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Effect of food to microalgal biomass ratio on the assimilation of ammoniacal nitrogen from the secondary treated tannery effluent coupled with bio-energy generation using grown algal biomass

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A B S T R A C T

Microalgae are receiving great attention towards biofuel and potential option for the removal of nutrients from wastewater as an alternative biological treatment. In the current study, five different food to algal biomass ratios (0.25, 0.5, 1.0, 1.5, and 1.75) experimented with *Chlorella vulgaris* and a ratio of 1.0 was found to be optimum. Further studies were carried out in a lab-scale tubular photobioreactor (PBR). The maximum removal efficiencies of ammoniacal nitrogen, total Kjeldahl nitrogen, and total organic carbon were found to be 66.60%, 62.50%, and 69.09%, respectively. Grown microalgal biomass was subjected to biomethanation for recovery of bioenergy in the form of methane. The specific methane yield obtained was 233 mL/g VS (VS – volatile solids) added using a standard biomethane production test using automated methane potential test system II. The results of the study are promising, as it was observed that the coupling of both processes, that is, algal treatment integrated with anaerobic digestion of grown algal biomass will benefit wastewater treatment for nutrient removal as well as helps in energy production.

Keywords: *Chlorella vulgaris*; Tannery wastewater; Nutrient removal; Energy production; Automated methane potential test system II (AMPTS II); Biogas

1. Introduction

Developing countries are the leading producer of finished leather and leather products due to the availability of raw materials and industrialization. Over the decades, there has been an improvement in leather production using cleaner production methods like the application of enzymes for leather processing [1]. Despite the development in the leather sector, the discharge of wastewater from tanning processes causes environmental pollution. Wastewater discharged has hazardous chemicals such as chromium, synthetic tannins, ammonium, sodium chloride, and phenolic compounds which contain a large number of organics that cause pollution to the environment if it is not properly treated and managed [2–4]. Conventional treatment plants are designed mainly for the removal of suspended solids and organic matter such as biochemical oxygen demand (BOD) to meet the discharge standards to protect ecosystems but not designed for removal of nitrogen. In the case of zero liquid discharge (ZLD) plants, employed with a membrane system for recycling of water from wastewater and evaporation of reject, nitrogenous substances present in biologically treated effluent reappeared along with condensate in the evaporator. However, the secondary treated effluents from wastewater treatment plants contain nutrients such as nitrogen and phosphorus (in the form of

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ammonium, nitrates, and phosphates). These nutrients are considered to be the major cause of eutrophication in natural water bodies. Conventional treatment using biological treatments, nitrification, and denitrification, for the removal of nutrients from wastewater results in a high sludge content and increase the operational cost. Hence, this bottleneck requires new technology which is cost-effective and energy-efficient. Biological treatment has more economic benefits when compared to chemical oxidation for the treatment of industrial effluent in the reduction of organic content [5]. Conventionally designed treatment plants, that is, the activated sludge process treat only the organics such as chemical oxygen demand (COD), BOD, and suspended solids but the nutrients, such as nitrogen in form of ammonia (NH_3^+) and nitrates (NO_3^-) are not resolved. In addition to this, due to the presence of sulfide, chromium, and chloride, and fluctuation in temperature have drastic effects on the nitrification process [6]. As ammonia nitrogen (NH_3–N) is one of the major pollutants in tannery effluent due to the high protein content in the effluent eluted from the collagen, amino acids from the skin, fatty aldehydes, and quinones from the tannins which lead to the ammonia-N concentration up to 300–400 mg/L in the effluents and leads to eutrophication in natural water bodies which results in the formation of algal blooms in water habitats that has several negative impacts on the human health and ecosystems such as the production of toxins in water bodies and hypoxic conditions and also affects the nature of aquatic systems [7–10]. In addition, ammonia-N from tannery effluents, which is highly toxic to aquatic organisms, may lead to death due to the toxic buildup in their blood and internal tissues [10].

Other conventional treatment methods for removal of ammoniacal nitrogen such as chemical precipitation, and adsorption result in a high sludge content; use of constructed wetland systems requires larger land area in addition to complicated operation to achieve nitrogen removal, and electrochemical treatment/air stripping consumes more chemical and electrical energy and lacks effective economic feasibility [11–17]. In addition to this, the excess sludge generated from these conventional wastewater treatments leads to further treatment and disposal which further increases the operational cost. To overcome these circumstances of the high cost of treatment and to meet stringent environmental regulations and standards [18–20], there is an urgent need to carry out the research through alternative methods for the development of effective treatment technologies.

