

Rapid and de-centralized model for municipal effluent reclamation using microalgae

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Abstract

Microalgal bioremediation is currently being venerated for its potential in municipal liquid waste (MLW) treatment. Algae-based water treatment with retention time competitive to the conventional water treatment processes is a challenge. The present study investigated the role of algal biomass concentration to improve treatment efficiency to reduce the time required for water treatment. Eighty percent removal of pollutants (in terms of chemical oxygen demand (COD), ammonia, phosphate and fecal coliforms) was obtained in 12 hours at a biomass concentration of 1 gL⁻¹. Further, continuous treatment of MLW using membrane-assisted photobioreactor was established. The treatment led to >95% removal of ammonia, >75% removal of COD and 100% removal of fecal coliforms within 12 hours. Physiological assessment of the algal culture showed that the cells did not manifest symptoms of stress throughout the reactor cycle, a consequence of continuous availability of the nutrients, maintaining the culture in continuous growth state.

Key words: algal bioremediation, culture perfusion, de-centralization, hydraulic retention time, sewage water treatment

Highlights

- De-centralization of water treatment.
- Rapid water treatment with retention time of 10 h.
- Photosynthetic systems for water treatment.
- Biomass produced which can be utilized for value-added products generation.
- Zero-waste discharge systems.

LIST OF ABBREVIATIONS AND ACRONYMS

MLW	Municipal liquid waste
ASP	Activated sludge process
COD	Chemical oxygen demand
UN	United Nations
STP	Sewage treatment plant
SRT	Solid retention time
m-PBR	Membrane-based photobioreactors
mg·L ⁻¹ h ⁻¹	Milligram per liter per hour

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MLD	Million liters per day
MJ	Mega joules
$\mu\text{M.m}^{-2}.\text{s}^{-1}$	Micromoles per square meter per second (micro Einstein)
USD	US dollar
t-PBR	Tubular photobioreactor
UASB	Up-flow anaerobic sludge blanket
NER	Net energy ratio
EL	Environment laboratory
HRT	Hydraulic retention time
g.L^{-1}	Grams per liter
KJ.L^{-1}	Kilo joules per liter
Mg	Mega grams
kL	Kilo liter

INTRODUCTION

Out of the total water available on Earth, only 2.5% is in freshwater form, of which only 21% is available for human use. Rapid increase in population and urbanization has already created a global water deficit due to imbalanced demand and finite supply and hence, restoration of the balance is necessary (Corcoran *et al.* 2010). There has been notable development in water supply and water treatment in urban areas in recent years, however, these efforts still lag behind in the current trend of urbanization and need for sanitation (Bajpai *et al.* 2019). These limitations of heavy economic investments, land availability and sustainability have pushed the need to explore and expand de-centralized water treatment systems (Bajpai *et al.* 2019).

Use of microalgae for wastewater treatment has been widely reported with limited exploration at a community and de-centralized scale (Pacheco *et al.* 2020). Microalgae-based wastewater treatment has the capacity to simultaneously capture COD and nutrients (N and P) and obliterate multiple steps involved in conventional methods (Figure 1). Typically, algae fix approximately 1.8 kg of carbon dioxide per kg of biomass (Sudhakar *et al.* 2011) and the biomass itself stores 12.24 MJ energy in a unit kilogram of dry biomass (Sturm & Lamer 2011). Thus, the use of algal biomass to capture energy from the waste sources and conversion to a re-usable form makes it an attractive option for environmentally friendly water treatment processes.

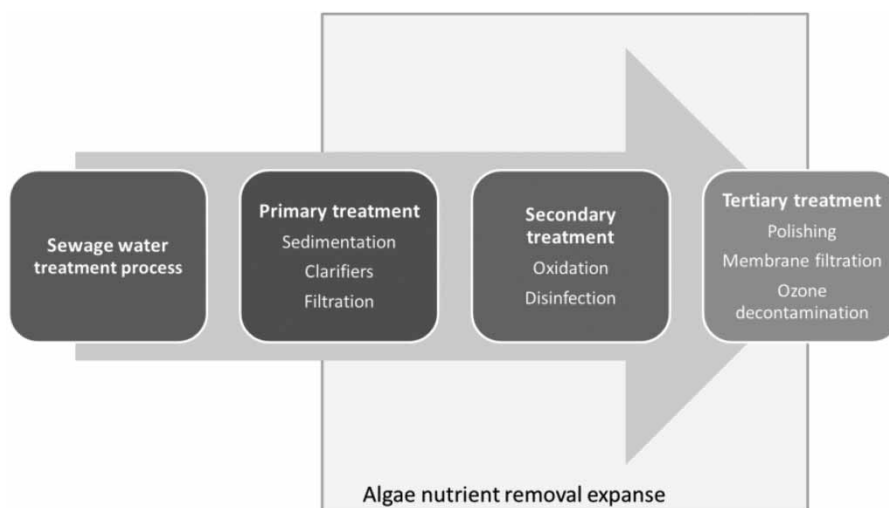


Figure 1 | The nutrient removal potential of algae: single step process.

