ARTICLE IN PRESS

Waste Management xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Waste Management

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

Anaerobic co-digestion of Tunisian green macroalgae *Ulva rigida* with sugar industry wastewater for biogas and methane production enhancement

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ARTICLE INFO

Article history:
Received 30 June 2016
Revised 20 October 2016
Accepted 30 November 2016
Available online xxxx

Keywords:
Anaerobic co-digestion
Green macroalgae
Sugar wastewater
Biogas and methane production
Denaturing gradient gel electrophoresis (DGGE)
Prokaryotic diversity

ABSTRACT

Ulva rigida is a green macroalgae, abundantly available in the Mediterranean which offers a promising source for the production of valuable biomaterials, including methane. In this study, anaerobic digestion assays in a batch mode was performed to investigate the effects of various inocula as a mixture of fresh algae, bacteria, fungi and sediment collected from the coast of Sfax, on biogas production from *Ulva rigida*. The results revealed that the best inoculum to produce biogas and feed an anaerobic reactor is obtained through mixing decomposed macroalgae with anaerobic sludge and water, yielding into 408 mL of biogas. The process was then investigated in a sequencing batch reactor (SBR) which led to an overall biogas production of 375 mL with 40% of methane. Further co-digestion studies were performed in an anaerobic up-flow bioreactor using sugar wastewater as a co-substrate. A high biogas production yield of 114 mL g^{-1} VS_{added} was obtained with 75% of methane. The co-digestion proposed in this work allowed the recovery of natural methane, providing a promising alternative to conventional anaerobic microbial fermentation using Tunisian green macroalgae. Finally, in order to identify the microbial diversity present in the reactor during anaerobic digestion of *Ulva rigida*, the prokaryotic diversity was investigated in this bioreactor by the denaturing gradient gel electrophoresis (DGGE) method targeting the 16S rRNA gene.

1. Introduction

Various problems linked to the exploitation of fossil fuels have revived interest in, and fostered efforts towards developing new technologies to obtain clean energy through the substitution of non-renewable fuels with renewable biomass, concerning both energy and environmental aspects (Morand et al., 1991; Bain and Overend, 1992; Park et al., 2009). Biomass generally contains a lower amount of carbon dioxide (CO_2) than fossil fuels. It is considered a carbon-neutral, since the CO_2 emitted by combustion balances the CO_2 previously fixed by photosynthesis (Yokoyama et al., 2007). The first generation of biofuels is made from edible feedstock like corn, soybean, sugarcane, and rapeseed. The use of these resources for energy production was blamed for a rise of food prices. The second generation of biofuels from waste and dedicated lignocellulosic feedstocks has advantages over those of first generation. The major benefits are higher stock yields and lower land requirements in terms of quality and quantity. The main problem associated with lignocellulose conversion to biofuels is its strong resistance to degradation. Thus, the second generation of biofuels still lacks economic viability at large scale (IEA, 2008; Smyth et al., 2010). Third generation biofuels feedstock is represented by micro- and macro-algae, which present further advantages over the previous two. This marine biomass shows the prospect of high yields requiring no use of arable land (Chisti, 2007; Ahmad et al., 2011; Daroch et al., 2012). Seaweeds share some similarities with terrestrial plants; they offer an attractive source for bioenergy production with several advantages over other terrestrial and lignocellulosic biomass. Compared to terrestrial biomass, marine algae have higher rates of carbon dioxide fixation, greater potential for carbon dioxide remediation, and, hence, higher production yields of algae per unit area (Saxena et al., 2009). Moreover, marine algae do not require arable land and can be grown in various environments, including wastewaters, with a three dimensional growth rate along height, breadth and length (Tedesco et al., 2014; Olabi, 2013). It has been proven that macroalgae can reach 2–20 times the production potential of conventional terrestrial energy crops (Bruhn et al., 2011).

The accumulation of seaweed in the near-shore areas, the piling of seaweed on beaches, and its subsequent decomposition can present a threat to the marine ecosystem. The accumulation of

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http://dx.doi.org/10.1016/j.wasman.2016.11.042

Please cite this article in press as: Karray, R., et al. Anaerobic co-digestion of Tunisian green macroalgae *Ulva rigida* with sugar industry wastewater for biogas and methane production enhancement. Waste Management (2016), http://dx.doi.org/10.1016/j.wasman.2016.11.042

macroalgae on the Tunisian coast is an important problem of pollution. Algal biomass accumulated on the coast of Sfax was 394.855 g/m^2 in 2009, which indicates poor water quality sea along 16 km (Ben said et al., 2009). The coastline of Sfax is 50 linear kilometers with a depth of about 5 km. This coastline is responsible for a large number of industrial activities, mainly related to the phosphate industry, which promotes the accumulation of macroalgae.

