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Mono and Co-Digestion of Laminaria Digitata with Simulated Food Waste (SFW)

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Abstract - *This study examined anaerobic digestion of mono and co-digestion of Laminaria digitata (LD) with a simulated food waste (SFW) in a continuous reactor experiments. Different mix ratios of LD and SFW, namely, $LD_{100:0\%}$, $LD_{90:10\%}$, $LD_{75:25\%}$, $LD_{50:50\%}$ were assessed. Results indicated that reactor, $LD_{90:10\%}$ was found to be optimal for the highest cumulative methane production (175 ± 0.17 L/ reactor) after 85 days and achieved a maximum biomethane efficiency factor BEF (0.93) at an OLR 4 $gVS.L^{-1}.d^{-1}$. The mono-digestion of $LD_{100:0\%}$ in continuous reactors was characterized by the accumulation of high total volatile fatty acids (tVFA), reduced pH, and an increased FOS: TAC ratio as the OLR was increased, which led to reactor failure.. Co-digestion of L digitata with SFW seem to cause the dilution of inhibitory components which was not evident in the mono-digested reactor.*

Key Words: Laminaria digitata; co-digestion; continuous reactor; anaerobic digestion; biomethane.

1. INTRODUCTION

The need to develop renewable energy has seen a recent increase in the amount of research on the use of waste material for anaerobic digestion technologies. Food waste, biodegradable municipal waste fractions, energy crops, and potentially seaweed (macroalgae), are used as feedstocks for these systems. Research into the potential of mixed co-digestion feedstocks is important as it can overcome some of the limitations of using single feedstocks such as generation of high concentrations of toxic products and extends access to greater quantities of potential feedstock material. Anaerobic co-digestion is regarded as a favourable option for increasing biogas production because of balanced nutrients and improved efficiency [1]. Co-digestion of several wastes has been increasingly applied in an effort to boost plant profitability [2] and has shown to improve biogas productivity[3]. Co-digestion has the ability to solve the problem of low C: N ratio and can dilute toxic compounds making them less toxic [4]. With respect to the feedstocks used in this study, macroalgae have been identified as a feedstock with sustainable potential for co-digestion with food waste having positive environmental and health benefits [5]. Macroalgae can be converted to biofuels from thermal, fermentation and various other processes [6]. Anaerobic digestion is the most direct route to obtaining biofuels from macroalgae [7]. Generally, organic fraction of stimulated food waste (OFMSW) is seen as a very attractive waste material for biogas plants as it is a readily available organic feedstock with a high biogas potential [8]. Food waste has been used as a co-substrate in a biowaste digester for equilibration of biogas production because of its steady availability, similar biodegradability and high methane potential [9]. This study investigated the potential of macroalgae as a possible feedstock for renewable energy via co-digestion with SFW.

1.1 Methodology

1) Collection, pre-treatment, and storage

Algal biomass *Laminaria digitata (LD)* used in this study were collected from shallow water during low tide at Culler coats Bay, 55.0342□N, 1.4309□W ,Tyne and Wear (NZ3572) and Seaton sluice, 55.0836□N, 1.4744 □W, Northumberland UK (NZ 3350) in December, 2017. The seaweeds were transported in 30-liter bags and were immediately washed to remove marine salts and sediments which can cause mechanical problems in digesters. The reactors feedstocks were prepared using only the frond; the stipe and holdfast were discarded. The fronds were roughly chopped by hand to particle size of about 10 mm. To obtain the dry algal substrate the roughly chopped frond was oven dried at 70 □C for 24 - 48 hrs. This was then pulverized with a Kenwood 100 coffee blender to particle size generally < 1 mm. All samples were stored at 4 □C in an airtight gas bag until required.

2) Synthetic food waste preparation.

The synthetic food waste (SFW) components were selected and prepared according to methods reported by [10][11]. A representative sample, 50g of each food substrate was weighed, then first chopped into small sizes (1 – 5 cm) with a kitchen knife before maceration and blending for approximately 2 minutes in a kitchen blender (James martin ZX 865) to produce a homogenous mixture of approximately 0.5 - 1 mm typical size.

3) Experimental design/reactor system

The setup of the CSTR as described by [12] but modified; the continues study were performed in 1 L Quick fit□ reactor vessels (800 ml working volume) with wide ground-glass necks. A multi-port head plate Quickfit□ flanged was fitted to the reactor vessel with a spring clamp. Five, 19/26 ground sockets on the head plate allowed gas lines to be fitted, and the impeller drive shaft to pass into the reactor through a Quickfit glass stirrer gland with a water-seal to ensure the reactor remained gas-tight. In order to ensure complete anaerobic conditions a feeding / sampling port was fitted with a PVC tube (12 mm in diameter, 80 cm long) into the

reactor vessel through one 19/26 sockets on the head plate to reach below the liquid level. Vacuum grease (Dow Corning, USA) was used to maintain the integrity of all ground glass seals and sockets pots not used were sealed with glass 19/26 stoppers. Mixing was achieved with a 40 □ 80mm rectangular impeller rotating at 90 rpm.