To economize the algal bioremediation process, it is essential to channelize the algal biomass for food, feed or fuels post its utilization as a remediating agent. This necessitates maximizing algal

biomass production without hampering the bioremediation process, essentially by un-coupling the hydraulic retention time (HRT) and solid retention time (SRT) (Marbelia *et al.* 2014). One of the successful routes to achieve this is, the use of membrane filtration technology of continuous removal of treated water, while retaining the biomass in the system (Low *et al.* 2016; Kumar *et al.* 2020). The membrane filtration technology, however, suffers from disadvantages of membrane fouling due to biomass and the pumping costs involved. Nonetheless, by optimizing the parameters, it is possible to achieve high biomass productivity and simultaneously treat sewage water to a higher degree of purity. Due to the drawbacks, the use of membrane filtration technology has been restricted to simulated sewage water with low biomass concentration and very few studies have been carried out using real domestic effluent (Luo *et al.* 2017; Solmaz & Işık 2019). The highest reported biomass productivity using these technologies for treating real domestic effluent is $2.5 \text{ gL}^{-1}\text{D}^{-1}$, however, with an HRT of 3.4 days.

Considering the advantages and drawbacks of algal bioremediation technology, the need of the hour is to fine-tune the balance between low HRT and high algal biomass productivity. With these challenges in focus, the key objective of our study was economizing the water treatment system by reducing the HRT and simultaneously generating high value algal biomass. Hence, we designed continuous system for treating real domestic effluent using suspended algal culture and membrane filtration technology. Reduction in HRT was obtained by optimizing the concentration of algal cell culture, without compromising the membrane performance. By optimizing the cell culture concentration, it was possible to reduce the SRT as well with high biomass productivity. Reduced HRT has an advantage of reducing land footprint and paving way for de-centralized water treatment in crowded urban set-up.

MATERIALS AND METHODS

Sewage water collection

Primary treated sewage water was acquired from the local sewage pumping station at Worli, Mumbai. Sewage water in batches of 10 L each was collected and stored at 4 °C until further experimentation. Usage of one batch of sewage water was restricted to a maximum period of one week. To ensure the uniformity in the sewage pollutant parameters, water samples were collected every month and tested in the lab for the pollutant levels. The average concentration of pollutants has been listed in Table 1.

Table 1 | Average concentration of pollutants in sewage water collected from local sewage pumping station

Pollutant	Concentration
Ammonia (mgL^{-1})	69.5 ± 3.1
COD (mgL^{-1})	166.6 ± 13.0
Phosphate (mgL^{-1})	5.0 ± 0.5
Fecal coliforms (MPN/100 ml)	20.0 ± 2.0

Culture maintenance, medium and growth

Microalga *Chlorella saccharophila* UTEX#247 was procured from the algal repository of the University of Texas at Austin, USA. The culture was maintained in BG-11 growth medium as described by (Stanier *et al.* 1971) and further modified to obtain optimum growth. Recipe for modified BG-11: NaNO_3 : $0.75 \text{ g}\cdot\text{L}^{-1}$; $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$: $75 \text{ mg}\cdot\text{L}^{-1}$; K_2HPO_4 : $40 \text{ mg}\cdot\text{L}^{-1}$; CaCl_2 : $36 \text{ mg}\cdot\text{L}^{-1}$; $\text{C}_6\text{H}_8\text{O}_7$: $6 \text{ mg}\cdot\text{L}^{-1}$; $\text{C}_6\text{H}_8\text{FeNO}_7$: $6 \text{ mg}\cdot\text{L}^{-1}$; $\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8$: $1 \text{ mg}\cdot\text{L}^{-1}$; Na_2CO_3 : $20 \text{ mg}\cdot\text{L}^{-1}$; H_3BO_3 : $2.86 \text{ mg}\cdot\text{L}^{-1}$; $\text{MnCl}_2\cdot 4\text{H}_2\text{O}$: $1.81 \text{ mg}\cdot\text{L}^{-1}$; $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$: $0.222 \text{ mg}\cdot\text{L}^{-1}$; $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$: $0.222 \text{ mg}\cdot\text{L}^{-1}$; $\text{Na}_2\text{MoO}_4\cdot 2\text{H}_2\text{O}$: $0.39 \text{ mg}\cdot\text{L}^{-1}$; $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$: $0.079 \text{ mg}\cdot\text{L}^{-1}$; $\text{Co}(\text{NO}_3)_2\cdot 6\text{H}_2\text{O}$: $0.049 \text{ mg}\cdot\text{L}^{-1}$.

Cultures were grown and maintained in polyethylene reactors of 1 L working capacity, continuously purged with air in specially designed sunlit environment laboratory (EL) with the temperature maintained at $\sim 30^{\circ}\text{C}$. The maximal natural light intensity for the exposure of culture was $1,500 \mu\text{M}/\text{m}^2\cdot\text{s}$ with natural diurnal variations. The culture was harvested in the active log phase and washed repeatedly to ensure complete removal of BG-11 salts. The biomass thus obtained was used as inoculum for all the experiments.

