ARTICLE IN PRESS
Alexandria Engineering Journal (2015) xxx, xxx–xxx

HOSTED BY
ELSEVIER

Alexandria University
Alexandria Engineering Journal
www.elsevier.com/locate/aej
www.sciencedirect.com

ORIGINAL ARTICLE

# Studies on reduction of inorganic pollutants from wastewater by *Chlorella pyrenoidosa* and *Scenedesmus abundans*

**B. Lekshmi, Rebekah S. Joseph, Anitta Jose, S. Abinandan, S. Shanthakumar** *

*Environmental Engineering Division, School of Mechanical and Building Sciences, VIT University, Vellore 632014, TN, India*

Received 3 September 2014; accepted 29 September 2015

**KEYWORDS**

*Chlorella pyrenoidosa*;
*Scenedesmus abundans*;
Wastewater;
Remediation

**Abstract** The aim of this study was to identify the potential for cultivation of *Chlorella pyrenoidosa* and *Scenedesmus abundans* in raw and autoclaved domestic wastewater (sewage) for nutrient removal, in a batch process. The growth was observed by measuring chlorophyll content. The inoculum size of 10% and 20% was used and the growth of microalgae and nutrient removal was monitored on daily basis. The maximum removal of ammonium nitrogen, phosphate and nitrates by *Chlorella pyrenoidosa* in raw samples was observed as 99%, 96% and 80%, respectively, whereas the maximum removal of ammonium nitrogen, phosphate and nitrates by *Scenedesmus abundans* in raw samples was observed as 98%, 95% and 83%, respectively. The maximum chlorophyll content was observed as 11.33 mg/l and 7.23 mg/l for *C. pyrenoidosa* and *S. abundans*, respectively, in raw samples. The experimental results reveal that both the microalgae are capable to grow and remove the nutrients from domestic wastewater.


## 1. Introduction

An alternative source for producing biofuel in present scenario helps in reducing environmental impact across the globe. Facilitating photosynthetic process of harnessing $CO_2$ for its growth and storing as energy source for biofuel production, compared to other microbes such as bacteria and fungi, establish algae as sustainable products for biofuel production [1]. Also, bio-energy production based on photosynthesis requires less water for cultivation of microalgae compared to other sources [2–4]. However, cultivating microalgae in freshwater for biodiesel production at large scale is still a challenging part as there is an increase in demand for freshwater for various purposes. The ideology of cultivating microalgae in wastewater is to reduce the growth media components for cultivation and also it helps in cleaning up (i.e., nutrient removal) the wastewater [5].

Although several other photosynthetic groups exist such as phototrophic bacteria, cyanobacteria, and diatoms, green microalgae are importantly used for cultivation in various wastewater sources. Microalgae also utilize inorganic nutrients such as Chlorides and sulfates that are needed to be removed from wastewater. Nitrogen and phosphates are important

* Corresponding author. Tel.: +91 416 2202209.
E-mail address: shanthakumar.s@vit.ac.in (S. Shanthakumar).
Peer review under responsibility of Faculty of Engineering, Alexandria University.

http://dx.doi.org/10.1016/j.aej.2015.09.013
1110-0168 © 2015 Faculty of Engineering, Alexandria University. Production and hosting by Elsevier B.V.

compounds that play a major role for algal growth in wastewater systems. Nitrates, Nitrites and Ammonium are the important nitrogen sources for microalgae in wastewater. Phosphates are also another major nutrient that is adequately present and plays a major role as intermediates in carbohydrate metabolic activity [6]. The main challenge in cultivating microalgae in raw wastewater is the presence of other microbes that affect the biomass productivity [7,8]. The wastewater produced from urban areas consists of major eutrophic compounds that affect other water bodies when discharged without meeting its desirable limits [9].

