Biodegradation of Phenolic Effluent of Producer Gas Plant using *Scenedesmus* sp.

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Highlights

- Degradation of phenolic effluent using the microalgae *Scenedesmus* sp.
- *Scenedesmus* sp. degrades more than 90 % of phenol in the effluent.
- Degradation study was performed in the ambient conditions.

Abstract

Phenol is a pollutant, usually found in effluents produced from industries such as coal processing plants, oil refineries, pulp and paper manufacturing plants, resins and coke manufacturing, steel industries etc. During last two decades, extensive attention has been paid on the management of environmental pollution caused by hazardous materials. A number of methods have been developed for removal of such substances like precipitation, evaporation, ion-exchange etc. However these methods have several disadvantages. The present work highlights the alternative biological agent abundantly present in nature i.e microalgae (*Scenedesmus* sp.) as a potential biological agent for removal of such toxic substances from the surrounding. The fresh water microalgae *Scenedesmus* sp. was investigated for the ability to degrade the phenolic compounds present in the effluent of producer gas plant. The effluent has 1024 ppm of initial phenol concentration. The biodegradation study was performed in two batches (with and without nutrients) both having four different concentrations of *Scenedesmus* sp. mixed with effluent. It was found that wet biomass of *Scenedesmus* sp., with nutrients, was able to reduce the phenol concentration(C_6H_5OH) present in the effluent by more than 90% for algal concentration of 2000 ppm (mg of wet biomass/l of effluent) and above. While for samples without nutrients the percentage reduction of total phenol was observed upto a maximum of 46%.

Key words

Phenol, Phycoremediation, Wastewater treatment, Phenolic effluent, Biodegradation, *Scenedesmus* sp., Microalgae

1.0 Introduction

Phycoremediation is a biodegradation method in which, either the microalgae or macro algae are used as a biological agent. Phycoremediation offers an interesting step for wastewater treatments, because they provide a tertiary bio-treatment coupled with the production of potentially valuable biomass, which can be used for several purposes. While degradation of phenol and its derivatives by conventional methods, releases toxic byproducts, degradation by microalgae is advantageous, as it eliminates toxic byproduct formation and favors useful byproducts, biomass production and CO_2 absorption [7]. Among the various industrial sectors, the coal and the petroleum industries generate significant quantities of wastewater containing large amount of phenolic compounds which may have a detrimental impact when exposed into the environment without any prior treatment ([6],[9]). Phenol or its derivatives are toxic to aquatic life if its concentration exceeds 2 ppm. Therefore, its effective decomposition and analysis of byproducts formed are necessary

([8], [13]). Algal strains can survive at higher concentration of phenol and its types [1]. Algae degrade organic matter present in the effluent by oxidation using the oxygen produced via photosynthesis. Algae utilize the released carbon dioxide and nutrients during aerobic oxidation of organic matter for their growth. In comparison to common treatment systems, oxidation pond supports the growth of some other species. High pH in algal ponds leads to pathogen disinfection other than algal species, so the degradation of organic matter by the specific algal species is achieved [4].

Growing algae in wastewater offers numerous economic and environmental merits, providing one of the most sustainable ways to produce biodiesel derived from microalgae. Wastewater usage eliminates competition for fresh water, saves cost of nutrients supplement since nutrients are in abundance in wastewater, provides the treatment of the wastewater by assimilating organic and inorganic pollutants into their cells, apart from eliminating the CO_2 emissions associated with wastewater treatment [17]. Algal strains are capable of metabolizing phenol in the environment. In the dynamic energy budget model proposed by Lika and Papadakis [14] for aerobic degradation, inhibition of phenol reduction was reported due to the presence of glucose (carbon source for algal growth).

Chlorella sp., *Scenedesmus obliquus* and *Spirulina maxima* degraded variety of Phenol and its types viz. 2,4-dimethylphenol, 2,4-dinitrophenol, 2,4-dichlorophenol, 2-chlorophenol, in which phenol was completely degraded by all the algal cultures up to 1000mg/l. *Scenedesmus obliquus* was able to degrade 2,4-dinitrophenol completely at a concentration of about 190 mg/l. *Chlorella* sp. dechlorinate 2-chlorophenol at a maximum concentration of 200 mg/l [1]. Pinto et al., 2002[4] reported that the two micro algae, *Ankistrodesmus braunii* and *Scenedesmus quadricauda* degraded more than 70% of olive mill wastewater having 400mg/l of phenol within 5 days.

Degradation of phenol by prokaryotic and eukaryotic organisms requires the presence of molecular oxygen to initiate enzymatic attack of the aromatic rings ([2] & [3]), hence the phenol degradation by aerobic oxidation is one of the most suitable methods. A typical pathway for metabolizing phenol is to hydroxylate the ring to form catechol and then to open the ring through ortho-or meta-oxidation ([3]&[11]).

Semple et al., 1996 [2] reported that the *O.danica* is able to metabolize phenol completely; and the algae accumulate the carbon in the nucleic acid and in the lipid content of the cells.

