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Bioconversion of algae to methane and subsequent utilization of digestate for algae cultivation: A closed loop bioenergy generation process

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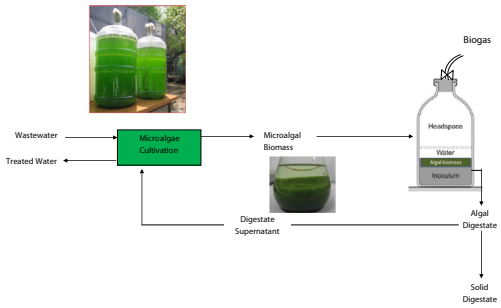
HIGHLIGHTS

- A novel "closed loop process" for algae to biomethane production.
- Algal biomethane production up to 317.31 ± 1.9 mL CH_4 g^{-1} VS added.
- Nutrient rich algal digestate recycled for biomass production.
- Good growth and nutrient removal at 30% digestate concentration.
- Significantly enhanced growth when diluted with rural sector wastewater.

GRAPHICAL ABSTRACT



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ABSTRACT

The present investigation was targeted on anaerobic digestion of *Chroococcus* sp. and utilization of resultant "Liquid Digestate" for its further biomass production. The algal biomass has biomethane potential of 317.31 ± 1.9 mL CH_4 g^{-1} VS_{fed}. Regular process monitoring revealed that process was stable throughout the experiments. The "Liquid Digestate" was explored as nutrient supplement for further algal growth. Diluted "Liquid Digestate" (30% concentration) was found optimal for algal growth (0.79 ± 0.064 g L^{-1}). Simultaneously, 69.99–89.31% removal in nutrient and sCOD was also recorded with algal growth. Interestingly, higher growth was observed when rural sector wastewater (1.29 ± 0.067 g L^{-1}) and BG11 broth (1.42 ± 0.102 g L^{-1}) was used for diluting the "Liquid Digestate". The current findings have practically proven the feasibility of hypothesized "closed loop process".

1. Introduction

Anaerobic digestion has long been practiced as an efficient technology for bioenergy generation from various wastes such as agriculture residues, industrial effluents and municipal solid wastes (Chanakya and Malayil, 2012; Rao et al., 2000). Relatively higher volatile solids (VS) content and favourable biochemical (lipid, protein and carbohydrates) composition of algal biomass makes it an ideal substrate for anaerobic digestion (Prajapati et al., 2013a,b). Recent studies on biogas production from algal biomass include the anaerobic digestion of *Chlorella* spp., (Prajapati et al., 2014), *Chroococcus* spp.,(Prajapati et al., 2013a) and mesophilic/thermophilic digestion of *Scenedesmus obliquus* and *Phaeodactylum tricornutum* (Zamalloa et al., 2012). Biogas production from *Scenedesmus* sp. AMDD in a continuous anaerobic reactor has also been reported recently (Tartakovsky et al., 2013). Furthermore, biomass

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residues from algal biodiesel production have been successfully used for biogas production (Alzate et al., 2014). Hence, it is evident that algae have good biomethane production potential and its anaerobic digestion can become commercially viable provided low cost cultivation methods are available (Dassey et al., 2014).

Ample literature is available on algae mediated pollutant remediation and recycling of nutrients from various wastewaters streams (Markou and Georgakakis, 2011; Prajapati et al., 2013b; Rawat et al., 2011). The ability of algae to grow and extract nutrients from wastewater streams further strengthens the feasibility and economic viability of algal based biofuels. Furthermore, there are reports on utilization of anaerobically treated manure and industrial effluents for algal biomass production. For instance, Wang et al. (2010) cultivated *Chlorella* sp. in 10–25 times diluted anaerobically digested dairy manure. Anaerobically digested effluent from sago starch factory has also been evaluated for *Spirulina* cultivation (Phang et al., 2000). Similarly, the "Liquid Digestate" from algal anaerobic digestion process which is rich in N&P can also be used for algal cultivation. Hence, there is a possibility to develop "a closed loop process" employing algal digestion for biogas production with subsequent utilization of resultant Liquid Digestate as nutrient source for algae cultivation. Nevertheless, to the best of our knowledge no previous attempts were made on the proposed process which could become an ideal bioenergy generation process with "zero waste discharge" to the environments.

Hence, in the light of above literature and identified research gaps, the present work was aimed to practically validate the hypothesized "closed loop process" for algal bioenergy generation. For this, anaerobic digestion of native algae *Chroococcus* sp. was conducted with regular monitoring of process parameters to identify the inhibitions, if any. The resultant Liquid Digestate was examined for its nutrient value and its utilization as growth medium for cultivating *Chroococcus* sp. was studied.

