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Biomethane Production from Macroalgae

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Thesis submitted for the degree of Doctor of Philosophy to the National University of Ireland, Cork

Supervisors: Professor Jerry D. Murphy

Head of School: Professor Nabeel Riza

June 2016

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Declaration

It is stated that this thesis is my original research work and that it has not been submitted for another degree, either at University College Cork or elsewhere. Any other sources of supporting information have been acknowledged.

Muhammad Rizwan Tabassum

June, 2016

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Abstract

Irish brown seaweeds have been identified as a potential bio-resource with potentially high specific methane yields. Anaerobic digestion is deemed the most feasible technology due to its commercial viability for handling such wet feedstock. However, the biomethane potential of seaweed is highly dependent on its chemical composition which can vary by species type, cultivation method, and time of harvest. This study aims to investigate and optimize the process for the production of biomethane from Irish brown seaweeds focusing on the key technology bottlenecks including for seaweed characterization, biomethane potential assessment, optimization of long-term anaerobic digestion and suitable pre-treatment technologies to enhance potential gas yields.

Laminaria digitata and *Ascophyllum nodosum* were tested for seasonal variation. From the characterization and batch digestion of *L. digitata*, August was found to be the optimal month for harvest due to high organic matter content, low level of ash and ultimately highest biomethane yield. The specific methane yield of 53 m^3 CH_4 t^{-1} wwt in August was 4.5 times higher than the yield in December (12 m^3 CH_4 t^{-1} wwt), with ash content the key factor in seasonal variation. For *A. nodosum*, the optimal harvest month was October with polyphenol content found to be a more influential factor than ash. The gross energy yields from both species were evaluated in the range of 116-200 GJ ha^{-1} yr^{-1}.

Continuous digestion trials were subsequently designed for *S. latissima* and *L. digitata* to optimize the key digestion parameters. Results from mono-digestion and co-digestion with dairy slurry revealed that both seaweeds could be digested at maximum biomethane efficiency to a loading rate of 4 kg VS m^{-3} d^{-1}. Accumulation of salt in the digesters was a concern for long term digestion and it was reasoned that suitable pretreatment may be required prior to digestion. Various pre-treatments were subsequently tested on *L. digitata* to enhance the gas yield. It was found that maceration after hot water washing yielded 25% more specific methane and up to 54% salt removal as compared to untreated *L. digitata*.

The experiments undertaken aim to assist in providing a basic guideline for feasible design and operation of seaweed digesters in Ireland.

Output of the thesis

The following chapters represent material which has been published or is currently under review in peer-reviewed journals:

Chapter 2:

Tabassum, M.R., Xia, A., Murphy, J.D. 2016. Potential of seaweed as a feedstock for renewable gaseous fuel production in Ireland. Renewable and Sustainable Energy Reviews, (under review).

Chapter 3:

Tabassum, M.R., Xia, A., Murphy, J.D. 2016. The effect of seasonal variation on biomethane production from seaweed and on application as a gaseous transport biofuel. Bioresource Technology 209, 213-219.

Chapter 4:

Tabassum, M.R., Xia, A., Murphy, J.D. 2016. Seasonal variation of chemical composition and biomethane production from the brown seaweed *Ascophyllum nodosum*. Bioresource Technology, (in press).

Chapter 5:

Tabassum, M.R., Xia, A., Murphy, J.D. 2016. Third generation gaseous biofuel generated through mono- and co-digestion of natural and cultivated seaweeds, with dairy slurry. Bioresource Technology, (under review).

Chapter 6:

Tabassum, M.R., Xia, A., Murphy, J.D. 2016. Comparison of pre-treatments to enhance the biomethane yield from *Laminaria digitata*. Bioresource Technology, (under review).

Chapter 7:

This chapter contains the summary of all conclusions and recommendations for the future research work.

Contribution to the papers

I was advised and mentored by Professor Jerry D. Murphy in compiling all chapters

Chapter 2: I was the first author of the paper and responsible for collecting, researching and processing the relevant data (Advised by Dr. Ao Xia).

Chapter 3: I was the first author of the paper and was responsible for the collection of the samples, processing the samples, experimental design, the undertaking of laboratory work and data analysis (Advised by Dr. Ao Xia).

Chapter 4: I was the first author of the paper and was responsible for sample collection and processing, experimental design and undertaking data analysis of the research work (Advised by Dr. Ao Xia).

Chapter 5: I was the first author of the paper and was responsible for the experimental design, operation of the continuous laboratory trials for approximately 11 months, analysis of the samples, data collection and evaluation. Collection and processing of raw materials were undertaken with my colleagues (Advised by Dr. David M. Wall).

Chapter 6: I was the first author of this paper and was responsible for the planning and execution of laboratory research work. I performed the experimental measurements, data analysis and evaluation (Advised by Dr. Ao Xia).

Nomenclature

AD	Anaerobic digestion
A:V	Ash to volatile solid ratio
B. Ef	biomethane efficiency
BI	biodegradability index
BMP	biomethane potential assay
BNG	Bio-natural gas
CH_4	methane gas
CNG	Compressed natural gas
CO_2	carbon dioxide gas
CSTR	continuously stirred tank reactor
TS	total solids (or dry solids)
EU	European Union
FOS:TAC	Flüchtige organische säuren /totales anorganisches carbonat
H_2	hydrogen gas
ha	hectare
HRT	hydraulic retention time
M t	million tonnes
NH_3	free ammonia
OLR	organic loading rate
RES-T:	renewable energy supply in transport
SMY:	specific methane yield
TAN	total ammonical nitrogen
TMP	Theoretical methane potential
VFA	volatile fatty acids
VS	volatile solids
wwt	wet weight

1. Introduction

1.1 Introduction and background to thesis

The impact of climate change and the depletion of fossil fuels are major concerns for future energy production systems; hence renewable energy alternatives are being sought. The European Commission directives propose a reduction in greenhouse gas emissions by using biofuels in transport at a level of 10% (Directive 2009/28/EC), with first generation biofuel substrates now limited at 7% (European Parliament, 2015). It has been proposed that the share of advanced third generation biofuel substrates (such as seaweed) should represent at least 1.25% in renewable energy supply in transport (RES-T). Biomethane production via anaerobic digestion can significantly contribute to the EU targets as the generated gas can be injected into the natural gas grid and supplied or stored off-site as a transport fuel. However, there is a very limited biogas industry in Ireland and no digestion of seaweed.

Macroalgae (seaweed) has been described as a promising alternative feedstock for biomethane production due to its high growth rates, superior gross energy yields and higher rates of carbon dioxide fixation as compared to traditional energy crops. With an easily degradable structure due to lack of lignin and low cellulose content, lack of arable land and fresh water requirement for its cultivation, macroalgae are being investigated as a potential feedstock for gaseous fuel production. The biomethane potential of seaweed is significantly dependent on its seasonal variation in chemical composition due to habitat, species type, cultivation technique, and harvest time. *Saccharina latissima* and *Laminaria digitata* have been described as the potentially highest biomethane yielding Irish brown seaweeds with gross energy yields in the range 38-384 GJ ha^{-1} yr^{-1}, depending on the type of species, harvesting time and cultivation method.

Anaerobic digestion is deemed the most suitable technology for the high moisture content feedstock; seaweed comprises of 85-90% moisture content. Drying seaweed for ethanol production would be a very energy intensive process. The digestion process biologically converts the organic matter into biogas (50-60 % methane) in an anaerobic environment. The generated biogas can be upgraded to biomethane gas (>97 % methane) for electricity generation, renewable heat applications or a compressed renewable transport fuel. Gas grid operators in Denmark, Sweden, Belgium, Netherlands, France and Switzerland are already in an agreement to supply 100 % carbon neutral gas by 2050. As mentioned, Ireland has currently no biogas industry to supply renewable gas. However, Gas Networks Ireland (gas grid owner) has committed to the development of renewable green gas by targeting 20% of natural gas demand to be renewable by 2030. Seaweed digesters may be applicable to coastal regions where there would be an availability of feedstock with minimum transportation. Such digesters could also be run in co-digestion with other abundant substrates in the locality such as dairy slurry. To initiate such a strategy, digestion operational parameters (such as loading rates and retention times) must be optimised for maximum biomethane production efficiency. High salt concentrations in the seaweed with low C:N ratio may, however, affect the digester performance.

This study is designed to analyse brown seaweed as a feedstock for biomethane production. The effect of seasonal variation on the chemical composition of the feedstock and its subsequent influence on potential gas yields will be investigated. Batch and continuous digestion trials will be carried out to calculate the biomethane production efficiency. Long term mono- and co-digestion trials will be undertaken to

optimize some key process parameters. Suitable pre-treatment technologies will also be tested to potentially maximize the gas yields by removing substantial amounts of salt from the feedstock.

1.2 Thesis aims and objectives

The aims and objectives of the thesis were as follows:

- To calculate the specific methane yields (L CH_4 kg^{-1} VS) of available Irish brown seaweeds based on seasonal variation in chemical composition.
- To calculate the gross energy yield from brown seaweeds and estimate the coastal area required to satisfy the EU 2020 target (1.25% from advanced biofuels; 2.35 PJ) for renewable energy supply in transport (RES-T) in Ireland using specific methane yields from seasonal variation data.
- To investigate the potential for mono- and co-digestion strategies for brown seaweeds to suggest offshore and onshore digesters.
- To optimize the organic loading rate and hydraulic retention time in mono-digestion of *L. digitata* and *S. latissima* and co-digestion of both seaweeds with the slurry in continuously stirred tank reactors (CSTRs).
- To investigate the effect of increasing the percentage of *L. digitata* and *S. latissima* in co-digestion systems to suggest the optimum blend.
- To find out the critical parameters which may inhibit the digestion process.
- To investigate a suitable pre-treatment technology to enhance the gas yield.

1.3 Thesis outline and link between chapters

The thesis consists of seven chapters. Chapter 2 is a review article that assesses the potential of seaweed as a feedstock for renewable gaseous fuel production in Ireland. The article examines the scientific literature and reviews previous work undertaken for the digestion of macroalgae. The chapter identifies the bottlenecks and suggests an integrated model to overcome such bottlenecks in the technology. Chapters 3 to 6 present the majority of the laboratory work undertaken over the research period. Each chapter describes a separate topic, however, a sequential theme combines the study into one unit. A summary of chapters 2 to 6 is given below:

Chapter 2: *Potential of seaweed as a feedstock for renewable gaseous fuel production in Ireland*

This chapter focuses on the potential of brown seaweed as a feedstock for biomethane production. Brown seaweed has been described as a promising feedstock due to its greater energy yields and a higher rate of carbon dioxide fixation than energy crops. Easily degradable structure, no freshwater and no arable land requirements for its cultivation make the seaweed a potential feedstock for gaseous fuel production. However, biomethane potential of seaweed is variable due to its chemical composition that changes round the year. *Saccharina latissima* and *Laminaria digitata* have been described as highest biomethane yielding Irish brown seaweeds. July was found the peak month to harvest the brown seaweed such as *L. digitata* and the gross energy yields were estimated in the range 38-384 GJ ha^{-1} yr^{-1} for five Irish brown seaweeds (*S. latissima, A. esculenta, S. polyschides, L. digitata and L. hyperborea*). Technology

bottlenecks were identified and an integrated model was suggested in which the seaweed may be mono- and co-digested with other suitable feedstock for the sustainable production of gaseous fuel. It was estimated that a range of 6,124 to 61,465 ha of the coastal area would be required to cultivate seaweed to meet Ireland's 2020 target by selecting high yielding seaweed species, applying cost-effective cultivation method, choosing peak season for harvesting, optimizing the parameters for long term digestion and developing the gas yield enhancing pre-treatment methods.

Chapter 3: *The effect of seasonal variation on biomethane production from seaweed and on application as a gaseous transport biofuel*

This chapter presents the effect of seasonal variation of *L. digitata* on the potential gas yields attainable. The seaweed was examined for proximate analysis, ultimate analysis, and biochemical composition. It was found that the characteristics in August were optimal having the lowest content of ash (20% of volatile solids), a C:N ratio of 32 and the highest specific methane yield (327 L CH_4 kg VS^{-1}) which was 72% of theoretical yield. The highest biomethane yield (per ton wet weight) was obtained in August which was 4.5 times higher than in December. In the peak month, a cultivation area of 11,800 ha would be required for seaweed to satisfy the 2020 target for advanced biofuels in Ireland (1.25% RES-T).

Chapter 4: *Seasonal variation of chemical composition and biomethane production from the brown seaweed Ascophyllum nodosum*

The Irish brown seaweed *Ascophyllum nodosum* has also shown a significant seasonal variation in its chemical composition and biogas production. It was observed that the polyphenol content was the key factor in biogas production rather than the ash content. Summer months were reported as peak month for the gas production but due to high polyphenol content in summer months, the gas yield was adversely affected. March and October were suggested two peak seasons for the seaweed harvesting due to relatively low level of polyphenols (2% of TS). October yielded the highest methane (215 L CH_4 kg VS^{-1}) due to lower ash (23% of volatile solids) and polyphenols (2% of TS). A gross energy yield of 116 GJ ha^{-1} yr^{-1} in October was calculated based on the optimal biogas production. Harvesting the seaweed at peak season, an area of 20,260 ha for cultivation could satisfy the renewable transport energy target in Ireland. Results from the seasonal variation of *L. digitata* (chapter 3) and *A. nodosum* (chapter 4) revealed that different peak harvesting months would be advantageous to run the reactors round the year to provide the continuous supply of the feedstock.

Chapter 5: *Third generation gaseous biofuel generated through mono- and co-digestion of natural and cultivated seaweeds, with dairy slurry.*

Biomethane potential results based on seasonal variation described in chapters 3 and 4 required further investigation in terms of the operational parameters in continuous digestion. This would present a more realistic interpretation of full-scale digestion processes. The emphasis was to examine digester performance whilst increasing the reactors organic loading rate (OLR). Long term mono-digestion of two Irish brown

seaweeds *L. digitata* (natural harvest) and *S. latissima* (farm cultivated young plants) and dairy slurry and co-digestion of both seaweeds with dairy slurry was investigated. It was observed that higher proportions of *L. digitata* in the co-digestion mix (66.6% *L. digitata*) allowed the digester to operate at a higher OLR of 5 kg VS m^{-3} d^{-1} achieving a higher specific methane yield (SMY) of 232 L CH_4 kg^{-1} VS, as compared to lower proportions of *L. digitata* (33.3%). For 66.6 % farm cultivated *S. latissima,* a higher SMY of 252 L CH_4 kg^{-1} VS was recorded but at a lower OLR of 4 kg VS m^{-3} d^{-1} as compared to the natural harvest of *L. digitata*. Optimum conditions for mono-digestion of both seaweeds were evaluated at 4 kg VS m^{-3} d^{-1}. Chloride levels were shown to increase to high levels in the digestion of both seaweeds that indicated further investigation to apply suitable pretreatment prior to digestion.

Chapter 6: *Comparison of pre-treatments to enhance the biomethane yield from Laminaria digitata*

Long-term continuous anaerobic digestion trials (chapter 5) indicated that high salt accumulation in seaweed digesters may be an issue if operated for longer periods. Thus, suitable pre-treatment may be required that can reduce the salt accumulation. Salt removal may also enhance the biomethane potential of seaweeds. However, seasonal variation in the biochemical composition of seaweed (results from chapter 3 and 4) may significantly affect the choice of pre-treatment used. Brown seaweed *L. digitata* harvested in March (with high ash content and low C:N ratio) and in September (with low ash content and high C:N ratio) were selected to suggest the optimal pre-treatment. Two pre-treatments, washing (tap water and hot water) and size reduction (4cm and 4mm) were investigated for the two different harvesting seasons (March and

September). Washing of *L. digitata* harvested in March with hot water at 40 ^{0}C removed 54% ash and improved VS content by 31%, thereby enhancing biomethane yield (282 L CH_4 kg VS^{-1}) by 16%. Size reduction after washing with tap water had little impact on the gas yield. Size reduction with scissors (4cm) after hot washing of the March harvest resulted in the removal of nitrogenous compounds from the seaweed which improved the C:N ratio. The gross energy yields were calculated as 60 GJ ha^{-1} yr^{-1} and 75 GJ ha^{-1} yr^{-1} for untreated and pre-treated seaweed, respectively.

2. Potential of seaweed as a feedstock for renewable gaseous fuel production in Ireland

Potential of seaweed as a feedstock for renewable gaseous fuel production in Ireland

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Abstract

Resource depletion and mitigation of climate change are the main driving forces to find alternatives to fossil fuels. Seaweeds (macroalgae) have been considered as a promising alternative source of biofuels due to higher growth rates, greater production yields and a higher rate of carbon dioxide fixation, than land crops. A comparatively easily depolymerized structure, lack of need of arable land and no fresh water requirement for cultivation, make seaweed a potential valuable feedstock for gaseous biofuel production. Biomethane potential of seaweed is greatly dependent on its chemical composition which is highly variable due to its type, habitat, cultivation method and time of harvest. *Saccharina latissima* and *Laminaria digitata* are the highest reported biomethane yielding Irish brown seaweeds. Seaweed harvested in July (northern hemisphere) was estimated to give gross energy yields in the range 38-384 GJ ha^{-1} yr^{-1}; the higher values are dependent on innovative cultivation systems. An integrated model is suggested where seaweed can be co-digested with other feedstock for the sustainable production of gaseous fuel to facilitate EU renewable energy targets in transport.

Keywords: Anaerobic digestion; Biofuel; Biogas; Pre-treatment; Seasonal variation

2.1 Introduction

World primary energy consumption is expected to double by 2050 with a 1.6% annual growth rate (Dudley, 2013). In 2001, energy consumption by 6.1 billion people was 13.5TW; with 86% derived from fossil fuels. In 2050, the population is expected to reach 9.4 billion with an energy demand of 40.8 TW. It is likely that environmental pollution will grow with population and energy growth. This has led to a focus on alternative and renewable sources of energy (Lewis & Nocera, 2006). The European Commission's Directive 2009/28/EC proposed a reduction in the greenhouse gas emissions through the use of transport biofuels at a level of 10% of primary energy in transport by 2020 (Murphy et al., 2013). In 2012, an EC proposal (Parliament, 2012) suggested limiting first generation biofuels (from food crops) to 5% RES-T (renewable energy supply in transport). In 2013, it was proposed to be raised to 6% (Parliament, 2013) and that advanced biofuels (such as from seaweed) should represent at least 2.5% of RES-T; In 2015 this target was reviewed and set to 1.25% (Parliment, 2015).

Life cycle analysis of biofuels benefits from utilization of carbon dioxide during growth of biomass (Davis et al., 2009). Biodegradability, low toxicity, and low pollutant emissions offer advantages of biofuels over petroleum-based fuels (John et al., 2011). Biofuels are classified on the basis of biomass feedstock. First generation biofuels are produced from food commodities (such as corn and sugar cane), second generation biofuels are from lingo-cellulosic biomass (including agricultural residues such as straw) and third generation biofuels (generated from algae) (Nigam & Singh, 2011). First generation biofuels suffer from the food-versus-fuel debate (Naik et al., 2010). Second generation biofuels do not use food crops, but may require land that could be used for

good. Technical barriers still exist in breaking down second generation substrates to fermentable sugars (Panagiotopoulos et al., 2013). Biological techniques may be (application of enzymes) used to overcome these barriers (Jones & Mayfieldt, 2012). Macroalgae (seaweed) can exhibit higher growth rates, greater production yields (Subhadra & Edwards, 2010) and higher rate of carbon dioxide fixation (Gao & Mckinley, 1994) than land-based energy crops. Seaweeds do not need arable land and fresh water for cultivation (Wei et al., 2013). Negligible quantities of hemicellulose and lignin (John et al., 2011) facilitate easy depolymerisation (Wargacki et al., 2012). Seaweeds are also amenable to co-digest with a variety of feedstock such as dairy slurry, agricultural waste, food waste and microalgae and are suitable for liquid and gaseous biofuel production (John et al., 2011). Seaweed biofuels can produce more gross energy yield per hectare than most land-based energy crops. For instance, rapeseed biodiesel (first generation) can generate 1350 L (44 GJ) of biodiesel per hectare per annum (Thamsiriroj & Murphy, 2009); willow biomethane (second generation biofuel) can generate a gross energy yield of ca. 130 GJ ha^{-1} yr^{-1} (Gallagher & Murphy, 2013). The yields of seaweeds (wet weight: wwt) per hectare are variable depending on species and growing conditions. Definitive yield data is not known or accepted yet but according to Christiansen (Christiansen, 2008), it can be up to 130 t wwt per hectare. The gross energy per hectare of seaweed biomethane can be estimated as 230 $GJ^{-1}ha^{-1}yr^{-1}$ if a specific methane yield (SMY) of 330 L CH_4kg^{-1} volatile solids (VS) is achieved (Vanegas & Bartlett, 2013b).

Ireland is an island with a temperate oceanic climate of the west coast of Europe with a very significant coastline (7500 km) allowing access to a large source of seaweed (Allen

et al., 2015). Irish brown seaweeds (such as *Laminaria digitata and Sacharina latissima*) are rich in organic matter. Existing harvesting potential of various seaweed ranges from 35 t wwt ha^{-1} yr^{-1} to 300 t wwt ha^{-1} yr^{-1} (Allen et al., 2015). Energy demand in the transport sector in Ireland is expected to be of the order of 188 PJ in 2020. According to EU target, 1.25% (2.35 PJ) should be from advanced biofuels, such as seaweed, by 2020 (European Parliment, 2015). The target may be achievable by applying innovative technologies using seaweed as an alternative substrate for gaseous fuel production.

The objective of this paper is to synthesise the literature on seaweed biomethane and assess the resource and applicability for a temperate island in the north Atlantic, Ireland. The review has an ambition of providing some clarity on classification, habitat, resource, and seasonal variation in selected seaweed species. This includes for analysis of the biomethane potential of selected seaweed species and assessment of viable biofuel technologies for these seaweeds. An integrated system coupling different substrates are proposed to overcome potential bottlenecks in the development of a bio-natural gas (BNG) market. The proposed model may facilitate coastal digesters for the BNG industry.

2.2 Seaweed as feedstock for biofuel production

2.2.1 Classification and worldwide availability

Seaweeds are multicellular photosynthetic organisms; they are classified as brown, green and red on the basis of the type of chlorophyll present in their thallus (Jard et al., 2013). Brown (such as *S. latissima*; *L. digitata*; *Ascophyllum nodosum*), green (such as *Codium tomentosum* and *Ulva lactuca*) and red seaweeds (such as *Palmaria palmate*

and *Gracilaria verrucosa*) are classified as *Phaeophyceae*, *Chlorophyceae,* and *Rhodophyceae*, respectively. The main photosynthetic pigments are chlorophyll a and c in brown seaweeds. They have plant-like structures, but they have frond, stalk, and holdfast instead of leaf, stem and root (such as Figure 1a). Green seaweeds have the same proportion of chlorophyll a to b as herbaceous land plants. They are closer to land plants as their biochemical compositions and chlorophyll type is very similar. The red colour in seaweed is due to chlorophyll a, phycoerythrin and phycocyanin (McHugh, 2003).

The marine environment, especially the absorption of light, determines the type of pigment, extent of growth and chemical composition of seaweed. Specific pigments present in various seaweeds absorb a specific wavelength of light. Temperature, salinity, nutrients and waves also affect seaweed composition (Adams et al., 2011a). Brown and green seaweeds (examples in Figure 1) are found in the littoral zone. Red seaweeds inhabit deep sea (up to 25 m below the surface) where availability of sunlight is limited (Santelices, 1991). Pigments in red seaweeds (such as phycoerythrin and phycocyanin) are capable of absorption of light in the deep sea (Shukun Yu et al., 2002).

Seaweeds are distributed worldwide. Seaweed harvest is dominated by Asia (China, Philippines, Japan, Korea and Indonesia) while in Europe, Norway is the leading producer (Roesijadi et al., 2010). Seaweeds can be naturally harvested (wild harvest) or cultivated. China harvests 3.2 and 11.2 million tonnes (wet weight) of seaweed from natural harvest and cultivation respectively (Table 1). Ireland is one of the largest producers of seaweed in Europe, producing 29,500 t per annum, of which 29,000 t per annum is *A. nodosum* (figure 1C). The seaweed industry takes place in the North West

of the country in counties Donegal and Galway (Murphy et al., 2013). This harvest is equivalent to 13% of the total European total harvest. In Ireland, *A. nodosum* is not harvested for biofuel but for food and feed (Burton, 2009). Ireland and Scotland have natural large seaweed forests consisting of brown seaweeds; these are not used for biofuel production. From an environmental perspective, these are better left in-situ. Cultivation of new seaweed is recommended for a biofuel industry; this limits the species (Jung et al., 2013).

Figure 2.1 Seaweeds found on the Irish coastline (Photograph: M.R. Tabassum).

Table 2.1 World production of wild and farm cultivated seaweed (Roesijadi et al., 2010).

Wild harvest (t wwt)			Farm cultivation (t wwt)		
Source	Production	Total (%)	Source	Production	Total (%)
World total	1,143,273	100.00	World total	15,075,612	100.00
China	323,810	28.32	China	10,867,410	72.09
Norway	145,429	12.72	Philippines	1,468,905	9.74
Japan	113,665	9.94	Indonesia	910,636	6.04
Ireland	29,500	2.58	Ireland	Techniques are at infancy	

t wwt = ton wet weight

2.2.2 Conversion technologies and fuel options

Seaweeds were first used as a source of acetone for military purposes during the First World War (Neushul, 1989). Production of gaseous biofuel from seaweed as a research topic was first considered in 1978 in the US (Chynoweth, 2002). Currently, seaweeds are utilized for human food and chemicals for industrial applications; seaweed in the food industry accounts for $5 billion per annum worldwide (Roesijadi et al., 2010). Various fuel production options from the seaweed, the process technology, and bottlenecks are summarized in Table 2.2. The gaseous biofuel options such as biomethane (Hinks et al., 2013) and bio-hydrogen (Duman et al., 2014) can be produced from seaweed. Alternatively, thermochemical conversion of seaweed may be used to produce syngas or bio-oil through gasification (Duman et al., 2014) or pyrolysis (Choi et al., 2014); however the technology readiness level (TRL) is such that these processes are not yet economically feasible.

Biomethane via anaerobic digestion (AD) would appear to be near commerciality, the main barrier is the high cost of seaweed cultivation (Burton, 2009). The technology is in infancy; biomethane production from seaweeds is proven predominately at laboratory scale. Biomethane yield is affected by species, seasonal variation and geographical location (Adams et al., 2011b). Yields can be increased by co-digestion with other substrates (Costa et al., 2012). Hydrogen can be produced through dark anaerobic fermentation and photofermentation (Panagiotopoulos et al., 2010). This is at a lower technology readiness level (TRL).

The liquid fuel options such as ethanol are still at a low TRL and far away from commercialization due to the bottlenecks in the technology (Table 2.2). Seaweeds contain carbohydrates (cellulose, starch laminarin, and agar) can be hydrolyzed into simple sugars and can be fermented into ethanol. Conversion of algal carbohydrates into ethanol requires suitable microorganisms that can ferment different sugars (such as mannitol, alginate, laminarin) into ethanol (Yanagisawa et al., 2013). Currently, scientific knowledge is limited to ferment laminarin and mannitol into ethanol (Horn et al., 2000). Molecular biology has proven a possibility to ferment all sugars present in brown seaweed into ethanol by a synthetic yeast (Enquist-Newman et al., 2014). Moreover, the drying process for the substrate is too expensive (Murphy & Thamsiriroj, 2011) while in the case of methane production (from seaweed), the drying step can be eliminated.

Table 2.2 A comparison of advanced biofuels from seaweed via 3G technology

Biofuels	Technology	Advantages	Disadvantages/Bottlenecks	Reference
Methane	AD	Mature environmental friendly technology suitable for wet feedstock. Drying of seaweed is not required for AD (low parasitic energy demand). The fuel can be blended with natural gas or hydrogen, feedstock cultivation does not require land and fresh water, abundant indigenous bio-resource, good socio-economic benefits especially in rural and coastal areas	Indigenous seaweed species are not fully characterized with respect to seasonal variation, cultivation costs of the substrate are high, high salt concentration in seaweed requires suitable pre-treatments, long-term digestion is still a question due to high ash content in the seaweeds, legislation and licencing for the substrate cultivation is still pending, government policy and legislation for the seaweed biogas industry in the country needs special attention.	(Allen et al., 2015; Murphy & Thamsiriroj, 2011; Tabassum et al., 2016a)
Hydrogen	DF	High energy value of H_2. No carbon emission on combustion. The technology is suitable for wet 3G-feedstock and can be blended with methane (termed hythane)	Salts in the seaweed adversely affect the gas yield. The yield is low and the process is energy intensive. H_2 requires high compression (700 bars) and cannot be used at low	(Murphy & Thamsiriroj, 2011; Xia et al., 2015b)

			compression (220 bars such as for methane) as a transport fuel. 13% parasitic energy demand for compression and safety issues at high compression	
Ethanol	EF	Environmental friendly 3G technology, can be blended with petrol	The substrate is partially fermentable into ethanol, hence, the yield is low, the process is considered energy intensive as a drying step is necessary.	(Horn et al., 2000; Murphy & Thamsiriroj, 2011; Yanagisawa et al., 2013)
Diesel	AD + MF	The 3G fuel can be blended with diesel without engine modification	The process is not fully understood and only tested in research laboratories	(Xu et al., 2014)
Butanol	ABE-F	Highly advanced fuel with better fuel properties than ethanol, can be blended with gasoline, infrastructure can be easily switched from gasoline to butanol.	Very low yields due to butanol toxicity for microbes, butanol is not recognized as a biofuel hence no subsidized benefits announced yet	(Huesemann et al., 2012; Potts et al., 2012)

AD: anaerobic digestion; DF: dark fermentation; EF: ethanol fermentation; MF: microbial fermentation; ABE-F: acetone-butanol-ethanol fermentation, 3G: third generation.

Algal biomass can be converted chemically into biodiesel through transesterification. In the presence of a catalyst, triglycerides in algal biomass react with methanol to give glycerol and methyl esters (termed biodiesel) (Chisti, 2007). Biologically, biodiesel can be produced from seaweed via AD and fermentation. In the first stage, seaweed is digested anaerobically to produce volatile fatty acids (VFAs); these VFAs are converted to biodiesel through fermentation using a yeast *Cryptococcus curvatus* (Xu et al., 2014). Biobutanol can be produced from seaweeds (through acetone-butanol fermentation) using anaerobic bacteria *Clostridium* sp. These species are capable of producing acetone, butanol, ethanol and organic acids from various substrates but are unable to effectively consume mannitol from brown seaweed (Huesemann et al., 2012). An attempt was made to convert the green seaweed *U. lactuca* to butanol using *Clostridium* sp. through acetone butanol ethanol (ABE) fermentation; the efficiency was greater than was expected indicating that other sugars were also fermented (Potts et al., 2012). Nevertheless, butanol production from seaweed is not a mature technology.

2.3 Anaerobic digestion: A viable technology for the seaweed biofuel

2.3.1 The digestion process

The digestion process is carried out by a complex microbial consortium that can be divided into four steps (Figure 2.2). The first step (hydrolysis) is the breakdown of complex organic macromolecules (carbohydrates, proteins, and lipids) into simple molecules (sugars, amino acids, and fatty acids) by hydrolytic microorganisms. In the second step (acidogenesis), the monomers are converted to gaseous metabolic products (such as H_2 and CO_2) and soluble metabolic products (VFAs and alcohols) through acidogenic microorganisms. Intermediate products from the previous step are converted

into acetic acid and hydrogen by the acetogenic microbial consortium. Finally, hydrogenotrophic methanogenic archaea convert hydrogen and carbon dioxide into methane while acetoclastic methanogenic archaea utilize acetate for methane production (Xia et al., 2015a).

The optimized process performance depends on upon the balanced activity of the each set of microbial consortia. For instance, if one stage (such as acidogenesis) works too fast the other stage (such as methanogenesis) becomes the rate limiting and vice versa (Weiland, 2010). The disturbance in this cooperation results in a drop in pH due to the accumulation of VFAs and ultimately the process either slows down or totally stops producing methane through inhibition of the methanogens. Slowly degrading substrates (such as cellulose, fats, and proteins) can make hydrolysis rate limiting (Weiland, 2010). Pre-treatment technologies facilitate the conversion of complex substrates into simpler monomers reducing the tendency of hydrolysis to be the rate-limiting stage. Brown seaweeds have slowly degradable compounds (alginates and laminarin) (Adams et al., 2011b) that may be responsible for making hydrolysis the rate-limiting step and should be the subject of suitable pre-treatment for conversion into monomers.

