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ORIGINAL ARTICLE

Exploiting Phosphate-Starved cells of *Scenedesmus* sp. for the Treatment of Raw Sewage

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Abstract Phosphate depletion is one of the favorable ways to enhance the sewage water treatment with the algae, however, detailed information is essential with respect to internal phosphate concentration and physiology of the algae. The growth rate of the phosphate-starved *Scenedesmus* cells was reduced drastically after 48 h. Indicating cells entered in the stationary phase of the growth cycle. Fourier Transform Infrared analysis of phosphate-starved *Scenedesmus* cells showed the reduction in internal phosphate concentration and an increase in carbohydrate/phosphate and carbohydrate/lipid ratio. The phosphate-starved *Scenedesmus* cells, with an initial cell density of, 1×10^6 cells mL^{-1} shows 87% phosphate and 100 % nitrogen removal in 24 h. The normal *Scenedesmus* cells need approximately 48 h to trim down the nutrients from wastewater up to this extent. Other microalgae, *Ankistrodesmus*, growth pattern was not affected due to phosphate starvation. The cells of *Ankistrodesmus* was able to reduce 71% phosphate and 73% nitrogen within 24 h, with an initial cell density of, 1×10^6 cells mL^{-1}.

Keywords Phosphate starvation · Fourier Transform Infrared · Sewage treatment · *Scenedesmus* · *Ankistrodesmus*

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Introduction

Phosphorus is the second most important nutrient in domestic waste-water. It is difficult to remove and hence along with nitrogen is responsible for eutrophication of water bodies, especially where treated sewage is discharged. The most common method of soluble phosphate removal from the wastewater is precipitation [1]. Disposal of the sludge generated by this method is both expensive and difficult to implement, considering low phosphate and nitrate concentrations present in sewage [2]. In contrast, employing algae for the removal of phosphates and nitrates from wastewater has several of several advantages viz.(a) Simultaneous production of O_2 and consumption of CO_2 in presence of light, (b)Obviating the need for an extraneous supply of organic or inorganic nutrients, (c) Providing a final effluent that is enriched in dissolved oxygen, (d) lesser sludge accumulation (e) absence of generation of secondary pollutants, effective uptake of N and P (g) ecologically safe, (h) generation of a biomass which can have potential use as feedstock for fertilizers, biogas and biofuel [3–6]. However, it is essential to enhance the nutrient uptake kinetics so that the process of sewage treatment will be faster and reduced footprints. The nutrient removal efficiency of the algal cell could be increased by intrinsic and extrinsic factors, which include culture density [7], appropriate algal species, and environmental factors like temperature, pH, CO_2 concentration [1] and starvation [8, 9] of algae, etc.

Higher algal inoculums of algae can be achieved with immobilized algae [10]. However, it may also introduce complexity in the operations at larger scale. The previous study reported that immobilized algal cells do not offer an advantage over free cells for removing nutrients from the wastewater [11].

Algal cells accumulate polyphosphate granules under different phosphate condition except the longer phosphate starvation [12]. Though algal cells with phosphate-starved for few days showed faster nutrient uptake rate than the phosphate- saturated algal cells, however how phosphate starvation results in an increase in phosphate uptake rate was not clear [13, 14]. Though some of the recent studies, successfully utilized phosphate-starved, immobilized algal cells for the rapid treatment of the sewage [8, 9, 15], the experimentation was done using either artificial wastewater or filter sterilized domestic wastewater, which makes it difficult for practical implementation.

Here we utilized FTIR spectroscopy to visualize the internal phosphate concentration associated with the various macromolecular pool in phosphate supplemented and starved algal cells and it's probable relation with the increased phosphate uptake rate under phosphate starvation condition. The changes in the macromolecular pool can be monitored by FTIR on the basis of infrared absorption of the vibrationally active functional group (including O–H, N–H, C=O, =C–H, –C–H, C–O–C and > P=O) in the biological sample. FTIR has been successfully used for algae like *Microcystis aeruginosa*, *Protoceratiun reticulatum*, *Pandina tetrastromatica*to study the physiological changes [16–20].

As algae isolated from the domestic wastewater, can adapt and execute better for treatment of municipal wastewater. Two different algae were isolated from the wastewater and identified as *Scenedesmus sp.* and *Ankistrodesmus sp.* Both the isolates were evaluated for phosphate and nitrogen uptake from the sewage. The objective of this study was to develop practically applicable, simple, economical primary treatment of the domestic wastewater using algal cells having accelerated nutrient uptake with minimum footprints. An effort was made to understand the underlying rationale behind this improved cellular mechanism. The depletion of the internal phosphate during starvation and other compositional changes were monitored by FTIR.

Materials and Methods

Algal Cultures and Growth Conditions

The unialgal cultures of *Scenedesmus sp.* and *Ankistrodesmus sp.* were isolated from local sewage contaminated water body, from suburban of Pune, India. The isolation was done by agar plating method. Once algae are obtained in pure form, they were cultivated under aseptic conditions and preserved for further experiments. The algae were grown on Bold's basal medium (BBM) containing following chemicals $NaNO_3$ (0.25 gL^{-1}), K_2HPO_4 (0.075 gL^{-1}), KH_2PO_4 (0.175 gL^{-1}), NaCl (0.025 gL^{-1}), $MgSO_4$ (0.075 gL^{-1}), $CaCl_2$ (0.025 gL^{-1}), and trace metals $ZnSO_4$ (5×10^{-6} gL^{-1}), $MnSO_4$. $4H_2O$ (1×10^{-5} gL^{-1}), H_3O_3 (5×10^{-5} gL^{-1}), $Co(NO_3)_2$-$6H_2O$ (5×10^{-6} gL^{-1}), $Na_2MoO_4{\cdot}2H_2O$ (5×10^{-6} gL^{-1}), $CuSO_4{\cdot}5H_2O$ ($0.025 \times 10^{-6} gL^{-1}$), $FeSO_4{\cdot}7H_2O$ ($3.5 \times 10^{-3} gL^{-1}$), Na·EDTA ($4 \times 10^{-3}$ gL^{-1}) [21]. The cultures were incubated in the average light intensity 7 Wm^{-2} at 30 °C temperature.