Experimental setup

Determination of optimum time for COD and pollutant removal

The culture concentration of *C. saccharophila* was varied and the time required for COD and ammonia removal from sewage water at these culture concentrations was assessed. The culture was inoculated in sewage water and the concentration was adjusted to 0.5 gL^{-1} , 1 gL^{-1} , 1.5 gL^{-1} and 2 gL^{-1} . The experiment was carried out in EL for 48 hours with set up similar to the set up similar to culture maintenance set-up using polythene reactors of the capacity 1 L. Samples for analysis were collected at regular intervals of 2 hours. All samples were centrifuged and supernatants were stored at -20°C till further analysis. The supernatants were analyzed for ammonia and COD content. From the data, the rate of COD and ammonia removal were calculated by the following equation:

$$X = C_t - C_{t+1} \quad (1)$$

where X is the pollutant removal rate (expressed as $\text{mgL}^{-1} \text{ h}^{-1}$), C_t is the concentration of a pollutant at time t (expressed as mgL^{-1}) and C_{t+1} is the concentration of a pollutant at the time one hour after t ($t + 1$), where t is the time elapsed (expressed in hours).

Continuous reactor setup for sewage water treatment

Continuous mode studies were carried out using customized, tubular photobioreactor (t-PBR) of the capacity 2 L as shown in Figure 2. The culture was circulated through a tubular array by a peristaltic pump (Watson Marlow 520-S) into a culture holdup tank. The pump flow rate was adjusted to maintain equal exposure of the cells in the light harvesting tubular array as well as the holdup tank (light:dark = 1:1). The holdup tank was provided with continuous sparging of air at the rate of 50 LPM for uniform mixing and stripping of the accumulated oxygen. Microfiltration polysulphone hollow fibre membrane unit with area 460 cm^2 , 0.65μ pore size and I.D. of 0.75 mm (GE Healthcare-Model-CFP-2-G-4X2MA) was placed at the interface of the light harvesting tubular array and the holdup tank. The hollow fibre membrane resulted in permeate (treated water in the current study) and retentate (algal cells, re-circulated back to the system). The flow rates of the sewage water entering the t-PBR and the treated water exiting the t-PBR were adjusted to equality using double head Watson Marlow-120S peristaltic pump. The cell concentration and flow rate of the inlet and outlet pump were set in accordance with the optimized values (cell concentration: 1 gL^{-1} ; retention time: 10 h). The samples were collected from inlet and outlet of the reactor at 2 hr time interval. The entire system was setup in EL exposed to the maximum natural irradiance of $\sim 1,500 \mu\text{M}/\text{m}^2\cdot\text{s}$ with a natural diurnal cycle and the temperature adjusted to an average of 30°C .

Monitoring for culture growth and cell recycle

The growth of culture was monitored by estimating the optical density of the culture at 750 nm on a UV-visible spectrophotometer (Model UV 3600 Plus; Shimadzu). Ash-free dry weights were

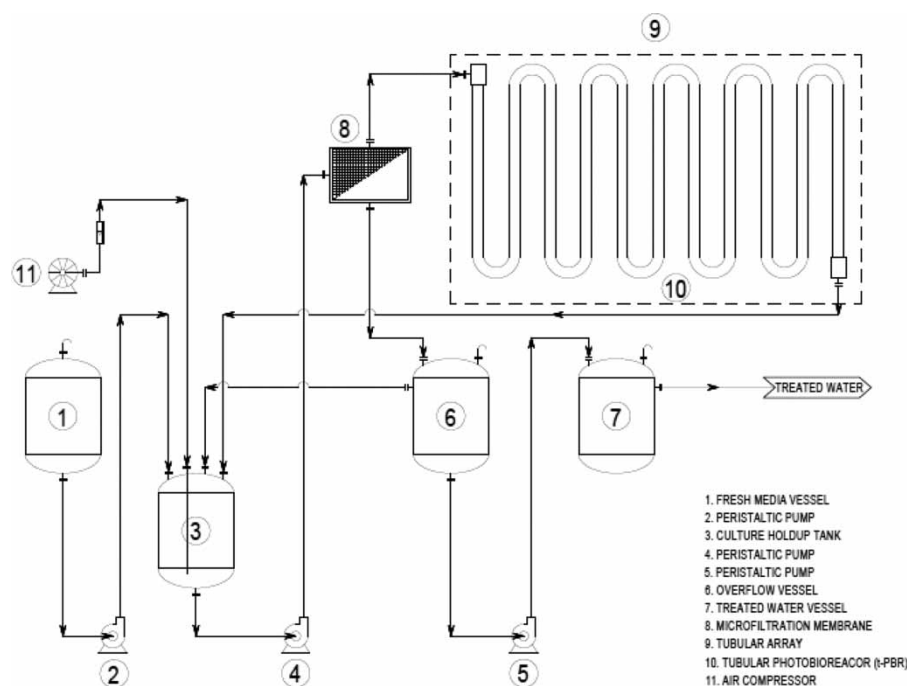


Figure 2 | Process flow diagram for the continuous system of t-PBR continuous photobioreactor.

determined on a daily basis throughout the growth cycle by drying the biomass at 60 °C until constant weights were obtained. To maintain constant culture concentration, the reactor was bled after every 24 h and the culture concentration was reset to 1 gL⁻¹ using fresh sewage water.