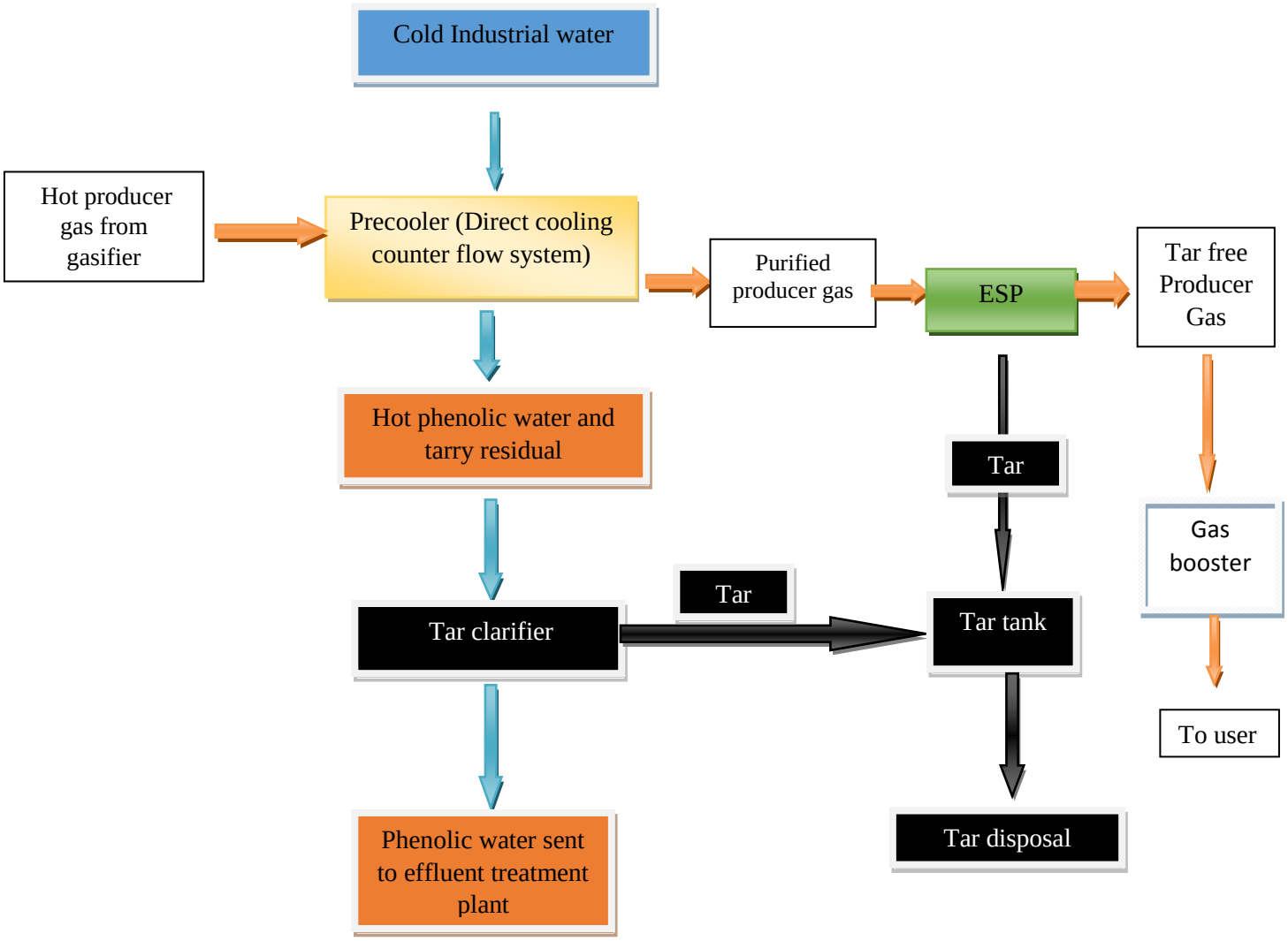
Many researchers demonstrated the biodegradation of synthetic phenol and its derivatives by algae; only few researchers reported degradation experiments on real effluents. The present study focuses on the degradation of phenol rich effluent of producer gas plant by the fresh water micro algae *Scenedesmus* sp.

2.0 Source of effluent

The effluent was collected from a producer gas plant located nearby. Coal is gasified using air and steam in this producer gas plant. The gasifiers used for the production of gas are of single stage with rotating bottom parts, water cooled jacket, coal charging and ash removal system. The water used for cooling and scrubbing the hot raw gas comes out from the bottom water seal of the pre-cooler. The schematic flow diagram of producer gas plant is depicted in Fig 1. The water released from the pre-cooler is contaminated with phenolic compounds. Due to stripping, various

phenolic compounds get dissolved in water, phenol being the major constituent. In addition, the effluent contains large amount of oil and grease. Due to the possibilities of enormous hazardous effects of phenolic compounds over the flora and fauna around, it becomes essential to characterize and degrade the effluent discharged by the industry.

Fig 1- Flow diagram of existing producer gas plant



3.0 Materials and Methods

The chemicals required are laboratory grade Bromate- Bromide solution, Starch powder, Potassium iodide, Sodium thiosulfate, Conc. HCl (M), Chloroform, Mercury (II) sulphate (in Sulphuric acid), Potassium dichromate (in Sulphuric acid), Ethyl acetate, Methanol, Urea and Potassium Bicarbonate (Nutrient source for the growth of *Scenedesmus* sp.)

3.1 Culture of *Scenedesmus* sp.

A culture of targeted algal species was procured from the Bioenergy lab, in the Department of Energy and environment at the National Institute of Technology, Trichy, India. The *Scenedesmus* sp. was cultivated in optimized culture medium comprising potassium bicarbonate and urea in the ratio of 2:1. The culture condition was atmospheric as it was kept in a closed room with sufficient openings via windows and doors for light and air. No artificial lamps were used. Temperature range varies from 30 -35° C. The pH of the culture was found out to be 7-8. The growth of microalgae was monitored by observing optical density at 600 nm and for the identification of contamination, the algae was monitored daily in fluorescent inverted microscope (Nikon DS-Fi2).

3.2 Characterization of phenolic effluent

The conventional parameters like pH, color, Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), Total Dissolved Solids (TDS) and Total Suspended Solids (TSS) were analyzed by using standard procedures listed in the Table 1. The concentration of total phenol present in effluent was analyzed by using Indian Standard, IS 3025 part no 43 method, the types of phenolic compounds present in the effluent was analyzed using gas chromatography. Total organic content present in the effluent was analyzed by TOC Analyzer (AJ- Analyzer multi N/C 3100; multiwin 4.09).

