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Co-digestion of municipal waste biopulp with marine macroalgae focusing on sodium inhibition

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ABSTRACT

The anaerobic digestion (AD) of municipal biopulp with two macroalgal biomasses (i.e. *Saccharina latissima* and *Fucus serratus*) was investigated at batch and continuously fed digesters at thermophilic conditions (54 ± 1 °C). At batch mono-digestion tests, municipal biopulp was associated with significantly higher methane production (549 ± 9 mLCH_4/gVS) compared to both *S. latissima* (210 ± 13 mLCH_4/gVS) and *F. serratus* (206 ± 37 mLCH_4/gVS). Regarding batch co-digestion tests, the highest methane yield was achieved when the feedstock consisted of 80% VS of biopulp and 20% VS of macroalgal biomass and it corresponded to the single methane contributions. The batch results were confirmed by continuous mode operation experiments, for the mono-digestion of biopulp and subsequently, the co-digestion with *S. latissima*. A specific challenge encountered with macroalgae biomethanation is the high sodium content. Therefore, mathematical modelling was followed to predict the performance of continuous mode experiments under increased salinity conditions by simulating the addition of more saline feedstock. The experimental results were used to calibrate and validate the model. Modelling simulations revealed that usage of saline feedstocks can drastically inhibit a well-performing AD reactor.

1. Introduction

Along with the worldwide increasing population, generation of biodegradable organic wastes was drastically increased during the last century. As a result, more than 120 million tonnes of biodegradable organic wastes are annually generated in European Union (EU) [1]. In the past, landfilling was the most common biowaste management practice in EU. However, EU by the Landfill Directive (1999/31/CE) has banned landfilling of biodegradable material and has put in priority re-usage, recycling or re-utilization of the generated municipal waste by alternative recovery technologies [2]. Hence, the alternative techniques for biowaste treatment were proliferated during the last decade to achieve the predetermined goals. Because the EU regulations prevent landfill application, the most common route for biowaste is incineration. However, due to a vivid discussion about nutrients and especially phosphorus recovery, anaerobic digestion (AD) has been pointed out as an efficient way to both recover energy and simultaneously, succeed benefits linked to recovery activities (i.e. phosphorus and biofertilizer production).

Indeed, there is high availability of municipal biowaste [3], which can be easily and rapidly degraded in a biogas reactor resulting in high biogas production [4]. For instance, Khoshnevisan et al. [5] reached more than 443 mLCH_4/gVS in triplicate continuously stirred tank reactors (CSTRs). The remarkable bioenergy output was attributed to the pretreatment of biowaste using biopulp technology. Specifically, a hydrapulper is equipped by a mechanical pulper that process the organic fraction of source separated municipal waste and rejects the hardly degradable (e.g. large particles of food and green waste) or non-degradable (i.e. plastic textiles) fractions found in the waste [6]. Finally, the processed organic fraction of municipal waste after biopulp technology (so-called "biopulp") is a well-homogenised and disrupted biomass with low content of intact organic materials and high content of easily accessible organic materials. Nevertheless, it should be noted that the researchers worked under mesophilic conditions at a hydraulic retention time (HRT) of 20 days, though similar or higher degradation of organic matter could be succeeded in thermophilic conditions setting a shorter HRT [7]. With respect to the degradation rates, the rapid AD is due to the microbial and physicochemical characteristics of biopulp. More specifically, homo- and hetero-fermentative bacteria are naturally presented in the biowaste and can metabolize the carbohydrates into

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intermediates as lactic acid and/or ethanol, acetic acid and formic acid [8]. Hence, the initially low degradable organic polymers are naturally transformed to smaller process intermediates by the indigenous microflora, enhancing also the degradation rates.

Despite the desirable biochemical composition of biowaste, the increased concentration of specific inorganic metal elements (e.g. Na^+) in the waste can be detrimental to the AD microbiome [9]. Notwithstanding cation's importance at low concentrations in order to be involved in building the Na^+/K^+ -ATPase to transfer glucose, amino acids and nutrients across the cell membrane; at high concentrations inhibit microorganisms growth due to the increased osmotic pressure or dehydration effect [10]. In general, AD inhibition from Na^+ is not an unknown phenomenon and it is directly connected with substrate characteristics and origin. Hence, an immense salty fraction into biowaste (e.g. wastewater from food processing industry) can markedly affect the overall efficiency of AD [11]. For example, sodium was measured at extremely high concentrations (i.e. 3.45 %TS) at raw food waste [12]. With respect to process inhibition, the methane production from raw food waste started to fall off after a threshold Na^+ concentration of 5.0 g/L [13].

