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Anaerobic co-digestion of Baltic seaweeds with wheat straw and straw pellets: synergetic effects on biomethane yield and kinetic biodegradability constant

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Abstract

Cladophora sp. and Ulva intestinalis are green macroalgae abundantly available in the Baltic Sea. Their low degradability could hamper the fully exploitation as resource for energy recovery through anaerobic digestion. For this reason, a co-digestion with lignocellulosic biomass represents a viable solution. This study aims to assess the potential effectiveness of the use of seaweed from Latvian nearshore co-digested with lignocellulosic biomass both in terms of increased BMP and kinetic constant of biodegradability. Specifically, biomethane potentials (BMP) in batch tests of 100 ml were assessed co-digesting *Cladophora sp.* and *Ulva intestinalis* from the Gulf of Riga with wheat straw and straw pellet from Brescia Region (Italy). The BMP tests were performed to evaluate the synergetic effect of co-digestion through a synergy index. A mechanical pre-treatment was also executed and its final effect assessed on the mono- and co-digestion experimental trials.

The co-digestion ratio (seaweed/lignocellulosic biomass) used based on the volatile solids content was 1:1. The used ratio substrate-to-inoculum was 1:3 on total solid base. The inoculum use was sewage sludge was collected waste water treatment plant in the Riga district. Two different rounds of experiments with duration of 30 days were performed in order to have a more consistent analysis of results. Results for mono-digestion show the lowest BMP for Ulva intestinalis (277.7 ± 8.6 mL CH_4/g_{VS}) and the highest for the *Cladophora sp.* 523.3 ± 23.24 mL CH_4/g_{VS}. The straw pellet and wheat straw present values in a range of 395.6 ± 25.07 mL CH_4/g_{VS} and 470.4 ± 7.40 respectively. The effect of the co-digestion results in a slight increase of the synergy index (i.e. from 1.9% to 4.7%) but not for all the co-digestion trials. The higher effect was detected for co-digesting of finely treated straw pellet and Ulva intestinalis. The kinetic degradation constant improvements are evident for all the tested trials with the most evident effect for finely treated Ulva intestinalis in the mono-digestion conditions (i.e. 53.8%). The experiments show that the co-digestion with seaweeds and lignocellulosic can be beneficial.

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1. Introduction

Biomass is playing a key role within the target set by the Directive 2009/28/EC. Differently from second generation biofuel substrate, seaweeds avoid ethical issue on use of food resources [1] and have and better adaptation to different growing conditions [2], can fix higher amount of carbon dioxide per unit of mass compared to terrestrial plant and produce a yields of algae per unit area 2–20 times the production potential of conventional terrestrial energy crops [3]. This makes such biomass a potential and feasible renewable resource for biofuel production including thermal processes, fermentation and anaerobic digestion (AD) to biogas [4].

In this context, different studies show that the high exploiting costs of seaweed respect to terrestrial biomass [5] can still undermine the use of marine biomass to make biogas economically and environmental feasible [4]. Using seaweed biomass as the sole feedstock has been reported to have practical limitations such as the high nitrogen and sulfur contents relative to its carbon content that specific seaweed can have, e.g. Ulva sp. [6]. Seaweeds can also have a low C:N ratio, which may lead to an increase of free ammonia, toxic to methanogens and thus inhibiting the methane production [7]. As well as the seasonality of seaweed and their composition could makes the feedstock supply not stable for continuous bioprocesses [8, 9]. Thus co-digestion with lignocellulosic biomass can provide a practical solution to the abovementioned problems as also reported in the study of Zhong et al. For anaerobic co-digestion is intended a digestion of two or more substrates within the same digestor aiming to optimize the nutrients share from co-substrates, diluting the potential toxic substances, and meantime stimulate synergistic effects on methanogens microorganisms [8, 9]. Important case studies are focusing on finding the optimal C/N ratio among algal feedstocks and lignocellulose substrate, such as corn and wheat straw as important unexploited synergy [10]. Published studies highlight the importance to avoid relying on the sole use of seaweeds-based feedstock due to the uncertain availability of the stock itself in producing high biomethane yields thus triggering an higher flexibility on the use of the available feedstocks [6].