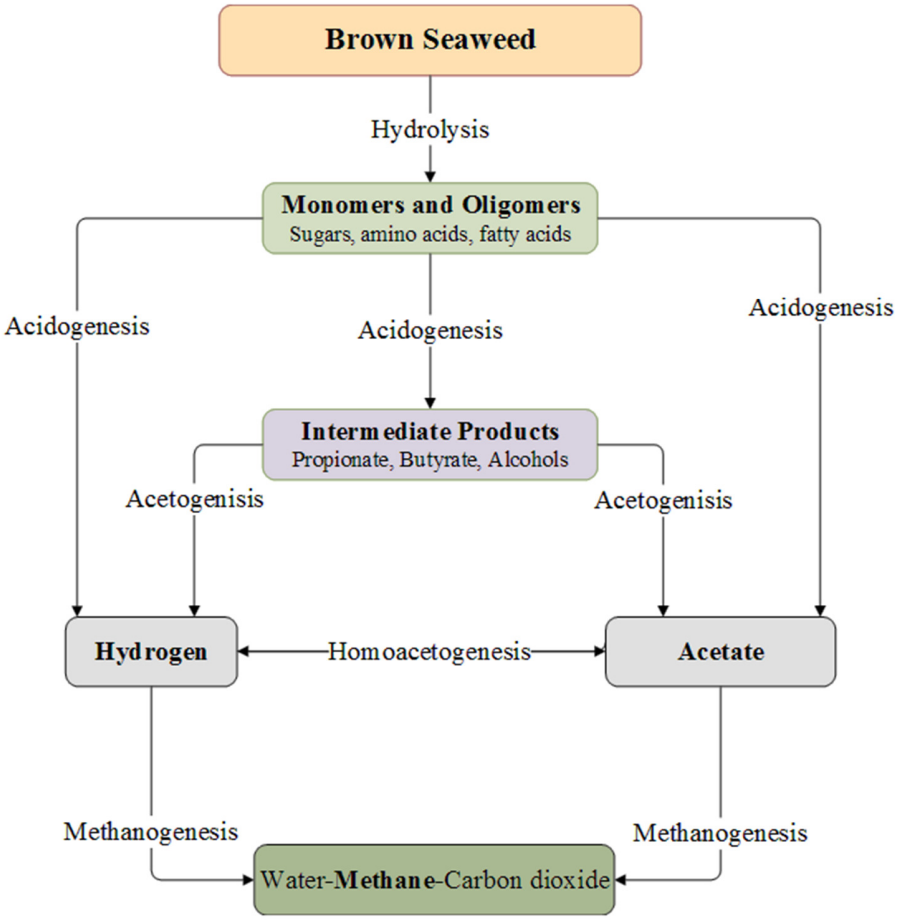

Figure 2.2 The anaerobic digestion process (adapted from (Xia et al., 2015a))

2.3.2 The key operational parameters

Hydraulic retention time (HRT) and organic loading rate (OLR) are the key parameters that can affect the gas yield. Temperature, pH, and micronutrients (trace elements) also affect the gas production significantly. Before setting up an industrial digester for biogas production, these parameters must be optimized for maximum biogas efficiency (Dinopoulou et al., 1988; Kinnunen et al., 2014; Sialve et al., 2009). HRT is equivalent to the capacity of the digester (m^3) divided by the volume of the fresh substrate added

per day (m^3d^{-1}). OLR can be started from a low value (such as 1 kg VS m^{-3} d^{-1}) and stepped up incrementally to the maximum value for optimization. Higher OLRs and shorter HRTs ultimately led to a decrease in the biodegradability achieved due to wash out of the microbial community from the digester and decrease in SMY (Dareioti & Kornaros, 2015). Seaweed has slowly degradable organic compounds (Adams et al., 2011a) that may require longer retention times and lower OLRs; however, due to seasonal variation (Tabassum et al., 2016a) this general principle may deviate.

The temperature for biomethane production (natural environmental) ranges from 0^0C to 97^0C (the lowest in ice fields and the highest in hot springs). Mesophilic (35-45^0C) and thermophilic (45-60^0C) temperatures have been studied for anaerobic digestion but the mesophilic range has been preferred due to low energy input (Kashyap et al., 2003; Wall et al., 2014b). However, thermophilic temperatures can result in faster hydrolysis and fermentation processes requiring shorter HRTs and smaller digester volumes than mesophilic conditions (Moset et al., 2015; Weiland, 2010).

It has been reported that along with macronutrients (such as nitrogen, phosphorus, potassium, calcium, magnesium, sodium), trace elements (such as cobalt, iron, molybdenum, nickel, selenium) are also critical for the stable and optimum performance of the process especially in mono-digestion systems (Demirel & Scherer, 2011; Wall et al., 2014a). These nutrients can be directly added to the digester or in co-digestion with mixed substrates (such as slurries and various kinds of wastes) and it was found that significantly more biogas can be generated by adding cobalt and nickel to mono-digestion of energy crops (Demirel & Scherer, 2011; Wall et al., 2014a).

2.3.3 Inhibition and control

Microbial activity during AD is adversely affected by various inhibitors such as ammonia, sulphide, metals and polyphenols (Chen et al., 2014a). Proteins, nucleic acids, and urea are converted into ammonia and provide buffer capacity, which can stabilize the process (González-Fernández & García-Encina, 2009). Elevated levels of nitrogen inhibit methanogens and the associated accumulation of VFAs lead to failure of the process. Unionized ammonia causes proton imbalance (potassium deficiency) while ionized ammonia directly inhibits enzymes that produce methane (Chen et al., 2008). Total ammonical nitrogen (TAN) is an important indicator of ammonia inhibition level. Ammonia inhibition can be controlled by adjusting the C:N ratio (in the range 20-30) (Montingelli et al., 2015) by adding glycerol (Resch et al., 2011) or co-digesting with substrates with higher C:N values (Wang et al., 2012; Zhong et al., 2012). It has been reported that TAN values in excess of 5 g L^{-1} are deemed to be in an inhibitory range (Allen et al., 2014).

Sodium and chloride may also be the major concern during anaerobic digestion of seaweed (Allen et al., 2014; Tabassum et al., 2016a). Sodium ions at low levels (350 mg L^{-1}) are required for cellular operations but show inhibitory effects at high levels (5-8 g L^{-1}) (Chen et al., 2008). Chloride levels have been reported inhibitory in the range 5-20 g L^{-1} (Lefebvre et al., 2007; Riffat & Krongthamchat, 2005). Acclimatized inoculum or inoculum sourced from the marine environment can stabilize the process (Aspé et al., 1997; Lefebvre et al., 2007; Riffat & Krongthamchat, 2005). Alternatively, application of suitable pre-treatment to remove the salts from the substrate can be a remedy for the accumulation of salts in the reactor (Tabassum et al., 2016a).

2.4 Bottlenecks in the technology

2.4.1 Characterization and assessment of biomethane potential

The first bottleneck for establishing a seaweed based biogas industry is the lack of knowledge of characterization and biomethane potential (based on seasonal variation) of the selected seaweeds. Studies on selected seaweeds indicated that due to seasonal variation in the chemical composition of the feedstock, the BMP greatly varies. In this section, the chemical composition of brown seaweed with reference to seasonal variation is discussed and biomethane potential of various seaweed species is gathered. Ultimate and proximate analysis may be assessed by well-known laboratory assessments (APHA, 2011). Such an analysis allows generation of a stoichiometric equation and hence theoretical methane potential (TMP). Allen et al. evaluated *Ulva* at 25% Carbon, 3.7% Hydrogen, 27.5% Oxygen and 3.3% Nitrogen. These values generated a TMP of 431 L CH_4 kg^{-1} VS at 51.5% methane content in biogas (Allen et al., 2015). Values for Irish cast seaweeds collected in 2013 are outlined in Table 2.3. The C:N ratio of the seaweeds were above 15 for all samples except *U. lactuca* (green seaweed). Many were close to the optimum range for AD (20 to 30:1). Biomethane potential is correlated to the chemical composition of seaweed, which varies with season (Adams et al., 2011b). The greater VS (carbohydrates, proteins, and lipids) in the seaweed, the greater biomethane production potential is expected. The chemical composition of representative seaweeds is listed in Table 2.4.

Table 2.3 Characteristics of natural Irish seaweeds (adapted from (Allen et al., 2015))

Seaweed	Proximate Analysis			Ultimate Analysis				
	TS (% of wwt)	VS (% of wwt)	Ash (% of TS)	C (% of TS)	H (% of TS)	N (% of TS)	O (% of TS)	C:N
A. nodosum	23.2	19.4	16.1	40.4	5.3	1.6	36.6	26.0
S. latissima	15.49	10.09	34.9	29.1	3.8	1.2	31.0	24.0
S. polyschides	15.25	13.11	14.0	36.1	5.0	1.6	44.3	23.2
L. digitata	14.20	10.34	27.2	34.2	4.8	1.5	32.3	22.3
H. elongate	12.65	8.10	36.0	30.8	4.1	1.4	27.7	21.4
F. vesiculosus	21.18	16.11	24.0	26.8	3.2	1.5	44.5	17.6
F. spiralis	19.72	13.92	29.4	36.1	4.7	2.1	27.7	17.3
F. serratus	20.07	14.74	26.6	37.1	4.8	2.4	29.1	15.5
A. esculenta	18.72	11.91	36.4	29.3	4.2	1.9	28.2	15.5
U. lactuca	18.03	10.88	39.7	30.0	4.4	3.5	22.4	8.5

Table 2.4 Chemical composition of seaweeds

Composition	*Gelidium amansii* (Red) (Yoon, 2010)	*Ulva lactuca* (Green) (Kim et al., 2011)	*Laminaria* (Brown) (Kim et al., 2011)
Carbohydrates (%)	75.2	54.3	51.9
Protein (%)	18.5	20.6	14.8
Lipids (%)	0.6	6.2	1.8
Ash (%)	5.7	18.9	31.5

Theoretical methane potential (TMP) can be calculated by data obtained from proximate and ultimate analysis and use of the Buswell equation (Eq. (1)) (Buswell & Mueller, 1952).

$$C_n H_a O_b + \left(n - \frac{a}{4} - \frac{b}{2}\right) H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right) CO_2 \quad \text{Eq. (1)}$$

The Buswell yield is a theoretical calculation that does not take into account the maintenance and anabolism of the microbial community. Therefore, an overestimation of biomethane yields occur. Biomethane potential (BMP) of seaweed is the experimental methane yield from a batch process under ideal conditions expressed in litres of methane per kilogram of volatile solids (L CH_4 kg VS^{-1}). The biodegradability index (BI) indicates the efficiency of biomass degradation. It is calculated by dividing BMP by TMP; the greater the index, the higher the digestion efficiency. In a study by Allen et al., (Allen et al., 2015) *S. latissima* was the highest biomethane yielding seaweed with the highest biodegradability index while *F. serratus* was the lowest in an analysis of Irish cast seaweeds collected in summer 2013 (Table 2.5).

Brown seaweeds, such as *L. digitata and S. latissima*, have high biomethane yields (Table 2.5). In Wales, the harvest of *L. digitata* in July provided the highest yield of biomethane (Figure 2.3). In July the seaweed had the highest proportions of carbohydrate and the lowest alkali metal and ash content (Adams et al., 2011a). Similar results were confirmed by Schiener et al. (Schiener et al., 2015) but not for year round harvest as was reported by Adams et al (Adams et al., 2011a).

Table 2.5 Biomethane potential of Irish seaweeds (adapted from (Allen et al., 2015))

Seaweed	BMP ($L\ CH_4\ kg\ VS^{-1}$)	TMP ($L\ CH_4\ kg\ VS^{-1}$)	BI	Methane potential ($m^3\ CH_4\ t^{-1}$ wwt)
S. latissima	342	422	0.81	34.5
S. polyschides	263	386	0.68	34.5
F. spiralis	235	540	0.44	32.7
A. nodosum	166	488	0.34	32.3
A. esculenta	226	474	0.48	26.9
L. digitata	218	479	0.46	22.5
H. elongate	260	334	0.78	21.1
F. vesiclosus	126	249	0.51	19.4
F. serratus	101	532	0.19	13.5
U. lactuca	190	465	0.41	20.9

BMP, Biomethane potential; TMP, Theoretical methane potential; BI, Biodegradability index; wwt, wet weight

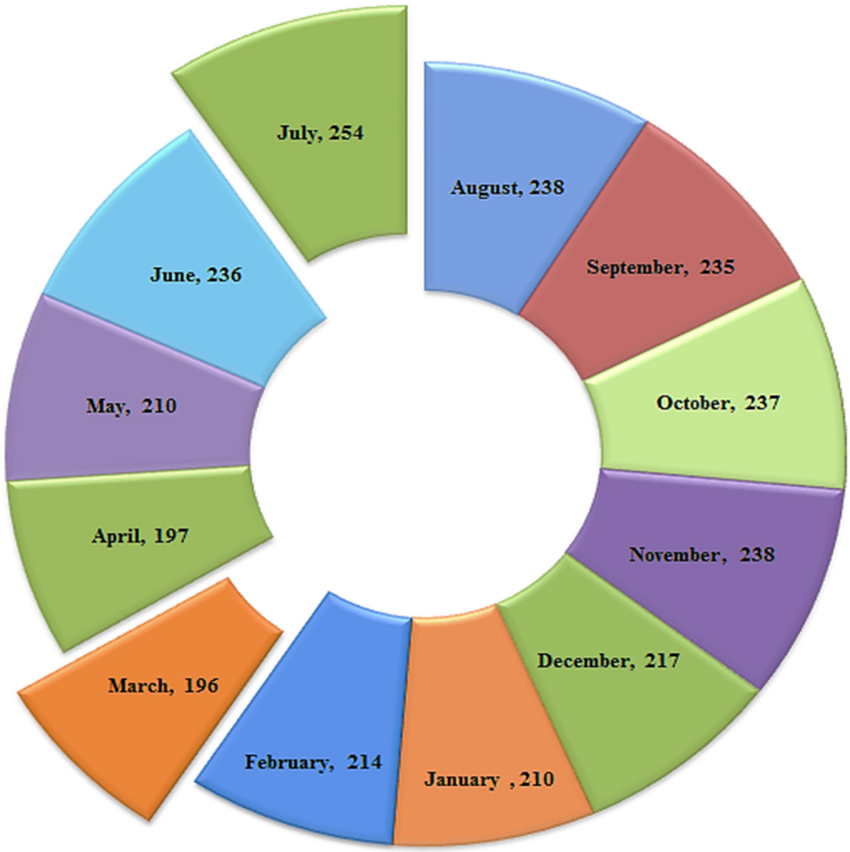

Figure 2.3 Seasonal variation in biomethane potential (L CH_4 kg VS^{-1}) of *L. digitata* (data collected from (Adams et al., 2011b))

2.4.2 Cultivation and harvesting cost

The cost of seaweed biomass is the key bottleneck in the technology. Seaweed can be harvested manually from beaches or cultivated at the farm scale. *A. nodosum* is reported as costing €330 dry t^{-1} in Ireland (Burton, 2009) when harvested off beaches. This expense may be associated with the small beaches, narrow roads between the beaches, and intensive use of manual labour, which is expensive in Ireland. The cost of *A. nodosum* may be compared with another AD substrate grass silage of €79 dry t^{-1} (Smyth et al., 2010). Cultivation is widely used in Asia (Table 2.1) but not in Ireland. This may

be associated with stricter planning legislation and requirement for environmental impact assessment. The cost per tonne of cultivated seaweed in Ireland is not known. The reported resource available for harvest in beaches in high concentrations of *U. lactuca* in Lannion Bay, Brittany, France is 100,000 t wwt yr^{-1} (Charlier et al., 2007) while, in West Cork, 10,000 t wwt yr^{-1} is cleared off one beach each year; this will be the cheapest source of seaweed for biogas production; but as noted is difficult to digest due to low C:N ratios and high levels of sulphur.

Seaweed cultivation is dominated by growing on ropes (or lines) with separation to allow boat travel between lines for harvest. If for example, 20 kg wet weight is produced per meter and the lines are at 5 m intervals then the maximum production per hectare (100 m by 100 m) is 40 t wet weight. Assuming a total solids content of 15% this equates to 6 t TS ha^{-1} yr^{-1} (Murphy et al., 2015). Brown seaweed can be cultivated with existing aquaculture systems. Integrated multi-trophic aquaculture concepts allow co-production of finfish, shellfish, and seaweed. The shellfish and seaweed can utilise the particulate and soluble nutrient discharge respectively, from the larger fish species reducing pollution load and associated eutrophication in the receiving waters. This may reduce the cost of production of seaweed. Jacob et al. suggested an EU industry of 2600 anaerobic digesters treating 168 Mt of seaweed per year (associated with fish farms) if advanced biofuels from seaweed are to satisfy 1.25% of the EU 2020 transport energy demand (Jacob et al., 2016). For Ireland alone, 2.7 Mt of brown seaweed would need to be digested in 41 anaerobic digesters, each treating 64,500 t wwt of seaweed per annum (Jacob et al., 2016). However, huge financial costs will be required in the development and maintenance of a seaweed farm. Alvarado-Morales et al. (Alvarado-Morales, 2013)

found that in Nordic conditions, the production phase was the most energy intensive in the life cycle analysis of a seaweed biofuel project, demanding 57% of energy input. Norway, France, and Denmark are the countries where seaweed cultivation is being studied at large scale; it is reported that a cost of seaweed of €20-21 t^{-1} wet weight is achievable (Jorunn et al., 2014; Karin et al., 2013).

2.4.3 Pre-treatment technologies

Salt accumulation in the digester over time (Tabassum et al., 2016a) may be another concern to establish long-term anaerobic digestion; the high ash content in the seaweed is associated with salt (Table 2.3). It requires suitable pre-treatment for salt removal with minimum material losses. Removal of salts may enhance biodegradability to give better biomethane yields. Biodegradability index of seaweeds (Table 2.5) are in the range of 0.19 (*F. serratus*) to 0.81 (*S. lattissima*) (Figure 2.1d and 2.1b respectively). Pre-treatments can increase digestibility. Intuitively, due to the absence of cellulose and lignin, harsh pre-treatment should not be required.

The literature outlines biomass pre-treatments such as: physical (Oliveira et al., 2014); mechanical (size reduction by cutting, chopping, maceration (Nielsen, 2011) and milling (Zhang, 2014)); biological (hydrolysis by enzymes (Borines et al., 2013)); chemical (hydrolysis by acid or alkali ((Borines et al., 2013; Oliveira et al., 2014)); thermal (heating (Passos & Ferrer, 2014) and steam explosion (Schultz-Jensen et al., 2013)); and hydrolysis by chemo-thermal methods (Oliveira et al., 2014). Size reduction (from a particle size of 1mm to 4mm) of dried seaweed was reported as a significant enhancer of biogas production from brown seaweeds (Vanegas et al., 2015), however, drying is considered to be energy intensive process (Alvarado-Morales, 2013). Mechanical pre-

treatments have proved most beneficial in pre-treatment of brown seaweed from Dublin, Ireland (Tedesco et al., 2013). In Denmark, maceration increased the BMP yield up to 56% when compared to untreated green seaweed (Bruhn, 2011). Washing improves specific methane production yield due to the removal of impurities or potential inhibitory compounds (Oliveira et al., 2014).

Thermochemical pretreatment was shown to have no positive effect on BMP, possibly due to the release of polyphenols and other toxic compounds on heating the substrate (Oliveira et al., 2014). Integrated storage of wheat straw at low temperature with bio-preservative yeast increased the biodegradability of the substrate and finally gave a higher ethanol yield on fermentation (Passoth et al., 2013). Seaweed can be stored at low temperature with the natural microbial community present on the substrate by using the same concept (Passoth et al., 2013) to study the effect of marine microbial community on the degradability of seaweed and ultimately the gas yield. Washing the seaweed with hot water may remove salts and impurities but the organic matter may be lost. Therefore, optimization of pre-treatment time and temperature may facilitate the removal of salts with minimum organic matter losses. Pre-treatments of different seaweeds and associated biogas yield are listed in Table 2.6.

Table 2.6 Effect of pre-treatment on biomethane potential of seaweed

Seaweed	Pre-treatment	BMP Before/after	Increase (%)	Country	Reference
U. lactuca	Untreated/macerated	174/271	56	Denmark	(Bruhn, 2011)
L. digitata	Untreated/Mechanical	103/156	51	Ireland	(Tedesco et al., 2013)
U. lactuca	Fresh/dried	183/250	37	Ireland	(Allen et al., 2013)
U. lactuca	Fresh/Macerated	190/250	33	Ireland	(Allen et al., 2015)
U. lactuca	Fresh/ Wilted	183/226	23	Ireland	(Allen et al., 2013)
U. lactuca	Untreated	128	-	France	(Peu et al., 2011)
U. lactuca	Washed/Milled	191	-	Ireland	(Vanegas & Bartlett, 2013b)
U. lactuca	Washed/Chopped	241	-	France	(Jard et al., 2013)
Gracilaria sp.	Washed/Macerated	481	-	Portugal	(Oliveira et al., 2014)
P. palmate	Dried and chopped	312	-	France	(Jard et al., 2012)
S. latissima	Steam explosion	268	-	Norway	(Vivekanand et al., 2012)
S. latissima	Dried and chopped	266	-	France	(Jard et al., 2012)
S. latissima	Washed/Wilted	335	-	Ireland	(Vanegas & Bartlett, 2013b)
L. digitata	Washed/Wilted	246	-	Ireland	(Vanegas & Bartlett, 2013b)

2.4.4 Digestion strategies

Application and feasibility of long-term anaerobic digestion technology have not been demonstrated for seaweeds to date and both mono- and co-digestion strategies may be investigated. There is limited literature available on mono-digestion of seaweeds in continuous processes. The one-stage CSTR system is probably the most simple and technical viability process for digestion of wet substrates (Nizami & Murphy, 2010). Allen et al. (Allen et al., 2014) compared mono and co-digestion of *Ulva* sp. in a CSTR; they did not recommend mono-digestion due to a low C:N ratio, high levels of VFA accumulation, high calcium and chloride concentrations and low levels of Selenium. Co-digestion of the seaweed with dairy slurry (25% seaweed: 75% slurry on a VS basis) gave significantly better results than mono-digestion (Allen et al., 2014).

The literature reports co-digestion of seaweed with a variety of substrates such as glycerol (Oliveira et al., 2014), bovine slurry (Vanegas & Bartlett, 2013b), wheat straw (Nkemka & Murto, 2013), cattle manure (Sarker et al., 2014), waste milk (Matsui & Koike, 2010), waste frying oil (Oliveira et al., 2015) and dairy slurry (Allen et al., 2014). Co-digestion of *G. vermiculophylla* with 2% glycerol increased the BMP by 18% (Oliveira et al., 2014). Brown seaweeds, *Saccorhiza polyschides, L. digitata, F. serratus* and *S. latissima*, were co-digested individually with bovine slurry (Vanegas & Bartlett, 2013b). S. *latissima* and *S. polyschides* were found to be the best seaweeds yielding 335 L CH_4 kg VS^{-1} and 255 L CH_4 kg VS^{-1}, respectively (Vanegas & Bartlett, 2013b). Co-digestion of seaweed and wheat straw was improved by 57% when straw was pretreated with enzymes and steam (Nkemka & Murto, 2013). Allen and co-workers (Allen et al., 2014) in co-digesting *U. lactuca* with dairy slurry found that mixes with

increased levels of dairy slurry and higher C:N ratios performed better. Reactor failure was observed when OLR was in excess of 2 kg VS m^{-3} d^{-1}.

Sarker and co-workers (Sarker et al., 2014) also found that high levels of VFA accumulation occurred when the reactor was loaded with increased level of *L. digitata* when co-digested with cattle manure; specific methane yields were lower than theoretical expectation. The same trend was reported by Hinks and co-workers (Hinks et al., 2013) in the continuous digestion of *L. hyperborea* at an OLR of 2 kg^{-1} VS m^{-3} d^{-1}.

Production of H_2S can be another concern in the long-term digestion of seaweed species with high sulphur content such as *U. lactuca*. Peu and co-workers reported that if the C:S ratio is less than 40, H_2S production will be significant in the biogas and may be at a level to cause a problem in digestion (Peu et al., 2012).

Brown algae like *S. latissima* and *L. digitata* may also produce H_2S; the C:S ratio for *S. latissima* was recorded as 24:1 (Jard et al., 2013) while for *L. digitata*, the values were recorded in the range 29 - 60.3 (Adams et al., 2011a). Matsui and Koike (Matsui & Koike, 2010) recommended co-digestion of a mixture of seaweeds (green and brown) with waste milk. Oliveira and co-workers (Oliveira et al., 2015) co-digested *Sargassum* sp. with glycerol and waste frying oil; an increase in gas production was obtained of 56% and 46%, respectively as compared to mono-digestion

2.5 Irish seaweed biogas industry: From sea to fuel

2.5.1 Potential seaweed species

Assessment of the potential seaweed species is the first step to develop the biogas industry in the country. The literature provides a wide range of BMP results with significant differences between species in Ireland. The best yielding Irish seaweed in a study by Allen and co-workers was *S. latissima* (Allen et al., 2015) with a BMP value of 341.7 $\pm$ 36.4 L CH_4 kg VS^{-1}; this is comparable to a value from Denmark of 333 $\pm$ 64.1 L CH_4 kg VS^{-1} (Nielsen, 2011) but a lower value was reported in Norway (223 $\pm$ 61 L CH_4 kg VS^{-1}) for *S. latissima*, harvested in August (Vivekanand et al., 2012). Carbohydrate content (alginic acid) in the seaweed is an important parameter in the BMP; higher quantities yield higher BMPs. *L. digitata* has been extensively reported as a potential biofuel feedstock due to the presence of easily fermentable sugars (laminarin and mannitol content can achieve 30% and 25% of TS, respectively) (Adams et al., 2011b). The C:N ratio of *L. digitata* harvested in Wales fluctuated from 10.9 (in January) to a peak of 31.9 (in August) (Adams et al., 2011a). *L. digitata* collected in August from West Cork (Allen et al., 2015) with a C:N ratio of 22.5 generated a BMP yield of 218.0 $\pm$ 4.1 L CH_4 kg VS^{-1} which was higher than *L. digitata* harvested in May (184 L CH_4 kg VS^{-1}) (Vanegas & Bartlett, 2013a) and in January (103.3 $\pm$ 19.8 L CH_4 kg VS^{-1}) (Tedesco et al., 2013).

The most abundant seaweed on Irish and Nordic coastlines is *Ascophyllum nodosum; A. nodosum* contains up to 33% of TS as degradable carbohydrates (Moen et al., 1997). High concentrations of degradable carbohydrates would suggest a good candidate for AD. However, unfortunately, it contains high levels of polyphenols (up to 14% of TS),

which are natural inhibitors of the AD process (Ragan & Jensen, 1978). A BMP yield of 166.3 $\pm$ 20 L CH_4 kg VS^{-1} and 110 L CH_4 kg VS^{-1} was reported in Ireland (Allen et al., 2015) and Norway (Hanssen et al., 1987), respectively. High polyphenol content leads to low biodegradability index (Table 4: BI = 0.34) and low kinetic decay values. This, however, would make *A. nodosum* a very good candidate for pre-treatment processes and assessment of seasonal variation. Some studies indicated that the polyphenol level in the seaweed changes during the year (Apostolidis et al., 2011; Tabassum et al., 2016b). Research is required to assess best harvesting month for *A. nodosum* and a suitable pre-treatment to enhance biomethane potential.

S. polyschides is another species that contains high alginate concentrations (up to 16% TS) (Jard et al., 2013). A BMP yield of 263.3 $\pm$ 4.2 L CH_4 kg VS^{-1} (Allen et al., 2015) and 255 L CH_4 kg VS^{-1} (Vanegas & Bartlett, 2013b) were reported from Ireland while a lower yield (216 $\pm$ 16 L CH_4 kg VS^{-1} in July) was obtained in France (Jard et al., 2013). *Laminaria hyperborea* and *Laminaria japonica* have the potential to be harvested at large scales due to high biomethane potentials of 260 L CH_4 kg VS^{-1} (Hinks et al., 2013) and 260-280 L CH_4 kg VS^{-1} (Chynoweth et al., 1993) respectively. Moreover, *Macrocystis pyrifera* can also be considered for farm cultivation due to its maximum growth size (up to 43 meters in length) and high growth rate (Chynoweth, 2002). High concentrations of organic content (mannitol levels of 5-16% of TS; alginate levels of 13-24% of TS) make it a good substrate for AD (Chynoweth, 2002). Low C:N values (11.7 – 17.5) can be a problem at high loading rate in continuous digestion but this can be overcome through co-digestion with other substrates such as *L. digitata*. The optimum growing temperatures (13 – 15°C) of *M. pyrifera* (Wheeler & North, 1981) make it a

suitable seaweed to cultivate in Irish waters during the months of June to October. In terms of natural stocks, the cheapest resource may be from algae blooms of the green seaweed *U. lactuca*. These blooms accumulate over the summer period and spoil the amenity of beaches (Allen et al., 2013). In France, it has become problematic for both shellfish production and amenity of beaches. *U. lactuca* contains a negligible amount of lignin (< 0.03 g kg^{-1}), high glucose concentrations (Ventura & Castañón, 1998) and high sulphur content (Hinks et al., 2013). BMP yield of the seaweed was 190.1 $\pm$ 3.1 L CH_4 kg VS^{-1} and 200 L CH_4 kg VS^{-1} as reported by Irish (Allen et al., 2015) and Danish researchers (Bruhn, 2011).

2.5.2 Energy yields of methane and the 2020 EU target

It was estimated that one beach in West Cork generating 10,000 t wwt yr^{-1} of *U. lactuca* has the potential to yield sufficient biomethane to fuel 264 cars each year (Allen et al., 2013). Smyth et al. reported the gross energy per hectare for a range of terrestrial crops (Smyth et al., 2009). Palm oil biodiesel (120 GJ ha^{-1} yr^{-1}) and sugarcane ethanol (135 GJ ha^{-1} yr^{-1}) were the best energy crops for liquid biofuel system. Maize and perennial ryegrass have biomethane yields (1,660 - 12,250 and 2,682 - 6,400 m^3 ha^{-1} yr^{-1} respectively) (Murphy et al., 2012); this equates to a yield in the range 60 to 441 GJ ha^{-1} yr^{-1}. Seaweeds may offer gross energy yields in the range 38-384 GJ ha^{-1} yr^{-1} (Table 2.7). The total coastal area required for brown seaweed species cultivation to satisfy the 2020 RES-T target (1.25% of energy in transport or 2.35 PJ) for Ireland is outlined in Table 7. Two scenarios are chosen. Scenario (1): a realistic current based on 20 kg wet weight per meter line with lines at 5 m c/c (40 t wet weight ha^{-1} y^{-1}) and scenario (2); optimistic future based on data from Allen et al., (Allen et al., 2015). The range outlines

the importance of developing techniques that allow high yields. The potential for sunlight to allow growth at such high intensities must be considered and the effect of the shadowing on the seabed below. A salmon farm (on 454 hectares) is planned in Galway by the Irish Sea Fisheries Board. There is potential for co-location of seaweed cultivation along with salmon and shellfish farms at a further 46 sites in Ireland (BIM, 2012).

Table 2.7 Energy yields of Irish brown seaweed based on BMP

Seaweed	BMP (L CH_4 kg VS^{-1})	Production (t wwt ha^{-1} yr^{-1})	Area Required for 2020 RES-T Target (Ha)	Gross Energy GJ ha^{-1} yr^{-1}
Scenario 1: Based on realistic current harvesting potential				
S. latissima	342 [b]	40 [d]	45,470 [e]	52 [e]
S. polyschides	263 [b]	40 [d]	45,105 [e]	52 [e]
A. esculenta	226 [b]	40 [d]	57,774 [e]	41 [e]
L. digitata	254 [a]	40 [d]	61,225 [e]	38 [e]
L. hyperborean	253 [c]	40 [d]	61,465 [e]	38 [e]
Scenario 2: Based on optimistic current harvesting potential				
S. latissima	342 [b]	297 [b]	6,124 [e]	384 [e]
S. polyschides	263 [b]	147 [b]	12,273 [e]	191 [e]
A. esculenta	226 [b]	302 [b]	7,655 [e]	307 [e]
L. digitata	254 [a]	100 [b]	24,490 [e]	96 [e]
L. hyperborean	253 [c]	100 [b]	24,586 [e]	96 [e]

[a] (Adams et al., 2011b); [b] (Allen et al., 2015); [c] (Sutherland & Varela, 2014); [d] (Murphy & Herrmann, 2015); [e] Authors calculated based on BMP; VS and t wwt ha^{-1} $year^{-1}$.