Apart from municipal biowaste, Na^+ is found in even higher concentrations in other biomasses. On top of this, it is out of the most dominant micronutrients into marine macroalgae (seaweed) [14]. These marine plant biomasses can be categorised in red, brown and green species, have diverse carbohydrate content and are easily available in coastal waters [15]. However, they are originated from marine and coastal environments and thus, the usage of macroalgal biomass in the feedstock of biogas reactors can also induce inhibition incidents due to the high cations content [16]. Despite the danger of provoking bacterial inactivation and/or death due to saline conditions, macroalgae species are widely examined as co-substrate for biogas production [17–19]. The rationale behind the usage of macroalgae are the potentially high degradable organic matter (i.e. 150–360 $NmLCH_4/gVS$) [20] and vice versa, low or zero lignin contents [21]. Furthermore, during the last decade the usage of algae for biofuel production was highly encouraged by European Union, as they do not create additional demand for land occupation [22]. In addition, they are easily available in high amounts and can be simply dried, stored and used when there is an imperative need for additional high energy-dense organic matter of low moisture content to counterbalance diluted substrates. However, as introduced above, the exploitation of macroalgae for biogas production should be always carefully considered to eliminate the risk of salt inhibition effects. Furthermore, the addition of saline biomasses in the feedstock of biogas reactor should be always carefully applied in cases where the digestate is going to be used as a biofertilizer, as sodium accumulation has negative effects on soil's structure, deteriorating plant's growth. On the other hand, positive effects are achieved through the utilisation of two dissimilar substrates in the feedstock. Specifically, the C/N ratio will be balanced and thus, higher process performance can be achieved [23]. In addition, the toxic effect of salinity could be eliminated using a co- substrate of lower inhibitor concentration [24].

The objective of the present study was to evaluate the co-digestion of municipal biopulp with marine macroalgae at thermophilic conditions. Primarily the co-digestion of biopulp with two macroalgal species (i.e. *Saccharina latissima* and *Fucus serratus*) was examined at batch tests and then, the most promising feedstock composition was tested in continuous process. Subsequently, the results from the continuous mode operation were validated through modelling simulations. Additionally, the course of co-digestion under stress conditions due to increased salinity was evaluated through the model simulations.

2. Materials and methods

2.1. Inoculum and substrates

The thermophilic inoculum was collected by a lab-scale reactor co-digesting biopulp and cattle manure. The effluent was sieved to remove the remaining organic matter and stored in thermophilic conditions (54 ± 1 °C) for one week before usage. Biopulp was collected from Gemidan Ecogi A/S biopulping facility in Holsted (South West Jutland, Denmark) and was mainly composed of source-separated organic waste and unsorted residual waste from households. After treatment the biopulp had an average size of ~0.1 mm. *S. latissima* was harvested from wild stocks at Aarhus Bay (Jutland, Denmark) during May 2013 and *F. serratus* was collected from Øresund (Zealand, Denmark) during March 2016. After collection the macroalgae samples were cut into > 0.5 cm length by a cutting mill (Retsch SM 2000) and stored at −20 °C prior usage. The main characteristics of the used inoculum and substrates are given in Table 1.

Table 1
Characteristics of inoculum and substrates.

Characteristics	Inoculum	Biopulp	*S. latissima*	*F. serratus*
pH	7.98	4.01	nm	nm
Total Solids, g/kg	10.6 ± 0.3	35.9 ± 0.4	963.7 ± 1.7	946.0 ± 2.5
Volatile Solids, g/kg	6.40 ± 3.2	30.7 ± 0.4	619.7 ± 4.2	666.2 ± 5.0
Chemical Oxygen Demand, g/kg	7.2 ± 0.6	49.5 ± 0.7	727.1 ± 2.1	769.0 ± 4.0
Total Kjeldahl Nitrogen, g/kg	1.03 ± 0.1	1.0 ± 0.1	31.7 ± 0.3	25.9 ± 1.0
NH_4^+, g/kg	0.77 ± 0.1	0.2 ± 0.0	4.8 ± 0.2	6.7 ± 0.2
Total volatile fatty acids, g/L	0.21 ± 0.01	3.4 ± 0.1	nm	nm
Glucose, g/kg	nm*	4.8 ± 0.7	44.0 ± 0.4	37.4 ± 1.9
Xylose, g/kg	nm	1.7 ± 0.6	7.7 ± 1.0	16.2 ± 0.8
Trace elements, g/kgTS				
Ca	nm	19.8 ± 1.5	17.3 ± 1.0	10.9 ± 0.5
Fe	nm	1.8 ± 0.2	0.9 ± 0.1	0.4 ± 0.0
K	nm	9.6 ± 0.3	78.5 ± 0.1	29.1 ± 1.1
Mg	nm	1.9 ± 0.1	7.4 ± 0.2	8.4 ± 0.4
Na	nm	9.5 ± 0.4	38.4 ± 0.8	29.1 ± 0.8
P	nm	2.8 ± 0.2	1.0 ± 0.0	2.1 ± 0.1
S	nm	2.3 ± 0.1	11.3 ± 0.4	24.0 ± 0.8
Sr	nm	0.0 ± 0.0	0.8 ± 0.2	0.5 ± 0.0
Al	nm	2.0 ± 0.2	0.4 ± 0.2	0.2 ± 0.0