2. Methods

2.1. Algal biomass production and characterization

The algal biomass used in the present work was obtained by growing previously isolated *Chroococcus* sp. (Prajapati et al., 2013a) in nutrient supplemented tap water under non-axenic conditions. Transparent plastic bottles (20 L) were used as outdoor photobioreactor for cultivating algae. Bottles were filled with 16 L tapwater medium containing 12.3 mg N L^{-1} (as $NaNO_3$) and 1.1 mg P L^{-1} (as KH_2PO_4) and inoculated using algal culture (OD_{680} ≈2.0) at inoculum size of 10% (v/v). To avoid settling of algae, aeration was provided (at 1 LPM) using aquarium pump. Bottles were incubated under direct sunlight and ambient temperature conditions with natural day–night cycle. The temperature fluctuated from 20–35 °C during the study period. After15 d, algal biomass was harvested through gravity settling by stopping the aeration and decanting the separated water. The harvested algal slurry was stored at 4 °C until further use in anaerobic digestion experiments.

The harvested algal slurry was characterized for total solids (TS) and volatile solids (VS) content as well as elemental composition. TS content of algal slurry was determined by drying the samples at 50 °C in hot air oven till constant weight. The VS was estimated through EPA Method1684 (Agency, 2001). Briefly, the oven dried samples (in a pre-weighted dish) were ignited for 2 h at 550 °C in a muffle furnace. The VS was then estimated as

$$\mathrm{VS(mg\ mg^{-1}sample)} = \frac{W_t - W_v}{W_t - W_d} \quad (1)$$

where, W_d = weight of dish (mg); W_t = weight of dried residue and dish (mg) and W_v = weight of residue and dish after ignition (mg). Elemental composition was determined using CHNS analyser (vario EL III, Elementar Analysensysteme GmbH). The stoichiometric methane potential (SMP) was then computed from the developed empirical formula for algal biomass as reported in our previous study (Prajapati et al., 2014).

2.2. Anaerobic digestion of algal biomass

2.2.1. Experimental design

For determination of biochemical methane potential (BMP) of algal biomass, batch anaerobic digestion was carried out using 1 L BOD glass bottles, hermetically sealed with stoppers and controlled sampling port for gas and slurry. Digested slurry from actively running cow dung based lab scale biogas digester was used as inoculum. The substrate concentration was kept at 5 g VS L^{-1} with inoculum to substrate ratio (*I*/*S* ratio) of 3.0 on VS basis. Initially, inoculum was aseptically transferred in to the bottle followed by the addition of substrate and then filled up to 700 mL with distilled water. Bottles with inoculum only (without substrate) were used as controls. After inoculation, the bottles were kept in stationary conditions under controlled temperature (36 ± 1 °C). Anaerobic digestion was carried out for 45 d. Gas volume was measured through acidic water (pH ≈2.0) displacement method after every 24 h (Angelidaki et al., 2009). Methane content in the biogas was determined through Gas Chromatograph equipped with stainless steel column packed with Porapack-Q 80/100 mesh (Supelco) and thermal conductivity detector (TCD) as reported earlier(Prajapati et al., 2014). Daily and cumulative biomethane yield was then calculated using Eqs. (2) and (3), respectively.

$$B_{net} = B_{exp} - B_0 \quad (2)$$

$$M_i = \sum_{i=1}^{i=i} B_i \quad (3)$$

Where, B_0 and B_{exp} are the daily biomethane produced (mL CH_4 g^{-1} VS_{fed} d^{-1}) from control and experimental flask, respectively; B_{net} is the net daily biomethane produced from the algal biomass; B_i and M_i are net and cumulative biomethane yield (mL CH_4 g^{-1} VS_{fed}) on *i*th day.

2.2.2. Data fitting and algal biomass digestibility computation

The cumulative biomethane data was fitted in Gompertz model for estimation of maximum rate of biomethane production (R_m), lag phase (λ) and ultimate methane yield (P). The used Gompertz model, adopted from Nopharatana et al. (2007) is given as

$$M = p \times exp\left\{-exp\left[\frac{R_m \times e}{p}(\lambda - t) + 1\right]\right\} \quad (4)$$

where M is the cumulative biomethane yield (mL CH_4 g^{-1} VS_{fed}) and e = 2.718.Hydrolysis rate constant (k_h) for algal biomass was also determined using first order hydrolysis kinetics model adopted from Angelidaki et al. (2009).

$$ln\left(\frac{p - M}{p}\right) = -k_h t \qquad or \qquad M = p\{1 - exp(-k_h t)\} \quad (5)$$

MATLAB (7.0) was used as the software platform to fit the experimental data in the models (Eqs. (4) and (5)). The digestibility of the algal biomass was calculated with BMP and SMP using following equation

$$Digestiblity(\%) = \frac{BMP}{SMP} \times 100 \quad (6)$$

2.2.3. Process parameters

Digestate samples were withdrawn after every 3 d during the anaerobic digestion for analysis of process performance indicator viz., pH, total volatile fatty acids (TVFA), soluble sugars and total ammoniacal nitrogen (TAN). The TVFA content of digestate (as equivalent mg L^{-1} of acetic acid) were estimated through spectrophotometric method as reported in previous study (Prajapati et al., 2013a). The TAN was estimated using multi-parameter (HQ40d, Hach) equipped with ammonium ion selective electrode (ISE). Magnesium acetate (0.25 M) with acetic acid (0.5 M) was used as ammonium ionic strength adjuster (ISA). The soluble sugar in the digestate samples was determined using phenol-sulphuric acid methods given by Dubois et al. (1951) and pH was measured with bench top pH meter (CyberScan PC510, Eutech). The digestate samples were also examined microscopically for determination of undigested intact algal cells. Microscopic analysis was done using light microscope (LIECA DM 2500) under phase contrast mode at magnification of 100×.