This study aims to assess the potential effectiveness of the use of seaweed from Latvian nearshore co-digested with lignocellulosic biomass both in terms of increase of the Biomethane Potential (BMP) and improvement of the kinetic constant of biodegradability (k). The effect of mechanical pre-treatment necessary for the lignocellulosic biomass, applied similarly to the seaweeds biomass, has been also evaluated. For the performed study seaweeds (i.e. *Cladophora sp.* and *Ulva intestinalis*) from the Baltic Sea in the region of the Gulf of Riga (Latvia) have been co-digested with straw and straw pellet from Brescia region (Italy). The synergetic influence of the co-digestion, rather than the sole mono-digestion, was calculated on the BMP through a synergy index (α) obtained by dividing the experimental BMP of the co-digestion tests by the mono-digestion on a volatile solid base.

The selection of a co-digestion of Baltic Sea seaweeds with Italian lignocellulosic was to address to have multiple objectives among within each regional context and to explore future direction of experimental research oriented towards low-carbon and circular economy solution both in the Baltic region and Italy.

2. Material and method

2.1. Substrate (collection and pre-treatment) and inoculum

Cladophora sp. and *Ulva intestinalis* collected near the city of Liepaja (West Latvia) during green tides on the summer of year 2016 and two types of lignocellulosic biomass, namely wheat straw and straw pellet, from Brescia region (Italy) were selected for the co-digestion. For each substrate two different levels of mechanical pretreatments were considered namely a rough treatment (in order to easily add the substrate to the batch reactor) and a fine treatment (in order to assess the real effectiveness of a pre-treatment at the scale of laboratory the batch tests):

- Rough and fine pretreatment for lignocellulosic biomass: straw pellets and wheat straw have been first mechanically crushed to a size of less than 0.5 cm; then through the use of a grinder more intensively comminuted. Using specific sieves, the granulometry 2.00-3.12 mm has been selected as dimension for the rough pretreatment while granulometry with particle's size lower than 1.00 mm as dimension for the fine pretreatment;

- Rough and fine pretreatment for lignocellulosic biomass: *Cladophora sp*. and *Ulva intestinalis* were chopped with a simple cutting blade up to a size of 0.5 cm in length (i.e. rough pretreatment). The size was then reduced using a pestle and mortar to <1 mm (fine pretreatment). For each substrate there are two different pretreatments: one rough treatment and one fine treatment so two different granulometries of the dry substrate input have been tested.

TS and VS values were determined prior to the experiments based on ISO Standards (ISO 14780:2017, ISO 18134–2:2017, ISO 18134–3:2015) as well for the sewage sludge used as inoculum. The results are presented in Table 1.

Table 1. Averaged TS and VS contents of inoculum and feedstocks, feedstock composition

	TEST 1		TEST 2						
	TS [%]	VS [% of TS]	TS [%]	VS [% of TS]	N [% of TS]	C [% of TS]	H [% of TS]	O [% of TS]	C/N ratio
Straw pellets	92.9	89.8	92.9	90.3	1.04±0. 12	41.83±0.71	5.51±0.05	51.63±0.73	40.1
Wheat straw	92.8	91.3	92.6	91.8	1.16±0. 03	42.59±0.31	5.72±0.04	50.53±0.31	36.6
Cladophora sp.	18.2	67.1	17,9	67.6	2.88±0.19	31.50±1.23	4.29±0.09	61.33±1.53	10.9
Ulva Intestinalis	-	-	7.0	78.2	1.18±0.06	34.6±0.19	5.28±0.04	59.09±0.28	29.3
Sludge	2.3	64.6	1.9	62.9	-	-	-	-	-

Sewage sludge was collected from local waste water treatment plant Daugavgriva (Riga district, Latvia) directly from the biogas reactors. Prior to the BMP experiments, the inoculum was incubated for 5 days at 37°C, with regular degassing. The carbon, hydrogen, nitrogen contents of the single feedstocks were analyzed by a certified Latvian Institute of Organic Synthesis according to the European Standard procedure EN 15104:2011. The oxygen content was calculated by difference of the given results [4].

