Anaerobic co-digestion of lipid-spent microalgae with waste activated sludge and glycerol in batch mode

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Abstract

Anaerobic co-digestion of lipid-spent *Botryococcus braunii* (LSBB) with waste activated sludge (WAS) and glycerol was studied. Different co-digestion mixtures were assessed in biochemical methane potential tests, performed at mesophilic temperature (35ºC). The experimental methane yields obtained were compared to theoretical values calculated from the methane individual yields. No significant increase in BMP was observed when mixing these substrates. A first-order exponential kinetic model was used to obtain the apparent kinetic constant of the processes assayed. The kinetic constant value of the mixture 25%WAS-75%LSBB was 116%, 16% and 43% higher than for WAS, LSBB and glycerol alone, respectively.

Keywords: Lipid spent microalgae; Waste activated sludge; Glycerol; Co-digestion; Biomethane.

1. INTRODUCTION

Biodiesel production from microalgae has been regarded as an important advance in the development of biofuels (Scott et al., 2010). However, sustainable biodiesel production from microalgae still faces several limitations. One of them is the energy requirement of the operations involved in the production of biodiesel itself, such as mixing, pumping, biomass harvesting, drying, and lipid extraction. The result is that, when traditional processes and technologies are considered, microalgae based biodiesel presents low or even negative energy yields (Scott et al., 2010). This situation can be addressed, at least partially, by anaerobic digestion of the lipid-spent microalgae. Produced biogas can be used as a source of energy, and released nutrients can be recycled for its use during the cultivation of microalgae (Ehimen et al., 2011; Torres et al., 2013). The study carried out by Lardon et al. (2009) indicates that 1.23 MJ of energy could be recovered from lipid-spent microalgae, per MJ of produced biodiesel. Indeed, Campbell et al. (2011) determined that production of biodiesel and biogas would represent a competitive fuel production scheme.

Of the wide variety of available species of microalgae, *Botryococcus braunii* presents great potential for the production of biofuels, especially biodiesel. This is mainly due to the capacity of this strain to accumulate lipids and hydrocarbons, under appropriate culture conditions. Moreover, an adequate profile of triglycerides for transesterification to biodiesel have been observed, comprising mainly palmitic, oleic

and linoleic acids (Sydney et al., 2011; Ashokkumar and Rengasamy, 2012). *Botryococcus braunii* is also characterized by the high presence of extracellular hydrocarbons, mainly terpenes (Wolf, 1983; Metzger and Largeau, 2005).

Torres et al. (2014) studied the feasibility of the anaerobic digestion of lipid-spent *Botryococcus braunii*. High protein content of oil-extracted microalgae may result in low C/N ratios, increasing chances of inhibition by ammonia. Co-digestion is a strategy that consists in mixing one substrate with another carbon source, in order to improve operational parameters such as C/N ratio, or to dilute inhibitors. As a result, a higher process performance can be achieved. Furthermore, co-digestion allows the treatment of different residues using the same installations. Indeed, co-digestion have been proposed as a promising approach for improving the methane yield and overall digestion performance (Fernández-Rodríguez et al., 2014). In this scenario, glycerol may be considered as a natural co-substrate for oil-extracted microalgae, since it represents the principal by-product of biodiesel production. About 0.1 gram of glycerol is generated per gram of produced biodiesel, and its ever growing supply had led to a drastic price reduction over the past years. Another possible co-substrate is waste activated sludge (WAS), produced during aerobic wastewater treatment. Anaerobic digestion of WAS is a common practice, since it needs to be stabilized prior to disposal. Microalgae production is likely to take place in arid geographical zones, since these are the ones providing high solar radiation and low land costs, requirements for economically feasible cultivation. Due to the lack of agriculture and other biomass-oriented industries, availability of biomasses suitable for co-digestion is unlikely. However, it is likely that cultivation facilities will be close to one or more urban centres with one or more wastewater plants. Then, activated sludge may be available as a potential co-substrate. The aim of this research was to study the co-digestion of oil-

spent *Botryococcus braunii* with WAS and glycerol, in order to provide information required to evaluate the potential technical benefits of the co-digestion of these substrates.

2. MATERIALS AND METHODS

2.1 Microalgae

Microalgae *Botryococcus braunii* race A was supplied by Antofagasta University (Chile), where it was grown in pilot scale batch reactors (Bazaes *et al.,* 2012). Microalgae was harvested by centrifugation, frozen and storaged until its utilization. Lipid-spent *Botryococcus braunii* (LSBB) was produced using a Soxhlet extraction unit for 16 h, with petroleum ether as solvent, at a solvent-sample ratio of 10:1. Proximate analysis of LSBB was done according to Babayan *et al*. (1978).