Macroalgal pollution in Tunisian coasts and the importance of this marine biomass as compared to the terrestrial biomass encouraged us to look for a valorization of this biomass. Macroalgae can be converted into biofuels by various processes including thermal processes and fermentation. The most energy efficient route to obtaining biofuel from macroalgae is via anaerobic digestion (AD) to biogas (Hughes et al., 2012). The production of biogas through AD offers significant advantages over other forms of bioenergy production. It has been considered as one of the most energy-efficient and environmentally beneficial technology for bioenergy production (Olabi, 2012). Biogas generation can drastically reduce greenhouse gases compared to fossil fuels by the utilization of locally available resources. In particular, macroalgae can be converted into biogas (60% methane) via (AD) (Burton et al., 2009). Biogas can then either be used to produce heat and electricity in combined heat and power (CHP) systems or up graded to biomethane. Biomethane is a gas chemically identical to natural gas that can be injected into the gas grid or used as a transport fuel (Browne et al., 2011). In fact, in previous studies of Montingelli et al. (2016), the methane yield was reached 34 ± 17 mL g^{-1} VS.

The digestate represents an improved soil conditioner that can substitute mineral (Weiland, 2010) and industrial fertilizers (Barbot et al., 2014). The anaerobic digestion of algal waste not only recycles the nutrients but also provides biomethane, an attractive renewable source of energy and offers the chance to spread the focus of feedstock materials to defuse the current tension regarding land use competition, while simultaneously managing the accumulating bio-wastes (Barbot et al., 2016). Compared to other fossil fuels, methane produces fewer atmospheric pollutants and generates less CO_2 per unit energy. As methane is comparatively a clean fuel, the trend is toward its increased use for appliances, vehicles, industrial applications, and power generation (Chynoweth et al., 1986). Reijnders and Huijbregts (2009) reported that methane presents the highestheating value when compared to the most common transport fuels, such as biodiesel, bioethanol and biomethanol. In fact, marine algae have been reported to contribute to higher biogas production yields in co-digestion with other organic wastes (Matsui and Koike, 2010). The co-digestion process, which can be defined as the simultaneous treatment of two - or more - organic waste streams by anaerobic digestion, offers great potential. One of the most recent outstanding examples of application of this technology is in Denmark, where a program regarding the common use of nine biogas plants, has promoted the possibilities of co-digestion of combined organic wastes (Ahring et al., 1992). Recent research indicates that anaerobic co-digestion offers a promising alternative, particularly because it involves the dilution of potential toxic compounds, an improved balance of nutrients, synergistic effects of microorganisms, and better yields of biogas production (Fernandez et al., 2005). In fact, several co-digestion studies have been performed using different organic fractions of industrial waste along with waste activated sludge to enhance biogas production (Yen and Brune, 2007).

The present work was aimed at exploring the potential of the green macroalgae *Ulva rigida* collected from the coast of Sfax, in terms of biogas and methane production by determining the best inoculum leading to the highest amounts of biogas production during anaerobic digestion. The study also provides an assessment of the viability of the anaerobic co-digestion of this macroalgae with sugar wastewater generated from the candy manufacturing industry for biogas and methane production. The analysis of bacteria and archaea species composition variation in an anaerobic biological up-flow reactor was also monitored using Polymerase Chain Reaction (PCR) and (DGGE) techniques.

2. Materials and methods

2.1. Substrate

The algal biomass samples used in the present study consisted of green macroalgae belonging to the *Ulva rigida* species collected from the coast of Sfax.

Sfax is a port city in the east located approximately 270 km from Tunis, the capital of Tunisia. It looks like a very large urban area (about 220 km^2, second largest city after Greater Tunis, which has four times the population).

The samples had up to 10 cm-long thalli, with ruffled or flat blades and small microscopic teeth on the margins. This macroalgal biomass is considered as a solid waste that generates intense marine pollution.

The algal sludge used in this study was a mixture of algae, bacteria, and fungi. The mixtures were collected from beach areas. The marine algae *Ulva rigida* was the dominant species in the mixture (>90%).

The wastewater sample used in this study was collected from a local candy manufacturing company in Sfax. It consisted of a washwater of candy preparation machines. The wastewater sample was stored at 4 °C until further use.

Fresh macroalgae was dried at 45 °C and ground to powder for the co-digestion of macroalgae with sugar industry wastewater using an anaerobic biological up-flow reactor with a working volume of 7 L.

2.2. Inocula

Three inocula were tested to identify the optimum inoculum composition from the anaerobic digestion of green macroalgae for biogas production. The different compositions assayed are described in Table 2:

- Inoculum 1: fresh macroalgae, seawater and sediment sludge collected from sediments.
- Inoculum 2: decomposed (chopped) fresh macroalgae collected from a beach area then decomposed manually to obtain small particles and anaerobic sludge collected from WWTP (Chotrana, TUNISIA) and water.
- Inoculum 3: fresh algae collected from beach area and supernatant of inoculum 2.

2.3. Preparation of bioreactors

2.3.1. Batch test

Batch tests were performed using serological bottles with a working volume of 50 mL, which were flushed with N_2 for 3 min to clear up any residual traces of oxygen from the flasks and pipes and to preserve anaerobic conditions and tightly closed with rubber stoppers.

All results were presented as averages of triplicates with standard deviation.

2.3.2. Semi-continuous digestion: sequencing batch reactor (SBR)

Serological bottles with a working volume 2500 mL were used. The (SBR) were then immediately sealed using rubber septa and

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