Algal Research 24 (2017) 309–316

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Use of rumen microorganisms to boost the anaerobic biodegradability of microalgae

Juan B. Giménez[a,*], Daniel Aguado[b], Alberto Bouzas[a], José Ferrer[b], Aurora Seco[a]

[a] CALAGUA – Unidad Mixta UV-UPV, Departament d'Enginyeria Química, Universitat de València, Avinguda de la Universitat s/n, 46100 Burjassot, Valencia, Spain
[b] CALAGUA – Unidad Mixta UV-UPV, Institut Universitari d'Investigació d'Enginyeria de l'Aigua i Medi Ambient – IIAMA, Universitat Politècnica de Valencia, Camí de Vera s/n, 46022, Valencia, Spain

ARTICLE INFO

Keywords:
Rumen microorganisms
Microalgae
AnMBR
Waste sludge production
Biodegradability potential

ABSTRACT

A laboratory bioreactor using rumen microorganisms to treat *Scenedesmus* spp. biomass was operated for 190 days. At first the bioreactor operated as a Rumen-like Fermenter (RF) with a Sludge Retention Time (SRT) of 7 days. The RF was subsequently transformed into an anaerobic digestion system including two configurations: continuously-stirred tank reactor and anaerobic membrane bioreactor in which different SRT values of up to 100 days were assessed. Methane production peaked at 214 mL CH_4 g^{-1} COD_{In} with a SRT of 100 days. COD removal and BDP peaked at above 70% and 60%, respectively, at the highest SRT, with no pre-treatment prior to microalgae digestion. The waste sludge production dropped to 0.133 mg VSS mg^{-1} COD_{In} after a SRT of 100 days.

1. Introduction

Microalgae biomass is an attractive feedstock for biofuel production for several reasons: they grow faster and have a higher biomass production than terrestrial crops and they can grow using wastewater as a medium [1]. Microalgae cultivation as a standalone treatment in photo-bioreactors or combined with activated sludge bacteria can be used in different phases of the wastewater treatment cycle depending on the nutrient composition of the wastewater [2]. In addition, the microalgae can use the CO_2 in the flue gases of combustion engines as a source of carbon. This helps reduce the carbon footprint of the biofuels obtained from the microalgae [3].

Microalgae can be used to produce different types of biofuels and by-products, including the increasingly attractive methane generated by Anaerobic Digestion (AD). The main reasons are that microalgae biomass enables wet AD [4], and all the macromolecules (i.e. proteins, carbohydrates and lipids) found in microalgae can theoretically be transformed into biogas after AD [1]. In addition, because some of the nutrients in organic form are mineralized during AD, they can be reused to cultivate new biomass [5–8].

The factor that influences the anaerobic biodegradability of microalgae most is cell wall composition. Sialve et al. [6] proposed a stoichiometric equation to predict the specific methane yield of a substrate with a known composition. The cell wall of some microalgae species, however, consists of complex carbohydrates with slow biodegradability and/or low bioavailability [9,10]. Such resilient cell walls hinder the digestion process because the organic matter retained in the cytoplasm is not easily accessible to anaerobic microorganisms [11]. The composition of the cell wall varies according to the species. Microalgae consist mainly of 25–30% cellulose, 15–25% hemicellulose, 35% pectin and 5–10% glycoproteins. Some species, such as *Dunaliella salina*, have no cell wall, whilst in others, the cell wall consists of glycoproteins (e.g. *Chlamydomonas* sp., *Euglena* sp. and *Tetraselmis* sp.), where AD is most effective (i.e. a high rate of biomass conversion) [9,11].In contrast, AD of some other microalgae species (e.g. *Chlorella* sp., *Nannochloropsis* sp. *and Scenedesmus* sp.) with cellulose-based cell walls and containing sporopollenin and polyterpene, is hampered due to their recalcitrant nature [9,11]. As a result, the cell walls must be broken down in order to release the organic compounds inside the cells into the surrounding culture medium and make them accessible to the microorganisms outside. This increases the digestibility of the microalgae by the anaerobic microorganisms. A variety of technologies can be used to break down cell walls: thermal, mechanical, chemical or biological. Thermal pre-treatments are the most widely used [12,13], and their effectiveness depends on the strain of microalgae. Unlike thermal pre-treatment, the effectiveness of mechanical pre-treatment does not depend on the characteristics of the microalgae species, although it is more energy intensive than thermal pre-treatment [14].