3.3 Analysis of phenolic compounds through gas chromatography

The types of phenolic compounds present in effluent were characterized by gas chromatography (GC 450 Varian).

3.3.1 Extraction Method

The phenolic compounds were extracted from the effluent using ethyl acetate as a solvent, dried over anhydrous sodium sulphate and analyzed by gas chromatography [16]. Extracted samples were analyzed in GC 450 Varian with area normalization.

3.3.2 Gas chromatography operating conditions

Capillary column VF-1ms (15m x 0.25 mm) and 0.25µm, Injector: 250°C, injection volume: 1µl (split), Flame Ionization Detector: 250°C, carrier gas: N_2, oven temperature: 100°C (hold 2 min.) to 230°C at 20°C/ min (hold 35min.)[16].

To identify the types of phenol present in the effluent, 11 phenolic standards were selected based on list enumerated by Environmental Protection Agency (EPA) [18]. The standard phenolic

compounds are Phenol, 2-Chlorophenol, 2-Methyl phenol, 2-Nitrophenol, 2,4-Dimethyl phenol, 2,4-Dichlorophenol, 4-Chloro-3-methyl phenol, 2,4,6-trichlorophenol, 2,4-Dinitrophenol 4-Nitrophenol, Pentachlorophenol. Out of which phenol constitutes 50.4% and 2,4,6-trichlorophenol constitutes 2.3%.

3.4 Experimentation

A 15 day old algal culture (*Scenedesmus* sp.) was centrifuged at 7000 g for 10 min to separate the biomass without disturbing the cells. The cells were washed with distilled water to remove the salts deposited on the cells. This step was repeated three times to accomplish complete removal of salts. The ability of *Scenedesmus* sp. on phenol degradation was analyzed by introducing four different concentration of algae viz. 1g, 2g, 3g and 4g per liter of raw effluent (without prior treatment) with nutrients(Potassium bicarbonate: Urea in the ratio of 2:1) (Batch I) and without nutrients (Batch II). The experiments were performed in 1000ml Erlenmayer flasks with cotton plugs. The two batches along with algal biomass were maintained at ambient condition same as maintained for the cultivation of *Scenedesmus* sp. over a period of 7 days. The effluent devoid of algae with and without nutrients served as a control and maintained as the same condition as maintained for the inoculum of *Scenedesmus* sp. The cultures were mixed three times a day to accomplish complete mixing. The growth of the algal species was analyzed by observing absorbance at 600 nm at UV-Vis spectrophotometer (Spectroquant pharo 300 IMERCK). Contamination and physical changes in the cells of *Scenedesmus* sp. due to the high phenolic stress was observed daily using fluorescent inverted microscope. The pH of the samples was analyzed by digital pH meter (Duralab).

3.5 Analyses

For the analysis of phenol, *Scenedesmus* sp. was separated from the culture broth through centrifugation at 10,000 g for 5 min. The supernatant was filtered through a 0.45 μm PTFE membrane filter and the filtrate was further analyzed in GC for identifying the phenolic compounds using the same method followed for the effluent characterization. The pellet was extracted with 1 mL ethyl acetate and then centrifuged at 10,000 g for 5 min. The ethyl acetate extract was filtered through a 0.45 μm PTFE filter and this was used for GC analysis in order to check for any possible phenol adsorbed. Analysis was performed using the same operating condition adopted for effluent analysis. GC analysis were performed using capillary column Vf-1ms, 15m, 0.25mm, 0.25μm, (Varian 450 GC), Detection was achieved by Flame Ionization Detector. Variation in Total Organic Content (TOC) was analyzed by using TOC analyzer (Analytikgena (multi N/C) 3100).

