

Removal and Biodegradation of Phenol by the Freshwater Microalgae *Chlorella Vulgaris*

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Abstract

The aim of this paper was to assess the capability of freshwater microalgae *Chlorella vulgaris* to remove and biodegrade phenol from wastewater. Microalgae was exposed to different concentrations of the chemical at during four days. Phenol concentration was measured using spectrophotometric method and cell growth was measured by neubauer chamber and optical density. The results showed the ability of *chlorella vulgaris* to remove the pollutant in short periods of time at both concentration but specially at high phenol concentration (100 ppm). The mean phenol removal value for 50 ppm solution was 75% and 98% for 100 ppm solution. Results also showed a growth inhibition for both phenol concentration. This study demonstrated that Colombian native *Chlorella vulgaris* could be used for treatment of contaminated aqueous systems.

Keywords: *Chlorella vulgaris*, phenol removal, microalgae, spectrophotometric methods

1. Introduction

Phenol is a natural substance found in air and water. It is also used in many manufacturing processes and for such use, it is produced synthetically. The chemical industry use phenol as an intermediate for the synthesis of more complex compounds (ortho-, para-, meta-halophenols, methylphenols, nitrophenols, etc.) [1] being the main sources of these phenolic compounds the petroleum processing plants, oil refineries, as well as pharmaceutical and agrochemical industries. Inappropriate disposal may result in an elevated presence in the aquatic environment, being charged with sometimes high concentrations of phenols. Phenolic compounds are hazardous pollutants in fresh and marine systems, including coastal sea waters[2][3]. The toxicity of phenolic compounds stems mainly from their hydrophobic character as well as their ability to form free radicals. Phenols may be removed from the environment and industrial effluents by physicochemical methods such as ozonation, activated carbon adsorption, chemical oxidation, Fenton's reagent, UV or hydrogen peroxide [4] but these treatments are limited because they are usually complex and expensive and produce hazardous end-products [5]. Various conventional methods such as physico-chemical, anaerobic digestion and biological have been adopted for removal of phenol. Amongst various treatment methods, biodegradation is gaining importance as versatile, inexpensive and potential alternative to the conventional treatment methods [6][7].

Microalgae have been reported to accumulate pollutants such as heavy metals, hexachlorobenzene, herbicides, insecticides and phenol [8][9]. In recent years, phytoremediation of contaminated waters by photoautotrophic aquatic organisms such as algae, has been demonstrated to be successful for the removal of both organic and inorganic pollutants [9]. Microalgae are capable of biotransforming phenolic compounds [10] and marine diatoms have been reported for the remediation of BPA [11], the microalga used the phenol as a carbon source to growth. Microalgal may biocumulate and biodegrade phenols. The contribution of microalgae in bioremediation of phenolic compounds has been proposed by Oswald et al. more than fifty years ago [12], but only relatively recently the capabilities of some algae for phenols biodegradation gained interest. While phenols show acute toxicity to some algae, both cyanobacteria and eukaryotic microalgae (e.g., *Chlorella sp.*, *Scenedesmus sp.*, *Selenastrum capricornutum*, *Tetraselmis marina*, *Ochromonas danica*, *Lyngbya gracilis*, *Nostoc punctiforme*, *Oscillatoria animalis*, and *Phormidium foveolamm*) are capable of biotransforming phenolic compounds [13].

Microalgae that are sensitive to phenolic pollutants when the cultures are grown under phototrophic or heterotrophic conditions reverse or alleviate the toxicity upon photoheterotrophic growth of the culture [14] or improve their ability to mineralize phenolic compounds under mixotrophic growth conditions [15]. This capability

enables them to survive under low light conditions and/or carbon dioxide availability and represent an alternative to other biological treatment used for the biodegradation of phenol-containing wastewater [16]. *Chlorella* and *Scenedesmus* species have been widely used for the biodegradation of phenolic compounds. Particularly *Chlorella* species are capable of biodegrading a variety of phenolic compounds, such as phenol, bisphenol-A, 4-nitrophenol, 4-chlorophenol, 2,4-dinitrophenol, and 2,4-dimethylphenol [17][18]. In the above-mentioned studies, the common characteristic was the presence of an alternative carbon source under illumination.

