**Improved methane yield from wastewater grown algal biomass**

Mohit Thawani, Nidhi Hans, Saurabh Samuchiwal and Sanjeev Kumar Prajapati

**ABSTRACT**

Methane production from the algal biomass cultivated in a laboratory scale continuous photobioreactor (PBR) using sewage was evaluated in the present work. During the preliminary experiments, algal biomass reached up to 1.69 ± 0.35 g L⁻¹ in 12 days' growth period. Besides, 65 to 100% removal in concentrations of total dissolved phosphorus (TDP), nitrate nitrogen (NO₃⁻N), total ammoniacal nitrogen (TAN) and soluble chemical oxygen demand (sCOD) was also recorded. The sCOD removal in the reactor was 100%, whereas removal of TDP, NO₃⁻N and TAN were up to 75, 40 and 92%, respectively. Upon anaerobic digestion, the fresh algal biomass showed methane yield of 180 mL g⁻¹ VSfed. Further, algal biomass was stored under natural conditions in open containers (aerobic conditions) in darkness at room temperature (27–30°C) for 72 h. Interestingly, >48% COD solubilization from algal biomass was observed during storage. Pretreatment through natural storage was further confirmed with qualitative observations including scanning electron and fluorescence microscopic analysis. Moreover, higher methane yield (284.38 mL g⁻¹ VSfed) was observed from the samples stored for 60 h. Thus, natural storage for a designated period may be recommended as a prerequisite stage in the process of methane production from wastewater-grown algal biomass.

**Key words** | algae, anaerobic digestion, native bacteria, pretreatment, sewage

**INTRODUCTION**

Rapid growth of energy demand against the limited availability of conventional fossil fuels has forced human beings to explore alternative renewable energy resources. In the recent past decades, biofuel has emerged as a potential alternative to fossil fuel. Among biofuel options, methane from anaerobic digestion of whole cell algal biomass is considered most feasible (Passos et al. 2014). However, the sustainability of the algae-based methane generation process is questionable due to the high cost of nutrients. This limitation is well addressed, and several reports have been published on algae cultivation in wastewaters including industrial effluents and sewage (Rawat et al. 2011; Chen et al. 2015). Besides, algae cultivation also results in nutrient and pollutant removal, leading to simultaneous wastewater treatment. Hence, through algae cultivation, it is possible to convert wastewater nutrient into useful biomass feedstock.

Management and treatment of sewage produced from human activities is one of the major challenges to avoid environmental pollution. In India, the estimated per capita sewage generation is around 98 L for rural/small town population and 220 L for populations in urban areas including Delhi (Kaur et al. 2012). As per the Central Pollution and Control Board (CPCB) report 2016, the estimated sewage generated in India in the year 2015 was more than 61,000 MLD, against the total sewage treatment capacity of around 22,000 MLD. Hence, around 39,000 MLD of untreated sewage is directly discharged to the environment, which is the major cause of environmental deterioration such as eutrophication in fresh water bodies. Interestingly, the nutrient-rich sewage may be utilized as a low cost media for algal cultivation. Several reports are available on utilization of sewage/municipal wastewater for algae cultivation coupled with wastewater treatment (Jiang et al. 2011; Cheah et al. 2016).

Poor digestibility of the algal cell wall is the major hurdle towards methane generation by anaerobic digestion (Passos et al. 2014). Recently, several reports have been published on improving the biodegradability of both micro- and
macro-algal biomass under anaerobic conditions through multifarious approaches including physico-chemical as well as biological pretreatment methods (Mendez et al. 2014; Passos et al. 2014). Moreover, attempts are being made worldwide for development of low cost biological algal biomass pretreatment.

Algae grow in close association with native microorganisms of wastewater, with inter-species transfer of CO2 and O2 (Ramanan et al. 2016). Moreover, most of the wastewater-grown microorganisms, especially bacteria, are hydrolytic (Gerardi 2006; Krah et al. 2016). Hence, it is possible that natural storage of wastewater-grown algae could lead to higher degree of algal cell disintegration due to the presence of hydrolytic bacteria. However, literature reports in this direction are scarce. To the best of our knowledge, a recent paper by Miao et al. (2015) reporting 36% improvement in methane yield from Taihu blue algae after 15 d of natural storage, is the only report on this particular research topic. On the other hand, similar biological pretreatment strategies involving activity of microorganisms or their endogenous enzymes during storage (aerobic/micro-aerobic) at temperatures ranging from 50 to 70 °C have been successfully applied for pretreatment of different wastes and biomass such as sewage sludge, food waste and animal waste (Carrere et al. 2016). However, for specific case of algae, research is mainly focused on the use of enzymes for biological pretreatment (Passos et al. 2014).