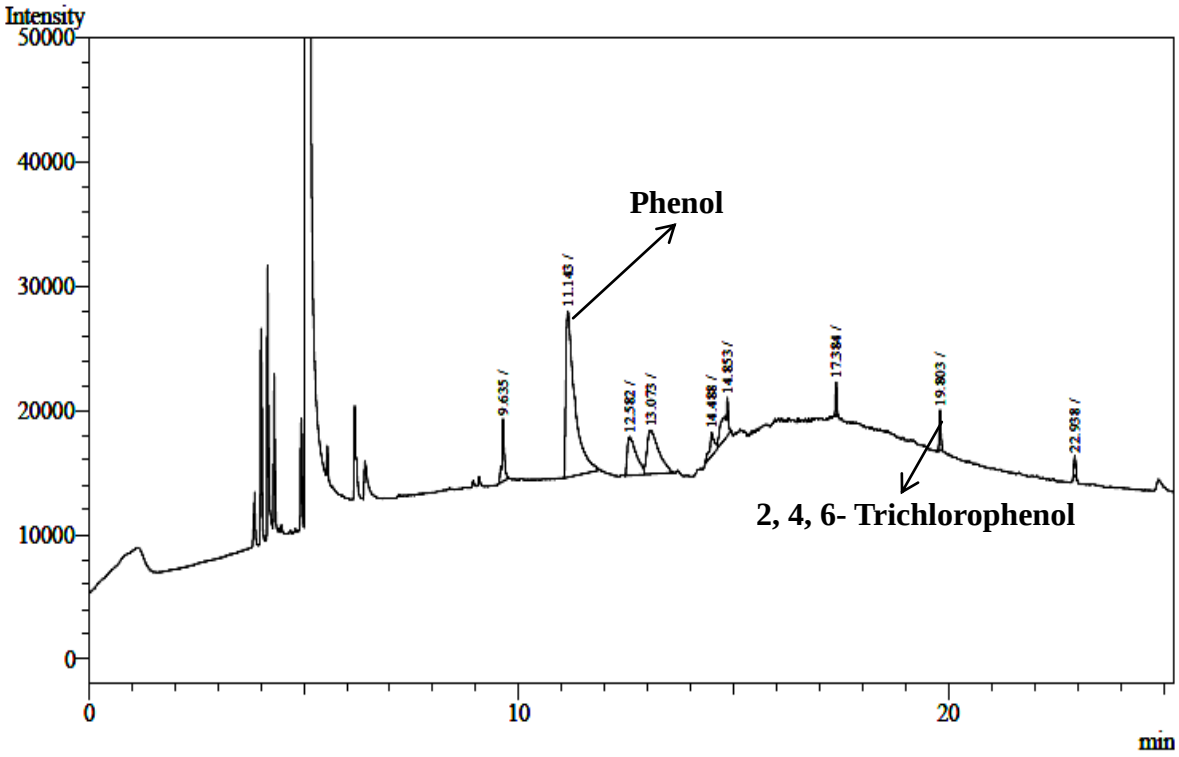
4.0 Results and Discussion

4.1 Initial characteristics of raw effluent

Conventional parameters like Colour, pH, Chemical Oxygen Demand (COD), Biological Demand (BOD), Total dissolved solids (TDS), Total Suspended Solids (TSS), Total Phenol concentration were analyzed using the standard procedure and the results were tabulated in Table 2. The GC Chromatogram for the raw effluent (Fig 2) showed that the raw effluent before treatment contains 9 different compounds. The results were compared with GC chromatogram for the known standard phenolic compounds. Out of the different compounds, Phenol (C_6H_5OH)

and 2,4,6-Trichlorophenol were confirmed by having the same retention times at the same operating conditions. According to the results, the effluent contains only two phenolic compounds listed by EPA. Phenol (C_6H_5OH) alone comprises more than 50% of raw effluent.

Fig-2 Characterization of types phenol present in the effluent using gas chromatography



4.2 Effect of phenol on the growth of microalgae

Growth of *Scenedesmus* sp. was monitored daily by analyzing optical density at 600 nm using UV –Vis spectrophotometer (Spectroquant) (Fig- 3,4,5,6) The increase in optical density was observed in both the batches. The increase in optical density is the measure of identification of algal growth. Increased growth rate for the *Scenedesmus* sp. was observed in batch I compared to batch-II. Optical density was gradually increased in all the samples of batch-I. Sudden lag in growth curve was observed in batch-I (with nutrient) due to the removal pollutant by the biological agent, which is involved in the degradation [10]. In case of batch-II (without nutrients) increased lag phase was observed because the *Scenedesmus* sp. requires adaptation period when it is exposed to high phenolic environment as reported in [1]. Increase in optical density was only observed after 2 days for batch II except for the sample having algal concentration of 4000 ppm.

Fig 3-6 Effect of phenolic effluent on the growth of *Scenedesmus* sp. (wet biomass of algae were inoculated at various concentration)

Fig 3 Growth of *Scenedesmus* sp. in 1000 PPM (1g of algae/1l of effluent)

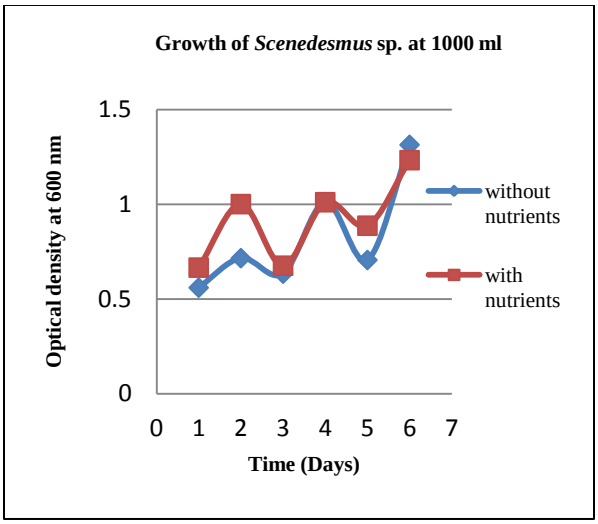


Fig 4 Growth of *Scenedesmus* sp. in 2000ppm (2g of algae/1l of effluent)

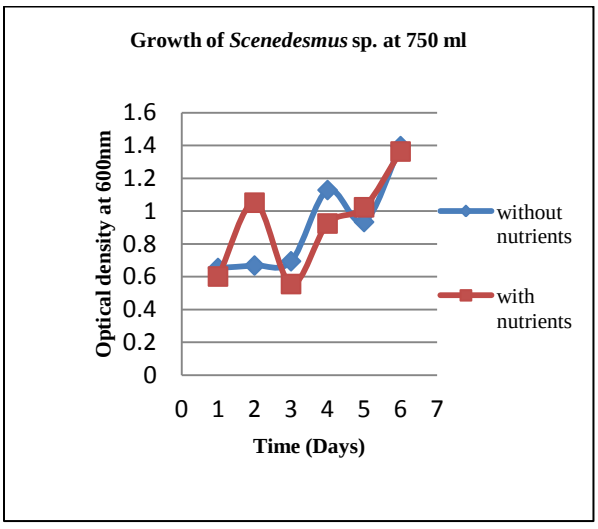


Fig 5 Growth of *Scenedesmus* sp. in 3000 ppm (2g of algae/1l of effluent)

