# Algal oil recovery from crepe cotton processing effluent using Scenedesmus dimorphus in comparison with activated sludge treatment

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## Abstract

Crepe cotton bandages or the surgical cotton fabrics output has risen dramatically in recent years as a result of rising demand from medical experts, particularly in nations like India, which is one of the top five cotton exporters in the world. For the manufacturing of crepe cotton bandages high quality of cotton fibres are used. The effluent released from crepe cotton textile units are distinguished from the other textile effluents by the absence of colouring agents. Suspended particles, COD, dissolved ions, organic carbon, and an alkaline pH characterise this effluent. Several degradation studies of colouring chemicals, organic and inorganic contaminants released from textile effluents have been conducted, but treatment studies on Crepe Cotton Textile Wastewater (CCTW) have been rare, despite their potential environmental threat. Biological treatment of wastewater is an indelible part of any industrial or domestic wastewater treatment plant. Although ASP (activated sludge process) is the most common, and primordial process used to treat wastewater, nevertheless generates ESP (Excess Sludge Produced) is the major disadvantage of this process. Conversely the burgeoning demand for energy has spurred scientists to test the potential of algae for treating wastewater, and energy production as well. Therefore, there is a paucity of knowledge of understanding, and selecting the right ecological, and economical system for effective treatment of cotton processing wastewater till date. In this research article a comparative batch study between activated sludge and microalgae *Scenedesmus dimorphus* with respect to different dilutions (100%, 50%, 25%, 10%) of CCTW was studied. From the results, it was observed that the strain *Scenedesmus dimorphus* could deplete > 83%, and > 97% of COD from 25%, and 10% of wastewater batches respectively during the microalgal batch study. On the contrary activated sludge microbes could remove maximum 76.40% of carbon from the initial concentration of 3560 mg/l of COD while cultured in 25% of wastewater. Likewise, the reduction efficiency of nitrate nitrogen ($NO^-{}_3$-N), ammonia nitrogen ($NH^+{}_4$-N), phosphate ($PO^{3-}{}_4$-P) was compared. Simultaneously the growth of *S. dimorphus* by measuring total chlorophyll showed a 1.28-fold increase in biomass in wastewater as comparison with pure culture medium within 15 days of batch experiment conducted in triplicates. After the batch experiment the total lipid content was estimated from the wet algal biomass with extraction efficiency of 90 ± 1.2%. From this study a significant decrease in organic and inorganic pollutants by *Scenedesmus dimorphus* was noticed as compare to activated sludge microbes.


## 1. Introduction

India is the one of the biggest exporters of textile and apparel in the world. Among the key textile zones of India, Tamilnadu, Punjab, and Gujarat contains 81% of total textile industries of the country. With 741 textile plants, Tamilnadu is at the top of the list. The wastewater discharges from textile industry, which contains harmful organic dyes, high biochemical oxygen and chemical oxygen demand (COD), total dissolved solids (TDS), other inorganic pollutants (Mishra and Maiti 2019) are of utmost concern to meet the Sustainable Development Goals (SDGs). Several villages surrounding the Rajapalayam town, located in Virudhunagar district of Tamil Nadu state, contains small and medium enterprises of surgical cotton manufacturing or crepe cotton textile industries (CCTW). Most of the people in this village maintain their livelihood by manufacturing and exporting these surgical cotton or crepe cotton bandages. These crepe cotton fabrics are the basic requirement of all pharmaceuticals, and health centres, and most commonly used in medical first aid box, which provides, warmth, insulation, and support in many medical scenarios.

Two biological systems have been commonly used, one is treatment of wastewater using activated sludge, in which degradation of organic and inorganic matter by the cell metabolism requires external aeration. Though it includes the most extensively used wastewater treatment technique, but the disposal and management of ESP (Excess Sludge Produced) are the major disadvantages of this system (Romero et al.,2015). Alternatively, phyco-remediation technique, where different species of micro, and macroalgae are not only used for the degradation of wastewater, but the treated algal biomass can be used to produce valuable bioproducts such as lipids, carbohydrates, proteins, used for animal feed stock, and also for biofuel production such as biodiesel, biohydrogen, biochar, and syngas, bioethanol (Goswami et al. 2020).

The autotrophic microalgae have the capability to produce biomass by using atmospheric $CO_2$ and sunlight. But in absence of sunlight some microalgae such as heterotrophic microalgae derive their carbon and energy source from organic substrates (Fig. 1), whereas both the organic, and inorganic carbon sources are used as energy source in case of mixotrophic growth of microalgae (Liu, and Hong, 2021). As microalgae are easily cultivable, they have the ability to grow in a large assortment as like in fresh, and marine water habitat. Apart from this the inherent potency of microalgae to take up inorganic contaminants like nitrogen, phosphorous from wastewater have made microalgal treatment of wastewater an eco-friendly and energy positive substitute to treat wastewater by removing contaminants and nutrients from municipal wastewater (Choi et al., 2015; Dvoretsky et al., 2017; Rani et al., 2020;), textile wastewater (Subashini and Rajiv et al., 2018; Anandhan et al., 2018; Wu et al., 2021), pulp and paper mill effluent (Bhatti et al.,2021;), dairy effluent (Choi et al., 2018; Pandey et al., 2019; Kumar et al., 2020), and even petroleum contaminated wastewater (Ugya et al., 2021). Although, the potential of microalgae in pollutant removal from several industrial wastewater has been well documented, there are only a few reports on real effluent treatment, especially the cotton processing wastewater. To the author's best knowledge, this is the first report of the production of algal-oil from crepe cotton processing effluent and microalgae *Scenedesmus dimorphus*. Herein, the microalgal treatment is compared with the conventional treatment process of activated sludge to demonstrate the recovery of valuable by-products with simultaneous pollutant removal from crepe cotton processing effluent.

Microalgal species such as Scenedsmus sp., Chlorella sp., Nostoc sp., and Anabena sp. have all been widely examined for their ability to remove contaminants from wastewater (Mustafa et al., 2021). Several studies have looked into employing microalgae to biologically remove nitrogenous, carbonaceous, and phosphorus compounds from effluents such as municipal effluents, agricultural, and brewery effluents with varying treatment efficacy and microalgae growth efficiency (Cai et al., 2013, and Chiu et al., 2015). The three main nutrients microalgae uptakes from wastewater are C(Carbon), N(Nitrogen), and P(Phosphorous). The strain *Scendesmus obliquus*, and *Chlorella pyrenoidosa* have been investigated successfully to remove nutrients such as C, N, P from piggery effluent, and diary production effluent respectively (Prandini et al., 2016, and Kothari et al., 2012). A huge decrease in biochemical oxygen demand (BOD)i.e., 88%, 82% of total nitrogen (TN), and 54% of Total phosphorous (TP) in brewery effluent was investigated (Choi 2016) by culturing *C. vulgaris*. The bioremediation potential of different species of microalgae namely *Nanochloropsis sp.*, *Chlamydomonas sp.*, *Spirulina sp.*, *Botryococcu sp.*, and *Dunaliella sp.*, have been demonstrated (Gonçalves et al., 2017; Ahmad et al., 2022). Likewise, the inherent potency of variety of microalgae to convert organic contaminants to useful bimass and bioproducts have been well documented (Yadav et al., 2021).