* nm = not measured.

2.2. AD experiments

Monitoring of biochemical Methane Potential (BMP) assays was conducted in order to define the maximum methane yield of pure substrates, according to Angelidaki et al. [25]. The co-digestion of macroalgal biomasses and municipal biopulp was examined using batch assays in order to define potential synergy or antagonism. Four different feedstock composition ratios were examined: 80/20, 60/40, 40/60 and 20/80 on organic matter basis. The batch assays were conducted in triplicate bottles with total and working volume of 547 and 150 mL, respectively. For both mono- and co-digestion trials the initial load was 2 gVS/L and a constant amount of inoculum was always added, 120 mL. All bottles were sparged with nitrogen gas to remove the remaining oxygen and ensure anaerobic conditions. Subsequently, the reactors were incubated at thermophilic conditions (54 ± 1 °C), until the daily biogas production was less than 1% of the accumulated biogas production.

Furthermore, the mono-digestion of municipal biopulp and the co-digestion with macroalgae were examined in continuous mode operation. A continuously stirred tank reactor (CSTR) with a total and working volume of 9.0 and 7.5 L was employed. During the first period, the CSTR was operated with biopulp as mono-substrate (0–45 days). Subsequently, the feedstock composition changed and consisted of 80% biopulp and 20% macroalgal biomass. The organic loading rate (OLR) of the initial phase was 2.3 gVS/L/d. After the addition of macroalgal biomass in the feedstock, the OLR was increased to 2.9 gVS/L/d. The hydraulic retention time (HRT) was constantly set at 15 days during the whole experimental period by feeding 250 mL twice per day using a peristaltic feeding pump. Silicone thermal jacket was used to ensure thermophilic operation. Samples directly from the CSTR were collected twice a week for pH and volatile fatty acids (VFA) determination. The water displacement principle was used to quantify daily the biogas production and the methane content in the biogas was determined twice a week.

2.3. Analytical methods

Total solids (TS), volatile solids (VS), pH, chemical oxygen demand (COD), ammoniacal nitrogen (NH_4^+) and total Kjeldahl nitrogen (TKN) were determined according to the standard methods [26]. Gas chromatography (GC-TRACE 1310, Thermo Scientific) was used to monitor the methane production of AD reactors and to determine the VFA composition (GC-TRACE 1300, Thermo Scientific) as previously described by Khoshnevisan et al. [5]. The content of total phenolic compounds (TPC) into the macroalgal biomasses was determined by spectrophotometric method according to the Folin-Ciocalteu method [27]. The trace element composition in the substrates was determined by inductively coupled plasma with optical emission spectrometry (ICP-OES). The C/N ratio of biopulp and macroalgae species was defined using an elemental analyser (vario MACRO cube, Elementar Analysensysteme GmbH). Triplicate samples were taken for all analyses.

2.4. Modelling approach and computational methods

The bioconversion model used in this work is the “BioModel” developed by Angelidaki et al. [28,29] and recently extended and validated by Kovalovszki et al. [30], Lovato et al. [31], Tsapekos et al. [32] for the dynamic simulation of anaerobic co-digestion of different organic substrates. Substrates in the model are described in terms of their basic organic components’ composition (carbohydrates, lipids, and proteins), organic acids (VFA and long chain fatty acids (LCFA)) and inorganic components (ammonia, phosphate, cations, anions, etc.). The BioModel includes three enzymatic hydrolytic processes and eight bacterial steps, and involves 19 chemical compounds, together with a detailed description of pH and temperature. Free ammonia, VFA and LCFA constitute the primary modulating factors. The current BioModel uses the optimal kinetic and yield parameters estimated by Kovalovszki et al. [30]. A detailed description of the equations and main pathways for anaerobic degradation of organic matter used in the BioModel is given in supplementary material (S1).

