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Carbon-dioxide biofixation and phycoremediation of municipal wastewater using *Chlorella vulgaris* and *Scenedesmus obliquus*

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Abstract The pure cultures of microalgae *Chlorella vulgaris* ATCC 13482 and *Scenedesmus obliquus* FACHB 417 were grown in municipal wastewater in 7-L airlift bubble column photobioreactor supplied with 5% CO_2/air (*v*/*v*). Batch experiments were conducted at 25 °C with 14-h light/10-h dark cycle for a period of 10 days. The CO_2 capture efficiencies for both the microalgae were monitored in terms of their respective biomass productivities, carbon contents, and CO_2 consumption rates. In the present study, the initial concentration of ammonia (43.7 mg L^{-1}) was decreased to 2.9 and 3.7 mg L^{-1} by *C. vulgaris* and *S. obliquus*, respectively. And, the initial concentration of phosphate (18.5 mg L^{-1}) was decreased to 1.1 and 1.6 mg L^{-1} by *C. vulgaris* and *S. obliquus*, respectively. CO_2 biofixation rates by *C. vulgaris* and *S. obliquus*, cultivated in municipal wastewater, were calculated to be 140.91 and 129.82 mg L^{-1} day^{-1}, respectively. The findings from the present study highlight the use of microalgae for wastewater treatment along with CO_2 uptake and biomass utilization for pilot scale production of biodiesel, biogas, feed supplements for animals, etc., thus minimizing the production costs.

Keywords *Chlorella vulgaris* · CO_2 uptake · Municipal wastewater · Photobioreactor · *Scenedesmus obliquus*

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Introduction

Energy dependence on fossil fuels and global climate change are the two pertinent issues posing serious environmental threats to the modern society. Hence, there is an alarming call to cut down global emissions of anthropogenic CO_2. Until now, major contribution for the electricity generation and transportation has been shared by coal and petroleum, respectively. According to an estimate by Boden et al. (2015), annual global carbon emissions from the use of fossil fuels crossed ~9 billion tons (or 33 Gt CO_2) in 2011. CO_2 alone contributes to more than two-thirds of total greenhouse gas emissions from human undertakings (Ho et al. 2011). CO_2 discharge from the fossil fuel burning in 2014 was 60% above the emissions in 1990 being the reference year for the Kyoto Protocol (Global Carbon Emissions 2016). Atmospheric CO_2 concentration even crossed an appalling figure of 407.9 ppm in May 2016 (Scripps Institution of Oceanography 2016) from the pre-industrial level of 280 ppm (Allas et al. 2007).

Researchers have tried with physical and chemical methods of CO_2 capture and storage into deep oceans, mineral formations, or enhanced oil recovery (Metz et al. 2005, Leung et al. 2014, Rao and Rubin 2002). But these technologies have inherent shortcomings and trigger further environmental risks

too. On the other hand, it has been suggested that biological method of CO_2 sequestration causes an overall negative greenhouse gas emissions and thus might prove to be a viable carbon sink. For the sustainable bioenergy options from algae, wastes are converted into methane (Hernandez et al. 2014; Ward et al. 2014), hydrogen (Ullah et al. 2014), biodiesel (Kligerman and Bouwer 2015; Galadima and Muraza 2014), and electricity (Gajda et al. 2015). In yet another novel approach, researchers are harnessing the potential of CO_2 sequestration by photosynthetically growing algae or cyanobacteria. Algae-mediated utilization of CO_2 is considered as one of the most promising negative emission technologies. Additionally, algae use the wastewater for nutrient supplementation and eventually cause bioremediation of the wastewater. Energy (biodiesel or methane) obtained from the harvested microalgae is comprehensibly carbon-neutral and such projects earn carbon credits too.

Although CO_2 emissions from petroleum and biodiesel are nearly equal, the foremost difference lies in carbon consumption during initial stages of the life cycle of these fuels. Edible vegetable oil fuels are more expensive than petroleum and have also continuously raised concern over "food versus fuel" debate. It has also been reported that traditional terrestrial plants (sugarcane and maize for ethanol; palm, groundnut, rapeseed, and sunflower for oil) have slower growth rates; they are seasonal and can contribute to only 3–6% reduction in global CO_2 emissions. In order to put forth a remedial measure, Chisti (2007, 2008) supported bioethanol and biodiesel from microalgae over oil crops as a renewable fuel having tremendous potential to replace the current conventional petroleum-based transport fuels. Many experimental findings suggest that there are numerous benefits in using microalgae-based systems for management of wastewater. Microalgae are 10–50 times more efficient in CO_2 sequestration than those by conventional terrestrial plants (Costa et al. 2000; Wang et al. 2008). Some microalgae like *Chlorella* can even consume combustion products such as NO*x* or SO_2 from flue gas (Costa et al. 2000; Cuellar-Bermudez et al. 2015). Algae require comparatively less water than the agricultural crops and they are also cultivated in saline/brackish water on non-arable lands as the case may be.