2.2 Co-substrates: WAS and glycerol

The WAS co-substrate was obtained from San Isidro sewage treatment plant (Labranza, Chile). The Glycerol co-substrate was obtained from a pilot scale plant for biodiesel production from rapeseed oil (Gorbea, Chile).

2.3 Batch co-digestion assays

Co-digestion of LSBB and co-substrates WAS and glycerol was evaluated through biochemical methane potential (BMP). The BMP assays were carried-out in batch tests, performed in sealed 117 mL vials. An initial substrate concentration of 5 g/L of volatile solids (VS) was applied. Anaerobic granular biomass from a full scale UASB reactor treating brewery wastewater was used as inoculum. Anaerobic biomass/substrate ratio

was 1:1, expressed as VS. Medium was supplemented with yeast extract (200 mg/L), NH_4Cl (65 mg/L), KH_2PO_4 (18.5 mg/L), $CaCl_2*2H_2O$ (4 mg/L) and $MgSO_4*7H_2O$ (5.7 mg/L). Sodium bicarbonate was also added (5 g/L), to provide pH buffer capacity. Temperature was kept constant at 35°C. Co-substrates were used at different proportions. WAS were added at LSBB/WAS ratios of 1:3, 1:1 and 3:1. Glycerol was mixed with LSBB at a ratio of 1:9. All previous ratios are expressed in terms of volatile solids (VS). Endogenous biogas production from anaerobic biomass was established by blank assays, where no substrate was provided. BMP tests were performed in triplicates.

Methane production was determined following pressure increase and biogas composition in the headspace of the vials. Pressure was measured through pressure transducer (Cole Parmer) and biogas composition was determined by gas chromatography coupled to thermal conductivity detector (Clarus 580, Perkin Elmer). The BMP value was expressed as methane volume per mass of substrate (mL CH_4 /g VS). Total solids (TS), VS and chemical oxygen demand (COD) were determined according to APHA (2005).

3. RESULTS AND DISCUSSION

3.1 Influence of co-digestion on biochemical methane potential

Proximate analysis of LSBB (Table 1) shows a high protein content, providing a low C/N ratio. Figure 1 presents the evolution of methane production for the BMP tests performed with WAS, LSBB, and the different tested mixtures. As can be seen in Figure 1, the rate of methane production became zero after 60 or 65 days. Latency periods and inflection points were not detected in the BMP curves. By contrast, important lag periods of up to 10 days have been reported during BMP assays of

mixtures of the microalgae *Dunaliella salina* and other organic substrates such as two-phase olive mill solid waste (OMSW) (Fernández-Rodríguez et al., 2014).

The methane yield obtained for LSBB (404 mL CH_4/g VS) was 18% higher than that obtained by Frigon et al. (2013), who used the same species, but without lipid extraction (343 mL CH_4/g VS). It is inferred that application of different cultivation procedures will result in different biomass compositions and characteristics, affecting BMP determination. The cell wall of this microalga is composed of complex carbohydrates, hardly biodegradable, that impede the degradation of intracellular organic molecules by anaerobic microorganisms (Frigon et al., 2013). Moreover, the cell walls of many species of microalgae, such as *Botryococcus braunii*, are multilayered and contain a relatively large proportion of cellulose (Muñoz et al., 2014). Cellulose is a linear polymer of β-1,4-D-anhydroglucopyranose units, which makes it very stable and resistant to degradation. The increase in methane production, despite lipid extraction, may be also consequence of a disruption or weakening of the cell walls, caused by the extraction of intracellular lipids (Ramos-Suárez and Carreras, 2014). Other authors have also observed an increase in the methane yield after lipid extraction from microalgae, attributing this increase to the disruption of the cell wall caused by the solvent-based oil extraction (Ward et al., 2014).

The experimental methane yield observed for each co-digestion mixture (Table 2) was compared to a calculated methane yield, based on the WAS and LSBB methane individual yields, using the Equation 1:

$$BMP_{calculated} = (f_{WAS} \cdot BMP_{WAS}) + (f_{LSBB} \cdot BMP_{LSBB}) \qquad (1)$$

Where, BMP_{WAS} and BMP_{LSBB} were 332 and 404 mL CH_4/g VS, respectively. The f_{WAS} and f_{LSBB} values correspond to the proportions of WAS and LSBB in each co-digestion mixture, respectively. A Student test reveals no statistical difference between observed experimental BMP of the different mixtures and calculated BMP, using α = 0.05. This means that no negative or positive effects of mixing the substrates were observed, when considering final BMP values. Co-digestion can still be a useful strategy, even in absence of positive effects, since it enables the treatment of different residues in the same installation, potentially providing lower costs.