Analytical methods

Cell-free supernatant was obtained by centrifugation and used to assess the amount of nutrient load in the media.

Estimation of ammonia content

Ammonia content was measured by the phenate method as described in the APHA manual (APHA-4500-NH₃ F) (Clesceri *et al.* 1998). In brief, appropriate dilution of the supernatant was mixed with a concoction of 95% phenol, 0.5% sodium nitroprusside and oxidizing solution (alkaline citrate in sodium hypochlorite) and was further subjected to dark incubation at room temperature for 1 hour. The optical density of the samples was measured at 640 nm on a UV-Visible spectrophotometer to determine the actual concentration of ammonia, subsequently.

Estimation of COD concentration

The chemical oxygen demand (COD) estimation of the liquid samples was done by HACH COD kit of range HR (20–1500 mg/l COD) and the digestion was carried out in the digester (HACH DRB 200) for 120 minutes at 150 °C. The optical density of the digested sample was measured at 620 nm after digestion and cooling of vials to room temperature. The COD of the samples were calculated, based on optical density and standard equation.

Estimation of fecal coliforms

The fecal coliform population was determined by the standard MPN test followed by a confirmatory test (Clesceri *et al.* 1998). For the MPN test, samples were serially diluted up to 10^5 dilutions and were inoculated in Lauryl tryptose broth with the bromocresol purple indicator at 37 °C for 48 h. Change of the color of the media from purple to yellow indicated the formation of lactic acid, and hence, the presence of fecal coliforms. These tubes were further subjected to the confirmatory test using MacConkey's agar. Red colonies with opaque borders confirmed the presence of fecal coliforms.

Physiological analysis of algal culture in t-PBR:

Photosynthetic measurements

The maximum quantum yield of PS II was measured using pulse amplitude modulated fluorometer (PAM-fluorometer) (Dual PAM 100, Walz, Effeltrich, Germany). The sample was dark adapted for 10 minutes to obtain initial chlorophyll fluorescence, F_0 , followed by saturating light pulses of the intensity $6,000 \mu\text{M}/\text{m}^2\cdot\text{s}$ to further obtain maximal fluorescence F_m . The variable for the maximum chlorophyll fluorescence ratio was calculated as yielding the maximum quantum yield of PS II.

$$F_v/F_m = [(F_m - F_0)/F_m] \quad (2)$$

Pigment estimation

Pigment estimation was carried out as described by Ritchie (2006). The samples were centrifuged and the pellet thus obtained was re-suspended in methanol (1:1 v/v) and incubated in dark at 50 °C for 60 minutes to extract the pigments. The samples were centrifuged at 10,000 rpm for 5 minutes post-incubation. The optical density of the supernatant was estimated at 652 nm, 665 nm, and 480 nm on Shimadzu Spectrophotometer Model UV 3600 Plus. The chlorophyll *a*, *b* and carotenoid concentrations were calculated as mentioned below (Ritchie 2006).

$$\text{Chlorophyll } a \text{ } (\mu\text{g}/\text{ml}) = -8.0962 \times A_{652} + 16.5169 \times A_{665} \quad (3)$$

$$\text{Chlorophyll } b \text{ } (\mu\text{g}/\text{ml}) = 27.4405 \times A_{652} - 12.1688 \times A_{665} \quad (4)$$

$$\text{Carotenoids } (\mu\text{g}/\text{ml}) = 4 \times A_{480} \quad (5)$$

Statistical analysis

One-way ANOVA was used to test the statistical difference between algae culture concentration and pollutant removal time. The initial pollutant concentration was maintained constant throughout the experimentation. A significance level of $p < 0.05$ was applied.

RESULTS AND DISCUSSION

Optimum time for COD and pollutant removal

Removal of COD and ammonia are the major parameters assessed to determine the efficiency of water treatment. The removal efficiency of these pollutants was observed to vary with the culture

concentration. For all the culture concentration (0.5 gL^{-1} , 1 gL^{-1} , 1.5 gL^{-1} and 2 gL^{-1}), the removal efficiency was 95% in 24 hours (Figure 3(a)). A lag in the COD removal was observed with culture concentration of 0.5 gL^{-1} with an average removal rate of $1.6 \text{ mgL}^{-1} \text{ h}^{-1}$ in initial 12 hours. The rate of COD removal then increased to $10 \text{ mgL}^{-1} \text{ h}^{-1}$ in 12–24 h amounting to 95% removal of COD. At biomass concentration 1 gL^{-1} , the time required for the 95% removal of COD was reduced to 12 h, increasing the removal rate by 50% as compared to the rate at biomass concentration 0.5 gL^{-1} . Further increase in the culture concentration did not affect the COD removal rate and was maintained at $20 \text{ mgL}^{-1} \text{ h}^{-1}$.

