Batch anaerobic co-digestion of waste activated sludge and microalgae (*Chlorella sorokiniana*) at mesophilic temperature

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Abstract

The microalgae *Chlorella sorokiniana* was used as co-substrate for waste activated sludge (WAS) anaerobic digestion in order to increase process stability, biodegradability and methane yield. Different co-digestion mixtures (0% WAS-100% microalgae; 25% WAS-75% microalgae; 50% WAS-50% microalgae; 75% WAS-25% microalgae; 100% WAS-0% microalgae) were studied. The highest methane yield (442 mL CH_4/g VS) was obtained for the mixture 75% WAS-25% microalgae. This value was 22% and 39% higher than that obtained in the anaerobic digestion of the alone substrates WAS and microalgae, respectively, as well as 16% and 25% higher than those obtained for the co-digestion mixtures 25% WAS-75% microalgae and 50% WAS-50% microalgae, respectively. The kinetic constant of the process increased 42%, 42% and 12% for the mixtures with 25%, 50% and 75% of WAS compared to the substrate without WAS. Anaerobic digestion of WAS, together with *Chlorella*, has been clearly improved by ensuring its viability, suitability and efficiency.

Keywords: Anaerobic co-digestion, waste activated sludge, microalgae, batch studies, mesophilic temperature.

Introduction

Large amounts of waste activated sludge (WAS) are produced in urban wastewater treatment plant (UWWTP) worldwide. The management of these wastes is an expensive and environmentally sensitive problem. The cost of treating WAS can account for around 50% of the total operating costs in a UWWTP. Moreover, there are several problems associated with the management of this waste, such as the presence of heavy metals, organic micro-pollutants and pathogens, which require its hygienization. [1]

Among the currently available management methods for treatment of this waste, anaerobic digestion is a highly efficient process which produces methane as a final product, which can be used as an energy source for electricity and on-site heating. Although anaerobic digestion of WAS as a single substrate is an attractive and interesting process, it has several important drawbacks, such as low methane production, poor biodegradability and the presence of heavy metals and other inhibitory compounds that make necessary the use of high retention times in the digesters with high mixing costs. [2] Therefore, it is necessary to improve the anaerobic digestion of WAS in order to ensure its viability, suitability and efficiency.

Microalgae, are characterized for an efficient conversion of the solar energy to biomass. They are a novel feedstock for biogas production especially considering their advantages over land-based energy crops. However, the low C/N ratio of microalgae hinders and inhibits anaerobic digestion. Recalcitrant cell walls and ammonia toxicity are usual cited causes of these low methane yields. [3] Specifically, *Chlorella sorokiniana* is a green microalgae, belonging to the phylum *Chlorophyta*, and has a stable and rigid cell wall with a high hemicellulose content. Its hemi-cellulosic cell wall accounts for the rigidity of the cells. [4] A recent research has shown a low methane yield coefficient (212 mL CH_4/g VS) in biochemical methane potential (BMP) tests of *Chlorela sorokiniana* as sole substrate. [5]

Anaerobic co-digestion consists in mixing substrates to improve operational parameters such as C/N ratio, or to dilute inhibitors. [6-8] Higher performance can be achieved compared to the sole substrates. [7- 9] Co-digestion has been shown to contribute to a more efficient use of anaerobic reactors by processing several wastes in the same installation. [9-12] Although there have been recent development in the field of co-digestion of some microalgae with WAS and other substrates, there is still a lack of knowledge regarding co-digestion of

Chlorella sorokiniana with WAS. *Chlorella sorokiniana* is a eukaryotic microalgae belonging to green algae from the class Chlorophyceae and is a dominant strain from natural habitats showing fast growth rates in both laboratory and nature.

The aim of this study was to assess the feasibility of improving methane production from anaerobic digestion of WAS in co-digestion with the specific microalga, *Chlorella sorokiniana*, based on an optimized mixture percentage. Different co-digestion mixtures were assayed by biochemical methane potential (BMP) tests, and the influence of the percentage of each co-substrate on the methane yield and kinetics were evaluated.

Materials and methods

WAS

The WAS used was collected from the urban wastewater treatment plant "El Copero" located in Seville (Spain). Its main characteristics were: volatile solids (VS), 14.9 ± 0.7 g/L; COD, 2.1 ± 0.3 g/g VS and C/N ratio, 4.9 ± 0.1.

Chlorella sorokiniana

Chlorella sorokiniana was provided by Huelva University (Spain). Its principal characteristics were: VS: 940 ± 16 g/kg; COD: 1.2 ± 0.1 g/kg and C/N ratio: 5.3 ± 0.1.