Several studies (de Morais and Costa 2007; Sydney et al. 2010) reiterate the possibility of microalgae-mediated capture of CO_2 from the simulated flue gases composition with CO_2 fixation efficacy within 28–53%. Microalgae exhibit faster growth rates than that of the terrestrial green plants, and therefore, they have better rates of CO_2 sequestration (Fulke et al. 2010). *Chlorella* and *Scenedesmus* species are widely accepted for the very promising potential of carbon sequestration (Fulke et al. 2010; Ho et al. 2010; Tang et al. 2011; Toledo-Cervantes et al. 2013). CO_2 is utilized as the carbon source by microalgae for their cellular growth, and hence, they act as micro-biofactories consuming greenhouse gas (CO_2). Other than feed supplements for animals, growing microalgae may also yield commercial products of high value like neutraceuticals, cosmetics, and pharmaceuticals (Cardozo et al. 2006; Gong et al. 2011) which can compensate for the capital investment and the operational costs. Therefore, coupling the commercial benefits of microalgae with CO_2 fixation and/or recycling process for wastewater treatment or biodiesel production/methane generation will help minimize environmental impacts of energy consumption during biodiesel production, and net energy gain (NEG) will surely be positive.

The objectives of the present work were to cultivate the microalgae, *Chlorella vulgaris* ATCC 13482 and *Scenedesmus obliquus* FACHB 417, in municipal wastewater, and to quantify their efficiencies for uptake of inorganic carbon CO_2. The wastewater was collected from the primary sedimentation tank of water reclamation plant (Ulu Pandan, Singapore). CO_2/air supply was maintained at 5% *v*/*v* during the light hours and only air was supplied during the dark hours. The final characteristics of the municipal wastewater treated simultaneously were also determined. Dry algal biomass and its elemental carbon contents during the experimental period were calculated to evaluate CO_2 uptake efficiencies of the two microalgae.

Materials and methods

Microalgae culture

The stock cultures of *Chlorella vulgaris* ATCC 13482 and *Scenedesmus obliquus* FACHB 417 were maintained in Bold's basal (BB) medium as recommended for freshwater algae (https://www.ccap.ac.uk/media/documents/BB.pdf) in the Corning cell culture flasks (surface area, 100 cm^2) maintained at 25 °C with fluorescent illumination of 90 ± 5 μmol m^{-2} s^{-1} operated in 14-h light/10-h dark cycle. The BB medium (per liter of DI water) contained the following macro-nutrients: 0.25 g $NaNO_3$, 0.075 g $MgSO_4 \cdot 7H_2O$, 0.025 g NaCl, 0.075 g K_2HPO_4, 0.175 g KH_2PO_4, 0.025 g $CaCl_2 \cdot 2H_2O$, and 1-mL trace elements solution. The trace elements solution was autoclaved to be dissolved and contained the following (per liter of DI water): 8.82 g $ZnSO_4 \cdot 7H_2O$, 1.44 g $MnCl_2 \cdot 4H_2O$, 0.71 g MoO_3, 1.57 g $CuSO_4 \cdot 5H_2O$, 0.49 g $Co(NO_3)_2 \cdot 6H_2O$, 11.42 g H_3BO_3, 50 g EDTA, 31 g KOH, 4.98 g $FeSO_4 \cdot 7H_2O$, and 1 mL H_2SO_4 (conc.). All the chemicals used for preparing the culture medium were of analytical grade (Sigma-Aldrich, Singapore).