Biological treatment using microalgae is receiving great attention for the removal of nutrients from wastewater. They have the capacity to consume simple organic compounds directly and assimilate them as carbohydrates and amino acids [21]. As a result of photosynthesis, microalgae can assimilate inorganic carbon, thereby reducing energy requirement for the treatment of effluent when compared to the conventional aerobic treatment technologies which need the presence of oxygen. The advantages of using microalgae in wastewater treatment are economic feasibility, less energy requirement, reduction of sludge formation, reduction of emission of greenhouse gases, and potential for utilization of algal biomass [22]. Not only was the developed microalgal technology useful for wastewater treatment applications, but also the biomass produced could be a source of raw material for biofuels and pharmaceutical and other industries [23].

Most of the nutrient removal studies using microalgae did not emphasize the nitrogen assimilation towards microalgae, that is, the food to microalgae ratio (F/M) which plays a major role in treatment efficiency. So the current study mainly focused on the effect of the F/M ratio in order to improve the treatment efficiency and to investigate the bio-methane potential (BMP) of grown algal biomass for bioenergy production through anaerobic digestion.

2. Materials and methods

2.1. Screening of microalgae

Pure cultures of *Chlorella vulgaris*, *Chlamydomonas* sp., *Chlorococcum* sp., and *Scenedesmus dimorphus* collected from Centre for Advanced Studies in Botany, Madras University, Chennai, India were screened to evaluate the efficiency based on their performance in nutrient removal from synthetic wastewater [24]. Based on their nutrient removal efficiency, freshwater microalga *C. vulgaris* was selected for the current study.

2.2. Media and cultivation conditions

The bold-basal modified (BBM) medium was used to develop the culture. The media was prepared with distilled water with an initial pH adjusted to 7.0–7.2, sterilized at 121°C for 20 min and maintained at 28°C [24].

2.3. Collection and characteristics of secondary treated tannery wastewater

Secondary treated tannery wastewater was collected from a common effluent treatment plant located in Tamil Nadu, India, and the parameters were analyzed as per the American Public Health Association (APHA) 2000. The concentrations of NH_3–N, total Kjeldahl nitrogen (TKN), and NO_3–N in secondary treated effluent were found to be in the range of 400–460 mg/L, 600–650 mg/L, and 2.5–5.0 mg/L, respectively.

2.4. Biological nutrient removal

2.4.1. Batch cultivation

The stock culture of *C. vulgaris* was cultivated in a tubular photobioreactor (PBR) with a capacity of 7 L and a working volume of 5 L of BBM at 27°C ± 2°C completely aerated (0.5 vvm) with light and dark cycles of 16h:8h at a light intensity of 5,000–6,000 lux [25,26]. Cool white fluorescent lights were used since they emitted radiance closer to the light spectrum of daylight and 45.65% of total light falls in the range of 400–700 nm, making it effective for studying algal biomass production rates [27]. The growth of *C. vulgaris* was frequently monitored by measuring the optical density at 675 nm using a UV-spectrophotometer (Shimadzu – 1800, Japan). The values were plotted to obtain the growth curve of *C. vulgaris*.

2.4.2. Batch scale studies on biological nutrient removal from secondary treated tannery wastewater using different F/M ratios

The initial batch studies were conducted based on the F/M ratio, that is, $TN_{Food}/TSS_{microalgae}$. Total nitrogen (TN) was considered here as food for microalgae where ammoniacal nitrogen and organic nitrogen are the major forms of nitrogen present in the secondary tannery effluent. The microalgal culture used for overall treatment studies has microalgal biomass ranging from 945–1,000 mg/L in terms of dry weight total suspended solids (TSS). The batch experiments with five different F/M ratios, that is, 0.25, 0.5, 1.0, 1.5, and 1.75, were performed. The batch cultivations were done in 250 ml Erlenmeyer flasks with a volume of 100–120 ml (Fig. 1). All experiments were conducted in duplicate with constant mixing at 120 rpm in a rotary shaker illuminated with 5,000–6,000 lux at 27°C ± 2°C for a period of 15 d. The samples were withdrawn at regular intervals and analyzed for nutrient removal efficiency.

Fig. 1. Batch scale studies with shake flask experiments of different F/M ratios.

2.4.3. Nutrient removal studies using tubular PBR

In order to study the nutrient removal efficiency in a tubular PBR, the secondary treated tannery wastewater was fed into the reactor. *C. vulgaris* was cultivated in a tubular PBR of 7 L capacity with a working volume of 5 L. The reactor was aerated with 0.5 vvm of air through an air pump. The PBR was illuminated with 5,000–6,000 lux with the light and dark cycles of 16:8 h (Fig. 2). The pH inside the reactor was adjusted using 1 N HCl and 1 N NaOH to maintain in the range of 7.0–7.5. Among the five different F/M ratios studied, the optimized ratio was considered for the scale-up study for a period of 7 d. The treated samples were withdrawn from the specific intervals for the analysis.