* Corresponding author.
E-mail addresses: juan.b.gimenez@uv.es (J.B. Giménez), daaggar@hma.upv.es (D. Aguado), alberto.bouzas@uv.es (A. Bouzas), jferrer@hma.upv.es (J. Ferrer), aurora.seco@uv.es (A. Seco).

http://dx.doi.org/10.1016/j.algal.2017.04.003
Received 2 February 2017; Received in revised form 23 March 2017; Accepted 10 April 2017

Chemical pre-treatments have been proven to be highly efficient, especially when combined with heat [15]. However, the presence of residual chemicals hinders downstream biological operations due to their toxicity [16]. Biological pre-treatments (i.e. enzymatic hydrolysis of the cell wall) increase the biodegradability of the microalgae whilst using little energy and employ operating conditions that are not very harsh [17]. In this context, the enzymatic hydrolysis of microalgae complex cell wall may be a promising alternative to energy-intensive mechanical and thermal pre-treatments and chemical hydrolysis because of its more favourable energy balance: a crucial factor for full-scale implementation.

In spite of studies regarding the effect of enzymatic hydrolysis of microalgae over the subsequent anaerobic digestion are scarce [18,19], it seems reasonable to state that the overall cost of enzymatic pretreatment of microalgae may be lower than that of thermochemical hydrolysis, since the energy expenses related to the biomass heating are avoided. Operating in less demanding conditions enables standard equipment to be used, resulting in a lower capital outlay. Similarly, enzymes can be produced by a wide range of bacteria and fungi [19,20].

For instance, several anaerobic microbial ecosystems, such as the digestive tract of termites and the rumen of ruminants, are very active in the conversion of lignocellulosic materials [21]. The controlled environmental conditions of rumen facilitate the growth of an extensive and complex microbial population which consists mainly of bacteria, many ciliate protozoa not found elsewhere in nature, flagellates and phycomycete fungi which are firmly attached to the solid substrate during degradation [21]. The physical coupling of the microorganisms to the substrate enables them to maximise their hydrolytic enzyme activities. In addition, the attachment of microbial cells to the solid digesta causes microbial biomass to be retained longer in the rumen, because the solid residence time has been shown to be much longer than the hydraulic retention time [21]. In artificial fermentation systems, biomass retention is achieved mainly by filtering techniques. In this regard, membrane bioreactors can be useful for retaining microorganisms whilst enabling a high quality effluent to be obtained. Some research involving biomass retention [21,22] has demonstrated highly effective degradation of Neutral Detergent Fibres (NDF) with Sludge Retention Times (SRTs) as short as 3 or 4 days, and Hydraulic Retention Times (HRTs) of 12 to 18 h. Longer HRTs decreased the degradation of NDF, probably due to the lower pH values caused by the accumulation of fermentation acidic end-products. Low pH values have been proven to affect “in vivo” and “in vitro” rumen fermentation negatively [21]. The way in which plant polymers are fermented by the microbial community in the rumen is comparable to the pattern observed in anaerobic digesters, but the acetate produced from Volatile Fatty Acids (VFAs) and the acetoclastic methane generated are far lower in the rumen because methane production reduces the potential substrate energy available for the animal. The use of rumen microorganisms in a rumen-like fermentation system might enhance the biodegradability of microalgae. However, the long-term cultivation of rumen microorganisms using artificial rumen in a simple, user-friendly construction is essential for such a purpose. Although rumen microorganisms have been used successfully in experiments to degrade lignocellulosic compounds including agricultural residues, the organic fraction of the municipal solid wastes and aquatic plants [22–25], rumen microorganisms have never been used, to the best of authors' knowledge, to digest microalgae anaerobically. Although most of the studies involving pre-treated biomass have been conducted in batch reactors, some long-term studies have already been undertaken in continuous digesters with SRTs ranging from 14 to 120 days [11].