2.5.3 Economics and biogas market development in Ireland

After a detailed analysis of the bottlenecks in the technology, it is obvious that establishment of the bio-natural gas market is still some way from commercial reality. However, by addressing key issues (especially the cost of the seaweeds), there is a potential to establish a seaweed biogas industry in Ireland. A comparison of the second generation (2G) and third-generation (3G) technologies is given in Table 8. Grass and seaweed are taken as representative of each class. Price calculations for the seaweed biomethane listed in the table are only for future predictions as currently, there is no data available on the topic for Ireland.

Grass and seaweeds are abundant in Ireland. Anaerobic digestion of grass silage at a yield of 55 t ha^{-1} yr^{-1} wwt in co-digestion with slurry at a mix of 50:50 on a VS basis would require 42,403 hectares (1.1% of grassland) to satisfy the EU 2020 target for RES-T (Wall et al., 2013). It was reported that this mix would yield 52m^3 CH_4 t^{-1},which is similar to seaweed yields at the optimum month for harvest (Table 2.8). Currently, the cost of cultivation for the seaweeds is very high but it is reported that the cost of seaweed may reduce to around 21 € t^{-1} wwt in the future (Jorunn et al., 2014; Karin et al., 2013). A yield of 40 - 100 t ha^{-1} yr^{-1} wet weight can be obtained by cultivating and harvesting at peak month (August). This could generate a maximum of 53 m^3 CH_4 t^{-1} [39]; this would suggest a requirement of 11,800 hectares of the coastal area to satisfy 1.25% RES-T (2020 EU target). For a simple comparison (if somewhat liberal) if the cost of seaweed cultivation and harvest is assumed to be the same as grass silage in the future (at 17 € t^{-1} wwt) and that seaweed yields an SMY of 53 m^3 CH_4 t^{-1} wwt (very similar to grass and slurry mix at 50:50 VS of 52 m^3 CH_4 t^{-1} wwt) the price of

Table 2.8. Price comparison of future gaseous fuel (BNG) from 2G and 3G technologies

Technology	Feedstock	Feedstock yield ($t\ ha^{-1}\ yr^{-1}$ wwt)	Area required to satisfy 2020 RES-T (ha)	Specific methane yields [a] ($m^3\ CH_4\ t^{-1}$)	Blend F:S ratio	Specific methane yield [b] $m^3\ CH_4\ t^{-1}$	Fuel Price (CNG) (€ m^{-3})	Fuel Price (BNG) (€ m^{-3})
2G	Grass	55 [c]	42,403 [c]	107 [c]	50:50 [c]	52 [c]	0.71 [d]	1.28 [d]
3G	Seaweed	100 [e]	11,800 [e]	53 [e]	100:0 [e]	53 [e]	0.71 [d]	1.26 [f]

2G: second generation; 3G: third generation; wwt: wet weight; RES-T: renewable energy system in transport; F:S ratio: feedstock to slurry ratio; CNG: compressed natural gas; BNG: bio-natural gas.

[a] Specific methane yield was obtained from digesting 100% grass or 100% seaweed (without blending with slurry); [b] specific methane yield was obtained from blending with slurry (only for grass) but digesting 100% seaweed; [c] (Wall et al., 2013); [d] (Browne et al., 2011); [e] (Tabassum et al., 2016a); [f] calculated by assuming that if cost of the feedstock and specific methane yield generated by the seaweed equals to grass, the predicted market price for BNG from the seaweed may be achievable in future.

biomethane (BNG) may be assessed as practically the same as from grass and slurry (Table 8). If the price of seaweed cultivation and harvest exceeds € 17 t^{-1} then it will be more expensive that grass biomethane.

According to Brown and co-workers [117], the price of CNG in the UK was of the order of € 0.71 m^{-3} in 2011. CNG is cheaper than petrol and diesel and as such blending of biomethane with natural gas can offer lower market price than petroleum and diesel (price range: 130-145 c L^{-1}). The blend of 10% to 50% of BNG (from seaweed or from grass silage and slurry) with natural gas could be pricing at a range from 0.77 € m^{-3} to 0.99 € m^{-3}, respectively. Currently, CNG vehicles and service stations are not readily available in Ireland. Government policy to encourage BNG in the country could lead to 3G biofuels based on seaweed.

2.6 Integrated system of bio-natural gas production

A four stage integrated system (including for cultivation, analysis, pre-treatment and the digestion) is proposed to address the key bottlenecks in the technology (Figure 2.4). The integrated system facilitates two routes of cultivation of the seaweed. First is mono-cultivation (CUL), where only seaweed may be cultivated. The second route integrated multi-trophic aquaculture (IMTA) allows co-cultivation of the seaweed with fish. The analysis before and after cultivation allows decisions on the co-digestion strategy and process optimization. Co-digestion strategies may attract substrates with gate fees. Seaweed can be mono-digested or co-digested with other substrates depending on the composition of the feedstock and the system requirements. Pre-treatment options (as discussed in section 2.4.3) can enhance biomethane yield by removing inhibitory compounds including salts and increasing the digestibility in the reactor. The produced

biomethane can be injected into the gas grid or used for power generation (P), domestic heating (H) and/or advanced transport biofuel.

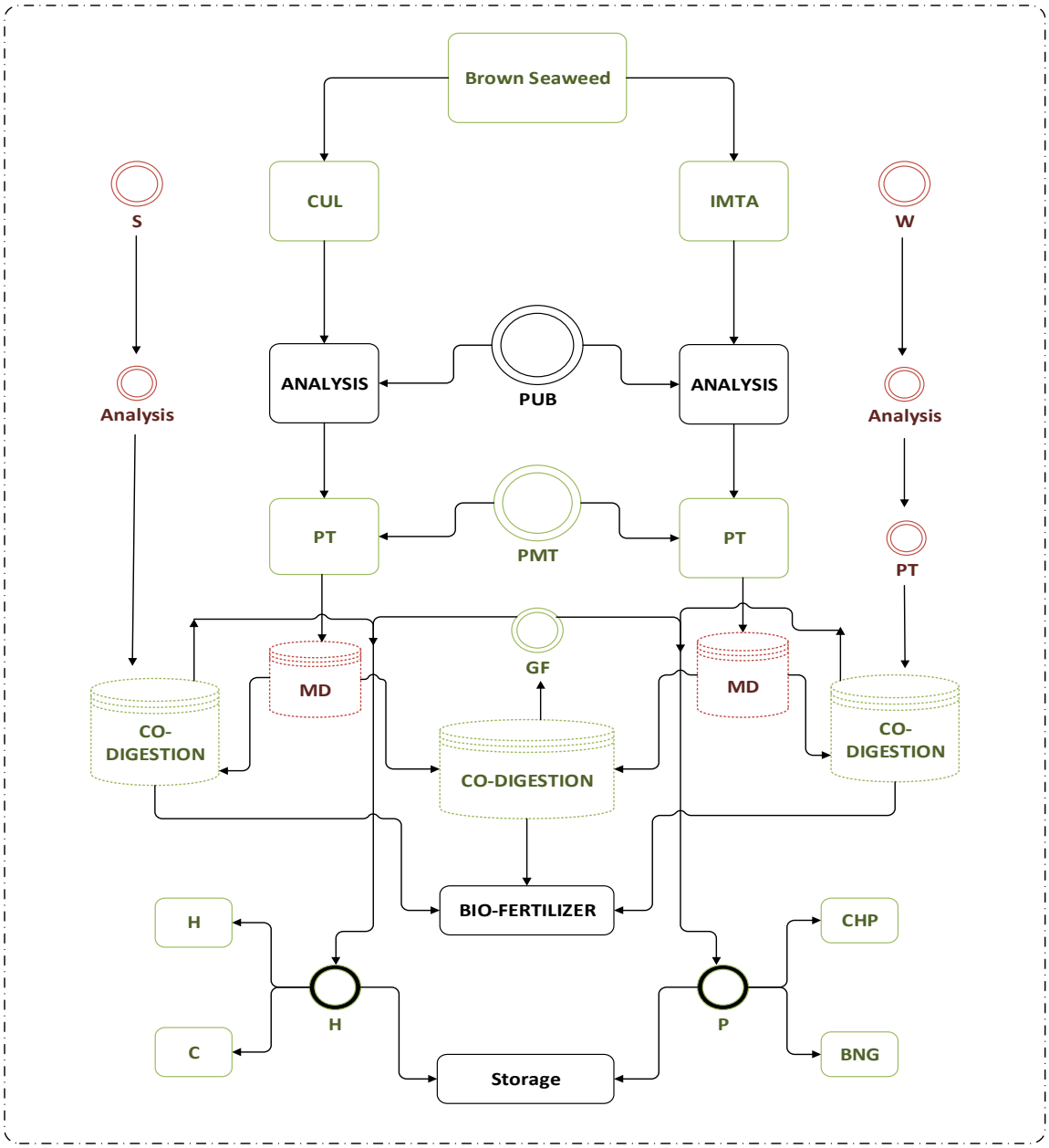

Figure 2.4. Integrated system of bio-natural gas (BNG) production

Where, W is waste, IMTA is integrated multi-trophic aquaculture, S is slurry, CUL is cultivation, PT is pre-treatment, PUB is proximate, ultimate and biochemical analysis, PMT is physical, mechanical and thermal PT, MD is mono-digestion, GF is gas flow, P is power, CHP is combined heat and power, E is electricity, BNG is bio-natural gas, T is transport, H is heating and C is for cooking.

2.7 Conclusion

Seaweed is an abundant resource that has the potential to satisfy targets for advanced biofuels in Ireland. A viable seaweed biomethane industry will require: a high yield of seaweed per hectare; a high biomethane yielding seaweed species; a cost-effective cultivation method; harvesting when the seaweed is optimal for digestion; enhancing pre-treatment methods; and optimal long-term continuous digestion operation. A range of 6,124 to 61,465 ha of the coastal area would be required to grow seaweed to meet Ireland's 2020 target in advanced biofuels.

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3. The effect of seasonal variation on biomethane production from seaweed and on application as a gaseous transport biofuel

The effect of seasonal variation on biomethane production from seaweed and on application as a gaseous transport biofuel

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Abstract

Biomethane produced from seaweed may be used as a transport biofuel. Seasonal variation will have an effect on this industry. *Laminaria digitata*, a typical Irish brown seaweed species, shows significant seasonal variation both in proximate, ultimate and biochemical composition. The characteristics in August were optimal with the lowest level of ash (20% of volatile solids), a C:N ratio of 32 and the highest specific methane yield measured at 327 L CH_4 kg VS^{-1}, which was 72% of theoretical yield. The highest yield per mass collected of 53 m^3 CH_4 t^{-1} was achieved in August, which is 4.5 times higher than the lowest value, obtained in December. A seaweed cultivation area of 11,800 ha would be required to satisfy the 2020 target for advanced biofuels in Ireland, of 1.25% renewable energy supply in transport (RES-T) based on the optimal gross energy yield obtained in August (200 GJ ha^{-1} yr^{-1}).

Keywords: Seaweed; Anaerobic digestion; Biogas; Seasonal variation; Gaseous transport biofuel

3.1 Introduction

Biogas derived from anaerobic digestion (AD) of biomass, can be used as renewable energy sources for transport fuel or combined heat and power (Chynoweth et al., 2001). Feedstock choice is considered as a critical issue for sustainable bioenergy production. Energy crops are not in favour due to the fuel versus food debate (Tenenbaum, 2008). Utilisation of marine biomass as a feedstock for bioenergy production is an attractive option, as it accounts for over 50% of the primary production of global biomass (McQuatters-Gollop et al., 2011) and does not compete for arable land. However, the potential of marine bioenergy has not been fully explored. Seaweeds (macroalgae) are marine photosynthetic, multicellular organisms that have higher growth rates and productivities than terrestrial crops (Gao and McKinley, 1994). Kelps (such as *Laminaria digitata*) are the largest growing brown seaweed in the Atlantic waters surrounding Ireland and UK (Smale et al., 2013). *L. digitata* is the very prevalent along the coast of Ireland (Hughes et al., 2013) and may allow commercial viability in biofuel production (Milledge et al., 2014).

Seasonal variation in the biochemistry of seaweed can alter the biogas yield dramatically. Changes in the composition of *L. digitata* were described by Black (1950) by assessment of dry solids, ash content, and measurement of carbohydrates. Laminarin and mannitol are the dominant carbohydrates and alginic acid is the structural polymer in *Laminaria* species. According to Black (1950), Laminarin and mannitol concentrations were highest in October and lowest in winter months, with the ash proportion showed a reverse trend. The highest alginate content in kelp species have been reported to occur in summer months (Rosell and Srivastava, 1984). Carbohydrates

have also been reported to accumulate during summer and autumn (Adams et al., 2011a; Rosell and Srivastava, 1984) and consumed during winter. In contrast, protein content was found to be highest in winter and lowest during summer (Adams et al., 2011a; Fleurence, 1999). It should be noted that brown seaweed contains relatively lower protein content (3–15 % of the dry weight) as compared with red and green seaweeds (Fleurence, 1999). Ash is a significant element of brown seaweed and can account for up to 35% of dry weight (Adams et al., 2011a; Schiener et al., 2015). In *L. digitata* the ash consists largely of sodium, potassium, calcium, magnesium and chloride. Seasonal variation in ash content can be of high impact on biomethane production; Adams et al. (2001b) reported the highest biomethane yield when the ash content was the lowest. Different geographical locations generate different growth conditions for seaweeds, depending on characteristics such as temperature, nutrients and sunlight. This results in significant differences in biochemical compositions of seaweeds and subsequent anaerobic digestion characteristics (Adams et al., 2011a; Black, 1950; Schiener et al., 2015). Ireland is a small island with a long coastline abundant in natural seaweed resources with potential for significant seaweed cultivation (Allen et al., 2015); cultivation may be coupled with salmon farms in multi-trophic aquaculture. Development of seaweed-based biogas may allow for the production of much needed third generation biofuel to allow compliance with European Directives and decarbonisation of transport fuel. Biogas is usually continuous produced thus a seaweed biogas industry requires a continuous supply of high-quality seaweed substrates. The harvested seaweed may be ensiled for continuous substrate supply (Herrmann et al., 2015). Determination of the optimal time for harvesting is crucial for a biogas industry.

The innovation in this paper is that it assesses the seasonal variation in composition and in biomethane production for *L.digitata*. It conducts a kinetic analysis of the cumulative biomethane production for twelve months of the year and evaluates the biomethane yields per unit mass wet weight. This level of assessment has not previously been reported for Irish seaweed. This paper assesses *L. digitata* (a representative Irish brown seaweed species) as a substrate in order to:

- Investigate the seasonal variation in the composition of *L. digitata*.
- Assess the seasonal variation in biomethane production from *L. digitata*.
- Undertake a kinetic analysis to detail the biodegradability of the seaweed across the year.
- Identify the peak season of Irish seaweed harvesting for biogas production.
- Calculate the gross energy yield per hectare from *L. digitata*.

3.2 Materials and Methods

3.2.1 Seaweed collection, preparation, and compositional analysis

Laminaria digitata samples were collected from their natural marine environment in Roaring Water Bay, Co. Cork, in the south of Ireland (51°N, -9°E) from January to December. Seaweed samples were washed with tap water to remove foreign objects. Washed samples were macerated in a Buffalo macerator to a particle size of less than 4 mm and packed in sealed transparent plastic bags with a mass of 500g in each bag. Packed samples were stored at -20°C prior to analysis and biomethane potential (BMP) assessment. Moisture content (MC), total solid (TS), volatile solid (VS) and ash were calculated using the standard method of drying of seaweed for 24 hours at 105 °C and then burning for two-hour at 550 °C (APHA, 2011; Xia et al., 2016b). For ultimate

analysis, samples were dried at 105 °C for 24 hours and then were ground to pass through a 500 µm sieve. Samples were analysed for C, H, N, and O (O calculated by difference) on an ash-free basis using a CE 440 elemental analyser. The protein content was calculated based on the data from ultimate analysis. A protein factor of 5.38 (multiplied by the nitrogen content) for brown seaweed (Lourenço et al., 2002) was used to calculate the total protein content in the seaweed sample. Polyphenol content was estimated by a modified version of the Folin Ciocalteu assay as described by Singleton and Rossi (Singleton and Rossi, 1965). Seasons were defined as winter (January), spring (March), summer (June) and autumn (October).

3.2.2 Biomethane yields of *L. digitata*

The data obtained from the ultimate analysis was used to calculate the theoretical biomethane yield using the Buswell equation (Eq. (1)). The output yields a maximum potential methane yield by conversion of VS to methane and carbon dioxide (Buswell AM, 1932). The molar volume of the gases was taken as 22.14 L at 0 °C and 1 atm.

$$C_n H_a O_b + \left(n - \frac{a}{4} - \frac{b}{2}\right) H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right) CO_2 \quad \text{Eq. (1)}$$

An automatic methane potential test system (Bioprocess AMPTS II® system) was used to assess the biomethane potential of seaweed. The inoculum was obtained from lab-scale continuous stirred-tank reactors (operated at 37 °C), which processed dairy slurry, grass silage, and seaweed. The Bioprocess AMPTS II® system incorporates 15 bottles which serve as the batch digester. The system has the capacity to accommodate five specimens at a time in triplicate. In each trial, three samples of seaweed, one of inoculum and one of cellulose were assessed in triplicate. The substrate to inoculum

ratio (S:I) on a VS basis, of 1:2 was used (Angelidaki et al., 2009; Chynoweth et al., 1993). Each bottle had a working volume of 400 ml with a head space of 250 ml. All bottles were filled with calculated amounts of inoculum and substrate and sealed with rubber stoppers. Nitrogen gas was flushed through each vessel to create anaerobic conditions. Each reactor was maintained at 37 °C by a water bath and continuously mixed at a speed of 45 rpm with an alternating time between on and off after every minute. Removal of CO_2, H_2S and other impurities in the gas was achieved by passing the gas through 3 M NaOH solution. Finally, biomethane was passed through a gas tipping device which recorded the volume of gas produced for each of the 15 reactors. The data was recorded every 15 minutes. As each BMP assay was run in triplicate the samples were assessed for standard deviation. In order to determine the specific biomethane production, the total average biomethane produced from the inoculum was subtracted from the average biomethane produced by each sample (Allen et al., 2015). Salinity (g/L), conductivity (milliSiemens/cm) and pH of the batch digestion were also recorded before and after each BMP assay in order to investigate the effect of the chemical composition of seaweed on the reaction performance and the gas yield. The salinity of sea water at the location was also recorded every month and reported the mean value.

3.2.3 Kinetic and statistical analysis

A kinetic assessment of the batch biomethane process allows assessment of the biodegradability and the rate of biodegradability of the substrate. Kinetic studies give data such as decay constant, lag phase, and half-life. The method of assessment involved taking data from the cumulative production curves and input to a MATLAB code. The

bespoke MATLAB programme used a first order differential equation (Eq. (2)) to determine the decay constant values and the modified Gompertz formula (Eq. (3)) to grow a list of variables to describe the decay course of organic matter in the batch process (Nopharatana et al., 2007). Statistical analyses were conducted using the software SPSS (IBM NY, USA). Analysis of variance (ANOVA) was performed to examine the effect of the chemical composition of the substrate (during various seasons) on biomethane yield. The significance of differences in methane yield between seasons was determined by multiple comparisons (Post Hoc test). The significance level was set at 0.05.

$Y(t) = Y_m \,.\left(1 - \exp^{(-kt)}\right)$ Eq. (2)

$M = P \cdot \exp\{-\exp[\frac{R_{max} \cdot e}{P}(\Delta - t)] + 1\}$ Eq. (3)

Where,

$Y(t)$ is the cumulative biomethane yield (L CH_4 kg VS^{-1}) at a time, t (days).

Y_m is the maximum biomethane potential (L CH_4 kg VS^{-1}) of the feedstock.

k (the decay constant in days $^{-1}$) is a measure of the rate of degradability of the substrate.

M is the cumulative biomethane yield (L CH_4 kg VS^{-1}) at a specified time t (days).

P is the maximum biomethane potential (L CH_4 kg VS^{-1}) of the substrate.

R_{max} is the maximum biomethane production rate (L CH_4 kg VS^{-1} day^{-1}).

Δ is the lag phase (a measure of how long (days) before the biomethane production starts to occur).

t is the time (days).

T_{50} is the half-life (a measure of how long (days) it takes to yield half of the maximum cumulative production of biomethane).

R^2 is a measure (in %) of how the kinetic model fits in the curve of biomethane production.

3.3 Results and Discussion

3.3.1 Proximate and ultimate analysis

Proximate and ultimate analysis (from January to December) of *L. digitata* are as shown in Table 3.1. The moisture content (MC) of *L. digitata* ranged from 80% to 92% with a peak in December and a through in August. Great variation was observed in the volatile solids; in August the value was three times higher (16%) than in December (5%). The Ash fraction was high in winter months and lowest in summer months. The carbon fraction peaked (37% of TS) in August due to the high concentration of storage carbohydrates (Adams et al., 2011a). Very low ammonia production in long term digestion is expected due to the high carbon to nitrogen ratio in the peak season. The ash to volatile solids (A:V) ratio along with the C:N ratio is crucial in suggesting harvest time. Low values of the A:V ratio will yield higher methane productions per unit of collected mass of seaweed. August is suggested as the peak month for harvest as the seaweed has the highest organic content (82% of TS), lowest ash content (18% of TS), lowest A:V ratio (0.2) and suitable C:N ratio (32). These results are comparable with the previous studies conducted in the UK on the same seaweed species (Adams et al., 2011a; Schiener et al., 2015). However, Irish seaweed was found to fix slightly more carbon (37%) than British seaweed (36%) in the same peak harvesting month (Schiener et al., 2015). This may be attributed to the different climate and seawater conditions.

3.3.2 Biomethane yields of *L. digitata*

Seasonal variation in chemical composition in the substrate significantly influenced the BMP yield. Multiple comparisons results from one-way ANOVA indicated that seaweed harvested in summer significantly produced more biomethane than in winter ($F=12.91$, $P < 0.002$). BMP yields of *L. digitata* were highest in August with values of 327 ± 26 L CH_4 kg VS^{-1} and lowest in April 203 ± 14 L CH_4 kg VS^{-1} (Figure 3.1).

L. digitata has been previously reported as a potential biofuel feedstock due to easily biodegradable sugars laminarin (up to 30% TS) and mannitol (up to 25% TS) (Adams et al., 2011b). Adams et al. found the C:N ratio of *L. digitata* ranged from 10.9 in January to a peak of 31.9 in August harvested in the UK resulting in a BMP yield of

Table 3.1 Proximate and ultimate analysis of *L. digitata* round the year

Harvest	Proximate Analysis						Ultimate Analysis			
	MC %	TS (%wwt)	VS (%wwt)	VS (% of TS)	Ash (% of TS)	A:V	C %	H %	N %	O %
January	88.57	11.43 (0.44)	7.00 (0.41)	61.24	38.82 (1.25)	0.63	26.06 (0.38)	3.38 (0.06)	3.95 (0.02)	27.85
February	88.53	11.47 (0.05)	7.34 (0.18)	63.99	36.05 (1.76)	0.56	26.47 (0.16)	3.62 (0.09)	3.95 (0.09)	29.96
March	90.26	9.74 (0.02)	6.49 (0.14)	66.67	33.33 (1.37)	0.5	30.41 (0.90)	3.97 (0.11)	3.7 (0.06)	28.58
April	85.58	14.42 (0.22)	9.31 (0.18)	64.56	35.44 (1.02)	0.55	30.02 (0.23)	3.83 (0.05)	2.28 (0.04)	28.43
May	87.63	12.37 (0.09)	8.91 (0.07)	72.73	28.04 (0.43)	0.39	32.36 (0.18)	4.74 (0.08)	2.18 (0.05)	33.44
June	85.83	14.17 (0.04)	10.37 (0.13)	73.15	26.85 (1.11)	0.37	34.5 (0.77)	5.02 (0.03)	1.82 (0.05)	31.81
July	85.64	14.36 (0.03)	10.87 (0.04)	76.6	24.3 (0.44)	0.32	33.2 (0.05)	4.95 (0.09)	1.53 (0.10)	36.92
August	80.28	19.72 (0.11)	16.12 (0.04)	81.72	18.28 (0.27)	0.22	36.76 0.10)	5.54 (0.02)	1.14 (0.04)	38.28
September	80.54	19.46 (0.26)	15.67 (0.25)	80.51	19.49 (0.44)	0.24	36.62 (0.17)	5.3 (0.05)	0.93 (0.03)	39.37
October	84.2	15.8 (0.24)	11.92 (0.24)	75.42	24.56 (0.37)	0.32	33.45 (0.22)	4.71 (0.01)	1.22 (0.05)	36.55
November	84.81	15.19 (0.12)	11.44 (0.09)	75.29	24.71 (0.09)	0.33	37.18 (0.42)	4.98 (0.06)	1.53 (0.11)	31.6
December	91.61	8.39 (0.95)	5.26 (0.92)	59.59	37.58 (4.05)	0.64	30.82 (0.50)	4.05 (0.01)	3.4 (0.09)	21.32

wwt = wet weight, Standard deviation is in parentheses.

254.14 ± 6.21 L CH_4 kg VS^{-1} (Adams et al., 2011a). Due to variation in location and season, different BMP yields were reported from various sites in Ireland. *L. digitata* collected in August (C:N ratio of 22.5) generated a BMP yield of 218.0 ± 4.1 L CH_4 kg VS^{-1} and a lower yield was recorded when harvested in May (184 L CH_4 kg VS^{-1}) (Vanegas and Bartlett, 2013) and in January (103.3 ± 19.8 L CH_4 kg VS^{-1}) (Tedesco et al., 2013). In this study, the BMP is higher than in previous studies (Table 3.2). It can be possibly due to the high C:N ratio (32), the low A:V ratio (0.2) (Table 3.1), the higher biodegradability (Table 3.2) and higher amount of storage carbohydrates. However, the high C:N ratio in September (40) slightly affected the gas yield, as the accumulated carbohydrates and the lack of proteins can result in an imbalance and a drop in pH and slight inhibition of methanogenesis (Wang et al., 2014).

Seaweed samples were washed with tap water and then kept in a vertical position for 15 minutes to remove the surface water. The biodegradability index (BI) is defined as the ratio of the BMP yield to the theoretical yield (Table 3.2). This value describes the efficiency of biodegradability of the substrate in the batch digestion. The highest biodegradability index was observed in August (0.72) while the lowest was in December (0.44). Low biodegradability may be attributed to the low carbohydrate content and imbalances in the C/N ratio in winter. The average BMP of cellulose in these trials was 335.3 ± 13.2 L CH_4 kg VS^{-1}, corresponding to a biodegradability index of 0.81 ± 0.03. This suggests a healthy inoculum condition and repeatable results between each BMP batch trials.

Table 3.2 Biomethane production over 12 months based on results of BMP analysis and theoretical analysis

Harvest	BMP yield ($L\ CH_4\ kg\ VS^{-1}$)	Harvest $kg\ VS\ t^{-1}_{wwt}$	Theoretical composition (CH_4 %)	Theoretical yield ($L\ CH_4\ kg\ VS^{-1}$)	Biodegradability index (BMP/theoretical)	Specific yield ($m^3\ CH_4\ t^{-1}_{wwt}$)
January	237 ± 4	70	42	421	0.56	17
February	259 ± 5	73	46	406	0.64	19
March	265 ± 4	65	48	469	0.57	17
April	203 ± 14	93	50	462	0.44	19
May	263 ± 11	90	52	407	0.65	23
June	294 ± 1	104	53	493	0.60	30
July	303 ± 12	110	50	435	0.70	33
August	327 ± 26	161	54	452	0.72	53
September	303 ± 19	160	50	450	0.67	47
October	267 ± 7	120	51	428	0.62	32
November	256 ± 11	114	53	510	0.50	29
December	235 ± 10	50	53	537	0.44	12

wwt = wet weight

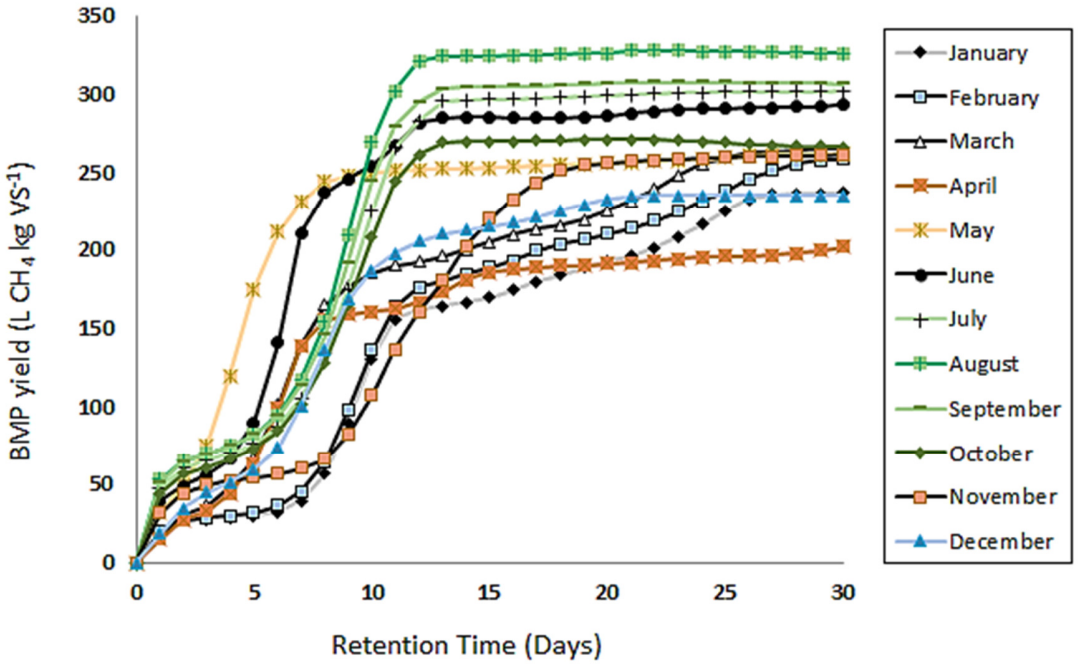

Figure 3.1 Biomethane potential cumulative yield curves for *L. digitata* collected throughout the year.

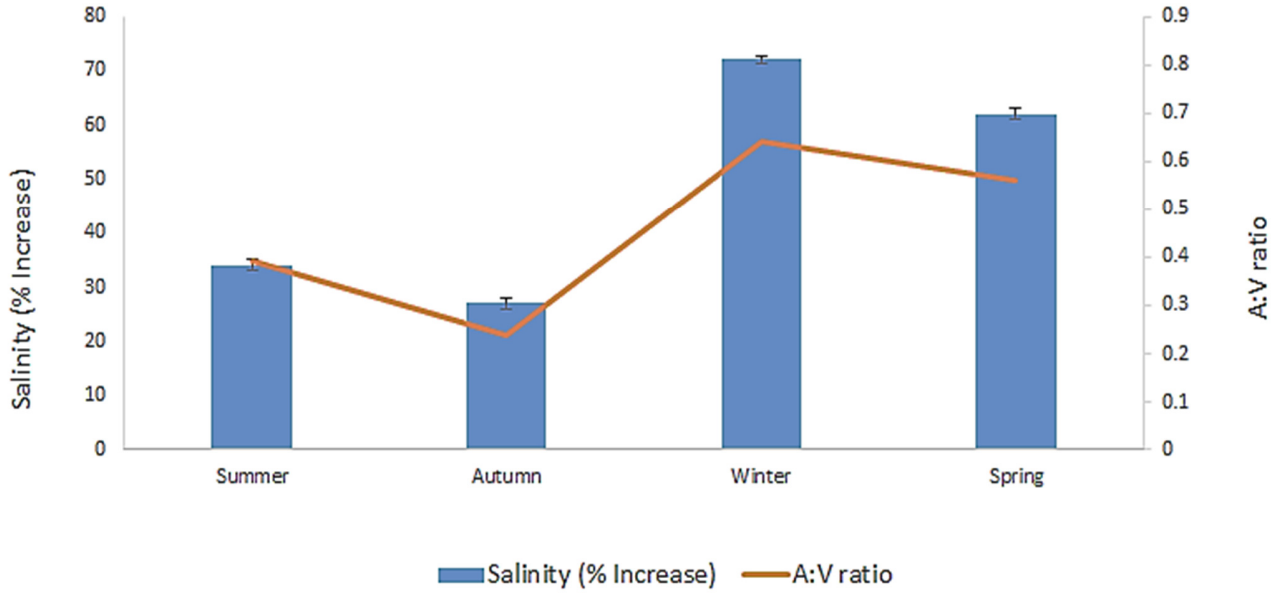

Figure 3.2 Salts accumulation in a batch reactor (summer to spring).

