



# 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<sub>4</sub> production up to 291.83 mL g<sup>-1</sup> VS<sub>fed</sub> 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<sup>-1</sup>) and outdoor conditions (4.44 g L<sup>-1</sup>) 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<sub>4</sub> g<sup>-1</sup> VS<sub>fed</sub> 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<sub>4</sub> g<sup>-1</sup> VS<sub>fed</sub> 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<sup>-1</sup> for a dairy farm with 100 adult cattle. However, further scale-up and testing is needed to make this process a reality.

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## 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 tricoratum* 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<sup>3</sup> y<sup>-1</sup>. 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:  $25 \pm 1$  °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 ( $n \geq 5$ ) 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  $\approx 0.5$ – $1.5$  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 ( $\text{NO}_3^-$ -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  $\approx 2.0$ ) 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  $\text{NO}_3^-$ -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

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