Algal Growth in Raw Sewage Water

The sewage was collected from a wastewater treatment plant located in Pune (India). Untreated 200 mL sewage was taken in 500 mL Erlenmeyer flasks with 10% algal inoculums. The flasks were incubated in the average light intensity 7 Wm^{-2} at 30 °C temperature. Sewage water then monitored for nutrients (nitrogen and phosphate) concentration, COD and number of algal cells for 3 days. The samples were centrifuged at $3000 \times g$ for 5 min before analysis. Total Kjeldahl nitrogen was analyzed by using KjelTron Nitrogen/Protein digestion system (KDIGB 6 M) by the standard procedure mentioned in American Public Health Association (APHA) 4500-NH_3 A,B,C [22]. Total phosphate were estimated by using thevanado-molybdo-phosphoric acid calorimetric method as mentioned in APHA 4500 [22]. Chemical oxygen demand (COD) was analyzed by using standard methods of APHA 5220B [22]. The number of algal cells was determined by counting in the hemocytometer. The experiment was done in triplicate.

Phosphate Starvation

The *Scenedesmus* and *Ankistrodesmus* growing in the early logarithmic phase were harvested by centrifugation, washed three times with sterile BBM medium without phosphate and nitrate, to remove the media impurity. Part of the washed cells was re-suspended in fresh 200 mL phosphate free BBM and remaining part suspended in phosphate containing BBM (regular medium), such that every flask has approximately 1×10^6 cells mL^{-1}. To determine intracellular phosphate, 10 mL sample was taken from each flask daily. The cells were harvested by centrifugation at the speed of $3000 \times g$ for 5 min and re-suspended in phosphate and nitrate free BBM. The cells were digested by boiling in an autoclave for 1 h with 5:1(v:v) alkaline potassium persulfate [23]. Intracellular phosphate was estimated by the phosphomolybdate-blue method as described in APHA 4500 E [22]. The number of cells was monitored by counting in the hemocytometer. All experiments were done in triplicate.

FTIR Analysis of the Phosphate-Starved and Supplemented Cells

The *Scenedesmus* cells were grown in BBM with and without phosphate for 120 h. The cells were harvested, washed and dried in the vacuumed oven at 100° C. This dried cell mass was utilized for FTIR analysis. For FTIR analysis, sample preparation was carried out as described earlier [18]. Dry algal sample, 2.5 mg was mixed with 150 mg potassium bromide (KBr) using mortar pestle. The mixture was filled in high press 13 mm diameter die to get the pellet. The IR of KBr –algae pellet was recorded at 23 ± 1 °C temperature in the mid-infrared range (4000–450 cm^{-1}) using FTIR (Perkin Elmer, Spectrum One). Thirty scans were single averaged for single spectrum. Each spectrum was displayed in terms of transmission. Analysis, of a peak area estimation was done by Spectrum One software. The carbohydrate-to-protein band ratio was given by the ratio of an area of the carbohydrate region (900–1200 cm^{-1}) and that of amide II band (1300–1500 cm^{-1}).

Utilization of Phosphate-Starved Cells for Sewage Water Treatment

The *Scenedesmus* culture was grown in 2 L flask containing 800 mL BBM. The culture was harvested by centrifugation in the early logarithmic phase. Cells were washed three times with sterile phosphate and nitrate free BBM medium to remove the media impurity and excess phosphate. Half of the cells were inoculated in phosphate free BBM and incubated in light for 48 h for phosphate starvation. The remaining half (untreated) were inoculated in three, 500 mL conical flasks containing 200 mL raw sewage at three initial inoculums sizes were 1×10^6, 5×10^6 and 10×10^6 cells mL^{-1}. Further, the sewage was monitored for its nutrient (nitrate and phosphate) concentrations and COD.

A similar procedure was adopted for the treated cells. Sewage was monitored further for the nutrients and COD. All experiments were conducted in triplicate.

Results and Discussion

Growth of Algae Isolates in Sewage Water

The algae isolated were checked for their growth rate in raw sewage water after isolation. In the first 24 h, the *Scenedesmus* was able to remove 72–76% phosphate; 93% phosphate removal occurred in 72 h. *Scenedesmus* was able to remove 84.5% of nitrogen in 24 h and 98% nitrogen was removal after 72 h. The COD was decreased by 87% during 72 h of incubation with *Scenedesmus* (Fig. 1a). *Ankistrodesmus* was able to remove 72% of phosphate in first 24 h, and up to 83 % of phosphate after 72 h. *Ankistrodesmus* consumed 75% of nitrogen in first 24 h while 100% nitrogen depletion occurred in 72 h. Also, 79% of COD was reduced by *Ankistrodesmus* culture in 72 h (Fig. 1b). However further incubation of *Ankistrodesmus* did not reduce the phosphate concentration in the raw waste-water, as nitrogen was completely utilized in 72 h. Values for phosphate, nitrogen, and COD in the control sewage are shown in Fig. 1c. In all these experiments the initial cell density for both of the culture was 1×10^6 cells mL^{-1}. In previous studies, it took 24 days for 97% removal of phosphate and 59% removal of nitrogen, when *Chlorella* was grown on filtered and autoclaved municipal waste-water [6]. Sing and Thomas [21] used membrane reactor permeated sewage water to grow four different local algal isolates viz. *Chlorella*, *C. vulgaris*, *Scenedesmus quadricauda*, and *S. dimorphus* and found that 66% PO_4 could be removed by the microalgae in first 24 h with an initial cell density of 1.2×10^6 cell mL^{-1}. *Scenedesmus obliquus* with the initial cell density around 2×10^6 cells mL^{-1} was able to remove 100% nitrogen and 83% PO_4 after 48 h from urban waste-water [11].