The salinity of seaweed was subtracted from the salinity of inoculum (6.9 ± 0.47 g/L) in order to present actual salinity increase in the batch digestion due to seaweed only.

Accumulation of salts can be a problem in the digestion of seaweed. It was observed that salinity was much higher in winter and spring as compared to summer and autumn (see Figure 3.2). A correlation between ash content and organic content (A:V) explains the tolerance level of salts during digestion. The salinity of inoculum and the seaweed was measured after completion of each batch (30 days). The salinity values were 11.0 ± 0.08 g/L, 10.4 ± 0.10 g/L, 9.8 ± 0.10 g/L and 9.6 ± 0.10 g/L in winter (January), spring (March), summer (June) and autumn (October) months, respectively (compared with the seawater salinity of 31 ± 0.90 g/L from the collection site). The salinity of seaweed was subtracted from the salinity of inoculum (6.9 ± 0.47 g/L) in order to present actual salinity increase in the batch digestion due to seaweed only. Salinity and A:V ratio had a significant effect on the gas yield during different seasons. Higher values of salinity and A:V led to lower values of gas production. It was observed that the optimum gas yield was obtained when salinity increased up to 30% (compared with inoculum salinity; in summer and autumn months). Lower yields were obtained when salinity increased by 60% or more in winter and spring. Excess levels of salinity level can adversely affect digestion performance. A low biomethane yield is expected at a high salinity level (Chen et al., 2008; Fang et al., 2011; Xia et al., 2016a).

Biomethane yields may be expressed based on per unit mass of volatile solid or per unit mass of wet weight (Figure 3.3). The yields per unit mass wet weight are more understandable to the biogas developer and also combine the effects of proximate analysis (change in VS content as indicated in Table 3.1) and BMP results (expressed in Table 3.2 as L CH_4 kg VS^{-1}). The December BMP value is 72% of the August value

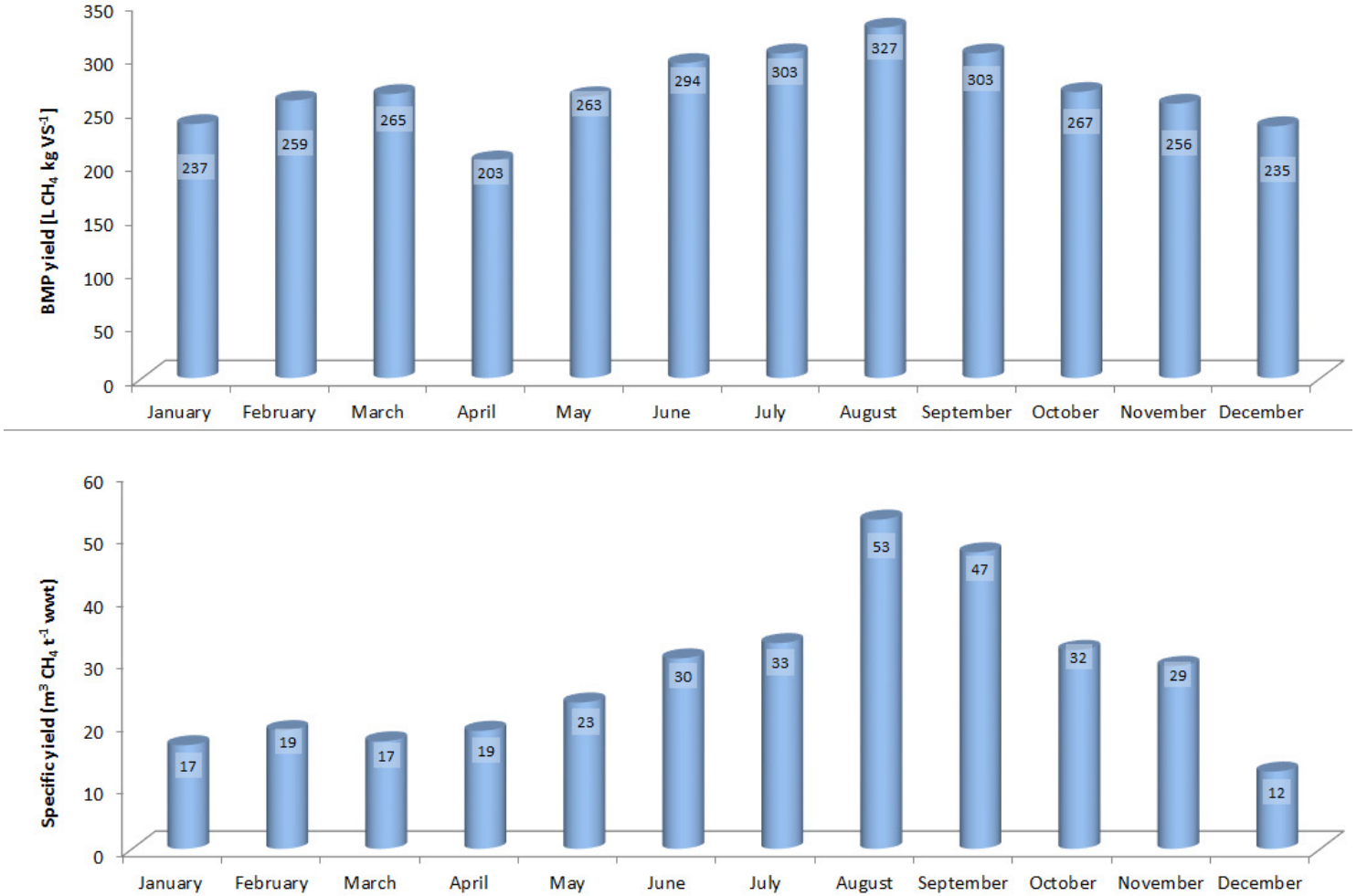

Figure 3.3 Calculation of methane yield expressed per unit mass of VS (A) and per unit mass wet weight (B)

(Figure 3.3A) but it is only 22.6% of the yield when expressed per unit mass wet weight (Figure 3.3B). More significant results were obtained when ANOVA was performed on the basis of wet weight ($F=5.75$, $P < 0.021$) as compared to dry weight basis ($F=4.90$, $P < 0.032$).

3.3.3 Kinetic analysis

The kinetic results of the BMP analysis are as shown in Table 3.3. The modified Gompertz equation presented very good correlation as values of R^2 were within an acceptable tolerance (R^2 greater than 0.95). All months indicated good kinetic decay (*k* value ranged from 0.13 to 0.21) except the winter months (around 0.08). This may be attributed to the high content of slowly degradable compounds (such as proteins) and low content of easily degradable carbohydrates (such as mannitol) in winter months (see Figure 3.4). Moreover, the high salinity level in winter can adversely affect methane production (see Figure2).

Kinetic decay values for perennial ryegrass (Wall et al., 2013), food waste (Browne et al., 2014) and brown seaweed harvested in late summer (Allen et al., 2015) were reported to be 0.11, 0.17 and 0.19, respectively. The half-life of the methane production (T_{50}) was less in summer (4-5 days) than winter months (6-9 days) probably due to the higher concentration of easily degradable laminarin and mannitol in summer (Adams et al., 2011a). These results were comparable with previous studies on *L. digitata* (Allen et al., 2015). The half-life for summer months was relatively low suggesting a short retention time of less than 20 days could be sufficient for long-term continuous digestion.

Table 3.3 Kinetic analysis of *L. digitata* based on seasonal variation

Month of Harvest	k decay constant ($days^{-1}$)	R^2	Δ lag phase (days)	T_{50} half-life of methane production (days)
January	0.08	0.97	2.80	8.91
February	0.08	0.97	3.09	9.02
March	0.10	0.97	1.60	6.81
April	0.13	0.98	1.60	5.19
May	0.21	0.99	1.17	3.23
June	0.15	0.98	1.91	4.48
July	0.13	0.95	2.16	5.54
August	0.13	0.95	2.46	5.21
September	0.13	0.95	1.95	5.24
October	0.14	0.95	2.00	5.12
November	0.09	0.97	2.91	7.69
December	0.13	0.99	1.94	5.52
Cellulose	0.17	0.99	2.17	4.09

The lag phase of 2 to 3 days for brown seaweed harvested at the same month was less than the 6 to 9 days reported by Gurung et al. (Gurung et al., 2012). This may be attributed to the higher I:S ratio of 2:1 in this study as compared to 0.8:1 in the study by Gurung et al. (Gurung et al., 2012). I:S of 2: 1 minimises potential inhibition during the digestion process (Allen et al., 2015).

3.3.4 Biochemical analysis

A full twelve-month biochemical profile of *L. digitata* is shown in Figure 3.4. The total carbohydrate content peaked in August and September. This result is consistent with the high BMP yield obtained from the samples collected in August and September. In January, the protein and ash content was highest and the carbohydrate content was lowest. Protein and ash content decreased from January until August and then dropped, while carbohydrates increased from January until September and then decreased. The carbohydrate trend followed the trend in the C:N ratio which rose from 7 in January to 40 in September.

The polyphenol content (data not shown) peaked in April (1.3 mg/g) and was lowest in August (0.02mg/g). A previous study (O'Sullivan et al., 2011) confirms that the polyphenol content of brown seaweed achieved the highest value (1.5 mg/g) in spring. High levels of polyphenol can adversely affect the digestion process and produce a low BMP yield (Allen et al., 2015).

3.3.5 Effect of seasonal variation on gross energy yields

The data from seasonal variation in *L. digitata* highlights that the peak month to harvest the seaweed is August. Gross energy yield from the seaweed in each month was calculated (Table 3.4). The table highlights the total coastal area in each month required for the seaweed species cultivation to satisfy the 2020 RES-T target (1.25% of energy in transport or 2.35 PJ) for Ireland. Two scenarios are chosen. Scenario (1): a realistic current based on 20 kg wet weight per meter line with each line at 5 m distance (40 t wet weight ha^{-1} y^{-1}) (Murphy et al., 2015) and scenario (2); optimistic analysis based on

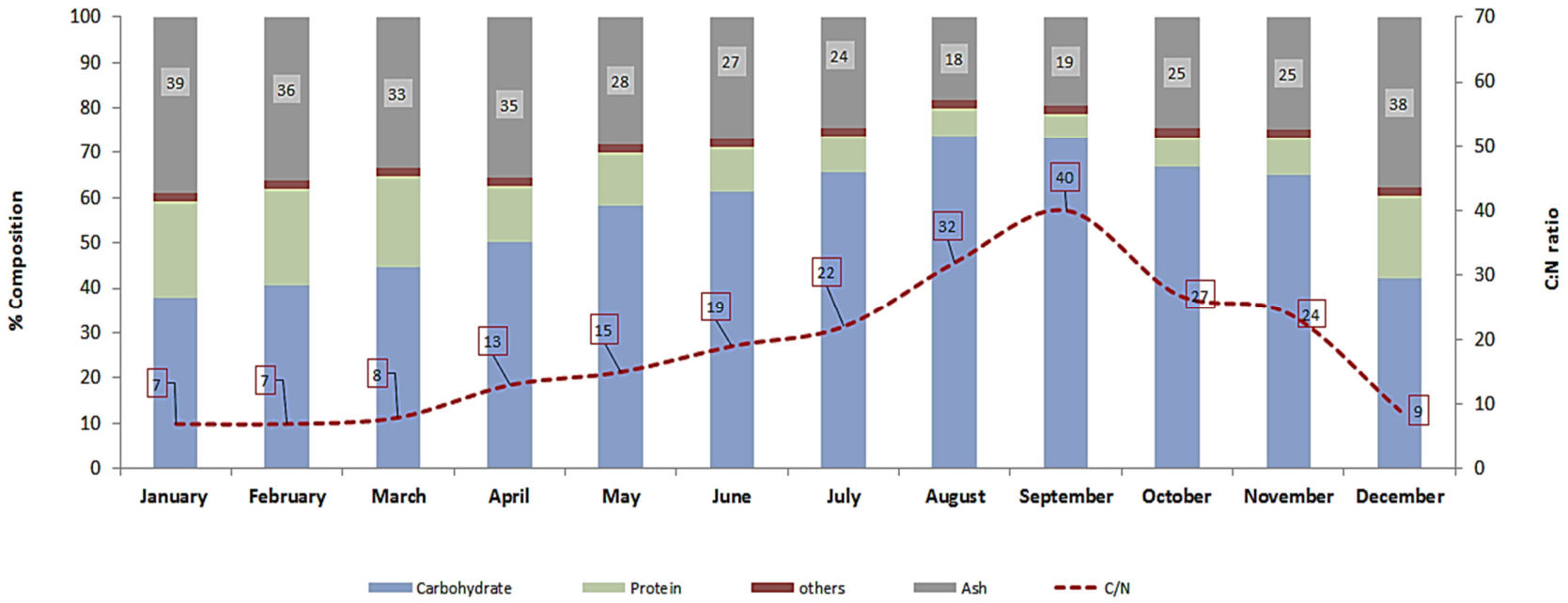

Figure 3.4 Full year biochemical profile of *L. digitata*.

Total carbohydrate content was calculated by difference by assuming TS equal to the sum of carbohydrates, proteins, ash and others (1% of TS).

Table 3.4 Calculation of gross energy of *L. digitata* based on various seasons and land area required to supply 1.25% RES-T of 2.35PJ

Harvest	Scenario 1 (40 t wet weight ha^{-1} y^{-1})			Scenario 2 (100 t wet weight ha^{-1} y^{-1})		
	Biomethane yield m^3 ha^{-1} yr^{-1}	Gross energy GJ ha^{-1} yr^{-1}	Area required Ha	Biomethane yield m^3 ha^{-1} yr^{-1}	Gross energy GJ ha^{-1} yr^{-1}	Area required ha
January	663	25	94000	1658	63	37422
February	760	29	81655	1901	72	32662
March	688	26	90195	1721	65	36078
April	756	29	82155	1890	71	32862
May	937	35	65777	2342	88	26310
June	1219	46	51000	3048	115	20400
July	1317	50	46600	3294	124	18640
August	2108	80	29500	5270	200	11800
September	1899	72	32100	4747	179	12840
October	1274	48	48500	3184	120	19400
November	1171	44	53000	2929	111	21200
December	494	19	132200	1236	47	52880

Sample calculation for January Scenario 1: Biomethane Yield, m^3 ha^{-1} yr^{-1} = 237 m^3 CH_4/ tVS (Table 3.2)* 40 t_{wwt} * 7% VS (Table 3.1) = 663.6 m^3 ha^{-1} yr^{-1};
Gross energy (GJ ha^{-1} yr^{-1}) = Biomethane yield * 37.8 MJ/m^3*0.001 (MJ to GJ) = 25 GJ ha^{-1} yr^{-1} , Area required: 2.35 PJ * 1,000,000 (GJ/PJ) / 25 GJ ha^{-1} yr^{-1} = 94,000 ha

future systems such as cultivation on mats generating up to 100 t wet weight ha^{-1} y^{-1} as suggested by Allen et al. (2015).

Selection of the optimum month for seaweed harvesting can give four times more energy than a least beneficial month (47 GJ ha^{-1} yr^{-1} in December and 200 GJ ha^{-1} yr^{-1} in August based on the optimistic scenario in Table 3.4). This value is higher than various land-based biofuels such as oil biodiesel (120 GJ ha^{-1} yr^{-1}), sugarcane ethanol (135 GJ ha^{-1} yr^{-1}) and grass (122-163 GJ ha^{-1} yr^{-1}) (Allen et al., 2015). A barrier to the industry may be the cultivation cost of the seaweed. A salmon farm (on 454 hectares) is planned in Galway off the west coast of Ireland and more farms are proposed at 46 sites in Ireland in the near future) (Irish Sea Fisheries Board, 2012).

There is potential for relatively low cost and high productivity cultivation of *L. digitata* when coupled with salmon farms allowing waste nutrients to provide fertiliser for enhanced seaweed growth and allowing seaweed to improve water quality levels.

3.4 Conclusions

In August the specific methane yield of *L. digitata* is 40% higher than that sampled in December. However, the volatile solids content per wet weight is a factor of 3.2 that of the December sample. Thus the biomethane potential yield is four times higher in August than in December. Gross energy yields from the seaweed in August can yield 80 GJ ha^{-1} yr^{-1}, based on long line cultivation with 20 kg harvested per meter length and lines at 5 m centres. At these cultivation levels, 29,500 ha of cultivation would satisfy 1.25% of renewable energy in transport in Ireland.

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4. Seasonal variation in the chemical composition and biomethane production from the brown seaweed *Ascophyllum nodosum*

Seasonal variation in the chemical composition and biomethane production from the brown seaweed *Ascophyllum nodosum*

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Abstract

Ascophyllum nodosum, an abundant Irish brown seaweed, shows significant seasonal variation in the chemical composition and biogas production. The polyphenol content is shown to be a more important factor in biogas production than ash content. High polyphenol content in summer months adversely affected biogas production; suggesting two potential harvest dates, March, and October. *A. nodosum* harvested in October showed a relatively low level of polyphenols (2% of TS) and ash (23% of volatile solids) and exhibited a specific methane yield of 215 L CH_4 kg VS^{-1}, which was 44% of theoretical yield. The highest yield per wet weight of 47 m^3 CH_4 t^{-1} was achieved in October, which is 2.9 times higher than the lowest value (16 m^3 CH_4 t^{-1}), obtained in December. The gross energy yield of *A. nodosum* based on the optimal biogas production was 116 GJ ha^{-1} yr^{-1} in October.

Keywords: Algae; Seaweed; *Ascophyllum nodosum*; Polyphenols; Biomethane

4.1 Introduction

Marine biomass, such as seaweed (macroalgae), accounts for over half of the global primary biomass production (McQuatters-Gollop et al., 2011); it has been considered as a potential source for bioenergy production (Adams et al., 2011; Allen et al., 2015; Xia et al., 2016a). Fermentative biomethane production from seaweed via anaerobic digestion may be exploited as an attractive energy source in future fuel systems. Compared with land-based crops, biofuels from seaweed may be more beneficial as seaweed can have high gross energy yields per hectare (Allen et al., 2015) and do not use arable land which would be used for food (Tenenbaum, 2008). Brown seaweed is reported as a major marine bioresource in the north Atlantic waters around Ireland and the UK (Smale et al., 2013). Ireland is one of the largest producers of seaweed in Europe with 13% (ca. 29,500 t yr^{-1})) of the total European harvest. This harvest is dominated by *A. nodosum* (ca. 30,000 t yr^{-1}) and mainly takes place in the north-west of the island in counties Donegal and Galway (Murphy et al., 2013).

Seasonal variation in the biochemical composition of the seaweed could have a significant impact on biogas production as shown for *L. digitata* in Welsh (Adams et al., 2011) and Irish waters (Tabassum et al., 2016). Carbohydrates, which are vital for the gas production, have also been reported to accumulate in brown seaweed during summer and autumn (Adams et al., 2011; Rosell and Srivastava, 1984; Tabassum et al., 2016) while consumption of storage carbohydrates may take place during winter. The lower concentration of carbohydrate in brown seaweeds in winter than in summer may be due to utilization of storage carbohydrates as an energy source for cellular activities (Adams et al., 2011; Tabassum et al., 2016). Ash is another significant component of brown

seaweed; changes of ash content around the year can account for up to 35% of dry weight in brown seaweed (Adams et al., 2011; Moen et al., 1997; Schiener et al., 2015). Brown seaweed such as *A. nodosum* contains polyphenols (such as phlorotannins) that are reported as inhibitory compounds for anaerobic digestion as they inhibit enzyme activities of various microbes including methanogens (Scalbert, 1991). For instance, polyphenols inhibit alginate lyase, an enzyme that breaks down alginic acid (Moen et al., 1997). Significant seasonal changes in polyphenol content of *A. nodosum* were described by Parys (Parys et al., 2009) and Apostolidis (Apostolidis et al., 2011). The polyphenol content in the seaweed is dependent on the location, harvesting time, light intensity, temperature, salinity and ambient nutrients (Parys et al., 2009). The biogas production from polyphenol-rich *A. nodosum* is rarely reported. A maximum yield of 176 ± 37.62 L CH_4 kg VS^{-1} was reported by MacArtain et al. (2015). The specific gas production from *A. nodosum* was reported as 50% less than brown seaweed *Laminaria* spp., due to the high polyphenol content (Hanssen et al., 1987). However, no study on the impact of seasonal variation on polyphenol content and the associated impact on biogas production from *A. nodosum* has yet been reported.

In 2015 the EU Environment Committee stated in a communication that advanced biofuels from seaweed or certain types of waste should account for at least 1.25% of energy consumption in transport by 2020 (European Parliament, 2015). Development of a seaweed biogas industry using an existing harvested seaweed such as *A. nodosum* would be very beneficial for achieving the EU 2020 target in Ireland. Biogas production is a continuous process that requires high-quality substrate supply. Thus ensiling of the harvested *A. nodosum* is required to provide a continuous supply of substrate for year

round digestion. Work by Herrmann et al. (2015) indicated that ensiled *A. nodosum* (collected in Ireland at the end of August) improved the gas yields as compared to fresh *A. nodosum* by 30% (45.7 m^3 t wwt^{-1} as compared to 35.1 m^3 $twwt^{-1}$). It is very important to identify the optimal season to harvest *A. nodosum*. This paper assesses *A. nodosum* as a feedstock for biogas production in order to:

- Examine the seasonal variation in composition with particular emphasis on polyphenol content of *A. nodosum*.
- Assess the effect of seasonal variation in biomethane production from *A. nodosum*.
- Identify the optimal season for the harvest of *A. nodosum* to maximise biomethane production.

4.2 Materials and Methods

4.2.1 Collection and processing of seaweed

A. nodosum was naturally grown and collected from Roaring Water Bay in Co. Cork (south of Ireland (51°N, -9°E)) from January until December. Approximately 30 plants were collected (sufficient to make the representative sample) at low spring tide each month from the same location. The samples were washed with tap water to remove foreign substances. The surface water was allowed to drain by keeping the samples in a vertical position for approximately 15 minutes. Subsequently, the samples were processed to reduce the particle size to less than 4 mm by using a "Buffalo" macerator; the processed seaweed was stored in plastic bags with a mass of approximately 500 g in each bag. Packed samples were sealed and frozen at -20°C for further analyses and biomethane potential (BMP) assessment.

4.2.2 Chemical and biological analysis

The contents of total solid (TS), volatile solid (VS) and ash were obtained by oven heating fresh seaweed at 105 °C for 24 hours and then burning at 550 °C for 2 hours (APHA, 2011; Xia et al., 2016b). The oven dried samples (moisture free) were ground and the powder was passed through a 500 µm sieve for ultimate analysis. The portions of dried substrate, which were C, H, N, and O (O calculated by difference) were obtained, using a CE 440 elemental analyser. The protein content was calculated based on the nitrogen content multiplied by a factor of 5.38 (for brown seaweed) (Lourenço et al., 2002). Polyphenol concentration was measured by a modified Folin Ciocalteu assay described previously (Singleton and Rossi, 1965).

4.2.3 Biomethane potential assessment

The theoretical biomethane potential (TMP) was calculated based on the elemental analyses using the Buswell equation (as shown in Eq. (1)) (Buswell AM, 1932).

$$C_n H_a O_b + \left(n - \frac{a}{4} - \frac{b}{2}\right) H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right) CO_2 \quad \text{Eq. (1)}$$

A Bioprocess Automatic Methane Potential Test System (AMPTS) II® system was used to assess the biomethane potential (BMP) of *A. nodosum*. The automatic methane potential test system facilitated the computer-aided measurement of the biomethane produced from the anaerobic batch digestion system. The mesophilic inoculum was sourced from a lab-scale continuous stirred-tank reactor, processing seaweed, dairy slurry and grass silage at 37 °C. The BMP system has 15 bottles which function as batch digesters. Each bottle has a total volume of 650 mL with a working volume of 400 ml. The inoculum to substrate ratio (I:S) was set as 2:1 based on VS (Angelidaki et al.,

2009; Chynoweth et al., 1993). All bottles were sealed with rubber stoppers and were purged with nitrogen gas for 5 min to ensure an anaerobic environment. The bottles employ a mixing system operating at 30 rpm and were kept at 37 °C using a water bath. Produced biogas was passed through 3 M sodium hydroxide solution to remove the impurities such as carbon dioxide and hydrogen sulphide. Gas flow was measured by a gas tipping. The biomethane volume was automatically normalised to standard temperature (0 °C) and pressure (1 atm) and zero moisture content by the Bioprocess AMPTS II® system. To determine the specific biomethane production of each seaweed sample, the biomethane produced from the inoculum was subtracted from the biomethane produced by each sample. Cellulose was used as a control to ensure a healthy inoculum condition (Allen et al., 2015). Batch BMP trials were conducted in triplicate, and the results were expressed as mean value ± standard deviation.

Salinity (g/L) and pH were also recorded before and after each BMP assay. Seasons were defined as winter (December), spring (March), summer (June) and autumn (October) for salinity (% change).

The gross energy yield of *A. nodosum* from each month was calculated by using the lower heating value of methane (37.8 MJ/m^3). The biomethane yield of *A. nodosum* for future scenarios was assumed with a 30% increase after an ensiling process compared with the BMP yield obtained from fresh *A. nodosum* in this study, as suggested by Herrmann et al. (2015).

4.2.4 Process dynamics and statistical analyses

The process dynamics were assessed by a first order differential equation (Eq. (2)) via MATLAB programme to obtain decay constant (days^{-1}) and maximum yield (Y_{max}). The

half-life time (T_{50}) was defined as the time taken to achieve half of the maximum cumulative production of biomethane (Nopharatana et al., 2007). The biodegradability index (BI) was defined as the ratio of BMP yield to the TMP yield.

Statistical analyses were carried out by using SPSS software (IBM NY, USA). Analysis of variance (ANOVA) was conducted to investigate the impact of seaweed chemical composition on the specific biomethane yield. The significance of differences in the specific biomethane yield between seasons was obtained by using multiple comparison (Post Hoc) test at a significance level of 0.05.

$$Y(t) = Y_m \cdot \left(1 - e^{(-kt)}\right) \qquad \text{Eq. (2)}$$

Where,

t is the fermentation time (days); k is the decay constant (days $^{-1}$), which indicates the rate of degradability of the substrate; $Y(t)$ is the cumulative methane yield (L CH_4 kg VS^{-1}) at time t (days); Y_m is the maximum methane potential (L CH_4 kg VS^{-1}).

4.3 Results and Discussion

4.3.1 Characterization of the seaweed

A. nodosum collected from January to December was characterized by proximate and ultimate analyses (Table 4.1). The total solids (TS) of the seaweed ranged from 19% to 34 % with a peak in September. Volatile solids were 71% higher in September than in May. The ash fraction was high in February (33%) and lowest in November (18%). Proximate analyses of *A. nodosum* in spring indicated higher VS content as compared to the same species harvested in March in the UK (23.1% versus 19.9%) (Obata et al., 2015). A study from Norway (Moen et al., 1997) on *A. nodosum* harvested in October yielded TS values of 26.5% (compared to 28.5% in this study).

Generally, low values of the ratio of ash content to volatile solid content (A:V) suggests a high organic matter content with a low salt accumulation (ash content is a good indicator of salt content). A low A:V is advantageous for substrate degradation and suggests avoidance of sodium inhibition.

The carbon to nitrogen (C/N) ratio was observed to vary from 16 to 46 which suggests the suitability of *A. nodosum* for mono-digestion or for co-digestion with other seaweeds, which would have a low C:N ratio such as U.*lactuca* (Allen et al., 2015). Increased C:N ratio in seaweed indicates carbohydrate accumulation, which may be easily degraded during digestion; the optimal C:N ratio for anaerobic digestion is usually suggested as being higher than 20 (Xia et al., 2016a). *A. nodosum* tends to accumulate carbohydrates and organic matter composition in summer, leading to higher C:N ratios and lower A:V ratios; this result is similar to the study by Adams et al. (2011). Based on the proximate and ultimate analyses of this study, November may be described as an optimal harvesting month, as the seaweed has the highest organic matter content of 82% of TS, lowest ash content of 18% of TS, lowest A:V ratio of 0.22 and suitable C:N ratio of 37. However, due to great seasonal variation in polyphenol content in *A. nodosum*, higher gas yields may not match typical indicators and highest biogas yields may not be observed in November (detailed discussion in section 4.3.2 and 4.3.3).

Table 4.1 Characterization of *A. nodosum* round the year

Month of Harvest	Proximate Analysis					Ultimate Analysis				
	TS (%)	VS (%)	OMC (%)	Ash (%)	A:V	C %	H %	N %	O %	C:N
January	24.87 (0.35)	17.46 (0.52)	70.19	29.82 (1.47)	0.42	37.57 (0.29)	4.59 (0.04)	2.09 (0.03)	25.94	17.98
February	26.02 (0.24)	17.42 (0.23)	66.94	33.06 (0.43)	0.49	39.78 (0.05)	4.91 (0.08)	2.42 (0.03)	19.83	16.44
March	33.22 (0.04)	23.14 (0.11)	69.64	30.36 (0.41)	0.44	40.70 (0.13)	5.07 (0.10)	1.74 (0.07)	22.14	23.39
April	20.99 (0.50)	15.94 (0.65)	75.94	24.06 (4.79)	0.32	36.14 (0.60)	5.03 (0.10)	2.47 (0.17)	32.30	14.63
May	19.18 (0.10)	14.76 (0.09)	76.93	23.07 (0.13)	0.30	33.66 (0.33)	4.58 (0.07)	1.20 (0.24)	37.50	28.05
June	27.65 (0.15)	18.88 (0.11)	68.26	31.74 (0.56)	0.46	40.12 (0.16)	4.93 (0.00)	1.23 (0.10)	21.99	32.62
July	28.37 (0.21)	21.81 (0.02)	76.88	23.12 (0.48)	0.30	37.02 (0.40)	5.44 (0.07)	1.32 (0.03)	33.10	28.05
August	29.98 (0.13)	22.19 (0.15)	74.02	25.98 (0.81)	0.35	39.71 (0.20)	5.21 (0.02)	1.22 (0.03)	27.87	32.55
September	34.46 (0.20)	25.19 (0.13)	73.12	26.88 (0.16)	0.37	40.90 (0.20)	5.14 (0.06)	1.16 (0.06)	25.92	35.26
October	28.52 (0.08)	22.01 (0.06)	77.17	22.83 (0.05)	0.30	40.67 (0.09)	5.11 (0.07)	0.89 (0.04)	30.51	46.75
November	23.80 (0.03)	19.46 (0.04)	81.74	18.26 (0.24)	0.22	40.83 (0.12)	5.05 (0.03)	1.11 (0.04)	34.74	36.78
December	23.49 (0.24)	16.65 (0.17)	70.87	29.12 (0.76)	0.41	39.45 (0.06)	4.83 (0.04)	2.01 (0.11)	24.58	19.63

TS, total solids; VS, volatile solids; OMC, organic matter content obtained dividing VS/TS; A:V, ash to volatile solid ratio.
Standard deviation is in parentheses.

4.3.2 Biochemical analyses

The whole year biochemical profile of *A. nodosum* is shown in Figure 4.1. The total carbohydrate content peaked in November and was lowest in February. A high carbohydrate content (or C:N ratio) of seaweed usually suggests a better degradability. However, in the case of polyphenol-rich *A. nodosum,* the general trend of high carbohydrates and low ash leading to a higher digestibility was not exactly followed as C:N ratios above 35 may be lacking in N and elevated polyphenols in the seaweed can inhibit digestion. Literature was not readily available to support this supposition as no detailed analysis is published on seasonal variation in biomethane production from A. *nodosum*. Obata et al. (2015) published biochemical analysis for a single month in spring (March) which was comparable to this data.

The polyphenol content was observed as the key influencing factor on the performance of anaerobic digestion and variation across the year (Table 4.2). Polyphenols peaked in June (4.9% of dry weight or 72.4 mg g VS^{-1}) and were lowest in April (0.2% of dry weight or 2.8 mg g VS^{-1}). Apostolidis et al. (2011) reported a similar seasonal variation in phenol content, with June and July having the highest and May the lowest. The reason for the variation in polyphenol content may be location, light intensity, temperature, salinity and ambient nutrients (Parys et al., 2009). The reproductive stage of the seaweed also significantly affects the variations in phenol content. Comparatively, lower polyphenol concentrations during the fertile period (April to June) were recorded than the time of shedding fruit bodies at the end of June (Ragan and Jensen, 1978).