Fig. 2. Batch scale studies in a tubular photobioreactor with the optimized F/M Ratio.

2.4.4. Analytical parameters and biochemical characterization

The treated wastewater samples were withdrawn at specific intervals and the concentrations of ammoniacal nitrogen (NH_3–N), TKN, and nitrate–nitrogen (NO_3–N) were analyzed according to standard methods [28]. The treated wastewater containing grown algal biomass was centrifuged at 3,000 g, for 10 min and the pellet was subjected to analysis of biochemical composition. The carbohydrate content, total lipid content, and total protein were estimated by the phenol–sulphuric acid method, gravimetric method, and Lowry's method, respectively [29–31]. The morphological identification was carried out using a clinical microscope (Olympus CH 20i) with Magnus image projection system (MIPS).

2.4.5. Biomethanation of harvested microalgal biomass for bio-energy production

The inoculum used in the bio methane potential (BMP) is anaerobic sludge collected from an anaerobic digester of the sewage treatment plant, Chennai. The pH of the anaerobic sludge was 6.8. Total solids (TS), and volatile solids (VS) were found to be 39.66 mg/g and 22.2 mg/g respectively. The moisture content and VS in TS were analyzed to be 96% and 55.9% respectively. The physicochemical parameters and biochemical composition of *C. vulgaris* are shown in Table 1.

The BMP of *C. vulgaris* was analyzed as per VDI standard 4630 (German method). The determination of specific methane (CH_4) yield and substrate degradation were performed using an automatic methane potential test system

Table 1
Initial characterization for *C. vulgaris*

Analytical parameter	*C. vulgaris*
pH	7.5
TS (mg/g)	73.9
VS (mg/g)	55.2
%VS in TS	74.6
Moisture (%)	92.6
Carbohydrates (% W/W)	30.8
Lipids (% W/W)	28.4
Proteins (% W/W)	14.5

(AMPTS) II (Bioprocess, Sweden) with an online monitoring system supported by software AMPTS v5.0 [32]. The substrate to inoculum ratio was fixed at 0.5 based on the VS. All the experiments were run in triplicate (Fig. 3).

3. Results and discussion

3.1. Batch scale studies on biological nutrient removal from secondary treated tannery wastewater using different F/M ratios

The pollutant concentration profile of secondary treated tannery wastewater and the removal efficiency using *C. vulgaris* were studied for a time period of 15 d. Among the 5 different F/M ratios, the degradation of ammoniacal nitrogen (NH_3–N), Nitrate nitrogen (NO_3–N) and TKN was found to be similar for F/M ratios of 1, 0.5, and 1.5 at which the removal efficiencies of NH_3–N were in the range from 55.48% to 57.76% whereas those of NO_3–N were in the range of 70.39%–78% and those of TKN were 50%–60%, respectively as shown in Table 2. Among these three ratios, there is no significant difference in removal efficiency. So the additional studies were done with ratios of 0.25 and 1.75. Further experimental studies with 0.25 ratio have the maximum removal efficiency of NH_3–N which was found to be 60.57% whereas 1.75 ratio has showed a lower nutrient removal efficiency of 37.5%. At all three ratios (1, 0.5, and 1.5) there is a gradual decrease in the pollutant concentration as shown in Fig. 4. The morphological change in *C. vulgaris* is shown in Fig. 5 in which the increase in cell size can be clearly seen. Fig. 5 shows the cell transformations with an increase in days which clearly shows the nutrient assimilation of *C. vulgaris* from the tannery wastewater.

3.2. Batch scale studies in a tubular PBR for the treatment of secondary treated tannery wastewater with optimized F/M ratios

Based on the treatment efficiency, retention time, and operational strategies, an F/M ratio of 1 was found to be the optimum, and further studies were carried out in a tubular PBR. The nutrient removal studies performed in the PBR have shown better results when compared with the shake flask experiments due to the complete mixing with the help of aeration, availability of CO_2, and surface area of the reactor for light transmission. From Fig. 6a, it was found that *C. vulgaris* has a very clear growth pattern in the composite secondary treated tannery wastewater. The removal efficiencies of NH_3–N were found to be 66.6% with an initial concentration of 252 mg/L whereas the maximum removal efficiency of TKN was 62.5% with an initial concentration of 448 mg/L as shown in Fig. 6b. The degradation profiles of NO_3–N, and PO_4–P are shown in Fig. 6c and the removal efficiencies were found to be 70.02% and 62.62%, respectively. As *C. vulgaris* is a mixotrophic species, its ability to consume carbon from the wastewater can be evidently seen in Fig. 6d with a total organic carbon (TOC) removal efficiency of 69.09% with the initial concentration in the reactor of 275.45 mg/L. Similarly, COD was degraded up to 61.49% as shown in Fig. 6d.