## 2. Materials And Method

# 2.1. Collection of Crepe Cotton Textile Wastewater, and its characterization

The wastewater used in this study were collected from a crepe cotton textile unit also known as the surgical cotton industry, located near Rajapalayam, of Southern region of Tamil Nadu. Figure 2.a, and 2.d demonstrate closed tanks used for bleaching, and the dry crepe cotton bandages after bleaching respectively. A lot of hazardous chemicals such as hypochlorite, chlorine, peroxide for bleaching, and caustic soda are used for mercerizing the crepe cotton bandages prior to effluent discharging (Babu et al., 2021). Mostly the crepe cotton effluent releasing from this unit is free of dye as shown in Fig. 2. b & 2. c. The wastewater was collected in sterilized 5 litres plastic cans, and stored at 4°C. pH and conductivity were analysed by using digital pH meter (Type: MK-VI, Systronic India), and digital conductivity meter (MK-509, Systronic India) respectively. Parameters such as BOD, COD, TDS, Sulphate, Phosphate, and Ammonia were determined shown in Table 1. by the standard methods suggested in APHA (2005).

Table 1
Initial characterization of collected crepe cotton textile wastewater.

| Parameters | Concentration in untreated crepe cotton effluent | I.S limit for textile (IS 201: 1992) (mg/l) |
|---|---|---|
| pH(mg/l) | 9.9 ± 1 | 5.5-9.0 |
| Conductivity(cm/cs) | 3.39 ± 0.07 | - |
| TDS (mg/l) | 2400 ± 50 | 500 |
| Total COD (mg/l) | 11520 ± 40 | 250 |
| BOD (mg/l) | 305 ± 5 | 30 |
| Nitrate(mg/l) | 46.193 ± 2 | 10 |
| Phosphate(mg/l) | 83.67 ± 6 | 5 |
| Ammonia(mg/l) | 121.59 ± 3 | 5 |

**Table.2 Composition of BBM medium for the growth of microalgae (*S. dimorphus*), and MSM medium for the growth of Activated Sludge Microbes.**

| BBM | | | MSM | |
|---|---|---|---|---|
| Chemicals | Stock solution | Quantity | Chemicals | Quantity |
| **Macronutrients** | (1L d.$H_2O$) | | **Macronutrients** | (1L d.$H_2O$) |
| KH2PO4 | 1.25 | 10 ml | $K_2HPO_4$ | 2.05 g |
| K2HPO4 | 0.5 | 10 ml | $KH_2PO_4$ | 0.75 g |
| $MgSO_4.7H_2O$ | 0.42 | 10 ml | $NH_4Cl_2$ | 0.6 g |
| $CaCl_2$ $2H_2O$ | 1.9 | 10 ml | Metal Solution | 1.5 ml |
| NaNO3 | 1.5 | 10 ml | **Metal solution** | |
| NaCl | 0.15 | 10 ml | $Na_2EDTA.2\ H_2O$ | 6.45g |
| EDTA solution | | 1 ml | $ZnSO_4.7\ H_2O$ | 0.15 g |
| EDTA | 4.5 | | $CaCl_2.2\ H_2O$ | 0.05 g |
| KOH | 3.1 | | $FeSO_4.7\ H_2O$ | 2.5 g |
| **Acidified Iron Solution** | | 1 ml | $NaMoO_4.2\ H_2O$ | 0.1 g |
| $FeSO_4.7H_2O$ | 0.0498 | | $CuSO_4.5\ H_2O$ | 0.2 g |
| $H_2SO_4$(ml) | 0.05 | | $CoCl_2.6\ H_2O$ | 0.15 g |
| Boron solution | | 1 ml | $MnCl_2.\ H_2O$ | 0.5 g |
| $H_3BO_3$ | 1.15 | | $MgSO_4.7H_2O$ | 0.55g |
| **Trace metals solution** | | 1 ml | | |
| $ZnSO_4.7H_2O$ | 0.89 | | | |
| $MnCl_2.4H_2O$ | 0.144 | | | |
| MoO3 | 0.071 | | | |
| $CuSO_4.5H_2O$ | 0.16 | | | |
| Co $(NO_3)_2.6H_2O$ | 0.05 | | | |
| Ph | 6.7±0.1 | | pH | 7±0.1 |

## 2.2. Microalgae and growth medium

The culture of *Scenedesmus dimorphus* was procured from Phycospectrum, Chennai, and maintained in BBM (Bold Basal Medium). The stock solution composition for BBM medium is shown in Table 2 (Brown et al., 1964; Nichols and Bold, 1965), and the pH was maintained to 7 ± 0.1. The culture was maintained at room temperature 25 ± 2°C in white fluorescent light (12:12h). The microalgae culture was allowed to grow for at least 10 days till the culture was visibly dark green in color.

## 2.3. Aerobic sludge microbes

The activated sludge or aerobic sludge for batch study was collected from a sewage treatment plant of Kalasalingam University, Krishnankoil, Tamil Nadu. Prior to the batch experiment, 100 ml of activated sludge microbes were cultured in MSM (Minimal Salt Medium) (Coleman et al., 2002; Hartmans et al.,1992) demonstrated in table.2. Continuous aeration was given to the bacteria culture, prior to batch experiments which were conducted at different concentration of wastewater in closed temperature controlled orbital shaker.

## 2.4. Experimental design

Due to high COD of CCTW, the batch study was carried out by diluting crepe cotton textile wastewater with BBM medium in case of microalgae, and with MSM medium in case of bacterial batch study. The batch studies for both microalgae, and activated sludge were done in triplicates and the mean values reported.

### 2.4.1. Microalgae batch study

The biodegradation experiment of pollutants with simultaneous biomass growth by measuring the total chlorophyll content of *scenedesmus dimorphus* in CCTW were done by using 250 ml Erlenmeyer conical flask in a closed orbital shaker (LTOSI- Referigerated) in which temperature was maintained at 25 ± 1°C, shaking speed at 110 rpm. Cultured test samples of microalgae were provided by white flourecent light with a dark/light period of 12:12 h. The initial pH of the test samples was maintained at 7 ± 0.3 and the pH was maintained though out the experimental period of 15 days. Similar to bacterial batch study, the batch experiment of microalgae was performed by taking blank i.e., B1, and four test samples i.e., R1, R2, R3, and R4 where B1 comprises of pure crepe cotton effluent without microalgae, and R1, R2, R3, and R4 are composed of 100%, 50%, 25%, 10% of CCTW respectively. Except B1, and R1, the test samples were diluted with BBM medium. 20 ml culture sample of *Scenedesmus dimorphus* was centrifuged for 10 minutes at 4000 rpm. The algae cells were washed thoroughly by distill water after decanting the supernatant and inoculated in the four test samples. After the batch study 500 ml of microalgal culture was centrifuged to obtain 1 g weight algal paste, which was sonicated prior to the lipid extraction, and then lipid was extracted by the solvents as mentioned in the below section.

### 2.4.2. Activated Sludge Microbes batch study

In batch of Activated Sludge Microbes, blank and test samples in batches were labelled as B1, T1, T2, T3, T4 where B1 referred to the blank, composed only of crepe cotton effluent, T1 referred to 100% wastewater sample with MSM salt, T2, T3, and T4 composed of 50%, 25%, and 10% of wastewater respectively, and were diluted with MSM medium. All the test samples were provided with 20 ml of activated sludge, and pH was maintained at 7 ± 0.5. Growth analysis of activated sludge microbes was done by measuring TSS (Total Suspended Solids) in each and every batch of bacteria.