Anaerobic inoculum

The anaerobic biomass used as inoculum in the BMP tests was obtained from an industrial-scale anaerobic reactor treating waste activated sludge from a municipal wastewater treatment plant operating at mesophilic (35 ºC) conditions. The main characteristics of the anaerobic biomass used as inoculum were: pH: 7.5; total solids (TS): 20.0 g/L and volatile solids (VS): 12.1 g/L.

Experimental procedure

Different mixtures of WAS/microalgae *C. sorokiniana* were assayed: 100% WAS, 75% WAS-25% *C. sorokiniana*, 50% WAS-50% *C. sorokiniana*, , 25% WAS-75% *C. sorokiniana*, and 100% *C. sorokiniana*.

BMP tests were performed in a multi-batch vessel system, which provides continuous agitation by magnetic bars, set at 300 rpm for this study. A thermostatic water bath kept the tests at mesophilic temperature (35±2 ºC).

For each reactor of 130 mL of effective volume, the required amounts of inoculum and the corresponding substrate were added to achieve an inoculum to substrate ratio of 2 (VS basis). In addition, 130 µL of a trace element solution were added to each reactor. The composition of this trace elements solution was given in a previous paper. [9]

The reactors were sealed and headspace flushed with N_2 at the beginning of the test. The produced biogas was measured by liquid displacement after going through a 2N NaOH solution to capture the produced CO_2; the remaining gas was expected to be only methane. The BMP tests lasted until the accumulated methane production was essentially unaffected, i.e. lower than 5% of the accumulated methane produced and c.a. 25-30 days. Each experiment was carried out in triplicate.

Analytical methods

Standard methods 2540B and 2540E were followed in order to determine TS and VS, respectively. [13] COD was determined as described by Raposo et al. [14], while SCOD was determined using the closed digestion and the colorimetric standard method 5220D. [13] pH was measured with a pH-meter model Crison 20 Basic. C and N were determined through an Elemental Analyser LECO CHNS-932 (Leco Corporation, St. Joseph, MI, EEUU).

Results and discussion

Figure 1 shows the variation of the methane yield obtained against digestion time for the BMP tests carried out with 100% WAS, 100% *C. sorokiniana* and the different mixtures tested. As can be seen, the highest methane yield (442 mL CH_4/g VS) was obtained for the mixture 75% WAS-25% microalgae. This value was 22% and 39% higher than that obtained in the anaerobic digestion of the alone substrates WAS (100%) (362 mL CH_4/g VS) and microalgae (100%) (318 mL CH_4/g VS), respectively, as well as 25% and 16% higher than those obtained for the co-digestion mixtures 25% WAS-75% microalgae (354 mL CH_4/g VS) and 50% WAS-50% microalgae (380 mL CH_4/g VS), respectively.

According to the increase in methane production, the biodegradability of the co-digestion mixtures were much higher than the biodegradability of the sole substrates, especially in the case of the mixture 75% WAS-25% *C. sorokiniana*. The maximum methane yield value for this mixture was also considerably higher than that obtained in the co-digestion of WAS with other solid substrates, (e.g. strawberry wastes) at 40%-60% for which a value of 171 mL CH_4/g VS was achieved. [6] A possible explanation for the synergetic effect of co-digesting WAS with microalgae when algal fraction is at low concentrations in the mixture is the enhanced alkalinity that the microalgae bring to the co-digestion, which showed a better process stability and enhanced methane yield by microalgae addition. [15] In addition to that, when the percentage of microalgae is high (50% and 75%) the methane yields decreased, which can be attributed to the hemicellulosic cell wall of this microalga, which present a high resistance to anaerobic bacterial degradation. [5] It has been also recently reported that the anaerobic co-digestion of *Chlorella* sp. with varying amounts of WAS increased the biogas yields of *Chlorella* by 73-79%, compared to the digestion of *Chlorella* as sole feed. Co-digestion mixtures with 4% and 11% of this alga have also significantly improved the dewatering rate than control digesters that processed only WAS or *Chlorella*. [16]

The methane yields observed for each co-digestion mixture (Figure 1) were compared to calculated or theoretical methane yields based on the WAS and *C. sorokiniana* methane yields separately according to the Equation (1):

$$\textit{Calculated methane yield}\ (\mathrm{mL\ CH_4/g\ VS_{added}}) = \%\,\textit{WAS} \cdot 362 + \%\,\textit{C. sorokiniana} \cdot 318 \quad (1)$$

where 362 and 318 are the experimental methane yields (mL CH_4/g VS_{added}) obtained from 100% WAS and 100% *C. sorokiniana*, respectively. *% WAS* and *% C. sorokiniana* are the percentages of WAS and *C. sorokiniana* in each co-digestion mixture.