The paper herein describes a simple, long-term, continuous system in which rumen microorganisms are used to degrade microalgae anaerobically. The effectiveness of the process is evaluated in terms of Chemical Oxygen Demand (COD) removal, Volatile Suspended Solids (VSS) removal, Waste Sludge Production (WSP) and BioDegradability Potential (BDP). The impact of SRT on reactor performance, using microalgae as substrate, is assessed.

2. Materials and methods

2.1. Source of microalgae

Microalgae were obtained from a pilot-scale membrane photobioreactor fed with nutrient-rich effluent from a pilot-scale, Anaerobic-Membrane BioReactor (AnMBR) treating municipal wastewater. Further details of the AnMBR pilot-scale plant can be found in [26]. Both pilot-scale plants are Calagua research group property and are located at the Barranco del Carraixet Wastewater Treatment Plant (Valencia, Spain). Before being fed into the acidogenic reactor, the collected microalgae were concentrated from 300 to 6000 mg COD L^{-1} on average, in a Cross-Flow, Ultrafiltration Hollow-Fibre, (CF-UHF) membrane unit (Koch Romicon 2″, 250 kDa MWCO). Once the COD concentration was adjusted to the desired value, the microalgae biomass was characterised. The microalgae biomass consisted mainly of *Scenedesmus* sp. (> 90%) except during an episode of cyanobacteria blooming around day 110 not taken into account when calculating plant performance. After being concentrated, the microalgae feedstock was stored at 4 °C for an average of 2 weeks depending on the original concentration of the microalgal liquor. Table 1 shows the average characteristics of the microalgae feedstock entering the anaerobic digester.

2.2. Source of rumen microorganisms

The rumen microorganisms used in this study were obtained from ruminal fluid extracted from a goat's rumen via the oesophagus and immediately transferred to a preheated, isolated flask. The ruminal fluid was strained through gauze to remove any coarse materials prior to inoculation.

The rumen ecosystem in goats is characterised by an almost constant supply of plant material, saliva and water, a constant temperature of 39 °C, an almost neutral pH (6–7), a low oxidation-reduction potential, and a higher removal rate of liquids than solids. These conditions favour the growth of a large and complex microbial population able to transform structural plant fibres [21].

2.3. Experimental set-up

The experimental set-up consisted of two continuously stirred anaerobic reactors: a 7-litre rumen-like fermenter (RF; 4-litre headspace) and a 13-litre Anaerobic Reactor (AnR; 4-litre headspace). Fig. 1a shows the lay-out of the RF, and the AnR. The RF had the same configuration than the RAn, the only difference being the volume of the

Table 1
Average characterisation of microalgae feedstock. Mean values ± standard deviation (SD) for the whole period (n = 28).

Parameter	Units	Mean ± SD
T-COD	mg COD L^{-1}	6093 ± 350
S-COD	mg COD L^{-1}	235 ± 141
TS	mg TS L^{-1}	5274 ± 324
% VS	%	75.4 ± 5.2
TSS	mg TSS L^{-1}	4201 ± 383
% VSS	%	91.0 ± 3.3
T-N	mg N L^{-1}	362 ± 67
T-P	mg P L^{-1}	71.8 ± 17.9
NH_4-N	mg N L^{-1}	43.5 ± 24.7
PO_4-P	mg P L^{-1}	7.7 ± 6.9
SO_4-S	mg S L^{-1}	91.6 ± 25.8
VFA	mg CH_3COOH L^{-1}	159.5 ± 111.9
Alk	mg $CaCO_3$ L^{-1}	361.6 ± 91.7