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Co-digestion of terrestrial plant biomass with marine macro-algae for biogas production

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Abstract

This paper investigates factors affecting anaerobic degradation of marine macro-algae (or seaweed), when used as a co-substrate with terrestrial plant biomass for the production of biogas. Using *Laminaria digitata*, a brown marine seaweed species and green peas, results showed that when only 2% of feedstock of a reactor treating the green peas at an organic loading rate (OLR) of 2.67 kg VS.m^3.day^{-1} was replaced with the seaweed, methane production was disrupted, whilst acidogenesis, seemed to be less adversely affected, resulting in excessive volatile acids accumulation. Reactor stability was difficult to achieve thereafter. The experiment was repeated with a lower initial OLR of green peas of 0.70 kg VS.m^3.day^{-1} before the addition of the seaweed. Although similar symptoms as in first trial were observed, process stability was restored through the control of OLR and alkalinity. These measures led to an increase in overall OLR of 1.25 kg VS.m^3.day^{-1} comprising of 35% seaweed. This study has shown that certain seaweed constituents are more inhibitory to the methanogens even at trace concentrations than to the other anaerobic digestion microbial groups. Appropriate adaptation strategy, involving initial low proportion of the seaweed relative to the total OLR, and overall low OLR, is necessary to ensure effective adaptation of the microorganisms to the inhibitory constituents of seaweed. Where there is seasonal availability of seaweed, the results of this study suggest that a fresh adaptation or start-up strategy must be implemented during each cycle of seaweed availability in order to ensure sustainable process stability.

Keywords: Anaerobic digestion; co-digestion; green peas; *Laminaria digitata*; methane production; methanogenic inhibition

1. Introduction

Anaerobic digestion is now commonly used for converting organic matter to bio-energy [1]. The underlying principles of anaerobic digestion are well established and advances in process control have put the method at the forefront of renewable energy solutions [2]. The interest in anaerobic digestion is further enhanced by regulatory incentives in many countries around the world and by the forecasted energy crisis with ramifications beyond natural resources exhaustion, fossil fuels shortages and geopolitical trends. However, the technology is still associated with high initial costs and a long-term return on investment. A systematic solution to mitigate the latter is to increase the net energy production and undertake an economic appraisal of anaerobic digesters. Consequently, innovative technological solutions associated with specific operational and feedstock preparation strategies have been, and are still being, developed for the enhancement of biogas yields. With the development of 'high-rate' systems and increased uptake of the technology, some challenges common to other conversion processes have emerged. The availability of suitable sources of organic matter is considered critical for the effective application of anaerobic digestion technology. Other challenges include the competition existing with alternative treatment solutions (such as aerobic composting), inadequate waste segregation practices and unavailability of sufficient fiscal inducements. The use of purposely grown energy crops is beginning to gain broad acceptance as an effective solution to securing sufficient sources of suitable organic matter for the generation of biofuels [3]. However, excessive use of energy crops to the detriment of food feedstocks can result in the increase in global food prices. Whilst the need for complimenting anaerobic digestion feedstock sources with other amenable organic matter is acknowledged, there is also a need to understand the effect changes in the preferred feedstock type brought about by seasonal variability in quantity and quality can have on the digestion process. Numerous studies [4-11] have addressed the co-digestion of two or more substrates, however, little has been reported on the full or part use of other feedstocks or co-substrates as a means of sustaining optimum conversion rates when the preferred feedstocks are unavailable. A typical example is the anaerobic digestion of vegetable and fruit residues, the availability of which is dependent upon both climatic conditions and the season of the year. Therefore assessing the potential contribution of co-digestion in alleviating any adverse economic impacts of feedstock seasonable variability will also require an in-depth understanding of the effects of operating anaerobic digesters in time variant cycles involving one or more of the contributing feedstocks.