In the original BioModel the cations concentration (such as Na^+) contributing to the charge balance is represented by Z^+ and is calculated iteratively to obtain a correct simulation for pH within the model [28,29]. Cation concentration (Z^+) for biopulp was calculated based on its elemental composition (Table 1); whilst Z^+ for the co-digestion feedstock was calculated based on mass balance taking into account the amount of macroalgal biomass added in feedstock to share 20% of the total VS and its trace elemental composition.

Inhibition due to Na^+ concentration –not represented in the original BioModel –was incorporated by means of a non-competitive inhibition term and it was solely considered to affect the aceticlastic methanogenic step [33].

$$I_{Z^+} = \frac{1}{1 + \frac{Z^+ f([Na^+])}{K_{i,Na^+}}} \quad (1)$$

where in Eq. (1) K_{i,Na^+} represents the sodium inhibition constant for aceticlastic methanogens and $Z^+ f([Na^+])$ represents the concentration of cations as solely function of the sodium concentration added in the reactor influent.

For simulation purposes Z^+ value was increased to consider the addition of extra Na^+ in the reactor influent (see Table S1.3 of supplementary material) and its dynamic mass balance was calculated as:

$$\frac{d}{dt} Z^+ f([Na^+]) = \frac{q_{in}}{V_L}(Z^+ f([Na_{in}^+]) - Z^+ f([Na^+])) \quad (2)$$

where q_{in} is the reactor influent, V_L the liquid volume. $Z^+ f([Na_{in}^+])$ and $Z^+ f([Na^+])$ are the initial concentrations of cations in the system as function of Na^+ concentration.

As starting point, the BioModel was set up to simulate the batch AD of biopulp and marine biomass. Biodegradability carbohydrate coefficient – given the chemical composition of the substrates – and the value of K_{i,Na^+} in Eq. (1) were estimated by fitting the model outputs to the methane production experimental data of the 80% biopulp/20% *S. latissima* co-digestion batch assay (data not shown).

Then, estimated values of biodegradability coefficient and K_{i,Na^+} were used as initial estimates to perform dynamic simulations where: (1) the model output is compared to the experimental data corresponding to operational periods PI and PII in continuous mode operation, and (2) a set of simulation scenarios are presented where the effect of sodium addition in the reactor feed is investigated after 90 days (period II).

In the first simulation scenario, the individual effect of different Na^+ concentrations in the reactor feed was simulated after day 90 (period III) to analyse the reactor performance under high salinity conditions. The Na^+ concentration in the reactor feed was increased to 4.5 g/L at day 90 and kept until either steady state was reached or methane production ceases (period III). This similarly was performed for 5.0, and 6.2 g/L concentrations, respectively. The later value (6.2 g/L) was selected based on the experimental determination of the IC_{50} at thermophilic (55 °C) conditions determined for a system with a non-acclimated methanogenic community [33]. Furthermore, the impact of sodium in AD not necessarily might come when treating saline feedstocks (like marine macroalgae), but also when treating wastewater from food, seafood, and leather processing industries where sodium levels can range from 10 up to 71 g/L [34].

In the second scenario, the reactor operation was simulated considering stepwise increased of the Na^+ concentrations in the reactor influent. First, the Na^+ concentration in the feed is increased and kept at 4.5 g/L through days 90–105 (period III). After this, the reactor was allowed to recover so that no Na^+ addition is performed through days 105–120 (period IV). Thereafter, the Na^+ concentration was increased and kept at 5.0 g/L during days 120–135 (period V). Afterwards during 135–150 days no Na^+ addition took place (period VI). Finally at day 150 the Na^+ concentration was increased and kept to 6.2 g/L through 150–320 days (period VII). Both simulation scenarios are summarized in Table 2.

2.5. Statistical analysis

One-way analysis of variance (ANOVA) followed by Fisher's Least Significant Difference test ($p < 0.05$) was conducted to determine the statistically significant differences among mono- and co-digestion samples using OriginPro 9.0.0 SR2 software (OriginLab Corporation,

Table 2
Overview of the different Na^+ addition strategies used for model simulations.

Feedstock	Scenario 1			Scenario 2		
	Period	Days	g-Na/L	Period	Days	g-Na/L
Experiments and BioModel simulations						
Biopulp	I	0–45	0.34	I	0–45	0.34
Biopulp + *S. latissima*	II	45–90	0.87	II	45–90	0.87
BioModel simulations						
Biopulp + *S. latissima* + Na^+	III	90–320	4.5, 5.0, 6.2	III	90–105	4.5
				IV	105–120	no-add
				V	120–135	5.0
				VI	135–150	no-add
				VII	150–320	6.2

USA). In addition, the positive or negative synergistic effects occurred as a result of the co-digestion process were evaluated as previously described [32].

