvested in May, thus it is likely that the polyphenol content was around 9% for *A. nodosum* and around 0.2% for *Laminaria sp.* Such difference in polyphenols content could explain the more suitability of *Laminaria sp.* for methane production. This explains the best performance of *A. nodosum* when the VS concentration was at the lowest level of 1% and the inhibition of methane production at the highest level of 4% of VS.

In the literature, few studies have compared these two brown species for biogas production. Hanssen *et al.* [92] carried out an AD of *Laminaria sp.* and *A. nodosum* for a retention time of 30 days by investigating the VS concentration. In the case of *A. nodosum*, it was recorded a methane production up to 140 ml g^{-1} VS at 6.2%. The present work showed a methane yield from *A. nodosum* in the same range (167 ml g^{-1} VS) at a lower VS concentration (1%), while an inhibition was observed at a higher VS concentration of 4%. The methane yield measured at 4% of VS was less than half of the yields obtained by Hanssen *et al.* at 6.2% [92]. Hanssen *et al.* [92] did not consider the polyphenol content of *A. nodosum*. However, considering that the harvesting times were close for both studies (September in Hanssen *et al.*'s study [92], August in the present work), it is likely that the content of polyphenols was quite similar. Nevertheless, the use of the beating pretreatment could explain the higher methane production at the lower VS concentration with respect to Hanssen *et al.*'s work [92]. The RSM analysis revealed that when the VS concentration was set at 1%, the pretreatment phase had a positive effect on the digestion as the methane production increased linearly with the time of pretreatment. The main effect of the beating pretreatment was to reduce the particle size of the substrate which allowed a better accessibility of the anaerobic microorganisms to the organic matter. Thus, according to these results, a VS concentration of 1% was sufficient in order to obtain methane production when the beating pretreatment was applied.

In the case of *Laminaria sp.*, Hanssen *et al.* [92] registered of up to 230 ml CH_4 g^{-1}VS at 5.8 % VS from *L. hyperborea* and at 3.6 % VS from *L. saccharina* [92]. The results reported in the present work showed a methane production for *Laminaria sp.* in the same range (240 ml g^{-1}VS), but similarly to *A. nodosum*, at a lower VS concentration of 2.5%. Thus, also for *Laminaria sp.*, the pretreatment phase determined a more efficient digestion, as similar methane yields were reached at lower VS concentrations. Nevertheless, it must be noted that Hanssen *et al.* [92] reported an initial failure of the digestion as a drop of pH (below 6.0) as well as high production of CO_2 were observed. Those were signs of an overloading of the digester, which was solved by adjusting the pH to 7.5, more suitable for the methanogenic population.

The main finding of this experiment was that the *Laminaria sp.* was the best option for biogas exploitation. The following research experiments dealt with an investigation of the methane production from *Laminaria sp.* by considering different harvesting periods in Ireland. In this case, the aim was to select the best periods of harvesting as well as optimising the pretreatment step and the organic substrate concentration.

4.4 Experiments 2-3-4: seasonality investigation

It is known that the chemical composition of *Laminaria sp.* undergoes a seasonal variation, which influences the methane yields and thus the exploitation of this kind of biomass for bioenergy purpose [94, 104]. Following the main results of the previous experiments, this series of experiments aimed to investigate the methane yields of *Laminaria sp.* when the seaweed was harvested as natural beach stock at different periods of the year in Ireland. An optimisation in terms of substrate concentration and pretreatment phase was also carried out in order to assess the best conditions for each harvesting time.

4.4.1 Methane production

Tables 32-33-34 and **Figure 31** report the cumulative methane yields achieved when the seaweed was subjected to AD for 14 days at different experimental combinations in May 2014, November 2014, and March 2015. The best results were achieved when the material was harvested in November (**Table 33**, **Figure 31-b**). In this period, the highest methane yield recorded was 342 ± 17 ml g^{-1} VS after 5 min of pretreatment and at a VS concentration of 1%. This corresponded to 59% and 49% more methane compared to the best yields achieved in March and May respectively. In the same period, an average of 220 ± 26 ml CH_4 g^{-1} VS was registered for the other experimental conditions. The lowest methane yields were registered in March (**Table 35**, **Figure 31-c**). In particular, at 4% of VS a failure of the digester was observed since negligible levels of methane and very high percentages (70-80%) of CO_2 were detected. This was also confirmed by the high levels of sCOD registered at 14 days of digestion as well as an average pH of 6.71 ± 0.04, which was too low in order to allow methane production. These were all signs of an unbalanced digestion caused by an overloading of the digester [74]. On the other hand, the other samples exhibited an average of 163 ± 28 ml CH_4 g^{-1} VS, with a peak of 215 ± 9 ml CH_4 g^{-1} VS.

Table 32: Methane, Biogas yields, sCOD for experiment 3, May 2014

Sample		Initial sCOD [g O L^{-1}]	Final sCOD [g O L^{-1}]	CH_4 [ml g^{-1} VS]	Biogas [ml g^{-1} VS]
VS [%]	BT [min]				
1	0	N.A.	N.A.	236±6	482±8
1	5	5.08±0.48	2.7±0.33	167 ± 23	402±20
1	10	4.78±0.28	2.08±0.38	210 ± 7	491±10
1	15	5.03±0.36	2.68±0.27	201 ± 20	463±25
2.5	0	N.A.	N.A.	221±26	451±24
2.5	5	5.63±0.61	2.80±0.46	208 ± 5	433±1
2.5	10	6.30±0.21	2.93±0.29	238 ± 20	494±22
2.5	15	5.53±0.96	2.2±0.55	240 ± 8	615±7
4	0	N.A.	N.A.	217±20	413±18
4	5	7.60±0.39	5.8±0.26	86 ± 12	222±23
4	10	7.53±1.13	4.63±0.49	139 ± 22	317±26
4	15	7.08±0.79	4.58±0.68	185 ±17	374±25

Table 33: Methane, Biogas yields, sCOD for experiment 4, November 2014

Sample		Initial sCOD [g O L^{-1}]	Final sCOD [g O L^{-1}]	CH_4 [ml g^{-1} VS]	Biogas [ml g^{-1} VS]
VS [%]	BT [min]				
1	0	N.A.	N.A	138±15	345±11
1	5	4.70±0.21	3.32±0.20	342±17	855±25
1	10	4.31±1.32	3.39±0.04	283±26	708±15
1	15	3.41±0.21	3.08±0.01	197±14	493±20
2.5	0	N.A	N.A.	172±20	430±22
2.5	5	8.23±0.53	3.46±0.03	220±3	523±6
2.5	10	10.15±0.39	2.91±0.02	207±7	467±21
2.5	15	9.41±0.56	3.26±0.38	204±10	493±8
4	0	N.A.	N.A.	209±17	502±20
4	5	11.75±0.84	3.67±0.06	212±17	512±12
4	10	12.43±0.28	3.55±0.23	202±23	485±21
4	15	12.30±0.39	3.10±0.15	212±16	514±5

Table 34: Methane, Biogas yields, sCOD for experiment 2, March 2015

Sample		Initial sCOD [g O L^{-1}]	Final sCOD [g O L^{-1}]	CH_4 [ml g^{-1} VS]	Biogas [ml g^{-1} VS]
VS [%]	BT [min]				
1	0	N.A.	N.A	139±10	490±22
1	5	6.48±0.15	4.75±0.35	157±13	506±16
1	10	6.04±0.02	3.5±0.70	182±11	564±23
1	15	6.20±0.03	2.35±0.25	169±7	533±19
2.5	0	N.A.	N.A.	146±3	418±23
2.5	5	9.43±0.01	3.45±0.85	120±6	314±12
2.5	10	9.88±0.02	4.20±0.50	177±15	540±7
2.5	15	8.80±0.08	1.85±0.05	215±9	576±20
4	0	N.A.	N.A.	20±5	269±24
4	5	12.78±0.20	16.15±1.05	20±5	228±19
4	10	12.35±0.13	18.45±0.25	25±3	224±6
4	15	12.80±0.38	14.25±0.65	15±3	227±23

In general, the effect of pretreatment is different according to the harvesting period and the VS concentration. In particular, results from May (**Figure 31-a**, **Table 32**) suggested that the use of a pretreatment step did not allow a high enhancement of methane yield during this period. The seaweed harvested during this month showed around 9% more methane than the untreated samples, only at 2.5 % of VS and after 10 and 15 min of beating.

The situation in November was different, when more than double of methane was obtained with respect to the untreated sample at 1% of VS and after treating for 5 min. At 2.5% of VS a general enhancement between 19-28% was observed, while negligible enhancements were recorded at 4% of VS.

Even though March was characterised by the lowest yields, a general enhancement of methane after beating pretreatment was recorded. Except for those samples at 4% of VS, that exhibited an inhibition of methane production due to an overloading of the digester, samples at 1% of VS showed an increase of methane with respect to the untreated sample from 13% up to 22%, while the best methane enhancement of 47% was achieved at 2.5% of VS and after 15 min of pretreatment.

