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# Algae mediated treatment and bioenergy generation process for handling liquid and solid waste from dairy cattle farm

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## HIGHLIGHTS

- An integrated approach for dairy farm waste management and bioenergy generation.
- Good biomass production potential of algae on neat livestock wastewater.
- Pioneering work on algae codigestion with cattle dung.
- Improved digestibility of algal biomass under codigestion.
- Enhanced $CH_4$ production up to 291.83 mL $g^{-1}$ $VS_{fed}$ under codigestion.

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## ABSTRACT

In the present work four algae were tested for their biomass production potential in neat livestock wastewater. *Chroococcus* sp.1 was found to be the best for biomass production under controlled (2.13 g $L^{-1}$) and outdoor conditions (4.44 g $L^{-1}$) with >80% of nutrients removal. The produced biomass was then digested with cattle dung as cosubstrate. Interestingly, up to 291.83 ± 3.904 mL $CH_4$ $g^{-1}$ $VS_{fed}$ was produced during codigestion studies (C/N ≈ 13.0/1). In contrast to this, only 202.49 ± 11.19 and 141.70 ± 2.57 mL $CH_4$ $g^{-1}$ $VS_{fed}$ was recorded with algae (C/N ≈ 9.26/1) and cattle dung (C/N ≈ 31.56/1) alone, respectively. The estimated renewable power generation potential of the investigated coupled process was around 333.79–576.57 kWh $d^{-1}$ for a dairy farm with 100 adult cattle. However, further scale-up and testing is needed to make this process a reality.



## 1. Introduction

Algal biomass is being considered as an attractive feedstock for biogas production. Recently, several studies targeting biogas production utilizing algal biomass have been reported. For instance, biogas production potential of algae including *Chlorella* spp. and *Chroococcus* spp. was explored during our previous studies (Prajapati et al., 2013a, 2014). Similarly, Zamalloa et al. (2012) have conducted digestion studies of *Scenedesmus obliquus* and *Phaeodactylum tricornutum* under mesophilic and thermophilic temperature conditions. However, high cost of nutrients required for algae cultivation is the major hurdle in commercialization of algal based biofuels including biomethane. This limitation could be overcome by utilizing wastewaters as growth medium. Various reports of algal biomass cultivation on wastewater has been summarized in a recent review (Prajapati et al., 2013b). Recently, anaerobic digestion of wastewater grown algal biomass in laboratory-scale accumulating-volume reactor has been reported by Kinnunen et al. (2014). Dairy farming is one of the fast growing agro based industry in India. In the recent years, India has emerged as the largest milk producing country in the world (≈94.5 million ton milk production per annum) and has the highest population of livestock (Srivastava, 2013). As the dairy farming is growing rapidly, the associated effluent is also increasing at fast rate. In 2007, the total livestock population of India was 529.7 million with 304.8 million bovines and 224.9 million other animals including goat, sheep etc. (Annual-Report, 2012). At least 30 gallons (≈113.55 L) per cattle wastewater is generated from the flush cleaning, milking and cattle washing practices from dairy farm (URL, 2013). Therefore, the estimated total wastewater generated from the livestock (bovines only) could be around 34.61 million $m^3$ $y^{-1}$. The dairy farm effluent termed as livestock

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wastewater (LSW) is characterized by high nutrient and organic loads (Cumby et al., 1999).

One of the possible utilization of LSW could be in energy (biomethane) generation through anaerobic digestion (Ming et al., 2007). However, during anaerobic digestion of livestock waste and wastewaters, high amount of ammonia is released. The released ammonia gets accumulated in the digester resulting in the inhibition of anaerobic microflora and subsequent failure of the whole process (Angelidaki and Ahring, 1993). Alternatively, LSW could be a potential nutrient source for food and fodder crops, but it can cause serious pollution problem of the surface and ground water if applied in excess to the crop's requirement (Ming et al., 2007). Hence, to reduce the loading of high nutrients and organics to natural water bodies, proper treatment of LSW should be done before its discharge to the environment.

Algal cultivation in LSW could provide an attractive approach for handling wastewater from dairy farming practices (Mulbry et al., 2008; Prajapati et al., 2013b). The advantage of phycoremediation is the production of algal biomass as byproduct which can be further used for bioenergy generation (Prajapati et al., 2013b). There have been some reports on treatment of anaerobically digested LSW and dairy manure using algae (Mulbry et al., 2008). However, reports on utilization of neat LSW are limited as majority of the existing studies have used diluted LSW for algal biomass production. One such study was cultivation of lipid rich algae on 10–25% LSW diluted with tap water (Woertz, 2007). Moreover, Mulbry et al. (2008) have demonstrated pilot-scale algal turf scrubbers for algal biomass production using raw or anaerobically digested dairy manure effluents. To the best of our knowledge, no previous work has been done for algal biomass production in suspended culture utilizing neat and undiluted LSW as growth medium.

Another major hurdle in the algal biomethane process is the low activity of anaerobic microflora due to imbalanced C/N ratio of the algae. The popular strategy to overcome low C/N ratio limitation is the codigestion with carbon rich substrate (Zhao and Ruan, 2013). For example, Zhong et al. (2012) observed significant enhancement in the biogas production by co-digesting Taihu blue algae with corn straw at C/N ratio of 20/1. Similarly, Zhao and Ruan (2013) optimized C/N ratio to be 15/1 for co-digestion of Taihu algae and kitchen wastes. Hence, it is obvious that anaerobic digestion of algal biomass can be enhanced by its codigestion with carbon rich substrate. Besides LSW, approximately 980 million tons solid waste (cattle dung) per annum is also generated from the Indian dairy farming (Vijay, 2007). The C/N ratio for fresh cattle dung is around 30/1 (Desai et al., 2013). Hence, it can be utilized as a cosubstrate to improve C/N ratio of algal biomass for anaerobic digestion. Previous studies also support utilization of cattle dung as cosubstrate in anaerobic digestion. For instance, Ali et al. (2010) have utilized cattle dung for anaerobic codigestion of *Jatropha curcas* defatted waste.

From the literature it can be concluded that algae can be cultivated utilizing LSW, resulting in production of algal biomass along with nutrient removal. Additionally, if the resultant biomass could be utilized with cattle dung as a substrate for biomethane production, it would further contribute to a technically and economically viable coupled process. Moreover, utilization of treated water for irrigation in agricultural applications would further strengthen the feasibility of the investigated process on sustainable basis. If successful, the proposed process can solve two major issues at one stroke (i) simultaneous wastewater treatment and solid waste management and (ii) to meet energy demand from biomethanation of algal biomass with solid manure, in dairy farm industries and rural sector.