To make the process more viable for practical application, raw, unsterile sewage was used in the present study, with inoculum of minimum initial cell density. However as it was unsterile, after 24 h, when algae reached their peak growth, zooplanktons (grazers) appeared and reduced the algal cell number. Depletion of nutrient and combating grazers reduced the number of algal cells after 24 h of incubation in the case of *Scenedesmus* (Fig. 1a). However, cells of *Ankistrodesmus* were exceptional to this phenomenon of reduction in the cell number, indicating an ability of growth in low concentration of nutrients like phosphate and nitrogen. It may possess some defence mechanism against grazers as the number of algal cells did not reduce like cells of *Scenedesmus* (Fig. 1b).

Effect of Phosphate Starvation on Algal Cells

When the cells of *Scenedesmus* were inoculated in phosphate supplemented and phosphate free BBM, the growth of *Scenedesmus* in phosphate-free BBM is hampered considerable only after 48 h of incubation. In this duration, the cell density was raised to approximately around, 5×10^6 cells mL^{-1}, from the initial 1×10^6cell mL^{-1} in both types of BBM. The cells growing in phosphate- free medium utilized their internally stored phosphate and able to multiply for first two generations in a similar way as that of normal growing cells. However after 48 h, the growth rate of the phosphate-starved cells was reduced as

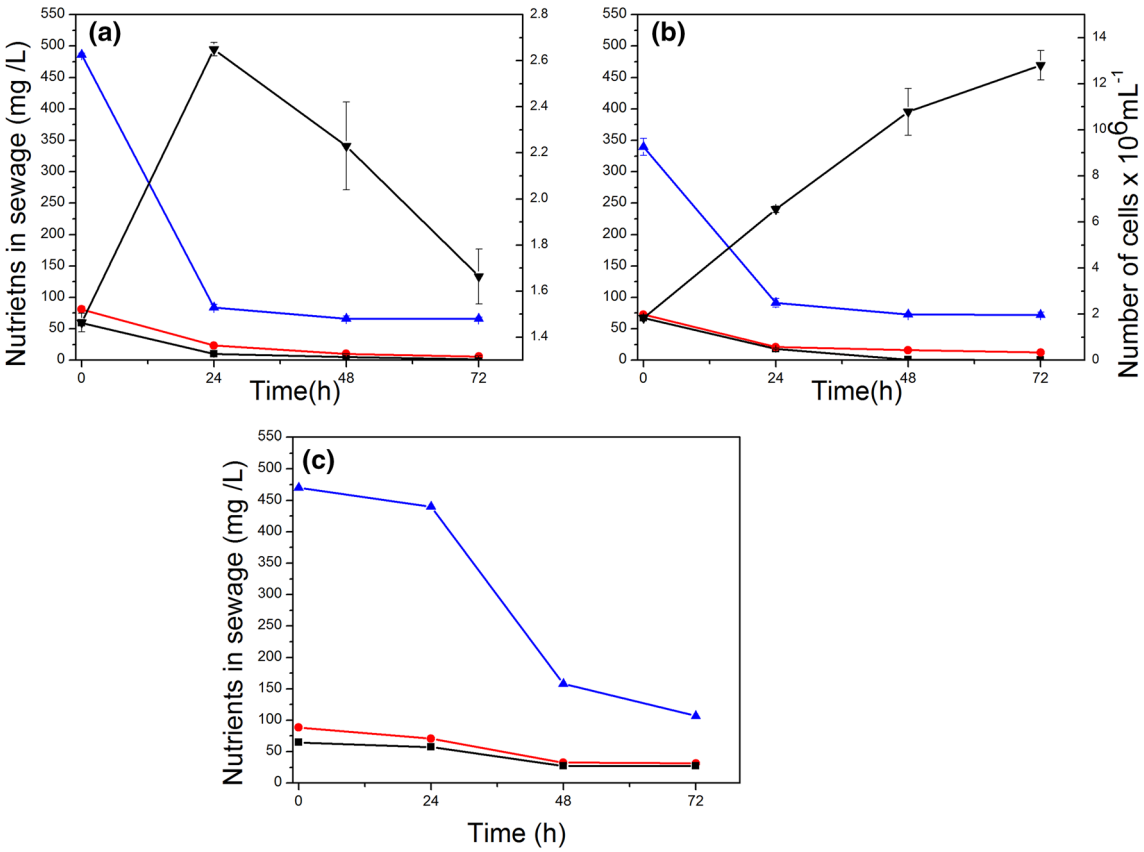

Fig. 1 Growth of algal isolates in sewage: Growth of algal isolates and concomitant reduction of total phosphate and nitrogen from sewage. **a** Growth of *Scenedesmus*, **b** growth of *Ankistrodesmus*, **c** control or un inoculated sewage. X axis shows time in h. Y axis shows *filled square* Kjealdal nitrogen in sewage (mg L^{-1}), *filled circle* total phosphate sewage (mg L^{-1}), *filled triangle* COD of sewage (mg L^{-1}), *filled inverted triangle* number of algal cells $\times$ 10^6 mL^{-1}

compared to the algal cells growing in the normal medium. At the end of 96 h incubation, the phosphate supplemented cells were able to multiply for more than four generation while the phosphate-starved cells showed only 2.5 generations. In other words, the phosphate-starved *Scenedesmus* cells showed the stationary phase of the growth cycle after 48 h (Fig. 2b). In a case of *Ankistrodesmus*, no reduction in the growth rate was not observed after giving phosphate starvation (data not shown). These algal cells were also observed growing in sewage when nitrogen was almost depleted and with 12 ppm of total phosphate indicating heterotrophic growth (Fig. 1b). Hence, cells of *Ankistrodesmus* were not selected for the treatment of phosphate starvation and utilizing further.