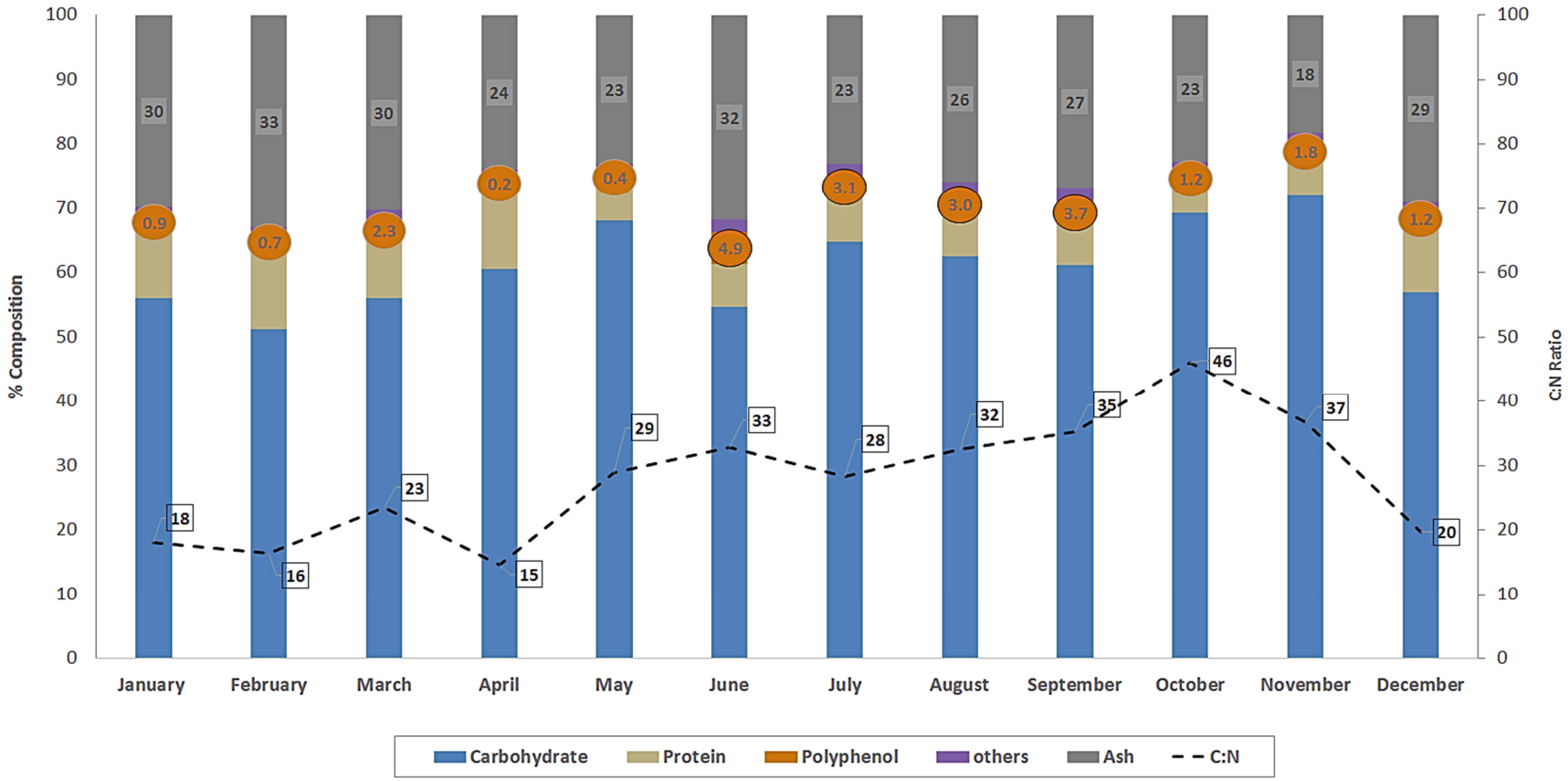

Figure 4.1 Full year biochemical analyses of *A. nodosum*

4.3.3 The gas yield

4.3.3.1 General description

Seasonal variation in chemical composition (especially phenol content) in the seaweed significantly influenced the biogas yield ($F = 4.72$, $P < 0.035$). BMP yields of *A. nodosum* were highest in April with values of 217 ± 14 L CH_4 kg VS^{-1} and lowest in December 95 ± 19 L CH_4 kg VS^{-1} (Figure 4.2). Moreover, it was observed that two peaks of the biogas were appeared per year (Figure 4.3). The two peak months per year raises the possibility to harvest *A. nodosum* twice in a year, in spring and again in autumn. A lower BMP yield of 176 ± 38 L CH_4 kg VS^{-1} was reported by MacArtain et al. (MacArtain et al., 2015).

According to Hanssen et al. (1987), the gas production from *Laminaria* species was almost double the figure obtained from *A. nodosum*. In the present study, the BMP is higher in April and October than reported in above studies (Table 4.2). This can be attributed to the lower polyphenol content, higher C:N ratio, lower A:V ratio (Table 4.1) in peak months obtained in this study. However, the months from June until September did not yield high biomethane as forecasted by the A:V ratio and the C:N ratio. The reason may be attributed to the polyphenol content (Figure 4.4). As phenolic compounds have been identified as strong inhibitors of the anaerobic digestion process, the high polyphenol contents in those months (30.2-49.4 mg g TS^{-1}) lead to the low BMP yields (144-170 CH_4 kg VS^{-1}). Inhibitory level for the process is not same for all microbes (Scalbert, 1991).

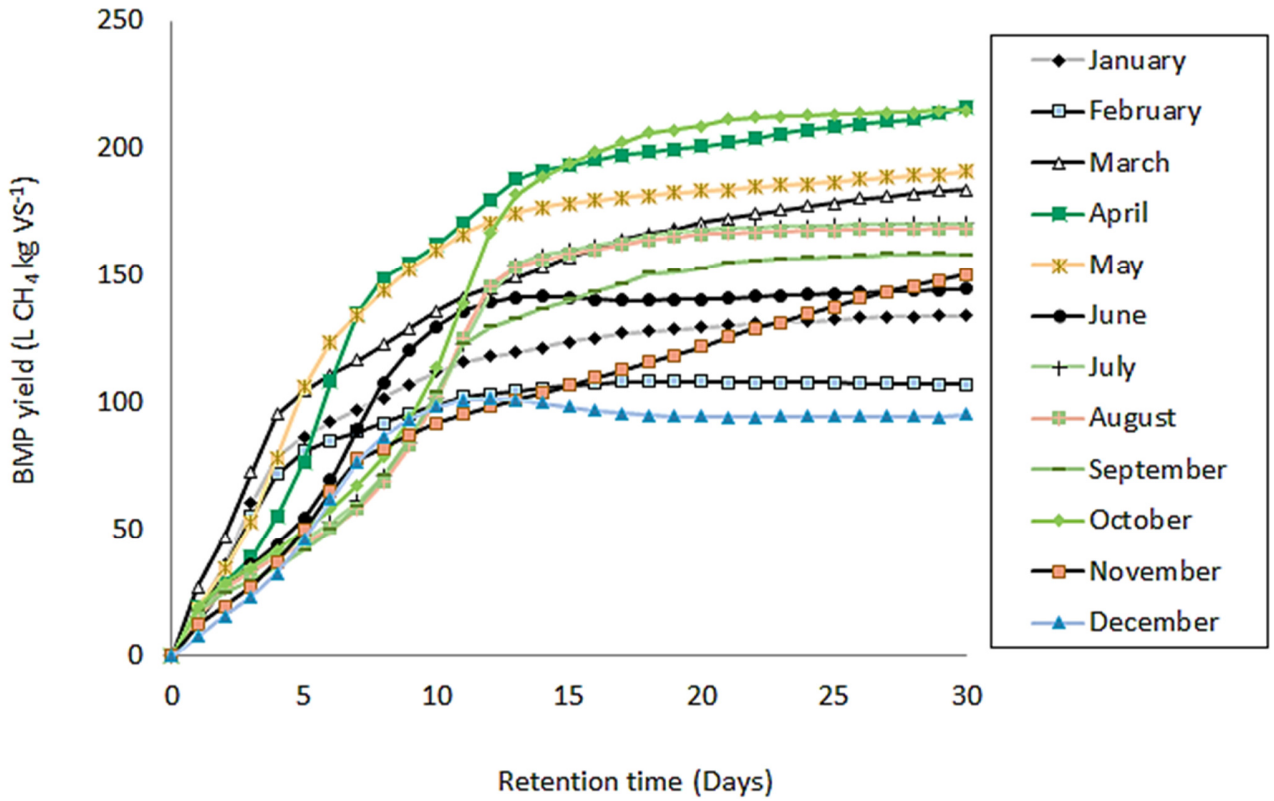

Figure 4.2 Biomethane potential cumulative yield curves for *A. nodosum* collected throughout the year

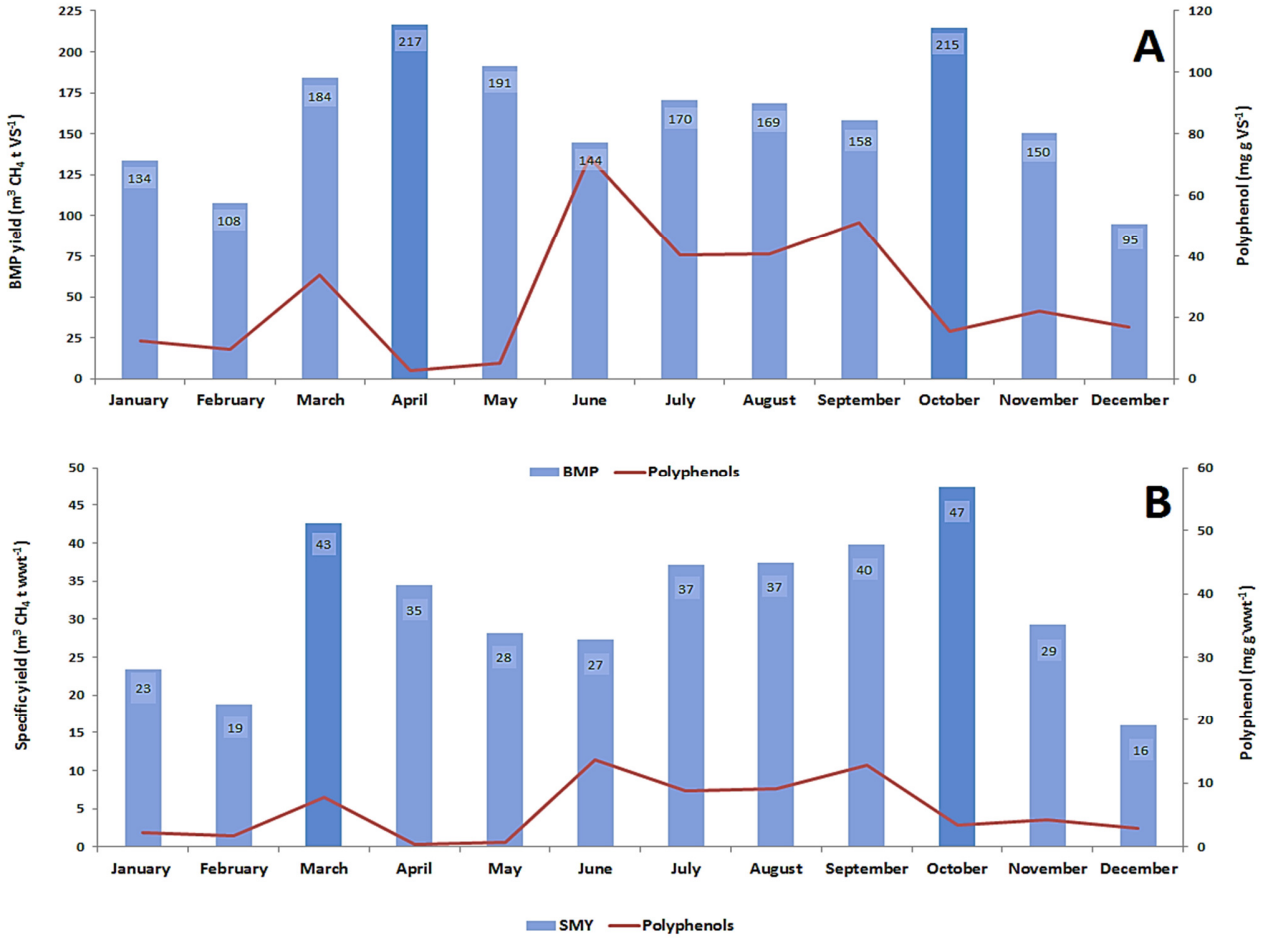

Figure 4.3 Gas yield expressed per unit mass of VS (A) and per unit mass wet weight (B).

The minimum inhibitory level reported for filamentous fungi was higher than 0.5 g L^{-1}. Yeast can resist a level of 10-20 g L^{-1} while for bacteria the inhibitory level was comparatively low (0.012-1.0 g L^{-1}) (Scalbert, 1991). Polyphenol inhibitory values extracted from *A. nodosum* (collected from Norway) were reported in the range of 0.2-1.3 g L^{-1} in the digester(Moen et al., 1997), but the study was based on harvest only in two months (April and October). In this study, the polyphenol concentration in the digester increased from 0.036 g L^{-1} (April) to 0.45 g L^{-1} (June) indicating elevated inhibitory levels for bacteria as reported by (Scalbert, 1991). Increased polyphenol levels can significantly inhibit the degradation of organic compounds (such as alginate) in *A. nodosum*, resulting in low BMP yields (Moen et al., 1997).

The polyphenol structures of *A. nodosum* may vary throughout the year (Parys et al., 2009); this may result in the different toxicity levels for the microorganisms during biogas production. Extraction of polyphenols prior to biogas production would be considered as an approach for seaweed harvested in summer months. Moreover, bio-refinery of seaweed may be set up by extraction of polyphenols and other valuable chemicals from the seaweed prior to anaerobic digestion. The BI value (Table 4.2) indicates the efficiency of degradability of the seaweed in the batch digestion. A high BI value of 0.81 for the control group (cellulose) indicates a healthy inoculum condition used in this study. The highest BI value for *A. nodosum* was observed in May (0.46), whereas the lowest value was obtained in December (0.16). Low BI values in winter may be mainly attributed to the low carbohydrate content, whereas low BI value in summer can be attributed to the high polyphenol concentration.

The high salt content of seaweed may inhibit the anaerobic digestion process (Xia et al., 2016a). Long-term continuous digestion of seaweed can lead to accumulation of salts in the reactor. In order to suggest salt tolerance level, % salinity increased during 30 days in the batch process was calculated. The values for increase in salinity were 17.0% ± 0.2%, 24.0% ± 0.10%, 26.0% ± 0.6% and 30.0% ± 0.10% in autumn (October), winter (December), spring (March) and summer (June) months, respectively. The salinity of seaweed was subtracted from the salinity of inoculum (6.8 ± 0.45 g/L) in order to calculate actual salinity increase in the batch digestion due to the seaweed only (Figure 4.4).

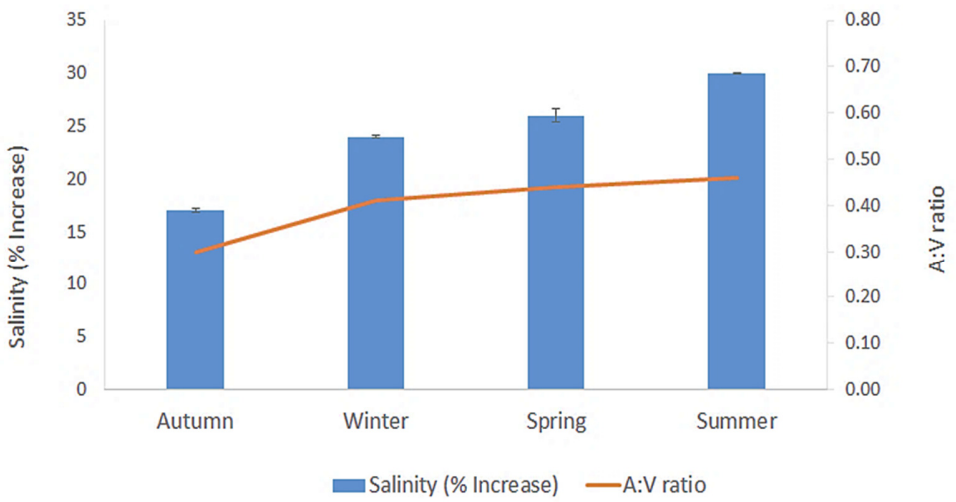

Figure 4.4 Salt accumulation in a batch reactor (autumn to summer)

Table 4.2 Biomethane yield over the period based on results of BMP analysis and theoretical analysis

Month of harvest	BMP yield ($L\ CH_4\ kg\ VS^{-1}$)	TMP ($L\ CH_4\ kg\ VS^{-1}$)	BI (BMP/TMP)	VS content ($kg\ VS\ wwt^{-1}$)	Specific yield ($m^3\ CH_4\ t\ wwt^{-1}$)	Polyphenol ($mg\ g\ TS^{-1}$)
January	134 ± 12	570	0.23	175	23	8.7
February	108 ± 2	681	0.16	174	19	6.5
March	184 ± 11	655	0.28	231	43	3.4
April	217 ± 14	497	0.44	159	35	2.1
May	191 ± 17	411	0.46	148	28	3.87
June	144 ± 4	650	0.22	189	27	49.4
July	170 ± 8	506	0.34	218	37	31.0
August	169 ± 9	576	0.29	222	37	30.2
September	158 ± 3	605	0.26	252	40	37.2
October	215 ± 9	543	0.40	220	47	12.0
November	150 ± 4	498	0.30	195	29	18.0
December	95 ± 19	606	0.16	166	16	10.0
Cellulose	335 ± 13	414	0.81	-	-	-

BMP, biomethane potential; TMP, theoretical methane potential; BI, biodegradability index; wwt, wet weight.

As expected the salinity increased with increasing A:V ratio. This can be explained by the ash content from seaweed was mainly composed of salt such as sodium chloride. As a result, the increase of ash content in seaweed would increase the dissolved salt level in the anaerobic digester, resulting in an increase in salinity.

Salinity, A:V ratio and polyphenol content had a significant effect on the gas yield. In autumn (October), the gas yield is high due to lower values of polyphenol content, salinity and A:V ratios. While, in summer (June), having higher values of all above parameters, the lowest BMP yield was recorded. Low biomethane yields were reported at high salinity levels, by others (Chen et al., 2008; Fang et al., 2011; Xia et al., 2016a). Biomethane yield can be described based on yield per unit VS or per unit wet weight (Figs. 4.3A and 4.3B). VS content (as indicated in Table 4.1) and BMP results (expressed in Table 4.2 as L CH_4 kg VS^{-1}) were used to develop the yield per unit mass (Figure 4.3B). The BMP value in April is highest when expressed per unit VS (Figure 4.3A) but is only sixth highest when expressed per unit mass wet weight (Figure 4.3B). October is the highest yielding month when expressed per unit wet weight followed by March; this emphasises the two-month harvest approach. In essence, phenol content influenced the gas yield. However, there are some exceptions where higher ash content and lower organic matter content became the influential factors (not polyphenols). For instance, in December polyphenol content was lower than in October but the gas yield was lower due to higher A:V ratio (0.30 in October and 0.41 in December). This trend was confirmed by statistical analysis that if the influence of polyphenols was not considered, the ash and VS content would become the critical factor (not polyphenols) for the gas yield.

4.3.3.2 Statistical description

The significance of the results was illuminated through the use of ANOVA. Multiple comparisons results from one-way ANOVA indicated that seaweed harvested in spring (April) and autumn (October) produced significantly more biomethane than in others ($P < 0.05$). Biomethane production was more related to dry weight ($F = 7.12$, $P < 0.012$) than wet weight ($F = 4.74$, $P < 0.035$). Ash content ($F = 2.2$, $P < 0.166$) and VS content ($F = 2.2$, $P < 0.158$) have a less significant impact than polyphenol content in the seaweed ($F = 4.72$, $P < 0.035$) on biomethane production. Moreover, there was no significant interaction (Pearson's correlation) of phenolic content with other factors as computed ($P < 0.550$ and $P < 0.570$ for Ash and VS, respectively). However, VS and ash content had a significant negative interaction with each other ($P < 0.000$), which means if one value increases the other will decrease (and vice versa) and with seasonality as well ($P < 0.037$) (if polyphenol is removed from the substrate). Therefore, in the light of statistical analysis, it can be concluded that variation in the polyphenol content was the critical factor for the biogas yield.

4.3.4 Process dynamics

The kinetic decay (k), half-life and maximum yield (Y_{max}) are the key parameters to understand the process dynamics. These parameters for the seaweed are listed in Table 4.3. All months show a good kinetic decay (k value ranged from 0.10 to 0.25). These values are comparable with *A. nodosum* (0.12) assessed by (Allen et al., 2015) and food waste (0.17) assessed by (Browne et al., 2014).

The half-life of the methane production (T_{50}) ranged from 3 to 8 days. These results were comparable with the values of Irish brown seaweeds (3-7 days) reported by (Allen

et al., 2015). The shorter half-life suggests a short retention time of less than 20 days could be appropriate for long-term continuous digestion of *A. nodosum.*

Table 4.3. The process dynamics of *A. nodosum* based on seasonal variation

Month of Harvest	K (days^{-1})	Y_{max} (L CH_4 kg VS^{-1})	T_{50} (days)	R^2
January	0.19	133	3.74	0.99
February	0.25	109	2.82	0.99
March	0.14	181	4.90	0.99
April	0.13	225	5.46	0.97
May	0.16	194	4.25	0.98
June	0.16	152	4.37	0.94
July	0.11	205	6.12	0.94
August	0.11	202	6.17	0.94
September	0.11	187	6.20	0.96
October	0.10	276	6.76	0.94
November	0.09	160	7.74	0.99
December	0.16	113	4.36	0.96
Cellulose	0.17	376	4.09	0.99

k, decay constant; Y_{max}, the maximum methane potential; T_{50} is the half-life.

4.3.5 Effect of seasonal variation on gross energy yields

Scenario 1: Based on standing crop harvest in Ireland of 30,000 t per year

A. nodosum has yet been reported as a cultivated marine crop in Ireland. This is a naturally occurring Irish seaweed and the current harvest from the standing crop is approximately 30,000 ton wet weight per year. After harvesting, the seaweed may be

ensiled for storage; ensiled *A. nodosum* can improve biomethane yield as compared to fresh *A. nodosum* by 30% (Herrmann et al., 2015).

An average car in Ireland travels approximately 15,000 km yr^{-1} (fuel efficiency of 5 L diesel per 100 km). Biomethane yield from the seaweed harvested in October (1.8 million m^3 of CH_4) has an energy equivalent of 1.8 million L of diesel and could fuel approximately 2,459 cars per year or 0.13% of the private car fleet in Ireland (Figure 4.5). In essence, 12.2 tonnes of *A. nodosum* can fuel 12 cars.

Scenario 2: Based on cultivation

Seaweed may be mass cultivated for the biofuel industry. According to Murphy et al. (2015), a future seaweed system would generate a biomass yield up to 100 t wet weight ha^{-1} yr^{-1} depending on the species of seaweed. *A. nodosum* is a naturally occurring seaweed found in shallow waters and is predominately exposed when the tide recedes; it has not been considered for farm cultivation thus far. However, Ugarte and Sharp (2011) state that yields of up to 71 t wet weight ha^{-1} yr^{-1} can be grown as a standing crop in summer in Canada. Allowing a more conservative seaweed yield of 50 t wet weight ha^{-1} yr^{-1}, the maximum gross energy yield from the seaweed in each month was calculated as shown in Figure 4.6. Selection of the optimal month for the seaweed harvesting can give three times more energy than the least beneficial month (39 GJ ha^{-1} yr^{-1} in December

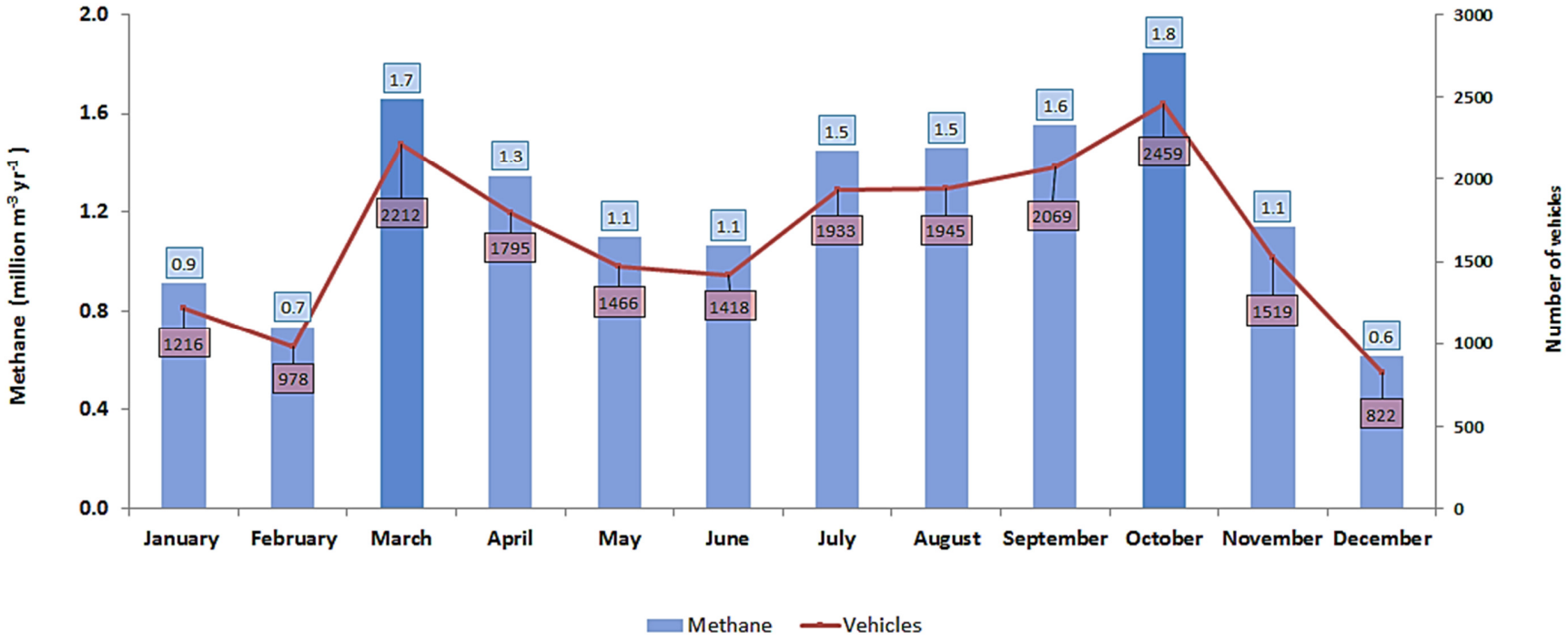

Figure 4.5 Number of vehicles fuelled from standing crop of *A. nodosum* based on annual harvest of 30,000 t in Ireland (Scenario 1)

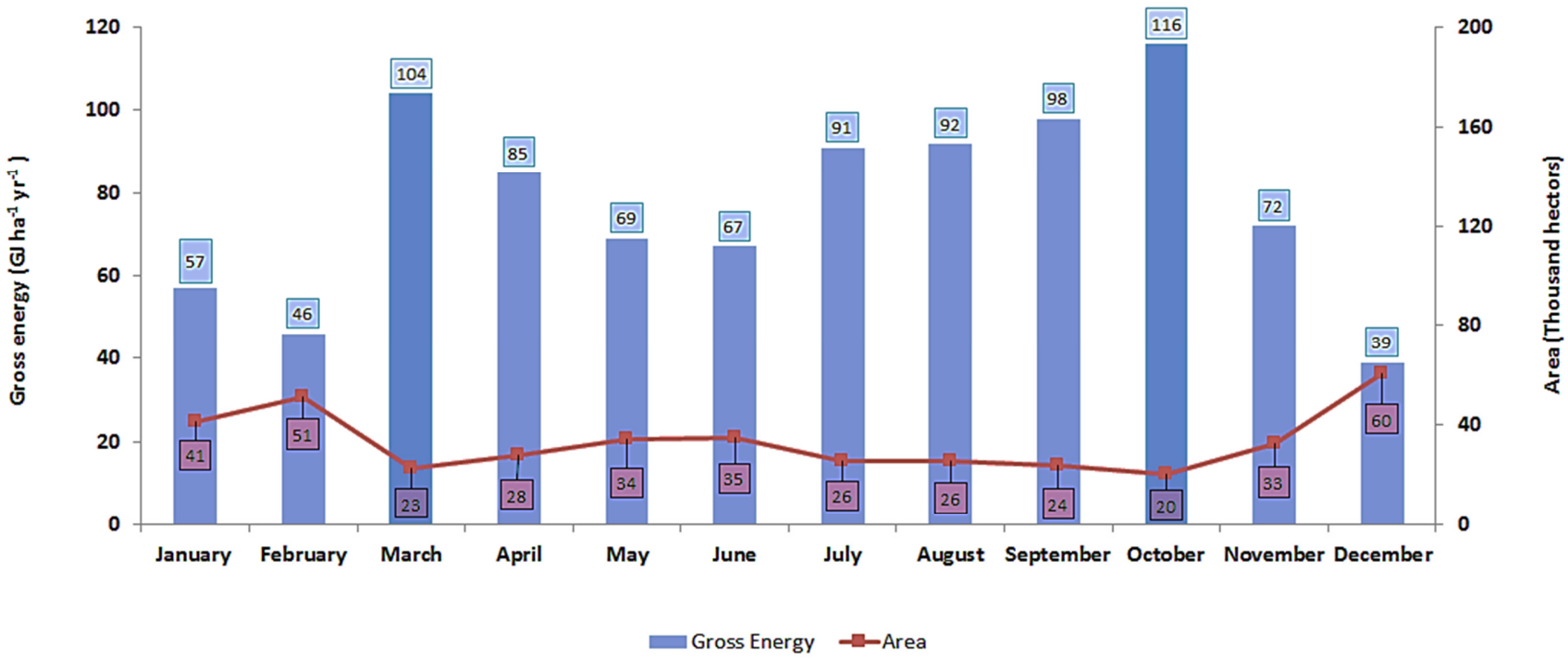

Figure 4.6 Gross energy of cultivated *A. nodosum* based on various seasons and land area required to supply 1.25% RES-T of 2.35PJ (Scenario 2)

compared to 116 GJ ha-1 yr-1 in October). These gross energy outputs may be compared with land-based biofuel systems such as wheat ethanol (66 GJ ha-1 yr-1) and rapeseed biodiesel (44 GJ ha-1 yr-1) (Allen et al., 2015). The cultivation area required to satisfy the 2020 target for renewable energy supply in transport (RES-T) for Ireland is 20,260 ha based on the optimal value in October (Figure 4.6).

4.4 Conclusions

The changes in polyphenol content of *A. nodosum* shows significant impact on biogas production. The summer months show low biomethane yield due to the accumulation of polyphenols in seaweed. The optimal biomethane yield of 217 L CH_4 kg VS^{-1} is achieved in April. The specific yield per wet weight of seaweed harvested in October (highest value) is three times higher than December (lowest value). Gross energy yields from *A. nodosum* in October may achieve 116 GJ ha^{-1} yr^{-1}. A total area of 20,260 ha for *A. nodosum* cultivation could satisfy 1.25% of renewable transport energy target in Ireland.

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5. Third generation gaseous biofuel generated through mono- and co-digestion of natural and cultivated seaweeds, with dairy slurry

Third generation gaseous biofuel generated through mono- and co-digestion of natural and cultivated seaweeds, with dairy slurry

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Abstract

The technical feasibility of long-term anaerobic mono-digestion of two brown seaweeds and co-digestion of both seaweeds with dairy slurry was investigated whilst increasing the organic loading rate (OLR). One seaweed was natural (*L. digitata*); the second seaweed (*S. Latissima*) was cultivated. Higher proportions of *L. digitata* in co-digestion (66.6%) allowed the digester to operate more efficiently (OLR of 5 kg VS m^{-3} d^{-1} achieving a specific methane yield (SMY) of 232 L CH_4 kg^{-1} VS) as compared to lower proportions (33.3%). Co-digestion of 66.6% cultivated *S. latissima*, with dairy slurry allowed a higher SMY of 252 L CH_4 kg^{-1} VS but at a lower OLR of 4 kg VS m^{-3} d^{-1}. Optimum conditions for mono-digestion of both seaweeds were effected at 4 kg VS m^{-3} d^{-1}. Chloride concentrations increased to high levels in the digestion of both seaweeds but were not detrimental to operation.