Characterization of wastewater parameters

The municipal wastewater (MW) was collected from the primary sedimentation tank of the Water Reclamation Plant at Ulu Pandan (Singapore) and stored at 4 °C until further

Table 1 Water quality indicators of the municipal wastewater

Parameters	Value (mean ± SD)
pH	6.6 ± 0.03
Chemical oxygen demand, COD (mg L^{-1})	293 ± 3.3
Total nitrogen, TN (mg L^{-1})	46.67 ± 0.27
Ammonia (mg L^{-1})	43.67 ± 0.72
Nitrate, NO_3^- (mg L^{-1})	1.64 ± 0.18
Total phosphorus, TP (mg L^{-1})	19.5 ± 0.24
Orthophosphate, PO_4^{3-} (mg L^{-1})	18.53 ± 0.05
C/N	6.7
N/P	2.3

n.d. not detectable, *C/N* COD/ammonia, *N/P* ammonia/orthophosphate

characterization. The wastewater sample was filtered through 0.45-μm filter to remove the suspended particles before the estimation of its quality indicators (given in Table 1) as per Standard Methods for the Examination of Water and Wastewater (APHA 2005). The chemical kits and DR900 colorimeter (Hach, USA) were used to estimate chemical oxygen demand (COD), total nitrogen (TN), ammonia, nitrate, total phosphorus (TP), and orthophosphate (PO_4^{3-}). pH of wastewater sample was measured using multi-parameter analyzer (3200M Agilent Technologies, USA).

Microalgae cultivation and acclimatization

Ready to use inoculum of the two algae strains were cultivated in 2-L cylindrical glass bottles maintained at 25 °C in BB medium. Sterile air passing through 0.2-μm PTFE filter was fed at 0.2 vvm, i.e., 0.2 L min^{-1} of gas per liter of culture. The microalgae culture was maintained at pH 7.0 using 1-M NaOH solution. *C. vulgaris* and *S. obliquus* were cultured for 10 days in BB medium and then acclimatized to municipal wastewater in conical flasks of 3 L working volume (Fig. 1) uniformly mixed at 100 rpm on a magnetic stirrer. The inoculum:wastewater ratio was 1:20, i.e., 5% *v/v* for starting the experiment of algal CO_2 utilization in 7-L airlift bubble column photobioreactors (PBRs).

Photobioreactor setup

The airlift bubble column PBR (Fig. 2) was used for this experiment. The PBRs (diameter 0.1 m, height 1 m, working volume 7 L) were fed with undiluted municipal wastewater and the algae inoculum. An artificial set of cool white fluorescent light (3 tubes ×24 W) was used to maintain 14-h light/10-h dark cycle. The reactors were supplied with gas (air +5% CO_2 *v/v*) at flow rate of 1.4 L min^{-1} (superficial gas velocity 0.0013 m/s) equal to 0.2 vvm (gas volume per liquid volume per min) through the bottom of the reactor. The input of gas (air + CO_2) at flow rate <0.2 vvm is insufficient to provide enough mixing, and sedimentation of the algae was the recurrent problem. Though the gas flow rates >1 vvm have better mixing and mass transfer effects to support higher biomass concentration, they have negative impacts considering greater power consumption and the resultant high shear stress on the algal cells. For adequate mixing of nutrients in the medium thereby disrupting diffusion barriers at the algal cell surfaces, gas flow rate in range 0.2–0.4 vvm has been reported in literature (Guo et al. 2015; Kargupta et al. 2015). Furthermore, proper mixing helps in uniform distribution of light to algae cells averting the dark zones, prevents buildup of the oxygen produced during photosynthesis, and subsequently, checks the potential oxidative stress. All the facilities were set up in temperature-stabilized laboratory at 25 °C throughout the experiment.

Analytical estimation

Dry algae weight

The biomass concentration of algae was calculated as its dry weight (g L^{-1}) as per the method described by Guo et al. (2015). The harvested algal biomass was centrifuged (Eppendorf, Germany) at 5600 rpm for 10 min. After

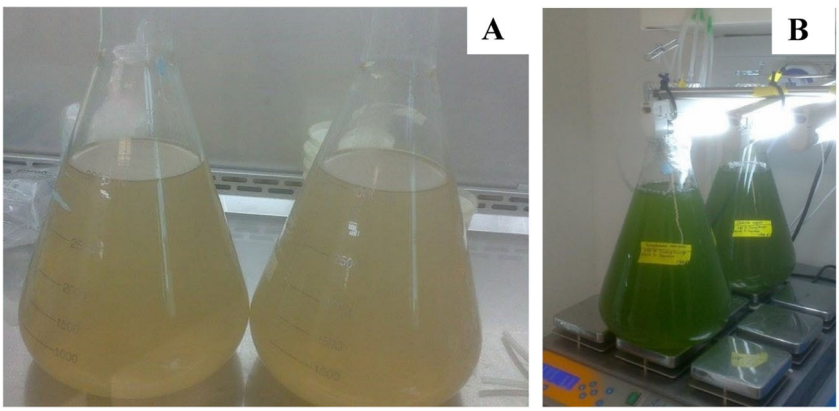

Fig. 1 Conical flasks with municipal wastewater (**a**) and algae inoculum cultivated in municipal wastewater (**b**)