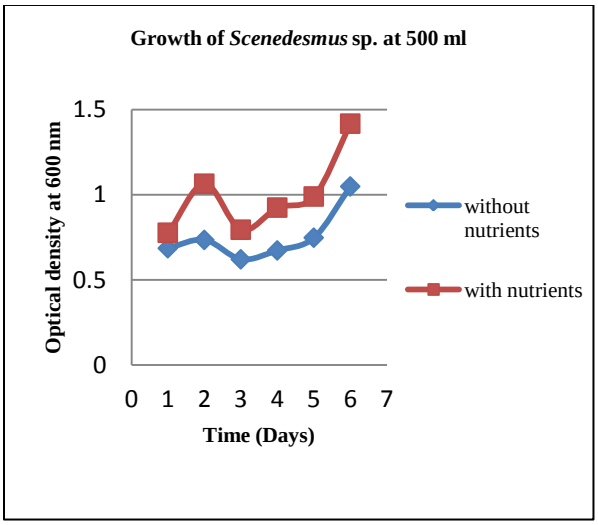
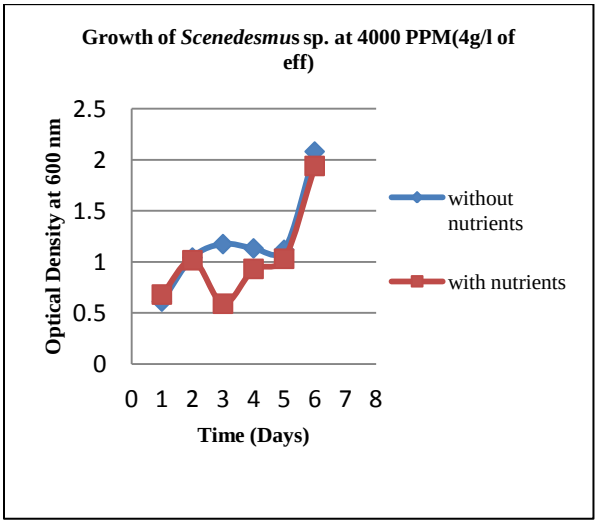


Fig 6 Growth of *Scenedesmus* sp. in 4000 ppm (4g of algae/1l of effluent)



4.3 Effect of potassium bicarbonate and urea on the removal of phenol

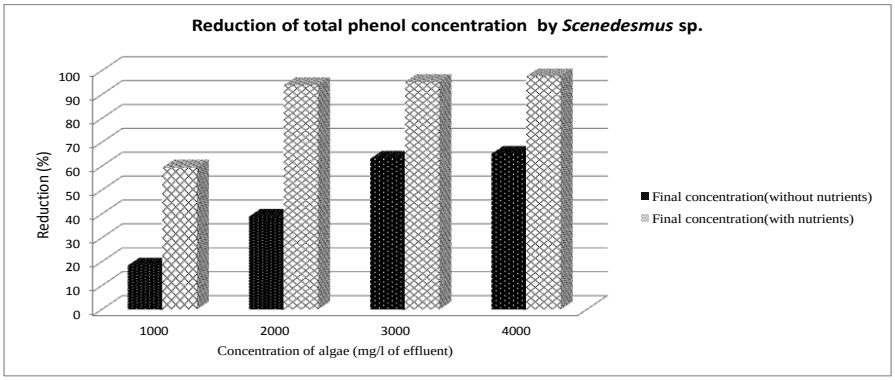
The effect of media components used in this study increases the growth of microalgae and also increases the removal of phenolic content present in the effluent by the microalgae. Petroutsos et al., 2007[12] reported that the increase in initial concentration of $NaHCO_3$, leads to increased biomass production and p-Chlorophenol removal by the microalga *Tetraselmis marina.* The meagre reduction of inorganic carbon observed through TOC analysis, reveals that the added salts are not involved in chemical degradation of phenol.

4.4 Removal of phenol by *Scenedesmus* sp.

In order to analyze the ability of phenol removal by the *Scenedesmus* sp., the algae was grown in two different batches. Each batch has four different concentration of algae viz. 1000, 2000, 3000 and 4000 ppm (mg of wet biomass/ liter of effluent). Potassium bicarbonate and urea were used as nutrient source in batch-I. All the experiments were conducted three times and the results were averaged. The algal cells were separated from the effluent by centrifuging the sample at 10000 g for 10 min and the phenol was extracted by the same method that was used for raw effluent characterization. And the total phenol concentration was analyzed using the IS 3025 part no 43 method and for phenol (C_6H_5OH) gas chromatography was used. The *Scenedesmus* sp. shows high phenol removal in batch-I having concentration of algae 4000 ppm (mg of wet biomass/l of effluent). No bioaccumulated (intracellular or cell surface adsorbed) phenol was detected with GC analysis of the micro algal pellet ethyl acetate extract. The degradation ability of the *Scenedesmus* sp. was comparatively identified by analyzing the batch without medium.

Reduction of total phenol observed using the IS 3025 part no 43 method was high at 4000 ppm (4g of wet biomass/ 1 liter of effluent) in batch-I and average of 87 % reduction was observed(Fig-7)) within 7 days and for without nutrients average of 46% reduction.

Fig-7 Comparison of concentration of total phenol presents in the effluent after treatment with nutrients and without nutrients samples



The reduction of phenol (C_6H_5OH) was determined by gas chromatography (GC 450 Varian) analysis with area normalization method (Fig 8-11 (batch-I)). The reduction of phenol (C_6H_5OH)

by the *Scenedesmus* sp. was high at 99 % at the algal concentration of 4000 ppm (mg/l of effluent) with nutrient batch (Fig 15). From the results, the degradation of phenol should be accomplished by the oxidation of phenol by the oxygen evolved during degradation. No change in the concentration of phenol was observed in the controls.