3. Results and discussion

3.1. Batch assays

The AD of biopulp and macroalgae was initially examined in batch assays. Among all feedstock combinations, the mono-digestion of biopulp had the highest methane productivity (549 ± 9 $mLCH_4/gVS$). The COD of the substrates was used in order to calculate the theoretically maximum methane potential [35]; and it was defined that 96% of the theoretical maximum methane yield was achieved based on biopulp chemical composition. Indeed, treating the organic fraction of municipal solid waste with a biopulper was previously proved to significantly boost the anaerobic biodegradability of municipal waste [6,36].

Regarding macroalgal biomass biomethanation, both species led to significantly low methane production ($p > 0.05$). Specifically, *S. latissima* had a methane yield of 210 ± 13 $mLCH_4/gVS$, which is in accordance with previous studies [16,20]. In contrast, *F. serratus* was markedly higher (206 ± 37 $mLCH_4/gVS$) compared to literature values ~100 $mLCH_4/gVS$ [37]. Despite the higher methane potential found in this study, only 51% of the theoretical methane yield was achieved for both macroalgae species. The limited biodegradability could be further improved applying a harsher pretreatment compared to the followed mechanical comminution to further reduce biomass particle size and improve the access of AD microbiome to the organics [38]. Nevertheless, even lower extent of biodegradability was previously achieved (~30%) for pretreated *Fucus* species [39]. The comparison of the chemical composition of substrates showed that the COD/VS ratio of biopulp was significantly higher (~1.61) compared to both macroalgae species (~1.17). Thus, the higher lipids and proteins content in the biopulp also augmented the methane yield compared to the macroalgae species [5].

Though the macroalgal biomass lack of lignin in the structure, they are not always connected with increased biodegradability, due to the presence of complex polysaccharides such as alginates [40]. Moreover, the used inoculum was derived from a lab-scale CSTR fed with biopulp and cattle manure; and thus, the presence of needed enzymes (e.g. alginate lyase) to disrupt the above mentioned structural sugars could not be expected. In addition, the carbohydrates' content was similar for both substrates (Table 1). In contrast, clear differences were observed for TPC. In particular, *F. serratus* was composed of 5.9 ± 0.5 mgTPC/gTS and *S. latissima* of 1.8 ± 0.1 mgTPC/gTS. On top of this, it is worth highlighted that *F. serratus* was collected from the shore and was not cultivated. In fact, *F. serratus* is naturally growing on shores on rocks and stones close to the water surface [37]. On the contrary, *S. latissima* was cultivated and harvested from a depth of 7.0–11.0 m, in which the light penetration is limited. Subsequently, light intensity might have affected biomass chemical composition and biodegradability. Hence, the higher light availability for *F. serratus* could favour the photosynthetic efficiency and thus, boost the TPC production [20]. Despite phenolic compounds can prompt inhibition to the AD process, the content into the macroalgae was extremely low to provoke stress conditions to the microbiome [41,42].

Regarding the co-digestion experiments, synergistic effects were revealed and are illustrated in Fig. 1. Indeed, the practical yields of the mixed feedstocks were always higher than the calculated values. In other words, the calculated production in absence of synergy (i.e. depicted as dash red line) is always below the obtained values from the batch assays. Data interpretation through the combination of mono- and co-digestion batch tests revealed that the methane yield was improved by 11% and 13% during the co-digestion of biopulp with *F. serratus* and *S. latissima* at 80% and 60% organic matter contribution