The experimental BMP values were higher than the calculated methane yields from Eq. (1) in each of the co-digestion mixture assayed: 7.6% for co-digestion mixture 25% WAS-75% *C. sorokinaina* (calculated value: 329 mL CH_4/g VS_{added}), 11.8% for co-digestion mixture 50% WAS – 50% *C. sorokiniana* (calculated value: 340 mL CH_4/g VS_{added}) and 25.9% for co-digestion mixture 75% WAS - 25% *C. sorokiniana* (calculated value: 351 mL CH_4/g VS_{added}). Synergy effect of the WAS and *C. sorokiniana* co-digestion was clearly shown with these results. In a similar way, Wang et al. [16] also obtained calculated biogas yields for different mixtures of WAS and the microalga *Chlorella* sp. with the assumption that biogas generation by WAS alone and algae alone can be applied to co-digestion with their different mass compositions. These authors obtained 23% higher biogas yield compared to the calculated value for the 41% algal addition (VS basis) set, demonstrating also this synergic effect. [16] These results mean that more efficient bioenergy harvesting from microalgae could be achieved when microalgae is co-digested with WAS. However, this synergy trend was not observed when microalgae and undigested sewage sludge were co-digested at thermophilic conditions (55 ºC). [17] The reason for this might be due to that some microalgae (i.e. *Chlorella vulgaris*, *Scenedesmus* sp.) contain high percentage (50-60%) of proteins. Degradation of proteins releases ammonium which at higher temperatures will be converted, to a higher extent, into ammonia. This substance can be toxic to methanogens at certain concentrations, which might explain why there was a lower biogas production at thermophilic conditions. [17]

The following first-order exponential model was used for assessing the kinetics of the anaerobic processes of the sole substrates and different co-digestion mixtures tested: [9, 10]

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

where B (mL CH_4/g VS_{added}) is the cumulative specific methane production, B_{max} (mL CH_4/g VS_{added}) is the ultimate methane production, k is the specific rate constant (days^{-1}) and t (days) is the time.

The non-linear regression adjustment of the pairs of experimental data (B, t) using the Sigmaplot software (version 11.0) allowed the calculation of the kinetic parameter, k, which value was 0.36±0.01, 0.27±0.01, 0.34±0.02, 0.34±0.03 and 0.24±0.01 days^{-1} for 100% WAS, 75% WAS-25% *C. sorokiniana*, 50% WAS-50% *C. sorokiniana*, 25% WAS-75% *C. sorokiniana*, and 100% *C. sorokiniana*, respectively. The high values of the R^2 (between 0.983-0.993) and low values of the standard error of estimate (between 9.7-17.3) demonstrate the goodness of the fit of experimental data to this model. In addition, the values of B_{max} were 361±3, 415±6, 345±5, 310±6 and 319±4 mL CH_4/g VS_{added} for 100% WAS, 75% WAS-25% *C. sorokiniana*, 50% WAS-50% *C. sorokiniana*, 25% WAS-75% *C. sorokiniana*, and 100%

C. sorokiniana, respectively. These calculated values were very similar to the experimental methane yields previously mentioned.

In all cases, the presence of WAS in the mixtures increased the kinetics of methane production with respect to the BMP assay without WAS (100% microalgae). Specifically, the kinetic constant of the process increased 42%, 42% and 12% for the mixtures with 25%, 50% and 75% of WAS compared to the substrate without WAS (100% microalgae). A decrease in the kinetic constant from 0.36 ± 0.01 to 0.27 ± 0.01 days^{-1} was observed when the percentage of WAS decreased from 100% to 75%, showing no significant difference for the mixtures with 50% and 25% of WAS.