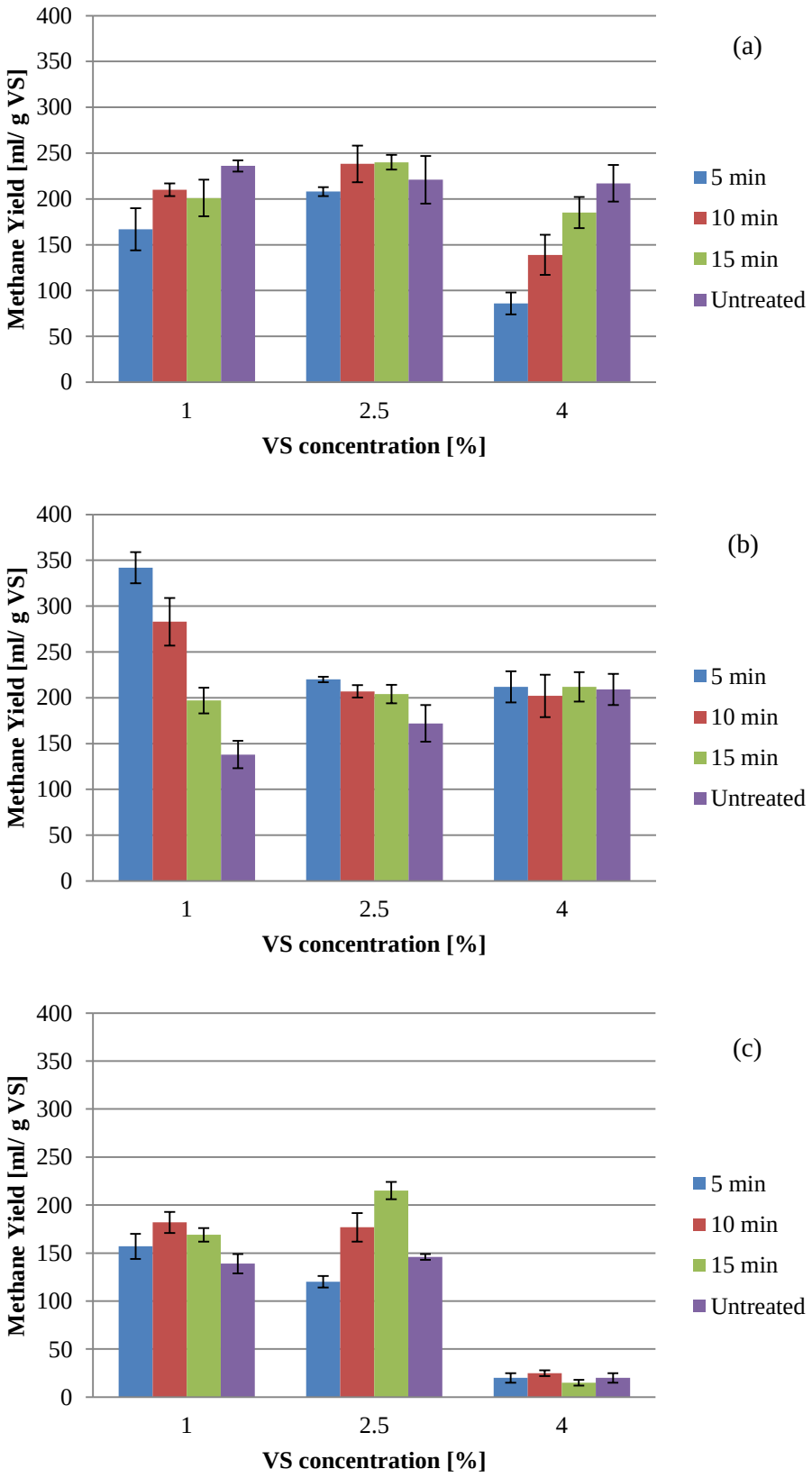


Figure 31: Methane yields in May 2014(a), November 2014(b), March 2015(c)

The statistical analysis for each harvesting time is presented in the next section. This provided a more comprehensive evaluation of the interaction between VS concentration and pretreatment on the methane yields.

4.4.2 Model estimation

The design matrixes of each experiment with the correspondent responses as yielded by the software are presented in **Tables 35-36-37** as well as the ANOVA tables for each experiment (**Tables 38-39-40**).

Table 35: Design matrix of experiment 2; May 2014

Exp. No.	***Factors***		
	X_1: VS concentration [%]	**X_2: beating time [min]**	**Response: Methane [ml g^{-1} VS]**
1	2.5	5	208
2	2.5	10	220
3	1	5	167
4	2.5	10	270
5	1	10	210
6	4	15	185
7	4	5	86
8	2.5	10	240
9	4	10	139
10	2.5	15	240
11	2.5	10	248
12	2.5	10	237
13	1	15	201

Table 36: Design matrix of experiment 3; November 2014

Exp. No.	*Factors*		
	X_1: VS concentration [%]	**X_2: beating time [min]**	**Response: Methane [ml g^{-1} VS]**
1	2.5	10	198
2	2.5	5	220
3	4	10	202
4	4	5	212
5	2.5	15	204
6	2.5	10	208
7	4	15	212
8	2.5	10	209
9	2.5	10	202
10	1	15	197
11	1	5	342
12	1	10	283
13	2.5	10	218

Table 37: Design matrix of experiment 4; March 2015

Exp. No.	*Factors*		
	X_1: VS concentration [%]	**X_2: beating time [min]**	**Response: Methane [ml g^{-1} VS]**
1	2.5	15	215
2	4	15	15
3	1	5	157
4	2.5	5	120
5	2.5	10	177
6	1	10	182
7	4	5	20
8	4	10	25
9	1	15	215
10	2.5	10	154
11	2.5	10	180
12	2.5	10	188
13	2.5	10	185

The ANOVA for each period estimated that the models adopted were significant. According to the harvesting period, different terms were estimated significant. It is worth noting that for all periods the A (VS concentration) and A^2 terms were estimated significant, while the B term (beating time) was significant only in May and a significant interaction AB was found only in November. For each experiment, the estimated model was able to fit the data since the 'Lack of Fit' P-value is <0.05. Also the values of R^2, adjusted R^2 and predicted R^2 were all close to 1 indicating good regression models.

Table 38: ANOVA experiment 2; May 2014

Source	***Sum of Squares***	***df***	***Mean Square***	***F Value***	***P-value Prob > F***	
Model	28262.97	5	5652.59	20.76	0.0005	significant
A: VS [%]	4704.00	1	4704.00	17.28	0.0043	
B: BT [min]	4537.50	1	4537.50	16.66	0.0047	
AB	1056.25	1	1056.25	3.88	0.0895	
A^2	12194.84	1	12194.84	44.79	0.0003	
B^2	793.34	1	793.34	2.91	0.1316	
Residual	1905.96	7	272.28			
Lack of Fit	577.96	3	192.65	0.58	0.6585	Not significant
Pure Error	1328.00	4	332.00			
Cor. Total	30168.92	12				

R^2 =0.9368; Adj. R^2 =0.8917; Pred. R^2=0.7421; Adeq. Precision=14.781

Table 39: ANOVA experiment 3; November 2014

Source	*Sum of Squares*	*df*	*Mean Square*	*F Value*	*P-value Prob > F*	
Model	20541.20	5	4108.24	63.08	< 0.0001	significant
A: VS [%]	6402.67	1	6402.67	98.31	< 0.0001	
B: BT [min]	128.00	1	128.00	1.97	0.2037	
AB	5256.25	1	5256.25	80.71	< 0.0001	
A^2	3498.03	1	3498.03	53.71	0.0002	
A^2B	1064.08	1	1064.08	16.34	0.0049	
Residual	455.88	7	65.13			
Lack of Fit	223.88	3	74.63	1.29	0.3931	Not significant
Pure Error	232.00	4	58.00			
Cor Total	20997.08	12				

R^2 =0.9783; Adj. R^2 =0.9628; Pred. R^2=0.8538; Adeq. Precision=26.643

Table 40: ANOVA experiment 4; March 2015

Source	*Sum of Squares*	*df*	*Mean Square*	*F Value*	*P-value Prob > F*	
Model	55938.59	4	13984.65	30.73	< 0.0001	significant
A: VS [%]	33450.67	1	33450.67	73.50	< 0.0001	
B: BT min]	1734.00	1	1734.00	3.81	0.0867	
A^2	15621.55	1	15621.55	34.33	0.0004	
B^2	346.88	1	346.88	0.76	0.4081	
Residual	3640.64	8	455.08			
Lack of Fit	2917.84	4	729.46	4.04	0.1026	Not significant
Pure Error	722.80	4	180.70			
Cor Total	59579.23	12				

R^2 =0.9389; Adj. R^2 =0.9083; Pred. R^2=0.7768; Adeq. Precision=13.898

For each group of data the software yielded the following model equations in terms of coded factors (**Table 41**).