Hence, in light of the above discussion, the present study aimed towards the continuous cultivation of algae in sewage for wastewater treatment coupled with algal biomass production. Further, attempts were made to study the effect of natural storage on algal biomass digestibility and methane yield during anaerobic digestion.

MATERIALS AND METHODS

Sewage collection and characterization

Sewage was collected from the continuously running open drainage line near Dwarka Sector 2, New Delhi, India, in the month of March (Supplementary Figure S1, available with the online version of this paper). The drainage line receives sewage from the local community and includes wastewater generated through domestic activities, local markets and some of the small-scale industries such as bakery, milk and dairy processing. The collected sewage was filtered through muslin cloth (pore size 0.5–0.8 mm) in order to separate insoluble particles and solid debris. The filtered sewage was then stored at 4 °C and was used for experiments whenever required. Homogenised samples of filtered sewage (in triplicate) were processed for characterisation through determination of soluble chemical oxygen demand (sCOD), total dissolved phosphorus (TDP), total ammoniacal nitrogen: NH3-N (TAN), nitrate nitrogen (NO3–N), total suspended solids (TSS), total dissolved solids (TDS) and pH.

Algal culture

The native algal culture for the present work was obtained from the sewage itself. The filtered sewage (50 mL) was mixed with equal amounts of nutrient media (BG11) in a conical flask and incubated at 25 °C under continuous illumination (≈6,000 lux) using cool fluorescent lights for 10 d. The culture flasks were manually shaken twice a day to provide mixing. During this incubation, the native algae started growing and culture optical density (measured at 680 nm) reached a stable value of around 1.8. An aliquot (5 mL) was withdrawn from the culture flask for morphological characterisation of algae through a phase contrast microscope (PCM) and scanning electron microscope (SEM). The PCM and SEM analyses were carried out using the methodology reported earlier (Prajapati et al. 2015).

Further, there were some bacterial strains associated with the native algal culture. These bacterial strains were isolated from algal cells through streaking on Luria agar plates. The isolated bacterial samples were then subjected to preliminary morphological and biochemical tests including Gram staining, etc., for their identification.

Batch scale studies

For batch studies, 90 mL filtered sewage was inoculated with 10 mL of inoculum culture in a conical flask (in triplicate) and incubated under controlled conditions (see previous section) for 12 d. The flasks containing sewage (100 mL) without inoculum were covered with aluminium foil to avoid algal growth by blocking light and incubated under similar controlled conditions (see previous section), used as a negative control. An aliquot (5 mL) of the sample was withdrawn after every 3 d (from both experimental and control flasks) for determination of algal growth, pH and residual nutrient concentrations.

Photobioreactor (PBR) studies

A laboratory scale PBR was fabricated using a 6.00 mm thick transparent acrylic cylinder (internal diameter:
14 cm, height: 30 cm) with a working volume of 3.0 L (Supplementary Figure S2, available with the online version of this paper). The PBR was filled with the filtered sewage, inoculated with a native algae culture (10% v/v) and kept under continuous illumination (~6,000 lux) using cool fluorescent lights placed on four sides. Air was also bubbled into PBR at 2.0 L min\(^{-1}\) using an aquarium pump to provide mixing and to prevent settling of the algae. The PBR was operated in batch mode during the first 12 d and then operated in continuous mode with 12 d hydraulic retention time (HRT) for the next 48 d (four HRT cycles of continuous operation). An aliquot (5.0 mL) from the PBR was withdrawn (after every 24 h for the first 30 d and after 5 days’ interval for the next 30 days) for determination of algal growth, pH, residual nutrient concentration and sCOD.