Marine macro-algae represent a unique and diverse reservoir of organic matter. Brown seaweed is of particular interest because of its abundance on the sublittoral zone of the British coastline and appealing conversion rates in anaerobic systems [12-15]. Furthermore, seawater as a growth medium results in high biomass productivity with a corresponding improvement of the feedstock sustainability in comparison with purposely grown terrestrial crops requiring a combination of water, fertiliser and extensive acreage. The reported major challenges associated with the anaerobic digestion of seaweed lies mainly in the possible antimicrobial compounds associated with the substrate [16-18]. Light metal salts, particularly sodium, have been reported to cause microbial inhibition in excessive concentrations [19-23]. However, bacteria are versatile organisms and have been found to be capable of adapting to severe environmental changes such as those resulting from sodium ion accumulation [24]. Polyphenolic compounds are another category of potential inhibitors and they typically affect anaerobic digestion through interactions with cell membranes and interference with microbial metabolism [17, 25, 26]. Despite these challenges, seaweed remains a potential source of biomass for biogas production [27, 28, 29], which even when utilised in relatively small amounts can enhance food waste digestion by providing deficient trace metals [30].

Although co-digestion of seaweed with purposefully grown terrestrial plant biomass can reduce these inhibitory components of seaweed to their non-inhibitory levels, little is known on how the system can cope with highly variable seasonable availability of each of the contributing feedstocks, an extreme scenario being the digestion of only one type of the feedstock at a given period. The aim of this work is therefore to investigate the potentials and challenges of using a brown seaweed species commonly considered as a relatively suitable anaerobic digestion feedstock, *Laminaria digitata*, as a co-substrate in the anaerobic digestion of vegetable residues, in this case green peas, in order to understand the key factors affecting the effective utilisation of seaweed as a feedstock for anaerobic digestion.

2. Material and Methods

2.1. Materials and start-up procedures

A single-stage anaerobic reactor was used for the study. The reactor had an 8 litre total and 5 litre effective capacity. The reactor was heated using an insulated electrical heating wire wrapped around the outside of the vessel, and the temperature monitored in real time using an electronic thermometer (Invensys controls, Italy). Intermittent mixing (15 seconds every 20 minutes) was achieved through the use of a propeller attached to a stepper motor (Igarashi IG33, Trident Engineering, UK). Feeding was carried out manually and occasionally automatically using a peristaltic pump (Masterflex L/S: Cole-Parmer, UK) through a port located at the top of the main vessel and connected to the liquid phase of the reactor to prevent gas leakage. Similarly, the effluent was withdrawn manually through a valve located at the base of the digester and occasionally automatically using a peristaltic pump connected to the bottom of the reactor through the feeding port. Peristaltic pumps used for daily feeding were controlled by electronic timers. Gas composition was monitored from the headspace through a gas-tight sampling port. The vessel was connected to a gas collector made of two cylinders and was based on the water displacement principle. Green peas (*Pisum sativum*) were obtained from commercially available sources. The brown seaweed, *Laminaria digitata*, was collected from Westhaven beach (56° 30′ N, 2° 42′ W) near Dundee, Scotland, UK in October 2010 and October 2011. After collection, the seaweed was washed with tapwater to remove debris, sand and excess seawater. Both feedstocks were oven-dried at approximately 75°C for 24 hours and milled in an industrial blender (Fritsch, Germany) to reduce particle size to a maximum of 1 mm and obtain a homogenised feedstock. The feedstocks were then stored in sealed containers at room temperature. Varying weight ratios of each substrate were mixed with 300 ml of tapwater before addition to the reactor. The reactor was firstly inoculated with anaerobically digested sludge obtained from the Hatton Wastewater Treatment Plant (Hatton, Angus, UK) operating at mesophilic temperature and initially fed with low quantities of green peas in batch start-up mode. Feeding was carried out once daily and the reactor was operated under mesophilic temperatures (37°C±1°C) with a 17 day hydraulic retention time (HRT). Two distinct experiments were conducted and these are detailed below.