Table 41: Variables coded factors for experiments 2-3-4

	Coded factors		
Variable	*-1*	*0*	*+1*
A: VS concentration [%]	1	2.5	4
B: Beating Time [min]	5	10	15

Each equation showed the CH_4 yield (Y) as a function of the independent variables A (VS concentration) and B (beating time) for the experiment in May (Eq. 16), November (Eq. 17) and March (Eq. 18) respectively.

$$Y = +242.41 - 28.00\,A + 27.50\,B + 16.25\,AB - 66.45\,A^2 - 16.95\,B^2 \quad (16)$$

$$Y = +208.43 - 32.67\,A - 8.00\,B + 36.25\,AB + 32.90\,A^2 - 28.25\,A^2B \quad (17)$$

$$Y = +177.34 - 74.67\,A + 17.00\,B - 75.21\,A^2 - 11.21\,B^2 \quad (18)$$

By considering the coefficients of each equation, it was possible to see that the extent of impact for each term was different according to the harvesting time. In May (Eq. 16) the highest impact was represented by the quadratic term A^2, while the impacts on methane yield of the A (VS concentration) and B (beating time) terms had the same magnitude. In November (Eq. 17), all the significant terms (A, AB, A^2, A^2B) had the same extent of impact, while in March (Eq. 18) the most important impacts were represented by the A term and the quadratic term A^2. In general, all experiments showed that the VS concentration had a strong impact, while the beating time had a relative minor impact on methane yield. The final equations in terms of actual factors in May (Eq. 19), November (Eq. 20) and March (Eq. 21) respectively are reported below:

$$Y = +35.88 + 107.33\,A + 13.64\,B + 2.17\,AB - 29.53\,A^2 - 0.68\,B^2 \quad (19)$$

$$Y = +648.05 - 268.79\,A - 29.38\,B + 17.39\,AB + 39.74\,A^2 - 2.52\,A^2B \quad (20)$$

$$Y = +14.05 + 117.35\,A + 12.37\,B - 33.43\,A^2 - 0.45\,B^2 \quad (21)$$

The graphs (**Figure 32**) of the normal probability of residuals are reported in order to check the assumption of normality distribution of errors. None of them

showed any outliers, thus the ANOVA for each group of data could be considered as an exact test of hypothesis of no difference in treatment means.

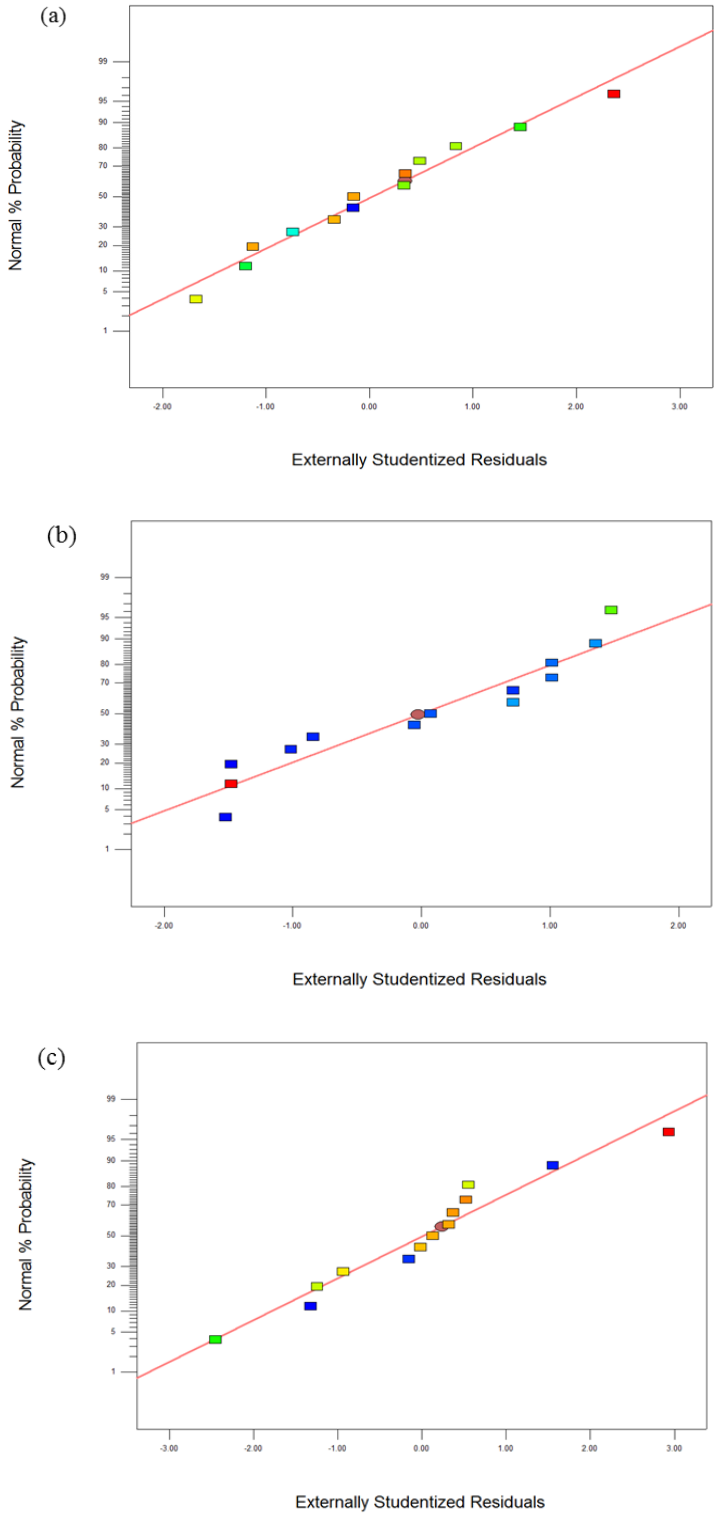


Figure 32: Normal plots of residuals in May (a), November (b) and March (c)

The resulting surfaces for each experiment and the correspondent contour plots are presented in **Figures 33-34-35**. All graphs showed better yields when the VS

concentration was below 2.5%. Besides, both contour surfaces related to May and March presented a similar curvature with longer treatment times having a positive effect on the response. This kind of trend was not detected for the material harvested in November as the optimum region was characterised by a shorter treatment time.