2.3. Liquid digestate characterization

After completion of anaerobic digestion experiments, the algal digestate was withdrawn from the reactor and allowed to stand for some time. Supernatant from the digestate was then collected and filtered using muslin cloth in order to remove suspended particulate matter. The obtained liquid portion of digestate termed as "Liquid Digestate" was analysed for determination of total suspended solid (TSS), total dissolved solids (TDS), TAN, nitrate nitrogen (NO_3–N), soluble chemical oxygen demand (sCOD) and total dissolved phosphorous (TDP). The sCOD was determined using HACH method 8000 utilizing reactor digestion method (Hach Digital Reactor DRB200) NO_3–N was estimated through Hach method 8039 and TDP was determined through Hach method 8114. TSS and TDS were estimated using standard methods of wastewater analysis (Eaton et al., 2005).

2.4. Algal growth and nutrient recycling from Liquid Digestate

The Liquid Digestate was dark (brown) in colour and hence was diluted with tap water (at concentration from 10 – 100% v/v) before its use as growth medium for algal cultivation. Keeping in mind that tap water addition may decrease nutrient levels, the selected Liquid Digested concentration (30%) was also tested by diluting it with BG11 broth. Furthermore, in our previous study (Prajapati et al., 2013b) it was observed that the low strength rural sector wastewater (RSW) possess ample nutrients (NH_3-N: 10 ± 0.32 mg L^{-1}; NO_3–N: 9.8 ± 0.02 mg L^{-1} and TDP: 26.89 ± 3.00 mg L^{-1}) to support the algal growth. Hence, RSW was also tested for diluting the Liquid Digestate (30%) to explore its effect on algal growth at optimal dilution.

The study on biomass production potential of Liquid Digestate was carried out in 250 mL flask containing 50 mL working volume using freshly growing *Chroococcus* sp. (OD_{680} = 2.0) as inoculum at 10% (v/v). After inoculation, flasks were incubated for 12 d under controlled conditions (temperature: 25 ± 1 °C, light intensity: 3.5 – 4.5 klux and light:dark cycle of 12:12 h). Algal growth was estimated in terms of biomass concentration and reported as g dry cell weight per litre.

After 12 d, algal grown digestate was analysed for residual pollutant and nutrient concentrations. The removal efficiency of the tested algae was then calculated as

$$\textit{Removal Efficiency}\ (\%) = \left(1 - \frac{X_t}{X_0}\right) x100 \quad (7)$$

where, X_0 and X_t are concentrations of pollutants (mg L^{-1}) in digestate before and after the algal growth.

2.5. Statistical analysis

All experiments were conducted in triplicates and results are presented as means of the replicates along with standard deviation (represented as mean ± SD or error bars).

3. Result and discussion

3.1. Characteristics of substrate and inoculum used for anaerobic digestion

The algal biomass harvested after growth in tap water was in the form of thick algal slurry. It contained more than 90% moisture. The TS and VS concentrations of the algal slurry were 59.47 ± 0.69 g L^{-1} and 54.00 ± 1.35 g L^{-1}, respectively. Similarly, the TS and VS concentration of inoculum used were 37.33 ± 0.80 and 38.57 ± 0.15 g L^{-1}, respectively. The specific methanogenic activity (SMA) of the inoculum estimated using BMP protocols (Angelidaki et al., 2009) was found to be around 0.192 g COD–CH_4 g^{-1} VSS d^{-1}.

The average carbon, nitrogen, hydrogen and oxygen contents (as % of TS) of the algal biomass were 58.04, 6.27, 7.57and 19.38, respectively. The elemental composition of algal biomass was used for development of its empirical formula and subsequent estimation of stoichiometric methane potential (SMP) using equation given by Symons and Buswell (1933). The developed empirical formula for the *Chroococcus* sp. biomass was $C_{4.83}H_{7.51}N_{0.45}O_{1.21}$, while SMP was 708.9 mL CH_4 g^{-1} VS. It is worth mentioning that the SMP estimated for *Chroococcus* sp. using empirical formula was significantly higher than biochemical composition based SMP (640 mL CH_4 g^{-1} VS) reported in our previous study (Prajapati et al., 2013a). Similar results were also obtained with biomass of *Chlorella* spp. (Prajapati et al., 2014).