3.3. BMP of C. vulgaris

The specific methane yield time period of 27 d from the anaerobic digestion of *C. vulgaris* is shown in Fig. 7.

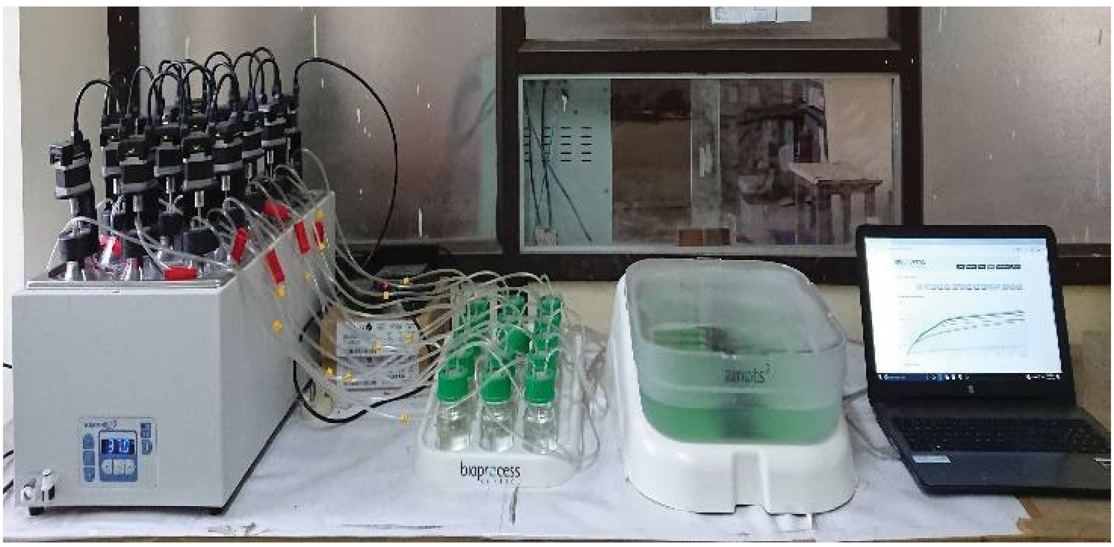

Fig. 3. Automated methane potential test system II (AMPTS II) experimental setup.