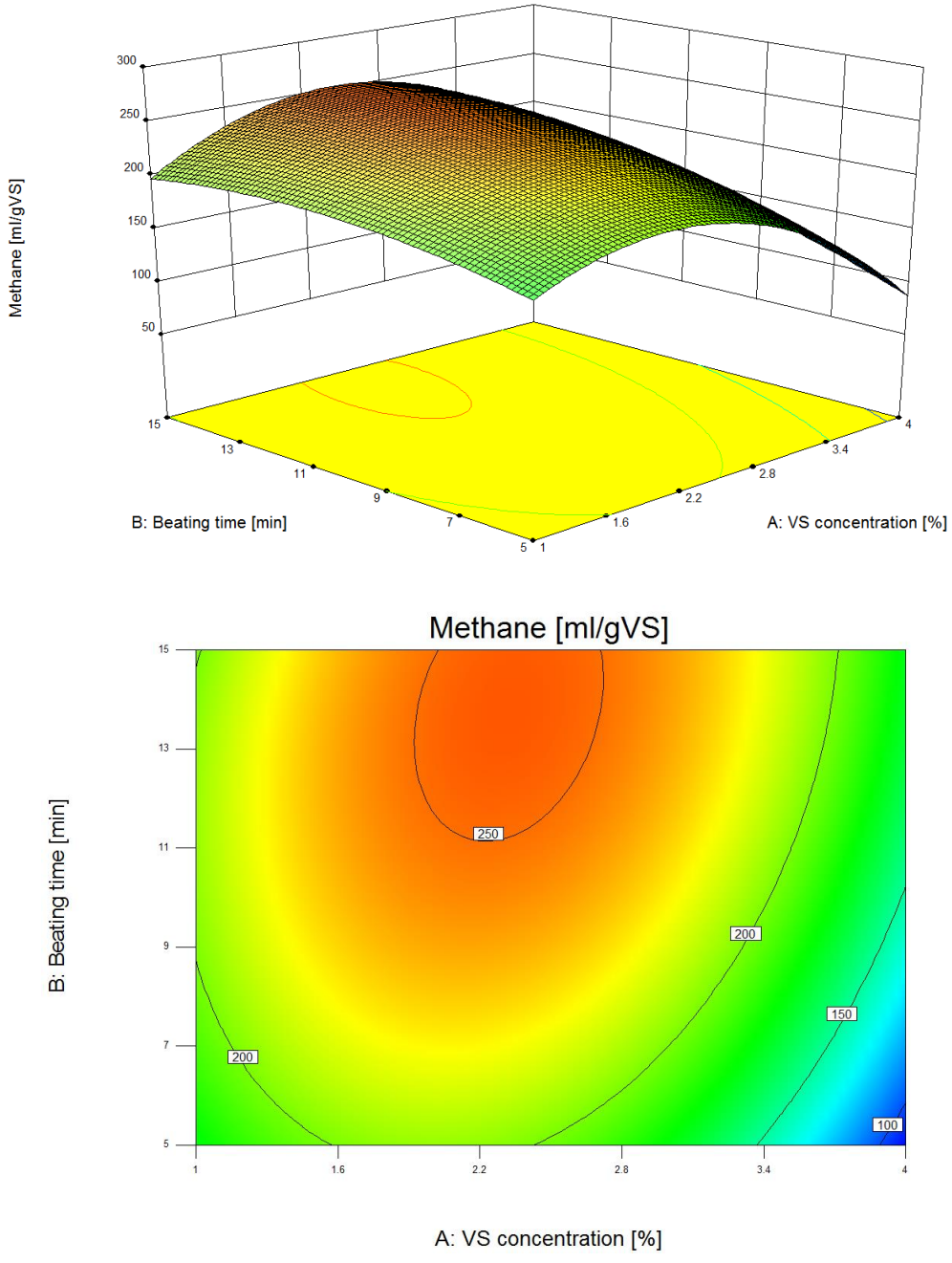


Figure 33: Response surface and contour plot of experiment 2, May 2014

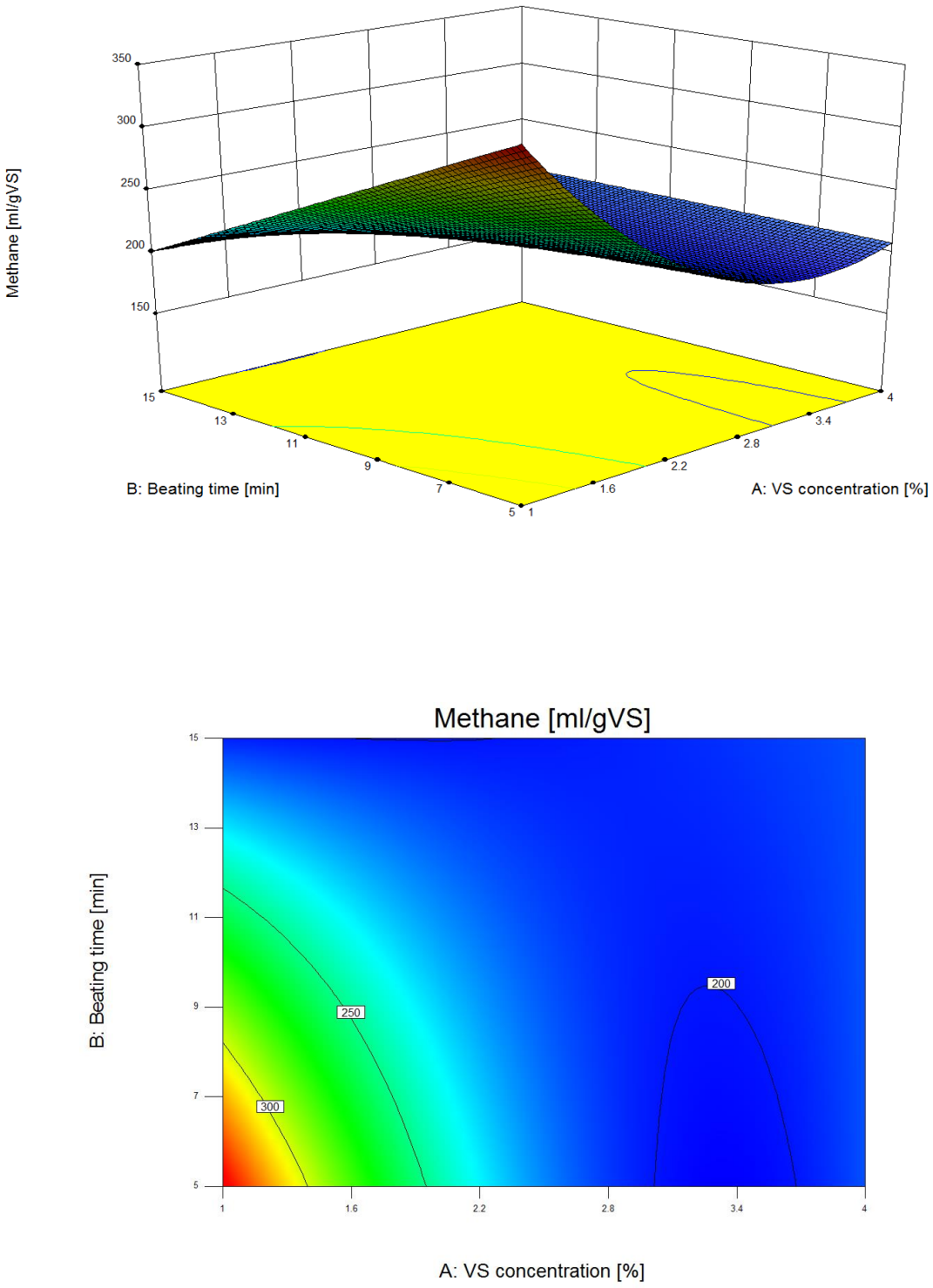


Figure 34: Response surface and contour plot of experiment 3, November 2014

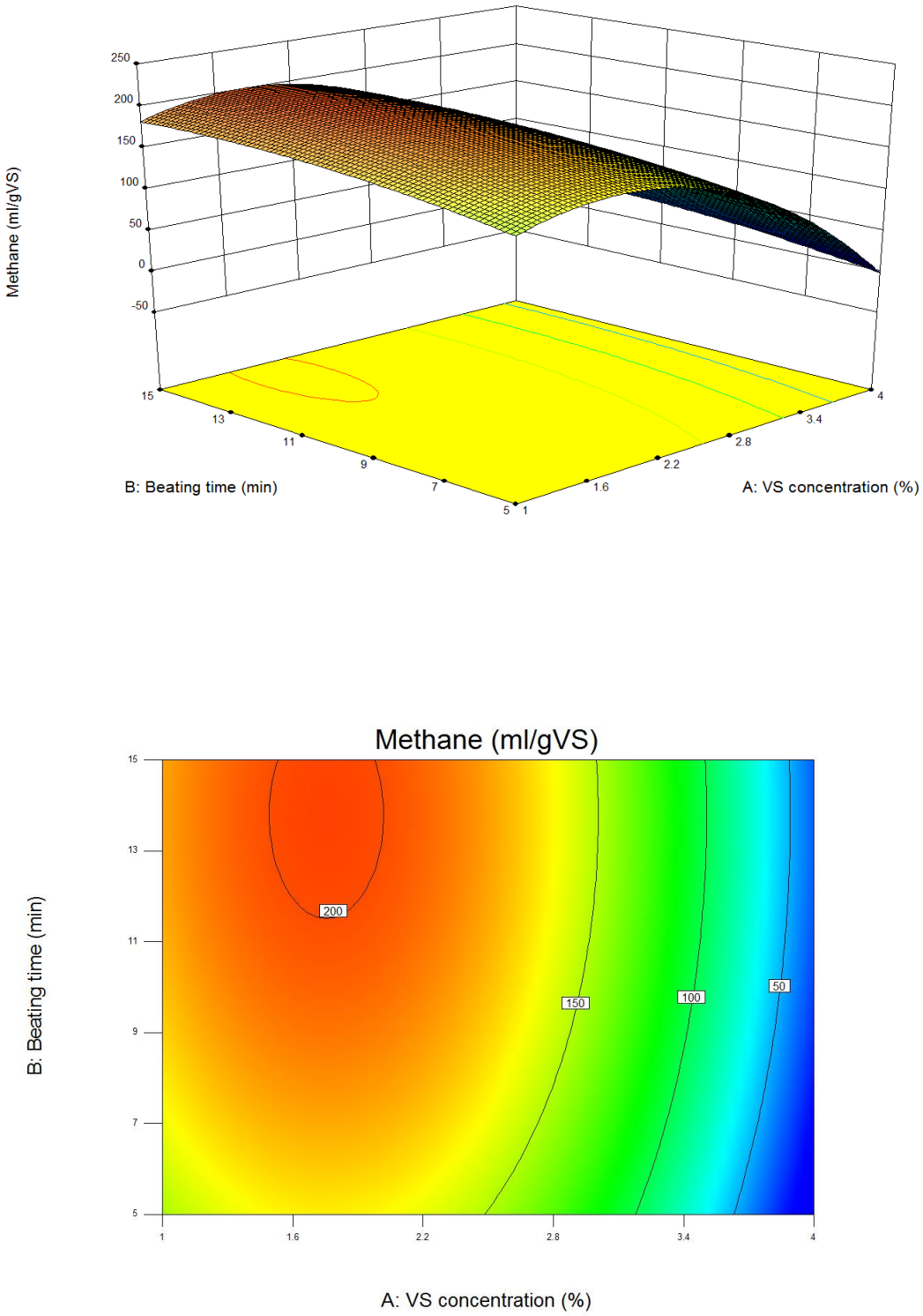


Figure 35: Response surface and contour plot of experiment 4, March 2015

The perturbation plots (**Figure 36**) showed similar trends for material harvested in May (**Figure 36-a**) and March (**Figure 36-c**), even though the methane yields were different. The curvature related to the VS concentration (A) meant that this factor impacted more on the response than the beating time (B). In particular, the methane yield was at the highest levels when the VS concentration was below the centre point (2.5%) while it decreased for higher values of VS. In November (**Figure 36-b**) there was a methane decrease while increasing the VS concentration over 2.5%; however such decrease resulted to be less important than in May and March.