4) Inoculum and operation

The reactors were inoculated with a mixed methanogenic sludge from a full-scale running anaerobic digester (Cockle Park Farm, Newcastle) operating on grass silage. It had following characteristics; pH 7.50, 21.2% TS, 60% VS (%TS), 0.019 Sulphur and C: N of 0.061. The CSTRs were operated in semi-continuous batch mode, with daily feeding event being initiated by the removal of an appropriate volume (Reactor Volume/hydraulic residence) of mixed liquors from the feeding/sampling point on the head plate of the reactor using a 100 ml plastic syringe. Stirring continued during sampling to prevent settling and fractionation of the reactor solids [12]), and the importance of mixing the reactors for efficient substrate conversion has been reported by many researchers [13]. An experimentally determined quantity (expressed as dry weight (g VS / L) was made up to a specified volume of water (water volume dependent on hydraulic residence), to replace exactly the sample volume that had been removed from the reactor, and added manually through a head plate port. All samples were carried out in duplicate.

5) Experimental procedure

The continuous reactor study comprised a series of 4 identical, 1-litre continuous stirred tank reactors (CSTR) (R 1 - R 4) operating simultaneously for 85 days under different mix ratios *($LD_{100\ \%}$, $LD_{90:10\%}$, $LD_{75:25\ \%}$, and $LD_{10:90\ \%}$)* but with the same daily feeding regime, with a hydraulic residence time of 25 days. The different mix ratios used for the reactors are given in Table 1.

Table 1 Ratios of LD with SFW used in both batch and continuous reactors study.

Ratios	Algae 100: 0 SFW	Algae 90: 10 SFW	Algae 75: 25 SFW	Algae 50: 50 SFW
Continuous reactors	R 1 (LD100 %)	R 2 (LD90:10 %)	R 3 (LD75:25 %)	R 4 (LD50:50 %)

The initial inoculum concentration was 10 $gVS.L^{-1}$, and was pre-acclimatised with macroalgae (1 gVS) feedstock daily for 9 days, then degassed for 3 – 5 days before the start of experiment. The organic loading rate OLR ($g\ VS.L^{-1}\ d^{-1}$) was increased stepwise after acclimatization from 2 $g\ VS.L^{-1}\ d^{-1}$ on day 1 of the experiment to 3 $g\ VS.L^{-1}\ d^{-1}$ on day 26, thereafter, to 4 $g\ VS.L^{-1}\ d^{-1}$ on day 39 and, finally to 5 $g\ VS.L^{-1}\ d^{-1}$ on day 55, till the end of the experiment. Biogas production rate was measured daily.

6) Analysis of Process Parameters

pH and solids

The pH was measured daily from the removed liquors (reactor effluent) at each feeding event using a Jenway 3010 pH meter.

Elemental composition (CNS) analysis

Samples (dried, powdered; ca. 50 mg) were weighed accurately into ceramic crucibles and analysed for carbon, nitrogen and sulphur content using an Elementar VarioMAX CNS analyser. The analysis involves combustion at 1145□C in an oxygen-enriched helium atmosphere. Sulfadiazine (%N = 22.37; %C = 47.99; %S = 12.81) was used as the calibration standard and was analysed at the start and end of the sample sequence and after every 5 - 10 samples. Raw data were corrected for analytical drift (based on the calibration standard data) during the analysis using the Elementar software.

Volatile fatty acids (VFAs)

Volatile fatty acids (VFAs) was analysed on a Dionex ICS 1000 with an AS40 autosampler (Dionex, USA). Separation was carried out on an ionpac ICE-AS1 4 □ 250 mm analytical column with a flow rate 16 ml min-1; 1.0mM heptafluorobutyric acid eluent; 5 mM tetrabutylammonium hydroxide suppressant regenerant; and a 10ul injection loop. Supernatant of centrifuged samples liquors were filtered through a 0.20 µl syringe filter (VWR, UK), 0.4 ml of filtered samples were then diluted 1:1 with octane sulfonic acid, and sonicated (FS200B Sonic Bath, Decon Laboratories, Sussex, UK) for 40 mins to remove carbonate, which caused interference. The prepared samples were then transferred to 1 ml tubes with filter caps (Dionex, USA) before analysis.

Biogas and methane measurement

The percentage (%) methane from the biogas content was determined using a GC-FID analyser (Carlo-Erba 5160 GC) in split mode with the injector at 150□C and FID at 300□C. Using a 100 µl sample Lock syringe (Hamilton, USA), duplicate headspace samples (100ul) were injected manually every 2 minutes into the GC with the split open 5 turns (100mls min^{-1}). After the initial injection, the GC temperature programme and data acquisition commenced. Separation was performed on an HP-PLOT-Q capillary column (30m x 0.32mm id) packed with 20um Q phase. The GC was held isothermally at 35□C for 90min and heated to 250 □C at 10 □C min^{-1} and held at final temperature for 10 minutes with Helium as the carrier gas (flow 1ml min^{-1}, pressure of 50kPa, split at 100mls min^{-1}. The acquisition was stored on an Atlas laboratory data system. Methane standard were prepared prior to each analysis from 100% analytical grade CH_4 (BOC Gases, UK) by injecting duplicate sample to make a five–point standard curve in the range 20 - 100% CH_4. The volume of biogas produced was measured using a 100 ml BD Plastipak syringe from the gas bags. The % methane

calculated was multiplied by the measured biogas volume giving the volume of methane produced [14].

2. Results and Discussion

2.1 Characterisation of macroalgae and food substrates

The chemical characteristics and elemental analysis of the macroalgae, food and inoculum samples used in this study are shown in Table 2. From Table 2, the total solids (%TS) of the algae feedstock is 86.8% with the organic fraction (% VS) constituting about 61.2 % of the TS. The %TS of the co-substrate (FW) is 10.1% with a %VS content of 61.2 %. The C: N ratio for both the macroalgae (11.7: 1) and food substrate (11.0: 1) are quite similar as shown in Table 2 but are still under the ideal range of 15:1 - 30 :1 suggested as optimum conditions for AD operation [15]. This low C: N ratio obtained for the substrates indicates they might be problematic during the digestion process leading possibly to accumulation of toxic level of total ammonia nitrogen (TAN) which inhibits methanogens [15]. *L. digitata* has been reported as having a range between 10.9: 1 - 31.9: 1 [14] while the foods substrate is 134: 1.