In the view of above discussion, it is clear that coupling of phycoremediation of dairy farm effluent followed by codigestion of resulting biomass with cattle dung holds great potential for dairy farm waste management with simultaneous bioenergy generation. However, to the best of our knowledge, no previous attempts have been made on such a coupled process. Hence, as the pioneering attempts in this direction, the present study was focused on the comparative evaluation of algae for their biomass production potential in undiluted and unsterile LSW under control as well as outdoor conditions. The best performing algal strain was then tested for its biomethane potential under codigestion with cattle dung. Theoretical model calculations were also done for estimation of renewable power generation and wastewater treatment potential of coupled process for a hypothesized 100 cattle dairy farm. Finally, the possible applied aspects of the investigated process have also been discussed under Indian scenario.

## 2. Methods

### 2.1. Algal cultures

Two procured algal strains namely *Chlorella vulgaris* and *Chlorella pyrenoidosa* and two native isolates, *Chroococcus* sp.1 and *Chroococcus* sp.2, available in our laboratory were used to test their potential for growth in high strength LSW. The algal cultures were maintained in sterile nutrient medium (BG11) under controlled conditions (light intensity: 4.5–5.0 Klux, dark: light of 12:12 h and temperature: 25 ± 1 °C).

### 2.2. Wastewater collection and processing

Dairy cattle based LSW was collected from the dairy cattle shed located in New Delhi (India). The LSW collected during alternate weeks ($n \geqslant 5$) were mixed together in order to get representative samples. Since there was periodical removal of cattle dung, the collected LSW was mainly dominated by flushing containing some residues of animal urine, cattle feeds and dung. The collected LSW was filtered through muslin cloth (pore size ≈ 0.5–1.5 mm) in order to remove the large particles and debris and stored in cold storage (<4.0 °C) until its use in the experiments. The filtered LSW was analyzed for determination of total suspended solids (TSS), total dissolved solids (TDS), total dissolved phosphorous (TDP), nitrate-nitrogen ($NO^{-3}$–N), total ammoniacal nitrogen (TAN) and soluble chemical oxygen demand (sCOD).

### 2.3. Biomass production potential of LSW

#### 2.3.1. Algae screening under controlled conditions

Indoor biomass potential assay (Chinnasamy et al., 2010b) was carried out using 250 mL conical flask with 50 mL working volume. Neat LSW (unsterile) was used as a growth medium. Freshly growing algal culture (OD at 680 nm ≈ 2.0) was used as an inoculum with inoculum size of 10% (v/v). One set of control flasks (without algae inoculation and covered with foil to avoid phototrophic growth of native algae) was also prepared. The flasks were incubated under controlled conditions (Section 2.1). After 12 d, content of the flask was withdrawn for estimation of algal growth. In order to estimate the nutrient and pollutant removal, residual concentrations of sCOD, TAN, TDP and $NO^{-3}$–N were determined.

#### 2.3.2. Biomass production potential under outdoor conditions

Biomass potential of selected algae under outdoor conditions was performed (during the month of October) under natural light and temperature conditions. One liter conical flask was used as an outdoor lab scale photobioreactor. Working volume was kept at 500 mL with 10% (v/v) inoculum. The outdoor experiment was conducted in triplicate. As the biomass obtained in control flask (during screening studies) was negligible, set of control flasks

was not used in the outdoor experiments. After inoculation, flasks were incubated under natural day: night cycle. During experiments, temperature was in the range of 18–34 °C and light intensities were <1.0 Klux (sunrise and sunset) and >80.00 Klux (mid-day time). Air was bubbled (≈0.5 LPM) in the flasks through aquarium pumps to provide proper mixing. After every 48 h, a homogenized aliquot (15 mL) was withdrawn from each flask for determination of algal growth and nutrient removal.

### 2.4. Anaerobic digestion of algal biomass with cattle dung

Freshly collected dung and the algal biomass were processed for determination of elemental composition and volatile solids (VS) content. Elemental composition (Carbon, nitrogen and hydrogen) was determined using CHN analyzer (vario EL III, Elementar Analysensysteme GmbH) and VS was estimated through EPA Method1684 (Agency, 2001).

The biochemical methane potential (BMP) test (Angelidaki et al., 2009) was carried out for the determination of methane yield for selected algal biomass; cattle dung and their mixture (1:1 on VS basis). BMP experiments were conducted in 500 mL BOD bottles (300 mL working volume), hermetically sealed with stoppers and equipped with controlled gas opening valves. The substrate concentration in the bottle was kept at 5 g VS $L^{-1}$ and freshly collected inoculum (from cow dung based lab scale biogas plant) was added at substrate to inoculum (S/I) ratio of 3.0. Bottles containing inoculum only were used as control. All the bottles were then incubated at 36 ± 1 °C for 30 days under stationary conditions.

### 2.5. Analytical methods

#### 2.5.1. Algal growth determination

Algal growth was determined in terms of biomass concentration and culture optical density measured at 680 nm ($OD_{680}$). For biomass estimation, sample containing grown algae (10 mL) was centrifuged at 8000 rpm (6869 g) for 10 min. The supernatant was collected for analyses of residual nutrients and sCOD. The obtained pellets were washed twice with distilled water to remove excess salts/solids, resuspended in distilled water and then filtered through pre-weighed dry 4.7 cm Whatman GF/C glass fiber filter. The filter paper containing algal biomass was dried overnight at 60 °C and weighed till constant weight. The actual biomass was determined by subtracting the initial solid content of the LSW from the estimated dry weight. Optical density of algal cultures was measured at 680 nm using UV/vis spectrophotometer (Lamda 35, PerkinElmer). Besides, an empirical relation (Eq. (1)) between biomass concentration and $OD_{680}$ was developed for the selected alga by growing it in nutrient medium (BG11) under control conditions.

$$\text{Biomass concentration } (\text{g L}^{-1}) = 0.399 \times \text{OD}_{680} - 0.1608 \; (r^2 = 0.9483) \quad (1)$$

Further, estimation of biomass was also done from $OD_{680}$ data, using the above empirical equation in order to roughly determine contribution of native microbes (other than algae) in the measured biomass concentration.