The BMP tests of glycerol, LSBB and their mixture (1:9 ratio) are shown in Figure 2. A Student tests for independent variables reveals no significant difference (α = 0.05) between observed BMP values for LSBB and glycerol-LSBB mixture, des´pite the lower BMP of glycerol alone. The possibility of using glycerol as co-substrate for the anaerobic digestion of lipid-spent microalgae has been previously reported. Ehimen et al. (2009), who observed an increase in the methane yield of 4-7%, depending on the lipid extraction method, when working with the microalgae *Scenedesmus*. Residual glycerol obtained during a biodiesel production process from waste cooking oil has been recently proven to be beneficial to the anaerobic digestion process of lipid-spent *Scenedesmus*, when added in low amounts (Ramos-Suárez and Carreras, 2014). Residual glycerol has also been used as co-substrate for pig-manure, increasing the methane yield in mixtures composed of up to 60% residual glycerol (fresh matter basis) (Astals et al., 2011).

A higher methane yield (540 mL CH_4/g VS) than those obtained in the present work has been reported by Park and Li (2012) during the anaerobic co-digestion of lipid-spent *Nannochloropsis salina* and lipid-rich fat oil and grease waste. By contrast, low methane yields were reported by Samson and LeDuy (1986) in the co-digestion

processes of mixtures of *Spirulina maxima* with peat hydrolyzate (280 mL CH_4/g VS), and with spent sulphite liquor (250 mL CH_4/g VS). Similar results were obtained by Ehimen et al. (2009), when studding the co-digestion of mixtures of *Chlorella* and post trans-esterified residues and glycerol (295 mL CH_4/g VS).

3.2 Influence of co-digestion on process kinetics

A first-order exponential model was used to correlate the evolution of the methane production with digestion time (Figures 1 and 2). This kinetic model is normally applied to assess the performance and kinetics of batch anaerobic digestion processes of easily biodegradable substrates (Li et al., 2012).

The first-order exponential model is given by the following expression:

$$B = B_{max} \cdot [1 - \exp(-k \cdot t)] \qquad (2)$$

Where: B (mL CH_4/g VS) is the cumulative specific methane production, B_{max} (mL CH_4/g VS) is the ultimate methane production, k is the specific rate constant or apparent kinetic constant (d^{-1}) and t (d) is the digestion time.

The adjustment by non-linear regression of the pairs of experimental data (B, t) using Sigmaplot software (version 11.0) allowed the calculation of the parameters k and B_{max} for methane production, which are summarized in Table 4. The high values of the R^2 and the low values of the standard error of estimate demonstrate the goodness of the fit of experimental data to the proposed model.

Table 4 shows that the highest kinetic constant was obtained for the co-digestion mixture 25% WAS-75% LSBB (0.106 d^{-1}), value 116%, 16% and 43% higher than for WAS, LSBB and glycerol alone, respectively. Co-digestion with glycerol did not

contribute to increase the kinetic constant of the digestion process. This agrees with the results presented by Ramos-Suárez and Carreras (2014) who did not observe an increase in the kinetic constant when increasing the glycerol fraction from 2.3% to 11.1%, in a mixture with lipid-spent *Scenedesmus* (0.105 d^{-1} and 0.106 d^{-1}, respectively).

4. CONCLUSIONS

No significant increase in BMP was observed when mixing these substrates. The kinetic constants of the mixture 25% WAS - 75% LSBB was much higher than for WAS, LSBB and Glycerol alone. Moreover, the mixture 10% Glycerol - 90% LSBB did not show a higher kinetic constant with respect to both substrates separately. Even though no benefits in term of increase of BMP was observed as a result of co-digestion, the increase in kinetic constant of one of the mixtures, plus the possibility of treatment of different residues using the same installations, turns co-digestion into an interesting alternative to provide adequate treatment of lipid-spend microalgae from a biodiesel producing process.

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Table 1. Proximate analysis for lipid-spent *Botryococcus braunii.*

	Lipid-spent Botryococcus braunii
Proteins (%)	46.0
Nitrogen (%)	7.4
Lipids (%)	2.71
Carbohydrates (%)	22.3
Ash (%)	23.9
Phosphorus (%)	0.76
VS/TS (g/g)	0.75
COD/VS (g/g)	1.6

Table 2. Calculated methane yield values obtained from Eq. 1 and the experimental data obtained through co-digestion of WAS and lipid-spent *Botryococcus braunii*. Values between parentheses represent standard deviation between triplicates.

f_{WAS}	f_{LSBB}	$BMP_{calculated}$	$BMP_{exp.}$
		(mL CH_4/g SV)	(mL CH_4/g SV)
1	0		332 (16)
0.75	0.25	350	374 (13)
0.5	0.5	368	355 (12)
0.25	0.75	386	393 (4)
0	1		404 (11)

Table 3. Calculated methane yield values obtained from Eq. 1 and the experimental data obtained through co-digestion of glycerol and lipid-spent *Botryococcus braunii*. Values between parentheses represent standard deviation between triplicates.