Klekner and Kosaric (1992) [17] reported that *Chlorella* sp., *Scenedesmus obliquus* and *Spirulina* sp., degraded phenol completely. *Chlorella vulgaris* and *Coenochloris pyrenoidosa* were found to degrade p-chlorophenol supplemented with zeolite up to 150 ppm and *Anabaena* cylindrical degrades 2,4-dinitrophenol completely. *Euglena gracilis* had been already mentioned capable to treat Phenol [19]. *Scenedesmus* species have been shown to be capable of removing acylated phenols and bisphenol-A [20], [21]. O. Danika was found to biodegrade phenol through the meta-cleavage pathway [22], while in *Chlorella* species, there is involvement of cytochrome P450 in the biodegradation of phenolic compounds [23]. In addition, NADH-dependent reactions were found to take place during the biodegradation of these compounds by the marine diatom *Thalassiosira* sp. [24]. Bioremoval of the phenolic compound enhances by increasing the light intensity, in a wide range of light intensities.

Biodegradation of phenol is sensitive to many factors which could affect the degradation ability/metabolism of microorganisms by preventing or stimulating growth of the organisms. These factors include inoculum size, pH, temperature, carbon and nitrogen sources and pollution load. It has been proposed that the biodegradation of phenolic compounds is a photoregulated process [25] and an aerobic process with high oxygen demands [26] as the enzymes implicated (e.g., phenol monooxygenase, catechol 2,3-dioxygenase, etc.) use oxygen as a substrate [22]. The aim of this research is to evaluate the efficiency of phenol removal using the freshwater microalgae *Chlorella vulgaris* at laboratory conditions as well as the impact of the contaminant on the microalgae growth.

2. Methodology

2.1. Materials

The native microalga *Chlorella vulgaris* was obtained from the collection of the National Learning Service (SENA). *Chlorella vulgaris* is unicellular green algae, its photoautotrophic growth is generally limited by depletion of nitrogen, light attenuation, pH change, carbon limitation, and accumulation of photosynthetic oxygen [27].

2.2. Culture conditions

Microalgae were scaled up from an algae batch. It was started with a petri dish, to a test tube, to a 250 ml Erlenmeyer and finally to a 1000 ml Erlenmeyer. Strains were maintained in Conway culture medium. Culture conditions included a temperature of 24 ± 2 °C, fluorescent lamps of 39W as a source of artificial illumination with irradiation of 5000 lux, photoperiod of 12 hours of light and 12 of darkness, aeration of 0.7 vvm using atmospheric air through of a mechanical ventilator, with no CO₂ injection. Each microalga was cultured four times for the time needed to achieve the reduction of the phenol concentration.

The modified Conway culture medium consists of the following components: FeCl₃.6H₂O, MnCl₂.4H₂O, H₃BO₃, EDTA, NaHPO₄.2H₂O, NaNO₃, Na₂SiO₃, H₂O, trace metal solution and vitamins solution. The solution of trace metals is composed of ZnCl₂, CoCl₂.6H₂O, (NH₄)₆Mo₇O₂₄.4H₂O, CuSO₄.5H₂O and distilled water, and the vitamins solution are composed by Decamyl compound and distilled water.

2.3. Phenol bioassays

In order to analyze the variation of the phenol concentration, a calibration curve was done to measure the concentration by spectrophotometry. The calibration curve was prepared using standard solutions of 0.1, 0.25, 0.5, 0.75, 1.0, 2.5 and 5.0 mg/L of reagent grade phenol using a Genesys spectrophotometer. To measure phenol degradability by *Chlorella vulgaris*, the microalga was contacted with phenol solutions of 50 and 100 mg/L and allowed to interact. The growth of biomass was analyzed at equal intervals of 24 h by spectrophotometry and by Neubauer chamber. Residual phenol was analyzed by spectrophotometry at 510 nm using the 4-aminoantipyrine method at equal 24 h time intervals.