The internal phosphate concentration in the phosphate supplemented *Scenedesmus* cells showed an increasing trend indicating the phosphate accumulation in the daughter cells while growing in the phosphate-rich medium. After 120 h the internal phosphate concentration in the phosphate supplemented *Scenedesmus* cells was raised up to 0.41 μg mg^{-1} of fresh weight (Fig. 2a). The cells growing in phosphate free BBM though did not showed significant reduction in the internal total phosphate content for first 96 h of incubation as the stored phosphate might be getting distributed to the daughter cells. However at the end of 120 h of incubation internal phosphate was reduced from 0.12 to 0.078 μg mg^{-1} of fresh weight in the phosphate-starved cells (Fig. 2a). Cells of *Phormidium*, *Sphaerocystis* and *Scenedesmus* when grown in phosphate supplemented medium showed an increase in the intracellular phosphate content [19]. When grown in phosphate-starved medium algae able to utilize the internal phosphate and could sustain for 3–4 generations under the starvation conditions [14].

FTIR Analysis of Phosphate-Starved and Supplemented *Scenedesmus* Cell

Effect of the phosphate starvation was further studied with FTIR. To visualize the effect of phosphate starvation stress, the integrated FTIR band of different spectral were studied. The dried cell mass showed transmission peaks over wave number 450–4000 cm^{-1}, are given in Fig. 3. The peaks were tentatively identified on the basis

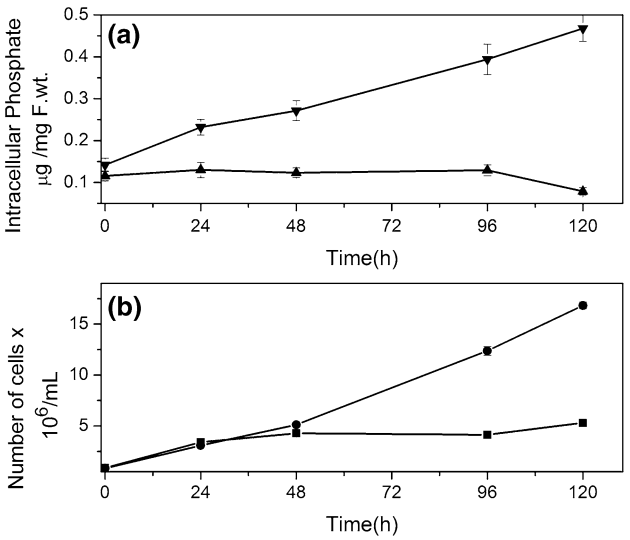

Fig. 2 Phosphate starvation effect on *Scenedesmus*. **a** Intracellular phosphate concentration in *Scenedesmus*. **b** Growth of *Scenedesmus* in phosphate free BBM and Phosphate supplemented BBM. X-axis shows time in h for **a** and **b**. For **a** Y axis shows *filled triangle* intracellular phosphate concentration in the phosphate-starved cells, *filled inverted triangle* intracellular phosphate concentration in the phosphate supplemented cells (µg mg^{-1} of fresh weight of the cells). In **b** Y axis have *filled square* number of phosphate-starved cells × 10^6 cells mL^{-1}, *filled circle* number phosphate supplemented cells × 10^6 cells mL^{-1}

of published FTIR spectra in relation to the specific molecular groups.

The FTIR spectra were compared by taking the ratios of areas, of the respective IR bands in the absorbance mode. The region from 900 to 1200 cm^{-1} are characteristics of C–C, C-O, C–O–C, C–O–P of polysaccharides stretching vibrations of polysaccharides [17, 18]. The cells grown in phosphate free BBM showed the peak at 1022, 1077, 1156 cm^{-1} (Fig. 3a) These peaks are due to various polysaccharides. Carbohydrate pool containing various polysaccharides increased during the phosphate-starved conditions. The increase in polysaccharides is another indicator of the stationary phase of the algae growth cycle. Cells were grown in phosphate supplemented BBM showed the peak at only at 1085, 1152 cm^{-1} (Fig. 3b). However, algae growing in phosphate supplemented BBM did not show the peak at 1022 cm^{-1}. Similar findings were reported in *Sphaerocystis* and *Phormidium*, where these algae showed strong bonds at 1024, 1080 and 1150 cm^{-1} under phosphate starvation, however, these bonds were disappeared within 24 h after supplementing cells with phosphate [19].

Phosphodiester bond stretching generates peak at 1245 and 1240 cm^{-1} in phosphate- starved and supplemented algal cells respectively. We found an increase in intensity and broadening of the aromatic phosphate bond (> P=O) at 1240 cm^{-1} and symmetric aliphatic phosphate bond (C–O–P) at 1085 cm^{-1} indicating phosphate storage in the phosphate supplemented *Scenedesmus* cells. This results of FTIR got confirmed when the intracellular phosphate was quantified with the phosphomolybdate-blue method, the phosphate supplemented cells showed 5.7 times more intracellular phosphate than the phosphate-starved cell (Fig. 2a). Collective effect of phosphate starvation and supplementation on the macromolecular pool is given in (Fig. 4).

The phosphate-starved *Scenedesmus* cells showed significantly increased in carbohydrate/phosphor (C/P), carbohydrate/lipid (C/L) and carbohydrate/protein (C/AII) ratio. When cells of *Microcystis aeruginosa* and *Phaeodactylum tricornutum* were grown in phosphate-limited conditions, they showed decreased growth rate, and increase in (C/AII) ratio [16, 24, 25]. *Phaeodactylum tricornutum* showed increased in lipid/phosphate after 3 weeks of phosphate starvation [24]. In this study L/P ratio of phosphate supplemented and starved *Scenedesmus* cell did not differ significantly. This may be due to a short starving period of 120 h. The Lipid/amide II (L/AII) and amide/phosphor (AII/P) did not show a significant difference after phosphate starvation. FTIR study confirms the stationary phase in phosphate-starved *Scenedesmus* cells as observed in growth curve study (Fig. 2). These starved, stationary algal cells might be able to consume nutrients at faster rate compare to the phosphate supplemented cells which might prove helpful for the faster treatment of raw sewage.

