Applied Energy 114 (2014) 790–797

Contents lists available at ScienceDirect

Applied Energy

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

Comparative evaluation of biomass production and bioenergy generation potential of *Chlorella* spp. through anaerobic digestion

Sanjeev Kumar Prajapati, Anushree Malik *, Virendra Kumar Vijay

Centre for Rural Development and Technology, Indian Institute of Technology, Hauz Khas, New Delhi 110016, India

HIGHLIGHTS

- Higher biomass production potential of *Chlorella pyrenoidosa* among tested species.
- Empirical formulae and theoretical COD estimation for algal biomass.
- Determination of maximum TMP and SMP of three species of *Chlorella*.
- Biogas production potential in range of 0.340–0.464 $m^3\ kg^{-1}$ VS added.
- Highest biogas production from *C. pyrenoidosa* biomass.

ARTICLE INFO

Article history:
Received 4 January 2013
Received in revised form 24 May 2013
Accepted 7 August 2013
Available online 29 August 2013

Keywords:
Chlorella
BMP
SMP
Anaerobic digestion
Biogas
Digestibility

ABSTRACT

Microalgae *Chlorella* spp. are being considered of great research interest for biofuel application. The current study was focused on the comparative exploration of biogas production potential of three *Chlorella* spp. namely *C. minutissima*, *C. vulgaris* and *C. pyrenoidosa*. Among the tested algae *C. pyrenoidosa* was found to be the best in both biomass production potential and biogas generation. After 12 days of cultivation, biomass productivity was found to be 0.90 ± 0.04, 0.98 ± 0.11 and 0.92 ± 0.01 $g\ L^{-1}$, respectively, for *C. minutissima*, *C. pyrenoidosa* and *C. vulgaris*. The corresponding estimated annual areal yields were 27.37, 27.98 and 29.20 tons dry biomass $ha^{-1}\ y^{-1}$, respectively. The elemental and biochemical composition of the algal biomass was also determined and the theoretical/stoichiometric methane potential (TMP and SMP) of respective algal biomass was estimated. The estimated TMP and SMP values ranged from 0.563 to 0.592 and 0.598 to 0.699 $m^3\ kg^{-1}$ VS, respectively. *C. pyrenoidosa* was found to have the highest TMP and SMP. Moreover, biogas production potential was also determined through BMP protocols. Relatively higher biogas yield of 0.464 ± 0.066 m^3 biogas kg^{-1} VS added with 57% (v/v) CH_4 content was obtained for *C. pyrenoidosa* biomass during 30 day digestion. Moreover, the digestate analyses showed that all parameters (pH, alkalinity, VFA and NH_3–N concentration) were in the stable range. In-contrast with the good biogas potential, the digestibility of the *Chlorella* biomass was around 50%. Current findings revealed that there is need of extensive comparative analysis in order to find out the interspecific variations of the algae with respect to biofuel production.

1. Introduction

Microalgae have been subjected to extensive investigations for biofuel production of liquid (e.g., bioethanol and biodiesel) and gaseous fuels such as biogas and bio-hydrogen [1]. The major advantage of using microalgae as biofuel feedstock include their ability to grow and replicate at faster rates, possibility of cultivation on non-arable lands as well as the less water uptake and land requirement compared to terrestrial biofuel crops [2–5]. Microalgae also have ability to grow in range of industrial and domestic wastewaters with simultaneous phycoremediation and biomass production [6]. Moreover, ability of microalgae to uptake and fix CO_2 from waste gas streams such as flue gases indicates the possibility of integration of algal biofuel production with CO_2 sequestration [7]. Reports on microalgal treatment of high strength wastewaters including livestock waters, biogas plant slurry and agro-industrial wastewaters, etc., are also available in the literature [8,9]. The phycoremediation potential of microalgae can also be utilized for the treatment of high strength wastewater or grey waters in rural areas [10] to make wastewater suitable for agricultural application with simultaneous production of substantial biomass for bioenergy production.

* Corresponding author. Tel.: +91 11 26591158; fax: +91 11 26591121.
E-mail addresses: sanjukec@gmail.com (S.K. Prajapati), anushree_malik@yahoo.com (A. Malik), vkvijay14@hotmail.com (V.K. Vijay).

0306-2619/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.apenergy.2013.08.021

Among the various microalgae, *Chlorella* sp. has been given very much attention in biofuel research. *Chlorella vulgaris*, owing to its high lipid content has been explored extensively for bio-diesel production [11]. *C. vulgaris* has also been widely reported for its application in phycoremediation of wastewater and flue gases [12]. Moreover, recently the biogas (286 mL CH_4 g^{-1} VS) and bio-hydrogen (10.8 mL H_2 g^{-1} VS) production potential of *C. vulgaris* biomass have also been reported [13]. Similarly, *C. minutissima* has been used in phycoremediation coupled biomass [14] and biofuel production [15]. *C. pyrenoidosa* has also been tested for phycoremediation of soybean processing wastewater [16]. However, the reports on application of *C. pyrenoidosa* in biofuel application are almost nonexistent.