3.2. Biomethane production from the algal biomass

The variation of daily and cumulative biomethane production during anaerobic digestion of algal biomass is shown in Fig. 1. The biomethane yield of *Chroococcus* sp. (317.31 ± 1.9 mL CH_4 g^{-1} VS_{fed} with 45 d digestion period) was either comparable or higher than the recently reviewed values for various algal biomass (Prajapati et al., 2013b). For example, methane yields of 178–387 mL CH_4 g^{-1} VS have been reported for various algae during anaerobic digestion carried out at 38 °C (Mussgnug et al., 2010). However, digestion time in that particular study (32 d) was relatively shorter than that used in present study. The obtained biomethane yield (at C/N ≈9.26) was also comparable to that obtained with Taihu blue algae at optimized C/N ratio (20) using corn straw as cosubstrate (325 mL CH_4 g^{-1} VS_{fed}) in 30 d digestion period (Zhong et al., 2012). Also, the observed biomethane yield was significantly higher than the previously reported yield of *C. vulgaris* and *Dunaliella tertiolecta* (286 and 240 mL CH_4 g^{-1} VS, respectively) digested at 37 °C for 49 d (Lakaniemi et al., 2011).

From the cumulative biomethane production profile (Fig. 1a), it was observed that around 52.69% and 32.93% biomethane was produced during 0 – 15 d and 16 – 30 d, respectively. Only 14.43% biomethane was produced during last 15 d of anaerobic digestion. Hence, it is clear that majority of biomethane (up to 85.62%) was produced within 30 d from the start of the experiments. As observed in the previous study with *Chlorella* spp. (Prajapati et al., 2013a), the biomethane production started from the first day of the experiments (Fig. 1a). This could be attributed to the available sCOD of the algal slurry (1087 mg L^{-1}) contributed by the damage and release of cellular content of some algal cells during the harvesting and handing stages as reported previously (Prajapati et al., 2013a). Moreover, fluctuations (ups and downs) in the daily

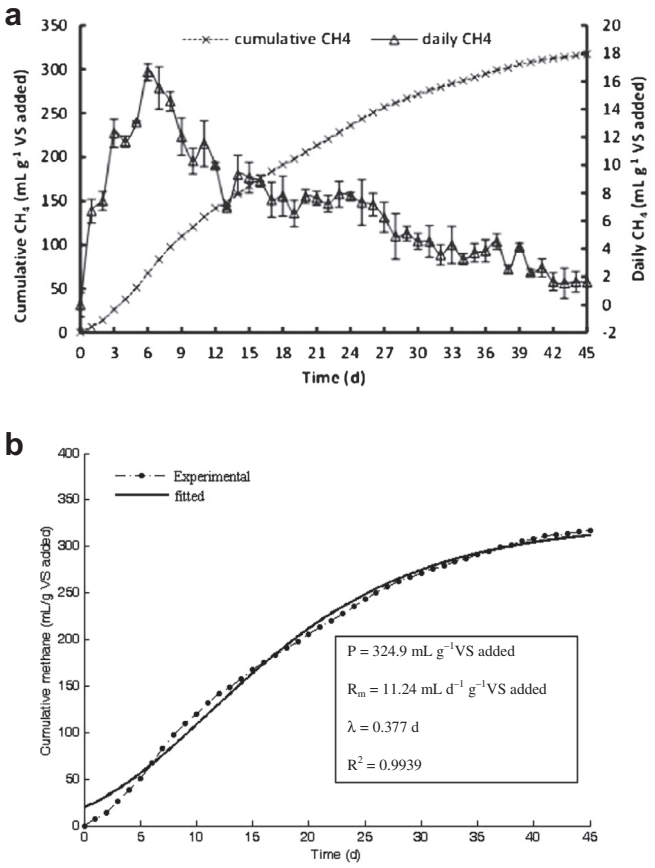

Fig. 1. Biomethane production from algal biomass (a) Variation of daily and cumulative biomethane with elapsed time and (b) fitting of cumulative biomethane data with the Gompertz model (Eq. (3)).

biomethane production were noticed (Fig. 1a). These fluctuations were probably due to the heterogeneity and improper contact of substrate with anaerobic microbial flora under stationery conditions as hypothesised earlier (Prajapati et al., 2014).

The fitting of experimental cumulative biomethane data to Gompertz equation is shown in Fig. 1b. There was a good agreement between the experimental data and the model (R^2 = 0.9939). The estimated maximum specific biomethane production rate (R_m) for algal biomass was 11.24 mL d^{-1} $g^{-1}VS_{fed}$ with lag phase (λ) of 0.377 d (7.54 h) and estimated ultimate biomethane yield (P) of 324.9 mL $g^{-1}VS_{fed}$. Recently, Miao et al. (2013) reported biomethane yield of 287.6 mL $g^{-1}VS$ from Taihu blue algae by using natural storage (15 d) as pretreatment stage. The values observed for P, R_m and λ in their study was 301.97 mL $g^{-1}VS$, 21.11 mL d^{-1} $g^{-1}VS_{fed}$ and 0.53 d (12.72 h), respectively. The digestion period for their study was 22 d in contrast to 45 d in the present work. The methane yield obtained with *Chroococcus* sp. was significantly higher than the value reported for Taihu blue algae (Miao et al., 2013), however, with longer digestion time. Moreover, the values obtained for P was also higher and the λ was relatively shorted in case of *Chroococcus* sp. In fact, Miao et al. (2013) used mechanical agitation (48 rpm) throughout the experiment, hence providing better interaction between the anaerobic microflora and the substrate. However, such agitation was not provided in our experiments. Hence, it is possible that the digestibility as well as biomethane yield of *Chroococcus* sp. can be further improved by providing proper interaction by agitation.