3. Results

3.1 Phenol removal

Figure 1 shows the variation of phenol concentration using an initial amount of 50 mg/L, it is shown that for all bioassays there was a decrease on phenol concentration after four days, which varied from 8 to 20 % in the end of the tests, the difference in the values of the first day is not significant in bioassays, this is probably owed to the low increase on cell density in the day 1, after day two, results varied for the individual experiments and presented the highest deviation, which can be related to the deviation of the cell densities for each bioassay after second day, in addition, for most of the cases at this time occurs the highest decrease of phenol concentration.

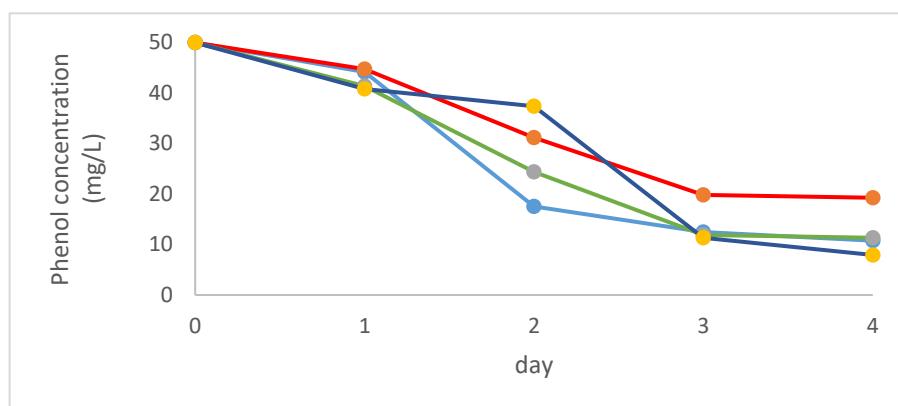


Figure 1. Phenol concentration variation in 50 mg/L phenol solution

Finally, for all cases, the rate of change phenol concentration decreases for day 4, which can be explained by the decrease on cell density for this time. In best case, a reduction of phenol concentration of 84% was achieved using local Chlorella vulgaris strain and the phenol removal mean was 75.34%.

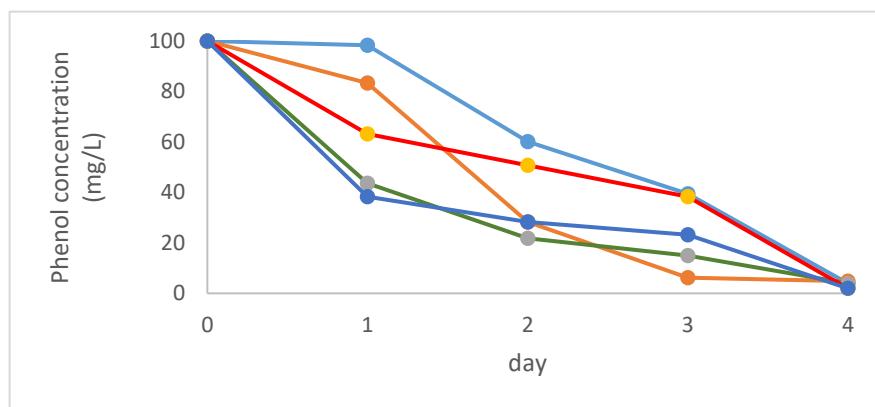


Figure 2 Phenol concentration variation in 1000 mg/L phenol solution

Figure 2 shows the variation of phenol concentration using an initial amount of 100 mg/L of phenol, it is shown that there are significant differences in the results of each bioassay for day 1, from 38.27 to 98.36 mg/L, however, it can be seen that for all cases there was a decrease of the phenol concentration, this trend remains for day 2, where differences were less significant between bioassays, and most of them are located in the range from 21.83 to 28.34 mg/L, rate of change of phenol decreased in most of cases of day 3, but there was still a diminishing in concentration, varying from 6.24 to 39.40 mg/L. The most interesting result was found by comparing the phenol concentration for all bioassays in day 4, it is clear that final concentration of phenol was similar in all bioassays, varying from 1.98 to 4.82 mg/L, although initial phenol concentration was twice the initial concentration of figure 1, removal percentage was higher, achieving a removal percentage of 98%

for best bioassay and an overall removal mean of 96.66%. In both cases (50 and 100 mg/L of initial phenol concentration), it may be concluded that local microalgae strain offers promising results in effective phenol removal.