$f_{Glicerol}$	$f_{B.braunii}$	$BMP_{calculated}$	$BMP_{exp.}$
		(mL CH_4/g VS)	(mL CH_4/g VS)
1	0	302	302 (13)
0.1	0.9	394	428 (16)
0	1	404	404 (11)

Table 4. Kinetic parameters obtained from Eq. 1 for the different co-digestion mixtures and substrates alone. Values between parentheses represent the standard error of each parameter.

	B_{max}	k	R^2	S.E.E.
	mL CH_4/g VS	d^{-1}		
WAS	340 (12)	0.049 (0.005)	0.992	12.003
WAS / LSBB - 75/25	368 (9)	0.071 (0.006)	0.993	12.839
WAS / LSBB - 50/50	346 (6)	0.097 (0.007)	0.995	10.257
WAS / LSBB - 25/75	394 (5)	0.106 (0.006)	0.997	9.104
LSBB	413 (13)	0.091 (0.010)	0.990	17.973
Glycerol/LSBB - 10/90	448 (16)	0.077 (0.009)	0.989	20.126
Glycerol	339 (45)	0.074 (0.026)	0.913	45.942

Where: B_{max} is the ultimate methane production, k is the specific rate constant or apparent kinetic constant. Parameters from the nonlinear regression fit: R^2: coefficient of determination; S.E.E.: Standard Error of Estimate.

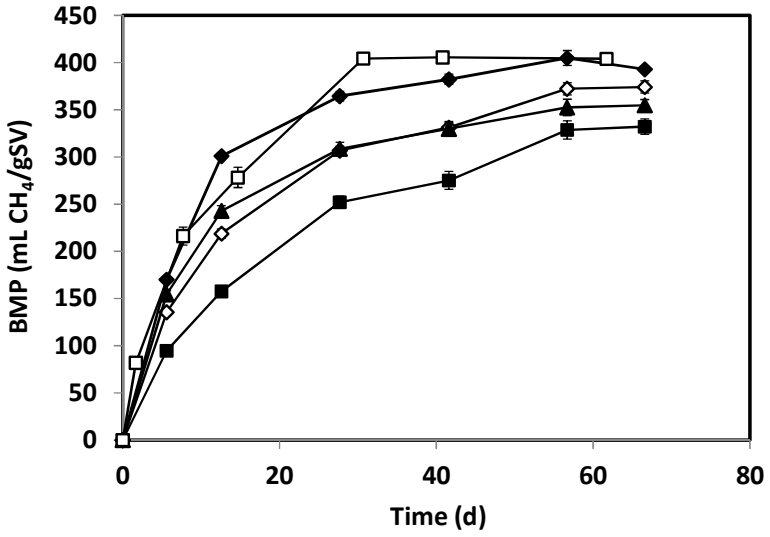

Figure 1. Biochemical methane potential (mL CH_4/g SV added) of 100% WAS (■), 100% LSBB (□) and different co-digestion mixtures tested: 75% WAS-25% LSBB (◊); 50% WAS-50% LSBB (▲) and 25% WAS-75 % LSBB (♦). Vertical bars represent standard deviation between triplicates.

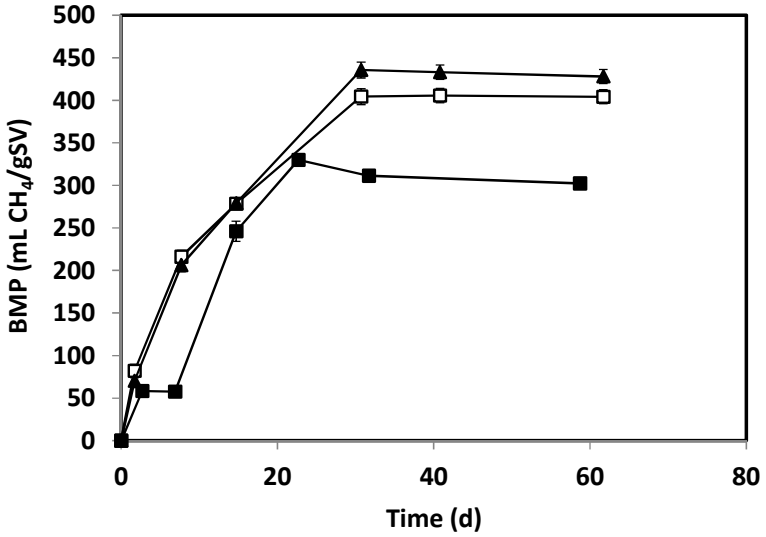

Figure 2. Biochemical methane potential (mL CH_4/g SV_{added}) for LSBB (□), Glycerol (■) and for the mixture glycerol-LSBB (1:9) (▲). Vertical bars represent standard deviation between triplicates.