Screening of fresh water algae from paper and pulp mill waste waters with the focus on high algal biomass production

Gurumoorthy P.* and Saravanan A.
Department of Chemical Engineering, Hindustan Institute of Technology and Science, Padur, Chennai, INDIA
*biogp77@gmail.com

Abstract

The industrial effluents are potential substitute for traditional media for algal cultivation. The effluents contain high amount of nitrogen and phosphorous microalgae has high removal efficiency which utilizes them and yields high algal biomass production. In this aspect, this research study focuses on high algal biomass production and ensures minimum levels of N and P in the treated water. Waters collected near TNPL Karur were able to support the growth of three fresh water microalgae to a limited extent; the three fresh water microalgae isolated from the paper and pulp waste water are Chlorella vulgaris, Scenedesmus and Spirulina platensis. The highest cell cultivation is reached when supplemented with nutrients. There was no noticeable change in growth. After two weeks of microalgae cultivation in paper and pulp waste water supplied with nitrates and phosphate, the highest biomass yield was achieved by Chlorella vulgaris (0.80 g/L-^{1}).

More than 90% of phosphate-P and around 70-85% nitrate-N was removed by these three fresh water microalgae. Chlorella vulgaris (COD: 92.4%, NO_3^- N: 80.1%, NH_4^+-N: 90.2%, PO_4^{3-}-P: 100%), Scenedesmus (COD: 72.4%, NO_3^--N: 81.1%, NH_4^+-N: 89.2%, PO43--P: 84%), Spirulina platensis (COD: 79.4%, NO_3^--N: 85.1%, NH_4^+-N: 96.2%, PO_4^{3-}-P: 98.3%). Results clearly indicated that specific solutions are required. Thus serious attention needs to be put on nutrient concentration levels while using pulp and paper mill waste water for microalgal biomass production while all the parameters went below the discharge limits in waste water. Paper and pulp waste water turned to be better nutrient media for algal biomass production

Keywords: Waste water, *Chlorella vulgaris*, *Scenedesmus* and *Spirulina platensis*, COD, Nitrate and phosphate removal.

Introduction

The algal waste water treatment of industrial effluents is very cheap when compared to chemical process. In recent years researchers have been working on primary treated waste water which does not favor the growth of algae and the biomass production went very low and algae utilized the nutrients and it does not produce algal FAME[1]. Now the trend has changed. de Souza et al[6] said that secondary treatment of waste water using algae is extensively used to reduce the cost of chemical process[2]. The microalgae can be used extensively to remove nitrogen, phosphorus and heavy metals from waste water and also microalgae has the capability to reduce the BOD. From the recent literature, it is very clear that the microalgae possess all qualities for waste water treatment and there is a need for a comprehensive comparison of multiple algal cultures in waste water.

Material and Methods

Sample Collection and Cultivation of microalgae: The microalgae cultures of 500 ml of *Chlorella vulgaris, Scenesdesmus sp, Spirulina platensis*, were procured from the Tamilnadu Agricultural University, Coimbatore and where respectively cultured in BG11 medium ($NaNO_3$ (1.5 g L^{-1}), K_2HPO_4 (0.01314 g L^{-1}), $MgSO_4.7H_2O$ (0.036 g L^{-1}), $CaCl_2.2H_2O$ (0.0367 g L^{-1}), Na_2CO_3 (0.020 g L^{-1}), Di-sodium magnesium EDTA (0.001 g L^{-1}), Citric acid (0.0056 g L^{-1}) and Ferric ammonium citrate (0.006 g L^{-1})[3]. The paper and pulp industry waste sample taken from secondary waste water treatment 5ml was mixed separately[9] in 250 ml Erlenmeyer conical flask containing 50ml of BG11 medium and kept in shaker for 40 minutes for proper mixing of paper industry waste water and then the 100ml of algal cultures of *Chlorella vulgaris and Scenesdesmus sp* 100 ml were cultured in the 250 ml conical flask and stored at 25^0c at cooling incubator and shaker.

Spirulina platensis was cultured in Zarrouk medium ($NaHCO_3$ (16.80 g L^{-1}), K_2HPO4 (0.50 g L^{-1}), $NaNO_3$ (2.50 g L^{-1}), K_2SO_4 (1.00 g L^{-1}), NaCl (1.00 g L^{-1}), $MgSO_4.7H_2O$ (0.20 g L^{-1}), $EDTA.Na_2.2H_2O$ (0.80 g L^{-1}), $CaCl_2.2H_2O$ (0.04 g L^{-1}) and $FeSO_4.2H_2O$ (0.01 g L^{-1}). The trace element mixture (1.0 mL L-1) contained following constituents: H_3BO_3 (2.86 g L^{-1}), $MnCl_2.4H_2O$ (1.810 g L^{-1}), $ZnSO_4.7H_2O$ (0.222 g L^{-1}), MoO_3 (0.015 g L^{-1}) and $CuSO_4.5H_2O$ (0.074 g L^{-1}). 100 ml *Spirulina platensis* were cultured in the 250 ml conical flask containing 5ml of paper waste water and 50 ml of Zarrouk medium and stored at 25^0C at cooling incubator and shaker and all the three microalgae growth was monitored for 12 days.

Waste water Characterization: The paper industry waste water were characterized for COD, chemical oxygen demand (COD), nitrate-nitrogen (NO_3-N), ammonia nitrogen (NH_3-N), phosphate (PO_4^{3-}) and pH using the standard protocols.