Comparison of Phosphate-Starved and Supplemented *Scenedesmus* for Sewage Water Treatment

The phosphate-starved and phosphate supplemented *Scenedesmus* cells were used for the treatment of sewage. The sewage water with initial phosphate concentration 124 mg L^{-1} when treated with phosphate-starved 1 × 10^6 cells mL^{-1}, the phosphate was reduced to 17.6 mg L^{-1}(86%) at the end of 24 h (Fig. 5a). Similar cell density of phosphate supplemented cells was able to reduce the phosphate concentration to 27 mg L^{-1} (76%) (Fig. 5a, b). Previously three days phosphate-starved and immobilized cells of *Chlorella sorokiniana* able to reduce the 23% phosphate from synthetic waste water in 48 h. This reduction was further enhanced by co- immobilizing *C. sorokiniana* with bacteria *Azospirillum brasilense* [8]. Zhang et al. [9] used two days starved cells of *Scenedesmus*, immobilized 2 × 10^8 cells mL^{-1} (cell intensity in the bead) and found 100% removal of PO_4 in just 135 min from filtered, sterilized secondary domestic waste water. Similar experimentation was done with 2 days phosphate-starved *Chlorella*, with immobilized 1.4 × 10^8 cells mL^{-1}(cell intensity

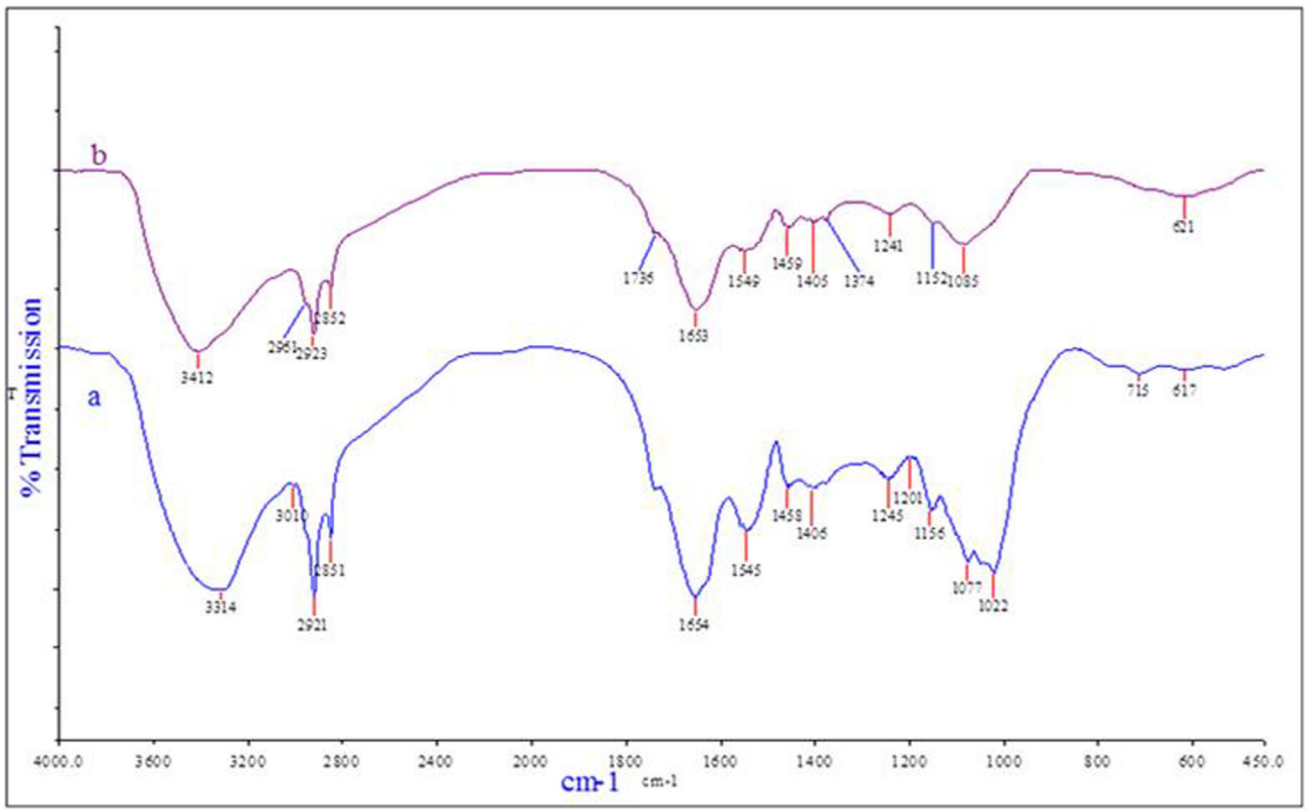

Fig. 3 FTIR spectrum: FTIR peak of phosphate *a* starved and *b* supplemented cells of *Scenedesmus*. At X axis—wavelength (cm^{-1}), Y axis represents—% Transmission every spectrum is average of thirty scans. The phosphate starved cells showing peaks at 1022 cm^{-1}. This polysaccharide peak is absent in phosphate supplemented cells, however these cells showed broad peak at 1240 and 1085 cm^{-1}

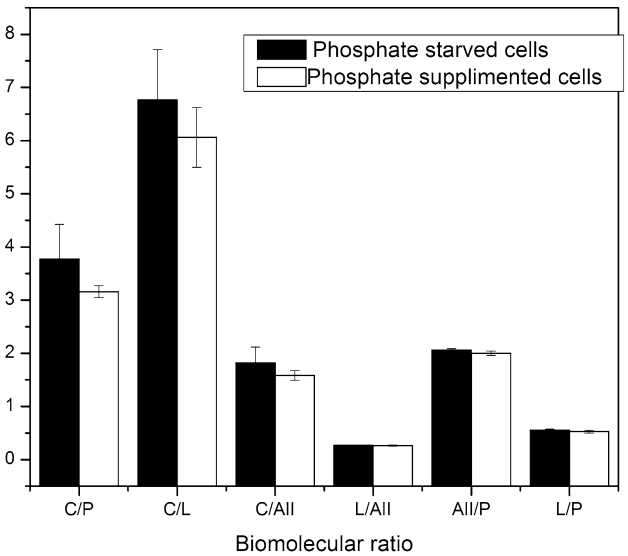

Fig. 4 Effect of phosphate starvation on macromolecular pool: The bond ratios of the various biomolecules of phosphate-starved (*gray bars*) and supplemented (*white bars*) *Scenedesmus* cells are plotted on X axis. Carbohydrate/phosphor (C/P), carbohydrate/lipid (C/L) and carbohydrate/protein (C/AII) ratio is increased in phosphate starved cells

in the bead), took 4 h for the complete removal of phosphate from filtered, sterilized secondary domestic wastewater [15]. In this study raw sewage water without any prior treatment like filtration and sterilization is used. Hence, it is more viable to the practical application. This unsterilized, uninnoculated sewage has it's own natural microbial flora which is responsible for reduction in phosphate, nitrogen and COD with time (Figs. 5, 6, 7).