2.2 Continuous co-digestion studies

Figure 2 and

Figure 3 outline the daily and cumulative biogas production profile, % methane content and cumulative methane production of the different mix ratios in the continuous digestion studies. **Error! Reference source not found.**- Figure 6 show the variation in the methane yield (MY) and the FOS: TAC ratio for tested OLRs for mono-digestion and co-digestion of the macroalgae and stimulated food waste. The daily biogas production for the mix ratios is shown in Figure 2A. The biogas production increased as the OLR was increased from 2 gVS.L^{-}.d^{-1} - 5 gVS.L^{-1}.d^{-1}, and achieved stable and steady production, except for the $LD_{100\%}$ reactor which showed signs of reactor instability from day 60 with reduction in biogas production at OLR 5 gVS L^{-1} d^{-1}. From Figure 2B, the $LD_{90:10\%}$ mix ratio produced the highest cumulative biogas production (175 ± 0.17 L / reactor) after 85 days of digestion followed by $LD_{100\%}$ (173 ± 0.27 L / reactor) with the lowest value from $LD_{50:50\%}$ (113 ± 0.07 L/ reactor). The cumulative methane production,

Figure 3 B, evaluated from the biogas production also followed similar trend with the highest for $LD_{90:10\%}$ (42.77± 0.19 L/ reactor, $LD_{100\%}$ (40.068 ± 0.20 L/ reactor) while the lowest was for $LD_{50:50\%}$ (28.86 ± 0.09 L/ reactor). The methane content of the biogas, (

Figure 3A), increased from 14 % for $LD_{100\%}$ and between 25% - 44% for the other reactors on commencement of the digestion process after acclimatization and remained in a steady range of between 45% - 60%. As the OLR was increased stepwise $LD_{100\%}$ reactor showed signs of inhibition (unsteady state) at OLR 5 with a sharp reduction of the methane content from day 75 to around 38% and continued to drop to around 16% by the end of the experimental duration. Generally, from Figure 2A, there was a reduction in biogas production in all the reactors on day 39 in the OLR 3 regime, as a result of an unplanned drop in temperature to about 22 ℃ (equipment failure) before recovering, this lead to a drop in pH in all reactors to around 7.0 - 7.1 and increase in tVFAs to between 15 g L^{-1}- 20 g L^{-1}, Figure 7 A and C.

2.3 Assessment of mono-digestion of $LD_{100\%}$ (100% L. digitata, 0% food waste)

The variation in CH_4 production and methane yield (MY) for R 1 ($LD_{100\%}$) with respect to increasing OLR from 2 - 5 gVS.L^{-1}.d^{-1} over the duration of the experiment is shown in **Error! Reference source not found.**. An assessment of the reactor process is given in Table 3. Generally, it is assumed for the continuous processes, stable digestion is achieved with a FOS: TAC ratio between 0.2 - 0.4 and when the MY value approaches the BMP value [16]. From Table 3, for $LD_{100\%}$ the biomethane efficiency factor (BEF) was estimated as 0.70, 0.61, 0.72 and 0.57 for OLR 2, 3, 4 and 5, respectively. The drop in BEF to 0.47 at OLR 5 is due to the higher loading rate which resulted in corresponding accumulation of tVFAs, reaching a maximum value of 15.5 g L^{-1} (Figure 7 C), a drop in pH to around 6.75 (Figure 7 A), and an increased FOS: TAC ratio to 2 at the end of the run (**Error! Reference source not found.**). Although the average pH observed at the different OLR is between 7.38 - 7.11 (Table 3), it then dropped to around 6.75 at OLR 5, indicating potential methanogen inhibition, which could lead to reactor failure if the process continued. The average methane content in the biogas also dropped from 59% - 47% and to 16% by day 85,

Figure 3A. The C: N ratio was 11.69: 1, a value that is regarded as non-optimal since AD process inhibition has been reported with C: N ratios less than 20: 1 and unbalanced ratios have been identified as a limiting factor during AD of algal biomass [17]. A feedstock with low C: N ratio could result in elevated tVFAs accumulation in the digester [18].

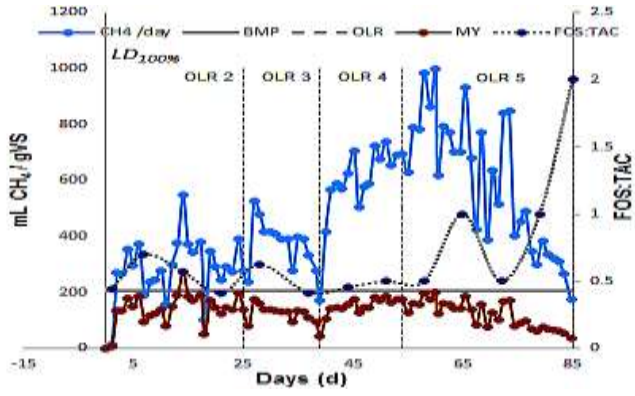


Figure 1: Continuous reactors operating with mono-digestion of *L. digitata* R1 ($LD_{100\%}$) at increasing organic loading rate (OLR) (gVS.L^{-1}.d^{-1}), showing CH_4 production,

MY, BMP (mL CH_4/ gVS), and FOS: TAC ratio. Vertical dashed line indicates change in OLR.