## 2.5 Extraction of lipid from wet algal biomass

The lipids from *Scenedesmus dimorphous* were extracted by successive mixture of polar and non-polar solvents in 3:1 ratio in 1 g of wet algal biomass (Fig. 3). The microalgae culture was centrifuged, and the obtained algal paste was sonicated before solvent extraction. The sonicated algal biomass was mixed with isopropanol, followed by n-hexane with vortexing. The vortexed mixture was centrifuged at 4000 rpm, and supernatant was collected. The same process was repeated again by mixing the weight algal biomass with hexane. The supernatant obtained from both polar, and nonpolar solvent were mixed, and heated at temp 60 °C to obtain algal lipid mixtures (Patel and Kannan, 2021). To determine the efficiency of lipid extraction, the above experimental procedure (solvent extraction) was compared with a standard analytical method i.e., Bligh, and Dyer method (Bligh, and Dyer 1959) by the following Eq. 1.

$$\text{Efficiencyofalgallipidextraction}(\backslash\%) = (\text{Lipidobtainedbyexperimentalmethod} / \text{Lipidobtainedbyanalyticalmethod}) * 100\backslash\%$$

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## 2.6. Analytical methods

The liquid test samples were analysed at one day interval for 15 days of incubation period for both microalgae and bacterial batch study. pH was maintained at 7 ± 0.8 for microalgae, and 7 ± 0.2 for bacteria ((Digital pH meter MK VI). The total chlorophyll, $NO_3$–N, $NH_4$-N, ($PO_4{}^{3-}$ -P) was measured by LABMAN Visible Spectrophotometer Model No LMSP V325 as recommended by APHA standard methods (APHA, 2005). The sample from each batch of microalgae and activated sludge microbes were first centrifuged at 4000 rpm for 10 minutes (REMI R-4C) to remove algal and bacterial cell prior to wastewater analysis. In order to investigate the organic matter present in CCTW and different batches, COD of each sample was measured as per Standard Method (APHA, 2005). Total chlorophyll of *S. dimorphus* was estimated by standard operating procedure for chlorophyll determination (Arnon 1945), and the growth of activated sludge microbes was observed by measuring TSS in each test sample by following the standard method (APHA, 2005).

# 3. Result And Discussion

## 3.1. Characteristics of crepe cotton wastewater

pH of the CCTW was found to be alkaline (9–11) due to excessive use of hypochlorites, peroxides and calcium carbonate. physico-chemical parameters such as TDS, COD, BOD were found to be above the permissible limit and if discharged untreated or partially treated will lead to ecological disturbances. CCTW was found to have a large organic load in this study, with BOD 10 times greater and COD 46 times than the International Standard limit for textile wastewater discharge (Table 1). Inorganic pollutants such as ammonia, nitrate, and phosphate were found to be increased as well, as shown in Table 1.

## 3.2. Growth pattern of *Scenedesmus dimorphus*, and activated sludge microbes in different concentration of CCTW

When cultivated in varied concentrations of CCTW, the microalgae *Scenedesmus dimorphus*, and activated sludge microbes exhibited an increasing growth trend with days three different phases of growth. In Fig. 4.a negligible growth of microalgae was marked in between 0th day and 3rd day. Then rapid increase in total chlorophyll concentration was observed within 5th day to 11th day, which denotes optimum production of algal biomass in every test sample within 15 days of incubation period. No further increase in chlorophyll was noticed with in 13th day to 15th day of growth period. Maximum increase in total chlorophyll was noticed in R4 i.e.,12.347 ± 0.57 mg/ml, which showed a 10.54-fold increase in biomass in comparison with initial day i.e., 1.177 ± 0.57 mg/ml, while in R1 minimum increase i.e., 3.291 ± 0.37 mg/ml in total chlorophyll was detected within 15 days of incubation period, which indicates low biomass growth. However, in control (B1), even though a very few quantities of total chlorophyll were observed initially, but no significant increase in total chlorophyll was detected within 15 days of incubation period. On other hand optimum growth of bacteria was noticed in T4 i.e., 88.24% within 11 days of batch study (Fig. 4. b). Leong et al., 2018 performed biodegradation experiment of nitrogen rich wastewater, and observed maximum biomass growth of 0.88 ± 0.0 g/l while co-cultivating microalgae, and activated sludge microbes in 1:1 ratio. This clearly indicates that the micro algal strain, and bacterial strains in the activated sludge is utilizing the pollutants present in wastewater as source of carbon and energy. A highest increase i.e 2.15-fold increase in chlorophyll a content was observed in *scenedesmus sp.* ISTGA1 while culturing it in the influent of sewage treatment plant (Tripathy et al., 2019). Chinnasamy et al (2010) observed the growth of five different algal species *Tetraselmis suecica, Dunaliella tertiolecta, Tetraselmis chuii, Chlorella saccharophila, Phaeodactylum carterae* and cultured in a carpet industry wastewater and observed 247, 16, 190, 36 and 118% increase in Chl a content respectively as compare to the controls.

## 3.3. Reduction of COD

In the present study, COD content of unfiltered CCTW is extremely high in the range of 11000–12000 mg/l (Table 1). Therefore, filtered CCTW has been used in batches to reduce the turbidity, toxicity, and to discard the suspended particles. The microalgae *Scenedesmus sp*. could significantly reduce the COD without the need of external sources of glucose, and carbon. As shown in Fig. 5. a, highest reduction of COD was noticed in R4 i.e., no COD was found after the completion of 15 days batch study, where as in R3 test sample 95.29% reduction of COD was noticed. Furthermore, lowest reduction i.e., 24.79% was observed in R1 (pure wastewater) in 15 days, which could be due to the presence high concentration of TOC (total organic carbon) in CCTW. Similar, biodegradation of organics from surgical cotton wastewater by microalgae have been reported by (Babu et al., 2021). The organic compound present in the wastewater could be removed by biodegradation, bioaccumulation, or biosorption, or an integration of all the mechanisms (Mustafa et al. 2021). Reduction in the values of COD in different test samples having different concentration of wastewater indicate that the microalgae *Scenedesmus dimorphous* was capable of utilizing organic contaminants present in the crepe cotton wastewater within 15 days of culture period. But in Bl (blank), no reduction in COD was observed due to absence of microalgae. Similar results have been noticed by Fazal et al., 2021, when *Chlorella vulgaris* was cultivated in diluted textile wastewater to remove 99.7 ± 4.2% of COD, and *Chlorella pyrenoidosa* was cultivated in TWW to remove 85% COD (Kumar and Pareek, 2019). on other hand, bacterial batch experiments Fig. 5. b indicates highest reduction efficiency of COD in T4, i.e., 89.87%, and lowest removal of COD i.e., 13.77% was found in T1 test sample which is very less as compare to the microalgae batches. Furthermore, in B1, no reduction in COD was noticed due to the absence of activated sludge microbes. Low performance in bacterial batch study as compare to microalgal batch study could be due to lesser ability of bacterial sludge to bioaccumulate or biodegrade the toxic pollutants. The inclusion of both hexadecenoic and octadecenoic acids, which are now known to breakdown slowly (Bhadani et al. 2020), could be responsible for the low performance of activated sludge bacteria.