Algal growth and biomass concentration: The growth of *Chlorella vulgaris, Scenesdesmus sp,* and *Spirulina platensis* was monitored thorugh estimation of dry weight and chlorophyll-a concentrations at the end of each experiment. To determine the dry cell weight, 10 ml sample is filtered and pre-weighed. Whatmann filter paper is dried at 50^0C for 24 hrs. The biomass concentration was determined by dry weight by the following formula:[7]

Biomass concentration(gL^{-1}) =[Initial wt-initial wt of filter] / Volume *1000

Results and Discussion

Microalgae cultures: The growth of mixed culture microalgae *Chlorella vulgaris, Scenesdesmus sp,* and *Spirulina platensis* shown in figure 1 was grown in waste water along with BG11 and Zarrouk medium.

Characterization of waste water: The microalgal cultures of *Chlorella vulgaris, Scenesdesmus sp,* and *Spirulina platensis* showed efficient phycoremediation by reducing the levels of chemical oxygen demand (COD), nitrate-nitrogen (NO_3-N), ammonia nitrogen (NH_3-N), phosphate (PO_4^{3-}) as shown in the table 1.

From the above table shows the reduction of COD, NO_3-N, NH_3-N and PO_4^{3-} by all three fresh water algae *Chlorella vulgaris* (COD: 92.4%, NO_3^- N: 80.1%, NH_4^+-N: 90.2%, PO_4^{3-}-P: 100%), *Scenedesmus* (COD: 72.4%, NO_3^- N: 81.1%, NH_4^+-N: 89.2%, PO_4^{3-}-P: 84%), *Spirulina platensis*(COD: 79.4%, NO_3^- N: 85.1%, NH_4^+-N: 96.2%, PO_4^{3-}-P: 98.3%).

Biomass production after algal waste water treatment: The algal growth in waste water was determined by biomass concentration and chlorophyll-a concentration and the biomass obtained is for 12 days *Chlorella vulgaris* (0.80 g L^{-1}), *Spirulina platensis* (0.79 g L^{-1}), *Scenesdesmus sp*(0.65 g L^{-1}) and the chlorophyll-a concentration *Chlorella vulgaris* had the highest content of chlorophyll-a (15.50 mg L^{-1}) followed by *Spirulina platensis* (14.68 mg L^{-1}) and *Scenesdesmus sp* (12.08 mg L^{-1}).

Fig. 1: Microalgal growth in waste water along with BG11and Zarrouk medium

Table 1
Physico-chemical properties of Paper industry waste water after algal waste water treatment

	Paper Industry Waste Water			
	COD	**NO_3-N**	**NH_4-N**	**PO_4^{-3}-P**
Discharge Limits**	250	10	5	50
Raw wastewater	823±9.84	13±0.4	169±2.64	80±1.01
Control	598.32±0.59	5.32±0.02	114.24±0.22	46.48±0.52
C.Vulgaris	245.01±0.015	2±0.24	48.59±0.295	7.34±0.055
S.Platnesis	202.05±1.7	3.5±0.26	50.01±0.005	6.78±0.11
Scenesdesmus sp	173.08±0.015	5.84±0.13	34.86±0.03	5.84±0.05

The algal phycoremediation worked very effective in paper waste water was proved through this experiment where all the three fresh water microalgae reduced the pollution load effectively *Chlorella vulgaris* (COD: 92.4%, NO_3^- N: 80.1%, NH_4^+-N: 90.2%, PO_4^{3-}-P: 100%), followed by the Spirulina platensis(COD: 79.4%, NO_3^- N: 85.1%, NH_4^+-N: 96.2%, PO_4^{3-}-P: 98.3%) and *Scenedesmus* (COD: 72.4%, NO_3^- N: 81.1%, NH_4^+-N: 89.2%, PO_4^{3-}-P: 84%). The above results conclusively prove that all three fresh water algae utilize the organic and inorganic nutrients present in the paper industry waste water and the nutrient load played a major role in determining the algal growth which in turn affects the treatment of algal potential.

Generally, the algae prefers ammonium over nitrates because elevated levels of ammonium promoted the algal growth effectively; high N concentrations are needed to ensure effective phosphorus removal from wastewater due

to the positive effect of N on the accumulation of phosphorus [4]. To ensure the simultaneous utilization of both nitrogen and phosphorus, the N/P ratio should be in the proper range. The ratio indicates the removal rate of nitrogen compared to the removal rate of phosphate. In wastewater, an optimal N/P ratio was reported to be 7 [5]. For instance, algae growth on dairy wastewater is usually limited by the ammonia and nitrogen deficiency but mixing of wastewaters can be done to get better balancing of ammonia. Mixing of wastewaters improved the biomass yield of *Chlorella vulgaris* (2.66 gL^{-1}) at low cost.

All the three fresh water microalgae *Chlorella vulgaris, Scenesdesmus sp,* and *Spirulina platensis* performed really well in paper industry waste water enriched along with BG11 and Zarrouk medium when compared to their growth in synthetic medium. Based on the analysis, it was concluded that specific algal cultures have to be selected for different wastewater given their preferences in nutrient uptake and other physio-chemical conditions like that of nutrient inhibition, pH etc. Hence, site specific solutions are required for different challenges in different waste waters.

Conclusion

The present study focused on the phycoremediation of *Chlorella vulgaris, Scenesdesmus sp,* and *Spirulina platensis* exhibiting positive results with respect to algal biomass generation and algal waste water treatment. The capability of all three fresh water algae was conclusively established through the experiments. There was varying degree of difference as noticed in biomass production and removal of nutrients in waste water. The presence of nitrogen and phosphorus favored the algal growth which resulted in increase in biomass.

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