Regarding the beating time, this had a stronger effect in May rather than in March, even though the general trend for these two months was an increase of methane with the time of pretreatment. Unlike May and March, the material harvested in November showed a decrease in methane yields while increasing the beating time. However the overall effect of the beating time was not statistically significant.

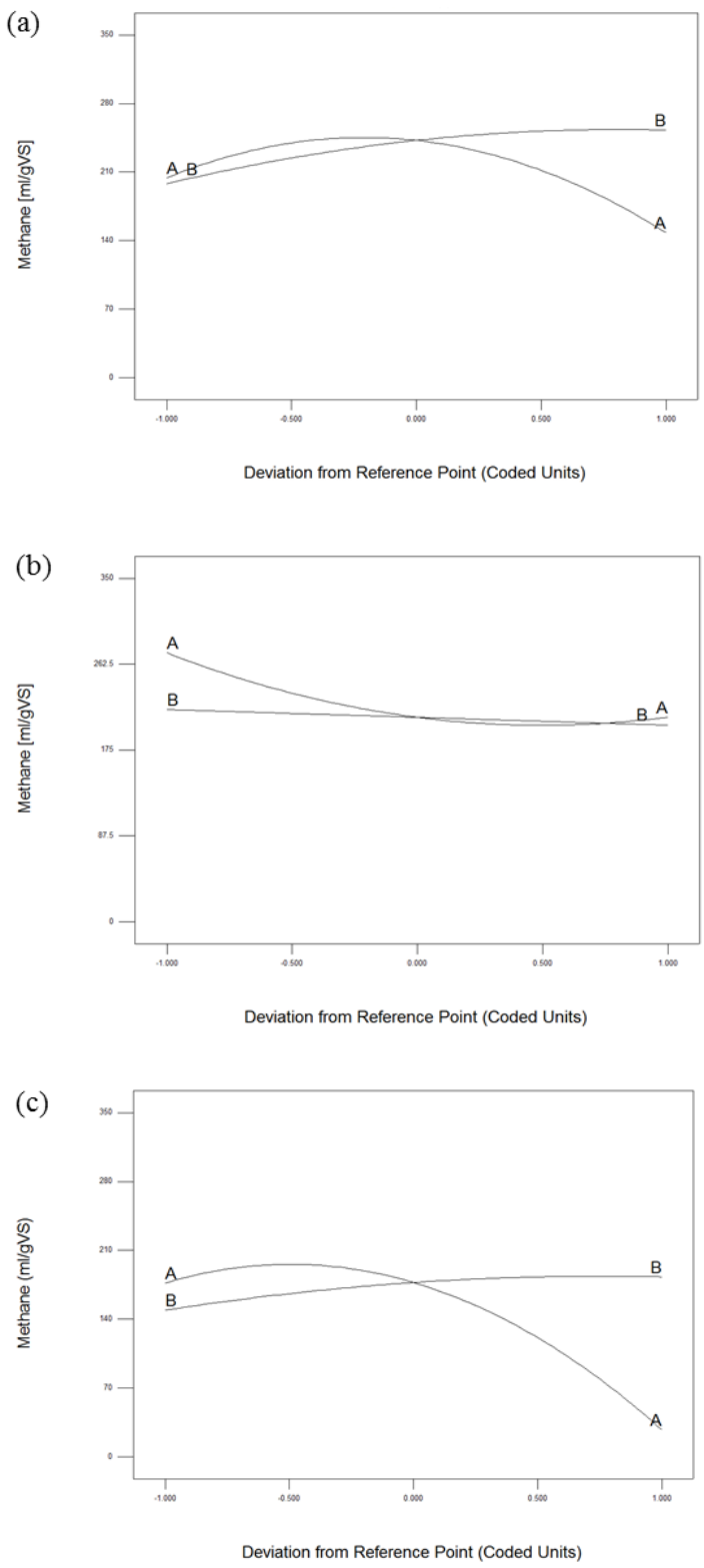


Figure 36: Perturbation plots in May 2014 (a), November 2014 (b), March 2015 (c)

From the AB interaction plots (**Figure 37**) relative to May and November experiments, it was interesting to note that in both months, the responses at 15

min were not affected by the VS concentration, while the predictions at 5 min of pretreatment were statistically significant. This means that when reducing the beating time up to 5 min, a reduction in VS concentration up to 1% was beneficial for the process, more in November than in May. When the VS concentration was set at 4%, in May an increase of beating time determined an increase of methane; while in November the pretreatment time did not have any significant effect.

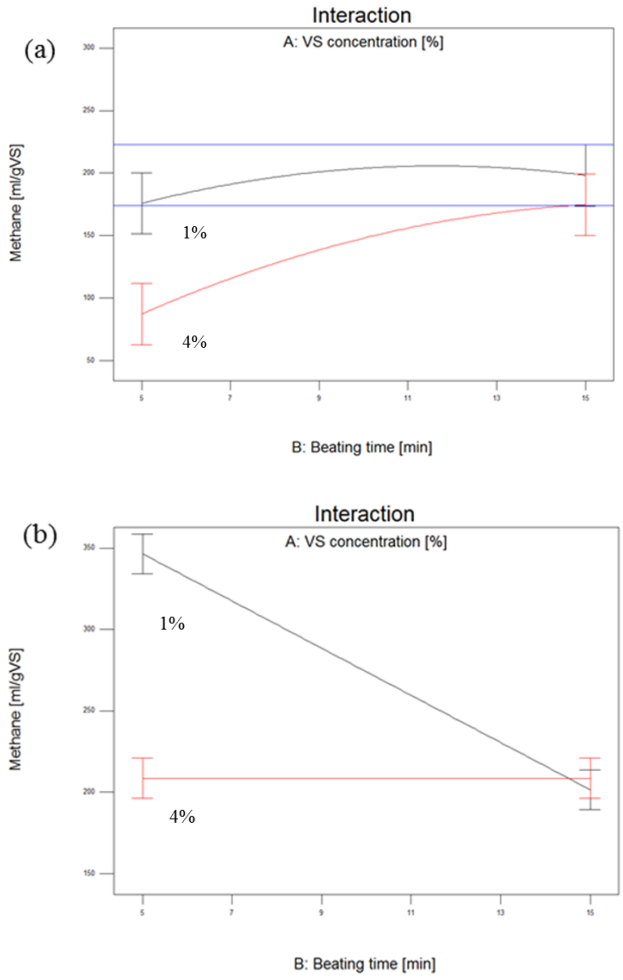


Figure 37: AB interaction plots in May (a), November (b)

4.4.3 Optimisation

For each experiment an optimisation analysis was carried out. The two goals of such analysis were to find:

- The optimum combination of VS concentration and beating time which maximised the methane yield,
- The optimum combination of VS concentration and beating time which maximised the methane yield while minimising the beating time.

4.4.3.1 Optimisation experiment 2-May 2014

For this month, the optimisation analysis predicted that the optimum combination of VS concentration and beating time which maximised the methane yield (**Table 42**) was at 2.5 % of VS and 14 min of treating (**Table 43**).

Table 42: Constraints optimisation 1, experiment 2-May

Variable	*Goal*	*Lower Limit*	*Upper Limit*
A: VS [%]	in range	1	4
B: BT [min]	in range	5	15
Methane [ml g^{-1} VS]	maximise	86	270

Table 43: Results optimisation 1, experiment 2-May

Solution	*A: VS [%]*	*B: BT [min]*	*Methane [ml g^{-1} VS]*	*Desirability*
1	2.322	13.777	254.446	0.915
2	2.328	13.825	254.444	0.915

When the goal was to maximise the methane and minimising the pretreatment time (**Table 44**), the software estimated that the best solution was to use 2% of VS for 5 min (**Table 45**). At the same time, this optimisation predicted a reduction of methane of 19% with respect to the previous optimisation. In general, decreasing the pretreatment time does not lead necessary to a better performance in terms of electricity consumption of the whole system, since using a lower pretreatment time determines a reduction in methane production as well. In *Section 4.3.3* it was calculated that a reduction of 10 min in beating time did not make up for a reduction of 17% of methane yield at 2% of VS. In this optimisation a 19% methane reduction was estimated at the same VS concentration, thus also in this case a reduction of beating time of 10 min was not sufficient in order to make up for a predicted reduction in methane of 19%.

Table 44: Constraints optimisation 2, experiment 2-May

Variable	*Goal*	*Lower Limit*	*Upper Limit*
A: VS [%]	in range	1	4
B: BT [min]	minimise	5	15
Methane [ml g^{-1} VS]	maximise	86	270

Table 45: Results optimisation 2, experiment 2-May

Solution	*A: VS [%]*	*B: BT [min]*	*Methane [ml g^{-1} VS]*	*Desirability*
1	2.000	5.000	205.332	0.805
2	2.014	5.000	205.327	0.805
3	1.967	5.002	205.318	0.805
4	2.040	5.000	205.289	0.805

4.4.3.2 Optimisation experiment 3-November 2014

During autumn, the best result was achieved at 1% of VS and for 5 min of pretreatment. Also the optimisation analysis predicted the same combination of factors' levels in order to achieve the maximum methane yield (**Tables 46-47**).