2.2. Analytical methods

Biogas production from the anaerobic reactor was measured by water displacement and its composition determined by gas chromatography using a Hewlett-Packard 5890 Series II gas chromatograph with dual thermal conductivity detector and an AT-Alumina stainless steel capillary column. Injector, oven and detector temperatures were 100°C, 75°C and 120°C respectively. The helium carrier gas flow rate was 7 $mL.min^{-1}$. Methane yield results were converted to standard temperature and pressure (STP: 273.15°K; 1013.25 hPa). Alkalinity was determined daily by titration according to standard methods [31]. Total and volatile solids were determined based on standard methods [31]. Concentration of ammonium nitrogen was determined by cuvette tests (LCK 304), total VFA were quantified by esterification [32] and colorimetric determination using a DR5000 spectrophotometer (Hach-Lange, USA).

2.3. Experimental design

2.3.1. High organic loading rate start-up

This study was carried out over a period of 190 days and it involved 6 different stages as shown in Figure 1. After start-up, the reactor was continually fed solely with green peas, but with a constant OLR of 2.67 kg VS.m^{-3}.day^{-1} for 15 days. Between Days 16 and 31, 2% by weight of the green peas was replaced with an equal amount of seaweed, whilst maintaining the overall OLR constant. The system was then operated until Day 66 in a sequence which alternated feeding at a loading rate of 2.67 kg VS.m^{-3}.day^{-1} and periods without feeding (Day 40-50) in an attempt to reduce the rate of accumulation of volatile fatty acids (VFA) (and hence prevent excessive fall in pH) which had increased sharply following addition of the seaweed. From Day 66 to Day 95, the OLR was gradually reduced, whilst maintaining the relative proportions of each feedstocks constant. Between Days 96 and 155, the reactor was fed with only green peas at much reduced OLRs that was varied from 0.89 to 1.78 kg VS.m^{-3}.day^{-1}. In the last stage of the experiment, both substrates were added daily at a further reduced total OLR of 0.19 kg VS.m^{-3}.day^{-1}.