3. BMP test method and experimental set-up

BMP tests were used to define the amount of methane produced per gram of volatile solids (VS), for an inoculum-to-substrate ratio equals to 3 based on TS base. A syringe piston-based BMP measuring method was selected. The tests were conducted in batch mode using 100 ml crimp neck with a working volume of 50 ml. Each bottle was filled with 30 ml of distilled water, 20 ml of inoculum and 1mL of 0.7 M$NaHCO_3$ buffer basal solution. Additionally, reference samples (blanks) containing only inoculum were prepared. Anaerobic conditions were guaranteed according to the procedure adopted by Gruduls et al. [11]. The tests were carried out in dark conditions at a mesophilic temperature for an incubation time of 31 days. The batches were manually shaken one time per day on average. Five mL of 3M NaOH alkaline solution was filled into the measuring syringes before each measurement [11].

3.1. Data analysis

A first-order kinetic model type was utilized to assess the changes on the biodegradability kinetic constant (k) and thus more properly asses the effect of the co-digestion. In this case an exponential equation was used to describe the progress of cumulative methane production for data processing, based on the following simple first-order rate equation due to the absence of phase lag in the biodegradation processes [12].

$$Y = Y_{max} \cdot (1 - e^{-kt}) \tag{1}$$

where: Y = cumulated methane yield at time; Y_{max}= ultimate methane yield; k = first order kinetical degradation constant; t = retention time.

The kinetic degradation constants for each trial were estimated by best fitting the experimental kinetics using MS Excel 2007 Solver. To evaluate the model's statistical indicators the relative root mean square error (rRMSE) based on Eq. (2) was calculated according to [1]:

$$rMSE = \sqrt[2]{\sum_{i=1}^{n} \frac{(Y_i - test_i)^2}{n}} \tag{2}$$

where: n=number of days; Y_i=forecast day i; $test_i$= measurement of the day i.

The synergistic effect of the co-digestion ratio was analyzed through a synergy index (α) [6] in fact dividing the observed BMP values in the co-digestion conditions by the theoretically calculated value of the mono-digestion according to the following formula 3.

$$\alpha_{BMP} = BMP_{co\text{-}digestion}/BMP_{mono\text{-}digestion} \tag{3}$$

Where: α = synergy index on the effect of BMP and $BMP_{co\text{-}digestion}$ and $BMP_{mono\text{-}digestion}$ the biomechmical potential in case of co- and mono- digestion calculated on volatile solid base.

Similarly, the effect of the synergy was evaluated towards the k (i.e. first order kinetical degradation constant) according to the following formula 4.

$$\alpha_k = k,_{co\text{-}digestion}/k_{mono\text{-}digestion} \tag{4}$$

4. Results and discussion

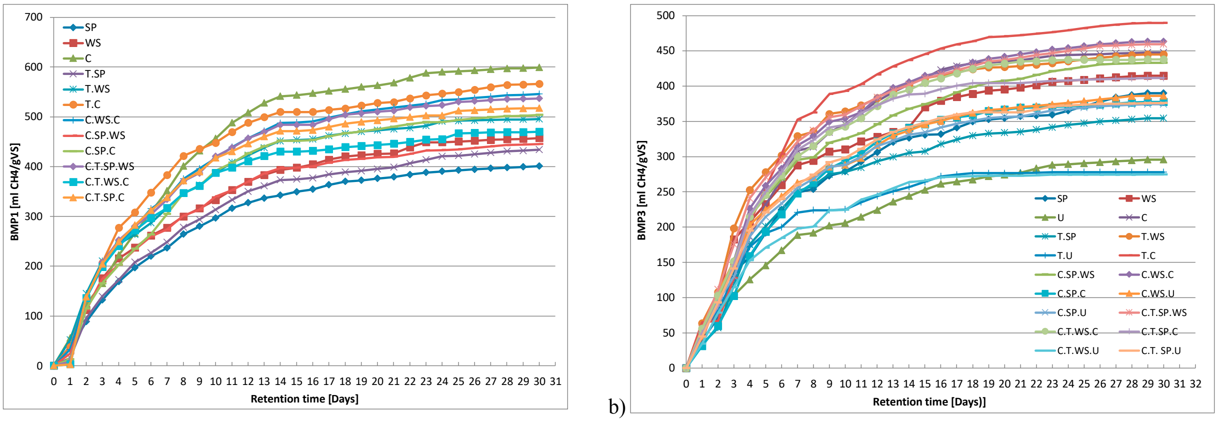
4.1. Inoculum and substrate characterization