Similar goodness of fit with Gompertz model was also observed for experimental data on biogas production from pretreated grass (Li et al., 2012). In comparison to their results, the P value of fresh algal biomass was significantly higher. Hence, the present investigation further proved that algal biomass has higher biomethane production potential than the non-algal terrestrial biomass as reported elsewhere (Chinnasamy et al., 2010; Heerenklage, 2010).

3.3. Variation of digestate properties during anaerobic digestion

3.3.1. Soluble sugar

Chroococcus sp. possess significant amount of carbohydrates (Prajapati et al., 2013a) that can be solubilised in to sugars and utilized by acidogens for production of VFAs. Hence, the soluble sugar levels were estimated in the digestate samples at regular time intervals. Initial soluble sugar concentration (on 0 d) was 333.33 ± 12.91 mg L^{-1}, while the highest concentration (627.78 ± 15.52 mg L^{-1}) was observed on 3rd day of experiment after which it constantly decreased to 0 mg L^{-1} on 39th day (Fig. 2). Hence, the rate of sugar solubilisation was higher than the utilization by anaerobic microflora during first 3 d of the experiment. This could be attributed to the easy degradation of the damaged algal cells and available sCOD of the feed as discussed in Section 3.2. Higher soluble sugar utilization after 3rd day of experiment could easily be explained by higher TVFAs concentration and biomethane production rate beyond 3rd day up to 6th day.

3.3.2. Total volatile fatty acids

Variation of VFAs concentration during the anaerobic digestion indicates the kinetics as well as the stability of the anaerobic digestion process. It reveals the comparative performance of the acid producer and consumer and also reflects the metabolic state of the process. VFA concentration is thus considered as an important variable in control of anaerobic digestion process (Horan et al., 2011). The variation of TVFAs concentration during anaerobic digestion of *Chroococcus* sp. is shown in Fig. 2. The initial TVFAs concentration (0 d) of the anaerobic reactor was around 190 mg L^{-1} which increased up to 796.67 ± 1.366 mg L^{-1} within first 3 d of the digestion. The maximum TVFAs concentration (986 ± 5.39 mg L^{-1}) was recorded on 6th d of digestion beyond which it started decreasing at very fast rate and attained lowest level of 290.67 ± 5.09 mg L^{-1} on 24th d. TVFA concentration again increased to 439.00 ± 7.95 mg L^{-1}on 27th d and staggered around 400 mg L^{-1} throughout rest of the experiments. It was noticed that the TVFAs variation with elapsed time follows the similar pattern as obtained for daily biomethane production. For instance, the highest biomethane production coincided with highest TVFAs concentration on 6th d. Similar VFA profile was also observed during anaerobic digestion of fresh and stored biomass of Taihu blue algae (Miao et al., 2013) and waste activated sludge (Amani et al., 2011).

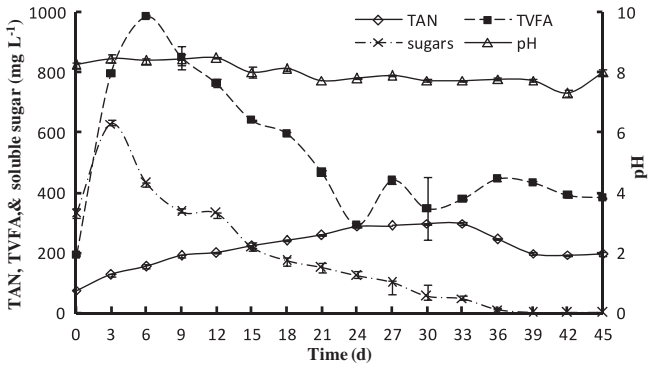

Fig. 2. Variation of anaerobic digestion process parameters (TAN, TVFA and pH) and soluble sugar with elapsed time.

It is worth mentioning that throughout the process, TVFA level was far below the inhibitory level (1500 – 2000 mg L^{-1}) reported earlier (Gooch, 2012). Hence, based on TVFA profile it can be said that the process of algal digestion was stable at the tested substrate concentration under batch mode.