The phosphate-starved *Scenedesmus* cells, with initial cell density 1×10^6 cells mL^{-1}, were able to utilize 100% nitrogen within 24 h (Fig. 6a) however the phosphate supplemented cells were able to reduce the nitrogen level up to 24 mg L^{-1} (52%) within 24 h (Fig. 6b). The initial total nitrogen concentration in the sewage water was 50 mg L^{-1}. Indicating fast uptake of nitrogen by the starved, stationary, *Scenedesmus* cells. Zhang et al. [9] report 99.1% NH_4 removal by two days phosphate-starved *Scenedesmus* from filtered, sterilized secondary domestic waste water. When 2×10^8 cells mL^{-1} immobilized (cell intensity in the bead) were used for the treatment. Two days phosphate-starved *Chlorella*, with immobilized 1.4×10^8 cells mL^{-1} (cell intensity in the bead), took 4 h for the 98.8% removal of NH_4 from filtered, sterilized

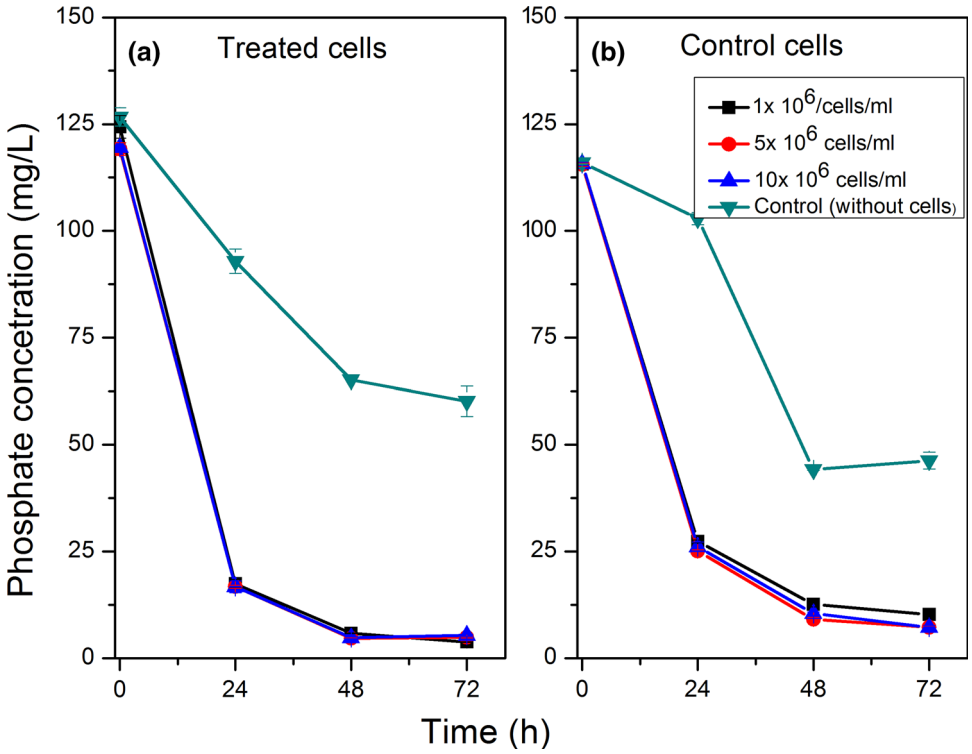

Fig. 5 Effect of algal phosphate starvation on phosphate uptake: the phosphate-starved (**a**) and phosphate supplemented (**b**) *Scenedesmus* cells inoculated in sewage. The X-axis represents time in h. Y-axis shows phosphate concentration in mg L^{-1} utilized by *filled square* 1×10^6 cells mL^{-1}, *filled circle* 5×10^6 cells mL^{-1}, *filled triangle* 10×10^6 cells mL^{-1}, *filled inverted triangle* un inoculated sewage