#### 2.5.2. Wastewater analysis and pollutant removal efficiencies

Wastewater samples (prior and after treatment collected from biomass estimation step) were analyzed either using Hach colorimeter DR890 or according to standard methods (Eaton et al., 2005). During the screening studies the pollutant removal was done in triplicate. However, for pollutant removal profiling during the outdoor experiments, the supernatant collected (from biomass estimation) from samples taken from different flasks were mixed together as the samples were not sufficient for all the pollutant analysis. For sCOD determination, centrifuged samples (2 mL) were digested in the Hach Digital Reactor DRB200 and COD was estimated using HACH method 8000. TSS and TDS were estimated according to standard methods of wastewater analysis (Eaton et al., 2005). The other parameters were analyzed using following methods: $NO_3$–N using Hach method 8039, TDP through Hach method 8114 and TAN using Hach method 1003. The pollutant removal efficiency was then calculated using following equations

$$\text{Removal } (\%) = \left(1 - \frac{X_t}{X_o}\right) \times 100 \quad (2)$$

where, $X_0$ and $X_t$ are concentrations of pollutants (mg $L^{-1}$) in wastewater samples before and after the algal treatment. The pH was measured using bench-top pH meter (Cyberscan PC510, Eutech).

#### 2.5.3. Biogas volume and composition analysis

Biogas volume was measured through acidic water (pH = 2.0) displacement (Angelidaki et al., 2009) after every 24 h. Composition of the biogas was determined using Gas Chromatograph equipped with stainless steel column packed with Porapak-Q 80/100 mesh (Supelco) and thermal conductivity detector (TCD) as reported previously (Prajapati et al., 2014). The actual $CH_4$ produced (mL) was then estimated by subtracting the $CH_4$ produced (mL) in control.

#### 2.5.4. Estimation of hydrolysis constant

The substrate hydrolysis constant was estimated assuming first order kinetics anaerobic digestion (Angelidaki et al., 2009). Thus, the relation between cumulative methane yield ($B_t$) at given a time ($t$) and ultimate methane yield ($B_0$) of the substrate can be expressed as

$$B_t = B_0 \times (1 - e^{-k_h t}) \quad (3)$$

where, $t$: the digestion time and $k_h$: the first order hydrolysis constant for substrate ($d^{-1}$). The model Eq. (3) was fitted with the experimental data reported in terms of cumulative biomethane yield. Curve fitting application of MATLAB 7.0 was used to fit the experimental data in the given mode for estimation of $k_h$ and $B_0$.

### 2.6. Statistical analysis

The standard deviation and means were analyzed for significance using biostatistics software SPSS 17.0 through one way ANOVA. Duncan multiple range test was used to compare the significance of differences among tested algae at $P$ values of <0.05. Results are reported as either mean, mean ± SD or error bars in graphs.

## 3. Result and discussion

### 3.1. Characterization of LSW

The collected LSW was light brown in color. The physiochemical properties of LSW listed in Table 1 shows that it was rich in sCOD (2965.00 ± 20.49 mg $L^{-1}$), TDS (4480.00 ± 29 mg $L^{-1}$), TDP (201.67 ± 6.83 mg $L^{-1}$) and TAN (160.67 ± 2.73 mg $L^{-1}$). Results were in agreement with the literature reports on high COD and nutrients levels in LSW. For instance, Cumby et al. (1999) reported average COD (probably total COD) and TAN values (for LSW collected from 20 different dairy farms) in the range of 6550–17,300 and 310–580 mg $L^{-1}$, respectively. Similarly, Woertz (2007) has reported TSS, TAN and phosphate in the range of 1132–1350, 122–163 and 10.2–18.0 mg $L^{-1}$, respectively (values calculated by multiplying with dilution factors). Since, the sCOD

**Table 1**
Physiochemical properties of LSW, permissible discharge standards and residual concentrations of nutrients/pollutants after algae cultivation (values reported are mean ± SD for $n \geqslant 3$).

| Parameter[@] | Initial concentration | Discharge and reuse standards | | Residual concentrations after algae cultivation | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Reuse for irrigation | Inland[#] surface water | *C. vulgaris* | *C. pyrenoidosa* | *Chroococcus* sp.1 | *Chroococcus* sp.2 | Control (un-inoculated) |
| COD | 2965.00 ± 20.49 | 500.00[&] | 250.00 | 923.00 ± 8.73 | 645.67 ± 4.23 | 693.33 ± 3.61 | 669.67 ± 2.25 | 2550.00 ± 18.50 |
| $NO_3$–N | 74.67 ± 1.37 | 45.00[&] | 10.00 | 14.00 ± 0.57 | 15.79 ± 0.87 | 12.17 ± 0.31 | 13.23 ± 0.35 | 69.00 ± 4.15 |
| TAN | 160.67 ± 2.73 | 15.00[a] | 50.00 | 10.80 ± 1.04 | 11.40 ± 0.68 | 3.14 ± 0.44 | 42.23 ± 0.91 | 134.000 ± 4.92 |
| TDP | 201.67 ± 6.83 | 30.00[&] | 5.00 | 95.54 ± 2.57 | 73.33 ± 2.46 | 31.16 ± 0.68 | 58.25 ± 2.59 | 197.24 ± 5.18 |
| TSS | 120.00 ± 5.26 | 200.00[#] | 100.00 | – | – | – | – | – |
| TDS | 4480.00 ± 29 | 2100.00[$] | 2100.00[$] | – | – | – | – | – |
| pH | 7.80 ± 0.56 | 5.5–9.0[#] | 5.5–9.0 | 9.20 ± 0.10 | 8.20 ± 0.34 | 9.00 ± 0.12 | 8.60 ± 0.52 | 8.10 ± 0.52 |

[@] All parameters are in mg $L^{-1}$ except pH.
[&] According to Jordanian Standard (JS: 893/2002) for effluent reuse for agricultural irrigation 1 and 2, adopted from "A compendium of standards for wastewater reuse in the Eastern Mediterranean Region" World Health Organization (WHO-EM/CEH/142/E).
[a] According to treated wastewater criteria for reuse in Kuwait, adopted from "A compendium of standards for wastewater reuse in the Eastern Mediterranean Region" World Health Organization (WHO-EM/CEH/142/E).
[#] According to General Standards for Discharge of Environmental Pollutants Part A: Effluents, The Environment (Protection) Rules, 1986 given by Central Pollution Control Board, India.
[$] Source: Schedule-I: Standards for Emission or Discharge of Environmental Pollutants from various Industries (Common Effluent Treatment Plants), The Environment (Protection) Rules, 1986 given by Central Pollution Control Board, India.

and nutrient levels in the LSW are significantly higher than the recommended limits for wastewater discharge or reuse in irrigation (Table 1), proper treatment needs to be done before its discharge to the environment.