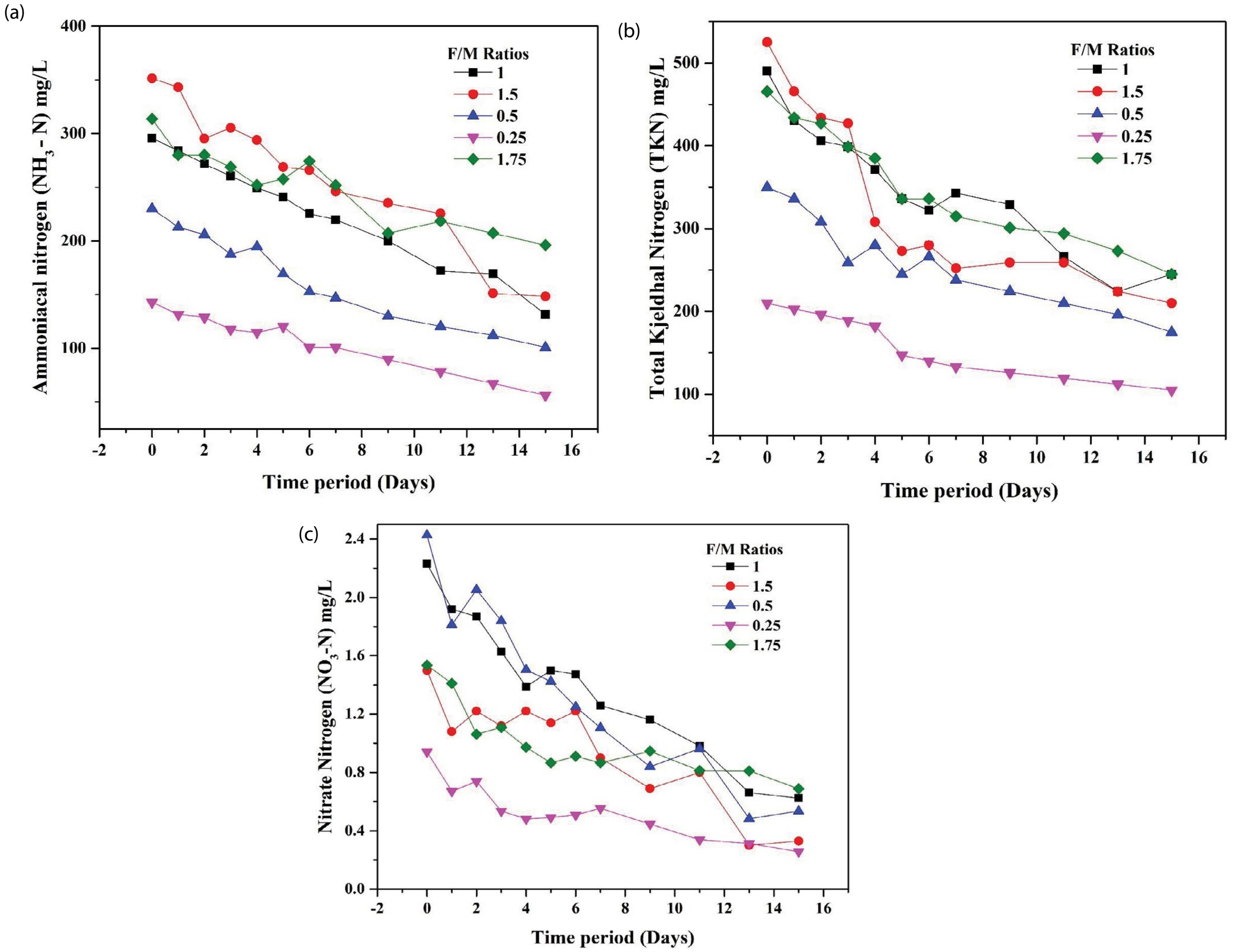

Fig. 4. The efficiency of *Chlorella vulgaris* in secondary treated tannery wastewater with different F/M ratios. Profile of (a) NH_3–N, (b) TKN, and (c) NO_3–N in secondary treated tannery wastewater with different F/M ratios.

The methane production from the inoculum was deducted from the methane produced in the reactor which contains the substrate and inoculum in order to get the true methane production from the substrate. The specific methane yield produced from microalgal biomass was found to be 233 ml/g VS added with a standard deviation of ± 27 ml. The BMP test showed a substrate VS reduction of 80.22% which clearly indicates the biodegradability of *C. vulgaris* as shown in Fig. 8.

Table 2
Removal efficiency (%) for different F/M ratios

Parameters	Food to microalgae ratio (F/M)				
	0.25	0.50	1.00	1.50	1.75
NH_3–N	60.57	56.17	55.48	57.76	37.5
TKN	50.00	50.00	50.00	60.00	47.36
NO_3–N	72.80	77.94	70.39	78.00	55.23

4. Discussion

The results from the current study confirmed that *C. vulgaris* has the ability to grow in ammoniacal nitrogen and total dissolved solids rich secondary treated tannery wastewater which remains even after the conventional treatment [32,33]. Most of the previous studies on the phycoremediation of tannery wastewater do not focus on the inoculum level during the treatment which is the most critical part of nutrient removal from effluents [34–37]. Nutrient removal performance may vary and depends on the effluent characteristics and the microalgal species. Most of the studies focus only on using mixed consortia in which the removal efficiency ranges from 60%–90% rather than focusing on individual microalgal cultures for nutrient removal [37,38]. Mixed cultures used for the treatment during the growth phase of the culture becomes unpredictable as each microalga in the consortium tries to dominate each other during the treatment which is evident from a reported study that *Stigeoclonium* sp. is dominant in the