Water content in the analyzed seaweeds ranged between 81.8% for *Cladophora sp.* and 93% for *Ulva intestinalis*, while for the lignocellulosic biomass the water content is rather similar ranging between 7.4% and 7.9%. *Cladophora sp.* also presented a significant TS value, making it a promising substrate for the use of this macroalgae compared to the range suggested by Montingelli et al. [13] as well as in other studies [14, 15] reporting water content in seaweeds in the ranges of 78% and 90%. The lignocellulosic biomasses are in the range as proposed by Wellinger et al [19].

Methane production is known to be directly positively affected by the biomass composition in terms of carbon and hydrogen, while a C/N ratio from 20 to 30 is considered an optimal set in order to avoid that a released of ammonium ion ($NH_4{}^+$) could occur in case of a C/N too low [16]. Results from the presented study show that C/N ratio range from 11 (*Cladophora sp.*) and 40 (Straw pellet) thus out of the optimal set. Only the feedstock of Ulva intestinalis is in the values of the proper range for anaerobic digestion processes. The obtained results suggested that combination of feedstock can be beneficial to optimize the C/N ratio thus supporting the co-digestion of seaweeds with lignocellulose biomass.

4.2. Biochemical methane potential and kinetics

The BMP results of the two rounds of experiments are presented in table 2 while the main kinetic results of the cumulative biomethane production are shown in Figure 1. Biomethane yields obtained from rough *Ulva intestinalis* (296 mL CH_4 $g_{VS}{}^{-1}$) were found similar to the values obtained by Heejung Jung [6]. Biomethane yields obtained from *Cladophora sp.* (523 mL CH_4 $g_{VS}{}^{-1}$) were found slightly above those obtained by Tedesco et al. 2013 [17]. The lower BMP of *Ulva intestinalis* respect *Cladophora sp.* it can be related to the type of cell wall composition of the *Ulva sp.* which contains ulvan, a polysaccharide with 1/5 of sulfate group respect the total mass. This characteristic could inhibit the anaerobic digestion process undermining the overall potential economic feasibility of the use of the algal feedstock as energy recovery [18]. Heejung Jung [6] defines *Ulva sp*. as a substrate with excessive concentrations of dissolved NH_3 with an inhibiting effect. The slightly higher value obtained of the *Cladophora sp*. respect the values identified in literature can be related to the inoculum used that in this case that could avoid the accumulation of volatile fatty acids (VFAs) in the batches [19]. Similarly, as mentioned by Hanssen et al. 1987 [20], the use of this sludge contribute to stabilize the digestion tackling the pH decrease due to a VFAs accumulation followed pH decrease, an aspect that can happen frequently when seaweeds are digested. Figure 1 shows that the BPM yields for wheat straw and straw pellet are close to the typical achievable values for lignocellulos biomass such as maize Miscanthus and corn straw [21, 22]. The results also show a dynamic without an evident phase lag in fact confirming the proper selection of the first order kinetic model.



a)

b)

Fig. 1. Averaged biomethane production kinetics of experimental test 1 and 2.