Table 2 Characteristics of inoculum, macroalgae, and food used for continuous processes.

Characteristics	Inoculum	Macroalgae	Food
% TS	25.6 (0.11)	86.8 (0.03)	10.1 (0.07)
% VS (% TS)	51.8 (0.08)	61.2 (0.07)	94.3 (0.12)
% Moisture	*	13.3 (0.10)	89.9 (0.08)
TKN (g/kg)	*	5.0 (0.18)	2.0 (0.22)
Ammonia (g/L)	1.76 (0.05)	1.68 (1.10)	0.42 (0.59)
Protein %TS	*	2.7 (0.18)	1.23 (0.45)
Alkalinity (g CaCO3/l)	10.5 (0.03)	*	*
TVFAs (g/L)	3.40 (0.16)	*	*
% C (% TS)		24.4 (0.36)	40.2 (0.30)
% H% (% TS)		5.0 (0.02)	7.1 (0.13)
% N% (% TS)		2.1 (0.44)	3.7 (0.85)
% S (%TS)		0.6 (0.15)	0.3 (0.02)
% O (% TS)		38.1 (0.02)	40.7 (0.15)
% Ash content		29.8 (0.01)	8.0 (0.18)
% TOC	7.4 (0.19)	29.5 (0.05)	5.3 (0.17)
C: N		11.7: 1 (0.21)	11.0: 1 (0.07)
C:S		40.7: 1 (0.11)	134: 1 (0.19)

* Not assessed

Table 3 Performance characteristics of the continuous reactors R1 - R4

OLR (kg VS / L / d)	BMP (L CH4 / kg VS)	SMY (L CH4 / kg VS)	Bio-methane efficiency factor (BEF)	CH4 (%)	HRT (days)	FOS: TAC	TAN	PH
R 1 $LD_{100\%}$ (100% L. Digitata, 0% Food waste)								
OLR 2	207 ± 0.07	143.99	0.86	59.86	25	0.42	1.43	7.38
OLR 3		126.55	0.76	57.2	13	0.51	1.12	7.36
OLR 4		148.36	0.89	52.97	16	0.47	1.07	7.34
OLR 5		118.96	0.71	47.87	31	1	0.7	7.11
R2 $LD_{90:10\%}$ (90% L. Digitata, 10% Food waste)								
OLR 2	167 ± 1.54	139.97	0.84	61.11	25	0.41	1.53	7.41
OLR 3		112.56	0.67	58.18	13	0.40	1.17	7.38
OLR 4		155.86	0.93	56.17	16	0.48	1.24	7.41
OLR 5		138.09	0.83	52.3	31	0.39	0.89	7.46
R3 $LD_{75:25\%}$ (75% L. Digitata, 25% Food waste)								
OLR 2	174.31 ± 1.24	132.05	0.76	65.48	25	0.28	1.42	7.39
OLR 3		108.35	0.62	57.52	13	0.4	1.09	7.37
OLR 4		127.48	0.73	55.18	16	0.43	0.98	7.38
OLR 5		121.49	0.70	52.99	31	0.43	0.74	7.42
R4 $LD_{50:50\%}$ (50% L. Digitata, 50% Food waste)								
OLR 2	115.31 ± 0.43	102.86	0.89	67.43	25	0.28	1.23	7.37
OLR 3		79.02	0.69	62.29	13	0.33	1.11	7.29
OLR 4		98.90	0.85	56.51	16	0.38	0.91	7.31
OLR 5		92.28	0.80	55.21	31	0.66	0.79	7.32