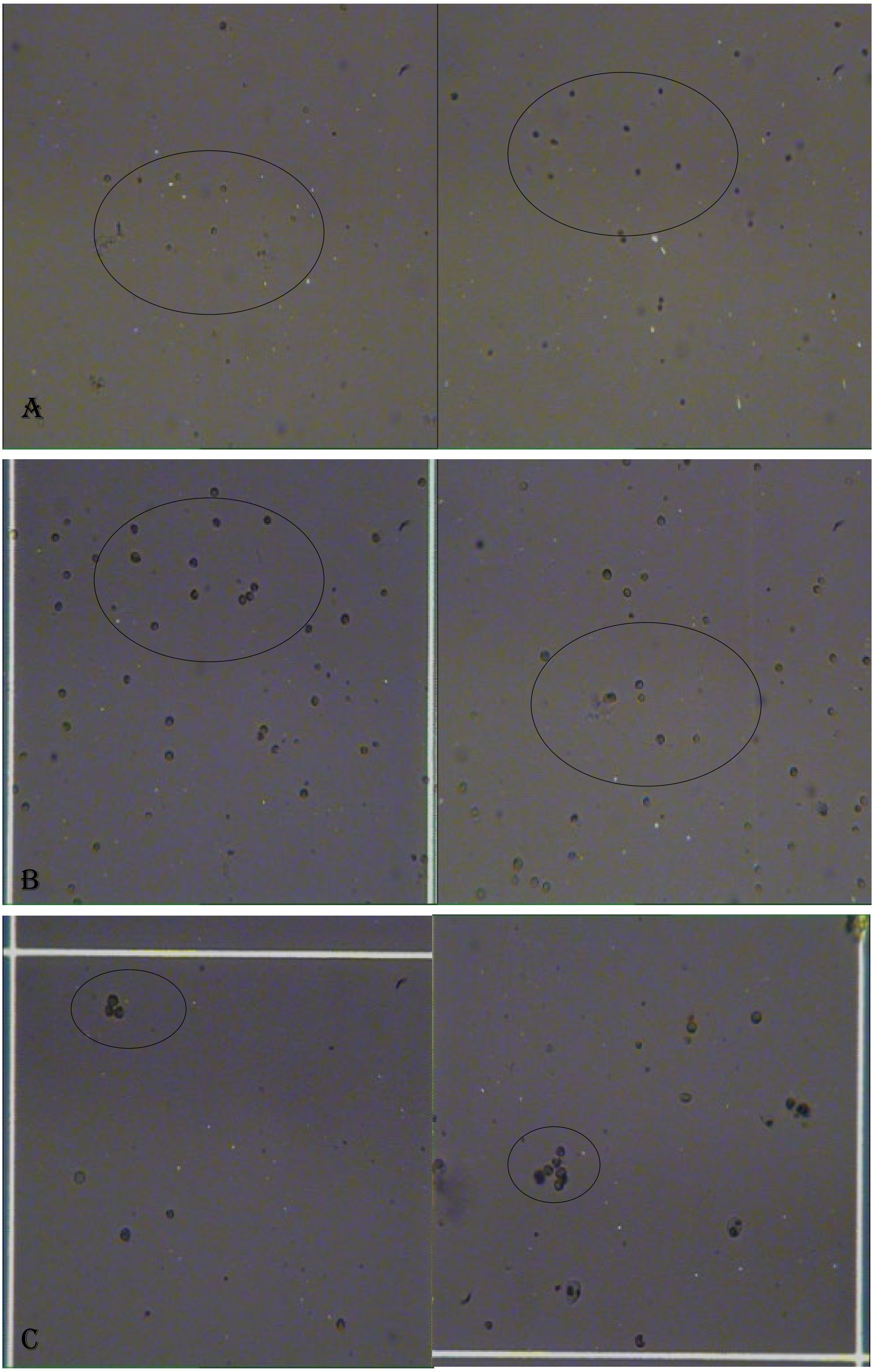

Fig. 5. Cell transformations with nutrient assimilation. (a) Cell size on the 2nd day, (b) 8th day, and (c) 14th day.

mixed culture in the initial treatment days while at the later stage, after the 10th day, *Scenedesmus* sp. becomes dominant. This could be mainly due to the influence of the N/P ratio in the reactor under the nutrient depletion condition as particular microalga shows a high growth rate [35]. It is reported that this variation in the biomass has an effect on the biochemical composition such as lipid accumulation in the consortium that varies frequently with the change in nitrogen and phosphorous concentrations [39,40] and ultimately results in fluctuations in the quantity and quality of the fuel produced due to the change in the biochemical composition of microalgae. The current study on the effect of nitrogen (food)/TSS (microalgae) has confirmed nutrient assimilation and removal from secondary treated tannery integrated with bioenergy production. Among the five different F/M ratios studied (0.25, 0.5, 1, 1.5, and 1.75), F/M ratio of 1 was found to be the optimum ratio for effective removal of nutrients from secondary treated tannery wastewater as 1 g of nitrogen (Food-F) is removed with 1 g of microalgae biomass (M) in the system. The corresponding removal efficiencies of nutrients in terms of ammoniacal nitrogen, TKN, NO_3–N, and PO_4–P were found to be 66.6%, 62.5%, 70.02%, and 62.62%, respectively for the F/M ratio of 1. In addition, it is observed that *C. vulgaris* not only has the capability of removing the nutrients but also can degrade the organic carbon present in the wastewater indicated by TOC and COD removal efficiencies of 69.09% and 61.49% indicating clear evidence that *C. vulgaris*, a mixotrophic species, has the potential to grow under both autotrophic and heterotrophic conditions. It is reported that *C. vulgaris* has fast growth rates since its doubling time is around half-a-day with intense photosynthetic activity [41], the harvested *C. vulgaris* biomass was subjected to biomethanation in which the specific methane yield was of 233 ml/g VS added was observed. Similarly, BMP obtained in this study is comparable with the results of other studies where 286 ml/g of VS was reported for *C. vulgaris* while methane yields of *Dunaliella tertiolecta* were reported to be 24 ml of CH_4 /g of VS. BMP result indicates that *C. vulgaris* has 10 to 12 folds of higher methane production than *Dunaliella tertiolecta* [42].