## 3.4. Nitrate nitrogen ($NO_{3-}N$) and ammonia nitrogen ($NH_4^+$-N) in CCTW

Removal of nitrogen from textile wastewater is a crucial factor in preserving surface water. Nitrogen is one of the key nutrients. Its overabundance can cause eutrophication in water bodies, and hence it needs to be reduced from wastewater such that the treated could be reused in agricultural field, and discharged to the nearby water bodies without affecting the aquatic lives. Microalgae have the ability to utilize nitrogen in both inorganic forms ($NO_2$, $NO_3$, $NH4^+$) and organic forms (nucleosides, amino acids, purine, urea) (Cai et al.,2013 and Ross et al., 2018). Inorganic N assimilation in microalgae requires the incorporation of carbon skeletons having form of keto-acids inside organic molecules (Falkowski and Raven, 2013). Inorganic N in the form of $NH_4{}^+$ is the most common N donor molecule in the anabolism of amino acids in microalgae. The two genetically conserved enzymes GOGAT (2-oxoglutarate amino transferase), and GS (Glutamate synthetase) catalyse N integration combinedly (Lu et al., 2005). GS catalyses the fixation of $NH4^+$ on a glutamate molecule to produce glutamine, and then extra amino group can act as a N donor to 2-oxoglutarate to produce two glutamate molecules in the NADPH-dependent conversion.

The CCTW was found to have higher concentrations of nitrate and ammonia as shown in Table 1. Microalgae have inherent potency to remove nitrogen from wastewater. Mayhead et al, 2018 observed 94.18% reduction of ammonium ($NH_4$-N) by *Chlorella vulgaris* while cultivating in domestic wastewater for 12 days. Figure 6.a demonstrates the batch study of CCTW with *Scenedesmus dimorphus* for nitrate reduction. Highest decrease in $NO_3$-N was observed in R4 test sample where the initial nitrate concentration was 16.99 ± 1.15 mg/l and the final concentration was 1.34 ± 0.76 mg/l, indicates a 92.09% decrease in nitrate nitrogen, and maximum decrease in $NH_4{}^+$-N also was detected in R4 sample i.e., 75.59% shown in Fig. 7.a. It could be concluded that *Scendesmus dimorphus* utilized both nitrate and ammonium nitrogen present in the wastewater for their growth, this has also been observed by Lam et al., 2017. Furthermore, least efficiency of reduction in nitrate and ammonia were shown in R1 test sample i.e., 25.72% and 31.23% respectively. As the nonionized form of ammonia can be transported at a faster rate as compare to ionized form of ammonia, the rate assimilation, and uptake of N directly corelate with the availability of N in wastewater (Kube etal., 2018; Alva et al., 2018; Gao et al., 2018). on other hand activated sludge microbes showed relatively lesser removal of nitrate nitrogen, and ammonia nitrogen as compared to *S. dimorphus* could be due to presence of high TOC (Total organic carbon) in CCTW as observed by Babu et al., 2021. Figure 6.b, and Fig. 7.b indicate the maximum reduction in $NO_3$-N, and $NH_4{}^+$-N concentration i.e., 83.35%, and 65.58% respectively in CCTW by using activated sludge microbes. Algae can synthesize oxygen through photosynthesis, while it uses sunlight as energy source and converts carbon sources into useful biomass. But the activated aerobic sludge microbes need oxygen, so that for nutrient removal from wastewater aerobics bacteria needs an oxygenated environment for cellular respiration and growth, and hence is considered energy intensive for the removal of both organics, and inorganics from wastewater.

## 3.5. Phosphate removal from CCTW

Microalgae shows two active transport mechanisms for the uptake of phosphorous(P) present in wastewater which finally contributes to its biomass growth i.e, by direct assimilation, where the additional phosphorous stored as polyphosphate, and another one is $PO_4{}^{3-}$ precipitation, both the mechanisms render microalgae a suitable candidate for wastewater treatment (Kube et al., 2018; Alva et al., 2018; Gao et al., 2018). A remarkable decrease in phosphate was observed in four different concentrations of wastewater by using *S. dimorphus* within 15 days of incubation period as shown in Fig. 8.a. Highest degradation of Phosphate i.e., 87.85%, in R4 and lowest degradation of Phosphate in R1 i.e., 18.05% was noticed. In contrary maximal reduction in phosphate in bacterial batch was noticed in T4 i.e., 67.16% (Fig. 8.b) which is less as compare to the microalgal batch experiment probably due to the dependency on oxygen, and less adaptability towards CCTW. In activated sludge-based wastewater treatment, alteration in aerobic condition without available organic matter or anoxic conditions with available organic matter allows the uptake of organic matter with the help of electron acceptor and by this process phosphate is released in anaerobic condition, and get absorbed in aerobic period or in presence of oxygen in a greater amount, and finally the wastewater concentration get decreased (Dorofeev et al., 2020). Inorganic phosphorus is readily available in environment, and most of the microalgae prefer to absorb $H(PO4)^{2-}$ and $H_2PO_4{}^-$ (Silva et al., 2015). $(PO)_4{}^{3-}$ enters into algal cell by active transport through a symporter channel mediated by $H^+$ or $Na^+$ ions which is produced by $H^+$-ATPase pump of plasma membrane (Falkowski and Raven, 2013).

## 3.6. Extraction of lipid

When 1 g of wet biomass of *Scenedesmus dimorphus* was sonicated, and centrifuged with successive mixture of polar, and nonpolar solvent, 0.09 g of lipid was obtained after heating the supernatant. The efficiency of extraction was calculated to be 90 ± 1.2%. The higher extraction efficiency could be due to the breakage of cell wall by ‘cavitation’ occurs in ultrasonication process (Araujo et al., 2013), where the high frequency sound wave results the formation of alternate compression, and rarefaction cycles, and the vacuum bubble formed will burst by radiating shock waves which finally rupture the membrane of microalgal cells. The lipids present inside the microalgal cell comes out, and the higher selectivity of the microalgal lipids toward the 2-propanol- n-hexane- water combination, might be linked to the higher lipid extraction capability of the above explained experimental method. Patel, and Kannan, 2021 developed wet algal lipid extraction methods by taking different polar, and nonpolar solvent mixture such as 2- propanol/hexane, and methanol/methyl tertiary butyl ether (MtBE), and reported maximum extraction efficiency by using the combination of solvents 2- propanol, and n-hexane. Regardless of whether an organic solvent is employed or not, the cells must be ruptured appropriately for microalgae lipid extraction. Pre-treatments of algal biomass can intesify lipid recovery and make cellular lipid extraction easier by weakening or by disrupting the microalgal cell walls (Cooney et al., 2009; Yoo et al., 2012). Saroya et al., 2018 compared the two-lipid extraction procedure while extracting trans-esterifiable lipid from pure culture of chlorella sp., and concluded lipid extraction of 25% by using wet lipid extraction procedure where as 20% of lipid extraction, from dry algal biomass using the Bligh and dyer extraction method.