Table 46: Constraints optimisation 1, experiment 3-November

Variable	*Goal*	*Lower Limit*	*Upper Limit*
A: VS [%]	in range	1	4
B: BT [min]	in range	5	15
Methane [ml g^{-1} VS]	maximise	197	342

Table 47: Results optimisation 1, experiment 3-November

Solution	*A: VS [%]*	*B: BT [min]*	*Methane [ml g^{-1} VS]*	*Desirability*
1	1.000	5.000	346.500	1.000
2	1.031	5.000	342.626	1.000
3	1.018	5.000	344.184	1.000
4	1.000	5.069	345.504	0.997
5	1.061	5.000	338.875	0.989
6	4.000	5.000	208.667	0.284
7	1.000	6.484	324.977	0.867
8	4.000	5.000	208.667	0.284

In this case, the result obtained by maximising only the methane yield corresponded also with the result obtained by maximising the methane yield while minimising the beating time (**Tables 48-49**).

Table 48: Constraints optimisation 2, experiment 3-November

Variable	*Goal*	*Lower Limit*	*Upper Limit*
A: VS [%]	in range	1	4
B: BT [min]	minimise	5	15
Methane [ml g^{-1} VS]	maximise	197	342

Table 49: Results optimisation 2, experiment 3-November

Solution	*A: VS [%]*	*B: BT [min]*	*Methane [ml g^{-1} VS]*	*Desirability*
1	1.000	5.000	346.500	1.000
2	1.023	5.000	343.581	1.000
3	1.034	5.000	342.189	1.000
4	1.076	5.000	336.996	0.983
5	4.000	5.000	208.667	0.284

4.4.3.3 Optimisation experiment 4-March 2015

The March experiment was characterised by the lowest results. The optimisation analysis predicted that the best yield of 202 ml CH_4 g^{-1} VS could be achieved by using less than 2% of VS and for less than 14 min of treatment time (**Tables 50-51**).

Table 50: Constraints optimisation 1, experiment 4-March

Variable	*Goal*	*Lower Limit*	*Upper Limit*
A: VS [%]	in range	1	4
B: BT [min]	in range	5	15
Methane [ml g^{-1} VS]	maximise	15	215

Table 51: Results optimisation 1, experiment 4-March

Solution	*A: VS [%]*	*B: BT [min]*	*Methane [ml g^{-1} VS]*	*Desirability*
1	1.755	13.791	202.324	0.937

When the goal was to minimise the beating time and maximise the methane yield (**Table 52**), the solution corresponded to 2% of VS, as the previous optimisation, but with 17% less methane (**Table 53**). Hence, also in this case, it was not convenient to reduce the beating time since the resulting methane yield reduction would cause a lower energy production with respect to the case that employs longer pretreatment time.

Table 52: Constraints optimisation 2, experiment 4-March

Variable	*Goal*	*Lower Limit*	*Upper Limit*
A: VS [%]	in range	1	4
B: BT [min]	minimise	5	15
Methane [ml g^{-1} VS]	maximise	15	215

Table 53: Results optimisation 2, experiment 4-March

Solution	*A: VS [%]*	*B: BT [min]*	*Methane [ml g^{-1} VS]*	*Desirability*
1	1.756	5.000	167.671	0.874
2	1.784	5.000	167.644	0.874
3	1.711	5.000	167.604	0.874
4	1.815	5.000	167.551	0.873
5	1.835	5.000	167.461	0.873
6	1.629	5.000	167.136	0.872
7	2.275	5.000	158.652	0.847

4.4.4 Energy Evaluation

The use of a mechanical pretreatment is justified when it benefits the system by increasing the methane yield or lowering the digestion time. The achieved advantages must be large enough in order to make up for the energy consumed by the pretreatment and eventually generate more energy with respect to the scenario without pretreatment [45, 129]. Thus, a simple calculation based on the electricity consumption measured during each experiment was carried out.

The average methane measured for each experiment was used as biogas methane content. The machine energy consumption was measured during the experiment and expressed in Wh for gram of VS, according to the optimum VS concentration found for each experiment. The results are reported in **Table 54**.

According to this analysis, May was the only month during which the use of the beating pretreatment was not convenient, while a positive energy gain was achieved both in November and March. As expected, November and more so in general the autumn season, was the most suitable period to harvest *Laminaria sp.* for biogas production. In the same period, optimised conditions of VS concentration and beating time (1%, 5 min), would allow the highest increase in terms of energy production.

Even though the lowest methane yield was observed in March, the energy evaluation showed that there was a benefit in using the beating pretreatment. The extra methane produced after pretreatment with respect to the untreated sample, could make up for the energy consumed during the treatment and produce an extra energy of 24%.

Table 54: Energy evaluation experiments 2 (May), 3 (November) and 4 (March)

	May	***November***	***March***
Treated			
Best treatment condition	VS=2.5% BT= 15 min	VS=1% BT= 5 min	VS=2.5% BT= 15 min
Biogas produced [ml g^{-1}VS]	615	855	576
Average CH_4 [%]	40	40	40
Bs: Biogas energy content [kWh m^{-3}]	3.99	3.99	3.99
Ep: Produced energy [Wh g^{-1} VS]	2.45	3.41	2.30
Ec: Machine electricity consumption [Wh g^{-1} VS]	0.24	0.20	0.24
Net Ep: Net produced energy [Wh g^{-1} VS]	2.22	3.21	2.06
Untreated			
Best untreated condition	VS=1%	VS=2.5%	VS=2.5%
Biogas produced [ml g^{-1}VS]	482	430	418
Average CH4 [%]	50	40	40
Bs: Biogas energy content [kWh/m^3]	4.98	3.99	3.99
Ep: Produced energy [Wh g^{-1} VS]	2.40	1.71	1.67
Gain/Loss [%]	-8	87	24

4.4.5 Discussion of the key findings

Very few studies in the literature investigated the methane production through AD from *Laminaria sp.* at different periods of harvesting [104]. It is known that the seasonal fluctuation of *Laminaria sp.* components influences the methane conversion of this kind of seaweed [94, 104]. In Ireland, the influence of the organic substrate concentration and the pretreatment phase when harvesting the seaweed at different periods of the year has not been investigated to date. In general, the main carbohydrates in *Laminaria sp.* are mannitol, laminarin and alginic acid. Alginic acid, also called alginate, is a polysaccharide widely distributed in the cell walls of brown seaweed while laminarin and mannitol are the major carbon storage compounds in monomeric (mannitol) or polymeric (laminarin) form [140]. During AD, mannitol is utilised more efficiently than the polymers laminarin and alginic acid [104]. According to Schiener *et al.* [94], the average mannitol content in *L.digitata*, *L.hyperborea* and *S.latissima* was at 19.4 ± 6.6, 17.5 ± 7.4, and 18.6 ± 4.7%, respectively and the average laminarin content for the same species accounted for 6.7 ± 6.0, 7.4 ± 8.0, 8.2 ± 5.3 % of the dry weight. During autumn, the highest mannitol levels of 24-27% were observed while the lowest levels of 6–8 % were recorded in early spring. Laminarin followed a similar trend rising to its highest levels during the summer and autumn months (25 % max. in *L. hyperborea*) and dropping to its lowest levels (1-3 %) in winter [94]. Alginate formed the majority of the carbohydrate content accounting for 34.6 ± 3.1, 33.2 ± 3.8 and 28.5 ± 3.9 % of the dry weight in *L.digitata*, *L.hyperborea* and *S.latissima*, respectively. In accordance with the levels of mannitol and laminarin reported by Schiener *et al.* [94], the highest yields of methane were recorded during autumn (November) which corresponded to the peak for laminarin and mannitol content, while the lowest recordings corresponded to early spring (March), when the carbohydrates content was reported at its minimum. In a study conducted in the UK by Adams *et al.* [104], the highest methane yield of 254.14 ± 6.21ml g^{-1} VS was reported in July when the macroalgae presented the highest combined proportion of mannitol and laminarin in conjunction with lowest concentration of ash and alkali metals. The current study found out a higher methane yield up to 35% of extra methane in November, by using the beating pretreatment for 5 min and at 1% of VS. According to Adams *et al.*'s result [104], July represented the best month for

harvesting while in this investigation higher methane yields were achieved in November, by optimising the beating pretreatment and the VS concentration. However, it must be noticed that this study did not consider an experiment in July. It could be interesting to apply the same experimental conditions for material harvested in July in order to verify if it is possible to reach a further increase in methane yield. During November the statistical analysis estimated that the joint action of the VS concentration and the beating time affected significantly the methane response. The RSM analysis showed that a reduction of beating time up to 5 min determines a dramatic enhancement of methane at 1% of VS, which is not detected at 4% of VS. This meant that during autumn, it was necessary to vary these two factors simultaneously in order to optimise the process.