Keywords: *L. digitata*; *S. latissima*, Continuous digestion; Biomethane

5.1 Introduction

The Renewable Energy Directive (European Parliment, 2015) has fixed a target of 10% of energy use in transport to be renewable by 2020 (Murphy et al., 2013). In 2015, it was proposed that advanced third-generation biofuels (such as seaweed) represent at least 1.25% of renewable energy supply in transport (RES-T) (European Parliment, 2015). Ireland's forecasted energy in transport for 2020 is 188 PJ (Murphy and Thamsiriroj, 2011), thus 2.35 PJ should originate from advanced biofuels.

Biofuels from seaweed (macroalgae) are referred to as third-generation biofuels as they do not require arable land. Hence, they do not fall into the food v. fuel debate as compared to land based energy crops. Additionally, the gross energy yield of seaweed per hectare per annum has been shown to be high when compared amongst other feedstock. For instance, rapeseed biodiesel generates approximately 44 GJ ha^{-1} yr^{-1} (Thamsiriroj and Murphy, 2009) while willow derived biomethane generates approximately 130 GJ ha^{-1} yr^{-1} (Gallagher and Murphy, 2013). The gross energy yield from seaweed has been reported to be as high as 199 to 365 GJ ha^{-1} yr^{-1} (Allen et al., 2015; Tabassum et al., 2016a) depending upon the species, location and seasonal variation of the seaweed (Tabassum et al., 2016b; Tabassum et al., 2016c).

The temperate climate and long coastline (7500 km) provide a large resource of seaweed in Ireland (Allen et al., 2015). Typical brown seaweeds in Ireland (such as *L. digitata* and *S. latissima*) are rich in organic matter. Ireland is one of the largest producers of seaweed in Europe, producing 29,500 t yr^{-1}, equivalent to 13% of the total European harvest (Murphy et al., 2013). At present, seaweed is harvested from natural stocks (not cultivated) typically for food and not for biofuels (Burton, 2009).

Potential exists for brown seaweed to be cultivated. Cultivation can be coupled with salmon farms as part of an integrated multi-trophic aquaculture system. This is a system whereby seaweeds can be cultivated by sequestering nutrients from fish farm waste and subsequently used as a feedstock for biogas production (Jacob et al., 2016). Up to 46 new sites for salmon farms have been identified in Ireland and a 454 ha salmon farm is already planned for the West of Ireland by the Irish Sea Fisheries Board (BIM, 2012). Cultivation of seaweed may be beneficial due to its multipurpose industrial applications and it has been reported that utilising existing infrastructure (salmon/mussel farms) is the most economic method for seaweed farming (Watson, 2013). A range of 40-150 tons wet weight ha^{-1} a^{-1} of brown seaweed is achievable in cultivation (Broch et al., 2013; Tabassum et al., 2016b; Watson, 2013) and such methods may potentially be more practical than the harvest of natural seaweed.

Ireland also has an abundance of dairy slurry. It has been reported that ca. 7.07 M t wwt (assuming approximately 1 million dairy cows, producing 0.33 m^3 of slurry per week with 20 weeks storage) of the dairy slurry is generated each year as a result of Ireland's intensive agricultural industry (Wall et al., 2013). Such a resource offers a great potential as a co-substrate in anaerobic digestion (AD) facilities. Currently, there a very small commercialised biogas industry in Ireland. Thus, potential exists to combine abundant dairy and marine feedstocks to establish a biogas industry that could boost the rural coastal economy and provide a third generation renewable transport fuel or renewable heat source in the form of biomethane.

Biomethane, bio-hydrogen, bioethanol and biodiesel may be derived from seaweed. However, apart from biomethane, the aforementioned technologies have proved difficult

in achieving economic feasibility (Tabassum et al., 2016b). Methane production via anaerobic digestion is a cost effective and commercially established technology (Hinks et al., 2013). According to IEA bioenergy task 37 reports, Germany had approximately 10,000 biogas plants including 8,000 agricultural biogas plants (IEA, 2015).

The scientific literature is relatively sparse for biomethane production from seaweeds, in particular, continuous anaerobic digestion of brown seaweeds (*L. digitata* and *S. latissima*). The majority of previous work investigated the biomethane potential at laboratory batch scale (Allen et al., 2015; Vanegas and Bartlett, 2013), which by their nature do not provide validation for the operating conditions in continuous digestion. Continuous co-digestion of seaweed with slurry has been reported in various studies (Allen et al., 2014; Peu et al., 2011; Sarker et al., 2012; Sarker et al., 2014), however, these studies did not establish the optimisation of key parameters such as the best co-digestion mix, the maximum organic loading rate (OLR), and minimum hydraulic retention time (HRT) for brown seaweeds. Allen et al. (2014a) investigated *U. lactuca*, a green seaweed rich in sulphur with a low carbon to nitrogen ratio (C:N); this seaweed is very different to brown seaweed. Co-digestion of dairy slurry (59% on a VS basis) with *L. digitata* (41% on a VS basis) in a continuous system at a maximum OLR of 2.9 kg VS m^{-3} d^{-1} was reported achieving a specific methane yield (SMY) of 139 ± 14 L CH_4 kg^{-1} VS; this study was only run for one HRT and as such did not investigate long-term digestion whilst increasing the OLR, nor did it vary the co-digestion mix (Sarker et al., 2014). A biogas production from *S. latissima* (wild harvest) was not found suitable due to the accumulation of sodium and potassium cations in digestion (Jard et al., 2012). The study recommended the use of farm cultivated *S. latissima* over wild harvest *S. latissima*

for biogas production as the possibility of accumulation of divalent ions may be reduced in younger plants. An essential parameter of the digestion process is the C:N ratio with optimum values reported in the range of 20-30 (Murphy et al., 2015). Such values can be obtained for seaweed if harvested at the correct time of year (Tabassum et al., 2016a). Farm cultivated seaweed has been reported to have a lower C:N ratio (ca. 15:1) as compared to wild species of brown seaweed (24:1 and 27:1 for *S. latissima* and *L. digitata*, respectively) (Allen et al., 2015; Tabassum et al., 2016a). Co-digestion with slurry can potentially optimise the C:N ratio and also enhance digestibility by providing important nutrients present in the slurry (Yangin-Gomec and Ozturk, 2013).

The innovation in this paper is that it is the first to investigate long-term continuous digestion of two carbohydrate-rich brown seaweeds *L. digitata* and *S. latissima* whilst increasing the OLR. The *L. digitata* was sourced from natural stock while the *S. latissima* was farm cultivated and is a younger plant. Profiles for various key digestion parameters such as volatile fatty acids, chloride concentration, FOS:TAC and TAN were developed. A co-substrate of the dairy slurry was used to assess for co-digestion. The objectives of the study were to compare and examine the:

(1) the potential for mono-digestion of both *L. digitata* (naturally occurring) and *S. latissima (cultivated);*

(2) effect of increasing the percentage of seaweed in co-digestion with the dairy slurry;

(3) optimal organic loading rate (OLR) for mono-digestion and co-digestion of both seaweeds;

(4) critical parameters which can inhibit the digestion process.

5.2 Materials and Methods

5.2.1 Substrates and inoculum

Approximately 250 kg of beach-cast *L. digitata* and 80 kg of farm cultivated *S. latissima* (harvested after four months) was sourced from a marine research facility in Bantry, West Cork, Ireland. Both seaweeds were washed with tap water, macerated to a particle size of less than 4mm, packed in sealed plastic bags (1.5kg in each bag) and stored at -20 ^{0}C until experimental use. Approximately 150 kg of the fresh dairy slurry was collected from a dairy farm, in Cork, Ireland. The slurry was stored in 25 L drums at -20 ^{0}C until required. The characteristics of both seaweeds and dairy slurry are listed in Table 5.1.

The inoculum used originated from an existing digester in Ireland that treated a combination of grease trap waste and slurry. In preparation, the inoculum was sieved through a 2 mm sieve to remove any larger particles and subsequently the reactors were filled. The sieved inoculum remained in all reactors for one week at 37^{0}C to remove any residual gas.

5.2.2 Analytical and chemical methods

Proximate analysis (total solids (TS), volatile solids (VS) and ash content) was analysed and calculated using standard methods (APHA, 2011). The pH was measured using a Jenway 3510 pH probe. The ratio of organic acid concentration to total inorganic carbon (FOS:TAC) was measured by a titration method (Nordmann titration method) using 0.1 N sulphuric acid with pH 5.0 and pH 4.4 as endpoints. TAN in each reactor was measured using Hach Lange cuvettes (LCK 303) coupled with a Hach Lange DR3900 spectrophotometer. Free ammonia (NH_3) was calculated by a standardised equation that

utilised the calculated TAN content with reference to pH and temperature (Banks and Heaven, 2013). Accumulation of total volatile fatty acid (tVFA) in each digester was measured using gas chromatography (Agilent HP 6890 Series) with a Nukol™ fused silica capillary column (30 m x 0.25 mm x 0.25 µm), argon as a carrier gas and flame ionisation detector (FID). Each digester was tested for acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, caproic and enanthic acid, twice a month. Samples for ultimate analysis (C, H N, O) were prepared by drying at 105^0C for 24 h and were ground to less than 0.5 mm particle size. Ultimate analysis of each substrate was carried out using an EAC CE 4500 elemental analyser. Hach Lange cuvettes (LCK 311) were used to determine chloride levels to evaluate the salt accumulation in each reactor. Biogas was analysed for methane, carbon dioxide, oxygen and nitrogen via an Agilent 6890 GC equipped with a Hayesep R packed GC column (3m x 2mm, mesh range of 80-100) and a thermal conductivity detector with Argon as the carrier gas.

5.2.3 Biomethane potential (BMP) assays

Bioprocess Control's AMPTS II system was used to assess the biomethane potential. Glass bottles (total volume 650 mL, working volume 400 mL) were used as batch digestion vessels having a semi-continuous stirring system operating at 45 rpm. The BMP assays were carried out in triplicate operated at 37^0C and included a cellulose standard and inoculum control. The inoculum to substrate ratio was set at 2:1 on VS basis (Angelidaki et al., 2009). Nitrogen gas was introduced to flush the headspace of each vessel to create anaerobic conditions prior to start up. Biogas produced in the bottles was passed through a 3M NaOH solution to remove carbon dioxide and any other trace gases. The resultant methane passed through to a flow measurement device,

which operates by water displacement. The gas was recorded continuously and automatically adjusted for standard temperature and pressure. The BMP was calculated as the sum of the biomethane volume after 30 days, minus the biomethane generated from the inoculum, with reference to the initial VS added. All assays were conducted in triplicate. The ultimate analysis provided the theoretical methane potential (TMP) of each substrate by applying the Buswell equation (Symons and Buswell, 1933). From this, the biodegradability index could be determined. The biodegradability index is defined as the ratio of the BMP to the Buswell TMP. Higher biodegradability indices corresponded to higher digestion efficiencies.

5.2.4 Continuous digestion system

Seven continuously stirred tank reactors (CSTRs) were used in the study. Each reactor was manufactured from PVC with a total volume of 5 L and a working volume of 4 L. Each reactor had a 25 mm diameter circular inlet feeding port, which was sealed by a rubber bung. A gas outlet was positioned at the top of the reactor and connected to a wet tip gas meter, which measured the gas production. Mixing was provided by a vertically mounted stirrer operating at 40 rpm, powered by a 12 V dc motor. The temperature was maintained at 37 ± 1^{0}C by heated water circulating continuously through brass coils around the reactors. A Labjack data recorder was used to count the number of tips from the gas-tipping device (each having a known volume). Gas volumes for each reactor were corrected for standard temperature and pressure at 0^{0}C and 101.325 kPa. A 5 L Tedlar gasbag was used to collect biogas from the gas outlet of the tipping device for subsequent biogas analysis.

5.2.5 Operation of continuous system

The 7 CSTRs used to assess the feasibility of mono-digestion of dairy slurry, mono-digestion both of the seaweeds and co-digestion of the seaweeds with dairy slurry were named as follows; DS (100% dairy slurry), LD_{33} (33.3% *L. digitata*, 66.6% dairy slurry), LD_{66} (66.6% *L. digitata*, 33.3% dairy slurry), LD_{100} (100% *L. digitata*), SL_{33} (33.3% *S. latissima*, 66.6% dairy slurry), SL_{66} (66.6% *S. latissima*, 33.3% dairy slurry) and SL_{100} (100% *S. latissima*).

The BMP yields evaluated from the batch BMP assays were set as target yields in the continuous digestion process. A parameter for biomethane efficiency (B.EF) was determined by dividing the SMY of each CSTR obtained in the continuous digestion trials by its respective BMP yield. Each reactor had an initial commissioning period of 4 weeks prior to start-up. The CSTRs started at an OLR of 2 kg VS m^{-3} d^{-1} and were run for at least two HRTs at each OLR to ensure that data could be collected at times when the reactors were considered stable. The initial HRT of each OLR was considered an acclimatisation period for the microbial consortia in each reactor, that is, facilitating the systems to reach steady state conditions to attain stable gas yields at the new imposed OLR. A wet weight quantity was calculated based on VS for the substrates and fed to each reactor to provide the required OLR. The wet weight quantity was also used to calculate an initial HRT. A calculated amount of sieved digestate liquor was recirculated only to reactors LD_{66} and LD_{100} as the TS content of the feed was higher than 10%. This was in order to ensure efficient mixing within the reactor. With respect to data obtained from the FOS:TAC ratio and biomethane efficiency, the OLR was increased gradually in a stepwise fashion on achieving process stability.

5.3 Results and Discussion

5.3.1 Batch and continuous digestion trials

A summary of each mix for *L. digitata and S. latissima* (including cellulose positive control) and the BMP yield attained in the batch digestion trials are shown in Table 5.2. Two BMP values are reported, allowing for both an initial inoculum (from an initial BMP) and an 'acclimatised' inoculum (from a second BMP named "BMP*"). The acclimatised inoculum represented digestate taken from the continuous trials used in a BMP undertaken when steady state conditions were in place after 14 weeks of operation (at OLR 3-4). With the acclimatised inoculum it can be seen that the yields from the BMP increased. This highlights the importance of using an acclimatised inoculum in conducting BMP assays. The acclimatised inoculum had little impact on BMP values obtained from the reactors with no or low seaweed (DS, LD_{33}, and SL_{33}).

Table 5.2 also documents the C:N ratio, TMP, and biodegradability index for each mix along with pro-rata yields (from mono-digestion) to seek for any potential synergy in co-digestion. However, pro-rata yields indicated no synergistic effects in co-digestion as evident by small negative % differences when compared to the BMP yields (Table 5.2). The biodegradability index increased with increasing proportions of seaweed for both *L. digitata* and *S. latissima*. The lowest biodegradability index was recorded for mono-digestion of the dairy slurry at 0.27. Figures 5.1 and 5.2 show the SMY and FOS:TAC for co-digestion and mono-digestion, respectively, of both seaweeds from the continuous digestion trials over the various OLRs tested. The key process parameters in continuous digestion (HRT, biomethane efficiency, TAN, methane composition, FOS:TAC and tVFA) for *L. digitata* (reactors LD_{33}-LD_{100}) are listed in Table 5.3.

Table 5.1 Characteristics of substrates for batch and continuous digestion

Characteristics	Dairy Slurry	*S. latissima*	*L. digitata*
Total solids (%wwt)	7.00 (0.15)	9.21 (0.27)	17.66 (0.34)
Volatile solids (%wwt)	5.60 (0.10)	5.27 (0.16)	14.42 (0.21)
Ash (% of TS)	20.23 (0.62)	42.80 (0.81)	18.31 (0.66)
C % (% of TS)	40.60 (0.18)	27.85 (0.16)	33.45 (0.22)
H % (% of TS)	5.32 (0.14)	3.58 (0.02)	4.71 (0.01)
N % (% of TS)	2.53 (0.10)	1.80 (0.28)	1.22 (0.05)
O % (% of TS)	31.32 (0.33)	24.02 (0.51)	42.31 (0.77)
C:N	16 (0.56)	15.84 (1.37)	27.42 (1.25)

Standard deviation is in parentheses

Table 5.2 Batch digestion results

Reactors	C:N (Ratio)	TMP (L CH_4 kg VS^{-1})	BMP (L CH_4 kg VS^{-1})	BMP* (L CH_4 kg VS^{-1})	Pro-rata (L CH_4 kg VS^{-1})	Difference (%)	BI
DS (100% Dairy slurry)	16.01	542	142 (2)	145 (2)	---	---	0.27
LD_{33} (33.3% *L. digitata*)	19.61	479	163 (7)	166 (7)	191	-13	0.35
LD_{66} (66.3% *L. digitata*)	23.40	422	197 (7)	231 (18)	238	-3.0	0.55
LD_{100} (100% *L. digitata*)	27.45	368	267 (7)	288 (13)	---	---	0.78
SL_{33} (33.3% *S. latissima*)	15.80	523	174 (7)	179 (10)	193	-7.0	0.34
SL_{66} (66.6% *S. latissima*)	15.70	509	218 (7)	246 (08)	243	1.2	0.48
SL_{100} (100% *S. latissima*)	15.80	500	258 (20)	296 (09)	---	---	0.60
Cellulose (control)	---	---	323 (04)	336 (16	---	---	---

BMP and BMP * are the yields using un-acclimatised and acclimatised inoculum, respectively. Biodegradability index (BI) was calculated by BMP*/TMP. Standard deviation is in parentheses

The same parameters for *S. latissima* (reactors SL_{33}-SL_{100}) are shown in Table 5.4. All data represents average values taken from the final HRT at each particular OLR. Figure 5.3 shows the accumulation of chloride over the lifetime of the continuous trials for all CSTRs; the green, grey, yellow and red colours signify the increase in OLR from 2 to 5 kg VS m^{-3} d^{-1} for each reactor. For LD_{66}, the OLR of 6 kg VS m^{-3} d^{-1} was signified by black, this was the only digester to operate at this OLR.

5.3.2 Mono-digestion of dairy slurry

The OLR for the reactor fed with 100% dairy slurry (DS) was increased from 2 to 4 kg VS m^{-3} d^{-1} over the lifetime of the reactor. Biomethane efficiency (SMY/BMP) was found to be 0.87, 0.95 and 0.38 for OLR 2, OLR 3 and OLR 4, respectively (Table 5.3). The same trend in biomethane efficiency was reported by (Wall et al., 2014). A greater biomethane efficiency (0.95) was obtained at OLR 3 by keeping the HRT for more than 20 days as suggested in previous studies (Wall et al., 2014). Biomethane efficiency dropped to 0.38 at OLR 4 due to the high loading rate and corresponding shorter retention time.

Methane composition in the biogas and SMY were both highest at OLR 3 at 57% and 138 L CH_4 kg VS^{-1}, respectively. These values dropped significantly when the OLR was increased to 4 kg VS m^{-3} d^{-1} (38% and 55 L CH_4 kg VS^{-1}). The average pH was observed in the range 7.3-7.4 at all OLRs. The TAN and FOS:TAC values were found within an acceptable range at all OLRs (Table 5.3). The TAN range peaked at 1.52 g L^{-1} and maximum values of FOS:TAC increased to 0.30 at OLR 3. However, these values indicated stable operation of the reactors at the various OLRs. The SMY was similar to

the BMP value obtained at an optimum OLR of 3 kg VS m^{-3} d^{-1} thereby illustrating the highest biomethane efficiency.

Due to high moisture content and short retention time (14 days) at a high loading rate of 4 kg VS m^{-3} d^{-1}, it was assumed that microbial washout was responsible for the drop off in SMY. Higher SMYs are conceivable in stable operation with longer retention times (in excess of 20 days) at the optimum OLR of 3 kg VS m^{-3} d^{-1}. Thus, mono-digestion of the dairy slurry was not deemed feasible above OLR 3. Chloride levels ranged from 1.5-3.0 g L^{-1} throughout the operation of the reactor.

5.3.3 Co-digestion at 33.3% seaweed and 66.6% dairy slurry

***L. digitata* (LD$_{33}$)**

LD$_{33}$ started at an OLR of 2 kg VS m^{-3} d^{-1} and was increased to an OLR of 5 kg VS m^{-3} d^{-1}. Co-digestion of *L. digitata* and dairy slurry operated efficiently up to an OLR of 4 kg VS m^{-3} d^{-1} over 30 weeks. This illustrated increased digester performance as compared to the 100% dairy slurry reactor, which failed at an OLR of 3 kg VS m^{-3} d^{-1}. The reactor's biomethane efficiency values were 1.02, 1.01 and 0.95 for OLR 2, OLR 3 and OLR 4, respectively (Table 5.3). At this range of OLR, the average pH was adequate at 7.4-7.5. The biomethane efficiency dropped significantly to 0.30 when the reactor increased to an OLR 5 due to the very high loading rate; this was followed by a drop in pH to 6.7. Biomethane composition was highest at 59% at OLR 3. The SMY was stable from OLR 2 to OLR 3 (168-170 L CH_4 kg VS^{-1}) but decreased marginally at OLR 4 (158 L CH_4 kg VS^{-1}). Both TAN and FOS:TAC values were adequate and did not increase above inhibitive threshold values for OLR 2-4 (Table 5.3).

Table 5.3 Key parameter results from continuous digestion of *L. digitata*

OLR kg VS m^{-3} d^{-1}	SMY (L CH_4 kg VS^{-1})	Biomethane efficiency (SMY/BMP)	CH_4 (%)	HRT (days)	FOS:TAC	TAN (g L^{-1})	pH
		DS (100% Dairy slurry)					
2	127	0.87	54	28	0.24	1.02	7.4
3	138	0.95	57	18	0.30	1.52	7.4
4	55	0.38	38	14	0.20	0.85	7.3
		LD_{33} (co-digesting 33.3% L. digitata, 66.6% dairy slurry)					
2	170	1.02	53	35	0.28	1.34	7.5
3	168	1.01	59	23	0.17	0.73	7.4
4	158	0.95	58	18	0.28	0.86	7.4
5	49	0.30	44	17	0.84	0.49	6.7
		LD_{66} (co-digesting 66.6% L. digitata, 33.3% dairy slurry)					
2	253	1.10	53	37	0.21	1.44	7.5
3	246	1.06	56	24	0.22	0.50	7.4
4	261	1.13	55	18	0.19	0.50	7.5
5	232	1.00	57	14	0.14	0.54	7.4
6	24	0.10	12	12	1.25	0.50	6.4
		LD_{100} (100% L. digitata)					
2	338	1.17	50	30	0.23	1.50	7.5
3	302	1.05	57	17	0.12	1.01	7.6
4	281	0.98	56	12	0.16	0.38	7.5
5	26	0.09	18	11	0.81	0.50	4.5

SMY: specific methane yield; BMP: biomethane potential; HRT: hydraulic retention time; FOS:TAC: the ratio of organic acid concentration to total inorganic carbon (max value); TAN: total ammonia nitrogen content; tVOA: total volatile organic acid content (max value).

However, at OLR 5, the reactor was severely inhibited and failed as the FOS:TAC value (0.84) increased above stability range (0.2-0.4) (Allen et al., 2014). This signified an accumulation of tVFAs in the reactor (Figure 5.4.A). LD_{33} proceeded to fail as indicated by low SMY, increased FOS:TAC value and a large accumulation of VFAs. Chloride concentration ranged from 1.5-7.0 g L^{-1} throughout the lifetime of the reactor.

***S. latissima* (SL$_{33}$)**

SL_{33} operated from an OLR 2 to an OLR of 5 kg VS m^{-3} d^{-1}. Biomethane efficiency was 0.92 at the initial OLR and started to drop gradually until failure of the reactor at an OLR of 5 kg VS m^{-3} d^{-1} (Table 5.4). A similar trend was observed for pH as to that of LD_{33} as values of 7.4-7.5 were evident up to OLR 4 but subsequently dropped at OLR 5 kg VS m^{-3} d^{-1} (Table 5.4).

Methane composition in the biogas was highest at 57% at OLR 3. A general trend of decreasing SMY was observed from OLR 2 to OLR 5 (165 to 53 L CH_4 kg VS^{-1}), with a significant reduction occurring at OLR 4. TAN and FOS:TAC values did not increase above inhibitory threshold values up to OLR 4 (Table 5.4). At OLR 5, TAN levels were higher for SL_{33} than that evident in LD_{33} (at the same OLR) potentially due to the lower C:N ratio associated with farm cultivated *S. latissima*, although values still remained low (<1 g^{-1}). The TAN ranged from 0.68 g L^{-1} to 1.24 g L^{-1} from OLR 2-4 and the average FOS:TAC value was 0.25 at OLR 4. The reactor became inhibited at OLR 5 as the FOS:TAC value increased, again signifying a build-up of tVFAs in the reactor (Figure 5.4.B). Hence, the reactor failed and the SMY diminished significantly. Chloride levels were within acceptable range (6.57 gL^{-1}) up to OLR 4 but increased sharply (12 gL^{-1}) at OLR 5 (Figure 5.3.B).

5.3.4 Co-digestion at 66.6% seaweed and 33.3% dairy slurry

***L. digitata* (LD_{66})**

LD_{66} started at an OLR of 2 kg VS m^{-3} d^{-1} but the TS content of the feed was in excess of 10%. This required a calculated amount of digestate liquor to be recirculated to keep the solids content below 10% in the CSTR to allow effective mixing to take place. It has previously been reported that recirculation of digestate liquor can facilitate lower FOS:TAC values and allow for shorter HRT (Wall et al., 2014). Biomethane efficiencies of 1.0, 1.06, 1.13 and 1.0 were observed as the OLR increased from 2 - 5 kg VS m^{-3} d^{-1} (Table 5.3). Biomethane efficiency was reported at a maximum even at a very short HRT (14 days) which can potentially be attributed to recirculation of the digestate liquor and higher C:N ratio of LD_{66} (23.40) as compared to LD_{33} (C:N=19.61). Biomethane efficiency dropped substantially to 0.10 at OLR 6 due to the accumulation of VFAs. The average pH was observed in the range 7.4-7.5 up to OLR 5 and dropped to 6.4 at OLR 6. Methane composition in the biogas of 53% at OLR 2 had improved to 57% by OLR 5 (Table 5.3). Average TAN and FOS:TAC values were observed to be low in comparison to the CSTRs that contained a lower concentration of seaweed; this was noteworthy particularly as the reactor was operating at a very high OLR of 5 kg VS m^{-3} d^{-1}. At OLR 6, the reactor failed as the FOS:TAC values (1.25) were far in advance of critical levels. Chloride levels were again found to have gradually increased as the OLR increased and concentrations were higher than reported for LD_{33} due to higher seaweed content (Figure 5.3.A).

In essence, LD_{66} can be considered a more suitable co-digestion mix than LD_{33} as it exhibited a very low level of VFA accumulation throughout digestion to a high OLR of

5 kg VS m^{-3} d^{-1} (Figure 5.4.A) and achieved higher SMYs (Table 5.3). Only at an OLR of 6 kg VS m^{-3} d^{-1} did the concentration of VFAs become critical (Figure 5.4.A). Reactor failure occurred at OLR 6 as indicated by very low SMYs.

***S. latissima* (SL$_{66}$)**

SL$_{66}$ operated from an OLR of 2 kg VS m^{-3} d^{-1} to an OLR of 5 kg VS m^{-3} d^{-1}. Biomethane efficiencies remained at near maximum at OLRs of 2, 3, and 4 kg VS m^{-3} d^{-1} (Table 5.4). However, the efficiency dropped very sharply as the OLR increased from 4 to 5 kg VS m^{-3} d^{-1} (Table 5.4). The average pH was recorded at 7.4 up to OLR 4 but subsequently dropped to 5.9 at OLR 5. Methane composition in the biogas was 55% at OLR 2 and increased to 57% at higher OLRs of 3 and 4 (Table 5.4).

TAN values were marginally lower as compared to SL$_{33}$ with the higher proportion of seaweed (Table 5.4). The FOS:TAC values were below the critical levels up to OLR 4 but the reactor failed at OLR 5 as the FOS:TAC values (2.01) were much higher than critical levels. Comparatively, chloride levels (9.71 gL^{-1}) were higher than SL$_{33}$ (6.57 gL^{-1}) due to higher seaweed proportion in the mixture (Figure 5.3.B).

SL$_{66}$ maintained maximum biomethane efficiency at OLR 4 (Table 5.4) and thus was deemed potentially more suitable as a co-digestion mixture than SL$_{33}$ as the SMY was much higher for SL$_{66}$ than SL$_{33}$.

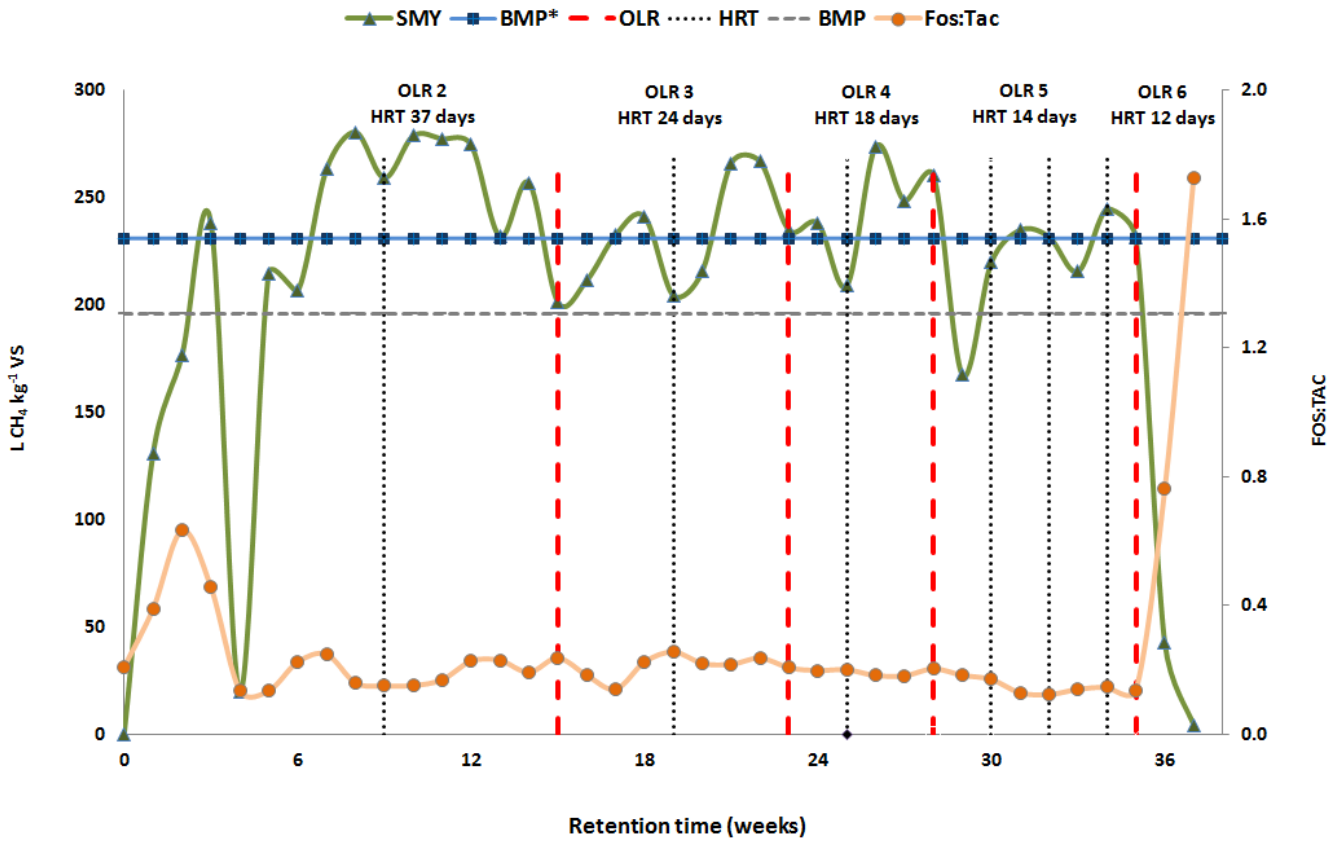

Figure 5.1.A Co-digestion of 66.6% *L. digitata* with 33.3% dairy slurry: Specific methane yields and FOS:TAC with increasing organic loading rate

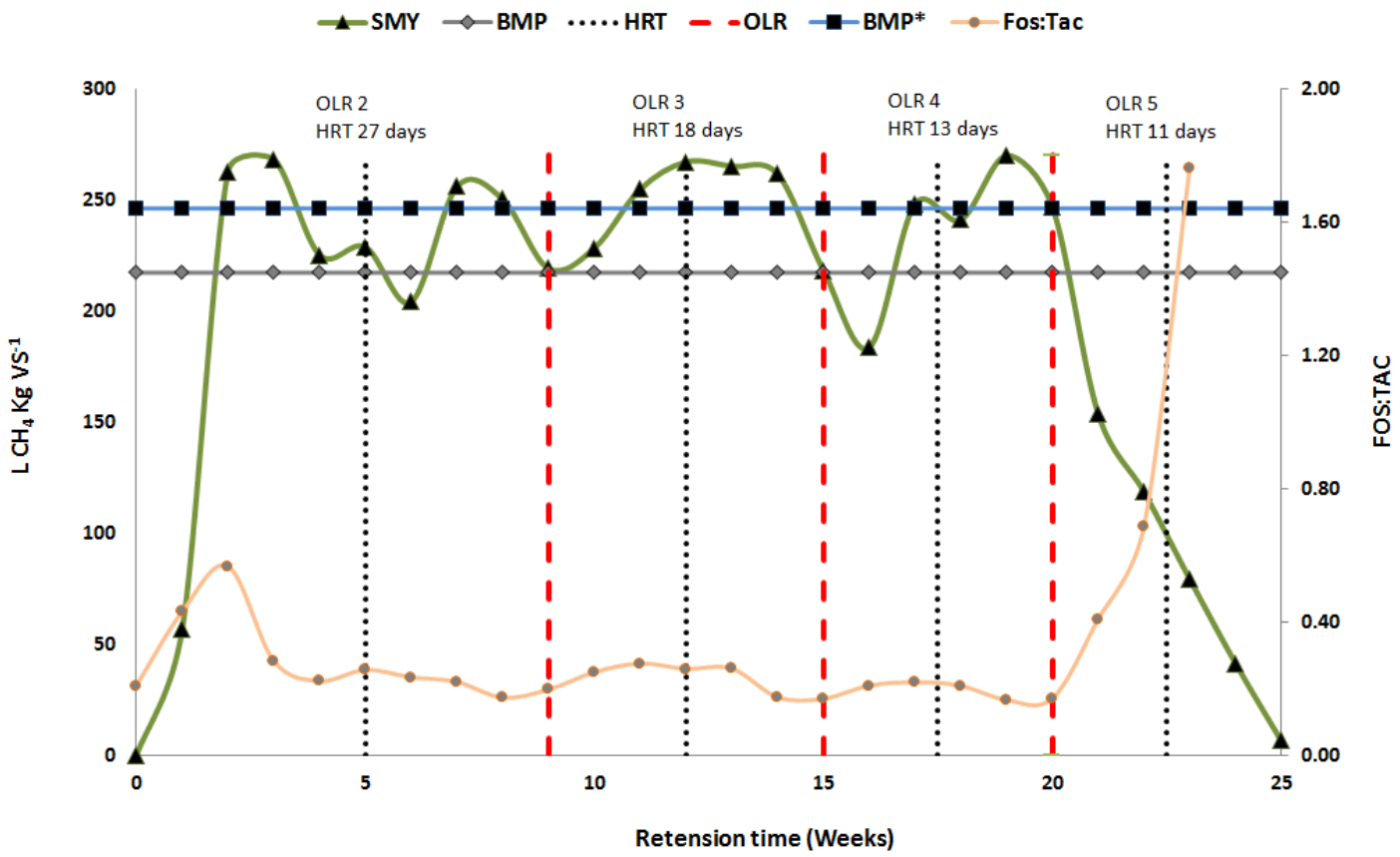

Figure 5.1.B Co-digestion of 66.6% *S. latissima* with 33.3% dairy slurry: Specific methane yields and FOS:TAC with increasing organic loading rate

Specific methane yield (SMY), biomethane potential before acclimatization (BMP), after acclimatization (BMP*), and the fermentation stability (FOS:TAC). Vertical darker lines indicate changes in organic loading rate (OLR), vertical small dashed lines indicate retention times (HRTs).