Present scenario using biological treatment has a setback in treating wastewater as the bacteria require fixed N:P ratio [10]. The effluent from the secondary treatment unit of domestic wastewater treatment plant can be used for large scale cultivation of microalgae for biofuel production [11,12]. Chlorophyll *a* is the photosynthetic pigment that is presented only in the green microalgae and contains four nitrogen molecules for each compound. Usually, all microalgae are photoautotrophs that absorb light and convert into Adenosine triphosphate (ATP), and Nicotinamide adenine dihydrogen phosphate (NADPH) to carry out photosynthesis [13]. In general, microalgae convert inorganic nitrogen into organic nitrogen and assimilate as chloroplastic nitrogen [6,14]. Reducing phosphate to lowest level in wastewater treatment plants is important to reduce chances of water pollution [15]. Green algae are prominently used for domestic wastewater treatment [5,8,12,16–20]. Although previously several studies have been conducted on microalgae for wastewater treatment, not many studies have been carried out on *Chlorella pyrenoidosa* and *Scenedesmus abundans* with emphasis on ability for inorganic pollutant reduction in raw and autoclaved sample with respect to inoculum sizes. Within this view an attempt has been made to study the effectiveness of *C. pyrenoidosa* and *S. abundans* for removal of pollutants in domestic wastewater (raw and autoclaved) by conducting batch studies and monitoring on daily basis.

## 2. Materials and methods

### 2.1. Culture preparation

*C. pyrenoidosa (NCIM 2789)* and *S. abundans (NCIM 2897)* were collected from National Collection of Industrial Microorganisms (NCIM), Pune, India. The cultures were separately grown in 250 ml Erlenmeyer flasks containing 100 ml of BG 11 medium [21].

### 2.2. Sample collection and its characteristics

The wastewater sample was collected from the aeration tank of sewage treatment plant in a pre-cleaned, sterilized polyethylene bottles. The collected samples were labeled, taken to the laboratory and stored in a laboratory refrigerator at 4 °C. All the chemicals used in the study are purchased from Thomas baker Ltd. (Mumbai, India) and the reagents were prepared using double distilled water. The analysis of various characteristics of the sample has been carried out as per standard procedure prescribed by American public health association [22] and the results are presented in Table 1.

**Table 1** Characteristics of sewage wastewater sample.

| Parameters[a] | Values |
|---|---|
| Turbidity (NTU) | 379 |
| pH | 7.7 |
| Alkalinity | 365 |
| Biological Oxygen Demand | 236 |
| Total Hardness | 19 |
| Chemical Oxygen Demand | 286 |
| Sulfates | 32 |
| Total solids | 3500 |
| Ammoniacal nitrogen | 992 |
| Nitrates | 197 |
| Phosphates | 286 |
| Chlorides | 268 |

[a] All parameters (except pH and turbidity) are in mg/l.

### 2.3. Experimental studies

Batch scale experiments were conducted on daily basis to understand the ability of microalgal cultures for reduction of inorganic pollutants. The main parameters such as Ammoniacal Nitrogen ($NH_3$-N), Phosphates ($PO_4^{3-}$), Nitrate ($NO_3$-N), and Chloride ($Cl^-$) were considered for nutrient removal study and measure of Chlorophyll *a* for growth measurement. All the experiments were conducted in 500 ml conical flasks containing 300 ml of the wastewater sample under continuous illumination of 1800 lux measured using TES Lux meter (TES CORP).

#### 2.3.1. Chlorophyll estimation

The growth of microalgae was monitored on daily basis by determining its chlorophyll content. The chlorophyll content of microalgae is determined by methanol extraction followed by spectrophotometric analysis [23]. 3 ml algae culture was centrifuged at 10,000 rpm for 10 min. The supernatant was drained off and the sample was re-suspended in ethanol/diethyl ether and kept boiling for 5 min. After boiling, the sample was made up to 5 ml with ethanol/diethyl ether. The optical density was measured at 660 nm and 642.5 nm with solvent as a blank. The chlorophyll content was determined using the following formula:

$$\text{Chlorophyll (mg/l)} = (9.9 * OD_{660}) - (0.77 * OD_{642.5})$$

## 3. Results

The chlorophyll profile of *C. pyrenoidosa* and *S. abundans* with different inoculum size is presented in Fig. 1. It can be noted from Fig. 1(a) that the maximum chlorophyll content in raw sewage sample for *C. pyrenoidosa* with inoculum concentration of 10% and 20% was observed as 11.4 mg/l and 7.73 mg/l in 4th day and 7th day, respectively. Similarly, from Fig. 1(b), it can be noted that the maximum chlorophyll content in raw sewage sample for *S. abundans* with inoculum concentration of 10% and 20% was observed as 7.23 mg/l and 7.12 mg/l in 6th day and 7th day, respectively.