Fig (8-11) Gas chromatographic analysis of samples in batch I

Fig-8 Gas Chromatogram of effluent having the concentration of 1000 ppm of algae with nutrients

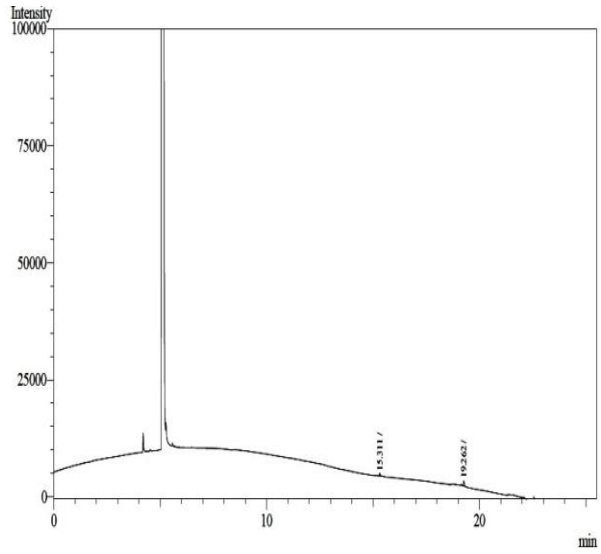


Fig-9 Gas Chromatogram of effluent having the concentration of 2000 ppm of algae with nutrients

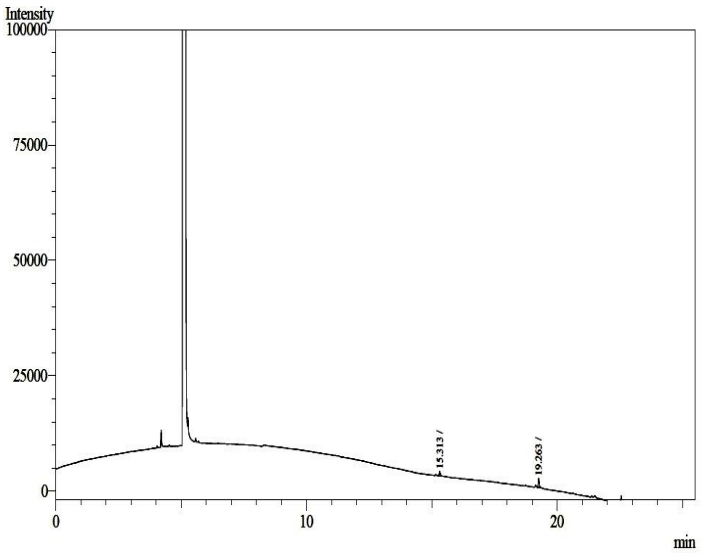


Fig-10 Gas Chromatogram of effluent having the concentration of 3000 ppm of algae with nutrients

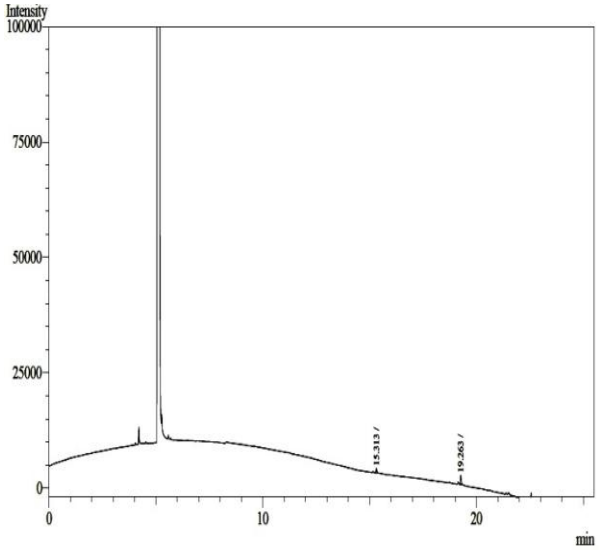


Fig-11 Gas Chromatogram of effluent having the concentration of 4000 ppm of algae with nutrients

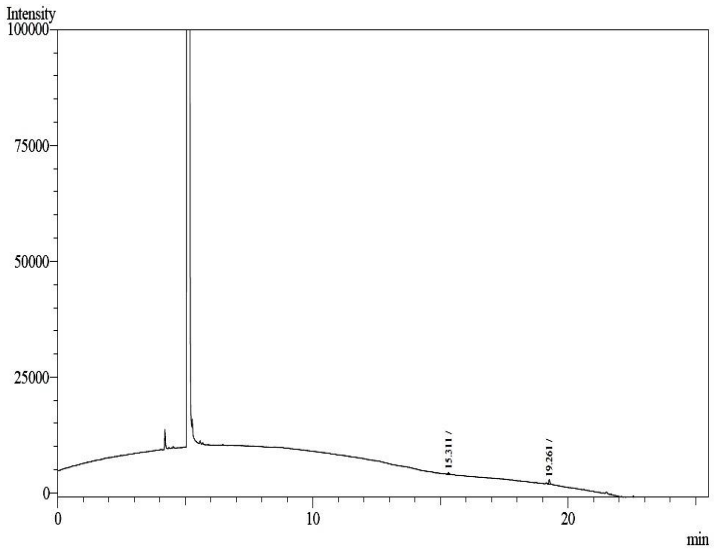


Fig (12-15) Gas chromatographic analysis of samples in batch II

Fig-12 Gas Chromatogram of effluent having the algal concentration of 1000(ppm) without nutrients

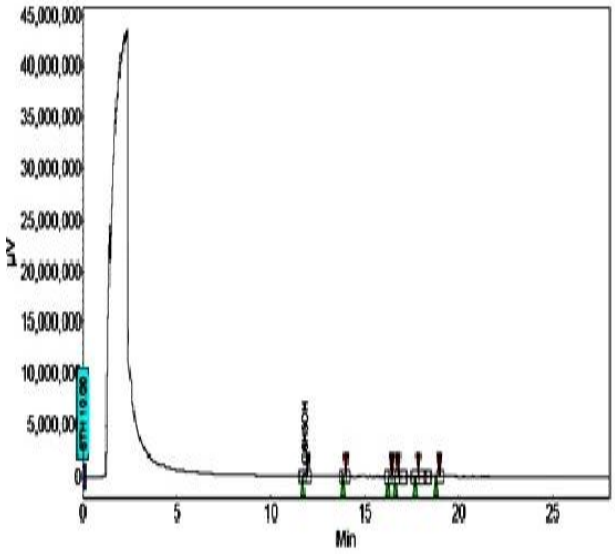


Fig-13 Gas Chromatogram of effluent having the algal concentration of 2000(ppm) without nutrients