**Pretreatment through natural storage**

After completion of PBR studies, algal biomass was harvested, in the form of slurry having >80% (w/w) water, through pH assisted auto settling. The algal slurry was then stored in multiple 100 mL open containers (~50 g) in the dark at room temperature (27–30 °C) for 72 h. The algal slurry was manually mixed 2–3 times daily (using a sterile spatula) in order to provide proper aerobic conditions. For qualitative assessment of pretreatment during storage, SEM and fluorescence microscopic analysis of algal samples was done. For fluorescence microscopy, algal samples were stained with Sytox Green® and processed according to the methodology reported earlier (Prajapati et al. 2015).

For quantitative analysis, COD solubilisation from algal biomass was studied. Homogenized samples were withdrawn after every 6 h for the first 24 h and after every 12 h for the rest of the storage period. The algal samples were centrifuged at 6,000 g for 10 min and the supernatant was collected for sCOD estimation. The COD solubilization was calculated using the equation adopted from Alzate et al. (2012) as given below:

\[
\text{COD solubilization (\%) = } \left( \frac{s\text{COD}_t - s\text{COD}_0}{\text{COD}_T - s\text{COD}_0} \right) \times 100
\]  

(1)

where 0 and t denote sCOD of algal slurry initially and sCOD after storage of time t, whereas T denotes the total COD of algal slurry before the storage.

To assess the contribution of cell natural lysis (through natural death and degradation under unfavourable conditions), in pretreatment during storage, a flask containing BF algal slurry (grown aseptically in BG11) was also stored under similar conditions (as mentioned above) and COD solubilization from algal biomass was determined using Equation (1). The bacteria free (BF) algal culture was obtained following the methodology reported earlier by Ferris & Hirsch (1991).

**Biomass composition and theoretical methane yield**

The sewage grown (SG) algal biomass, collected as a concentrated slurry, was centrifuged at 6,000 g for 10 min at room temperature. The pellet thus obtained was suspended in distilled water and centrifuged again in order to remove slats or nutrients deposited on the algal biomass. Finally, the pelletized algal biomass was dried at 60 °C until it reached a constant weight and then ground (using a mortar and pestle) to obtain a fine powder of algae. The powdered algal biomass was used for determination of its lipid, protein and carbohydrate content. Lipid was extracted and estimated using the modified Bligh and Dryer’s method (Lee et al. 2010). Carbohydrate content of the algal biomass was determined through the phenol sulfuric acid method (Dubois et al. 1951). Protein was estimated using the elemental nitrogen content and the nitrogen-to-protein conversion factor i.e. 6.25. The biochemical composition based theoretical methane yield was calculated using the equation adopted from Prajapati et al. (2015):

\[
\text{Theoretical methane yield (m}^3\text{ kg}^{-1}\text{ VS}) = \left( \frac{0.851 \times F_P + 1.014 \times F_L + 0.415 \times F_C}{100} \right)
\]  

(2)

where \(F_P\), \(F_L\) and \(F_C\) are the protein, lipid and carbohydrate content (% total solids, i.e. TS) of algal biomass.

**The biochemical methane potential (BMP)**

For determination of the methane potential of fresh algal biomass and improvement in methane yield through natural storage of algal biomass (0–72 h), BMP tests (in triplicates) were performed. BMP experiments were carried out in 500 mL amber color reagent bottles with 300 mL working volume. The initial substrate concentration, in terms of algal volatile solids (VS), in the BMP bottles was 5 g L\(^{-1}\), whereas the substrate to inoculum (S/I) ratio was kept at 1:3 on VS basis (Angelidaki et al. 2009). The digested slurry collected from a laboratory scale cattle dung-based biogas plant
(working volume 18 L; semi-continuous feed with 30 days’ HRT) was used as anaerobic inoculum. The VS content and specific methane yield of the inoculum was used 60% (of TS) and 0.178 g COD–CH₄ g⁻¹ VS d⁻¹, respectively. The inoculum was aseptically transferred to BMP bottles followed by addition of substrate (biomass), and the volume was made up to 300 mL using tap water whenever required. All the experimental bottles were incubated at 37 ± 1 °C under stationary conditions in a benchtop incubator (Holliger et al. 2016). The BMP test was carried out for a 30 d anaerobic digestion period.

An aliquot (10 mL) from each BMP bottle (at the beginning and end of the experiment) was withdrawn and centrifuged at 6,000 g for 40 min. The supernatant thus collected was analyzed for determination of TAN and total volatile fatty acids (TVFA) concentration. TVFA concentration was determined spectrophotometrically (Siedlecka et al. 2008). The pH of the digestate was also determined both at the beginning and immediately after the completion of the BMP test.