### 3.1. Nutrient removal from sewage

#### 3.1.1. Nitrogen removal

Nitrogen is one of the important compounds that are mainly present in wastewater that exists in different forms viz., nitrite,

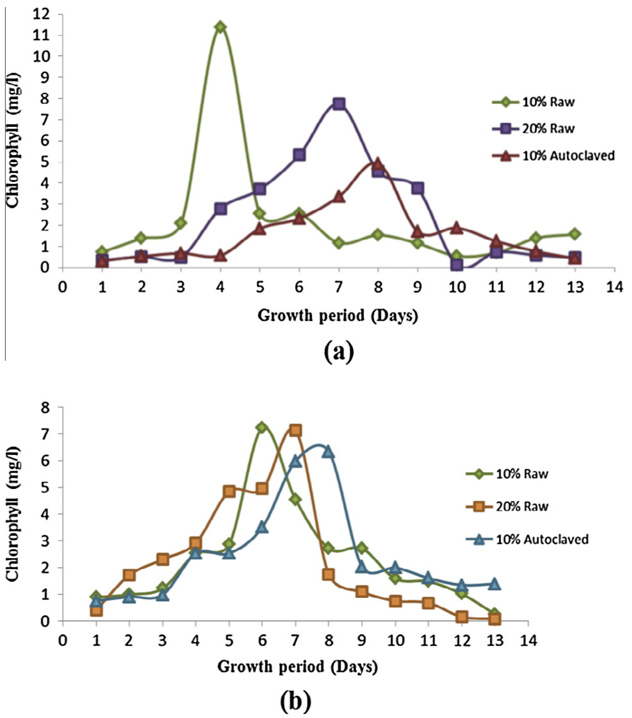


**Figure 1** Growth curve of (a) *Chlorella pyrenoidosa* and (b) *Scenedesmus abundans*.

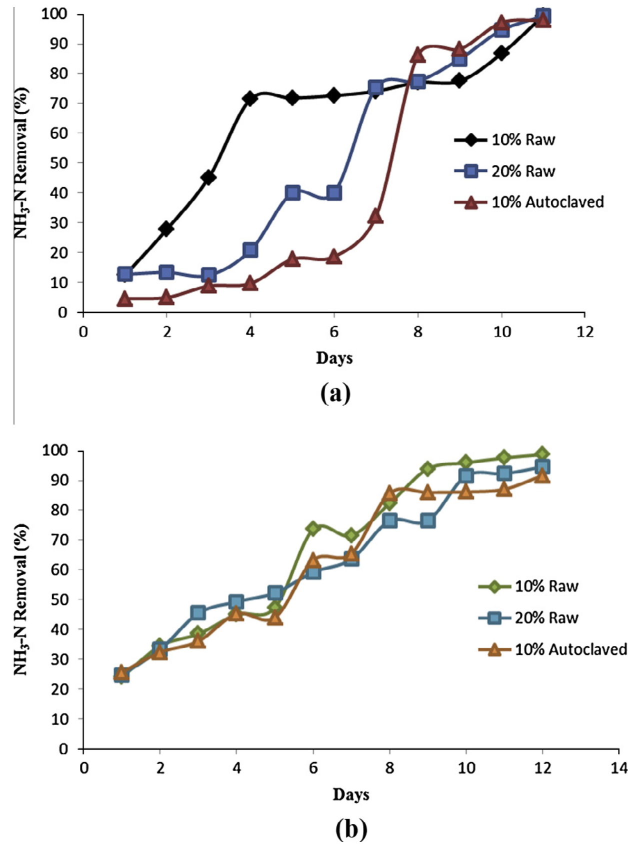


**Figure 2** Ammoniacal –Nitrogen ($NH_3$–N) removal by (a) *Chlorella pyrenoidosa* and (b) *Scenedesmus abundans*.

nitrate and ammonium and that plays a vital role for survival of microorganisms. The results of batch study conducted for removal of Ammoniacal-nitrogen ($NH_3$-N) are presented in Fig. 2a and b for *C. pyrenoidosa S. abundans*. It can be noted from Fig. 2a that the maximum removal of Ammoniacal-Nitrogen ($NH_3$-N) by *C. pyrenoidosa* cultivated in raw sample with the inoculum size of 10% and 20% was observed as 99.46% and 99.3%, respectively, whereas, in autoclaved sample with the inoculum size of 10%, it was observed as 98.86%. Similarly, it can be noted from Fig. 2b that the maximum removal of Ammoniacal-Nitrogen ($NH_3$-N) by *S. abundans* cultivated in raw sample with the inoculum size of 10% and 20% was observed as 98.68% and 94.6%, respectively, whereas, in autoclaved sample with the inoculum size of 10%, it was observed as 91.66%.