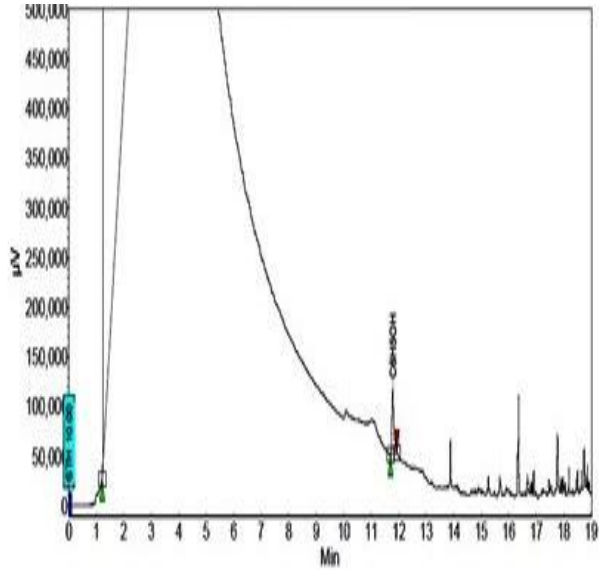


Fig-14 Gas chromatogram of effluent having the algal concentration of 3000(ppm) without nutrients

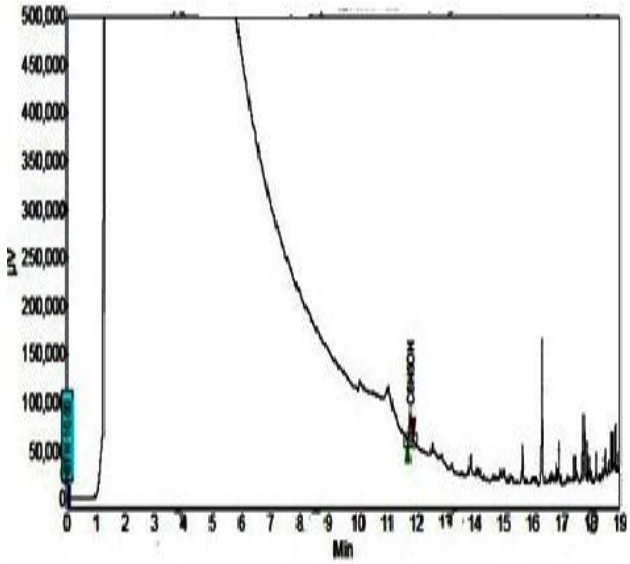
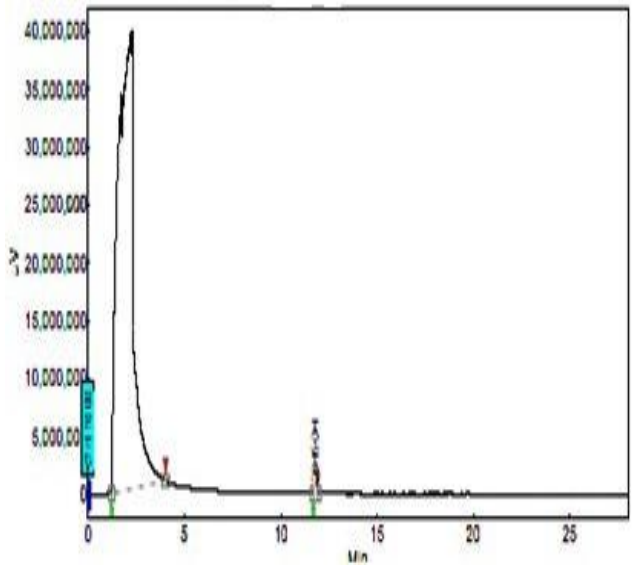


Fig-15 Gas Chromatogram of effluent having the algal concentration of 4000(ppm) without nutrients



The reduction of phenol (C_6H_5OH) by *Scenedesmus* sp. in the batch II also shows the same degradation observed in the batch I. Disappearance of peak corresponds to the phenol (C_6H_5OH) was observed in all samples in batch I. Interestingly in batch I the microalgae *Scenedesmus* sp. degrades the phenol (C_6H_5OH) as well as other contaminants present in the effluent. The gas chromatogram results of batch II showed that the reduction of phenol was less comparatively than batch I.

Effect of *Scenedesmus* sp. on total carbon (Organic (OC), Inorganic (IC) and Total (TC))

The variation in total organic content present in the effluent was analyzed using TOC Analyzer (Fig 16 & 17), in which the effluent with nutrients devoid of algae and the effluent without nutrients devoid of algae served as control to monitor the actual reduction of carbon content present in the effluent by the microalgae *Scenedesmus* sp. The reduction of organic and inorganic carbon has linear relationship with the concentration of algae in batch-I. The results showed that the *Scenedesmus* sp. was able to reduce the organic carbon content present in the effluent to a maximum of 91 % in batch- I(Fig-16) having 4000 ppm of algal concentration (mg of wet biomass/l of effluent) and the 80% in batch-II(Fig-17) having algal concentration of 4000 ppm within 7 days. No change the concentration of carbon (OC, IC and TC) was observed in the controls.