2.3.2. Low organic loading rate start-up

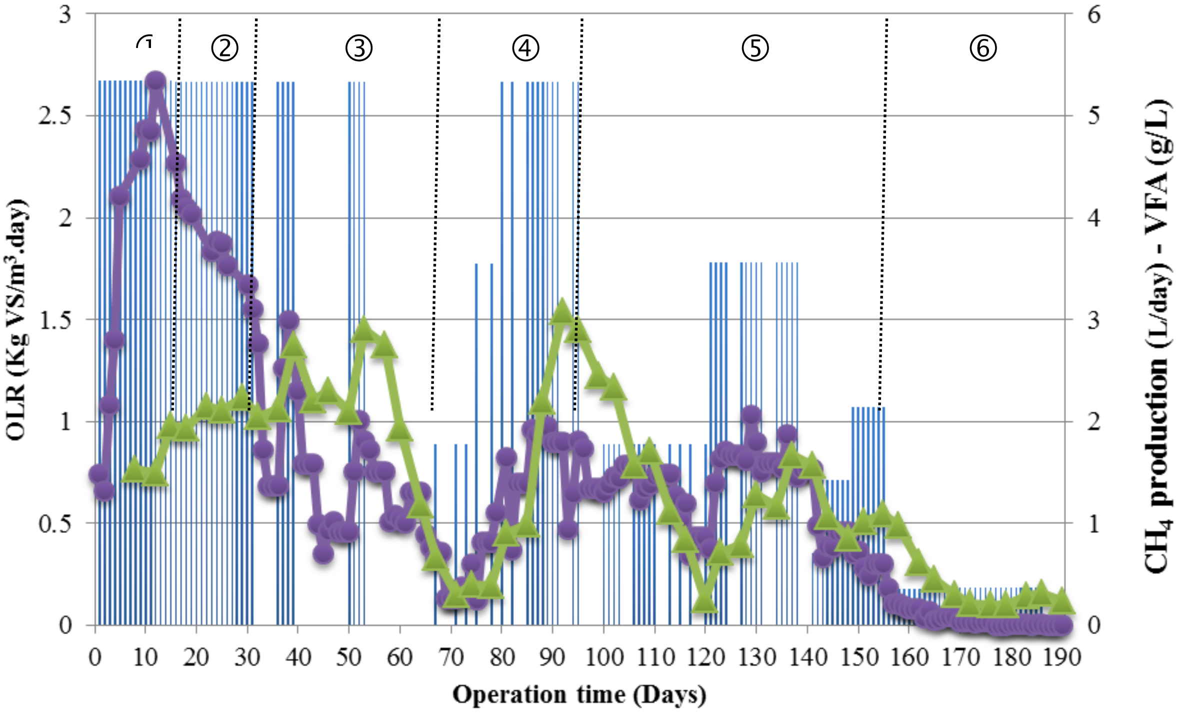
This second trial came about following experiences obtained from the earlier high OLR study and was aimed at improving the stability of the co-digestion process. The experiment was conducted over a period of 220 days and it also involved 6 distinct stages following the start-up period as shown in Figure 2. Start-up procedure was similar to the first experiment and involved the use of mesophilic sludge inoculum and feeding with a solution of green peas in a feed batch mode. After start-up, the reactor was fed solely with green peas at a constant OLR of 0.71 kg VS.m^{3}.day^{-1} for 10 days and thereafter seaweed was gradually added to increase the loading rate to 0.77 kg VS.m^{-3}.day^{-1} until Day 54. VFA accumulation was observed following the addition of the seaweed. Consequently between Days 55 and 74, the reactor was operated with a lower fraction of seaweed in the influent feedstock in a sequence which alternated feeding at OLRs of 0.74 and 0.72 kg VS.m^{-3}.day^{-1} and periods without feeding. On Day 75, calcium carbonate (16.5 g as $CaCO_3$) was added to the reactor with the aim of increasing the alkalinity of the system and hence it’s buffering capacity. From Day 76 onwards 0.1 g of $CaCO_3$ was added daily into the reactor to bolster its alkalinity and hence reduce the fall in pH. Between Days 88 to 102, the loading rate was decreased from 0.72 kg VS.m^{-3}.day^{-1} to 0.18 kg VS.m^{-3}.day^{-1} with seaweed representing less than 2% of the total OLR in a further attempt to reduce VFA levels. The loading rate was then kept constant at 0.18 kg VS.m^{-3}.day^{-1} for a further 20 days during which the proportion of seaweed was increased to up to 35% of the total OLR. At steady state performance, the OLR was gradually increased along with the percentage of the seaweed in the feedstock over a period of 98 days to a maximum value of 1.25 kg VS.m^{-3}.day^{-1}.

3. Results and Discussion

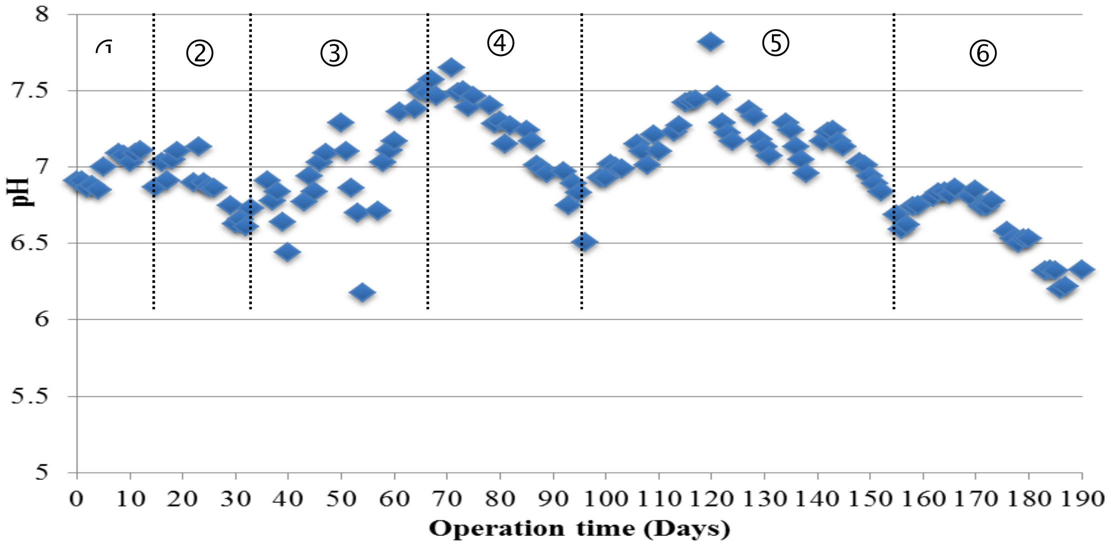
3.1. System performances and recovery at high start-up OLR