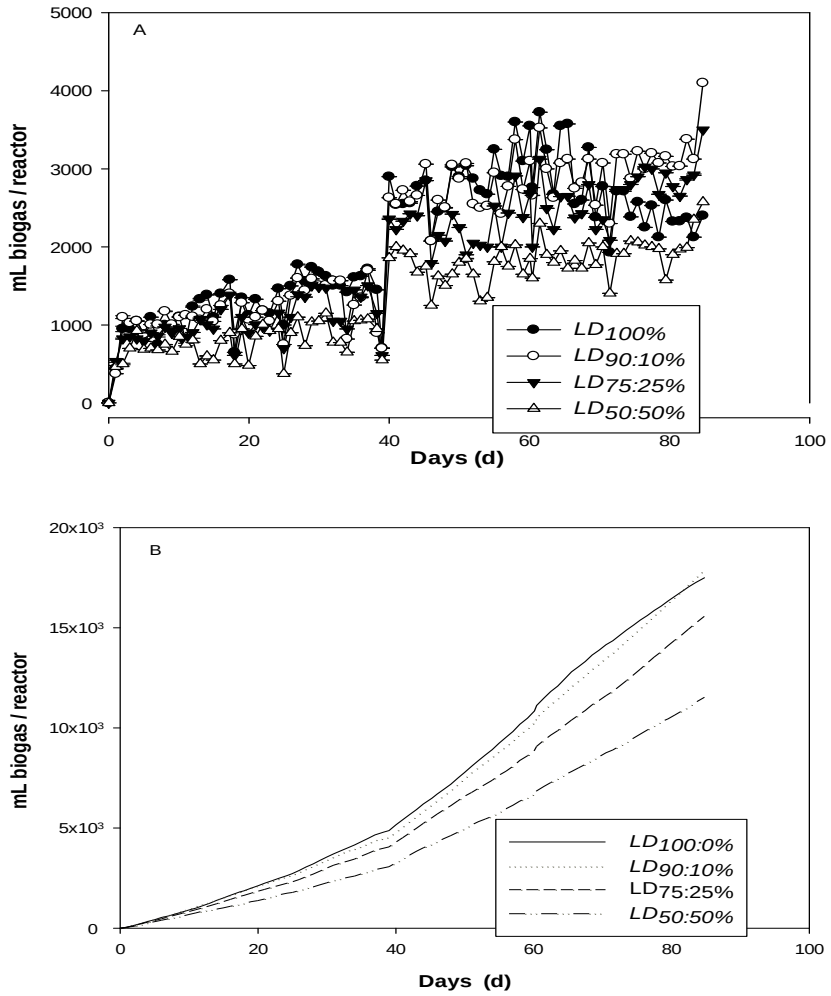
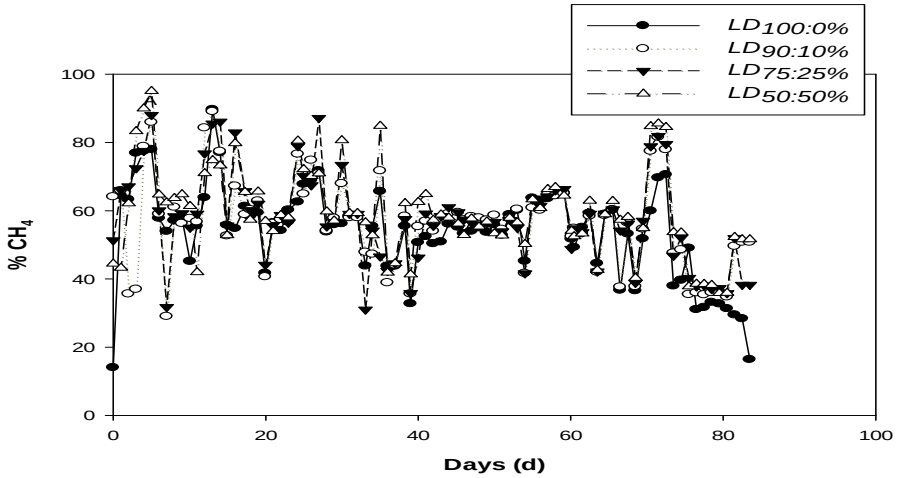


Figure 2: Continuous reactors operating with different co-digestion mixtures; A), Daily biogas production; B), Cumulative biogas production.



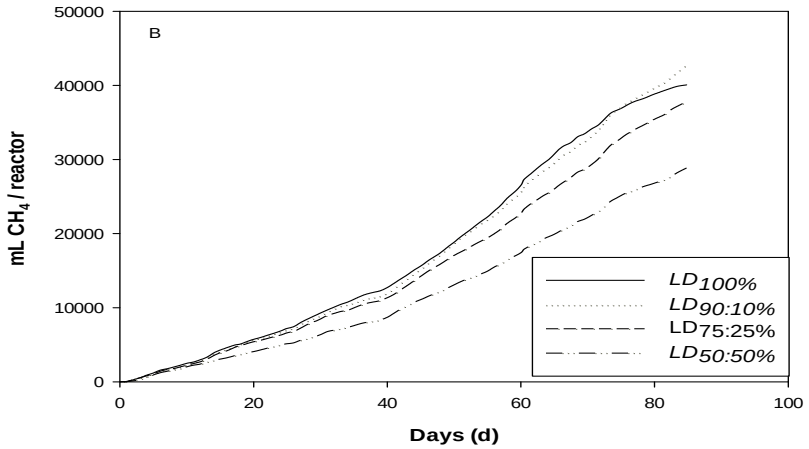


Figure 3: Continuous reactors operating with different co-digestion mixtures; A), % Methane; B), Cumulative methane production.

2.4 Assessment of co-digestion of $LD_{90:10\ \%}$ (90% L. digitata, 10% food waste)

Reactor R2, operating with feeding regime $LD_{90:10\%}$, contained the lowest feed component of food waste among the mixed ratio reactors (R2 – R4), and produced both the highest cumulative biogas and methane content (Figure 2A and

Figure 3 B). This enhanced efficiency can be attributed to acclimatization of the reactor sludge microorganisms to the food waste and macroalgal biomass co-digestion feedstock. Synergy can be brought about by improved and balanced C: N ratio, which can be achieved by blending feedstock components, preventing ammonia inhibition, and by improving the bioavailability of nutrients [19], and this could be attributed for the performance of R2.

Figure 4 shows the variation in CH_4 production together with the MY and FOS: TAC ratio. The co-digestion process operated steadily, with increase in methane yield occurring as the OLR was increased, with OLR 4 $gVS.L^{-1}.d^{-1}$ producing the highest MY yield close to the BMP value (Table 3). Under these conditions the process was efficient in biogas production, and was operationally stable, with only short period of reactor instability during the first 10 days, since the FOS: TAC ratio fluctuated between 0.1 -.0.6 throughout duration of the experiment. Although stable digestion is characterized by FOS: TAC ratio of ≤ 0.4 or given as ≤ 0.3, between 0.3 - 0.8 indicates risk of instability and ≥ 0.8 suggests instability [20]. This demonstrates better performance of $LD_{90:10\%}$ compared to $LD_{100\%}$, which failed at OLR 5 g VS L^{-1} d^{-1} with a FOS: TAC > 0.8. The bio-methane efficiency factor (BEF) obtained at OLR 2, OLR 3, OLR 4 and OLR 5 were 0.84, 0.67, 0.93, and 0.83 respectively (Table 3). At OLR 4, the average BEF value of 0.93 was close to maximum, signifying an acclimatized inoculum and better performance of the reactor. The average pH was between 7.41 - 7.46 over the OLR tested, which probably resulted from good ammonia buffering capacity in the reactor [21]. High buffering results in less accumulation of tVFA at increased OLR. The % methane content in the biogas reduced from 61% - 52% as the OLR was increased.