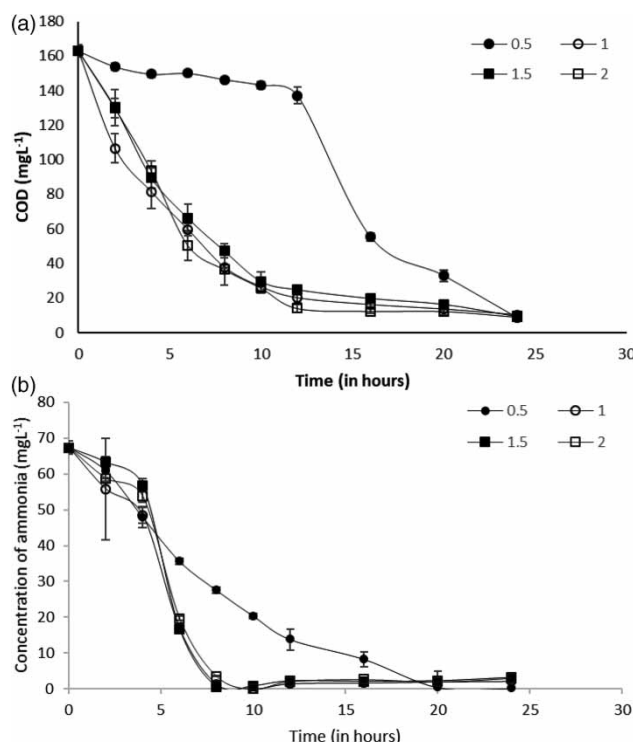


Figure 3 | Time required for COD capture (a) and ammonia capture (b) from sewage water with variation in culture concentration. The culture concentrations were varied from 0.5 gL^{-1} to 2 gL^{-1} . Each curve indicates the corresponding culture concentration in gL^{-1} (0.5 , 0.5 gL^{-1} (closed circles); 1 , 1 gL^{-1} (open circles); 1.5 , 1.5 gL^{-1} (closed squares); 2 , 2 gL^{-1} (open squares)).

Simultaneously, the capture of ammonia was achieved following a similar trend as COD removal. Hundred percent ammonia could be internalized by the cells within 20 h at culture concentration of 0.5 gL^{-1} (Figure 3(b)). The time required for complete ammonia capture was lowered by ca. >50% (i.e. 9 h) when the biomass concentration was increased to 1 gL^{-1} . Similar to COD removal, a further increase in the culture concentration did not increase in ammonia capture rate. The average rate of ammonia removal was $3.5 \text{ mgL}^{-1} \text{ h}^{-1}$ at culture concentration of 0.5 gL^{-1} , which increased to $7.7 \text{ mgL}^{-1} \text{ h}^{-1}$ at cell concentration of 1 gL^{-1} . The ammonia removal rate at culture concentration of 1.5 gL^{-1} and 2 gL^{-1} was $8.7 \text{ mgL}^{-1} \text{ h}^{-1}$. However, increase in the concentration from 1 gL^{-1} to 1.5 gL^{-1} did not significantly reduce the ammonia removal rate ($p = 0.04$). The optimum culture concentration for COD as well as ammonia uptake was 1 gL^{-1} . Hence, biomass concentration of 1 gL^{-1} and retention time of 12 h was chosen for the subsequent studies.

Our results demonstrate a pivotal role of biomass concentration in increasing the treatment efficiency. Lau *et al.* (1995) have shown that water treatment by algae is directly proportional to the growth and physiological state of algae, which in turn is proportional to the initial inoculum

concentration (Lau *et al.* 1995). There have been many reports regarding water treatment using algae with retention time ranging from 4 hours to 10 days (Wang *et al.* 2008). Algae immobilization experiments conducted by Zhang *et al.* (2008) have demonstrated N and P removal within 4 hours from wastewater when the cell density of *Scenedesmus* sp. in the immobilized matrix was 2×10^8 cells·ml⁻¹ (Zhang *et al.* 2008). In yet another study by Ruiz-Marin *et al.* (2010) reports, the time required for N and P removal from wastewater was 48 hours, when the cell density inside the immobilized beads was 2.3×10^7 cells·ml⁻¹ (Ruiz-Marin *et al.* 2010). From these reports, it is evident that faster pollutant removal from water can be achieved by increasing cell density. However, these studies have employed cell immobilization techniques, which has limited scope at higher scales. To date, this is the first report to our knowledge to treat sewage water in 12 h using suspension culture. Similar work was carried out by McGriff & McKinney (1972) with a resultant water treatment time of 10 h, however, a consortium (called algae sludge) consisting of algae, bacteria, protozoa and rotifers was used (McGriff & McKinney 1972). The disadvantage of such consortia is the shift in the dynamic composition of the consortia during the studies, with time and/or feed, which subsequently may affect the retention time. Our treatment strategy uses a monoculture of algae and thus offers advantages over algae-bacterial sludge-based treatment. However, our approach is confined by free monoculture suspension, resulting in uniform culture and treatment conditions, irrespective of change in feed.