3.3.3. TAN concentration and digestate pH

Algae are known to possess significantly higher amount of nitrogen as their cellular constituent. Hence, regular monitoring of the ammonia concentration during anaerobic digestion is necessary as it may accumulate in the reactor resulting in failure of the whole process. The initial TAN of the reactor content was 75.56 ± 0.27 mg L^{-1}, which increased to 126.91 ± 5.48 mg L^{-1} within first 3 d of the experiments. TAN accumulation in the reactor continued up to 33rd d (297.9 ± 2.27 mg TAN L^{-1}) beyond which it declined and reached 196.33 ± 2.37 mg L^{-1}at the end of experiment. Decrease in TAN levels could be attributed either to its evolution (as NH_3) or less ammonia production as digestion process slowed down at this stage. It is worth mentioning that TAN in the reactor were much below the levels (>2000 mg L^{-1}) reported inhibitory for anaerobic digestion (Gooch, 2012). The pH of the digestate remained nearly constant with initial and final values at 8.26 ± 0.09 and 8.01 ± 0.10, respectively.

Hence, as reflected from the variation profiles of the digestate properties, the anaerobic digestion process was stable at the tested substrate concentration. There was no accumulation of VFAs or TAN during the process. Moreover, pH remained in the vicinity of neutral range (7 – 8) throughout the experiment. Similar process stability during anaerobic digestion of various algae has also been reported recently (Frigon et al., 2013).

3.4. Algal biomass digestibility

The digestibility of the selected algal biomass was estimated to be around 44.76% for 45 d digestion period. Low digestibility of the algal cells can be explained with the first order hydrolysis rate constant (k_h) estimated from the model (Eq. (4)) as 0.035 d^{-1} (R^2 = 0.9967). In contrast to this, Mendez et al. (2013) reported significantly higher k_h for untreated biomass of *C. vulgaris* (0.1 d^{-1}) during 30 d digestion under similar conditions. They also observed significant enhancement in k_h values (up to 0.23 d^{-1}) for thermal pretreated algal biomass. Moreover, the k_h values obtained for *Chroococcus* sp. was relatively lower than those for cattle manure (0.13 d^{-1}) and food waste (0.41 d^{-1}) as summarized by Wolfsberger (2008). Hence, it is clear that *Chroococcus* sp. 1 displayed relatively poor digestibility over recently reported *C. vulgaris* as well as other substrates.

The poor digestibility of the algal cells was also reflected from the microscopic examination of the digestate sample collected at different time intervals. Significant amount of intact cells were observed in the microscopic images of digestate samples including that from 45 d (Supplementary file, Fig. S1). Similar presence of intact algal cells was also observed during anaerobic digestion of *Chlorella* spp. previously (Prajapati et al., 2014). Mussgnug et al. (2010) also reported presence of intact algal cells after 28 d anaerobic digestion of various algae including *Chlamydomonas reinhardtii*, *C. kessleri*, *Dunaliella salina*, *Euglena gracilis* and *S. obliquus*. The reasons for low digestibility of the algal biomass could be twofold. Firstly, it could be the resistant algal cell wall which generally contain more 70% cellulose on dry weight basis (Baldan et al., 2001). Moreover, algal cellulose may also contain sugar other than glucose (commonly xylose) and hence are possibly tough to digest by anaerobic microflora (Baldan et al., 2001). However, it is possible to enhance the algal biomass digestibility by suitable pre-treatment methods targeting cell wall disruption (Ehimen et al., 2013). Another reason for poor digestibility could be the low activity of anaerobic microflora due to imbalanced C/N ratio (≈9.26 for *Chroococcus* sp. biomass) as the reported optimal C/N ratio for anaerobic digestion is 20 (Zhong et al., 2012). There have been some successful attempts on improving the algal biomass digestibility as well as the C/N ratio by co-digesting with carbon rich waste (Zhao and Ruan, 2013). Hence, by following the proper pre-treatment and/or codigestion strategies, the methane production form *Chroococcus* sp. biomass can be enhanced significantly.

3.5. Liquid Digestate characteristics and utilization for algal growth

3.5.1. Nutrient and pollutant level of Liquid Digestate

After completion of digestion experiments, obtained Liquid Digestate was evaluated for its nutrient value. It was rich in nutrients (N & P) needed for algae cultivation. TAN, NO_3–N and TDP concentration in the Liquid Digestate was 196.63 ± 2.366, 46.34 ± 1.48 and 45.2 ± 2.16 mg L^{-1}, respectively (Table 1). Apart from the nutrients, sCOD level of the Liquid Digestate was also high (1927.5 ± 45.23 mg L^{-1}). It can be noted that the nutrient as well as the sCOD level was significantly higher than the discharge limits for in land surface water given in Table 1. Hence, it is necessary to treat the Liquid Digestate before its discharge into the environment. *Chroococcus* sp. has already been proved as efficient candidate for nutrient sequestration from rural sector wastewater (Prajapati et al., 2013a). Hence, it could be successfully used for nutrient recycling and treating the Liquid Digestate to disposable limits.