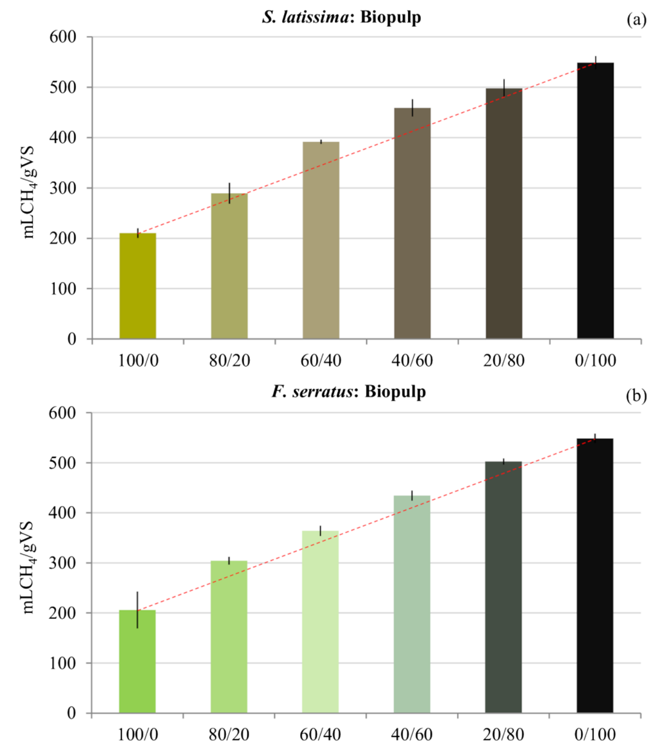

Fig. 1. Methane yields of mono- and co-digestion BMP tests using biopulp with (a) *S. latissima* and (b) *F. serratus*. The dash red lines illustrate the calculated methane yields based on the absence of synergy.

from macroalgae, respectively. The results are in accordance with a recent study showing that the synergistic effect of dissimilar substrates do not exceed 15% difference in most cases and that an improved micro- and macro- nutrient (i.e. C/N ratio) balance cannot fully overcome substrate's limited biodegradability [23]. Specifically, the C/N ratio of biopulp was 19 in contrast with the lower values of *F. serratus* and *S. latissima* that were equal to 11 and 9, respectively. Hence, the C/N ratio was only slightly augmented.

The higher the share of biopulp in the feedstock the higher the methane yield. For co-digestion perspectives, 80% share of biopulp in the feedstock was connected with the highest methane production for both macroalgae and did not differ significantly ($p > 0.05$). Taking into consideration the abundance of *S. latissima* in macroalgae cultivation parks and the high sodium content, settled them more preferable compared to *F. serratus* for practical and modelling aspects. Hence, *S. latissima* was used for the subsequent continuous mode experiments, modelling and simulation purposes.

3.2. Continuous reactor operation

To better evaluate the AD of municipal biopulp and macroalgae a lab-scale CSTR was used. During the first period (i.e. mono-digestion of biopulp), the CSTR was associated with relatively high and stable bioenergy output (Fig. 2a). Specifically, the methane productivity was 0.86 ± 0.02 NL/L/d and the methane yield was 379 ± 8 $NmLCH_4/gVS$ or 69% of the theoretically maximum based on substrate's chemical composition. In addition, pH was relatively unchanged (7.46 ± 0.11) (Fig. 2c) and no significant accumulation of VFA was detected (< 0.4 g/L) (Fig. 2b). The obtained results (i.e. pH values, VFA accumulation/degradation and bioenergy output) are in accordance with a recent study examining the AD of biopulp [4]. From both studies, it is indicated that a biopulper can produce homogenous municipal biopulp that can lead in rapid and high methane production and also, contribute on the avoidance of operational problems (i.e. clogging due to large particle size).

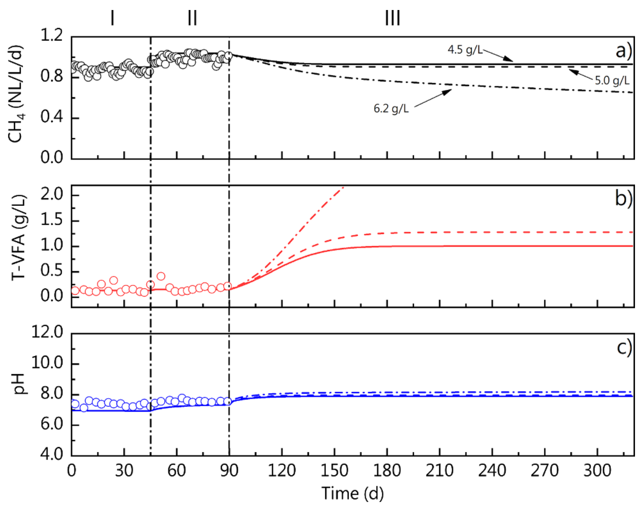

Fig. 2. Comparison between experimental values (open circles) and simulation results (periods I and II) and simulation results of post-experimental period III of scenario 1.