## 3.2. Algal biomass potential in LSW and nutrient removal

### 3.2.1. Algal screening under controlled conditions

*3.2.1.1. Growth potential of tested algae in LSW.* Growth potential of tested algae (biomass concentration and $OD_{680}$) in LSW is shown in Fig. 1. Under controlled conditions, biomass potential (g $L^{-1}$) of *Chroococcus* sp.1 (2.13 ± 0.12) and *C. vulgaris* (2.15 ± 0.059) were close to each other. However, performance of *C. pyrenoidosa* and *Chroococcus* sp.2 was relatively poor. Similar pattern of algal growth was also observed with $OD_{680}$ data. Moreover, the $OD_{680}$ in case of *C. vulgaris* (≈6.86) was relatively higher than that for *Chroococcus* sp.1 (≈6.11). It is worth mentioning that the biomass concentration in control flask was negligible (<0.01 g $L^{-1}$). The biomass concentration of *Chroococcus* spp. obtained in LSW was significantly higher than the values obtained during our previous study conducted using BG11 and rural sector wastewater as growth medium (Prajapati et al., 2013a). Similarly, biomass concentration of tested *Chlorella* spp. was higher than that observed during their cultivation in tap water medium (Prajapati et al., 2014). Overall results reflected that under controlled conditions only *C. vulgaris* and *Chroococcus* sp.1) have good biomass potential utilizing LSW as growth medium.

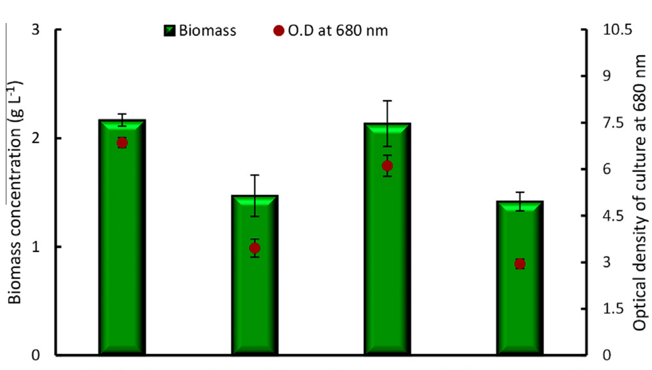


**Fig. 1.** Biomass potential ($OD_{680}$ and biomass concentration) of tested algae grown in LSW under controlled conditions.

*3.2.1.2. Nutrient and COD removal from LSW.* From nutrient removal data (Table 1), it was observed that all the tested algae were equally good in terms of sCOD removal with highest removal of 78% shown by *C. pyrenoidosa* in 12 d. The $NO_3$–N reduction ranged from 78% to 83% for all the tested algae. Further, *Chroococcus* sp.1 was relatively efficient in TAN removal (up to 98%), while *Chroococcus* sp.2 performed poorly (<74%). Moreover, only *Chroococcus* sp.1was found efficient in TDP removal (with >84% TDP reduction) followed by *Chroococcus* sp.2 (71% TDP removal in 12 d). Poorest TDP removal (≈56%) was observed with *C. vulgaris*. Although nutrient reduction (%) was considerably good during algal growth, the residual concentrations of sCOD and TDP were significantly higher than the standards prescribed for discharge (inland surface water) or reuse for irrigation (Table 1). On the other hand, residual TAN and $NO_3$–N (for all tested algae except *Chroococcus* sp.2) were reduced below the standards given for wastewater reuse in irrigation. Moreover, the final pH of the treated water was also in line with discharge standards (Table 1).

Previous investigations have indicated similar pollutant removal efficiencies for various algal strains cultivated in range of wastewaters including industrial effluents, domestic wastewater and LSW, listed in recent review (Prajapati et al., 2013b). For example, de Godos et al. (2010) observed 56% COD and 98% $NH_4^+$ removal from pretreated piggery wastewater. Similarly, 77.8%, 89.1% and 70.3% removal of sCOD, TAN and total phosphorous, respectively, was observed with *C. pyrenoidosa* cultivated on high strength wastewater from soybean processing plant (Hongyang et al., 2011). Moreover, relatively higher removal efficiencies (up to 98% TDP, 98% $NO_3$–N and 100% $NH_3$–N) of *Chroococcus* spp. were observed under similar controlled conditions from rural sector gray water (Prajapati et al., 2013a). From the present results and literature reports, it is obvious that algae are potential remediation agents to reduce the pollution levels below discharge limits in case of low strength wastewater. However, in case of high strength wastewater including LSW, further treatment is needed in order to meet the discharge standards.

From the screening studies it is clear that biomass production potential of *Chroococcus* sp.1 and *C. vulgaris* was at par but the nutrient and sCOD removal efficiency of *Chroococcus* sp.1 was

significantly higher. Hence, *Chroococcus* sp.1 was selected for further studies under outdoor conditions to access the performance in more realistic situations.

*3.2.2. Algal growth and pollutant removal in outdoor photobioreactor*

*3.2.2.1. Biomass production profile.* The selected alga was grown under outdoor conditions with aeration. Growth profile of *Chroococcus* sp.1 (in terms of biomass and $OD_{680}$) is shown in Fig. 2a, which shows a lag phase of about 2 d. Beyond 2nd day, the algal biomass started increasing exponentially and the stationary growth phase was achieved on 12th day. The estimated biomass production rate (or volumetric productivity) during early exponential phase (2–8 d) was around 0.217 g $L^{-1}$ $d^{-1}$ and dramatically increased to 0.558 g $L^{-1}$ $d^{-1}$ during 8–12 d. The average biomass production rate was found to be around 0.353 g $L^{-1}$ $d^{-1}$ during the exponential phase. The growth profile in terms of $OD_{680}$ also had similar pattern (Fig. 2). The final biomass concentration at the end of the exponential phase (12 d) and at the end of experiment (16 d) was 3.83 and 4.34 g $L^{-1}$, respectively. The corresponding algal culture densities ($OD_{680}$) were 8.89 and 9.53, respectively.