3.2 Cell Growth

According to figure 3, for most of cases there were an uptake in cell concentration in day 2, but after that day, cell concentration decreased, this behavior cannot be related to presence of phenol, taking into account that the trend was also present in control test.

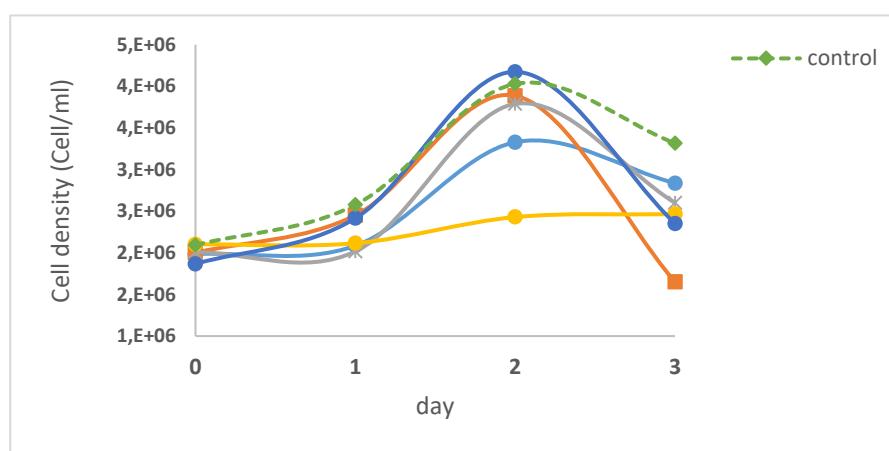


Figure 3. Chlorella vulgaris Growth in 50 mg/L phenol solution

More significant differences in cell growth were found in bioassays with initial phenol concentration of 100 mg/L, where higher cell concentrations varied between days 2 and 3, for figures 3 and 4. It is also shown that all final cell concentrations were lower than control test, which allow to conclude that despite phenol removal and degradation by microalgae, the presence of this chemical specie affects the increase of cell concentration.

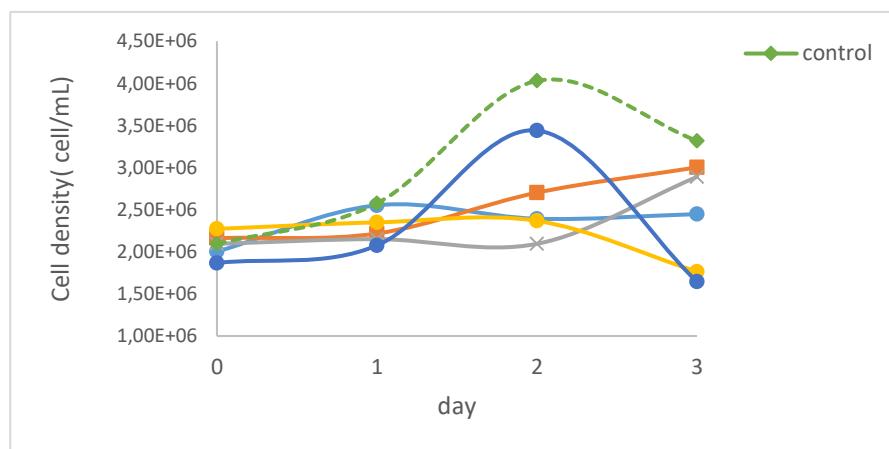


Figure 4 Chlorella vulgaris Growth in 100 mg/L phenol solution

4. Conclusions

Experimental results showed that for both concentration microalgae *chlorella vulgaris* was effective to remove phenol. It also showed a best removal performance at the highest concentration of phenol. An inhibition of micro-algae growth at both concentration of phenol was evidenced; this inhibition was clearer at the higher phenol concentration (100 mg/L). This study suggests that *Chlorella vulgaris* has the ability to biodegrade phenol compounds, and might be used to treat contaminated water streams.

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