This study reported the lowest yields in March in accordance with Adams *et al.* [104]. The best yield of 215 ± 9 ml CH_4 g^{-1} VS was measured at 2.5% of VS and after 15 min of beating treatment with an increase of almost 10% with respect to Adams *et al.*'s result [104]. During this month, *Laminaria sp.* is generally characterised by low concentrations of carbohydrates. In proportion, high concentrations of alginic acid were observed in conjunction with low mannitol and laminarin concentrations. Alginic acid is known to have a slower hydrolysation rate than mannitol and laminarin [104]. Therefore, low levels of mannitol, laminarin, and slow alginate hydrolysation rate were likely to be the reasons of the lower methane yields observed during this month. Unlike other months, in March, the only parameter which had a strong impact on the methane response was the VS concentration. This was the only experiment characterised by a severe failure of the digester at 4% of VS. This suggested that in March, the choice of the VS concentration was a major issue in order to optimise the process.

In May, an increased methane yield of 14% with respect to Adams *et al.*'s result [104] was observed after 10 min of pretreatment and at 2.5% of VS. However, it must be noticed that it was not observed much improvement with respect to the untreated sample. The yields during this period resulted to be higher than those registered in March, but still lower than the yields measured in autumn. This trend is also confirmed by Adams *et al.*'s work [104]. Also in this case, the methane yield reflected the levels of the algal carbohydrates, which were

observed to be not as high as during autumn and not so low as in winter or early spring.

In general, for all the harvesting periods, the higher methane yields were observed at an optimum VS concentration below 2.5%. Autumn was the best harvesting period in order to exploit *Laminaria sp.* as feedstock for AD. During this period, the system would benefit the most by applying the beating pretreatment. The energy balance calculated an energy gain of 87%, in accordance with Tedesco *et al.* [120] who also used the beating pretreatment. In particular, short pretreatment times were sufficient in order to obtain the best methane yields; this would also be beneficial for the economics of the process. Even though early spring represented the worst period for harvesting *Laminaria sp.*, it was possible to improve the system performance by applying the beating pretreatment and optimising the VS concentration. In particular, an interesting finding is that the extra methane produced after pretreatment could make up for the energy consumed during the treatment and produce an extra energy of 24%.

In this discussion, it was underlined that the seaweed carbohydrates levels affect the methane production. For a better understanding of such matter, the next section provides a brief discussion about the environmental factors which seasonally affect the seaweed carbohydrates levels by considering the Irish context.

4.4.5.1 Discussion on environmental factors

It is known that amongst the major environmental factors affecting seaweeds chemical composition are light, temperature, salinity, water motion and nutrient availability [141]. High light intensities increase the rate of photosynthesis and the polysaccharide production [107] and a positive correlation exists between temperature and carbohydrates content [142]. Light quantity and quality depend on season, depth and turbidity. The turbidity affects negatively the seaweeds carbohydrates content since determines a reduction in irradiance [141, 143]. This factor is influenced by fast tidal motions [144], nutrient availability and pollution [141]. In particular, the Irish Sea is characterised by very high turbid seawater, especially during winter [144, 145] due to the strongest winds generally registered in this season. The data regarding the solar radiation registered in

Dublin over several years revealed a peak between May and August, while declining from December up to February (**Table 55**). Regarding the sea temperature, highest temperatures were registered between July and November and the lowest between winter and spring (**Figure 38**). In this case, it is worth noting that highest and lowest temperatures occur later in the year at sea than overland since water takes longer to warm up and cool down. In general, sea temperatures are higher than those of the air during winter, while the reverse is the case during summer months. By comparing the air temperatures with sea temperatures (**Figures 38-39**), the temperature trends are shift of one month between each other. For instance, while for the air temperature the peak is generally registered in July, for sea temperatures the peak is registered in August.

Table 55: Global Solar Radiation in Joules cm^{-2} for Dublin [146]

	Year				
Month	***2012***	***2013***	***2014***	***2015***	***Mean***
Jan	7580	5909	6508	8749	7228
Feb	12456	12106	14654	13203	12761
Mar	28991	19993	25421	29537	25705
Apr	37313	40281	42869	47485	39407
May	51564	55706	45343	51364	52530
Jun	46884	59657	57067	n/a	52648
Jul	48889	61855	54042	-	50860
Aug	40767	43342	42419	-	42506
Sep	33093	31714	31993	-	30043
Oct	16838	15960	19354	-	18168
Nov	10753	10184	8050	-	8935
Dec	6187	6146	6317	-	5550
Annual	341315	362853	354037	-	346340

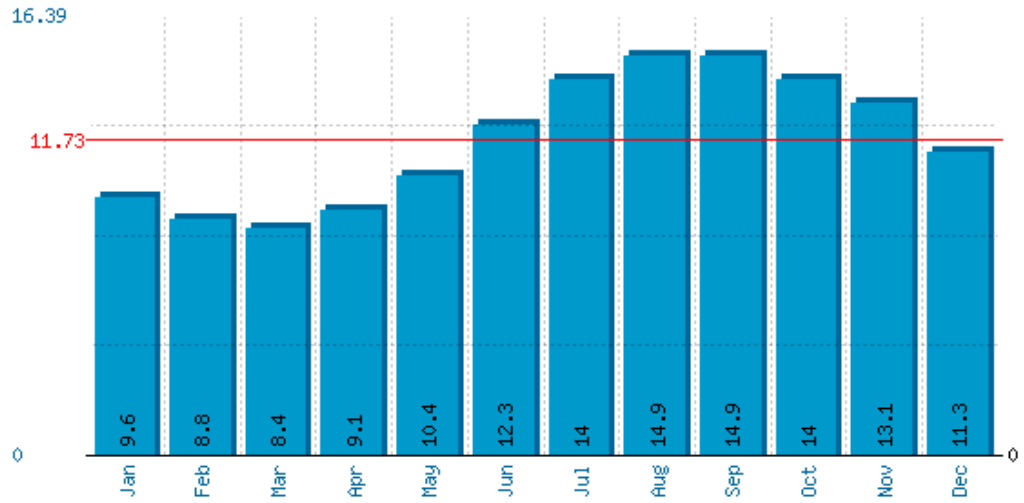


Figure 38: Average sea temperatures for Dublin over several years of archived data [147]

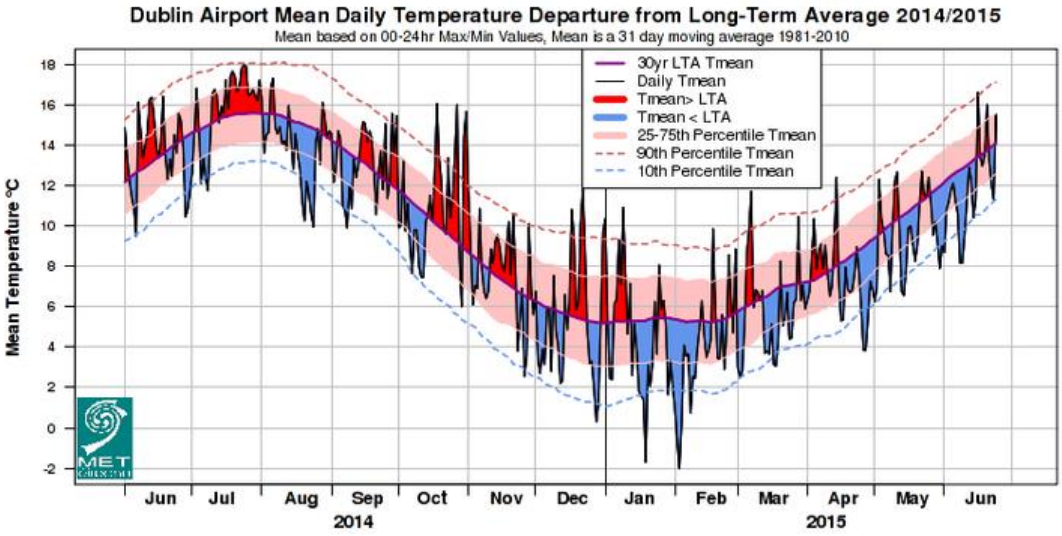


Figure 39: Dublin Mean Daily Temperature Departure from Long-Term Average 2014/2015 (mean based on 00-24hr Max/Min Values, Mean is a 31 day moving average 1981-2010) [146]

According to these data, it is likely that the seaweed biomass generally stores the main carbohydrates during summer due to high solar radiations and temperatures, and then consumes them during winter for tissue growth. Therefore, by considering the Irish climate, summer and autumn are in general the best harvesting periods of *Laminaria sp.* for bioenergy exploitation, since the seaweed is rich of carbohydrates stored during summer months thanks to higher solar

radiation and temperatures that are generally recorded in these months, while in early spring (March) the biomass is poor of nutrients due to the consumption during winter months.