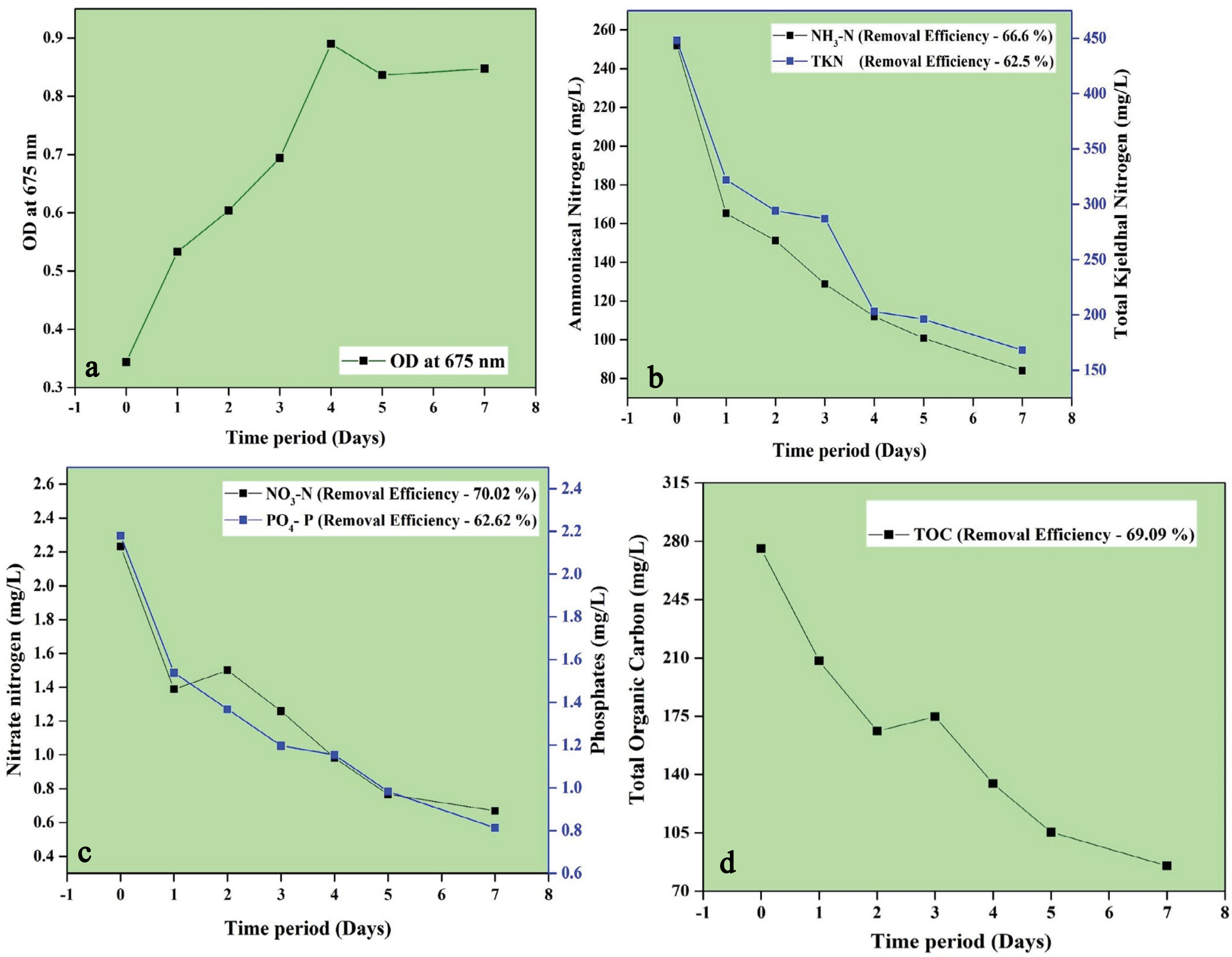

Fig. 6. The efficiency of *Chlorella vulgaris* in secondary treated tannery wastewater with the optimized F/M ratios. (a) Growth profile of *Chlorella vulgaris* in the tubular PBR, (b) profile of NH_3–N and TKN in the tubular PBR, (c) profile of nitrate and phosphate in the tubular PBR, and (d) profile of TOC in the tubular PBR.