**Analytical methods**

**Wastewater parameters**

The sCOD was determined through the colorimetric method (Hach method 8000). The samples were first digested in Hach Digital Reactor DRB200, and the sCOD concentration was measured through reading the sample in the Hach colorimeter DR890. The TDP and NO₃−N were determined colorimetrically through the molybdovanadate method (Hach method 8114) and cadmium reduction method (Hach method 8114), respectively. TAN was measured using multi-parameter (HQ40d, Hach) equipped with an ammonium ion selective electrode, and pH was measured with a bench top pH meter (CyberScan PC510, Eutech). TSS and TDS were determined through standard methods for wastewater analysis (Eaton et al. 2005).

**Biogas volume and composition**

The volume of biogas produced from BMP experiments was measured through acidic water (pH <2.0) displacement in every 24 h (Krishania et al. 2013). Methane content in the biogas was determined using a gas chromatograph (Agilent 7890A) equipped with a stainless steel column packed with Porapack-Q 80/100 mesh (Supelco) and thermal conductivity detector (TCD). The net methane production (mL g⁻¹ VSfed) was determined by subtracting the methane yield of the control bottle (with inoculum only) from the methane yield obtained in experimental bottles.

**Algal biomass digestibility during BMP**

The biomass digestibility was estimated in terms of % of volatile solids reduction (VSR). VSR was calculated after determination of volatile solid (VS) content of the BMP bottles before and after anaerobic digestion using the following equations:

\[
\text{VSR (\%) = } \left( \frac{\text{VS}_f - \text{VS}_d}{\text{VS}_f} \right) \times 100
\]

where VSf and VSd are the VS concentration (g/L) in the experimental bottles before and after anaerobic digestion (30 d), respectively. The VS content of the feed and digestate were estimated using the standard EPA protocols (Telliard 2001) by burning the oven dried samples at and above 550 °C.

**RESULTS AND DISCUSSION**

**Algal culture and remediation studies**

The microscopic analysis showed that the algal culture obtained is a consortium of more than one type of algal cell as well as bacteria (Supplementary Figure S3, available with the online version of this paper). The dominating algal species in the consortium were morphologically identified as *Chlorella* and *Chroococcus* spp. Moreover, preliminary biochemical tests indicated the presence of both gram positive and gram negative bacteria including *Azotobacter*, *Bacillus* and *Actinobacteria* in the algal consortium. The native algal consortium thus obtained was used as inoculum for further experimentations.

To assess the native algal biomass production in sewage, batch studies were conducted using sewage as the sole growth medium. The composition of the sewage is shown in Table 1. Under illumination, the growth of algae in the sewage started, and the final biomass concentration after 12 d was 1.69 ± 0.35 g dry weight L⁻¹. Apart from the biomass production, removal of nutrients and sCOD by native algal growth was also studied (Figure 1). A drastic reduction in the sCOD was observed and the sCOD concentration reduced to 0 within 3 d due to the algal growth in sewage under illuminated conditions. Moreover, in the control flask (incubated under similar conditions but covered with aluminum foil to avoid light penetration) the change in sCOD was almost negligible (Figure 1). Further, the concentrations of NO₃−N, TDP...
and TAN in sewage reduced to 14.15, 11.05 and 10.56 mg L\(^{-1}\), respectively, during native algal growth for 12 d. Also, pH of sewage changed from its value near neutral (before algal growth) to alkaline (9.20), which could be due to utilization of carbonates (or dissolved CO\(_2\)) from wastewater during native algae growth. Overall, the observed removal efficiency of the native algae were 100, 79, 64 and 82\%, for sCOD, NO\(_3\)-N, TDP and TAN, respectively. However, the corresponding removal efficiencies observed in the control flask were only 6, 40, 15 and 28\%, respectively, which could be due to the growth of native microorganisms other than algae. Similar removal efficiencies for various pollutants from wastewater have been reported for different algal cultures (Chinnasamy et al. 2014; Paskuliakova et al. 2016).