The maximum tVFA concentration of 6.6 g L^{-1} was obtained on day 79 at an OLR of 5 $gVS.L^{-1}.d^{-1}$ (Figure 7 C). At this tVFA concentration, reduction in methane yield was evident (Figure 4), but not sufficient to cause failure, and the MY fluctuated from 221 mL CH_4 gVS^{-1} on day 74 to 121 mL CH_4 gVS^{-1} on day 75 and, continued in this trend before recovering on day 80, (Figure 4). It is generally accepted that the performance of an AD process has a direct correlation with concentration of the tVFA [18], and above 6 g L^{-1}, both biogas and the ratio of methane to CO_2 produced is greatly inhibited [22].

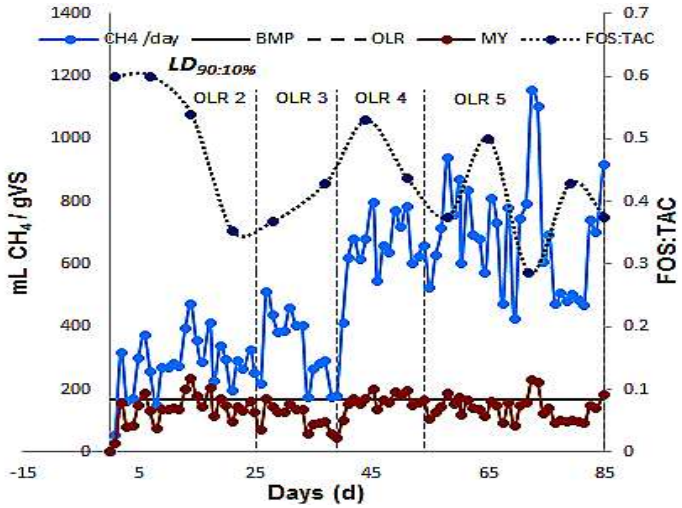


Figure 4: Continuous reactors operating with co-digestion of *L.digitata* R2 ($LD_{90:10\%}$) at increasing organic loading rate (OLR) ($gVS.L^{-1}.d^{-1}$), showing CH_4 production, MY, BMP (mL CH_4/ gVS), and FOS: TAC8 ratio. Vertical dashed line indicates change in OLR.

2.5 Assessment of co-digestion of $LD_{75:25\ \%}$ (75% L. digitata, 25% food waste)

The continuous fermentation data of R 3 $LD_{75:25\ \%}$ are shown in Figure 5 and Table 3. The methane production rate fluctuated from an average value of 132 mL CH_4 $gVS^{-1}.d^{-1}$ to 122 mL CH_4 $gVS^{-1}.d^{-1}$, which coincided with an increase in OLR from 2 g $VS.L^{-1}.d^{-1}$ - 5 g $VS.L^{-1}.d^{-1}$ over 85 days of operation. Figure 2 B shows the cumulative biogas production for $LD_{75:25\ \%}$ was 156 ± 9.20 L biogas, while

Figure 3 B shows the cumulative methane production was 38 ± 1.72 L CH_4. These are less by 11% and 5.6%, respectively, compared to the cumulative biogas and methane produced for $LD_{100\%}$ with no co-digestion mix. Comparing $LD_{75:25\ \%}$ *to* $LD_{100\ \%}$, it is evident that the former performed better because it continued to produce biogas after day 75 with no sign of instability or reactor failure, as experienced in $LD_{100\%}$. From Table 3, Reactor R3 BEF was 0.76, 0.62, 0.73 and 0.70 at OLR 2, OLR 3, OLR 4 and OLR 5, respectively. The average pH ranged between 7.39 – 7.42. The FOS: TAC ratio fluctuated only slightly (0.28 - 0.43) as the OLR was increased, indicating good stability of the process.

The average % methane concentration of the biogas in $LD_{75:25\ \%}$ reactor was highest (66%) at OLR 2 and lowest (53%) at OLR 5,

Figure 3 B. This decreasing trend was reflected in average MY value of 132 mL CH_4 gVS^{-1}, 128 mL CH_4 gVS^{-1} and 122 mL CH_4 gVS^{-1} for OLR 2, OLR 4 and OLR 5, respectively, except OLR 3 with 108 mL CH_4 gVS^{-1} which experienced drop in temperature from 35 ℃ to around 22 ℃ on day 39, hence the low average MY obtained for OLR 3.

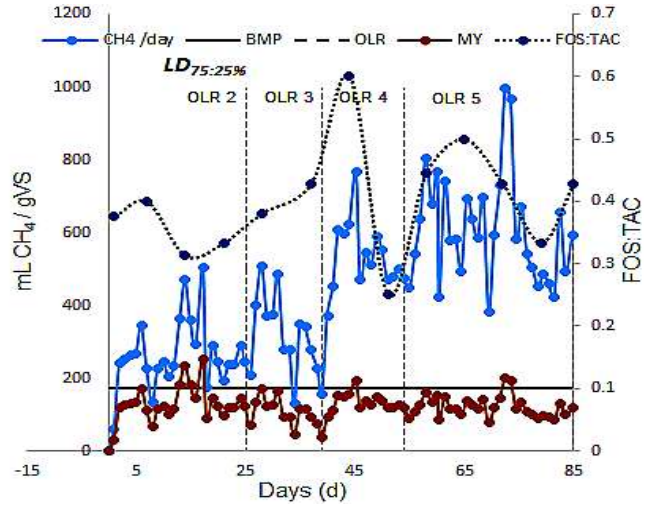


Figure 5 Continuous reactors operating with co-digestion of *L.digitata* R3 ($LD_{75:25\%}$) at increasing organic loading rate (OLR) ($gVS.L^{-1}.d^{-1}$), showing CH_4 production, MY, BMP (mL CH_4/ gVS), and FOS: TAC ratio. Vertical dashed line indicates change in OLR.