Nitrates are one among the supportive nitrogen sources for microalgae. Fig. 3a and b presents the nitrates removal by *C. pyrenoidosa* and *S. abundans* in raw sample and autoclaved sample, respectively. It can be noted from Fig. 3(a) that the maximum nitrate removal by *C. pyrenoidosa* cultivated in raw sample with the inoculum size of 10% and 20% was observed as 78% and 71%, respectively, whereas, in autoclaved sample with the inoculum size of 10%, it was observed as 80%. Similarly, it can be noted from Fig. 3(b) that the maximum nitrate removal by *S. abundans* cultivated in raw sample with the inoculum size of 10% and 20% was observed as 81% and 74%, respectively, whereas, in autoclaved sample with the inoculum size of 10%, it was observed as 83%.

### *3.1.2. Phosphate removal*

Phosphate is one of the important macronutrients for microalgae that are presented in lipids and nucleic acids and also act as intermediates for carbon metabolism [6]. Also, the metabolic uptake of phosphate begins with phosphorylation which converts ADP to ATP [24]. The removal of phosphate by *C. pyrenoidosa* and *S. abundans* is presented in Fig. 4a, Fig. 4b respectively. It can be noted from Fig. 4(a) that the maximum phosphate removal by *C. pyrenoidosa* cultivated in raw sample with the inoculum size of 10% and 20% was observed as 97% and 96%, respectively, whereas, in autoclaved sample with the inoculum size of 10%, it was observed as 86%. Similarly, it can be noted from Fig. 4(b) that the maximum phosphate removal by *S. abundans* cultivated in raw sample with the inoculum size of 10% and 20% was observed as 96% and 97%, respectively, whereas, in autoclaved sample with the inoculum size of 10%, it was observed as 96%. Notably, *C. pyrenoidosa* with inoculum size of 10% showed variation compared to 20% inoculum size in raw sample which is due to prolonged acclimatization period.

### *3.1.3. Chlorides*

The chloride removal efficiency by *C. pyrenoidosa* and *S. abundans* in raw sample and autoclaved sample is presented in Fig. 5a and b. The batch studies conducted in raw sample using *C. pyrenoidosa* (Fig. 5a) with the inoculum size of 10% and 20% showed the chloride removal of 91% and 86%, respectively, whereas, in autoclaved sample with 10% inoculum size, it was observed as 88%. Similarly for *S. abundans* (Fig. 5b) the chloride removal efficiency was observed as 85% and 91% for raw samples with inoculum size of 10%

Please cite this article in press as: B. Lekshmi et al., Studies on reduction of inorganic pollutants from wastewater by *Chlorella pyrenoidosa* and *Scenedesmus abundans*, Alexandria Eng. J. (2015), http://dx.doi.org/10.1016/j.aej.2015.09.013