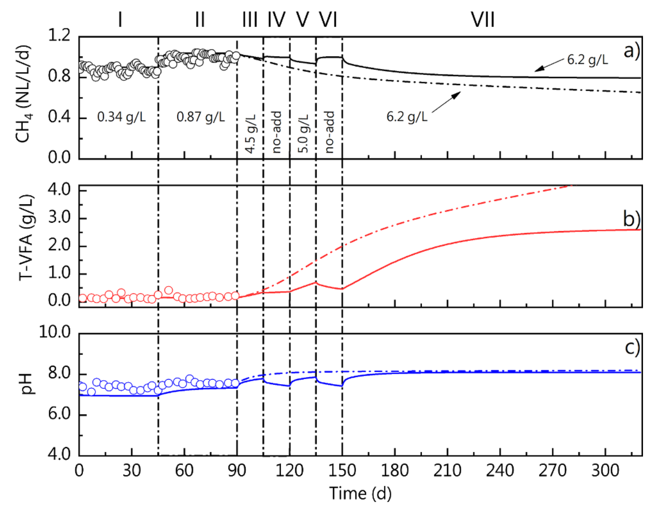

Fig. 3. Comparison between experimental values (open circles) and simulation results at the highest saline conditions of scenario 1 (direct Na^+ increase, dash dot line) and 2 (stepwise Na^+ increase, solid line).

At the second period, the co-digestion of municipal biopulp with *S. latissima* was examined adding macroalgal biomass in the feedstock. Due to the enriched feedstock and the increased OLR, the biogas productivity was rapidly enhanced by 13% compared to the first period at steady state conditions. In contrast, the methane yield was 336 ± 16 NmLCH$_4$/gVS or 12% lower than the mono-digestion of biopulp, which in agreement with the results obtained from the batch assays. Furthermore, the addition of saline biomass had no significant impact ($p > 0.05$) in the rest monitored biochemical parameters. Specifically, pH was only slightly increased (7.66 ± 0.04) due to the higher cations concentration. Also, the VFAs remained in the same range and only a temporary increment (~0.4 g/L) was observed at the beginning of the second period. However, substantial VFA accumulation was not observed at steady conditions.

Overall, both experimental periods showed stable AD process without perturbations. Thus, the biochemical parameters of the well-performing continuous mode operation were used to calibrate and validate the model; and subsequently, to reveal the potential inhibitory performance of CSTR under increased salinity levels.

3.3. BioModel validation and simulations

The experimental trends of both mono- and co-digestion periods were adequately captured by the BioModel. To begin with the AD of municipal biopulp, the monitored biochemical parameters were adequately predicted and at steady conditions, the calculated values did not differ markedly with the experimental values (Fig. 2a). On top of this, the BioModel has been suitably validated with the AD of similar source separated municipal biowaste in a recent study.

As regard to the co-digestion period, the trend of boosted bioenergy output was efficiently forecasted since the first days of enriched feedstock. In addition, the bioenergy productivity experimental trend was also very well captured. This predicted increase in biogas could find use dynamic energy planning, when dedicated increase need for energy is needed in specific periods. Hence, acetogens and methanogens efficiently processed the produced intermediates leading to lower total volatile fatty acids (T-VFA) accumulation and higher methane productivity (Fig. 2a).

The slightly higher accumulation of T-VFA, experimentally observed during the first HRT (days 45–60) of the co-digestion period was not adequately captured by the BioModel (Fig. 2b). After this period, the T-VFA experimental trend was suitably predicted during the entire experimental period.

Furthermore, pH differences between the first two periods were also well fitted (Fig. 2c). The surplus of cations in the marine biomass slight lifted up the pH values and so, the experimentally observed increment was adequately depicted through the simulations. With the exception of the biodegradability carbohydrate coefficient and the sodium inhibition constant K_{i,Na^+} the same set of parameters of the BioModel was used to simulate the impact of Na^+ addition. The biodegradability coefficient, was estimated to be 0.75 $g_{carb,\ sol}/g_{carb,\ is}$, whilst the value for K_{i,Na^+} was estimated to be 4.83 g/L.

It was observed that when the Na^+ concentration in the reactor feed was increased to 4.5 and 5.0 at day 90, the system was able to reach a stable operation, although with a slight drop in methane production at the steady state. This drop in methane production rate corresponded to 10.4% (for the case of 4.5 g/L) and 12.8% (for the case of 5.0 g/L), when compared to period II, respectively (Fig. 2a). The concentration of T-VFA in the system was still not excessive to cause a process failure in long-term operation (Fig. 2b). A more severe effect was observed when the Na^+ concentration was increased to 6.2 g/L. The methane production rate gradually decreased resulting in a reduction of 37% respect to period II. The concentration of T-VFA in the system increased up to 4.65 g/L (Fig. 2b), thereby resulting in pH levels that the process still can tolerate (pH = 8.2).