As in the case of batch flask scale studies, good algal growth and nutrient removal was also observed in PBR (Figure 2). During the batch growth phase, drastic reduction in sCOD was observed. The residual sCOD concentration in the PBR reached zero within 5 d of the experiments. The observed residual concentrations of NO\(_3\)-N, TDP and TAN (on 12th day) were 18, 14 and 6.5 mg L\(^{-1}\), respectively. Interestingly, the biomass concentration in the PBR (3.29 ± 0.42 g L\(^{-1}\)) during batch growth was higher than the value observed in flask scale studies (1.69 ± 0.35 g L\(^{-1}\)). The higher growth rate in the aerated PBR could be due to better light availability, mixing, and the proper CO\(_2\) availability from the bubbled air.

A sudden increase in sCOD along with other nutrients was observed as soon as the PBR was converted to continuous mode. With the addition of fresh sewage (during the first 2 d of continuous operation), the sCOD reached up to 21 mg L\(^{-1}\) and again reduced to zero within the next 4 d and remained almost zero thereafter. Similarly, a significant increment in the TAN was also observed with

### Table 1

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Parameter</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Total suspended solids (TSS)</td>
<td>1656.40 ± 32.25</td>
</tr>
<tr>
<td>2.</td>
<td>Total dissolved solids (TDS)</td>
<td>3509.34 ± 37.52</td>
</tr>
<tr>
<td>3.</td>
<td>Total ammoniacal nitrogen (TAN)</td>
<td>58.65 ± 4.20</td>
</tr>
<tr>
<td>4.</td>
<td>Soluble chemical oxygen demand (sCOD)</td>
<td>101.55 ± 6.58</td>
</tr>
<tr>
<td>5.</td>
<td>Nitrate nitrogen (NO(_3)-N)</td>
<td>67.02 ± 3.56</td>
</tr>
<tr>
<td>6.</td>
<td>Total dissolved phosphorus (TDP)</td>
<td>30.50 ± 2.54</td>
</tr>
<tr>
<td>7.</td>
<td>pH</td>
<td>7.15 ± 0.56</td>
</tr>
</tbody>
</table>

Values reported are mean ± Std. Dev. (All values are in mg L\(^{-1}\) except pH).
concentrations of up to 18 mg L\(^{-1}\) recorded on the 13th day (i.e., the first day of continuous mode). However, there was relatively small change in the concentrations of NO\(_3\)-N and TDP, and the respective values observed on the 13th day were 19 and 16 mg L\(^{-1}\) (Figure 2(a)). The residual concentration of NO\(_3\)-N, TDP and TAN remained in the range of 11–18, 14–20 and 4.8–8.2 mg L\(^{-1}\), respectively, for rest of the period of the continuous mode. The pH in the PBR was raised to 9.2 from an initial value of 7.15, within 3 days of the start of operation, and then remained in the vicinity of 9.0 throughout the experimentation. Moreover, there was a slight decrease in algal biomass concentration (3.15 ± 0.22 g L\(^{-1}\)) observed on the 15th day. However, the algae again started growing and reached a maximum biomass concentration of up to 5.45 ± 0.21 g L\(^{-1}\) recorded on the 23rd day of the experiment (Figure 2(b)). The average biomass concentration during the continuous mode was 4.64 ± 0.92 g L\(^{-1}\). The observed biomass concentration is in accordance with previously observed values for native algae grown in dairy farm effluent (4.44 g L\(^{-1}\)) under outdoor PBR (Prajapati et al. 2014).

**Methane production from SG algae**

The biochemical compositional analyses revealed that the algal biomass harvested after sewage treatment contains up to 22, 16 and 44% carbohydrate, lipid and protein, respectively. Theoretical methane yield calculated using Equation (2) was 628 mL g\(^{-1}\) VS. To predict the bioenergy generation potential, anaerobic digestion of harvested algal biomass was carried out. The methane profiles of the algal biomass are shown in Figure 4(b) (shown as a graph for 0 h stored algal biomass). Methane yield from algal biomass was only 180.40 ± 8.92 mL g\(^{-1}\) VS\(_{fed}\). Further, the maximum rate of methane production was only 18.92 mL CH\(_4\) d\(^{-1}\), which was observed on the seventh day of the BMP experiment. In contrast, higher cumulative and daily methane yield have been reported in literature (Dębowski et al.)
For instance, methane yield in the range of 188–295 mL g\(^{-1}\) VS\(_{\text{fed}}\) was observed for anaerobic digestion of the microalgal mixture (Alzate et al. 2012). The low methane yield observed in the present work could be attributed to slow digestibility and degradation of algal biomass. Hence, there are possibilities to improve the methane yield by improving the degradability of the algal biomass.