# 4. Conclusion

In this study the potency of microalgae *Scenedesmus dimorphus* and activated sludge microbes to treat crepe cotton wastewater was investigated. Biomass increase of *Scenedesmus dimorphus* was governed by measuring total chlorophyll in different concentration of wastewater. COD removal efficiency, and inorganic nutrients such as nitrate, ammonia, phosphate removal from CCTW by microalgae and activated sludge microbes was well compared in this study. The growth of algae depends on light source but no any external aeration is needed to grow in wastewater whereas the growth of aerobic bacteria is highly dependent on oxygen. The treatment efficiency of both microalgae *S. dimorphus* and activated sludge microbes to treat CCTW were observed to be different. Even though no dying chemicals are used in the manufacturing of surgical cottons, yet the effluent is loaded with highly toxic organic and inorganic contaminants. From the comparative study of microalgae *Scenedesmus dimorphus*, and activated sludge microbes it was observed that *Scenedesmus dimorphus* showed high > 92%, > 75%,>87%, and > 95% removal efficiency for $NO_3$-N, $NH_4$-N, $(PO4)_3$-P, and COD respectively which was significantly higher in comparison to activated sludge microbes. Along with pollutant removal, a 0.09 g of lipid was extracted from 1g of wet algal biomass of *S. dimorphus*. The result of this work suggests that growing *Scenedesmus dimorphus* in real effluents provides an option for energy recovery with simultaneous environmental protection.

# Declarations

### Ethics approval and consent to participate

Not applicable

### Consent for publication

Not applicable

### Availability of data and materials

All data generated or analysed during this study are included in this published article

### Competing interests

The authors declare that they have no competing interests


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### Authors' contributions

Rajanandini Meher: Research and first draft writing. NKS: Methodology, writing, review and editing.  MM: Supervision, review and editing.