Fig. 2 Airlift bubble column 7-L PBR

discarding the supernatant, the cell pellets were washed thrice with DI water and dried in an oven at 105 °C for 24 h, and the weight was estimated by gravimetric method. The biomass productivity (g L^{-1} day^{-1}) denoted by $P_{biomass}$ is given by Eq. (1):

$$P_{biomass} = \frac{W_t - W_o}{t}, \quad (1)$$

where W_t and W_0, respectively, represent the dry algae mass and the initial biomass concentration; and t represents the cultivation period.

Elemental analysis of algae biomass

The dried algal biomass was pulverized into fine powder using a mortar and pestle and then analyzed for their elemental carbon contents (C_{carbon}, wt.%) using Elementar Vario Micro Cube (GmbH, Germany) at the Department of Chemistry, National University of Singapore.

CO_2 utilization and conversion into biomass

According to the method described by de Morais and Costa (2007), the CO_2 biofixation rate (mg CO_2/L·day) denoted by F_{CO2} is given by Eq. (2):

$$F_{CO_2} = C_{carbon} P_{biomass} \left(\frac{M_{CO_2}}{M_C} \right), \quad (2)$$

where M_C was the molecular weight of carbon, M_{CO2} was the molecular weight of CO_2, and C_{carbon} was the carbon content (wt.%) in the algal biomass.

The percentage efficiency (E_{CO2}) of conversion of CO_2 into algae biomass is given by Eq. (3):

$$E_{CO_2} = \frac{F_{CO_2} V_{column} t}{\rho_{CO_2} V_{CO_2}} \times 100, \quad (3)$$

where V_{column}, V_{CO2}, and ρ_{CO2} represent the working volume of the PBR, total CO_2 consumed (vol.) during the experimental period, and the density of CO_2, respectively.

Results and discussion

Microalgae growth measurement

Municipal wastewater (7 L) was inoculated with the above cultured *C. vulgaris* and *S. obliquus* into PBRs (triplicates for each algae species). The initial inoculum density of the microalgae was adjusted to be at 0.1 g L^{-1} for each reactor. The cell growths of the two microalgae grown in municipal wastewater with normal air (0.03% *v*/*v* CO_2) and 5% *v*/*v* CO_2 were estimated by cell density measurement using UV/Vis spectrophotometer (Shimadzu, Japan). The relationship between optical density (OD_{680}) and the dry cell weights of *C. vulgaris* ATCC 13482 and *S. obliquus* FACHB 417 was established by linear regressions given in the Supplementary Material (Figs. S1 and S2). Biomass productivities and specific growth rates calculated for *C. vulgaris* and *S. obliquus* are given in Table 3.

Table 2 Comparison of total CO_2 consumption in microalgae cultivations previously reported in the literature with our present study

Microalgae species	CO_2 (vol%)	Culture medium	Cultivation time (day)	PBR working volume (L)	CO_2 consumption (L/day)	Reference
C. vulgaris	5	Wastewater with high ammonia	20	1.5	10.8	He et al. (2013)
C. vulgaris	15	Wastewater with high nitrate	10	1	43.2	Jin et al. (2006)
Chlorella sp.	20	Modified f/2 medium	7	50	240–1440	Kao et al. (2012)
C. vulgaris	4	BG-11 medium	10	1	8.64	Mujtaba et al.(2012)
C. vulgaris	2	BB (3N+V) medium	7	7.5	34.04	Guo et al. (2015)
C. vulgaris and *S. obliquus*	5	Municipal wastewater	10	7	58.8	This study

Total CO_2 consumption = CO_2 (vol%) × vvm × PBR working volume × cultivation time

Table 3 Mean values for biomass productivity, CO_2 biofixation rate, and CO_2 sequestration rate of *C. vulgaris* and *S. obliquus* grown in PBR for 10 day