2.6 Assessment of co-digestion of $LD_{50:50\%}$ (50% L. digitata, 50% food waste)

Reactor R 4 had a feedstock mixture $LD_{50:50\%}$ consisting of equal amount of *L. digitata* and food waste. During continuous operation, the cumulative biogas and methane production were 113 ± 2.43 L biogas and 29 ± 2.01 LCH_4, respectively, (Figure 2B and

Figure 3B).

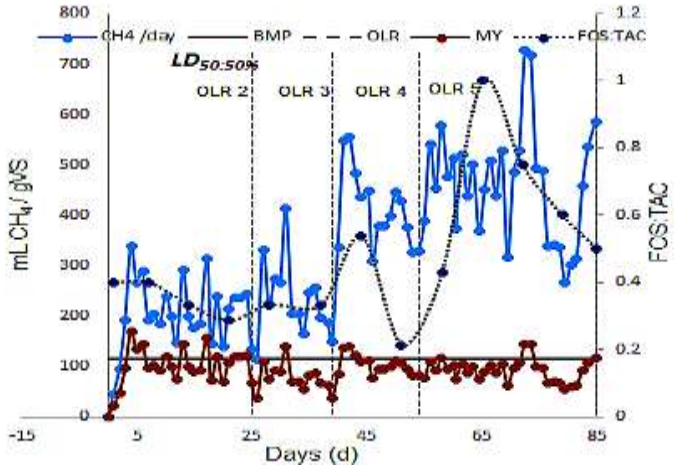


Figure 6 Continuous reactors operating with co-digestion of *L.digitata* R4 ($LD_{50:50\%}$) at increasing organic loading rate (OLR) ($gVS.L^{-1}.d^{-1}$), showing CH_4 production, MY, BMP (mL CH_4/ gVS), and FOS: TAC ratio. Vertical dashed line indicates change in OLR.

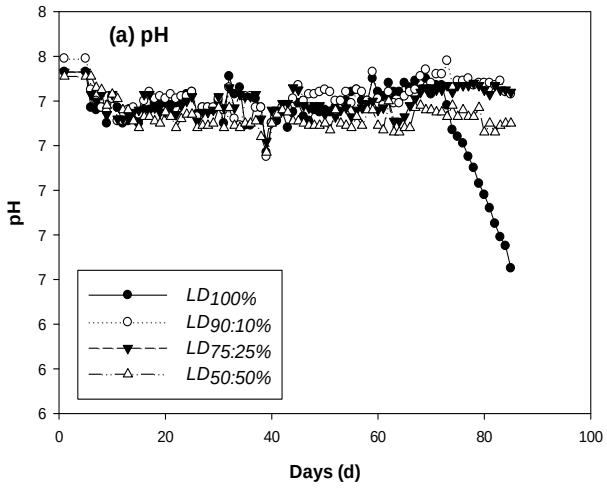
The MY were 103 mL CH_4 gVS^{-1}, 79 mL CH_4 gVS^{-1}, 99 mL CH_4 gVS^{-1}, 93 mL CH_4 gVS^{-1} at OLR 2, OLR 3, OLR 4 and OLR 5, respectively (Figure 6). Comparing $LD_{50:50\%}$ to the mono-digested reactor ($LD_{100\%}$), at OLR 5 it continued to produce gas with no sign of the instability that was experienced in $LD_{100\%}$. Nutrients supplemented from the food waste and better acclimatization of the microbial community to the mixed feedstock at the higher OLR were considered the main reasons for the high stability of reactor R4.

The % methane composition in the biogas continued to decline slightly as the OLR was increased stepwise, from 67% at OLR 2, 62% at OLR 3, 56% at OLR 4 and to 55% at OLR 5, (Table 3). The BEF was 0.89 at the initial OLR 2 after 25 days within the first HRT, but dropped to 0.69 at OLR 3, and improved again to 0.85 at OLR 4. Similar stable pH trend (7.37 - 7.32) was observed, as seen in other co-digestion mixture ratios ($LD_{90:10\%}$ *and* $LD_{75:25\%}$), with the exception of $LD_{100\%}$ (7.38 - 6.75). The FOS: TAC ratio increased from 0.4 – 1.0 indicating reactor imbalance at OLR 5 feeding regime but declined to 0.5 before the end of the 3.5 HRT period. This normalised the instability in the reactor which was reflected by the recovery and continuous production of biogas from day 75, (Figure 6). There was no tVFA accumulation which averaged between 2.6 g. L^{-1} - 2.3 g. L^{-1} (Figure 7).