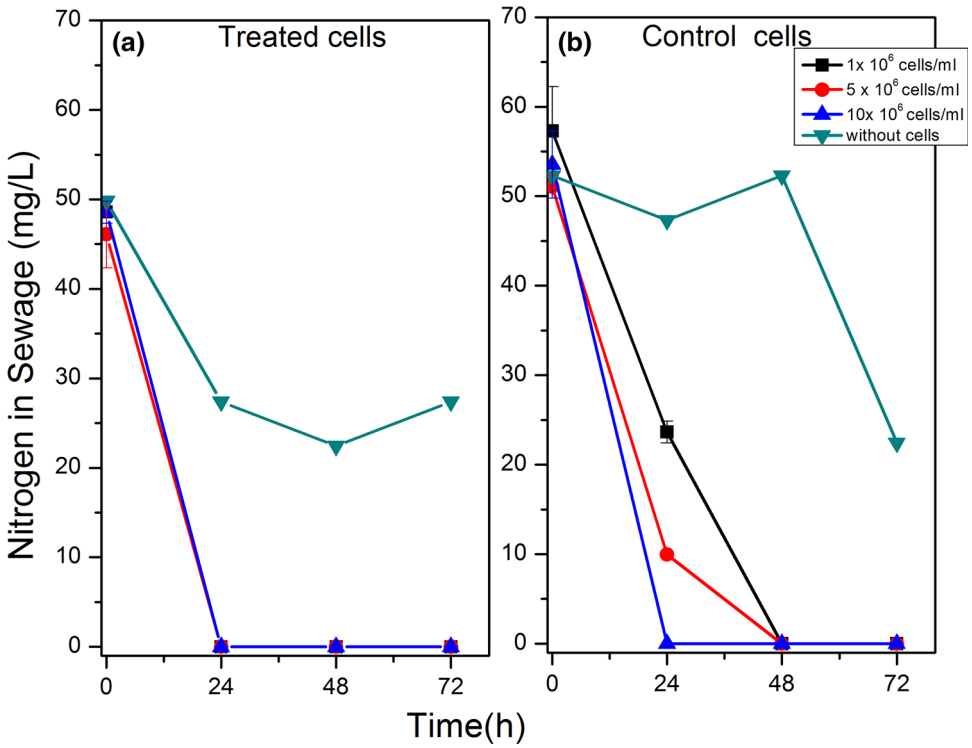

Fig. 6 Effect of algal phosphate starvation on nitrogen uptake: The phosphate-starved (**a**) and phosphate supplemented (**b**) *Scenedesmus* cells inoculated in sewage. The X axis represents time in h. Y axis shows nitrogen concentration in mg l^{-1} utilized by *filled square* 1×10^6 cells mL^{-1}, *filled circle* 5×10^6 cells mL^{-1}, *filled triangle* 10×10^6 cells mL^{-1}, *filled inverted triangle* un inoculated sewage

secondary domestic wastewater [15]. In the present study P-starved *Scenedesmus* isolate showed no significant increase in phosphate and nitrogen reduction with the increase in a number of free cells (1×10^6 to 10×10^6 - cell mL^{-1}) in the sewage water (Figs. 5a, 6a). However, the phosphate supplemented cells at higher cell density showed faster nitrogen reduction with increasing thenumber of free cells in the sewage (Figs. 5b, 6b). Reduction of the COD was similar for both phosphate-starved and supplemented cells (Fig. 7a, b).

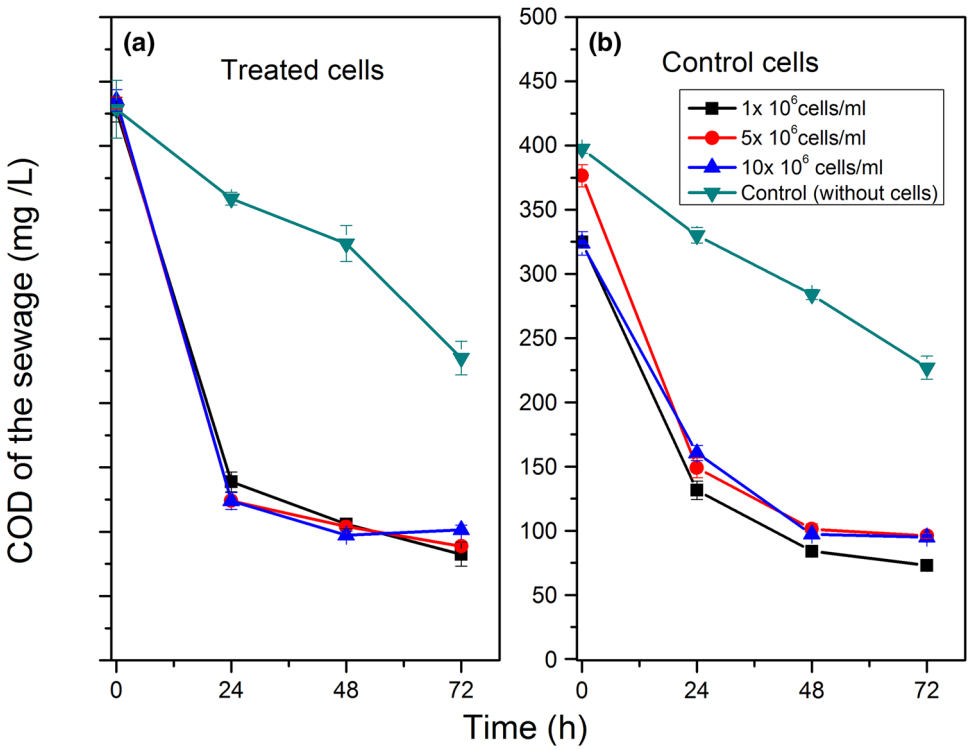

Fig. 7 Effect of algal phosphate starvation on COD reduction: The phosphate starved (**a**) and phosphate supplemented (**b**) *Scenedesmus* cells inoculated in sewage. The X axis represents time in h. Y axis shows COD mg L^{-1} utilized by *filled square* 1×10^6 cells mL^{-1}, *filled circle* 5×10^6 cells mL^{-1}, *filled triangle* 10×10^6 cells mL^{-1}, *filled inverted triangle* un inoculated sewage

Conclusions

The local algal isolate *Scenedesmus* was entering in the stationary phase of life cycle due to the phosphate starvation. These stationary phase cells of *Scenedesmus* showed effective phosphate and nitrogen utilization from the untreated sewage water. However, the phosphate starvation did not induce any stationary phase in the cells of *Ankistrodesmus*. Phosphate starvation of *Scenedesmus* for 120 h showed a reduction in the internal phosphate and rise in carbohydrate pool. Phosphate starvation of algal cultures appears to be a good technique for an enhanced rate of removal of the total phosphorus and nitrogen contents in sewage. Additional detailed studies need to be conducted under steady state conditions in a chemostat to determine how phosphate starvation triggers an enhanced rate of nitrogen and phosphate.

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Compliance with Ethical Standards

Conflict of interest No potential conflicts of interest was reported by the author(s).