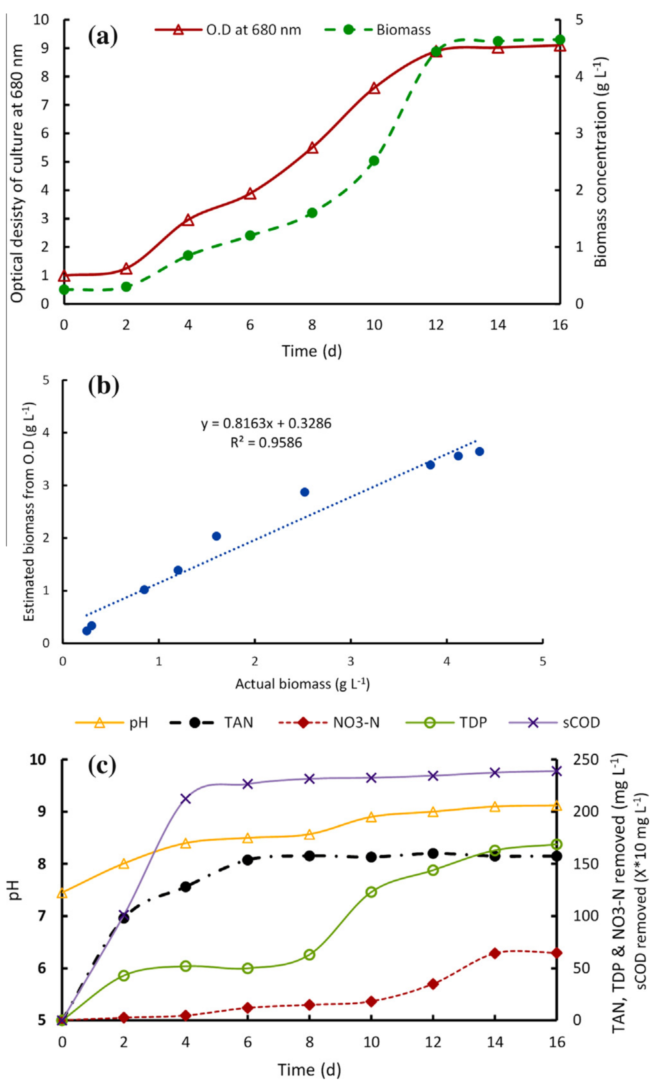


**Fig. 2.** Growth and nutrient removal profiles of *Chroococcus* sp.1 under outdoor conditions. (a) Variation of biomass concentration and $OD_{680}$, (b) correlation of measured biomass (dry weight basis) with the optical density based estimated biomass and (c) pollutant removal profile and pH variation with elapsed time of algal growth.

The biomass production rate obtained in the present study was almost double of the value observed during cultivation of *Chroococcus* sp.1 in rural sector gray water (Prajapati et al., 2013a). Similarly, the biomass production rate of *Chroococcus* sp.1 (in LSW) is much higher than the reported values for algal consortium grown in polybag photobioreactor using carpet industry wastewater as growth medium (Chinnasamy et al., 2010a). The observed higher biomass productivity could be due to significantly higher nutrient concentration in LSW as compared to gray water and carpet industry wastewater. Further, the growth of native microbes cannot be neglected as the growth medium (LSW) was unsterile. There exist a synergetic interaction between bacteria and algae in the unsterile wastewater (de-Bashan et al., 2004; Munoz and Guieysse, 2006). This interaction benefits both the agent of the system and results in enhanced growth of both algae as well as bacteria (Rawat et al., 2011). The provided aeration during outdoor experiments could also be oxygen source for native bacterial growth. The correlation of measured biomass (dry weight basis) with the $OD_{680}$ based estimated biomass was positive ($R^2 = 0.959$). Also, the measured biomass concentration (4.34 g $L^{-1}$) was relatively higher than the estimated biomass (3.64 g $L^{-1}$). The present observation further strengthened the hypothesis of biomass contribution by native bacteria in the biomass concentration measurement. Overall, it can be concluded that under outdoor conditions, *Chroococcus* sp.1 had significantly higher biomass production potential compared to controlled condition and reported values in literature. Considering the application potential in terms of biomethanation of resultant biomass, algal cultivation in unsterile wastewater would be advantageous as it results in higher biomass production.

*3.2.2.2. Pollutant removal profile of Chroococcus sp.1.* The pollutant removal profile and pH variation during the growth of *Chroococcus* sp.1 under outdoor photobioreactor is shown in Fig. 2b. It was interesting to notice that almost 71% of sCOD was removed with first 4 d of experiments at an average removal rate of 531 mg $L^{-1}$ $d^{-1}$. This rapid removal of sCOD could be attributed to two factors. Firstly, in the high ammonia containing wastewaters (like LSW), ammonia is the major contributor to the sCOD. Further, as depicted from Fig. 2b, the removal of TAN was very fast (up to 80% reduction in TAN at removal rate of ≈36.04 mg $L^{-1}$ $d^{-1}$) during the first 4 d. Another reason could be the rapid accumulation and adsorption of organic carbon on the surface of alga and native bacteria during the early phase of their growth. However, this needs to be explored further. Further, the rapid reduction of TAN could be attributed to its volatilization into free ammonia due to aeration (Liao et al., 1995). Shifting of pH towards alkaline due to algal growth could also be a possible reason for rapid TAN removal from LSW (Posmanik et al., 2013). The final removal of TAN and sCOD at the end of the experiments (16 d) was 98 and 80%, respectively. In contrast to sCOD and TAN removal, the removal of TDP and $NO_3$–N were significantly lower. The corresponding removal of TDP and $NO_3$–N was only 25.87% and 6.34%, respectively, for first 4 d. However, almost 86.40% and 84.05% removal of TDP and $NO_3$–N were achieved at removal rate of 10.55 and 4.05 mg $L^{-1}$ $d^{-1}$, respectively, during the 16 d of algal cultivation. As reflected from the current observations, in the *Chroococcus* sp.1 treated LSW, the residual nutrient concentration ($NO_3$–N, TAN and TDP) and pH values were suitable for reuse as irrigation water (Table 1). However, sCOD was still above the irrigation water quality standards. The removal efficiencies of selected algae under outdoor conditions were in line with value reported previously for algal consortium cultivated in diluted (10% and 25%) dairy effluent in outdoor pond bioreactor (Woertz, 2007). Although, previous reports showed phycoremediation potential of algae in diluted wastewaters, the present investigations revealed that *Chroococcus* sp.1 was equally efficient

in removal of nutrients and sCOD from untreated and undiluted LSW.