The kinetic constants obtained in the present work for the different co-digestion mixtures of WAS-*C. sorokiniana* were much higher than that achieved in BMP tests of mixtures of WAS with tannery carving fat and bovine rumial content (at a ratio of 1:2:4) for which a value of 0.089 ± 0.010 day^{-1} was achieved. [15] In the same way, these kinetic constant values were also much higher than that reported recently for the anaerobic co-digestion of WAS with lipid-spent *Botryococcus braunii* (at 75%/25% and 50%/50%: 0.071±0.006 and 0.097±0.007 days^{-1}, respectively). [10]

Conclusions

Anaerobic co-digestion of WAS and *Chlorella sorokiniana* with a mixture of 75%-25% respectively, increased the methane yield by 22% and 39% compared to the anaerobic digestion of the alone substrates WAS and microalgae, respectively. By comparing the experimental BMP values with the calculated methane yield values obtained from the sole WAS and microalgae methane yields a clear synergy effect was observed, especially for the mixture 75% WAS – 25% *C. sorokiniana*. The kinetic constant of the process increased 42%, 42% and 12% for the mixtures with 25%, 50% and 75% of WAS respectively, compared to the substrate 100% microalgae.

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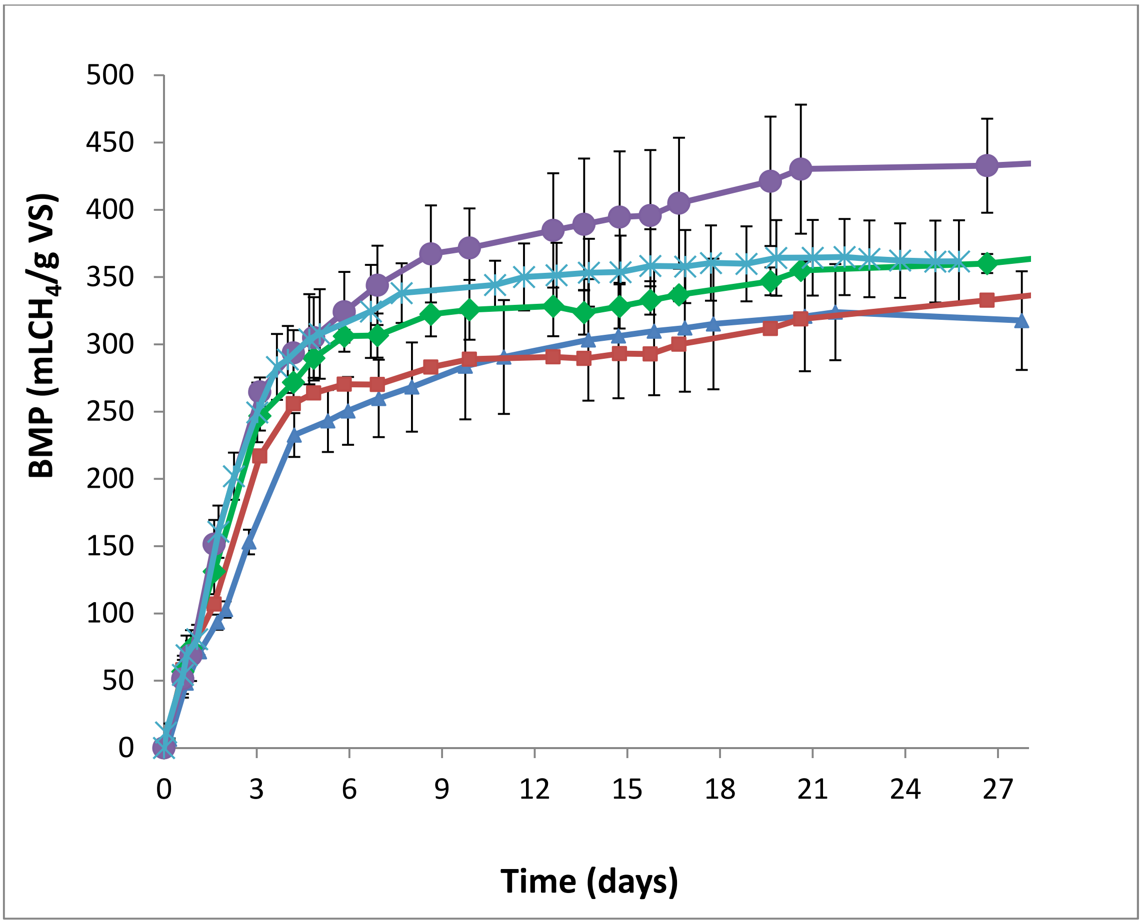

Figure 1. BMP (mL CH_4/g VS_{added}) of 100% WAS (*), 100% *C. sorokiniana* (▲) and different co-digestion mixtures: 75% WAS-25% *C. sorokiniana* (●); 50%WAS-50%*C. sorokiniana* (♦); and 25% WAS-75% *C. sorokiniana* (■).