There have been various attempts on anaerobic digestion of algal biomass. For instant, biogas production potential in the range of 287–587 mL g^{-1} VS have been reported for various algal biomass including *Chlorella kessleri*, *Scendesmus obliquus* and*Chlamydomonas reinhardti* [17]. Recently, Zamalloa et al. [18] have reported the biomethane potential of around 0.36 and 0.24 L CH_4 g^{-1} VS added for *P. tricornutum* and *S. obliquus* biomass, respectively. Moreover, Ehimen et al. [19] have worked towards the optimization of anaerobic digestion of *Chlorella* biomass residue resulting from bio-diesel production process. Ras et al. [20] have also reported the methane production of 147 (during 16 days HRT) and 240 mL g^{-1}VSS (during 28 days HRT) from anaerobic digestion of *C. vulgaris* biomass under semi-continuous mode. However, despite the good theoretical biomethane potential (0.8 L g^{-1} VS) reported by Sialve et al. [21], there is no previous experimental attempt on exploring the biogas production potential of *C. pyrenoidosa* and *C. minutissima*.

Apart from the biogas production potential, determination of COD of biomass or organics solids by experimental methods is often considered very tedious and prone to produce erroneous results due to incomplete oxidation of solids or biomass residues [22,23]. Alternatively, estimation of theoretical COD has been reported to be a better approach [22]. Estimation of theoretical specific methane potential also provides quick and easy insight into the bioenergy generation potential of any organic substrate including algal biomass. Demonstration of such theoretical estimation has systematically been done by Sialve et al. [21] using biochemical composition of algal biomass. However, this may result in the wrong estimation as in spite of similar composition, the biochemical profiling among the different algal strains may vary significantly. Therefore, empirical formulae based estimation of methane potential may be appropriate alternative to the biochemical estimation approach as demonstrated elsewhere [22].

The current study was focused on the determination and comparison of bioenergy potential of three commonly used *Chlorella* strains through anaerobic digestion in order to identify the best suitable algal substrate for biogas production. In addition, two different methods for estimation of theoretical COD and methane potential were also tested for their consistency, reliability and suitability for further applications.

2. Materials and methods

2.1. Algae culture and growth medium

Three species of *Chlorella* namely *C. vulgaris*,*C. minutissima* and *C. pyrenoidosa* were used in the present study. Pure cultures of algae were procured from algal culture collection of Vivekananda Institute of Algal Technology (VIAT), Chennai (India); Centre for Conservation and Utilization of Blue Green Algae, IARI New Delhi (India) and National Collection of Industrial Microorganisms (NCIM), NCL Pune (India), respectively. BG11 broth (HIMEDIA, M1541-500) was used as standard growth medium. After receiving, the aliquots of each alga was transferred to separate sterile BG11 agar slants (2% agar) and broth. The cultures were than maintained in a plant growth chamber (Daihan Labtech, LGC-5101) under cool fluorescent light (≈2500 Lux) at 25 ± 1 °C with 12:12 h light:dark cycle.

2.2. Biomass production and harvesting

Biomass production potential was estimated in 250 mL flask with 50 mL working volume under controlled conditions as reported in our previous study [10]. In order to get sufficient biomass for biochemical analysis and anaerobic digestion studies, algae were cultivated (under non-axenic conditions) in fabricated photobioreactor (PBR) with 20 L working volume. Tap water medium [10] having 12.3 mg L^{-1} nitrogen (as $NaNO_3$), 1.1 mg L^{-1} phosphorous (as KH_2PO_4) and sodium carbonate (20 mg L^{-1}) was used as growth medium. The initial pH of the growth medium was adjusted at 7.0. Inoculum (10% v/v) was taken from the algal culture (optical density ≈2.0 at 680) maintained in plant growth chamber (Section 2.1). After inoculation, the culture bottles were incubated under natural atmospheric conditions (direct sun light and temperature ≈30–42 °C) during day time and under illumination of ≈1000 lux using cool fluorescent light during night. In order to prevent the settling of algal biomass, mixing was provided by bubbling air (0.5–1.0 L min^{-1}) through aquarium pump. After incubation for 12–14 days, the air bubbling was stopped and bottles were kept overnight for harvesting of algal biomass by auto (gravity) settling [24].

2.3. Biomass composition analyses

After harvesting, algal biomass were dried overnight at 65–70 °C and grinded in a mortar pestle to make fine powder for elemental and biochemical analyses. The elemental analysis (CNH) was done using CHN analyzer. Volatile solids (VS) and ash content of biomass was determined through standard methods [25].

Total carbohydrate was determined through phenol–sulfuric acid method [10,26]. Briefly, the powdered biomass (100 mg) was hydrolyzed with 2.5 N HCl (5 mL) in boiling water bath for 3 h, cooled at room temperature and neutralized with solid sodium carbonate. After neutralization, an aliquot of 0.1 mL was pipette out in a clean test tube and diluted to 1 mL. After dilution, 1 mL phenol solution and 5 mL 96% sulfuric acid were added, well mixed and cooled to 25–30 °C in a water bath. The color intensity of the samples was measured at 490 nm and the total carbohydrates were than calculated using standard calibration curve. Protein content was calculated by multiplying the total nitrogen (obtained through CHN analyzer) by 6.25 [27].

Total lipid was extracted using chloroform–methanol mixture (1:1 v/v) mixed with the sample in the proportion of 1:1 and than estimated through modified Bligh and Dryer's method [10]. For efficient extraction of lipids, algal cell wall was disrupted through heat treatment at 100–110 °C up to 5 min (with heat pulses of 30 s to avoid sample loss by over boiling) in a microwave oven [28].

2.4. Estimation of methane production potential

After biochemical and elemental composition analysis, the maximum possible methane potential of selected algal biomass was estimated through two different methods. In the first method, the elemental composition of the algal biomass was utilized. Based on the elemental composition, empirical formula of algal biomass was developed and the maximum possible stoichiometric methane potential (SMP) was calculated through the equation given by Symons and Buswell [29] adopted from Sialve et al. [21].