Overall, the outdoor conditions were found to have positive effect on the growth of *Chroococcus* sp.1. Under outdoor conditions, the biomass production of *Chroococcus* sp.1 was almost double of that observed under controlled conditions. The phycoremediation potential of the selected algae was also significantly affected by the cultivation conditions as noticeable increments in removal efficiencies were observed under outdoor conditions. For instance, the residual concentrations of $NO_3$–N, TAN and TDP under outdoor conditions were relatively lower (9.96 ± 0.70, 0.98 ± 0.76 and 29.00 ± 0.89 mg $L^{-1}$, respectively) than those observed under controlled conditions (12.17 ± 0.31, 3.14 ± 0.44 and 31.16 ± 0.44 mg $L^{-1}$, respectively), in *Chroococcus* sp.1 treated LSW. Hence, the nutrient load of LSW could be significantly reduced (sCOD, $NO_3$–N and TDP by more than 80% and TAN more than 98%) by the algal treatment. Although algal cultivation could not bring nutrient levels below discharge standards, the residual, TAN, TDP and $NO_3$–N were in the range recommended for wastewater reuse in irrigation (Table 1). Literature reports also support the possibility of treated wastewater utilization in irrigation (Sacks and Bernstein, 2011).

### 3.3. Biomethane production potential of selected algae

Since *Chroococcus* sp.1 had the highest biomass production potential in LSW under outdoor conditions; it was selected for further biogas production studies. The characteristics of *Chroococcus* sp.1 biomass (S1) and fresh cattle dung (S2) are summarized in Table 2. The observed C/N ratio for S1 (9.258 ± 0.061) was much lower than the reported optimal C/N ratio of 15.0 in anaerobic digestion of algal biomass (Zhao and Ruan, 2013). Hence, co-digestion with cattle dung (C/N: 31.556 ± 1.569) was performed to overcome the C/N ratio limitation. *Chroococcus* sp.1 biomass and cattle dung were mixed in the ratio of 1:1 VS basis (C/N ≈ 13.0) for co-digestion assay.

#### 3.3.1. Biomethane profiles of algae during codigestion with cattle dung

The daily biomethane production profiles of algal biomass (S1), cattle dung (S2) and their mixture (S1 + S2) are shown in Fig. 3a. Gas production started from the first day of experiment. Interestingly, two sharp peaks were observed in the daily gas production profile of S1. The first peak (on 2nd day) in S1 gas profile could be attributed to rapid digestion of the soluble cellular content of the algal slurry resulting from cell damage during harvesting stages (Prajapati et al., 2013a). From 3rd day onward the digestion of algal biomass slowed down with first minima on 4th day. The gas production rate again started increasing with second peak observed between 10th and 11th day. After 11th day, gas production continued with relatively slower rate. This variation in daily gas profile of S1 indicates the poor and improper digestion of algal biomass (Prajapati et al., 2014). On the other hand, the gas production from S2 (cattle dung) showed that significant gas was produced during first 6 d of experiment with its maxima on 4th day. From 6th day onward, the gas production from S2 was relatively

**Table 2**
Characteristics of algal biomass and fresh cow dung ($n \geqslant 3$; represented as mean ± SD).

| Parameters | Algal biomass (S1) | Cow dung (S2) |
|---|---|---|
| C (% of TS) | 58.041 ± 0.112 | 36.183 ± 0.370 |
| H (% of TS) | 7.575 ± 0.113 | 5.060 ± 0.048 |
| N (% of TS) | 6.269 ± 0.047 | 1.149 ± 0.046 |
| VS (% of TS) | 91.26 ± 0.756 | 83.040 ± 0.288 |
| C/N ratio | 9.258 ± 0.061 | 31.556 ± 1.569 |

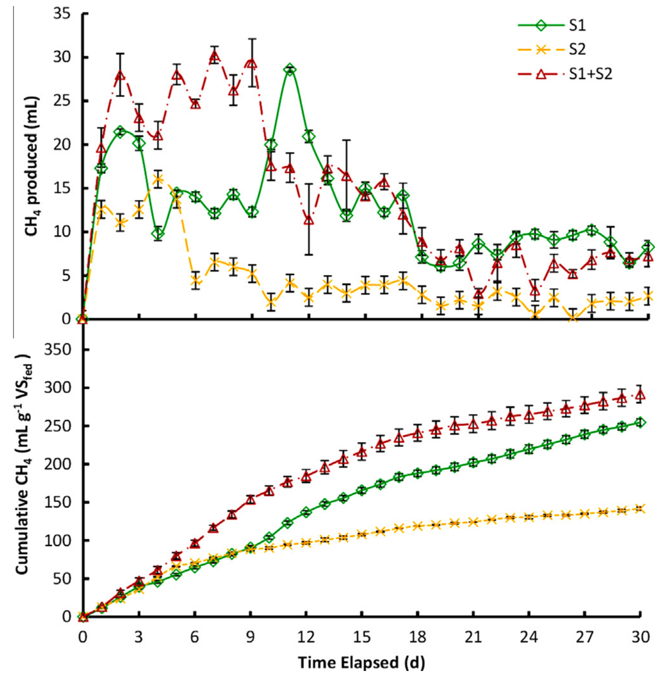


**Fig. 3.** Daily and cumulative biomethane production profiles of algal biomass (S1), cattle dung (S2) and their mixture (S1 + S2).

slower. The current findings revealed that cattle dung has faster digestion rates than that for algal biomass. Interestingly, significantly higher biomethane production was recorded with the codigestion setup i.e., from S1 + S2 (Fig. 3a). It achieved up to 30 mL daily biomethane production on 2nd day of the anaerobic digestion. The biomethane production remained around 30 mL till 9th day except some ups and downs recorded intermittently. After 9th day, the biomethane production decreased gradually but at relatively lower rate in comparison with S1 or S2 alone.

The cumulative biomethane yield from S1, S2 and S1 + S2 was 223.88 ± 4.02, 141.7 ± 2.035 and 291.83 ± 12. 51 mL $g^{-1}$ $VS_{fed}$, respectively. Fig. 3b depicted that the rate of biomethane production from codigestion was higher as compared to S1 and S2 alone. During codigestion the rate of gas production was relatively higher for first 9 d and slowed down slightly thereafter. However, the gas production rates were relatively lower for bottles containing individual substrates S1 and S2 (Fig. 3b). Hence, cattle manure had synergistic effect on algal biomass digestion and resulted in significant enhancement in biomethane production. Similar synergistic effects of balancing C/N on biomethane production from algae, by adding carbon rich substrate such as corn straw (Zhong et al., 2012) and kitchen waste (Zhao and Ruan, 2013), have also been reported in literature. Our results were in agreement with these reports.