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# Figures

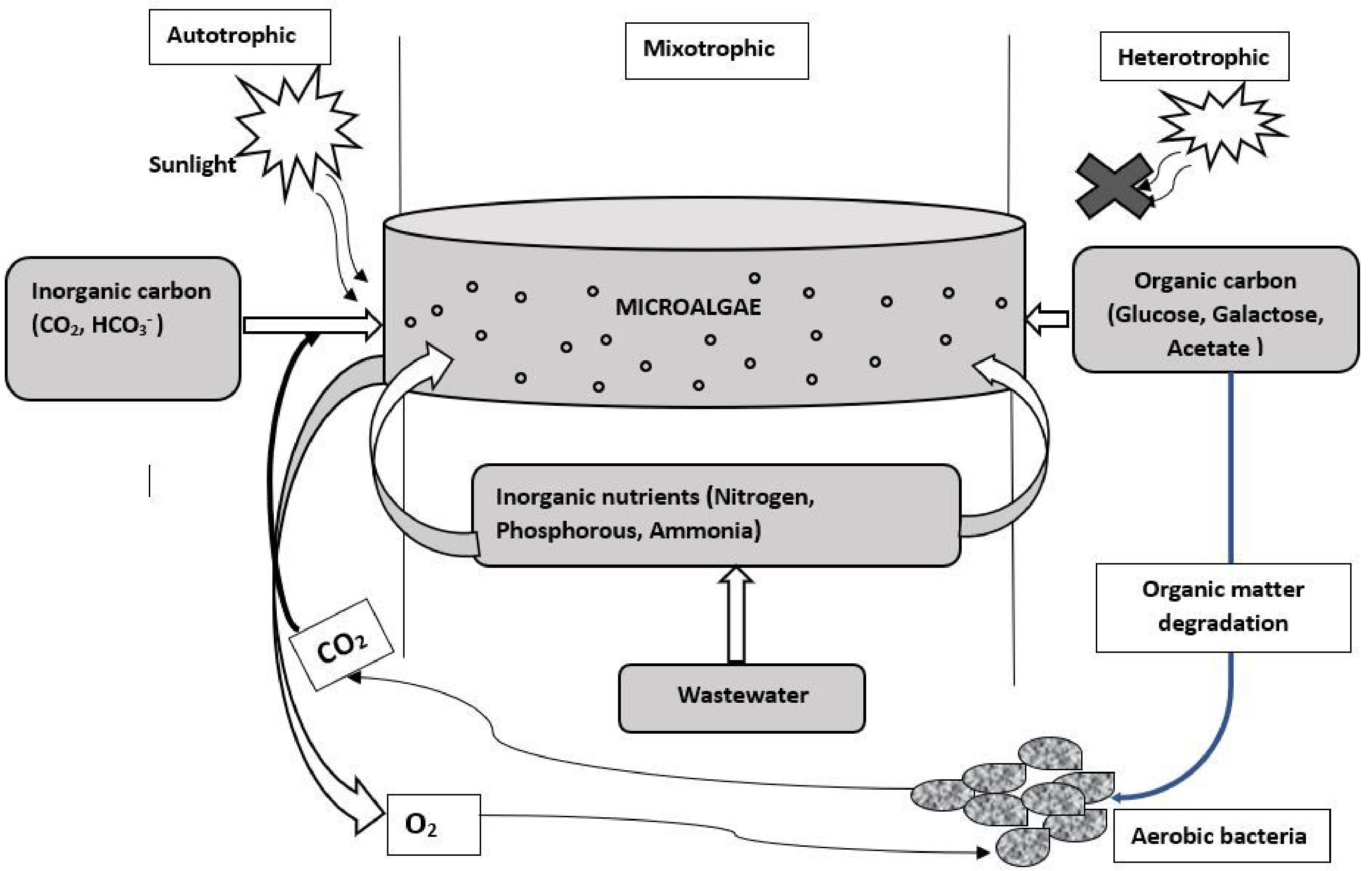


Figure 1

**Diagrammatic representation of Nutrients uptake by microalgae in autotrophic, mixotrophic, and heterotrophic growth.**

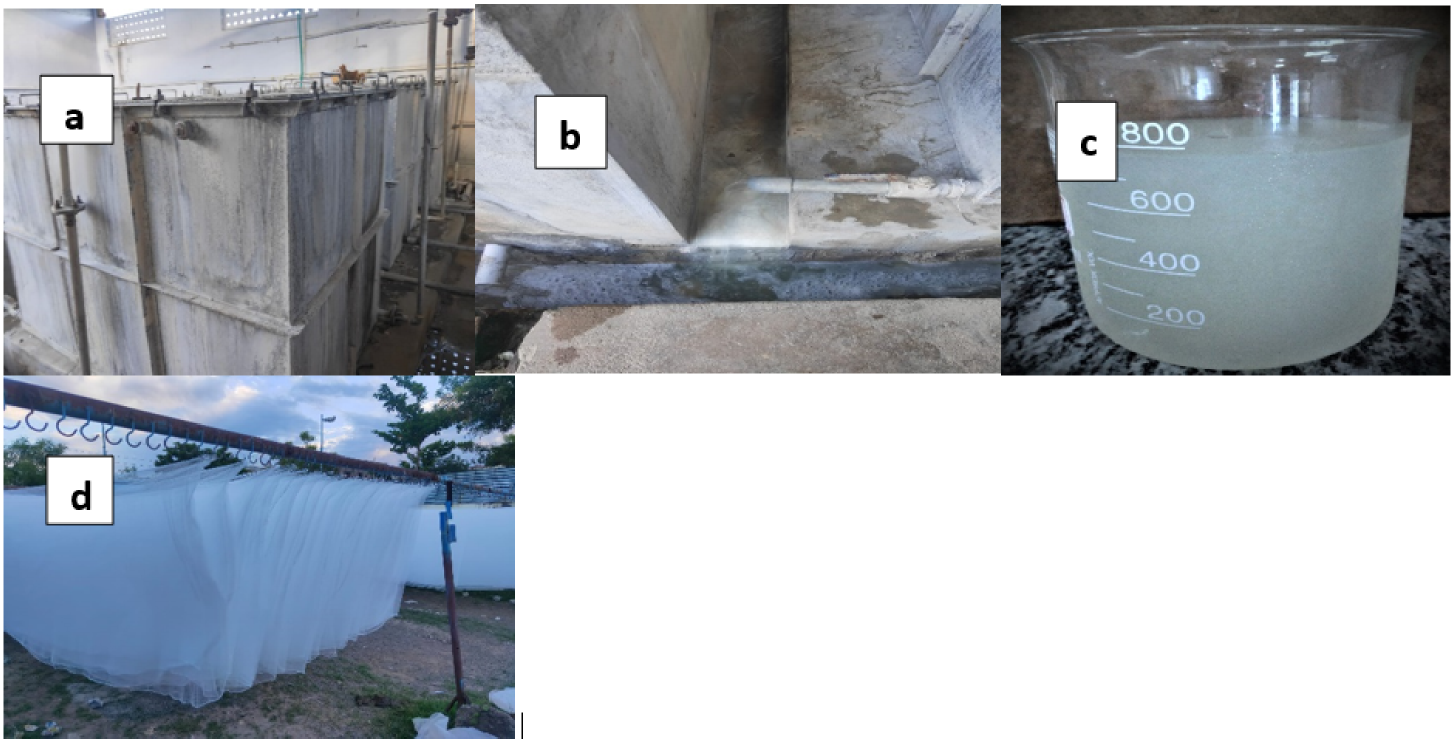


Figure 2

a. The closed tank used for bleaching. b. The image of effluent coming after bleaching. c. collected wastewater. d. Dry crepe cotton after bleaching and washing.

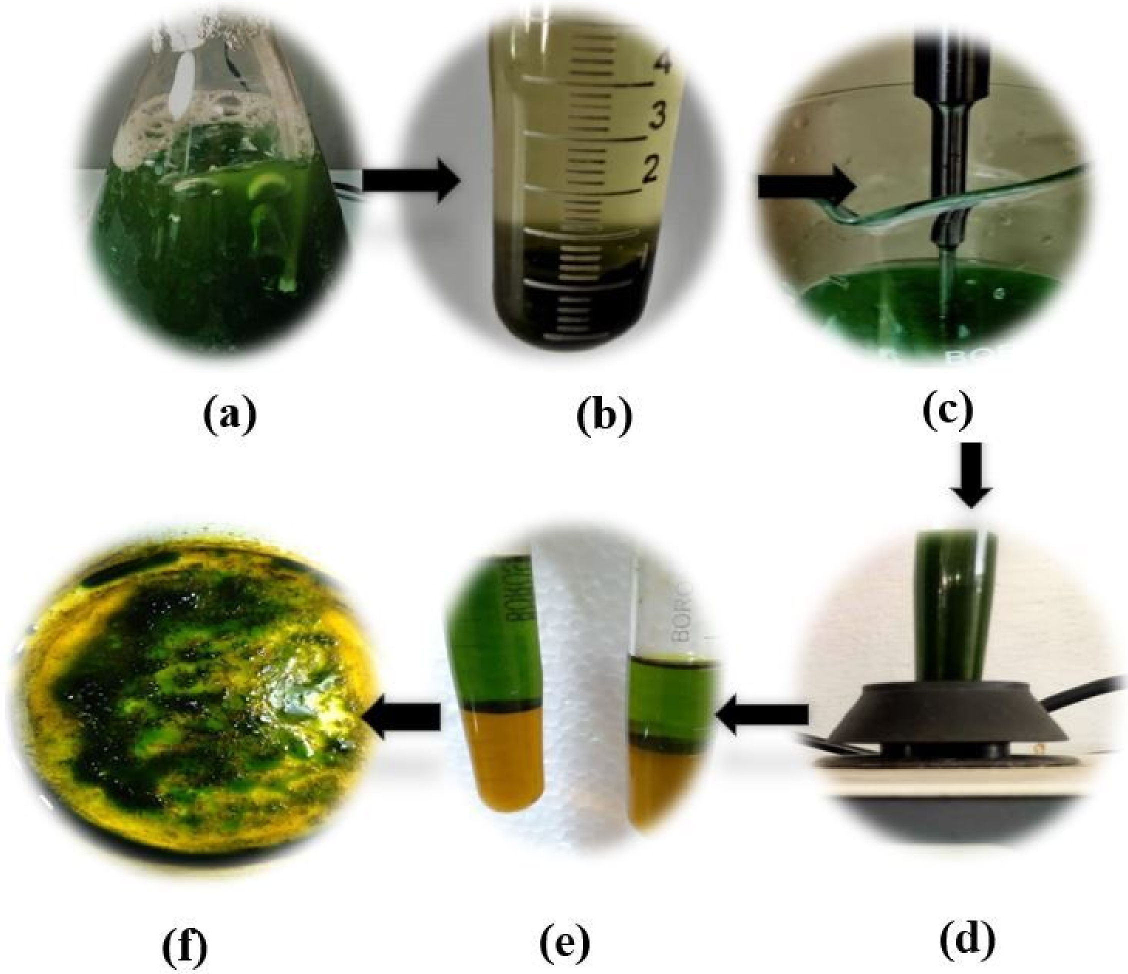


Figure 3

Flow diagram of lipid extraction from wet biomass of *S. dimorphus*. (a) Pure culture of *Scenedesmus dimorphus* (b) Wet microalgal biomass after centrifugation (c) Sonicated wet algal paste (d) Mixture of wet microalgal paste with solvents isopropanol/methanol by using vortex mixer (e) Supernatant having algal lipid after centrifugation of microalgae, and solvent mixture (f) Extracted impurified algal lipid after heating the supernatant