5.3.5 Mono-digestion of seaweeds

***L. digitata* (LD_{100})**

The general trend in biomethane efficiency for LD_{100} was to decrease as the OLR increased from 2 to 4 kg VS m^{-3} d^{-1} (Table 5.3). Biomethane efficiency dropped to 0.09 at OLR 5 due to the high loading rate that resulted in an accumulation of tVFAs and a corresponding increase in FOS:TAC. Even at a very short HRT (12 days) at OLR 4, biomethane efficiency was close to maximum, likely due to better acclimatization in the reactor as a result of digestate liquor recirculation. The average pH observed was 7.5 at an OLR of 4 kg VS m^{-3} d^{-1} but this dropped to 4.5 at OLR 5, which inhibited methanogenesis.

Methane composition in the biogas improved from 50% to 56% as the OLR increased from 2 to 4 kg VS m^{-3} d^{-1} (Table 5.3). Maximum TAN and FOS:TAC values were again not above the critical thresholds for inhibition at this OLR (Table 5.3). However, FOS:TAC values rapidly increased to 0.81 as the OLR increased to 5 kg VS m^{-3} d^{-1} and led to reactor failure (Figure 5.2.A). Chloride levels in LD_{100} were highest among all *L. digitata* reactors (Figure 5.3.A). Concentrations in excess of 10 g L^{-1} were recorded, significantly higher than that of LD_{66} at ca. 6 g L^{-1}. Although difficult to define, such high concentrations have been shown to inhibit methane production (Herrmann et al., 2016).

***S. latissima* (SL_{100})**

Mono-digestion of *S. latissima* was operated from an OLR of 2 kg VS m^{-3} d^{-1} to 5 kg VS m^{-3} d^{-1}. Biomethane efficiency remained above maximum up to the optimum OLR of 4

Table 5.4 Key parameter results from continuous digestion of *S. latissima*

OLR (kg VS m^{-3} d^{-1})	SMY (L CH_4 kg VS^{-1})	Biomethane efficiency (SMY/BMP)	CH_4 (%)	HRT (days)	FOS:TAC	TAN (g L^{-1})	pH
		SL_{33} (co-digesting 33.3% S. latissima, 66.6% dairy slurry)					
2	165	0.92	56	27	0.20	0.68	7.5
3	156	0.87	57	18	0.22	0.93	7.4
4	103	0.58	55	14	0.25	1.24	7.4
5	53	0.30	22	11	2.86	1.08	6.8
		SL_{66} (co-digesting 66.6% S. latissima, 33.3% dairy slurry)					
2	232	0.94	55	27	0.22	0.70	7.4
3	248	1.01	57	18	0.20	0.81	7.4
4	252	1.03	57	13	0.18	0.89	7.4
5	60	0.25	23	11	2.01	0.88	5.9
		SL_{100} (100% S. latissima)					
2	298	1.01	52	26	0.17	0.72	7.5
3	343	1.16	52	18	0.24	0.72	7.4
4	330	1.12	53	13	0.26	0.65	7.4
5	61	0.21	16	11	1.20	0.61	5.6

SMY: specific methane yield; BMP: biomethane potential; HRT: hydraulic retention time; FOS:TAC: ratio of organic acid concentration to total inorganic carbon (max value); TAN: total ammonia nitrogen content

kg VS m^{-3} d^{-1} (Table 5.4) and only dropped to 0.21 at OLR 5 due to a build-up of tVFAs. The pH profile ranged from 7.4 to 7.5 up to an OLR of 4 kg VS m^{-3} d^{-1} and dropped to 5.6 at OLR 5, too low for effective digestion. Methane composition in the biogas was consistent up to OLR 4 at 52 to 53% (Table 5.4). TAN levels and FOS:TAC values did not exceed the critical thresholds for inhibition in the OLR range up to 4 kg VS m^{-3} d^{-1} (Table 5.4). However, FOS:TAC values increased to 1.20 as the OLR was increased to 5 kg VS m^{-3} d^{-1} and ultimately resulted in reactor failure (Figure 5.2.B). Chloride levels in SL_{100} were highest among all reactors (including for *L. digitata* and *S. latissima*) at 17 g L^{-1} (Figure 5.3.B). Failure of the reactor occurred through the accumulation of VFAs at the high loading rate of 5 kg VS m^{-3} d^{-1} (Figure 5.4.B).

5.4 Discussion

5.4.1 Natural *L. digitata* as feedstock for AD

This paper proposes rural coastal digesters co-digesting seaweeds with the dairy slurry to produce third generation gaseous biofuel. The present study suggests an optimal mix for harvested natural *L. digitata* of 66.6% seaweed and 33.3% dairy slurry (on a VS basis), which can be employed at a high OLR of 5 kg VS m^{-3} d^{-1} whilst maintaining maximum biomethane efficiency. The methane yield generated was 232 L CH_4 kg^{-1} VS and matched closely that of the BMP utilising acclimatised inoculum. Although this yield was lower than that achieved at an OLR of 4 kg VS m^{-3} d^{-1} (261 L CH_4 kg^{-1} VS), the higher OLR allows for a reduction in the digester size and capital cost of such a facility.

Mono-digestion of *L. digitata* was proven to be stable at an OLR range of 2–4 kg VS m^{-3} d^{-1} with maximum biomethane efficiencies throughout this period.

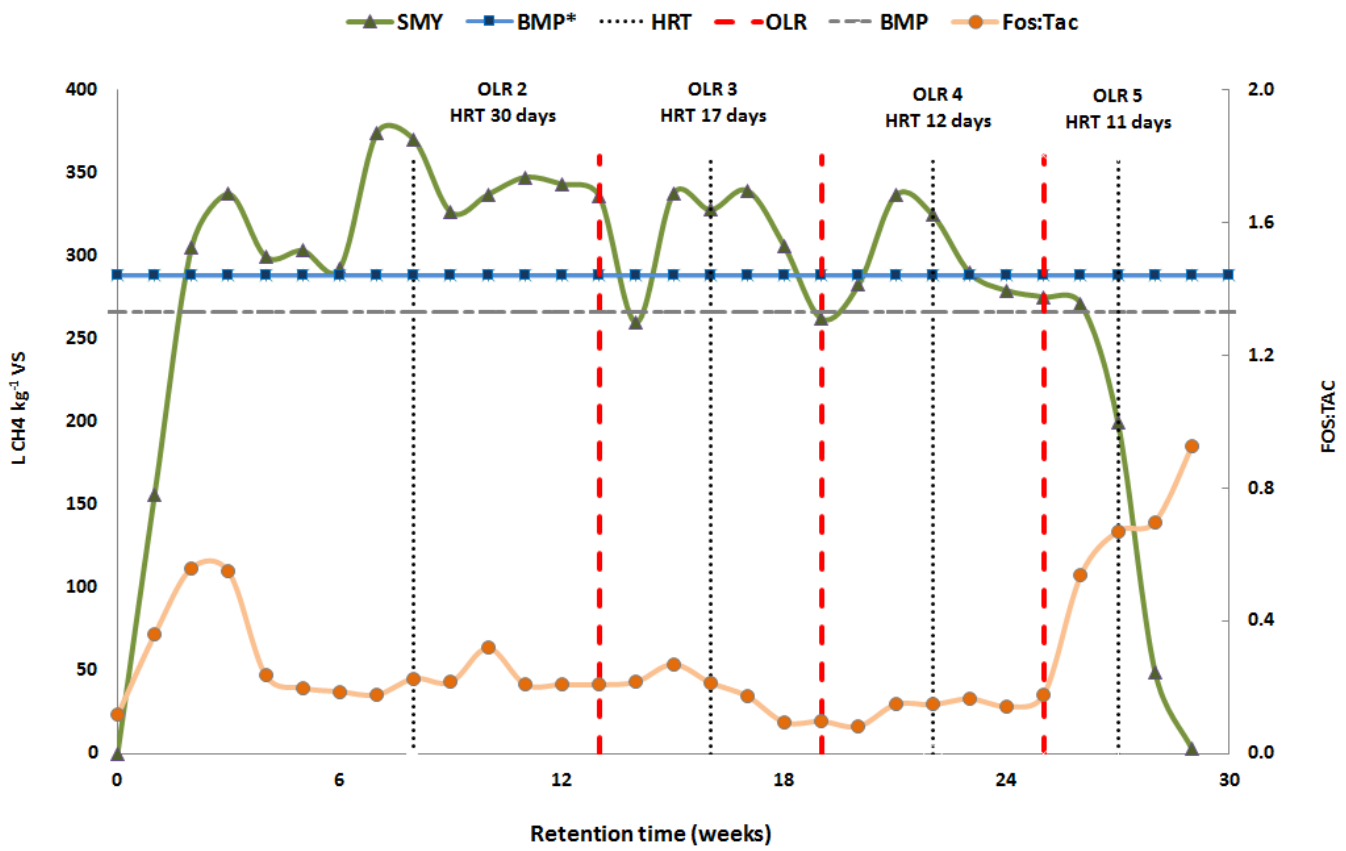

Figure 5.2.A Mono-digestion of *L. digitata*: SMY and FOS:TAC with increasing organic loading rate

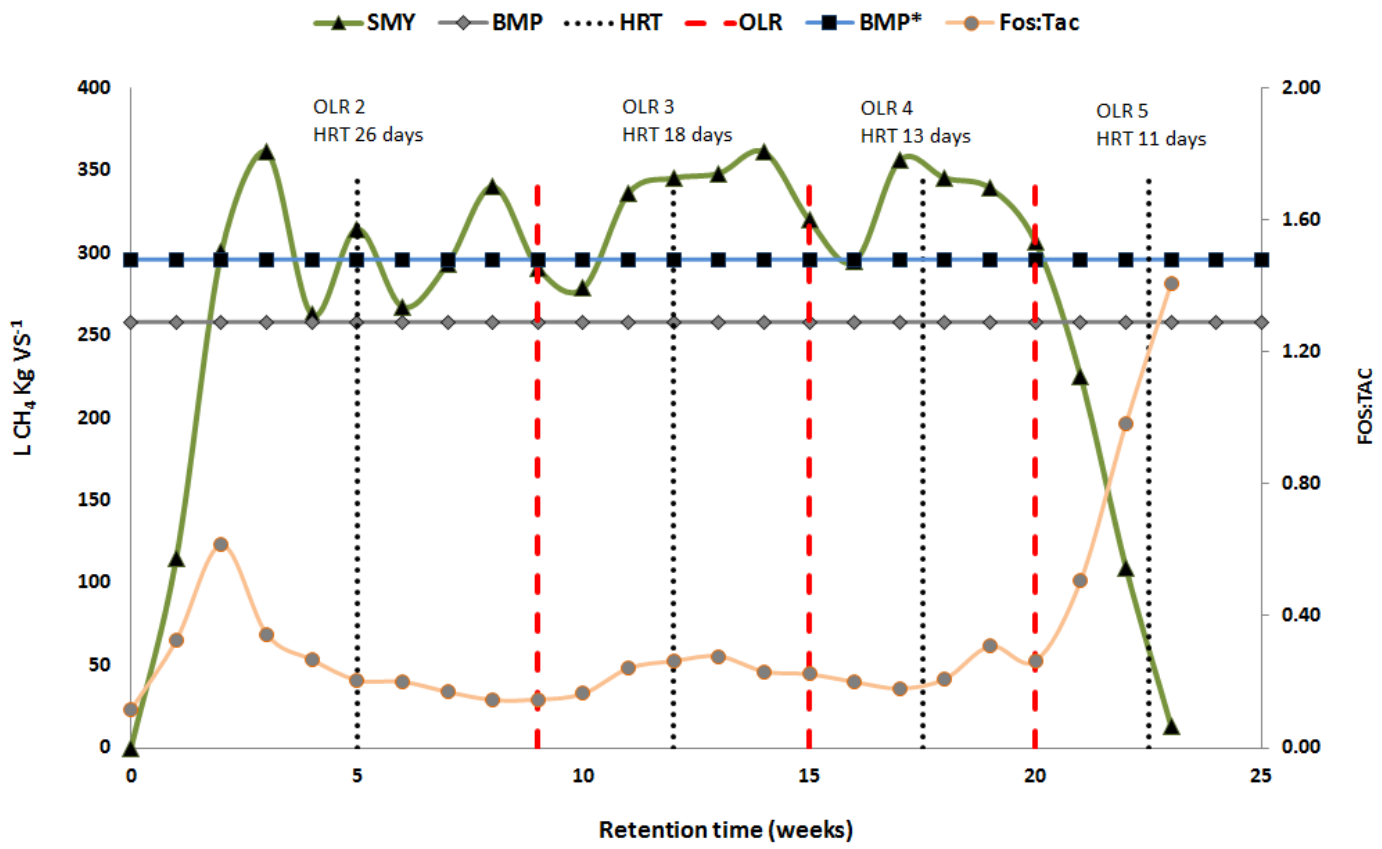

Figure 5.2.B Mono-digestion of *S. latissima*: SMY and FOS:TAC with increasing organic loading rate

Specific methane yield (SMY), biomethane potential before acclimatization (BMP), after acclimatization (BMP*), and the fermentation stability (FOS:TAC). Vertical darker lines indicate changes in organic loading rate (OLR), vertical small dashed lines indicate retention times (HRTs).

The ability to increase the OLR was achievable owing in part to recirculation of digestate liquor which the authors believe kept the FOS:TAC value at a low level. This is a similar concept in theory to the acclimatised inoculum in the second BMP allowing for higher SMYs. The steep drop in SMY, evident at OLR 5, was a result of the HRT becoming too short at the corresponding high loading rate. Thus, it was found practical to operate a 100% seaweed-fed digester at an OLR of 4 kg VS m^{-3} d^{-1} and an HRT of 12 days, provided recirculation of digestate liquor, generating an SMY of 281 L CH_4 kg^{-1} VS. Higher SMYs were achievable operating at a reduced OLR.

5.4.2 Comparison of cultivated with natural seaweed harvest

An optimal co-digestion mixture for *S. latissima* of 66.6% seaweed and 33.3% dairy slurry (on a VS basis) is proposed at a high OLR of 4 kg VS m^{-3} d^{-1} maintaining maximum biomethane efficiency. Digestion of wild harvest *L. digitata* with dairy slurry at the same percentage mix was found to be feasible to a higher OLR of 5 kg VS m^{-3} d^{-1}. Reaching the higher OLR for *L. digitata* may potentially have been due to a more optimal C:N ratio (23.40) than *S. latissima* (15.70). A combination of a short HRT (11 days) and sustained high chloride content (in excess of 15 g L^{-1}) is proposed as the reason for failure at OLR 5, ultimately leading to tVFA accumulation.

Mono-digestion of *S. latissima* was observed to be stable maintaining maximum biomethane efficiency up to an OLR 4 kg VS m^{-3} d^{-1}. A sudden drop in SMY recorded at OLR 5 that may be due to very short HRT (11 days). Failure of the reactor was due to high OLR of 5 kg VS m^{-3} d^{-1}, short HRT of 11 days and elevated levels of chloride (17 g L^{-1}). In essence, it was observed that a stable operation of a reactor fed with 100% seaweed was possible at a high OLR of 4 kg VS m^{-3} d^{-1} at a short retention time of 13

days. Ultimately, the continuous digestion trials indicated the potential application for both brown seaweeds at industrial scale. Although mono- and co-digestion strategies were found efficient in operation, the selection or preference of one seaweed over another will depend on upon various factors such as location, feedstock availability with respect to seasonal variation, cultivation or harvesting methods, government policy, and licencing issues. Mono-digestion of farm-cultivated seaweeds can be recommended for coastal areas. Farm cultivation may be preferred over harvest of natural seaweed due to reduction in environmental impact in removing natural stocks of seaweed from disperse coastlines; and the advantages of integrated multi-trophic aquaculture where cultivated farmed seaweed removes waste nutrients from fish farms and can achieve higher harvest yields due to this natural fertilisation.

5.4.3 Chloride risks in seaweed digesters

The issue of chloride is a concern for the development of mono-seaweed digesters. This study confirmed that chloride content in digesters containing seaweed increase over time (Figure 5.3.A), yet no correlation could be explicitly established between the level of chloride and the SMY. Sodium chloride has been recognised as a possible inhibitor in anaerobic digestion but is still essential in small concentrations for microbial activity (Suwannoppadol et al., 2012). A wide range of chloride toxicity levels has been prescribed for anaerobic digestion (5-20 g L^{-1}) (Lefebvre et al., 2007; Riffat and Krongthamchat, 2005). This disparity in chloride toxicity levels may be dependent on various factors. One possibility is the substrate type, that is, more complex substrates may have potentially lower salt tolerances (Lefebvre et al., 2007). In this study, mono-digestion of beach cast *L. digitata* reached a

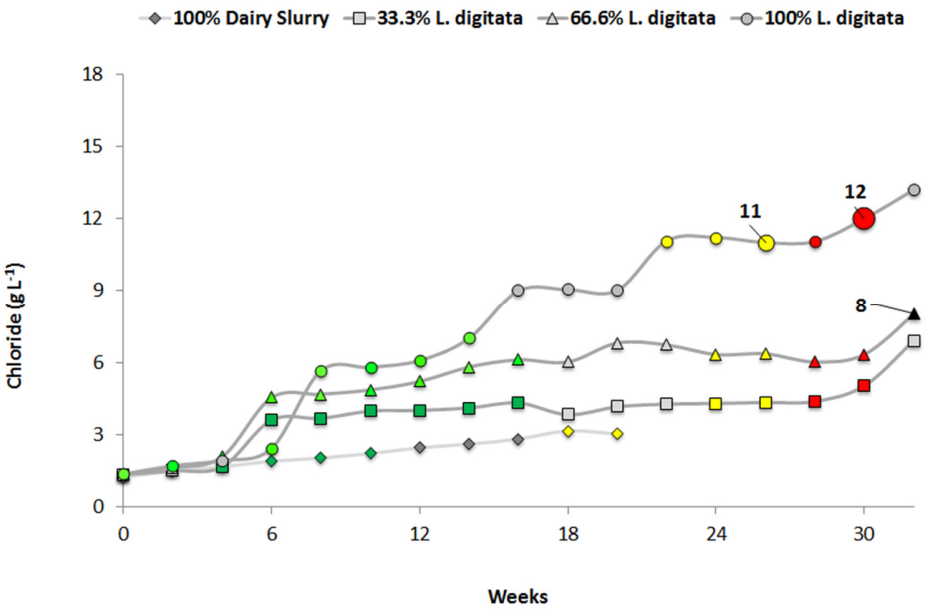

Figure 5.3.A Chloride accumulation over the period digesting *L. digitata.*

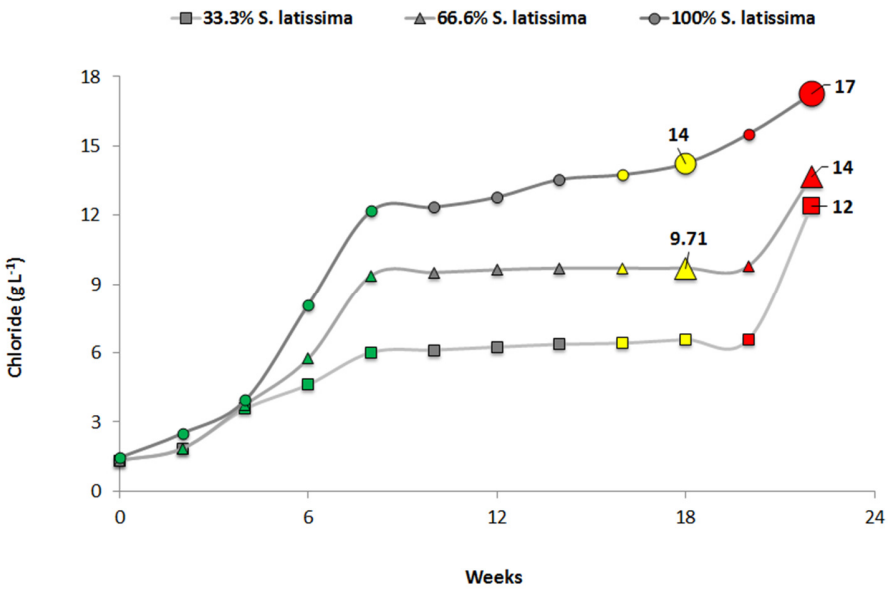

Figure 5.3.B Chloride accumulation over the period digesting *S. latissima.*

Green, grey, yellow, red and black coloured dots represent OLR ranged from 2-6, last black coloured triangle (Figure 5.3.A) is only for LD_{66} at OLR 6. Chloride level increases as the quantity of seaweed in digestion mix increases.

chloride concentration of 11 g L^{-1} when the reactor suffered inhibition. However, greater levels (>14 g L^{-1}) were experienced in stable mono-digestion of farm cultivated *S. latissima* at an OLR of 4 kg VS m^{-3} d^{-1} (Figure 5.3.B). Thus, the increase in OLR (and a corresponding reduction in HRT) had potentially more impact than the concentration of chloride for the digestion of *L. digitata.*

It should be noted that recirculation of digestate took place in the reactors treating *L. digitata* due to the higher solids content of this seaweed (17.7% versus 9.2% for S. *latissima*). Operating digesters at lower OLRs has previously been shown to result in higher salt tolerance (Lefebvre et al., 2007). It is evident from this study that increasing the OLR accelerates chloride accumulation within a digester. For mono-digestion of *S. latissima* at an OLR of 5 kg VS m^{-3} d^{-1}, chloride levels rose to 17 g L^{-1}. Thus, it is again difficult to interpret whether the increase in OLR, the very high concentration of chloride or a combination of both ultimately lead to the reactors demise.

It is proposed that for the safe operation of a mono-seaweed reactor that the chloride must be consistently monitored. However the acclimatisation process should not be underestimated as evidenced in this study by the stable operation at high OLRs (up to 4 kg VS $m^{-3}d^{-1}$) with high chloride levels (up to 14 g L^{-1}) whilst providing biomethane efficiencies at or close to maximum. There may be a need to run such digesters for longer than the 30 weeks assessed here to assess longer term effects of increasing chloride concentrations.

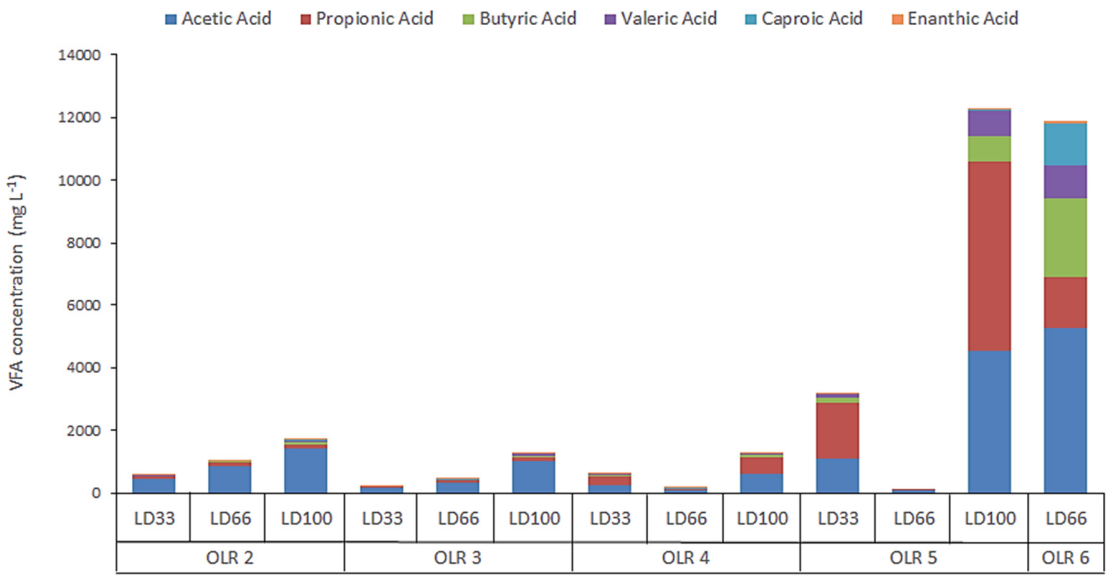

Figure 5.4.A Volatile fatty acid (VFA) profile for the reactors for LD_{33}-LD_{100} at increasing OLR

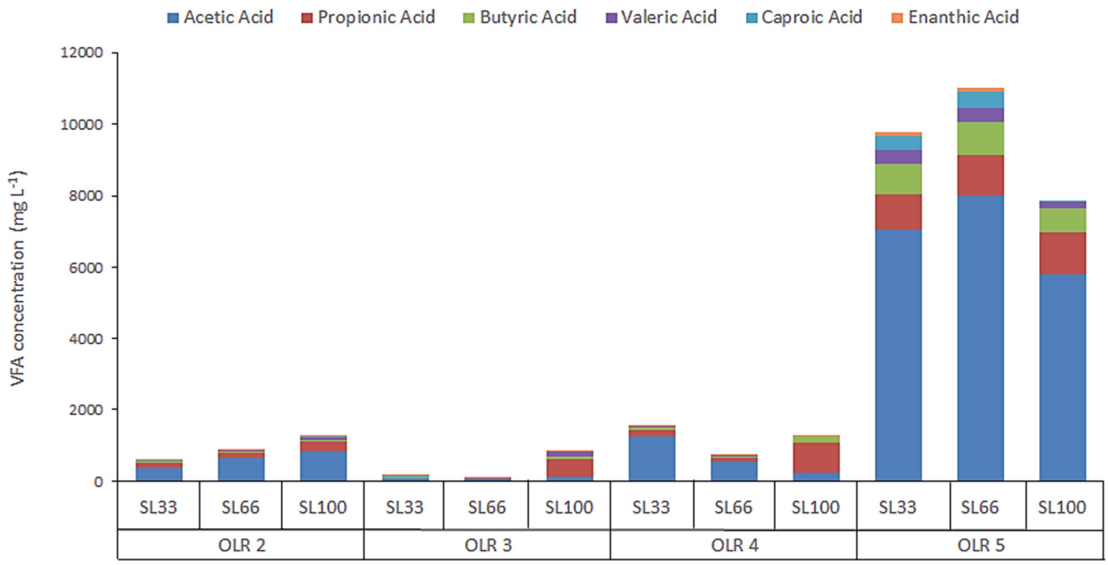

Figure 5.4.B Volatile fatty acid (VFA) profile for the reactors for SL_{33}-SL_{100} at increasing OLR

(Butyric acid: sum of iso-butyric acid and n-butyric acid; Valeric acid: sum of isovaleric acid and n-valeric acid; Caproic acid: sum of iso-caproic acid and n-caproic acid). Each bar represents average VFAs at a final hydraulic retention time (HRT).

5.5 Conclusion

A mix of 66.6% seaweed and 33.3% dairy slurry was found optimal for co-digestion for *S. latissima* and *L. digitata* achieving maximum biomethane efficiency in long-term continuous digestion up to an OLR of 4 and 5 kg VS m^{-3} d^{-1}, respectively. Mono-digestion of cultivated *S. latissima* and natural *L. digitata* generated similar methane yields (330-338 L CH_4 kg^{-1} VS) but digestion of *S. latissima* could achieve such yields at a higher OLR of 4 kg VS m^{-3} d^{-1}. Accumulation of salts was evident and was accelerated at higher loading rates. Acclimatisation of the digestion process to marine algae was significant.

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6. Comparison of pre-treatments to enhance the biomethane yield from *Laminaria digitata*

Comparison of pre-treatments to enhance the biomethane yield from *Laminaria digitata*

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Abstract

Pre-treatment may enhance the anaerobic digestion of seaweed for biomethane production. However, seasonal variation in the biochemical composition of seaweed has a significant impact on the pre-treatment choice. In this study, various pre-treatments were employed for brown seaweed *Laminaria digitata* harvested in March (with high ash content and low C:N ratio) and September (with low ash content and high C:N ratio) to suggest the optimal pre-treatment. Washing of *L. digitata* harvested in March with hot water at 40 ^{0}C removed 54% ash and improved the VS content by 31%, enhancing biomethane yield to 282 L CH_4 kg VS^{-1}. This pre-treatment effected a 16% increasing in biodegradability with 54% less salt accumulation in the digestate as compared to an untreated sample. This effect was not noted for seaweed harvested in September.

Keywords: *Laminaria digitata*; Seaweed; Pre-treatment; Anaerobic digestion; Biogas

6.1 Introduction

Brown seaweed is an attractive feedstock for gaseous biofuel production through anaerobic digestion due to availability of the technology (Gebrezgabher et al., 2010), high gross energy yield per hectare compared with land-based biomass (Tabassum et al., 2016a) and lack of competition with food for arable land (Tenenbaum, 2008). Brown seaweed is reported as an abundant marine bioresource in Irish waters (Smale et al., 2013). The feedstock received more attention after the European Parliament communication that advanced biofuels (such as from seaweed) should represent at least 1.25% of RES-T (renewable energy supply in transport) (European Parliment, 2015). Studies suggest that brown seaweed (*L. digitata*) has significant seasonal variation in chemical composition (Adams et al., 2011a; Tabassum et al., 2016a; Tabassum et al., 2016d; Tabassum, 2016b). Biodegradability of *L. digitata* is lower in winter and spring; this can be attributed to lower levels of readily digestible carbohydrates (laminarin and mannitol), higher ash contents (mostly salts) and a higher level of process inhibitors (Adams et al., 2011b).