#### 3.3.2. Enhancement in algal biomass digestibility under codigestion with cattle dung

For assessing the effect of codigestion on digestibility of algal biomass, the cumulative biomethane data was fitted in first order digestion kinetics model (Eq. (3)). The data was found to fit well ($R^2$ values between 0.9909 and 0.9944) with digestion kinetics model for all sets of experiments (Fig. 4). In line with the cumulative biomethane yield, the ultimate methane yield (estimated from Eq. (3)) was significantly higher for codigestion setup (346.20 mL $g^{-1}$ VS). Whereas, corresponding estimated values were 312.20 and 143.0 mL $g^{-1}$ VS for substrates S1 and S2, respectively. The value of first order hydrolysis rate constant ($k_h$) depicted

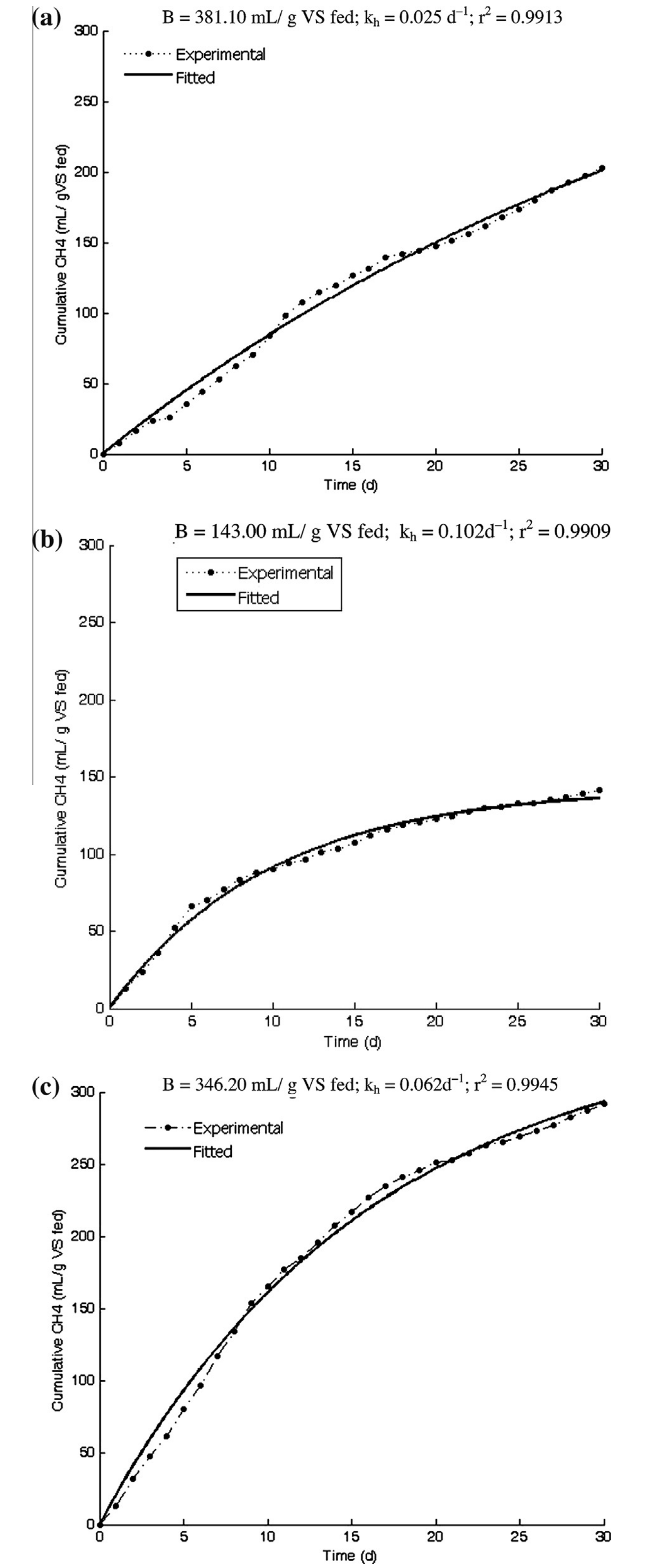


**Fig. 4.** Fitting of cumulative methane data (a) S1, (b) S2 and (c) S1 + S2) with the first order kinetics model for anaerobic digestion (Eq. 3).

significant enhancement in the algal biomass digestibility under codigestion. It is noticeable that the $k_h$ for algal biomass increased from 0.040 to 0.064 $d^{-1}$ when codigested with cattle dung. The $k_h$ for cattle dung (0.102 $d^{-1}$) was relatively higher than algal biomass and the mixture. Enhancement in the digestibility and biomethane yield during codigestion could be attributed to the relatively improved C/N ratio (≈13.0/1) as compared to algal biomass as sole substrate for anaerobic digestion (9.26/1). Furthermore, cattle manure contains ample amount of anaerobic microbes and is often used as inoculum for start-up of anaerobic digestion (Budiyono et al., 2010). Hence, the enhancement in digestibility under codigestion can also be attributed to the availability of native anaerobic microbes in cattle dung in addition to inoculum. Overall results indicate that the selected algal biomass has good biomethane potential which was further enhanced by 30.35% when codigested with cattle dung.