Growth conditions	Algae biomass (mg L^{-1})	Specific growth rate μ (day^{-1})	Biomass Productivity (mg L^{-1} day^{-1})	Carbon content (wt.%)	CO_2 biofixation rate (mg L^{-1} day^{-1})	CO_2 sequestration rate (mg CO_2/L CO_2)
Cv + air	178.95	0.06	17.9	49.96	+	+
Cv + 5% CO_2	941.48	0.22	94.1	50.4	140.91	16.78
So + air	166.67	0.05	16.7	50.1	+	+
So + 5% CO_2	865.44	0.21	86.5	50.67	129.82	15.45

+not applicable

Estimation of CO_2 biofixation rate

C. vulgaris and *S. obliquus* were grown in undiluted MW (filtered and autoclaved MW). Initial inoculum of the algae was obtained from the acclimatization study for 10 days. Suspended solids (78 mg L^{-1}) was determined after filtering MW through 50-μm stainless steel filter mesh sieve (GmbH, Germany), and its value was subtracted from the dried algal biomass to get the net algae dry weight. The amount of CO_2 consumed (L/day) during the cultivation period in our study has been compared with similar observations from previously reported studies (Table 2).

The estimation of CO_2 biofixation rates for *C. vulgaris* and *S. obliquus* was done using Eq. (2). The stepwise calculation of CO_2 sequestration (denoted as S_{CO2}) using Eq. (4) is given in the Supplementary Material (Appendix A).

$$S_{CO_2} \approx \frac{F_{CO_2}}{NetCO_2 \text{supplied}} \tag{4}$$

The results from the elemental analysis showed that the carbon contents (C_{carbon}, wt.%) of *C. vulgaris* and *S. obliquus* were marginally higher when 5% CO_2 was supplied during aeration than that of bubbling only air (Table 3). In our work, CO_2 biofixation rates for *C. vulgaris* and *S. obliquus* were found to be 140.91 and 129.82 mg L^{-1} day^{-1}, respectively. The efficiencies of CO_2 conversion into biomass (E_{CO2}) were calculated to be 14.9% by *C. vulgaris* and 13.8% by *S. obliquus* given in the Supplementary Material (Appendix A).

It is very well documented in literature that dissolution of gaseous CO_2 into water induces principally three carbon species: CO_2, HCO_3^-, and CO_3^{2-}, which are inorganic carbon sources for microalgae. Owing to the low solubility of inorganic carbon species in water, CO_2 availability is limiting factor for the microalgal photosynthesis, and hence, microalgae have evolved carbon concentrating mechanisms (CCMs) that augment CO_2 concentration by the enzyme RubisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) (Trimborn et al. 2009). However, microalgae vary widely in their preferences to the utilization of carbon sources (Trimborn et al. 2009). For some species, major flux of dissolved inorganic carbon into microalgal cells is the direct CO_2 uptake across the plasma membrane (Spalding 2008).

The CO_2 input conditions in the present study were manually adjusted as per the light and dark conditions. Instant online control of CO_2 input is henceforth necessary based on the feedback from CO_2 and light sensors. Moreover, a feedback sensor connected with pH variations of the microalgal culture is needed to regulate on/off for CO_2 inlet. When pH exceeds 9.0, there will be inlet of CO_2 into the algal culture whereas when pH drops below 6.0, CO_2 supply will be cut off. This feedback regulated operation will also save the unnecessary loss of CO_2.

The importance of the present study is also relevant for the production of fatty acid methyl esters (FAME) which are starting materials for biodiesel. A study on CO_2 input conditions by Guo et al. (2015) has brought forward the point that

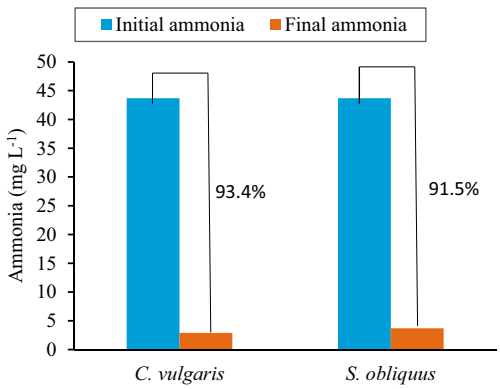

Fig. 3 Ammonia removal by *C. vulgaris* and *S. obliquus* in 7-L PBR for period of 10 days