2.7 Comparison of $LD_{100\%}$ with other $LD_{LD\%:\ FW\%}$ mix reactors

Process operational parameters: pH, VFA, and FOS: TAC ratio

The pH of all the co-digested mixture reactors ($LD_{90:10\%}$, $LD_{75:25\%}$, and $LD_{50:50\%}$) fluctuated between 7.60 - 7.20, compared to the mono-digested reactor ($LD_{100\%}$), which started to drop sharply from 7.10 on day 78 to 6.65 by day 85, (Figure 7A). pH is regarded as one of the critical indicators for digester performance because it promotes favorable conditions for growth of microorganisms and determines the overall performance of anaerobic digesters [23], Optimum pH range has been suggested as between 6.8 – 7.2 for methanogens and the VFA produced in the acidogenesis phase can induce a drop in pH [24]. The $LD_{100\%}$ reactor produced the highest tVFA which increased from 2.7 g L^{-1}- 15.5 g L^{-1} as the OLR increased from 2 gVS. L^{-1}. d^{-1} – 5 gVS. L^{-1}.d^{-1}, this was followed by $LD_{90:10\%}$ (3.3 g L^{-1} – 6.6 g L^{-1}), $LD_{75:25\%}$ (2.6 g L^{-1} – 2.3 g L^{-1}), with the lowest being $LD_{50:50\%}$ (1.7 g L^{-1} – 2.21 g L^{-1}). Accumulated levels of undissociated VFA cause the greatest detrimental effects on AD process by allowing VFA to penetrate cell membranes and damage intracellular macromolecules [25]. Consequently, a VFA range of 2.0 g L^{-1}– 3.0 g L^{-1} is regarded as the optimum level for metabolic activity [26]. At OLR 5 gVS. L^{-1}.d^{-1}, for $LD_{100\%}$ a maximum VFA concentration of 15.5 g L^{-1} contributed to reactor failure, while at the maximum concentration of 6.63 g L^{-1} for $LD_{90:10\%}$ it caused low gas production at the same loading rate. As can be seen from Figure 7 C, on day 39 there was an increase in VFA concentration in all reactors to between (14 g L^{-1} - 21 g L^{-1}) with a corresponding decrease in pH (to 7.0 - 7.1). This was due to an unplanned drop in



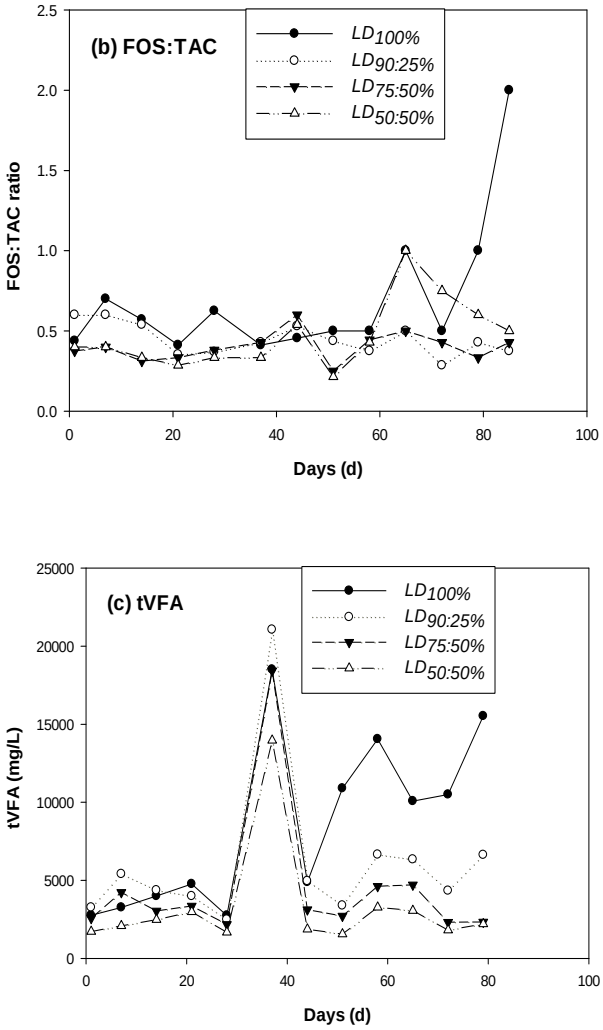


Figure 7 Performance indicators in reactors fed with different co-digestion mixtures, (A), pH; (B), FOS: TAC ratio; (C), Total volatile fatty acid (tVFA)

Reactor temperature to around 22 ℃, caused by equipment failure. Once back at normal temperature, performance recovered again. The temperature of the reactors plays a critical role for the AD microorganisms as the conversion of acetic acid to methane is highly temperature dependent [26].

The FOS: TAC ratio showed the largest increase for $LD_{100\%}$, reaching up to 2 at OLR 5. The other co-digested mixture reactors were all within the stable digestion ratio of 0.2 - 0.5, except for $LD_{50:50\%}$ which showed transient signs of instability with a FOS: TAC ratio of 1 at OLR 5, before normalising.

3. CONCLUSION

Continuous reactor studies were carried out using mono-$LD_{100:0\%}$, and co-digestion of *L. digitata* macroalgal biomass with food waste at several mixture ratios. The $LD_{90:10\%}$ was found to be optimal for the highest cumulative methane production after 85 days of fermentation when the OLR was

increased step-wise. The continuous mono-digestion of $LD_{100\%}$ was characterised by the accumulation of high tVFA and an increased FOS: TAC ratio as the OLR was increased, leading to reactor failure. This study shows that co-digestion has inherent advantages when using *L. digitata* macroalgal biomass as feedstock, making it the preferred option for long-term continuous digestion. Synergy and beneficial effects were observed with co-digestion mixture feedstocks, which enhanced continuous gas production at high loading.

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