The methane production and volatile acid concentrations in the system are shown in Figure 1. During the first phase of the assay (green peas only)①, the system produced a daily methane yield of 5.5 litres, and the pH values varied from 7 to 7.7. With the addition of seaweed ②, methane yield immediately dropped sharply with a corresponding increase in VFA levels and lower pH values of between 6.8 and 7.0. To reduce excessive VFA accumulation, the reactor was fed intermittently. However, the VFA concentrations continued to fluctuate resulting in a substantial drop in pH to about 6.5 between Days 31 and 35③.

Feeding was stopped on Day 40 and resumed again on Day 50, and this led to further increases in VFA concentrations. Thereafter, feeding was stopped on Day 53 and the digester was left to recover until Day 66. A gradual rise in the OLR accompanied by an alternating feeding regime resulted in an increase in biogas production but VFA levels again reached concentrations of up to 3 g/l, as can be observed in stage ④, which impacted directly on pH values. Stage ⑤, which consisted of intermittent feeding with relatively high OLR made up of only green peas, seem to have brought about improved system performance as shown in the figure. This stage was followed by a further reduced OLR comprising of both substrates⑥.



Methane production (●), VFA (▲) and OLR (■)



①. Green peas only: continuous feeding
②. Green peas + 2% (dry wt) seaweed: continuous feeding
③. Green peas + 2% (dry wt) seaweed: intermittent feeding
④. Green peas + 2% (dry wt) seaweed: intermittent feeding
⑤. Green peas only: intermittent feeding
⑥. Green peas + 2% (dry wt) seaweed: continuous feeding