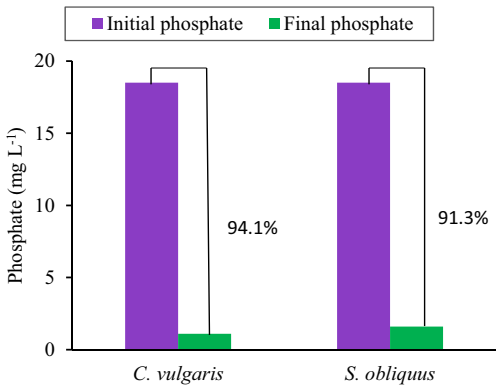

Fig. 4 Phosphate removal by *C. vulgaris* and *S. obliquus* in 7-L PBR for period of 10 days

Table 4 Water quality of the municipal wastewater after treatment by *C. vulgaris* (Cv) and *S. obliquus* (So) in 7-L PBR

Parameters	Treatment[a] by Cv	Treatment[a] by So	Discharge standards in Singapore (National Environment Agency 2016)		Discharge standards in India (CPCB 1986)
			Watercourse	Controlled watercourse	Inland surface water
pH	8.1	8.2	6–9	6–9	5.5–9
COD (mg L^{-1})	69.4	70.5	100	60	250
Ammonia—N (mg L^{-1})	2.9	3.7	*	*	5
Phosphate—P (mg L^{-1})	1.1	1.6	5	2	5

* means not given

[a] Present study (using 5% *v*/*v* CO_2)

augmented CO_2 concentration with air supply into microalgal culture increases polyunsaturated fatty acids (PUFA). Tang et al. (2011) also highlighted that CO_2 concentration higher than the ambient air was favorable for the accumulation of PUFAs in microalgal cells.

Quality indicators of treated wastewater

The removal percentages of ammonia and phosphate were measured over the experimental period of 10 days (Figs. 3 and 4). In the present study, ammonia was decreased by 93.4 and 91.5% and phosphate was decreased by 94.1 and 91.3% by *C. vulgaris* and *S. obliquus*, respectively. Lau et al. (1996) reported the removal of 86% inorganic nitrogen and 70% inorganic phosphorus using *Chlorella* sp. The removal of nitrogen and phosphorus by both the microalgae bubbled with 5% *v*/*v* CO_2 in our work is comparable with similar studies by Feng et al. (2011), Sydney et al. (2011), McGinn et al. (2012), Ji et al. (2013), and Discart et al. (2014).

The increase in pH with growth of algal biomass induces precipitation of phosphorus as calcium phosphates (Hammouda et al. 1994). Microalgal photosynthesis is associated with increase in pH of the culture medium which further enhances NH_3 stripping or P precipitation thereby causing nutrient removal (Nunez et al. 2001; Nurdogan and Oswald 1995; Oswald 2003). Alkaline condition (pH >8) also inhibits coliform bacteria (Lefyedi and Taylor 2006; Nilsson et al. 2013). Alkaline pH is also suitable for the growth of green microalgae (Olaizola et al. 2004; Suryata et al. 2010) allowing better capture of inorganic CO_2 (dissolved in liquid) and uptake by algae (Suryata et al. 2010). Alkaline pH (range 8–9) was observed over the experimental duration (data not shown) which meets the effluent discharge standards set by the Environment (Protection) Rules, India (CPCB 1986). Final characteristics of the wastewater treated by the microalgae in our study (Table 4) follow the range for trade effluents discharged into watercourse/controlled watercourse set by Public Utilities Board of Singapore (National Environment Agency 2016).

Conclusion

In the present study, the two species of green microalgae *C. vulgaris* ATCC 13482 and *S. obliquus* FACHB 417 were grown in the municipal wastewater. Both of the species effectively treated undiluted municipal wastewater. *C. vulgaris* proved to be better than *S. obliquus* in terms of wastewater treatment efficiency, biomass generated, and CO_2 fixation rate over the test period. As compared with PUB effluent discharge standards, final wastewater after algal treatment in the present study had significantly lower ammonia and phosphate. The results in our work demonstrated that *C. vulgaris* ATCC 13482 and *S. obliquus* FACHB 417 can be potential microalgae species to integrate the approach of wastewater treatment with CO_2 fixation thereby scoring positive points over conventional chemical methods of CO_2 capture. Harvested algae biomass after treatment could be used for biomethane production under anaerobic digestion and/or be harnessed for lipid/biodiesel extraction. The study presented in our work is environmentally more sustainable as it does not use synthetic culture medium to cultivate microalgae and also takes into account the wastewater treatment.

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