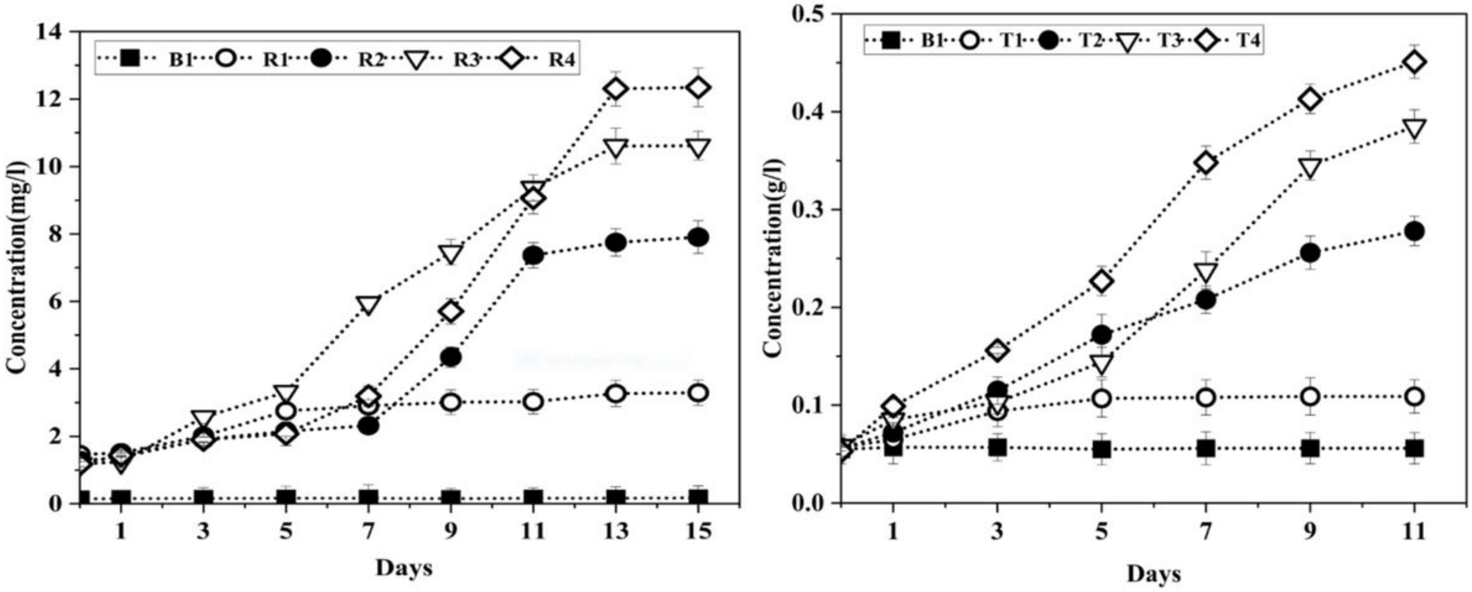


Figure 4

(a) Total chlorophyll content of microalgae

(b) Growth of activated sludge microbes

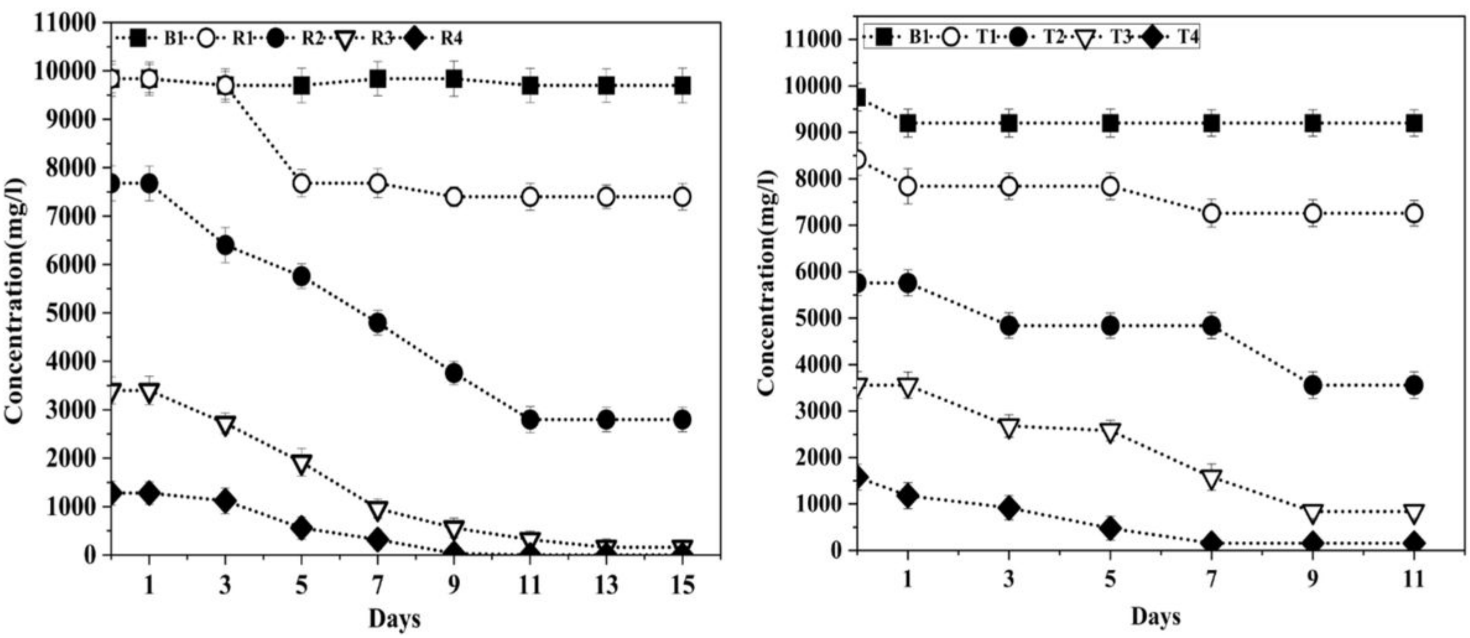


Figure 5

(a) Removal of COD by microalgae

(b) Reduction of COD in activated sludge microbes

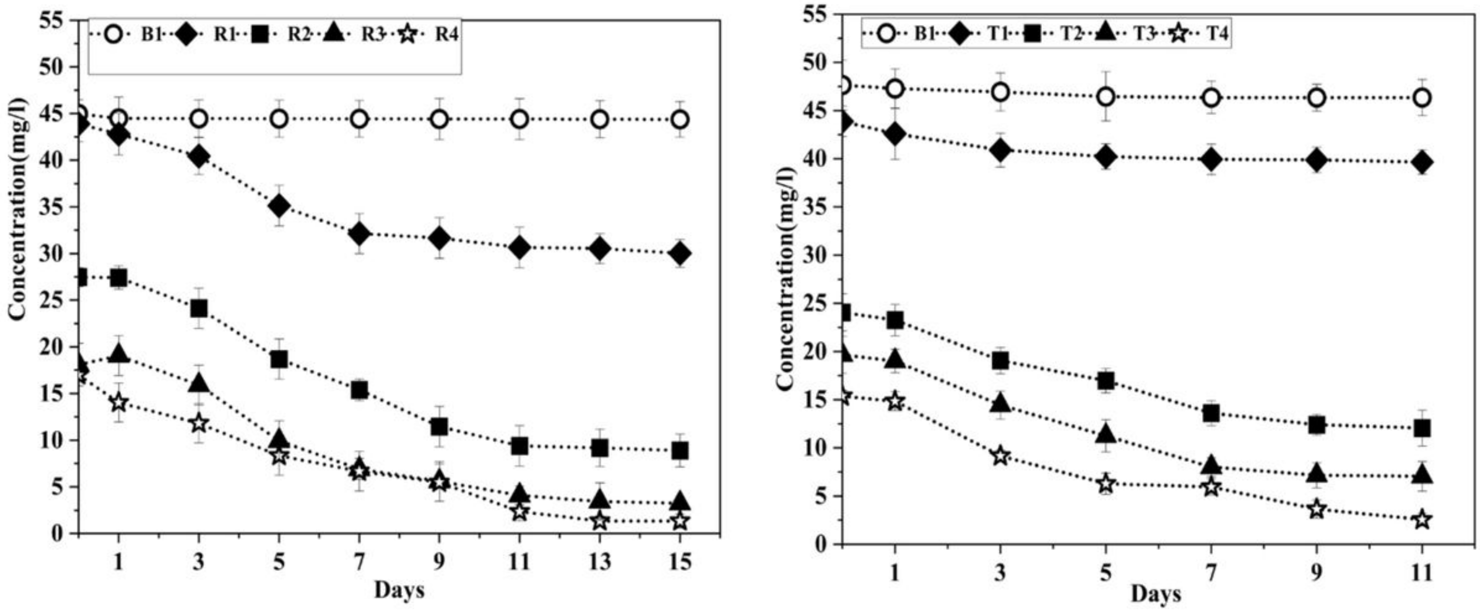


Figure 6

(a) Removal of nitrate ($NO_3$-N) by microalgae