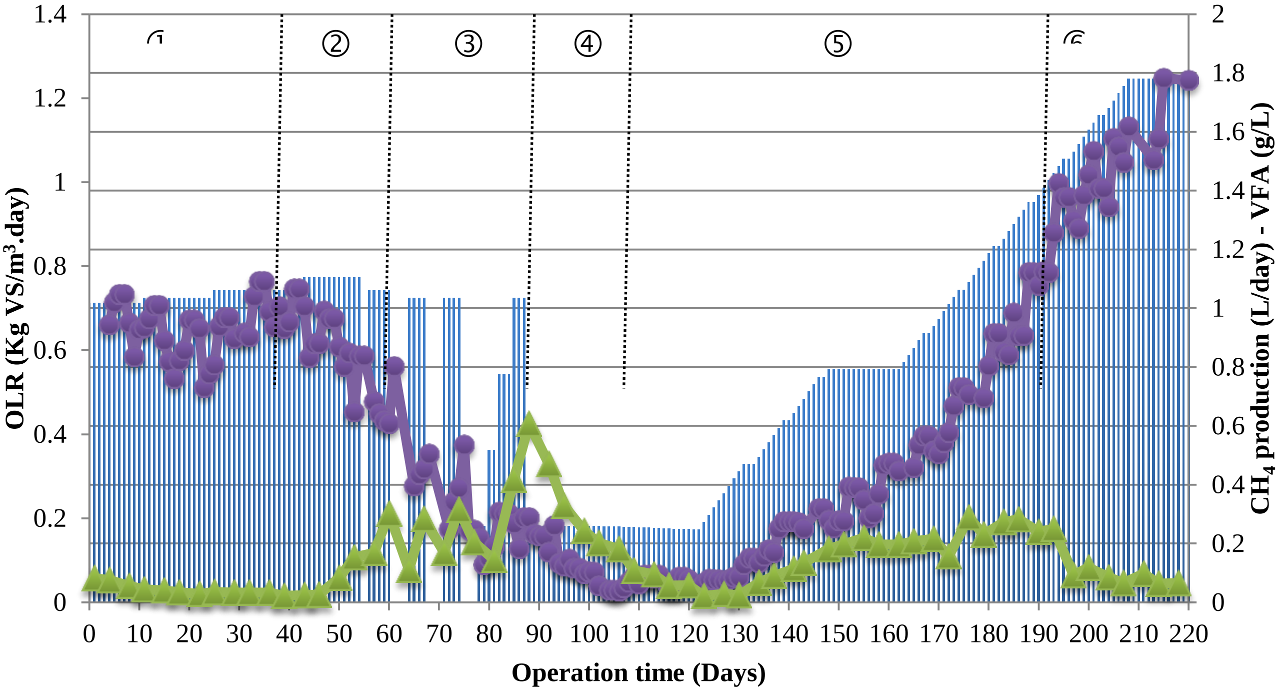
Figure 1. Performance of high start-up OLR co-digestion over 190 days.

In general, the results suggest that the addition of a small proportion of brown seaweed to a digester treating vegetable residues can bring about a significant perturbation of the anaerobic processes, to the detriment of methanogenesis. This perturbation appears to be reversible, that is, reactor stability can be restored once seaweed is no longer part of the feedstock mixture. This suggests that certain constituents of the seaweed pose proportionately higher levels of inhibition to the methanogenic anaerobes than their fermentative counterpart. Consequently, at relatively high OLR, and in the presence of seaweed, the resultant lower methanogenic activity can lead to a build-up of VFA, with the production the latter apparently less affected by the new feedstock mixture.

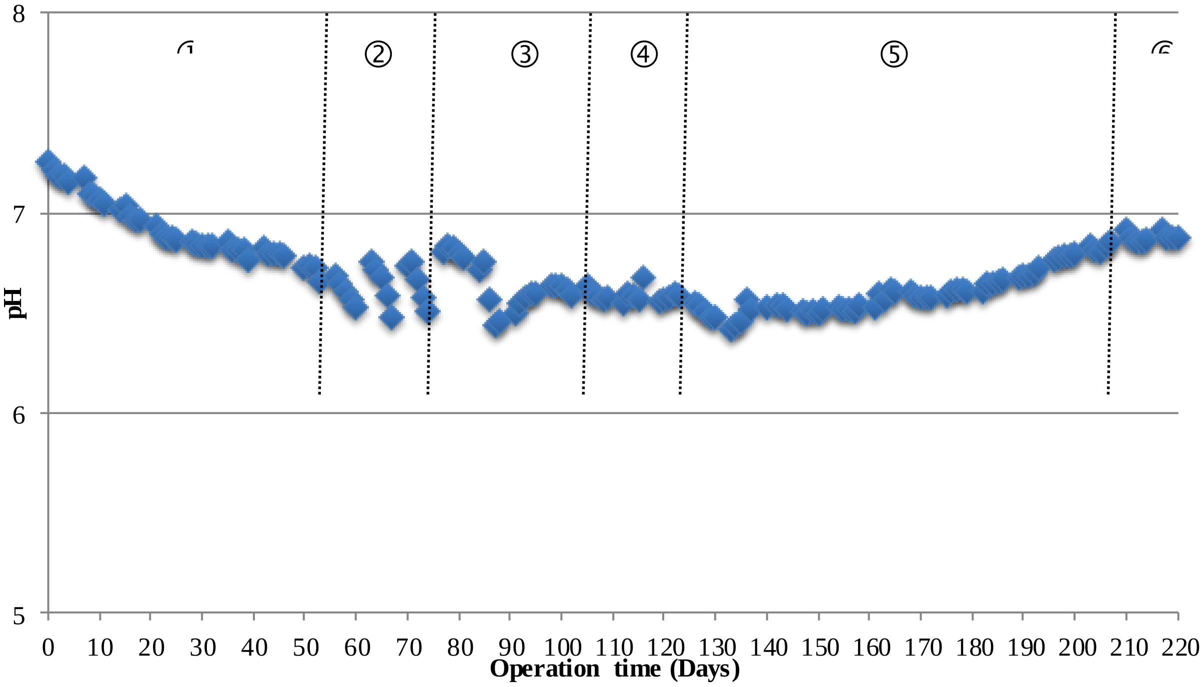
3.2. System performances at reduced start-up OLR