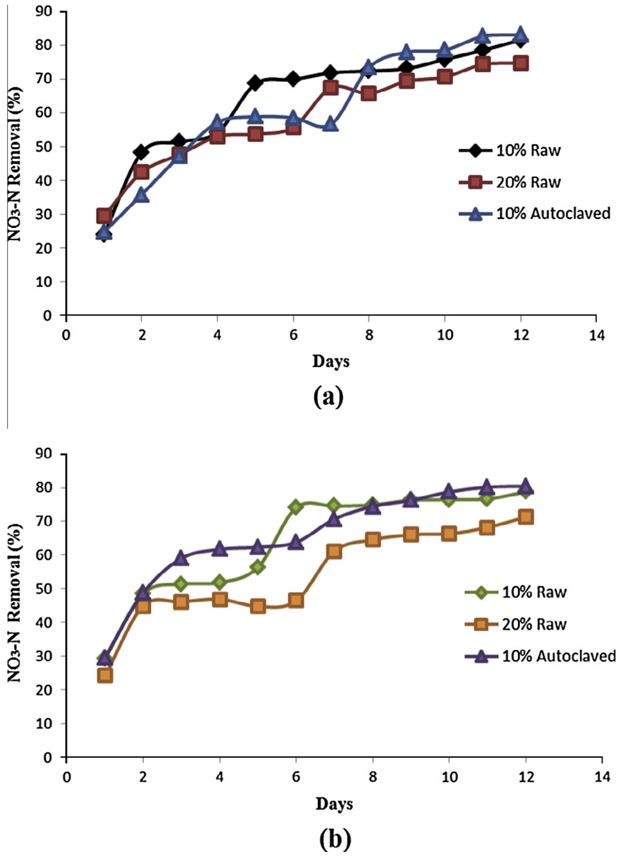


**Figure 3** Nitrate ($NO_3$–N) removal efficiency by (a) *Chlorella pyrenoidosa* and (b) *Scenedesmus abundans*.

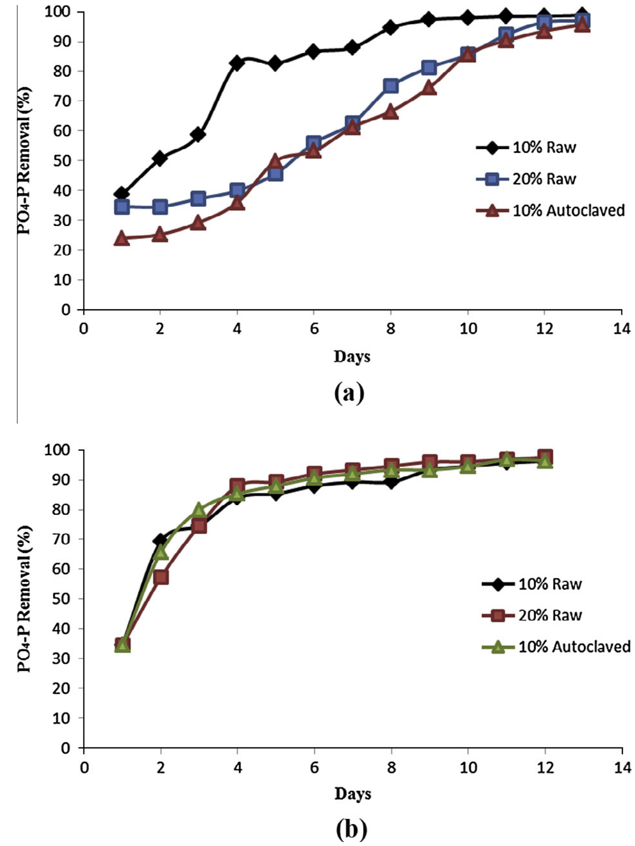


**Figure 4** Phosphate ($PO_4^{3-}$) removal by (a) *Chlorella pyrenoidosa* and (b) *Scenedesmus abundans*.

and 20%, respectively, whereas, it was observed as 90% for autoclaved sample with 10% inoculum size.

## 4. Discussion

The growth factor (Chlorophyll) is an important parameter to evident the photosynthetic activity of algae growing in sewage. Additionally, varying inoculum size helps in determining the behavior of algae in the sewage. The accumulation of chlorophyll is shown in Fig. 1a which depicts *C. pyrenoidosa* in raw sewage with 10% inoculum showed higher growth (Chlorophyll accumulation) when compared to 20% inoculum whereas there was not much significant change for *S. abundans* in Fig. 1b by varying inoculum sizes. Thus, increase in inoculum size leads to decrease in chlorophyll which may be due to the fact that the lesser availability of C:N ratio as nitrogen is the important source for accumulation of chlorophyll. Although autoclaved swage is not economical at practical scale, this condition gives us better picture in microalgae ability to degrade the pollutants. In this study, *S. abundans* exhibit better growth (chlorophyll accumulation) when compared to *C. pyrenoidosa*. However, the maximum chlorophyll content observed in the autoclaved sewage sample was low as compared to raw sewage cultivated by both the microalgae *C. pyrenoidosa* (4.92 mg/l) and *S. abundans* (5.98 mg/l) which proves that for growth of microalgae, nutrition condition is one of the key factors that control their growth [25,26]. Microalgae preferentially use inorganic nitrogen for growth, particularly nitrates and ammonium but some of them are able to grow on organic nitrogen [27]. However the growth rate (chlorophyll) increment with 10% inoculum may be due to lesser energy needed to uptake into cell for its metabolic activity. On the other hand inorganic sources such as nitrates, ammoniacal nitrogen, phosphates, and chlorides are some important pollutants in a wastewater so it is necessary to reduce the levels before discharging. It can be noted from Fig. 2a that not much variation was occurred on ammoniacal nitrogen removal exhibited by *C. pyrenoidosa* in autoclaved sample and raw sewage but, removal was slightly high in case of raw sample compared to autoclaved sample, whereas, from Fig. 2b it can be easily observed that *S. abundans* on raw sewage is higher compared to autoclaved sewage. The possible reason in depleting ammoniacal nitrogen in raw sewage would be due to synergistic effect which is lacking in autoclaved sample. Furthermore, it can also be noted that as ammonia removal increases, chlorophyll accumulation increases for all samples. This result exhibits that changes in nitrogen source can alter the pigment composition in microalgae [28].