Ash is the significant component of brown seaweed that changes greatly through the whole year and can be up to 35% of dry weight (Adams et al., 2011a; Moen et al., 1997; Schiener et al., 2015). Due to the seasonal variation, accumulation of salts in long-term digestion can be a problem when the seaweed is harvested (for digestion) in winter or early spring (Tabassum et al., 2016a) (Allen et al., 2014). To increase the digestibility and degradability of brown seaweed, some pre-treatment may aid biogas production; however due to the absence of cellulose and lignin, harsh pre-treatment may not be required (Tabassum, 2016b). The literature outlines pre-treatments employed on

biomass such as physical (washing) (Oliveira et al., 2014), mechanical (size reduction by cutting, chopping, maceration) (Nielsen, 2011), chemical (Borines et al., 2013), hydrothermal (heating) (Schultz-Jensen et al., 2013) and thermochemical processes (Oliveira et al., 2014). According to the literature, mechanical pre-treatment is most suitable for biogas production (Tedesco et al., 2013; Tedesco et al., 2014), whereas chemical pre-treatment was found inhibitory (Oliveira et al., 2014). However, none of the above considered salt accumulation.

The objectives of the current study was to:

- Examine the effect of pre-treatments on seasonal harvests;
- Assess the seaweed biochemical composition before and after pre-treatment;
- Study the improvement in the process dynamics of pre-treatment;
- Investigate the effect of pretreatment on salt accumulation in the batch reactor.

6.2 Materials and Methods

6.2.1 Collection and processing of *L. digitata* for pre-treatments

L. digitata was collected from Roaring Water Bay, Co. Cork, in the south of Ireland (51°N, -9°E) during March and September. Combinations of various pre-treatments were applied (Figure 6.1) to investigate the effects on the biogas yields based on seasonal variation in chemical composition (March and September). Washing with tap water at room temperature was a basic physical pre-treatment to remove any foreign particles. After washing, two mechanical pre-treatments (cutting and maceration) were applied to reduce the particle size. The washed samples were cut by scissors to a size of approximately 4 cm (CC: cold cut). Subsequently, the samples were macerated in a Buffalo macerator to further reduce the size less than 4 mm (CM: cold macerated).

The seaweed samples were washed with hot water (40 ± 1 ^{0}C) for 3 minutes and then cut with a scissor (HC: Hot cut) to compare it with CC. The samples washed with hot water were macerated (HM: hot macerated) to the same size (4mm) to compare it with CM.

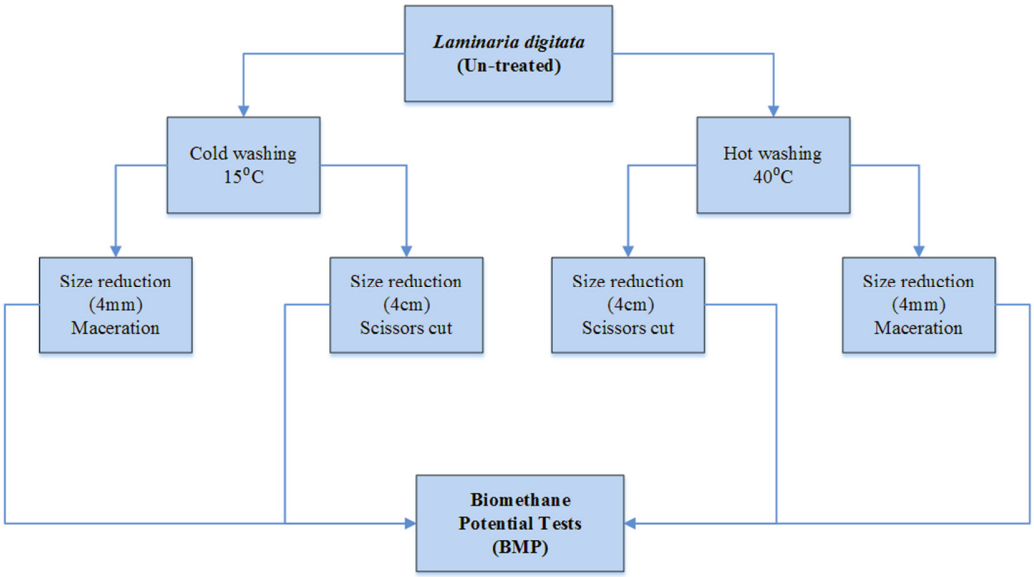

Figure 6.1 Schematic diagram of various pre-treatments

6.2.2 Analytical methods

Total solid (TS), volatile solid (VS) and ash were analysed by using the standard method of drying of the seaweed for 24 hours at 105 °C and then was combusted for 2 hours at 550 ^{0}C (APHA, 2011; Xia et al., 2016b). Carbon, hydrogen, nitrogen were measured by preparing the seaweed samples by drying at 105 ^{0}C for 24 hours and then grinding to pass through a 500 µm sieve. Dried samples were analysed for C, H, N, and O (O calculated by difference) using a CE 440 elemental analyser.

6.2.3 Anaerobic digestion of the seaweed

The theoretical methane potential was calculated by putting the values of C, H, and N into the Buswell equation (Eq. (1)). The output from this equation provides a maximum potential methane yield by converting VS to methane and carbon dioxide (Buswell AM, 1932). The molar volume of the gases was taken as 22.14 L at 0 ^{0}C and 1 atm.

$$C_n H_a O_b + \left(n - \frac{a}{4} - \frac{b}{2}\right) H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right) CO_2 \quad \text{Eq. (1)}$$

The biomethane potential (BMP) tests of the seaweed were conducted in a bioprocess system (Bioprocess AMPTS II® system). The Bioprocess AMPTS II® system is an automated methane potential test system with output to a software package. The inoculum was sourced from lab-scale continuous stirred-tank reactors (operated at 37 ^{0}C), processing various substrates such as grass, dairy slurry, and seaweed. The system was operated as described by (Allen et al., 2015; Tabassum et al., 2016a). The substrate to inoculum ratio (I:S) on a VS basis, of 2:1 was used (Angelidaki et al., 2009; Chynoweth et al., 1993). To calculate the specific biomethane production, the total average biomethane produced by the inoculum was subtracted from the average biomethane produced by each sample (Allen et al., 2015). Salinity (g/L) and pH of the batch digestion processes were also recorded before and after each BMP assay to investigate the effect of pre-treatment on the reaction performance and the gas yield.

6.2.4 Process dynamics and statistical analysis

The study of the process dynamics is beneficial for the understanding the changes in the biodegradability and the rate of biodegradability of the substrate before and after pre-treatment. The kinetic parameters such as change in decay constant (days^{-1}), maximum

yield (Y_{max}) and half-life (days) were obtained by taking data from the cumulative methane production curves (after 30 days) and analysing in MATLAB software using a first order differential equation as described previously (Nopharatana et al., 2007; Tabassum et al., 2016a). The biodegradability index (BI) was defined as the ratio of the BMP yield to the theoretical value as expressed by the TMP. Statistical significance of each pre-treatment was determined by using a statistical software (SPS, IBM NY, USA). Analysis of variance (ANOVA) was performed to examine the effect of various pre-treatments on different parameters (such as ash removal, improvement in gas yields and enhancement in bio-degradability of the substrate). The significance level was determined by multiple comparisons (Post Hoc test).

6.3 Results and Discussion

6.3.1 Effect of pre-treatment on the seaweed chemistry

L. digitata was characterised for compositional and elemental analysis. March and September harvest of the seaweed were compared before and after each pre-treatment (Table 6.1). After pre-treatment, it was revealed that washing with cold water did not remove a substantial amount of salts and hence did not improved the VS composition of the substrate. However, the VS content of the seaweed harvested in March was improved from 6.49% to 7.02% on pre-treatment with hot water washing and macerated (HM) to a particle size of less than 4 mm (Table 6.1). For samples harvested in March, HM pre-treatment succeeded in reducing ash content from 33.33% to 15.62%, resulting in increasing organic matter content from 66.66% to 84.33% and decreasing A:V ratio from 0.51 to 0.19. The substantial removal of ash (salt) content should make the seaweed more easily degradable (Adams et al., 2011b; Tabassum et al., 2016a).

Removal of ash and improvement of VS content for the March harvest can be advantageous for long-term continuous digestion as salt accumulation was reported as high in batch and continuous digestion processes (Allen et al., 2014; Tabassum et al., 2016c; Tabassum et al., 2016a).

The C:N ratio and the ash to volatile solids (A:V) ratio were described as the key factors for digestion of seaweed (Tabassum et al., 2016a). Washing with cold water did not show any improvements in the C:N ratio as compared to hot water pre-treatment. It may be explained that low temperature was not enough to disrupt the seaweed structure as compared to higher temperatures (40^0C). On the other hand, hot water pre-treatment may cause the removal of nitrogenous compounds (proteins, lectins, and alkaloids) (Pérez et al., 2016) and ultimately lead to an improvement in the C:N ratio (from 8.22 to 13.83 in this study). It also facilitated the reduction in the A:V ratio from 0.51 to 0.20 due to substantial removal of ash (Table 6.1). Improvement of the C:N ratio was greater in the March harvest than the September harvest due to the higher protein content in March seaweed than September (Tabassum et al., 2016a). Removal of ash, improvement of VS content and the C:N ratio for the March harvest can be advantageous for long-term digestion as salt accumulation was reported as significant in batch and continuous digestion processes (Allen et al., 2014; Tabassum et al., 2016a).

Table 6.1 Change in the chemistry of *L. digitata* on different pre-treatments

Harvest	Compositional Analysis					Elemental Analysis				
	TS (%)	VS (%)	OMC (%)	Ash (%)	A:V	C %	H %	N %	O %	C:N
March (U)	9.74 (0.02)	6.49 (0.14)	66.67	33.33 (1.37)	0.51	30.41 (0.90)	3.97 (0.11)	3.70 (0.06)	28.58	8.22
Cold cut	9.04 (0.20)	5.72 (0.20)	63.26	36.73 (0.50)	0.58	28.09 (0.38)	3.46 (0.07)	3.55 (0.34)	28.16	7.91
Hot cut	6.47 (0.06)	5.37 (0.09)	83.09	16.91 (0.06)	0.20	39.29 (0.12)	4.83 (0.05)	2.84 (0.05)	36.12	13.83
Cold macerated	9.76 (0.12)	6.56 (0.07)	67.19	32.81 (0.05)	0.49	30.41 (0.90)	3.97 (0.11)	3.70 (0.57)	29.10	8.22
Hot macerated	8.32 (0.10)	7.02 (0.02)	84.38	15.62 (0.7)	0.19	39.53 (0.01)	4.88 (0.12)	2.91 (0.29)	37.06	13.58
September(U)	19.46 (0.26)	15.67 (0.25)	80.51	19.49 (0.44)	0.24	36.62 (0.17)	5.30 (0.05)	0.93 (0.03)	39.37	39.38
Cold cut	19.44 (0.34)	15.60 (0.35)	80.27	19.43 (0.43)	0.24	36.74 (0.17)	5.03 (0.11)	1.18 (0.08)	37.32	31.14
Hot cut	15.51 (0.36)	13.42 (0.32)	86.56	13.44 (0.30)	0.16	38.98 (0.17)	5.21 (0.11)	0.97 (0.25)	41.39	40.19
Cold macerated	19.46 (0.26)	15.67 (0.25)	80.51	19.49 (0.44)	0.24	36.62 (0.17)	5.30 (0.05)	0.93 (0.03)	37.66	39.38
Hot macerated	16.82 (0.10)	14.42 (0.10)	85.75	14.25 (0.14)	0.17	38.20 (0.07)	5.39 (0.02)	0.93 (0.10)	41.23	41.08

U is untreated sample; TS is total solids; VS is volatile solids; OMC is organic matter content obtained dividing VS/TS; A:V is ash to volatile solid ratio; while C, H, N, O and C:N are carbon, hydrogen, nitrogen, oxygen and carbon to nitrogen ratio, respectively; Standard deviation is in parentheses

6.3.2 Impact of pre-treatment on the biogas yield

Seaweed harvested in spring (March) displayed a higher ash content and lower organic matter content, which would adversely affect anaerobic digestion (Tabassum et al., 2016a). To investigate the effect of pre-treatment on the gas yield, various pre-treatments were designed and compared (Figure 6.1). The BMP results revealed that size reduction (4cm and 4mm) after washing with tap water had a little effect on the gas yield as compared to same size reduction after hot washing (Table 6.2).

It was observed (Table 6.2) that the effect of hot washing was more significant on the March harvest (from 245 L CH_4 kg VS^{-1} to 283 L CH_4 kg VS^{-1}) than the September harvest (from 280 L CH_4 kg VS^{-1} to 326 L CH_4 kg VS^{-1}). The rationale for this difference can be explained by the difference in seasonal chemical composition. Seaweed harvested in March had high ash content as compared to September (Table 6.1), hence there is more significant potential for ash removal (Tabassum et al., 2016a). Size reduction (from a particle size of 1mm to 4mm) of dried seaweed was reported as a significant pre-treatment for biogas production from brown seaweeds (Vanegas et al., 2015), however, drying is considered to be an energy intensive process on an industrial scale. In the current trials maceration to a particle size of 4 mm is deemed unnecessary as compared to size reduction by scissors to 4 cm particle size (Table 6.2). The gas yield was almost the same for both particle sizes for the March harvest (282 L CH_4 kg VS^{-1} and 283 L CH_4 kg VS^{-1}). However, the gross energy calculations calculated based on specific methane yield based on wet weight highlights that maceration is an essential step (Table 6.2).

Table 6.2 Effect of pre-treatments on the gas yield and gross energy production

Harvest	BMP yield ($L\ CH_4\ kg\ VS^{-1}$)	TMP ($L\ CH_4\ kg\ VS^{-1}$)	B. Index (BMP/TMP)	Specific yield ($m^3\ CH_4\ t^{-1}_{wwt}$)	Gross energy ($GJ\ ha^{-1}\ yr^{-1}$)
March (U)	245 (10.86)	469	0.52	16	60
Cold cut	258 (12.42)	469	0.55	15	56
Hot cut	283 (6.24)	468	0.60	15	57
Cold macerated	265 (3.56)	469	0.57	17	66
Hot macerated	282 (2.33)	462	0.61	20	75
September (U)	280 (28.76)	450	0.62	44	165
Cold cut	303 (22.55)	450	0.67	47	179
Hot cut	326 (26.25)	424	0.76	44	165
Cold macerated	307 (18.98)	450	0.68	48	182
Hot macerated	308 (5.63)	403	0.76	44	168

U is untreated sample; BMP is biomethane potential; TMP is theoretical methane potential; BI is biodegradability; wwt is wet weight; the standard deviation is in parentheses.

Sample calculation for specific methane yields and gross energy calculations.

Specific methane yield = 0.245 $m^3\ CH_4$/ tVS * 64.94 (kgvs/t wwt) = 16 $m^3\ CH_4\ t^{-1}_{wwt}$

Biomethane Yield, $m^3\ ha^{-1}\ yr^{-1}$ = 245 $m^3\ CH_4$/ tVS * 100 t_{wwt} * 6.49% VS = 1590 $m^3\ ha^{-1}\ yr^{-1}$;

Gross energy ($GJ\ ha^{-1}\ yr^{-1}$) = Biomethane yield * 37.8 MJ/m^3*0.001 (MJ to GJ) = 60 $GJ\ ha^{-1}\ yr^{-1}$

The gross energy yield, improved by 25% (75 GJ ha^{-1} yr^{-1}) for maceration after hot washing as compared to the untreated sample (untreated yielded 60 GJ ha^{-1} yr^{-1}) (Table 6.2). The same trend was observed in term of specific methane yield (Table 6.2). On the other hand, size reduction by scissors (4cm) after hot washing decreased the yield both in terms of gross energy (60-57 GJ ha^{-1} yr^{-1}) and specific methane yield (16-15 m^3 CH_4 t^{-1} wet weight). This may be attributed to material loss during manual cutting with scissors and can be avoided on an industrial level using mechanical instruments.

6.3.3 Effect of pre-treatment on the key process parameters

Ash content, organic matter content and C:N ratio is key process parameters, which affect biodegradability and the gas yield. Washing with hot water is a low energy intensive pre-treatment. Seaweed with lower ash content, higher organic matter content, and C:N ratio in the optimum range are advantageous for biogas production (Adams et al., 2011a; Tabassum et al., 2016a).

Ash content in marine biomass can be an issue in long-term anaerobic digestion through the accumulation of salts in the digester. The salt build-up was higher in winter and spring samples of seaweed as compared to summer and autumn samples (Tabassum et al., 2016a). Hot water washing reduced the ash content by 54% (March) and 31% (September) when cut to a particle size of 4 cm by scissors; values of were 47% and 27% when achieved when macerated (Figure 6.2). Ash removal resulted in increased organic matter content of 31% and 8% in March and September, respectively when the seaweed was cut by scissors (Figure 6.2).

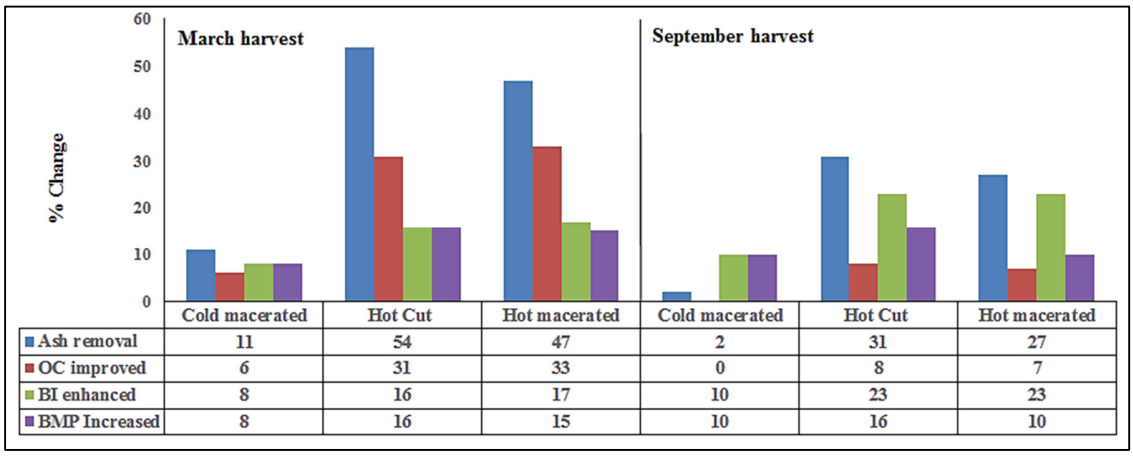

Figure 6.2 Impact of various pre-treatment on the process parameters of *L. digitata*

The biodegradability index (defined as the ratio of the BMP yield to the theoretical yield) explains the process efficiency in terms of degradability of the substrate in the reactor. The index was improved from 0.52 to 0.61 of the seaweed harvest in March while it was increased from 0.62 to 0.76 for the September harvest (Table 6.2). The substrate was 16% and 23% more biodegradable as compared to untreated seaweed for March and September harvest, respectively (Figure 6.2). The higher biodegradability index in September as compared to the March harvest may be due to higher concentrations of easily degradable organic matter content in the substrate (Adams et al., 2011a; Adams et al., 2011b).

Accumulation of salts was recorded before and after pre-treatments to examine the reduction of salts in the reactor. Salinity and the A:V ratio were reported as key factors affecting the gas yield during different harvesting seasons (Tabassum et al., 2016a). According to Tabassum et al., higher values of salinity and A:V led to lower values of biomethane production (Tabassum et al., 2016a). The salinity of seaweed was subtracted from the salinity of inoculum (6.85 ± 0.46 g/L) to calculate the salinity increase in the

batch digestion due to the seaweed only. During current studies, it was observed that hot water pre-treatment successfully resulted in lowering the A:V ratio and salinity (Figure 6.3). However, reduction in % salinity was comparatively higher for the March harvest (Figure 6.3). A low biomethane yield was expected at a high salinity level (Chen et al., 2008; Fang et al., 2011; Xia et al., 2016a). Application of hot water washing (Hot cut) as a pre-treatment technology before anaerobic digestion of the seaweed may result in 54% less salt accumulation in the reactor as compared to the untreated March harvest.

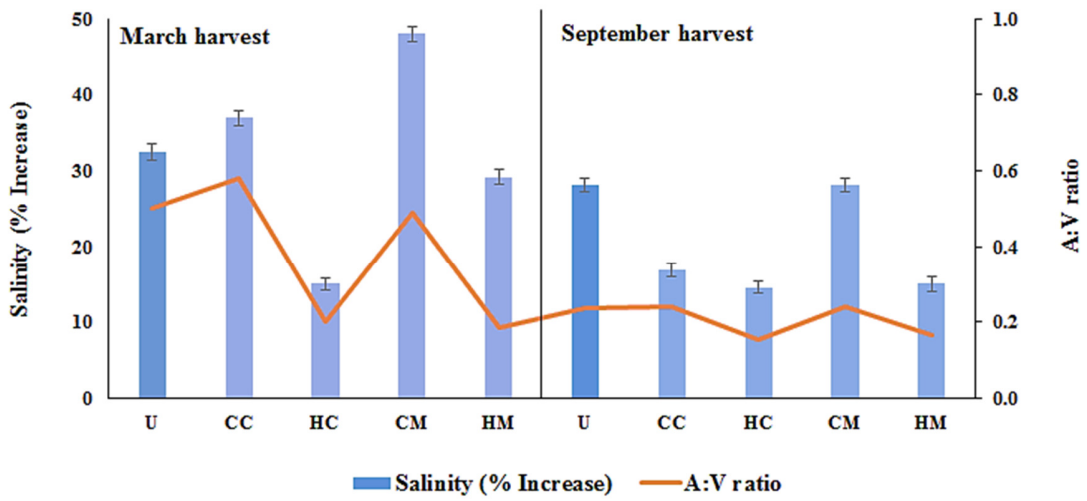

Figure 6.3 Effect of pre-treatment on salts accumulation in the batch process.
U is untreated seaweed; CC is cold cut. HC is hot cut; CM, cold macerated; HM is hot macerated

After the process parameter studies, it was revealed that the March harvest has the greater impact on the process. While, for the September harvest, the impact is lower due to the already higher organic matter content, lower ash content and lower levels of inhibitory compounds in the substrate as compared to the March harvest. For both

harvests, further research is required in the calibration of the specific temperature of the hot water treatment and the duration of the treatment time.

6.3.4 Process dynamics and statistical analysis

The changes in the process dynamics after each pre-treatment are listed in Table 6.3. Maceration after hot water washing (HM) indicated significant kinetic decay (*k* value doubled 0.10 to 0.20) for the March harvest and the biomass was degraded efficiently (half-life was shortened from 6.8 to 3.4 days). This may be attributed to the removal of a substantial amount of inhibitory compounds (such as furfural, hydroxyl methyl furfural, polyphenols) from the substrate that may be responsible for slower degradation of the seaweed in the untreated sample (Monlau et al., 2014).

The decay values as the result of other pre-treatments for the same harvest remained close to the untreated sample (Table 6.3). The decay constant for perennial ryegrass (Wall et al., 2013), food waste (Browne et al., 2014) and brown seaweed (Allen et al., 2015) were reported to be 0.11, 0.17 and 0.19, respectively. After employing the current pre-treatment method, (Hot Maceration of March Harvest) the degradability of the substrate was 100% improved both in terms of the decay constant and half-life.

The half-life of the methane production (T_{50}) was reported as 4-5 days in summer and 6 – 9 days in winter (Gurung et al., 2012) probably due to the higher concentration of easily degradable laminarin and mannitol in summer (Adams et al., 2011a).

Improvement in kinetic parameters for the March harvest (after pre-treatments) delimited the utilization of the substrate for biogas production and may reduce the retention time for digestion of the substrate to less than 20 days, which is suggested

sufficient for long-term continuous digestion (Allen et al., 2015; Tabassum et al., 2016c).

Table 6.3 The process dynamics of *L. digitata* based on different pre-treatments

Pre-treatments	k decay constant ($days^{-1}$)	R^2	Y_{max}	T_{50} (half-life, days)
March (U)	0.10	0.98	245	6.81
Cold cut	0.08	0.97	258	8.89
Hot cut	0.08	0.96	283	8.43
Cold macerated	0.10	0.97	265	6.81
Hot macerated	0.20	0.99	282	3.46
September (U)	0.13	0.95	282	5.24
Cold cut	0.15	0.99	338	4.68
Hot cut	0.08	0.95	326	8.20
Cold macerated	0.13	0.96	307	5.24
Hot macerated	0.09	0.93	308	7.49
Cellulose	0.17	0.99	356	4.09

U is untreated sample; k is the decay constant; R_2 is a measure that how the kinetic model fits the biomethane potential curve (%); Y_{max} is the maximum methane potential; T_{50} is the half-life (days)

The experimental results were supported by statistical significance by conducting an ANOVA analysis. Multiple comparisons results from one-way ANOVA indicated that pretreatment applied to the March harvest were significant ($F=8.39$, $P < 0.001$) as compared to the September harvest ($F=1.68$, $P < 0.23$). The samples harvested in March were analysed further in comparison with different factors affecting the gas yield. These factors were particle size (4mm and 4cm) and washing method (hot water and cold

water) in comparison with the removal of salts (ash), biodegradability and ultimately the BMP enhancement. After comparison, it was revealed that the most significant factor for the process was A:V ratio ($F=11.97$ and $P < 0.001$) ash removal ($F=14.09$ and $P < 0.001$). Particle size and washing method comparison indicated that the most significant pretreatment was maceration to a particle size of 4mm after hot washing ($P < 0.002$). After comparison of statistical results, it can be concluded that maceration after hot washing was the most efficient pretreatment method to enhance the gas yield by substantial removal of salts (ash) from the substrate. However, it requires further optimization of pre-treatment time and temperature.

6.4 Conclusions

Size reduction after cold washing has little impact on the gas yield as compared to washing with hot water. Higher ash content removal occurred in the March harvest than the September harvest. Scissor cutting after hot washing of the March harvest removed 54% ash, improved the VS content by 31% and increased biomethane yield by16% with a 68% enhancement in the C:N ratio. However, maceration after hot washing yielded a 25% higher specific methane and gross energy yield as compared to the untreated sample. Hot washing requires optimization of pre-treatment time and temperature to facilitate the continuous supply of the seaweed even in March for biogas production.

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7. Conclusions and recommendations

7.1 Conclusions

- Brown seaweeds are an abundant resource that exhibits great seasonal variation but have the potential to satisfy the targets for advanced biofuels in Ireland.
- Seasonal variation studies of brown seaweed indicated that peak month for harvesting brown seaweeds to get optimum biomethane yield varies. In August, the specific methane yield (327 L CH_4 kg VS^{-1}) of *L. digitata* is 40% higher than harvested in December. Gross energy yields from the seaweed can yield up to 200 GJ ha^{-1} yr^{-1}, assuming 100 tons wet weight per hectare per year. At these cultivation levels 11,800 ha of cultivation would satisfy 1.25% of renewable energy in transport in Ireland (RES-T).
- Seasonal variation studies of *A. nodosum* indicated that polyphenolic content has a significant impact on biomethane production. The optimal biomethane yield of 217 L CH_4 kg VS^{-1} is achieved in April but is lower than *L. digitata* in August (327 L CH_4 kg VS^{-1}). The two optimum harvesting months based on SMY (March and October) of *A. nodosum* were different from *L. digitata* (August) due to high polyphenol in summer months. Gross energy yields from *A. nodosum* in the optimum harvesting month was 116 GJ ha^{-1} yr^{-1} and a relatively greater cultivation area (20,260 ha) is required as compared to *L. digitata* (11,800 ha) to satisfy the renewable transport energy target in Ireland.
- Variation in peak harvesting months of *L. digitata* and *A. nodosum* are advantageous for the biogas industry as it would potentially ensure a continuous supply of seaweed feedstock to produce biomethane.

- Long-term anaerobic digestion of natural harvest and farm cultivated brown seaweed shows that both mono- and co-digestion strategies are feasible to establish offshore and onshore digesters near coastal areas.
- Long-term mono-digestion of farm cultivated *S. latissima* and naturally harvested *L. digitata*) generated similar methane yields (330-338 L CH_4 kg^{-1} VS) but the digestion of *S. latissima* could achieve the yields at a higher OLR of 4 kg VS m^{-3} d^{-1}.
- The digester performance was found stable at short hydraulic retention times for both species. The retention time at OLR of 4 kg VS m^{-3} d^{-1} was just 11 days for mono-digestion of *S. latissima* and *L. digitata*. While, there was a 13 and 14 day (at OLR of 4 and 5 kg VS m^{-3} d^{-1}, respectively) retention time for co-digestion of *S. latissima* and *L. digitata* with dairy slurry, respectively.
- The optimum blend for co-digestion is 66.6% seaweed and 33.3% dairy slurry for *S. latissima* and *L. digitata* with maximum biomethane efficiency in continuous digestion up to an OLR of 4 and 5 kg VS m^{-3} d^{-1}, respectively.
- Accumulation of salts in the digester is the key concern at higher loading rates. However, the tolerance of salts may be higher in the case of farm cultivated young plants as compared to natural harvest.
- Pretreatment studies indicated that seasonal variation in the biochemical composition of brown seaweed has a significant impact on the choice of suitable pre-treatment.
- Size reduction after cold washing has little impact on the biomethane yield as compared to washing with hot water.

- Higher ash content was removed from *L. digitata* harvested in March as compared to September harvest. Size reduction (particle size of 4cm) with scissors after hot water washing for March harvest successfully removed 54% of ash content, improved the VS content by 31% and increased the gas yield by 16% with an improvement in the C:N ratio as compared to untreated sample. However, hot water washing and subsequent maceration yielded a 25% higher gross energy yield (75 GJ ha^{-1} yr^{-1}) as compared to the untreated sample (60 GJ ha^{-1} yr^{-1}).

7.2 Recommendations

The research undertaken in this study demonstrates that high specific methane yields can be generated from mono- and co-digestion of brown seaweeds. However, some further research recommendations are suggested below for the potential initiation of a seaweed-based biogas industry in Ireland.

1. Characterization of other potential brown seaweeds (such as *S. latissima*) with reference to seasonal variation should be carried out to select the optimal harvesting month. This would further facilitate the makeup of different seaweeds for harvest in supplying feedstock to the digesters year-round.
2. A comparison in the characterization of natural harvest and farm cultivated seaweeds in biomethane potential tests would be beneficial to select the best species and peak harvesting times.
3. Biomethane potential assessments of polyphenol-rich brown seaweeds (such as *Ascophyllum nodosum*) after polyphenol extraction should be investigated for its potential as a digestion feedstock.

4. For the continuous digestion of seaweed, monitoring of trace elements (through analysis of the digestate) would help to understand the role of trace elements in the digestion process. Analysis of trace element concentrations at different organic loading rates may indicate deficiency levels of some trace elements, which furthermore if added to the reactors may help to increase performance.
5. Continuous monitoring of salts (chloride) is required for the long-term operation of brown seaweed at a high OLR (greater than 3 kg VS m^{-3} d^{-1}). Alternatively, digesters with elevated salt levels (in excess of 15g L^{-1}) may be investigated for co-digestion with other substrates (such as with various types of slurries, grass, straw, food waste) to potentially dilute salts so that the digesters can maintain steady operation.
6. Hot washing pretreatment (for salt removal) prior to continuous digestion may also be applicable to achieve better process stability. Hot water pretreatment was performed at a single temperature point (40^0C) in this study and was applied to only one species (*L. digitata*). Such a pre-treatment needs to be investigated for other potential brown seaweeds (*S. latissima*) with the calibration of pre-treatment time and temperature. This again may result in higher specific methane yields due to salt removal.
7. Seaweed storage technologies may be required at industrial scale after bulk natural harvesting or farm cultivation. The brown seaweeds of interest may be investigated for suitable storage methods such as anaerobic storage at lower temperatures. This would facilitate the continuous supply of the seaweed

feedstock year-round. It may also be investigated as a potential pre-treatment technology.

8. The cost of seaweed may be a challenge in employing digestion technologies. The potential for an integrated multi-trophic aquaculture (IMTA) system should be investigated to potentially reduce the cultivation costs. Support from Government in policy and legislation is required for expansion of research that focuses on such innovative cultivation techniques.