However, when the Na^+ addition was performed as described in scenario 2 (stepwise increase), the process managed to lower the T-VFA accumulation in c.a. 44% at steady state operation (2.60 g/L) (Fig. 3b – red[2] solid line) which is reflected in an marginal gain of 22% in the methane production at steady state (Fig. 3a – black solid line). As observed in Fig. 4(a), the increased of Na^+ concentration in the reactor feed is associated with a slight increase in the pH for periods III and V to finally be stabilized at 8.0 in period VII where the concentration of Na^+ was increased to 6.2 g/L. (Fig. 3c – green solid line). Moreover, acetic and propionic acid concentrations predicted by the model at steady state (Fig. 4b and c) were also significantly lowered than the inhibition threshold to cause a process failure [4].

The key factor determining the fate of the process in scenario 2 (stepwise Na^+ increase) could be linked to periods (IV and VI) at which Na^+ addition was ceased in the reactor influent. After each Na^+ addition there was a slight increase in T-VFA (Fig. 3b – periods III and V), therefore the methane production dropped since Na^+ inhibition was considered solely to affect the aceticlastic methanogenic step. During

[2] For interpretation of color in Figs. 2 and 3, the reader is referred to the web version of this article.

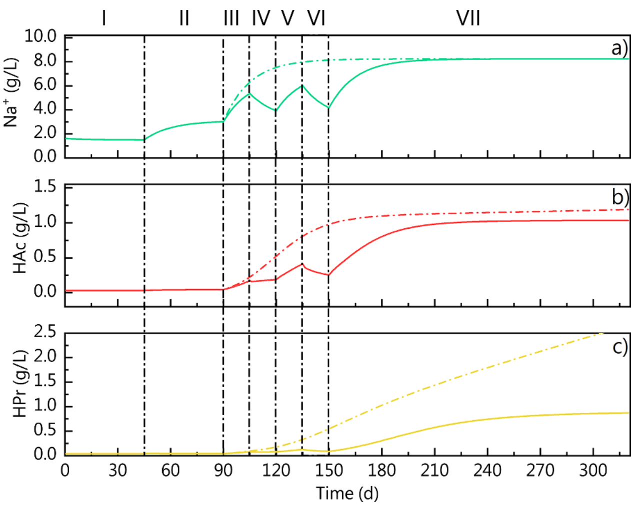

Fig. 4. Comparison of simulation results for experimental periods I, II and post-experimental period III-VII for: (a) sodium, (b) acetate and (c) propionate contents of scenario 1 (direct Na^+ increase, dash dot line) and 2 (stepwise Na^+ increase, solid line).

periods IV and VI, methanogens would have sufficient time to uptake the VFA accumulated in previous periods respectively; resulting in a marginal boost in the methane productivity (Fig. 3a – periods IV and VI). As a result, higher methanogenic activity (Fig. S1.2 – pink solid line) could be the responsible to counteract the final Na^+ concentration in the system (c.a. 8 g/L) at period VII (Fig. 4a – green solid line). It is interesting to note the lower steady state concentration of T-VFA reached in period VII (Fig. 3b – red solid line) which might be the result of some degree of microbial "acclimation".

It should be pointed out that the degree of acclimation of the microbiome depends among other factors on reactor configuration, substrate composition, inoculum characteristics and presence of other ions that might cause either antagonistic or synergistic effect. On the other hand, Na^+ inhibition was only considered to affect only aceticlastic methanogens, but it has been reported that can also affect propionic acid-utilizing microorganisms [43]. Therefore, these simulation results should be interpreted as merely qualitative indication of the performance of the process and not as absolute indication of concentration thresholds that might cause inhibition.

4. Conclusions

The present study demonstrated that anaerobic digestion of biopulp can lead to significantly high methane production. The synergistic benefit of co-digesting biopulp with macroalgal biomass e.g. *Saccharina latissima*, was shown in batch assays and continuous mode operation. Specifically, the partial replacement of biopulp with macroalgal biomass by 20% in terms of organic matter in the reactor influent improved the volumetric bioenergy production by 13%. The applicability of the BioModel was extended to consider Na^+ inhibition when feedstocks with high salinity content are preferred. Mono- and co-digestion strategies were properly simulated. Finally, simulations to examine the effect of Na^+ addition in reactor feed, clearly indicate that a proper dosage strategy could potentially lead to acclimation of the microbial communicate, minimizing the risk of process failure.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.enconman.2018.11.048.

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