### 3.4. The coupled process involving codigestion has higher bioenergy generation potential

The present investigation revealed the suitability of coupling algae treatment of LSW and biomethane production. The minimum daily generation of LSW and solid manure per animal head is around 113.35 L $d^{-1}$ (URL, 2013) and 10 kg $d^{-1}$ (fresh dung with ≈85% moisture content), respectively (Vijay, 2007). Based on these facts, four scenarios of hypothesized process model were evaluated for estimation of total biomethane yield and LSW treatment potential for dairy farm having 100 adult cattle (Table 3). During the first scenario, hypothesized process was assumed without algae and hence no LSW treatment or codigestion was possible in this process. In this particular process a net biomethane yield from anaerobic digestion of cattle dung only was up to13.28 ± 0.12 $m^3$ $CH_4$ $d^{-1}$. In second process algal treatment of LSW was included but the anaerobic digestion of available dung and algal biomass was assumed to be carried out separately i.e., no codigestion. The estimated biomethane yield in this hypothesized process was 23.05 ± 0.87 $m^3$ $CH_4$ $d^{-1}$ with LSW treatment rate of approximately 10768.25 L $d^{-1}$ (Table 3). Furthermore, in the scenario assuming codigestion of resultant algal biomass (from LSW treatment) with cattle dung in 1:1 ratio on VS basis, a net biomethane yield was estimated to be 32.58 ± 0.83 $m^3$ $CH_4$ $d^{-1}$ along with treatment of approximately 10768.25 L LSW $d^{-1}$. Lastly, the fourth hypothesized process was assumed involving codigestion of available dung with equal amount of algal biomass (i.e. ≈94.13 kg VS $d^{-1}$ of each). The rest algal biomass (≈50 kg VS $d^{-1}$) could be produced using any other wastewater or nutrient medium. Under this scenario, the estimated methane yield was found to be around 54.94 ± 2.19 $m^3$ $CH_4$ $d^{-1}$.

As can be seen from the Table 3, biomethane production in scenario 2 was approximately 1.73 times of that obtained in scenario 1. Hence, it is clear that coupling of both process viz., algal treatment of LSW and anaerobic digestion is profitable. Moreover, codigestion of algal biomass with cattle dung further enhances the process feasibility with methane production up to 2.45 times to that obtained from cattle dung alone. Additionally, the coupled process also results in treatment of significant amount of generated LSW. Also, as reflected from the scenario 4, there is still a possibility of enhancing the biomethane generation through codigestion of available dung with algal biomass, if additional algal biomass is available. There are reports on enhanced biomass production of algae at elevated $CO_2$ levels (Prajapati et al., 2013b). Hence, the biomass production potential of selected algae can be enhanced by supply of $CO_2$ to the algal cultivation system in order to meet the demand of additional algal biomass in codigestion under scenario 4.

Considering the energy content of $CH_4$ (37.78 MJ $m^3$), the produced biomethane has the energy potential of 1.231 and 2.076 GJ $d^{-1}$, respectively, for scenario 3 and 4. Whereas, the uncoupled process (scenario 1 and 2, respectively) have significantly lower energy generation potential (0.502 and 0.87 GJ $d^{-1}$). The equivalent renewable power generation potential of the

**Table 3**
Comparison of three possible scenarios for live stock based solid and liquid manure management with generation of biomethane through anaerobic digestion. Calculation basis: a dairy farm of 100 adult milking cattle (results shown as mean ± SD; $n \geqslant 3$).

| Scenario | Process involved | Available dung VS (kg $d^{-1}$) | Available algal VS (kg $d^{-1}$) | $CH_4$ from cattle dung ($m^3$ $d^{-1}$) | $CH_4$ from algae ($m^3$ $d^{-1}$) | $CH_4$ from codigestion ($m^3$ $d^{-1}$) | Net $CH_4$ ($m^3$ $d^{-1}$) | Treated LSW (L $d^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| 1 | No algae | 94.13 ± 0.39 | – | 13.30 ± 0.12 | – | – | 13.30 ± 0.12 | – |
| 2 | Coupled process without codigestion | 94.13 ± 0.39 | 43.63 ± 0.43 | 11.83 ± 0.07 | 9.77 ± 0.21 | – | 23.05 ± 0.87 | 10768.25 |
| 3 | Coupled process with codigestion | 94.13 ± 0.39 | 43.63 ± 0.43 | 6.35 ± 0.09 | – | 25.46 ± 0.80 | 31.85 ± 0.82 | 10768.25 |
| 4 | Codigestion of total dung with equal amount of algae | 94.13 ± 0.39 | 94.13 ± 0.39 | – | – | 54.94 ± 2.19 | 54.94 ± 2.19 | 10768.25 + X[#] |

[#] Volume of treated wastewater from some other source used for production of algal biomass for codigestion of complete dung with algae.

processes is 139.4, 241.91, 341.97 and 576.57 kWh $d^{-1}$, respectively, for scenario 1–4. Additionally, considering the total LSW available (34.61 million $m^3$ $y^{-1}$), the Indian dairy farming industries have annual algal biomass production potential of $1.4 \times 10^5$ tons VS $y^{-1}$. The available algal VS if digested with cattle dung could generate $8.14 \times 10^7$ $m^3$ $CH_4$ $y^{-1}$ (equivalent annual power generation of $\approx 3.08 \times 10^6$ GJ $y^{-1}$).

Hence, the current observations and theoretical calculations have proven the significance of coupling algal treatment of LSW and biomass production with anaerobic codigestion. The investigated process was found efficient for simultaneous managements of dairy farm based solid and liquid wastes with generation of considerable renewable power. Hence, such integration of phycoremediation and anaerobic digestion technology with dairy farming could turn the waste management practice into an efficient bioenergy generation process.

### 3.5. Applied aspects of the investigated process under Indian scenario

The Indian dairy farming is operated in the decentralised manner either through small cooperative societies or very small dairy farms in rural habitats. Also, there is lack of adequate facility for treatment of dairy effluent and the untreated wastewater is discharged to the environments. Further, in India and other developing countries, biogas production from cattle dung is well established technology. Indian government had taken appreciable steps for the adaptation of biogas technology. The Ministry of New and Renewable Energy (MNRE) is leading agency in the promotion of biogas technology. Various subsidy schemes and polices have been launched by MNRE to promote cattle dung based family type as well as large scale biogas plants to provide renewable energy fuel for cooking and electricity in rural habitants (Vijay, 2010). The proposed process showed potential to not only enhance biogas yield but also the treatment of LSW by algae cultivation. Hence, the investigated process can further be explored for integration with such decentralised biogas plants for waste (LSW and cattle dung) management and enhanced biogas generation from codigestion of algal biomass and cattle dung.

## 4. Conclusion

The present study demonstrated algae cultivation in neat LSW followed by codigestion of produced biomass with cattle dung. Among the tested algae, *Chroococcus* sp.1 was found to be best for biomass production and nutrient recycling from LSW. Interestingly, significant enhancement in biomass production was observed in outdoor PBR. Furthermore, codigestion with cattle dung had synergistic effects on biomethane production from algae. Theoretical calculation revealed promising potential of investigated process for efficient bioenergy generation utilizing dairy farm wastes. However, further process scale-up is needed to integrate it with the dairy farming and cattle dung based biogas plants worldwide.

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