References

1. de-Bashan LE, Bashan Y (2004) Recent advances in removing phosphorous from wastewater and its future use as fertilizer (1997–2003). Water Res 38:4222–4246. doi:10.1016/j.waters.2004.07.014
2. Powell N, Shilton AN, Pratt S et al (2008) Factors influencing luxury uptake of phosphate by microalgae in waste stabilization pond. Environ Sci Technol 42:5958–5962. doi:10.1021/es703118s
3. Woertz I, Feffer A, Lundquist T et al (2009) Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. J Environ Eng 135:1115–1122. doi:10.1061/9ASCE0EE.1943-7870.0000129
4. Xin L, Hong-Ying H, Ke G et al (2010) Effect of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of fresh water microalga *Scenedesmus* sp. Bioresour Technol 101:5494–5600. doi:10.1016/j.biortech.2010.02.016
5. Olguin E (2012) Dual purpose microalgae–bacteria-based systems that treat wastewater and produce biodiesel and chemical products within a Biorefinery. Biotech Adv 30:1031–1046. doi:10.1016/j.biotechadv.2012.05.001
6. Wang C, Yu X, Lv H et al (2013) Nitrogen and phosphorus removal from municipal wastewater by the green alga *Chlorella* sp. J Environ Biol 34:421–425
7. Lau SP, Tam FYN, Wong SY (1995) Effect of algal density on nutrient removal from primary settled wastewater. Environ Pollut 89:59–66. doi:10.1016/0269-7491(94)00044-E
8. Hernandez JP, de-Bashan LE, Bashan Y (2006) Starvation enhances phosphorus removal from wastewater by microalga *Chlorella* spp. co-immobilized with *Azospirillum brasilense*. Enzyme Microb Technol 38:190–198. doi:10.1016/j.enzmictec.2005.06.005

9. Zhang E, Wang B, Wang Q et al (2008) Ammonia–nitrogen and orthophosphate removal by immobilized *Scenedesmus* sp. isolated from municipal wastewater for potential use in tertiary treatment. Bioresour Technol 99:3787–3793. doi:10.1016/j.biortech.2007.07.011
10. Mallick N (2002) Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review. BioMetals 15:377–390. doi:10.1023/A:1020238520948
11. Ruiz-Marin A, Mendoza- Espinosa LG, Stephenson T (2010) Growth and nutrient removal in free and immobilized green algae in batch and semi continuous culture treating real wastewater. Bioresour Technol 101:58–64. doi:10.1016/j.biortech.2009.02.076
12. Eixler S, Karsten U, Seling U (2006) Phosphorus storage in *Chlorellavulgaris* (Trebouxiphyceae, Chlorophyta) cells and it's dependence on phosphate supply. Phycologia 45:53–60. doi:10.2216/04-79.1
13. Prieto B, Pardo MA, Garbisu C et al (1997) Phosphate uptake by phosphate starved cells of cyanobacterium *Phormidium laminosum*. World J Microbiol Biotechnol 13:699–705. doi:10.1023/A:1018583224294
14. Yao B, Xi B, Hu C et al (2011) A model and experimental study of phosphate uptake kinetics in algae considering surface adsorption and P-stress. J Environ Sci 23:189–198. doi:10.1016/S1001-0742(10)60392-0
15. Zhang E, Wang B, Ning S et al (2012) Ammonia–nitrogen and orthophosphate removal by immobilized *Chlorella* sp. isolated from municipal wastewater for potential use in tertiary treatment. Afr J Biotechnol 11:6529–6534. doi:10.5897/AJB11.4281
16. Jebsen C, Norici A, Wagner H et al (2012) FTIR spectra of algal species can be used as physiological fingerprints to assess their actual growth potential. Physiol Plant 146:427–438. doi:10.1111/j.1399-3054.2012.01636.x
17. Duygu D, Udoh UA, Ozer T et al (2012) Flourier transform infrared (FTIR) spectroscopy for identification of *Chlorella vulgaris* Beijerinck 1890 and *Scenedesmus obliquus* (Turpin) Kützing 1833. Afr J Biotechnol 11:3817–3824. doi:10.5897/AJB11.1863
18. D'Souza L, Devi P, DivyaShridhar MP et al (2008) Use of Fourier transform Infrared (FTIR) spectroscopy to study cadmium induced changes in *Padina tetrastromatica* (Hauck). Anal Chem Insights 3:135–143
19. Beardall J, Breman T, Heraud P et al (2001) A comparison of methods for detection of phosphate limitation in microalgae. Aquat Sci 63:107–121. doi:10.1007/PL00001342
20. Devi MP, Subhash GV, Mohan SV (2012) Heterotrophic cultivation of mixed microalgae for lipid accumulation and wastewater treatment during sequential growth and starvation phases: effect of nutrient supplementation. Renew Energ 43:276–283. doi:10.1016/j.renene.2011.11.021
21. Singh G, Thomas PB (2012) Nutrient removal from membrane bioreactor permeate using microalgae and in a microalgae membrane photoreactor. BioresourTechnol 117:80–85. doi:10.1016/j.biortech.2012.03.125
22. Clescir LS, Greenberg AE, Eaton AD (1999) Standard methods for the examination of water and wastewater, 20th edn. Americal Public Health Asscoiation, American Water Work Association, Water Environment Federation (APHA, AWWA, and WEF). Washington, DC, USA
23. Maher W, Krikowa F, Louie HW et al (2002) Determination of total phosphorus and nitrogen in turbid wasters by oxidation with alkaline potassium peroxodisulfate and low pressure microwave digestion, autoclave heating or the use of closed vessels in a hot water bath: comparison with Kjeldahl digestion. Anal Chem Acta 463:283–293. doi:10.1016/S0003-2670(02)00346-X
24. Stehfest K, Toepel J, Wilhelm C (2005) The application of micro-FTIR spectroscopy to analyze nutrient stress related changes in biomass composition of phytoplankton algae. Plant Physiol Biochem 43:717–726. doi:10.1016/j.plaphy.2005.07.001
25. Ponnuswamy I, Madhavan S, Shabudeen S (2013) Isolation and characterization of green microalgae for carbon sequestration, waste water treatment and bio fuel production. Int J Biosci Biotechnol 5:17–26