The total methane and VFA production are shown on Figure 2. From Day 11 to 42, the methane yields remained stable when seaweed represented 2% to 5% of the total organic mass input. On Day 43, the proportion of seaweed in the feedstock was increased to represent 10% of the total OLR (0.77 kg $VS.m^{-3}.day^{-1}$) and the methane production dropped substantially (①) with an increase in the total VFA levels and a sharp decrease in pH values from near neutral levels to about 6.5.

Consequently, the system was operated in a sequence which alternated feeding at varying loading rates and periods without feeding between days 54 and 75 (②). During this period, VFA concentrations and pH levels fluctuated rapidly, maximum and lowest values of 0.3 g/l and 6.5 respectively. On Day 75, $CaCO_3$ was added daily in order to increase the system buffering capacity, and hence to reduce the fall in pH. Feeding was started again on Day 78 (③), but a sharp increase in the OLR resulted in the accumulation of VFA and a decrease in the production of methane, with the maximum VFA concentration of 0.6 g/l. The OLR was thereafter reduced to 0.18 kg VS.m^{-3}.day^{-1} between Day 90 and Day 122. During this period, (④), the OLR was kept constant whilst the percentage of seaweed was increased, resulting in a slight augmentation in methane gas production and stable pH levels. From Day 122, the gradual raising of the OLR resulted in an increase in methane production to 1.8 l/day with relatively low VFA levels (⑤).



Methane production (●), VFA (▲) and OLR (■)



①. Green peas only, first 10 days, followed by gradual addition of seaweed up to 10% (by dry wt)
②. Green peas with gradual reduction in the proportion of seaweed to about 2% (by dry wt)
③. Green peas + about 2% (by dry wt) of seaweed: decrease in overall OLR
④. Green peas with gradual increase in the proportion of seaweed: constant low OLR
⑤. OLR increase and increase in the proportion of seaweed to up to 35% (by dry wt)
⑥. Green peas + 35% (dry wt) seaweed

Figure 2. Performance of low start-up OLR co-digestion over 220 days.

These results suggest that the addition of seaweed to a system digesting terrestrial biomass is likely to bring about a ‘shockload’ effect on the anaerobic digestion process, symptomatic by excessive accumulation of VFA. Since the methanogens appear to require a longer time to adapt to some constituents of the new substrate, the continued build-up of VFA and the resultant low pH can contribute further to the inhibition of methanogenic activities. This study has shown that low initial OLR and high alkalinity are required in order to ensure a satisfactory environmental condition during methanogenic adaptation to certain inhibitory constituents in seaweed.