Fig-16 Effect of *Scenedesmus* sp. on the carbon content (Organic Carbon (OC), Inorganic carbon(IC), Total carbon (TC)) of batch-I

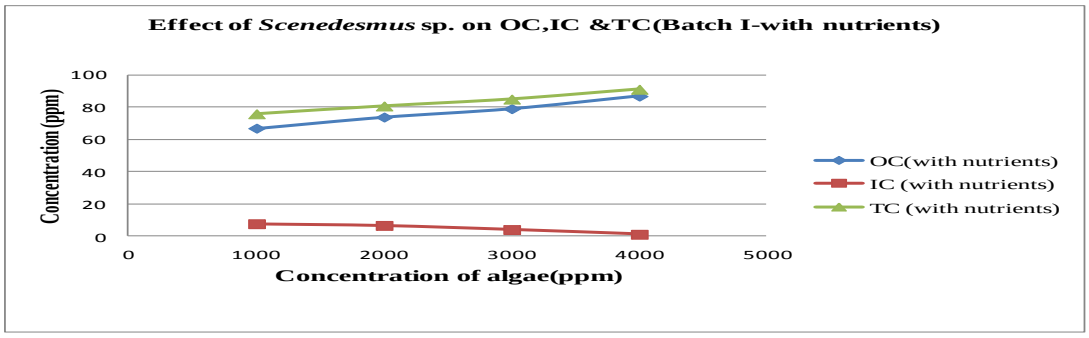
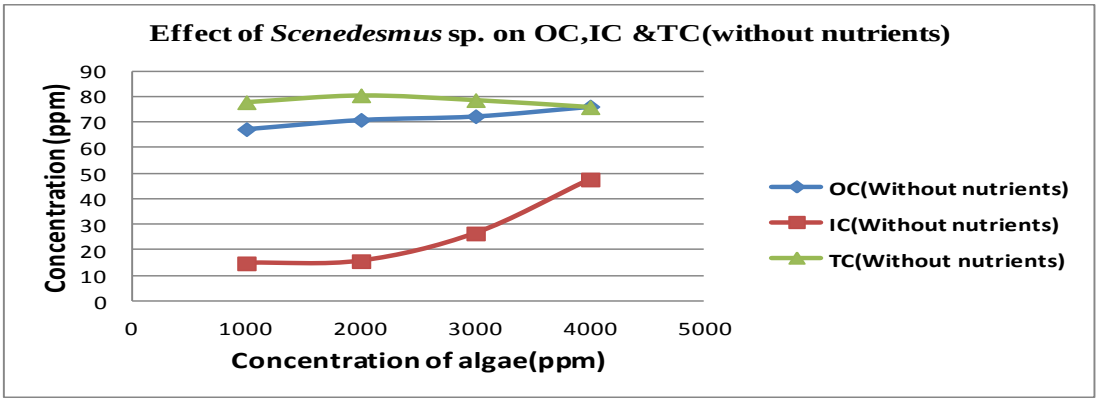


Fig-17 Effect of *Scenedesmus* sp. on the carbon content (Organic Carbon (TOC), Inorganic carbon(IC), Total Carbon (TC)) of batch-II



The reduction of inorganic carbon decrease with increase in organic carbon reduction was noted in the degradation study carried out in batch-I (with nutrients) (Fig-15). But the reduction of inorganic carbon present in the effluent is negligible in batch-I. The results demonstrate that the algae require initially some amount of inorganic carbon for their growth [4]. The oxygen evolved from the photosynthesis initiates the breakdown of phenol and makes the algae to utilize it further. In case of external nutrient starved condition(batch-II), the reduction pattern was reversed (Fig- 17); which clearly indicates that the utilization of inorganic carbon by the algae enhanced when the species is in nutrient starved condition, since, the algal cells utilize the carbon easily from inorganic carbon than from the organic carbon donor such as phenol.

On comparing both the batches, it can be observed that the fresh water microalgae *Scenedesmus* sp. is able to utilize the organic carbon present in effluent as their carbon source for their growth rather than the inorganic carbon supplemented externally. Further, the reduction was only by the microalgae *Scenedesmus* sp. as confirmed by the results obtained for the controls.

4.6 Discussion

Chlorella sp., *Scenedesmus obliquus* and *Spirulina maxima* degraded about 1000 ppm of synthetic phenol in less than 6 days. *Chlorella* and *Scenedesmus* needs an adaptation period of a few days before starting the degradation beyond 400 ppm [1]. Wet biomass of fresh water microalgae *Scenedesmus* sp. exhibits average of more than 95 % degradation of phenol (C_6H_5OH) present in the effluent of producer gas plant in with nutrient batch having concentration of algae 4g of wet biomass/liter of effluent within 7 days. The analysis of total organic content indicates that the media constituents (carbonates and nitrate) added in the effluent enhances the growth and enhances the phenol removal by the microalgae. Due to this, the reduction of phenol increases with the addition of nutrients to the effluent during degradation. The degradation of phenol requires molecular oxygen to initiate the aromatic cleavage [2], [3]; that oxygen is gained by the phenol from the byproduct of photosynthesis of microalgae. The addition of nutrient externally is required by the algae as confirmed by analyzing the batch II in which the reduction of inorganic carbon is significantly more compared to the nutrient rich batch. The increase of pH demonstrates that utilization of carbon dioxide (CO_2) leads to disinfection [3]. There were no physical changes identified during the degradation in the cells of *Scenedesmus* sp. Removal of contaminants present in the effluent other than phenol could also be noticed when the *Scenedesmus* sp. was externally supplied with nutrients.

5.0 Conclusion

Phycoremediation of phenolic effluents is gaining attention globally. Compared to conventional wastewater treatment (physical and chemical) processes, Phycoremediation can potentially achieve nutrient removal in a less expensive and ecologically safer way with the added benefits of resource recovery and recycling [15]. The unique properties of the algae in waste water treatment integrated with conventional methods present great opportunities to the water and wastewater treatment technologies. The present study proves that the *Scenedesmus* sp. is a promising alternative to the conventional methods for phenol removal from the industries producing phenol rich effluents without prior treatment.

6.0 Acknowledgement

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Abbreviation

ppm- parts per million
mg/l- milligram per liter
OC-Organic carbon
IC-Inorganic carbon
TC-Total carbon
TOC –Total organic carbon
TSS-Total dissolved solids
TDS-Total Dissolved solids
COD-Chemical Oxygen Demand
BOD-Biological Oxygen Demand