May is generally characterised by high solar radiation and high sea temperatures. In this case though, methane yields as high as in autumn were not observed probably due to the fact that the seaweed had not already reached high levels of carbohydrates. This resulted in higher TS (19 ± 2% Wt on wet basis), VS (84 ± 1% of TS) contents and methane production during November, while lower levels were seen in March and May, with May having a higher VS content standard deviation, as during this period the seaweed was starting to accumulate nutrients.

Therefore, according to the low levels of algal carbohydrates both in winter and early spring, it is likely that the winter months would be characterised by similar yields as those observed in March. On the other hand, both summer and autumn are characterised by high levels of carbohydrates, thus it is possible that during summer similar yields as in November are likely to be observed.

4.5 Summary of the research findings

In this section, an overview of the main findings achieved by all the experimental investigation is reported. This experimental research aimed to investigate and optimise the use of macroalgal biomass for biogas production through AD. The main findings of the experimental study could be summarized as follows:

1. Amongst the three mechanical pretreatments tested such as beating, milling, and microwave, the best performance in terms of methane production was achieved when beating pretreatment was applied. Microwave and milling influenced negatively the digestion process, thus confirming that harsh pretreatments are not suitable for algal biomass,
2. The main effect of the beating pretreatment was to boost the start of the digestion process while a marginal methane enhancement was recorded at the end of digestion,
3. Between the two macroalgae species tested, *Laminaria sp.* exhibited a general enhancement in methane yield of 40% with respect to *Ascophyllum nodosum*,
4. *Ascophyllum nodosum* exhibited 30% more methane after pretreatment and at a low organic substrate concentration (1% of VS),
5. It was found out that the organic substrate concentration had a major impact on methane production with respect to the beating time. Low organic substrate concentrations (below 2.5% of VS) enabled higher methane yields,
6. Regarding the seasonality investigation, it was confirmed that the autumn represented the most suitable season in order to exploit *Laminaria sp.* as feedstock for methane production. General higher methane yields (between 50-60%) with respect to other periods were observed at low substrate concentrations (1% of VS) and short beating time (5 min),
7. Early spring exhibited the lowest methane yields, nonetheless a better performance of 50% extra methane after pretreatment was observed,
8. The energy balance showed the highest energy production in November, while an energy loss was registered in May, when the methane yield produced after pretreatment was similar to the methane yield obtained without pretreatment.

Following these main findings, some conclusions and recommendations are provided in the next chapter. The contribution to the field provided by this research work is also discussed. Finally, the next steps that this research should focus on are reported.

Chapter 5: Conclusions and Future Work

5.1 Conclusion

The main conclusions of this research are summarised in this section. The issues that emerged from the literature were addressed as follows:

1. Amongst the physical pretreatments tested, namely beating, ball milling and microwave, the beating pretreatment showed the best performance. Thus, in the case of seaweed biomass, the beating pretreatment represents a valuable option for biogas conversion,
2. Amongst the two Irish seaweeds species tested, namely *Laminaria sp.* and *Ascophyllum nodosum*, the *Laminaria sp.* performed the highest yields, proving to be a better feedstock for biogas exploitation with respect to *Ascophyllum nodosum*,
3. From the seasonal investigation, it can be concluded that in Ireland, summer and autumn are the best harvesting periods of *Laminaria sp.* as feedstock for biogas production. In particular, low organic substrate concentration (1%) and short pretreatment times are recommended in order to achieve optimised methane yields. In winter and early spring, the use of beating pretreatment is recommended at low substrate concentrations (below 2.5%), while at the end of spring and start of summer, the use of the beating pretreatment is not recommended,
4. From an energy point of view, it was confirmed that autumn is the best period for harvesting, as it allows a higher energy gain compared to winter and early spring.

5.2 Research contribution

The research work contribution is presented as follows:

1. This experimental research was able to provide new knowledge and understanding about the use of a mechanical pretreatment when the scope was to exploit Irish seaweed biomass as feedstock for biogas production. This was the first study that dealt with the investigation of different mechanical pretreatments, namely beating, microwave and milling, applied to the seaweed *Laminaria sp.* for methane production,
2. Very few studies were found in regards with the most abundant and commercially important Irish seaweed species such as *Laminaria sp.* and *Ascophyllum nodosum*. None of these studies dealt with the optimisation of both pretreatment and organic substrate concentration. Therefore, the current research generated new data on the employment of these two seaweed species for biogas production while optimising a mechanical pretreatment as well as the organic substrate concentration,
3. For the first time in Ireland, crucial data were generated on the exploitation of *Laminaria sp.* for methane production at different periods of harvesting. The knowledge provided was challenged in terms of energy consumed as well as the optimisation of the digestion process which represents a fundamental basis in order to investigate the employment of seaweed as biomass for bioenergy purpose at an industrial scale.

5.3 Future Work

In conclusion, new research is needed in order to exploit the full potential of seaweed biomass, in Ireland:

1. The energy evaluation only concentrated on the beating pretreatment. The aim was to provide a preliminary understanding of the economics of the process. A more comprehensive understanding of the economics of the process can be achieved by evaluating its energy consumption related to the digestion process (*e.g.* digester's heating). This would generate essential data to evaluate the economic feasibility of the use of seaweed biomass for biogas production at a larger scale,

2. This was the first study conducted in Ireland which dealt with the seasonality of seaweed biomass. In particular, the study depends on the biogas conversion of beach cast seaweed, which is the most readily available biomass in Ireland. For this reason, more effort is required to generate more data which would validate the findings achieved. The aim is to exploit this biomass for biofuel conversion at a small, localised scale, over a short period,
3. In this work it was discussed as the seasonality of the seaweed chemical composition was related to environmental factors. However, it must be said that if the aim was to exploit seaweed biomass at a large scale, the wild harvesting would not be a valuable option as a constant and homogeneous feedstock supply would not be guaranteed due to variable environmental conditions. Thus, the best option is to develop farming systems which ensure a constant supply of seaweed biomass. One of the main drawbacks related to seaweed farming, especially in Ireland, is its economic viability. On this matter, a possible option is to couple the seaweed cultivation with other renewable energy activities. For instance, considering that Ireland has a flourishing wind sector, wind farms can be used as platform for seaweed farming. By using this approach, the costs of seaweed production are covered by the wind generation plant capital and operational costs. Also the biorefinery concept represents a viable route. In Ireland, the seaweed sector is mainly focused on hydrocolloid and alginic acid production. Once these high-value products are extracted, the seaweed residue can be potentially exploited as feedstock for AD. Future research should focus on biogas conversion of cultivated seaweed as well as residue from other Irish industrial sectors. A comprehensive evaluation of the economics and logistics of such concepts should also be addressed.

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Appendix A: How can seaweed heat my home?

This research contributed with an article [148] in the *"Science of Summer"* supplement of the *Irish Independent Newspaper*, printed on June 18th. The article is reported in this section and briefly describes the potential of seaweed biomass for bioenergy conversion in Ireland.

How can seaweed heat my home?

Depending on its colour, seaweed is classified as red (*Rhodophyceae*), brown (*Phaeophyceae)* and green (*Chlorophyceae)*. In Ireland, approximately 500 species of seaweeds have been documented within these three classes.

Seaweed can be harvested from beaches, or cultivated in "seaweed farms". Globally, production of seaweed was estimated at 19 million tonnes in 2010, with the Japanese kelp species, *Saccharina latissima/Laminaria japonica* accounting for 99% of that. China is the world's biggest producer of seaweed, at over 11 million tonnes annually.

In Ireland, seaweed production is much smaller, around 45,000 tonnes annually and it is mainly used as fertiliser and as a source of alginate, a gum-like compound widely used in the food, pharmaceutical and cosmetic industries.

Seaweed is highly valued as a property in food because it is a valuable source of minerals and vitamins. Seaweed extracts can be found in a wide range of everyday products such as toothpaste, shaving foam, ice cream, cheese, body products, printing inks and even beer.

Seaweed also represents a huge and renewable resource for the generation of bioenergy, the term for energy derived from organic materials such as plants, animals, wood or waste.

Despite all its benefits, when it accumulates in large quantities on our beaches, seaweed can represent a big nuisance. It can make access to beaches difficult, while the presence of seaweed in the water and the rotten-egg type smell that follows its decomposition (caused by the production of hydrogen sulphide (H_2S) tends to drive people from beaches.

While many people regard decomposed seaweed as waste product, for others it is a valuable resource. After harvesting, seaweed can be subjected to a biological process called anaerobic digestion**,** which means that it takes places in an oxygen-free environment. This process is very similar to human digestion, which happens in our stomach, thanks to the action of a series of different bacteria.

When seaweed undergoes anaerobic digestion, the bacteria produce a biogas that is about 60pc composed of methane (CH_4). When the biogas is stripped of by-products of the anaerobic digestion process, other than methane, the result is another gas called biomethane. This is composed of about 97%-985 methane and is similar to natural gas used to generate heat, electricity and also as transport fuel.

Among all seaweeds, one species particularly suitable for this kind of process is the *Laminaria* spp., which is very easy to see while walking along the beach, but maybe without being aware of what amazing resource it represents.