One of the remarkable attributes of the current study is the time required for pollutant removal. Ninety-five percent removal of COD and 100% capture of ammonia was achieved within 12 h. Thus, the resultant treatment time is reduced significantly as compared to other studies using domestic sewage with free culture suspension. Our results presented a noteworthy aspect of culture performance for designing nutrient removal systems, where, constant culture concentration would be required for constant pollutant removal at steady state. It is evident from our results that, the culture concentration of 1 gL⁻¹ is a threshold concentration, beyond which, the rate of nutrient removal is unaffected. Also, higher cell densities results in self-shading, thus restricting photosynthesis and consequently affecting growth (Shigesada & Okubo 1981). Thus it is imperative to create a balanced micro-environment which not only ensures repletion of sewage water after 12 h (to avoid nutrient deplete condition) but also maintain the culture concentration of 1 gL⁻¹ (to manage the limitations imposed by high cell density cultures). To maintain such micro-environment necessitates to design a system which can support continuous influx of sewage water at a rate to maintain HRT of 12 hrs and intermittent bleeding of algal biomass at a rate to maintain cell density of 1 gL⁻¹.

Continuous efficient capture of pollutants from sewage water using tubular photobioreactor (t-PBR)

For continuous systems involving live culture, the specific growth rate of the culture has to be maintained in tandem with the dilution rate of the media. Our previous studies have shown that the doubling time of *C. saccharophila* is 2.3 days (data not shown). In contrast, algae capture COD and N from sewage water within 12 h. Based on the doubling time, studies were undertaken by adjusting the dilution rate to 2.3 days, which resulted in nutrient starvation, causing aberration of algal growth. Affected algal growth, in turn, impaired sewage treatment. This shortcoming was addressed by using perfusion culture in t-PBR, where sewage water was added to the system continuously and simultaneously treated water (cell-free) was removed from the system at the same rate. The tubular array of t-PBR allowed maximum light penetration in the system, further stimulating algal growth and pollutant capture. In the present study, the algal cells were retained into the reactor by using a hollow fibre membrane. Intermittent bleeding of algal biomass ensured maintenance of culture concentration and avoided self-shading and membrane fouling due to biomass deposition. There have been reports on the use of membrane-based photobioreactors (m-PBRs) for treatment of water, but, with treatment time ranging from 24 to 48 h (Luo *et al.* 2017). Under the current study, the t-PBR system has been improvised to reduce the retention time to 12 h.

In accordance with the results obtained in section Continuous efficient capture of pollutants from sewage water using tubular photobioreactor (t-PBR), the cell density of t-PBR was maintained to 1 gL^{-1} with the retention time of sewage water adjusted to 12 h. The reactor was monitored simultaneously, for nutrient capture as well as an increase in the cell density. The increase in biomass was 0.5 gL^{-1} after 24 h. The culture was diluted after every 24 h to restore the biomass concentration to 1 gL^{-1} (Figure 4). Thus, biomass productivity as well as constant pollutant removal rate was maintained. Use of osmotic membrane photobioreactor for continuous removal of pollutants was attempted by Praveen *et al.* (2016), however, the resultant treatment time was 24 h (Praveen *et al.* 2016). Yang *et al.* (2018) have integrated low-cost filtration unit constituting of denim fabric and other support materials in the PBR to enable continuous treatment of water (Yang *et al.* 2018). This strategy also resulted in water treatment in 24 h. Both the studies though achieve continuous nutrient removal, the retention time was more than 12 h and simultaneous biomass production was not observed. The current study has un-coupled the HRT and SRT to 12 and 24 h respectively and resulted in biomass production of 0.5 gL^{-1} per day without compromise on the water treatment.

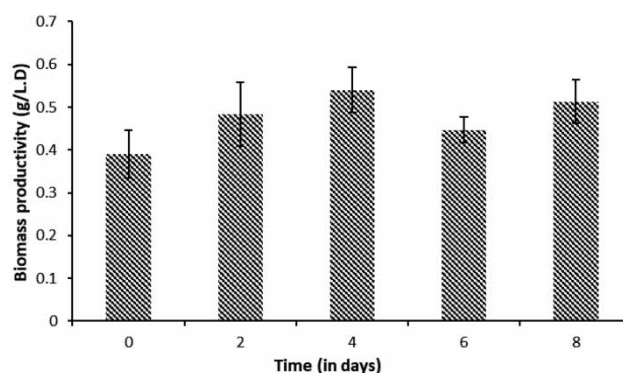


Figure 4 | Biomass generated per day in t-PBR shown as the biomass productivity of *C. saccharophila*.

Rapid membrane fouling was observed at the end of 24 h as described by Zhang *et al.* (2006). However, the average transmembrane pressure (TMP) remained in the range of 35–40 Kpa in the 10 days of reactor cycle. After 10 days, a 10 minute backwash was required to restore the TMP and normal functioning of the membrane.