In the presence of ammonia nitrogen, uptake of nitrate nitrogen is slow in microalgae as the energy required for nitrate assimilation is very high compared to ammonium [14,29]. It can be observed from the Fig. 3 as both microalgae exhibited higher removal of nitrates in autoclaved sewage compared to raw sewage. The greater efficiency is due to nitrate grown cells in growth medium that is responsible in the conversion of photosynthetically generated reducing power for its metabolic activity as mentioned by Rhee and Lederman [30]. Among the other

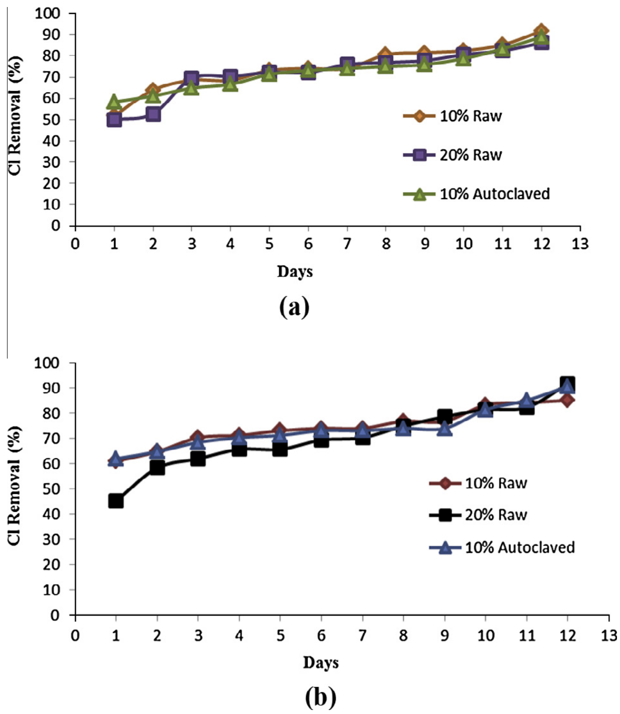


**Figure 5** Chloride ($Cl^-$) removal by (a) *Chlorella pyrenoidosa* and (b) *Scenedesmus abundans*.

inorganics, Phosphates are also an important element that plays a main role in microalgae growth rates in higher production [28]. It can be noted from Fig. 4a *C. pyrenoidosa* with 10% inoculum in raw sewage exhibited higher removal whereas from Fig. 4b there is not much significant difference in removal rates by *S. abundans* in raw and autoclaved sewage. However, inoculum concentration of 20% exhibited higher removal of phosphates. Overall, it is clearly visible that algal cultures could utilize the inorganic compounds efficiently. It is also important to note that, N/P ratio in the wastewater is 4.15 whereas the optimal requirement of growth of microalgae is suggested to be in the range of 6.8–10 [31] which indicates that N:P ratio is lower than the optimal range. Nevertheless, despite lower N:P ratio algal growth was found significantly more than 10 days after which the growth starts declining. The one plausible reason for declining stage after 10 days is the depletion of nutrients such as nitrogen and carbon that results in unfavorable nutrient ratio [32]. Furthermore, a green microalga requires more nitrogen and phosphates than any other species that helps to utilize nitrogen compounds when the phosphate is relatively very high [33]. It is also important to note other inorganic salts such as chloride are assimilable by microalgae. The reason for chloride uptake may be due to the fact that both algae may have highly permeable membrane that helps in accumulation of $Cl^-$ ions due to external osmotic pressure and also at low light intensities [34].