(b) Removal of ($NO_3$-N) by activated sludge microbes

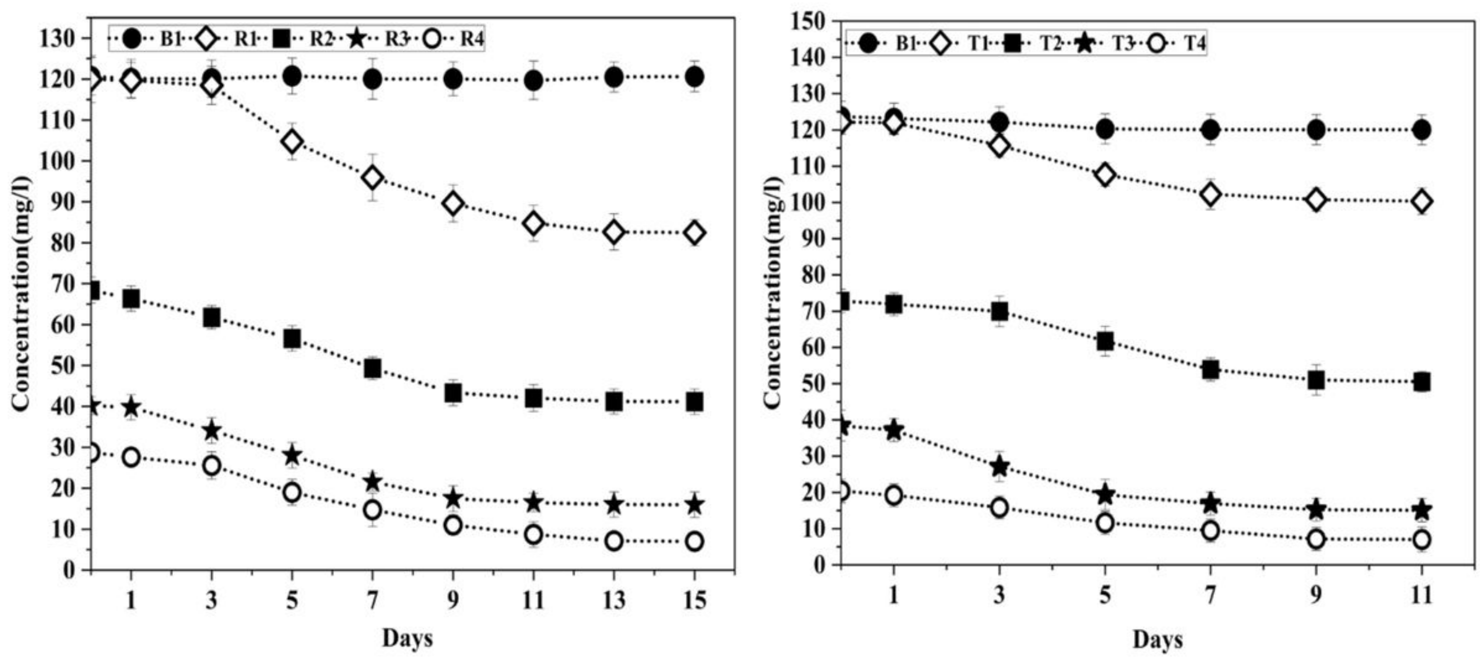


Figure 7

(a) Removal of ammonia by microalgae

(b) Removal of ammonia by activated sludge microbes

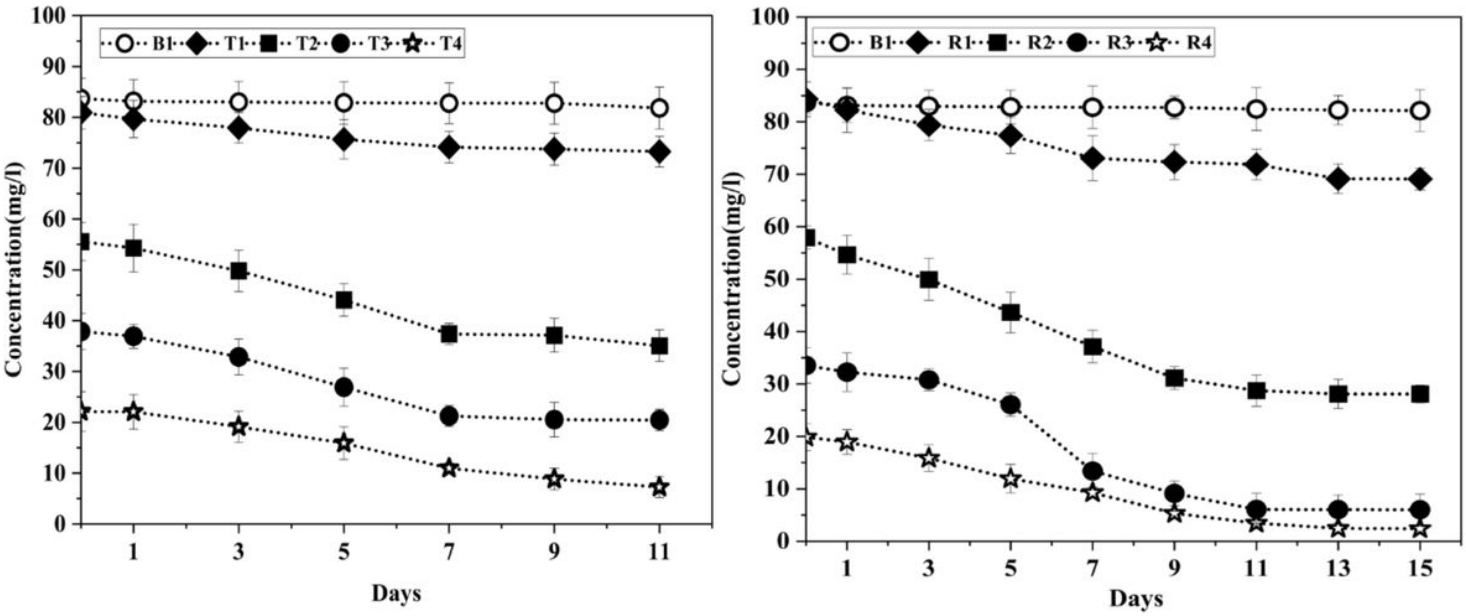


Figure 8

(a) Removal of phosphate in microalgae

(b) Removal of phosphate in activated sludge microbes