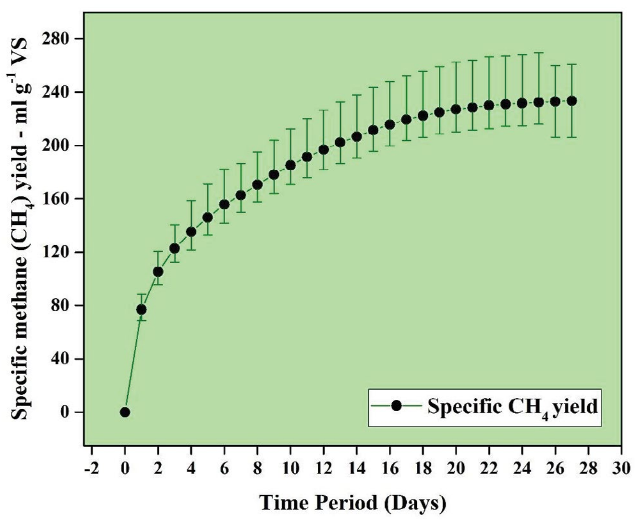

Fig. 7. Specific CH_4 production during anaerobic digestion of *Chlorella vulgaris* in the AMPTS II.

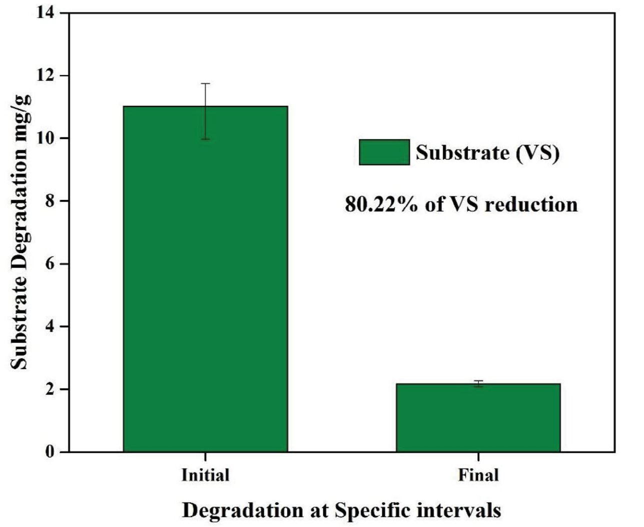

Fig. 8. Substrate degradation during the anaerobic digestion of *Chlorella vulgaris* in the AMPTS II.

This variation in the production of biogas is mainly due to its digestibility of algal biomass. It is also reported that some species of *Chlorella* sp., possess sporopollenin which has highly resistant outer layers and needs some pre-treatment in order to make the cell components digestible. However, few species including the species studied in the present work do not have the resistant cell wall component sporopollenin, which has relatively high digestibility (up to 81.7%), thus adding an additional advantage to make the process easier and less expensive [43,44]. From the results of the current study, it is observed that sufficient biomass could be retained throughout the treatment in order to improve the removal efficiency and the excess biomass could be used for bio-methane production. Based on the results of this study, it is suggested that *C. vulgaris* (phycoremediation) could be a better alternative option to remove nutrients from secondary treated tannery effluents, and the microalgae grown in the effluent could be used as an energy source through biomethanation.

5. Conclusion

This study concludes that there is a promising aspect in the assimilation of ammoniacal nitrogen from secondary treated tannery effluents by *C. vulgaris*. Moreover, the harvested microalgal biomass from the treatment has a high economic value for the production of bio-energy as a potential source of renewable energy.

Regulatory requirements for the discharge of tannery effluent are becoming stringent. To meet the discharge standards for ammoniacal nitrogen, the study suggests that *C. vulgaris* could be a better alternative for the significant removal of nutrients from secondary tannery effluents coupled with energy production. Since phytoremediation is being an upcoming research area with promising aspects in wastewater treatment and biofuel production, further studies will be dealing with other operational parameters such as variation in light intensity, CO_2 levels, and also design parameters of photo-bioreactors for the betterment of treatment efficiency.

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