3.5.2. Algal growth and biomass production on Liquid Digestate

Since the Liquid Digestate was dark brown in colour, to overcome the light availability problem, different dilutions (10 – 100% Liquid Digestate concentration in tap water) were made and tested for algal growth. The comparison of algal biomass concentration on dry weight basis (12 d growth) at different Liquid Digestate concentration is shown in Fig. 3a. Biomass concentrations of 0.41 ± 0.025 and 0.57 ± 0.019 g L^{-1} were obtained at Liquid Digestate concentration of 10% and 20%, respectively. Optimal algal growth (biomass concentration ≈0.79 ± 0.064 g L^{-1}) was obtained at Liquid Digestate concentration of 30%, beyond which the growth was suppressed due to the dark colour of the digestate.

Hence, the low nutrient concentration (below 30% Liquid Digestate) and the dark colour (above 30% concentration) hindering the light availability were the limiting factors for the algal growth. However, the optimal biomass concentration obtained at 30% Liquid Digestate (0.79 ± 0.064 g L^{-1}) was significantly lower than that obtained with conventional BG11 medium (1.320 g L^{-1}) or other wastewaters (0.95 – 1.05 g L^{-1}) as reported previously (Prajapati et al., 2013a).

3.5.3. Phycoremediation of diluted Liquid Digestate by algae

The residual nutrient concentration & sCOD (after algal growth) was analysed in case of 30% Liquid Digestate in tap water (Table 1). It was observed that *Chroococcus* sp. could reduce the TAN level

Table 1
Characteristics of Liquid Digestate, diluted Liquid Digestate before and after algal growth with the discharge standards for in-land surface water. The values shown in table are average of at least three replicates. (All data is in mg L^{-1} except pH).

Parameter	Discharge limits[$]	Liquid Digestate	30% Liquid Digestate	After algal growth	Removal (%)
TAN	5.0	196.63	58.98	8.73	85.21
NO_3–N	10	46.34	13.90	3.15	77.34
TDP	5.0	45.20	13.56	1.45	89.31
sCOD	250	1927.5	578.25	173.5	69.99
pH	5.5 - 9.0	8.01	7.92	8.23	–

[$] According to General Standards for Discharge of Environmental Pollutants for inland surface water, The Environment (Protection) Rules, 1986 given by Central Pollution Control Board, India.

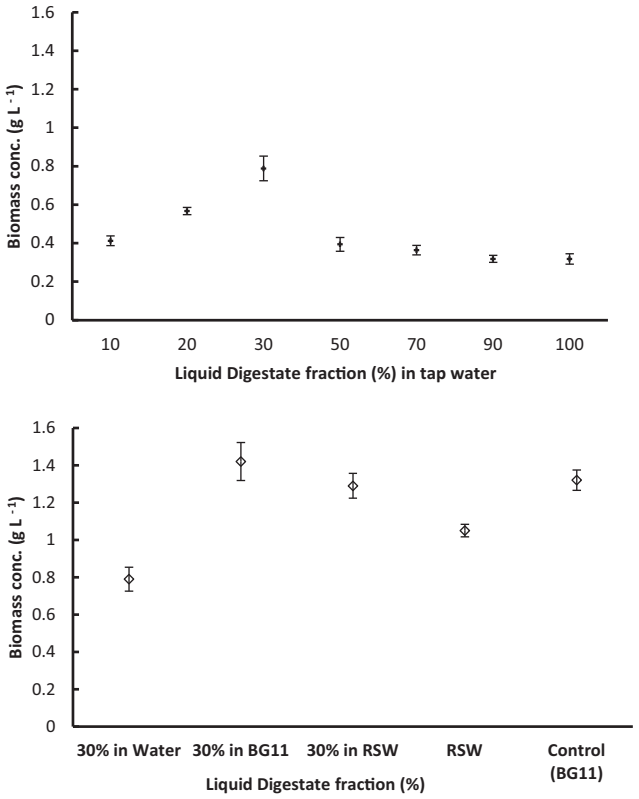

Fig. 3. Biomass production potential of *Chroococcus* sp. (a) in diluted Liquid Digestate supernatant (10 – 100% concentration) and (b) effect of dilution with BG11 and RSW (at 30% digestate concentration) on algal growth.

from 58.98 to 8.72 mg L^{-1} (≈85% removal) during 12 d of cultivation. Simultaneously, 77.34% and 69.99% reduction in NO_3–N and sCOD, respectively, were also observed. *Chroococcus* sp. was found most efficient in removing TDP as it resulted in 89.31% TDP reduction. The nutrient and sCOD levels of Liquid Digestate (even at 30% concentration) were relatively higher. Overall, cultivation of *Chroococcus* sp. eventually reduced all the parameters in the diluted Liquid Digestate below discharge limits (Table 1). Hence, it can be concluded that utilization of the Liquid Digestate (obtained from anaerobic digestion of algal biomass) for further biomass production not only provides cheaper alternative to expensive growth medium but also reduces its pollution levels.