## 5. Conclusion

The present study showed that both microalgae *C. pyrenoidosa* and *S. abundans* are highly adaptable in sewage water. The maximum nutrient removal was observed with 10% inoculum size and found to be as follows: Ammoniacal nitrogen – 99.4%, 98.6%; Nitrates – 78%, 81%; Phosphates – 97%, 96%; Chlorides – 91%, 85% for *C. pyrenoidosa* and *S. abundans* respectively. Growth in sewage appeared to be very effective for microalgae cultivation that reflected in chlorophyll accumulation. The maximum chlorophyll content obtained for 10% inoculum size was 11.4 mg/l and 7.23 mg/l for *C. pyrenoidosa* and *S. abundans*, respectively. Based on the study it can be concluded that both *C. pyrenoidosa* and *S. abundans* with 10% inoculum size can be used for wastewater treatment with respect to nutrient removal.

## References

[1] Rawat, R. Ranjith Kumar, T. Mutanda, F. Bux, Dual role of microalgae: phycoremediation of domestic wastewater and biomass production for sustainable biofuels production, Appl. Energ. 88 (2011) 3411–3424.
[2] J. Sheehan, T. Dunahay, J. Benemann, P. Roessler, A Look Back at the US Department of Energy's Aquatic Speices Program – Biodiesel from Algae, National Renewable Energy Laboratory (NREL), USA, 1998.
[3] Y. Chisti, Biodiesel from microalgae, Biotechnol. Adv. 25 (2007) 294–306.
[4] M.K. Lam, K.T. Lee, Renewable and sustainable bioenergies production from palm oil mill effluent (POME): win–win strategies toward better environmental protection, Biotechnol. Adv. 29 (2011) 124–141.
[5] E.B. Sydney, T.E. Da Silva, A. Tokarski, A.C. Novak, J.C. De Carvalho, A.L. Woiciecohwski, C. Larroche, C.R. Soccol, Screening of microalgae with potential for biodiesel production and nutrient removal from treated domestic sewage, Appl. Energ. 88 (2011) 3291–3294.
[6] T. Cai, S.Y. Park, Y. Li, Nutrient recovery from wastewater streams by microalgae: status and prospects, Renew. Sust. Energ. Rev. 19 (2013) 360–369.
[7] J.K. Pittman, A.P. Dean, O. Osundeko, The potential of sustainable algal biofuel production using wastewater resources, Bioresour. Technol. 102 (2011) 17–25.
[8] S. Cho, N. Lee, S. Park, J. Yu, T.T. Luong, Y.K. Ohc, T. Lee, Microalgae cultivation for bioenergy production using wastewaters from a municipal WWTP as nutritional sources, Bioresour. Technol. 131 (2013) 515–520.
[9] B.S.M. Sturm, L.S. Lamer, An energy evaluation of coupling nutrient removal from wastewater with algal biomass production, Appl. Energ. 88 (2011) 3499–3506.
[10] K. Lee, C. Lee, Nitrogen removal from wastewaters by microalgae without consuming organic carbon sources, J. Microbiol. Biotechnol. 12 (2002) 979–985.
[11] S. Cho, T.T. Luong, D. Lee, Y.K. Oh, T. Lee, Reuse of effluent water from a municipal wastewater treatment plant in microalgae cultivation for biofuel production, Bioresour. Technol. 102 (2011) 8639–8645.
[12] J. Yang, X. Li, H. Hua, X. Zhang, Y. Yu, Y. Chen, Growth and lipid accumulation properties of a freshwater microalga, *Chlorella ellipsoidea YJ1*, in domestic secondary effluents, Appl. Energ. 88 (2011) 3295–3299.
[13] T. Kim, Y. Lee, S. Han, S. Hwang, The effects of wavelength and wavelength mixing ratios on microalgae growth and nitrogen, phosphorus removal using *Scenedesmus* sp. for wastewater treatment, Bioresour. Technol. 130 (2013) 75–80.
[14] O.P. Garcia, F.M.E. Escalante, L.E. de-Bashan, Y. Bashan, Heterotrophic cultures of microalgae: metabolism and potential products, Water. Res. 45 (2011) 11–36.
[15] M. Sidat, H.C. Kasan, F. Bux, Laboratory-scale investigation of biological phosphate removal from municipal wastewater, Water. SA 25 (1999) 459–462.