Physiological status of *C. saccharophila* in t-PBR

In the current study, algae uptake the ammonia and COD from sewage water and utilize for growth and physiological activities. The active physiological status of microalgae is desired to achieve rapid and efficient sewage water treatment. The physiological status of the culture in t-PBR was evaluated from the carotenoid:chlorophyll ratio (caro:chl) (Figure 5(a)) and by monitoring the effective light utilization by PS II estimated as the changes in the F_v/F_m (Figure 5(b)). The caro:chl ratio in the initial two days was observed to be 0.25 which subsequently increased to 0.35 and was maintained over a period of 8 days. Constant availability of nutrients enabled maintenance of caro:chl ratio and consequently the log phase of the culture. In typical batch cultures, the availability of nutrients declines as the cell density increases, consequently leading to the down-regulation of chlorophyll pigments and other proteins (Jiang *et al.* 2012). Despite the down-regulation, the concentration of carotenoids is maintained due to their key role in photo-protecting the cells (Gill & Tuteja 2010). Thus, monitoring the fluctuation in the caro:chl ratio can be used to assess the probable stress to the culture. Increase in the caro:chl ratio was observed by Pancha *et al.* (2014) when microalga *Scenedesmus* sp. CCNM 1077 was subjected to nitrogen starvation conditions. In addition to the starvation conditions, stress due to pollutants (which is likely to happen in sewage water) also leads to the increase in the level of

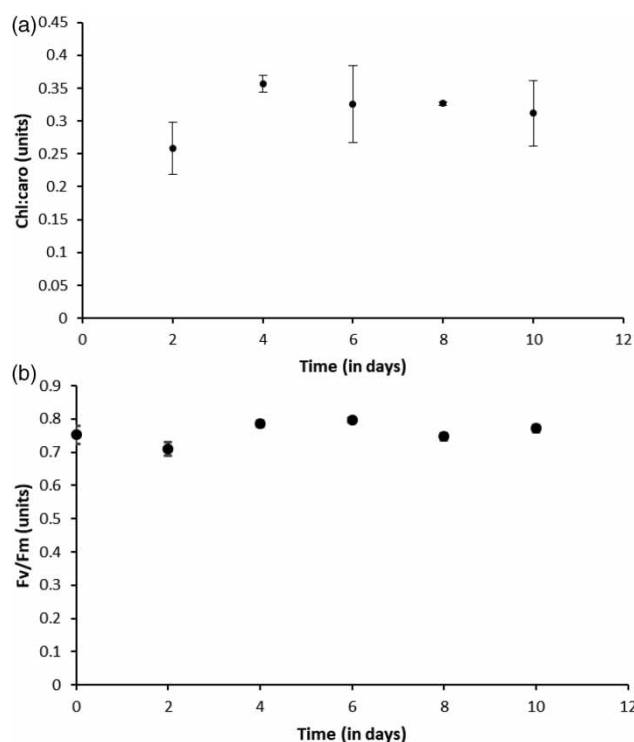


Figure 5 | Physiological status of *C. saccharophila* in t-PBR throughout the reactor cycle; (a) Carotenoid:chlorophyll ratio of *C. saccharophila*; (b) Profile of the changes in the maximum quantum efficiency of PS II (F_v/F_m) of *C. saccharophila*.

carotenoids (Pancha *et al.* 2014). Polyak *et al.* (2013) have reported a 6-fold increase in the carotenoid ratio when the culture of *Microcystis aeruginosa* was exposed to 0.04 mgL^{-1} of copper (Polyak *et al.* 2013). In the current study, the pigment balance of the culture was maintained, indicating maintenance of a steady state of growth of the culture.

These findings were further augmented by the steady state of the primary photosynthesis as seen by the maintenance of the F_v/F_m values. The effective light utilization (F_v/F_m) remained constant and close to the ideal value for a healthy cell (at ca. 0.75). Progressive photoinhibition and hence, a decrease in the efficiency of PS II has been reported in conditions of nutrient stress, high light, cold conditions or exposure to toxicants (Vonshak *et al.* 1994; Parkhill *et al.* 2001; Guan *et al.* 2015). Simionato *et al.* (2011) observed diminished F_v/F_m in *Nannochloropsis gaditana* in a batch mode of growth as the culture progressed towards the stationary phase at different light intensities (Simionato *et al.* 2011). Inhibition in the primary photosynthesis has also been reported by Matorin *et al.* (2009) due to the toxic action of methylmercury on the culture of *Chlorella vulgaris* (Matorin *et al.* 2009). Incessant photosynthetic efficiency, as seen in our study, suggests healthy cellular physiology, which in turn leads to active pollutant capture. Continuous mode of the growth system thus had an advantage of maintaining the culture in incessant log phase due to a continuous supply of nutrients (pollutants internalized as nutrients in the present case) and maintenance of constant cell density.