3.5.4. Biomass production potential of Liquid Digestate diluted with BG11 and low strength wastewater

The hypothesis of nutrient limitation in tap water diluted Liquid Digestate was confirmed by diluting it with BG11 instead of tap water. Ample biomass production (1.42 ± 0.102 g L^{-1}) occurred in flasks containing Liquid Digestate diluted with BG11, which was even higher than that in control i.e. BG11 alone (Fig. 3b). These findings further supported the hypothesis that the digestate can be used as nutrient supplement for algal cultivation in low strength (colour less) wastewater such as rural sector wastewater (RSW) used in the previous study (Prajapati et al., 2013a). To validate this, experiments were conducted by utilizing diluted Liquid Digestate (30% concentration) with RSW. Interestingly, biomass production level (1.29 ± 0.067 g L^{-1}) closer to control (BG11 alone) were obtained with RSW diluted Liquid Digestate. Moreover, the biomass production was significantly higher than that obtained with RSW only (1.05 g L^{-1}) in the previous study (Prajapati et al., 2013a). Hence, it can be concluded that the algal Liquid Digestate have good potential to be utilized as nutrient supplement for biomass production in low strength wastewater.

Table 2
Basic calculations for "closed loop process" (Basis: 1.0 m^3 CH_4 d^{-1}).

Parameter	Value	Remarks
Biomethane yield from algal biomass (m^3 CH_4 g^{-1} VS fed)	0.317	
Daily algal biomass needed (kg VS d^{-1})	3.15	Calculated from biomethane yield from algal biomass
Biomass production potential (g VS L^{-1}) on digestate diluted with RSW	1.21	90.00% VS content of algal biomass
Total growth medium required (L d^{-1})	2603	Calculated from the biomass yield on diluted digestate
Liquid Digestate produced for 1.0 m^3 CH_4 d^{-1} (L d^{-1})	567	Considering 90% water recovery form digestate
RSW needed for dilution (L d^{-1})	1323	RSW required to dilute Liquid Digestate at 30% concentration
Total available medium for algae growth (L d^{-1})	1890	
Additional wastewater needed (L d^{-1})	713	

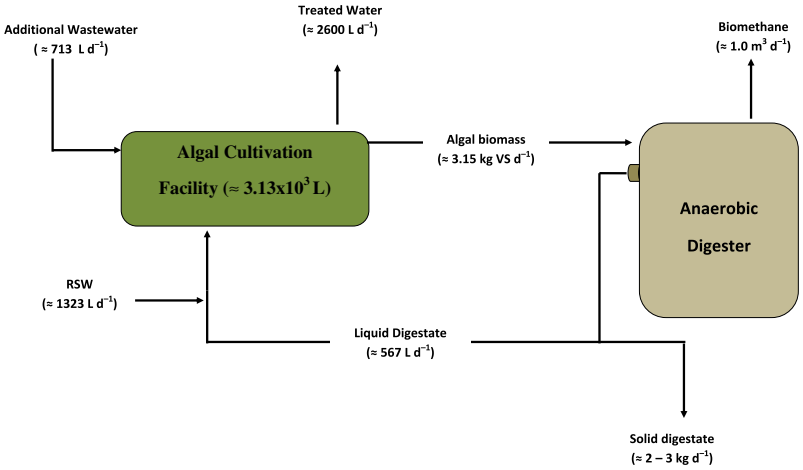

Fig. 4. Schematic flow diagram for hypothesised closed loop process (Basis: biomethane production capacity of 1.0 m^3 d^{-1}).

Based on the experimental data, the feasibility of the proposed "closed loop process" was determined. A hypothesized closed loop process with biomethane generation potential of 1.0 m^3 CH_4 d^{-1} was used as calculation basis (Table 2). The process flow diagram with basic material balance is shown in Fig. 4. The anaerobic digestion process generates ≈567 L d^{-1} Liquid Digestate which is then utilized with 1323 L d^{-1} RSW (at 30% concentration) to produce algal biomass at a rate of 2.3 kg VS d^{-1}. However, as can be estimated from the biomethane yield, ≈3.15 kg VS d^{-1} is needed to achieve biomethane production of 1.0 m^3 CH_4 d^{-1}. Therefore, additional wastewater (preferably of equal strength) is also needed at a rate of ≈713 L d^{-1} to fulfil the deficit in required algal biomass. Thus, apart from the biomass and bioenergy generation potential, the investigated process has the total capacity to treat ≈2600 L d^{-1} of wastewater including algal digestate and RSW. Hence, the current observations indicate that the closed loop process is feasible for bioenergy generation coupled with simultaneous utilization and treatment of resultant Liquid Digestate along with low strength wastewaters.

4. Conclusion

Biomethane yield of 317.31 ± 1.9 mL CH_4 g^{-1} VS_{fed} was obtained with *Chroococcus* sp. The real-time analysis indicated process stability with neutral pH. The results showed good biomass potential (0.8 g dry biomass L^{-1}) at 30% Liquid Digestate concentration. Higher biomass potential (1.29–1.42 g dry biomass L^{-1}) was obtained by diluting it with BG11/RSW. The added advantage of utilizing Liquid Digestate for algal growth is its ability to reduce nutrient/pollutant below discharge limits. The study validated the hypothesized closed loop process. However, further scale up/optimization is needed to develop a realistic economically viable process.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2014.02.023.

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