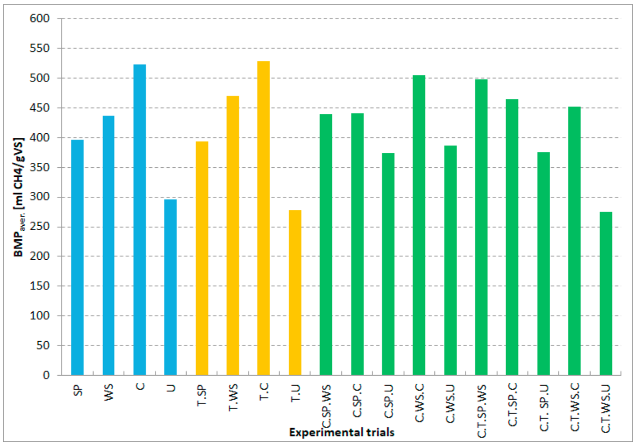


Fig. 2. Averaged BMP

From the results of figure 2 table 2 the most suitable C/N ratio is in connection with *Ulva intestinalis* with a value of 29.3 and wheat straw with a value (i.e. 36.6) slightly above the optimal range. From the interpretation of this output it is possible to understand how a synergy among the different feedstock can have an effect on the overall effectiveness of the digestion. The results of the tests were considering the co-digestion only of mixing ration of 50:50 on volatile solid based. The synergistic effect of the co-digestion ratio was analyzed through a synergy index (α) [6and calculated according to the equation 3. Increases of the BMP due to the synergy effect within the co-digestion of algae and lignocellulosic substrates have been noticed for: C.WS.C (α=1.052), C.WS.U (α=1.055) and C.T.SP.C (α=1.007); the most important improvement was for C.SP.U with an α equal to 1.083. Eventough the difference is not clearly exhibited for all the co-digestion combination, more evident it is the contribution of the co-digestion synergy on the degradation constant k with improvement in the range of i.e. 0.1% to 20.8% respect to the mono-digestion, the most important improvement was for C.SP.U.

Table 2. Averaged BMPs values and kinetic constants of biodegradability

	Substrate	**Trial acronym**	**BMP1*** **(lCH$_4$ kg$_{VS}^{-1}$)**	k_1**	**BMP2*** **(lCH$_4$ kg$_{VS}^{-1}$)**	k_2**	**Average BMP (lCH$_4$ kg$_{VS}^{-1}$)**	$k_{average}$	α_{BMP}	α_k
Rough treat.	Rough straw pellets	SP	401.0 ± 15.65	0.14	390.1 ± 25.07	0.13	395.55 ± 25.07	0.14	-	
	Rough wheat straw	WS	457.6 ± 0.13	0.14	414.6 ± 27.35	0.15	436.1 ± 27.30	0.15	-	
	Rough Cladophora sp.	C	598.8 ± 11.9	0.14	447.8 ± 23.34	0.15	523.3 ± 23.34	0.15	-	
	Rough Ulva Intestinalis	U	-		295.7 ± 18.05	0.13	295.7 ± 18.05	0.13	-	
Fine treat.	Finely mechanically treated straw pellets	T.SP	433.7 ± 5.43	0.13	354.2 ± 29.92	0.15	394.0 ± 29.90	0.14	-	
	Finely mechanically treated wheat straw	T.WS	495.7 ± 1.53	0.15	445.1 ± 7.43	0.18	470.4 ± 20.80	0.17	-	
	Finely mechanically treated with use of pestel/mortar on Cladophora sp.	T.C	566.4 ± 4.15	0.16	489.8 ± 39.69	0.16	528.1 ± 39.70	0.16	-	
	Finely mechanically treated and use of pestel/mortar on Ulva intestinalis	T.U	-	-	277.7 ± 8.64	0.20	277.7 ± 8.64	0.20	-	
Codigestion	Rough straw pellets and rough wheat straw	C.SP.WS	445.6 ± 0.13	0.15	433.1 ± 20.81	0.15	439.4 ± 20.80	0.15	1.057	1.071
	Rough straw pellets and Cladophora sp.	C.SP.C	503.7 ± 10.65	0.14	377.3 ± 16.39	0.15	440.5 ± 16.39	0.15	0.959	1.036
	Rough straw pellets and Ulva intestinalis	C.SP.U	-	-	374.4 ± 32.69	0.16	374.4 ± 32.69	0.16	1.083	1.208
	Rough wheat straw and Cladophora sp.	C.WS.C	545.9 ± 5.06	0.15	463.1 ± 10.90	0.15	504.5 ± 10.9	0.15	1.052	1.034
	Rough wheat straw and Ulva intestinalis	C.WS.U	-	-	386.1 ± 14.07	0.16	386.1 ± 14.07	0.16	1.055	1.164
	Finely treated straw pellet and wheat straw	C.T.SP.WS	537.1 ± 11.29	0.15	459.7 ± 13.25	0.16	498.4 ± 13.3	0.16	1.153	1.016
	Finely treated straw pellet and Cladophora sp.	C.T.SP.C	517.1 ± 8.36	0.16	411.6 ± 23.68	0.18	464.4 ± 23.7	0.17	1.007	1.133
	Finely treated straw pellet and Ulva intestinalis	C.T. SP.U	-	-	375.8 ± 16.18	0.17	375.8 ± 16.18	0.17	1.119	1.001
	Finely treated wheat straw and Cladophora sp.	C.T.WS.C	470.0 ± 0.90	0.17	434.0 ± 36.61	0.16	452.0 ± 36.60	0.17	0.905	1.015
	Finely treated wheat straw and Ulva intestinalis.	C.T.WS.U	-	-	274.5 ± 16.34	0.19	274.5 ± 16.34	0.14	0.734	1.041