Nutrient capture and pathogen removal efficiencies in t-PBR

The dynamics of ammonia capture profile are shown in Table 2. The capture of ammonia was obtained within 10 h, where the concentration of ammonia decreased from ca. 70 mgL^{-1} to $<5 \text{ mgL}^{-1}$ resulting in an average ammonia capture rate of $6.5 \text{ mgL}^{-1} \text{ h}^{-1}$. This removal efficiency was maintained for 10 days throughout the reactor cycle. Similarly, COD reduced from 160 mgL^{-1} (untreated) to $<50 \text{ mgL}^{-1}$ in continuous t-PBR (Table 2) with average COD capture rate of $11 \text{ mgL}^{-1} \text{ h}^{-1}$. In addition, phosphate removal was achieved within 2 h and constitutive phosphate

Table 2 | Dynamics of pollutant uptake profiles of ammonia, COD, phosphate and fecal coliforms by *C. saccharophila* in t-PBR

Time (in days)	Ammonia (mgL ⁻¹)	COD (mgL ⁻¹)	Phosphate (mgL ⁻¹)	Fecal coliforms (MPN/100 ml)
0	69.5 ± 3.1	166.6 ± 13.0	5.0 ± 0.5	20.0 ± 2.0
2	6.1 ± 3.4	100.4 ± 01.0	<LOD	0
4	4.4 ± 1.0	41.3 ± 08.5	<LOD	0
6	3.9 ± 2.9	37.8 ± 08.0	<LOD	0
8	3.8 ± 0.5	35.2 ± 07.5	<LOD	0
10	2.5 ± 0.4	36.6 ± 05.3	<LOD	0

LOD: Limit of detection (0.1 mgL⁻¹).

removal was seen throughout the reactor cycle (Table 2) with an average phosphate removal rate of 0.5 mgL⁻¹ h⁻¹. One of the remarkable findings is the complete disappearance of fecal coliform within 2 h of inoculation. The system was evaluated time and again for the presence of fecal coliforms during the reactor cycle, but MPN studies did not show the presence of fecal coliforms (Table 2). This could be due to the fact that bacterial population in the vicinity of algal cells face multi-pronged attack by the algae, eventually leading to the death of the bacteria. Algae scavenge the nutrients (in the form of N and COD) from the media, creating depletion in nutrients causing bacterial death by starvation. Additionally, oxygen produced during photosynthesis gets reduced to reactive oxygen radicals, which damages the cell membrane of bacterial cells thereby leading to fatality (Ansa 2013).

Algal bioremediation as a potential technology for water treatment

Based on the results, the continuous mode of culture growth in t-PBR turns out to be a promising strategy for MLW treatment using microalgae. Pollutant removal in terms of capture of COD, ammonia, phosphate as well as fecal coliforms was accomplished within 10 h in a single step making it highly effective as a MLW treatment strategy when compared with conventional modes of water treatment like activated sludge process (ASP) and upward anaerobic sludge blanket (UASB) (Zahid 2007). Removal of N and P within 4 hours was observed with *S. obliquus* and *Scenedesmus* sp. when immobilized microalgae were used (Zhang *et al.* 2008; Ruiz-Marin *et al.* 2010). This water treatment was not accompanied by biomass production. Continuous system for pollutant removal from sewage water in closed PBR was demonstrated by Robinson *et al.* (2012), however, the study employs the use of algae-bacterium consortium and an average retention time of >1 day. The striking aspect of the current study is continuous pollutant removal with simultaneous algal biomass production. The uncoupling of HRT and SRT resulted in rapid pollutant removal (<12 h) without compromising on the biomass production (biomass productivity obtained 0.5 gL⁻¹D⁻¹). Kong *et al.* (2010) have reported continuous pollutant removal system from municipal centrate using *Chlamydomonas reinhardtii* with biomass productivity of 2 g/L.D. The time required for complete replacement of reactor volume is >3 days (Kong *et al.* 2010). Additionally, most of the reported studies have been carried out under artificial illumination. This energy expenditure was eliminated in the current study by exposing the t-PBR to sunlight and harness the natural illumination. This ensured rapid water treatment additionally being sustainable in terms of energy usage.

CONCLUSION

Use of algae for sewage water treatment has a triple action of water cleansing, atmosphere cleansing (CO₂ utilization during photosynthesis) and biomass production. The major highlights of this study are high biomass production (0.5 gL⁻¹D⁻¹) with rapid sewage water treatment (<12 h). Also, COD and nitrogen was removed in a single step, unlike conventional treatment processes. t-PBRs used in

this study are vertical structures and help in reducing the land footprint required for water treatment, and can be positioned at sites of higher population densities for de-centralized water treatment.

CONFLICT OF INTEREST

The authors hereby declare that there is no conflict of interest pertaining to the current manuscript. All the authors have approved their names for the manuscript and are aware of the content of the manuscript.

CONTRIBUTION OF THE AUTHORS

The idea was conceptualized by A. Lali. Actual work was executed by J. Palkar and M. Navale. Manuscript writing was done by J. Palkar and R. Pandit. Critical evaluation of the manuscript was done by R. Pandit.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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