[16] P.J. McGinn, K.E. Dickinson, K.C. Park, C.G. Whitney, S.P. MacQuarrie, F.J. Black, J.C. Frigon, S.R. Guiot, S.J.B. O'Lear, Assessment of the bioenergy and bioremediation potentials of the microalga *Scenedesmus* sp. AMDD cultivated in municipal wastewater effluent in batch and continuous mode, Algal Res. 1 (2012) 155–165.
[17] Y. Li, W. Zhou, B. Hu, M. Min, P. Chen, R. Ruan, Integration of algae cultivation as biodiesel production feedstock with municipal wastewater treatment: strains screening and significance evaluation of environmental factors, Bioresour. Technol. 102 (2011) 10861–10867.
[18] I.T.D. Cabanelas, J. Ruiz, Z. Arbib, F. Alexandre, C.C.G. Perez, F. Rogalla, I.A. Nascimento, J.A. Perales, Comparing the use of different domestic wastewaters for coupling microalgal production and nutrient removal, Bioresour. Technol. 131 (2013) 429–436.
[19] B. Wang, C.Q. Lan, Biomass production and nitrogen and phosphorus removal by the green alga *Neochloris oleoabundans* in simulated wastewater and secondary municipal wastewater effluent, Bioresour. Technol. 102 (2011) 5639–5644.
[20] S. Sawayama, T. Minowa, Y. Dote, S. Yokoyama, Growth of the hydrocarbon-rich microalga *Botryococcus braunii* in secondarily treated sewage, Appl. Microbiol. Biotechnol. 38 (1992) 135–138.
[21] UTEX. BG-11 medium composition. Houstin (USA): University of Texas. <http://web.biosci.utexas.edu/utex/mediaDetail.aspx?mediaID=26> [Accessed on 22/11/2012].
[22] APHA. Standard methods for the examination of water and wastewater. 21st ed., USA; 2005.
[23] E.W. Becker, Microalgae: biotechnology and microbiology, Cambridge University Press, 1994.
[24] M.E. Martinez, J.M. Jimenez, F. El-Yousfi, Influence of phosphorus concentration and temperature on growth and phosphorus uptake by the microalgae *Scenedesmus obliquus*, Bioresour. Technol. 67 (1999) 233–240.
[25] A. Vonshak, A. Richmond, Mass production of blue–green alga Spirulina – an overview, Biomass 15 (1988) 233–247.
[26] B.L. Faintuch, S. Sato, E. Aquarone, Influence of the nutritional sources on the growth rate of cyanobacteria, Arch. Biol. Technol. 34 (1991) 13–30.
[27] G.E. Fogg, W.D.P. Stewart, P. Fay, A.E. Walsby, The blue–green algae, Academic Press, London, 1973.
[28] E.S. Mostert, J.U. Grobbelaar, The influence of nitrogen and phosphorus on algal growth and quality in outdoor mass algal cultures, Biomass 13 (1987) 219–233.
[29] J. Peccia, B. Haznedaroglu, J. Gutierrez, J.B. Zimmerman, Nitrogen supply is an important driver of sustainable microalgae biofuel production, Trends. Biotechnol. 31 (2013) 134–138.
[30] G. Rhee, T.C. Lederman, Effects of nitrogen sources on P-limited growth of Anabena flos-aquae, J. Phycol. 19 (1983) 179–185.
[31] L. Wang, M. Min, Y. Li, P. Chen, Y. Chen, Y. Liu, Y. Wang, R. Ruan, Cultivation of green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment plant, Appl. Biochem. Biotechnol. 162 (2010) 1174–1186.
[32] G.W. Prescott, The algae: a review, Houghton Mifflin Company, Boston, 1968, pp. 279–294.
[33] P.S. Lau, N.F.Y. Tam, Y.S. Wong, Effect of algal density on nutrient removal from primary settled wastewater, Environ. Pollut. 89 (1995) 59–66.
[34] P.E. Chimiklis, E.P. Karlander, Light and calcium interactions in *Chlorella* inhibited by Sodium chloride, Plant Physiol. 51 (1973) 48–56.