3.3. Comparing biogas production at high and low start-up OLR

Figure 3 shows similar rates of biogas production at the beginning of both studies when only green peas were fed into the reactor. At high OLR start-up, the specific gas production varied during the whole experiment, which is an indication of process instability. However, for the low OLR start-up, the specific gas production varied widely initially before becoming stable from Day 130, and thereafter increased steadily with increase in OLR to reach a maximum value of approximately 0.5 m^3/kg VS_{added} on Day 220, with methane content in the range of 55-65%.

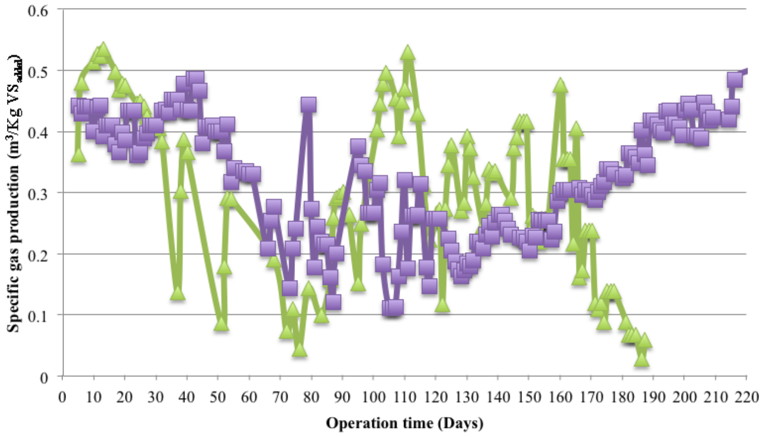


Figure 3. Specific gas production: ▲ high OLR (190 days); ■ low OLR (220 days).

3.4 General Discussion

Both studies demonstrate that the addition of even a small amount of seaweed can cause process instability leading to a rapid build-up of VFAs, which is indicative of a proportionately higher inhibitory impact of seaweed on the methanogens. The fact that process instability observed in this study was triggered by a very small proportion of seaweed in the feedstock (in this case, by as small as 2%), shows that the inhibitory compound is disruptive even in trace amounts. The effect of this instability was also expressed in the gas composition. The results also show that VFA accumulation and rapid falls in pH values are the key indicators of seaweed-induced inhibition, however, the magnitude of the inhibition seem to depend on various factors such as the operational OLR prior to the addition of the seaweed, the proportion of seaweed in the feedstock mixture and the alkalinity of the reactor contents. Process instability caused by co-digestion with seaweed can therefore be reduced by operating the system at very low OLR, that is, lower than the operating OLR when seaweed is not part of the co-digestion feedstock. It is also essential to ensure that the buffer capacity of the reactor contents is maintained at an appropriate level prior to the addition of seaweed. Operating a co-digestion system at low OLR, and with initial low concentrations of seaweed, can ensure that any consequent instability created such as VFA accumulation and fall in pH do not present additional challenges to the microbial community during their adaptation to the inhibitory constituents of the seaweed. This study has also shown that it may be necessary to augment reactor alkalinity by chemical addition during the initial stages of co-digestion with seaweed to ensure stable optimum pH values, necessary for faster and effective adaptation of the methanogens to the seaweed. This approach has, in this study, led to an eventual increase in both the OLR and the proportion of seaweed that can be added by up to 35% of the total organic input.

4. Conclusions

This study has shown that seaweed contain certain compounds that even at trace concentrations appear to be inhibitory to the anaerobic microbes. These compounds appear to have more adverse effects on methanogenesis than on the stages of the process. Effective microbial adaptation and start-up procedures, involving initial very low seaweed addition, have been shown to enhance sustainable microbial adaptation of the inhibitory compounds contained in the seaweed. Where seaweed is only available during certain periods of the year, the results of this study indicate that fresh start-up procedures may be necessary each period that the seaweed is available as substrate before co-digestion. More investigation is thus required to understand the limits of microbial adaptation to seaweed and the microbial responses to variable periods of presence and absence of seaweed in the feedstock, before commercial-scale co-digestion with seaweed is viable.

5. Acknowledgements

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6. References

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