**Algal biomass disruption during storage**

Algal biomass solubilization during natural storage was measured in terms of sCOD concentration. The sCOD of stored samples increased dramatically, which indicates release of intracellular constituents from algal biomass, probably due to disruption of the algal cell wall. The SEM image of the culture showed the presence of bacteria with algae (Figure 3(a)), which could have played a major role in pretreated of algal biomass during natural storage. The disruption of the algal cells was clearly observed with SEM and fluorescence microscopy (Figure 3(b)–3(d)). During the first 18 h, the solubilisation of biomass COD was negligible (1–4%). However, after 24 h, the algal biomass solubilization started increasing and the highest algal biomass solubilization with sCOD concentration of up to 7,298 ± 188 mg L\(^{-1}\) (≈48% COD solubilization) was observed with a sample incubated for 60 h (Figure 4(a)). Storage beyond 60 h had a negative effect on algal biomass solubilization (sCOD concentration reduced to 6,331 ± 210 mg L\(^{-1}\)). This could be due to the utilization of ruptured algal cell content by the native bacteria present in the biomass. In contrast, the BF algal samples, stored under similar natural conditions showed very low change in sCOD (≈3,918 ± 78 mg L\(^{-1}\)), with only 10% COD solubilisation even after 72 h.

One of the possible underlying mechanisms for algal cell disruption during storage could be natural cell death under unfavourable conditions for growth, leading to apoptosis (Miao et al. 2013). Hence, cell lysis under rugged environmental conditions could have led to an increased sCOD concentration due to release of the cytoplasmic constituents of stored algal samples. However, the slow rate of biomass solubilisation in the case of BF samples indicated a minor contribution of natural cell death in algal biomass pretreatment. Another explanation for algal biomass disruption during storage could come from the fact that there was a presence of native bacteria along with the wastewater-grown algal biomass as confirmed with the SEM image (Figure 3(a)). The storage conditions were not favourable for algae growth, as no light and nutrient were supplied.
but there was optimal environment (temperature 27–30 °C, moisture content >80% and dead algal biomass as nutrient and carbon source) for native bacteria to grow. Hence, it is possible that native bacteria could have grown on the algal biomass, leading to further algal cell disruption and release of cellular constituents. Indeed, native bacterial cultures isolated from the algal consortium showed good cellulolytic activity on carboxymethylcellulose (CMC) agar plates (results not shown). Hence, the combined effect of algal cell natural death and cell degradation by native bacteria could have led to the high rate of COD solubilization from naturally stored biomass. Similar observations on biomass pretreatment were also made when the algal biomass was stored with the fungi under natural conditions (Prajapati et al. 2015). Indeed, biomass solubilization in the present work was in line with the previously reported values for different algal cultures pretreated through different thermal, chemical or biological methods (Passos et al. 2014). Indeed, biomass solubilization in the present report is higher than that observed during thermal and biological pretreatment of different algal biomass (Alzate et al. 2012). Hence, based on these observations it can be concluded that the present method is a simple and efficient approach to replace traditional physico-chemical or biological methods including enzymatic pretreatment of algal biomass.

Natural storage improves the methane yield

The natural storage had a positive impact on methane yield (Figure 4). Significant improvement (as compared to fresh algal biomass i.e., 0 h stored biomass) was observed in the methane production, which reached up to 284.38 ± 11.52 and 276.78 ± 10.24 mL g⁻¹ VSfed for samples stored, respectively, for 48 h and 60 h. It was also noticed that storage beyond 60 h has a negative effect on methane yield, and the values observed for samples stored for 72 h were relatively low (265.27 ± 6.20 mL CH₄ g⁻¹ VSfed). The BMP of the algal samples stored for less than 24 h was not determined, as the biomass COD solubilization in these samples was very small. The effect of natural storage on algal biomass digestibility was also determined through estimation of VSR. As in the case of methane yield, the highest VSR (78%) was also observed with the samples stored for 60 h. Further, an almost similar VSR (≈76%) was observed for samples stored for 72 h. In contrast, the anaerobic digestion of fresh algae showed only 38% VSR. The VSR for other samples stored for 24–48 h was in the range of 47–65%. Moreover, the digestate obtained after anaerobic digestion was analysed for pH, TAN and TVFA content in order to predict the process stability. The characteristics of the digestate are given in Table 2. The values observed for TAN (209–285 mg L⁻¹) and TVFA (895–1,265 mg L⁻¹) for all the sets