*BMP1 –experimental test 1; BMP 2 – experimental test 2; ** $k_{1,...}$ = first order kinetic constant of biodegradability (k). of test nr. 1, 2.

The results show that considering the same amount of algae feedstock and lignocellulos feedstock in volatile solid basis more bioenergy (in terms of biomethane) can be generated through the formation of positive synergism during the digestion. Similar results were also found by Mata-Alvarez et al. [23]. In the presented case study, the reason is lying probably on the structural changes of the lignocellulose feedstocks during the co-digestion due to the greater alkalinity and buffering capacity of the digested biomass. Another reason could be in line with the results from Yen and Brune [24]. The authors explain that adding algae feedstock to cellulose feedstocks can increase the cellulase activity in turn having a benefit for the biomethane yields. A similar behavior could be the reason of the better degradation of the algal biomass thus with enhancing the methanogenic activity.

From table 2 it is possible to see how the effects of the pre-treatment are not evident for all the proposed trial in terms of BMP increases, nevertheless it is possible to notice how this is more reflected within the kinetic degradation constant with increased values ranging from 3.7% (SP) and 53.8% (U) for the mono digestion trials and form 3.3% (C.T.SP.WS) and 18.8% (C.T.WS.U). This highlights how mechanically pretreatment more generally affect the biodegradation rather than the overall biomethane yield.

4. Conclusions

This study aims to assess the potential effectiveness of the co-digestion of *Cladophora sp.* and *Ulva intestinalis* from Baltic Sea and lignocellulosic feedstock (wheat straw and straw pellet) both in terms of increase of the Biomethane Potential (i.e. BMP) and improvement of the kinetic constant of biodegradability (i.e. k) prior a mechanical pre-treatment. The effectiveness of co-digestion rather than the mono-digestion was calculated on the BMP through a synergy index (α). Specifically, important increases have been noticed for the co-digestion of: C.WS.C (α=1.052), C.WS.U (α=1.055), C.T.SP.C (α=1.007) and C.SP.U (=1.083). More evident is the contribution of the co-digestion synergy on the degradation constant k with improvement in the range of 0.1% to 20.8% respect to the mono-digestion, the most important improvement was for C.SP.U.. The results from this study clearly indicate that increases in volumetric biomethane production can be reached by adding lignocellulos feedstock to the co-digestion process with seaweeds in fact generating more bioenergy in terms of biomethane production. This increase is depending on the formation of positive synergism during the digestion that brings to structural changes of the lignocellulose feedstocks during the co-digestion. The pre-treatment effect, in term of higher kinetic biodegradability constants, is evident for all the tests with increased values ranging from 3.7% (SP) and 53.8% (U) for the mono digestion trials and form 3.3% (C.T.SP.WS) and 18.8% (C.T.WS.U). This highlights how mechanical pretreatment more generally affects the biodegradation rather than the overall biomethane yield.

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