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**Figure 4** | Effect of natural storage on: (a) algal biomass disruption reported in terms of variation in sCOD concentration with storage time for sewage grown (SG algae) as well as nutrient media grown bacteria free algal biomass (BF algae) and (b) cumulative methane production profile of sewage grown algal biomass stored for 0 h (i.e. freshly harvested algae) to 72 h.
tested were lower than those reported as inhibitory (TVFA > 2,000 mg L⁻¹ and TAN > 1,500 mg L⁻¹) for the anaerobic digestion process (Labatut & Gooch 2012). Moreover, that pH was also in the neutral range (6.80–7.82) for all the tested sets.

The observed improvements in the methane yield and the digestibility were in line with algal biomass solubilization measured in terms of sCOD. Further, the observed methane yield and enhancement was at par with the values reported for algal biomass pretreated through various methods. For instance, Alzate et al. (2012) reported up to 62% enhancement in the methane yield through thermal hydrolysis of algal biomass. Ben Yahmed et al., (2016) reported methane production up to 260 mL g⁻¹ VS through crude enzyme-based pretreatment of Chaetomorpha linum biomass. Similarly, more than 50% enhancement in the digestibility and methane yield was observed during novel simultaneous harvesting and pretreatment of Chroococcus sp. biomass using fungi as a bioagent (Prajapati et al. 2016). Based on these facts, natural storage (for 2–3 d) of wastewater grown algae may be recommended as a prerequisite stage in algae to methane generation by anaerobic digestion.

**CONCLUSIONS**

It is well documented that in wastewater treatment systems algae and bacteria grow together, with higher growth rates (high biomass production) leading to higher nutrient removal from wastewaters. High removal of nutrients and sCOD (65–100%) along with substantially higher biomass production was observed in both flask and reactor scale during the present studies. Further, up to 48% COD solubilization from SG algal biomass was observed through aerobic treatment when stored for designated periods under natural conditions. The biomass solubilization resulted in >57% enhancement in methane yield from algal biomass stored for 60 h. Overall, the wastewater grown native algal biomass seems to be a good substrate for methane production through natural storage followed by anaerobic digestion. This may further be explored to develop decentralised algae mediated wastewater treatment coupled bioenergy generation systems.

**ACKNOWLEDGEMENT**

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**REFERENCES**


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**Table 2** Values recorded for pH, total ammoniacal nitrogen (TAN) and total volatile fatty acid (TVFA) concentrations in the content of BMP bottles at the start (initial) and after completion (final) of anaerobic digestion as well as volatile solid reduction (VSR) during BMP test

<table>
<thead>
<tr>
<th>Sample</th>
<th>Parameter</th>
<th>pH</th>
<th>TAN (mg L⁻¹)</th>
<th>TVFA (mg L⁻¹)</th>
<th>VSR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
<td>Final</td>
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<tr>
<td>Fresh (0 h)</td>
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<tr>
<td>Stored (24 h)</td>
<td>pH</td>
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<td>7.21</td>
<td>172.12</td>
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<td>Stored (36 h)</td>
<td>pH</td>
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<td>7.54</td>
<td>155.14</td>
<td>259.36</td>
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<tr>
<td>Stored (48 h)</td>
<td>pH</td>
<td>7.12</td>
<td>6.85</td>
<td>162.83</td>
<td>220.06</td>
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<tr>
<td>Stored (60 h)</td>
<td>pH</td>
<td>6.89</td>
<td>7.44</td>
<td>158.15</td>
<td>209.50</td>
</tr>
<tr>
<td>Stored (72 h)</td>
<td>pH</td>
<td>7.14</td>
<td>6.80</td>
<td>156.23</td>
<td>219.62</td>
</tr>
</tbody>
</table>

Values reported are mean of at least three replicates.


Prajapati, S. K., Bhattacharya, A., Kumar, P., Malik, A. & Vijay, V. K., 2016 A method for simultaneous bioflocculation and


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