Bioremoval Capacity Of Phenol By Green Micro-Algal And Fungal Species Isolated From Dry Environment

Abdullah T. Al-fawwaz, Jacob H. Jacob, Turkia E. Al-Wahishe

Abstract: Phenol is an organic hazardous pollutant that exerts toxic effects on living cells at relatively at low concentrations. Moreover, accumulation of phenol exhibit toxicity towards the biotic components of the environment. Phenol bioremoval is a very useful approach to clean up the residual phenol from the environment. This study aims at isolating green microalgae and fungi from local dry environment to test their ability to remove phenol. Subsequently, two green microalgal species have been isolated and identified as Desmodesmus sp. and Chlamydomonas sp.. Also, two fungal species have been isolated and identified as Rhizopus sp. and Mucor sp. Phenol bioremoval capacity as well as the effects of some physicochemical factors on the bioremoval process were then studied. These factors include initial phenol concentration, contact time, and the synergistic effect (Desmodesmus sp. and Rhizopus sp.) on the bioremoval process. Both microalgae and fungi showed phenol bioremoval capacity. The highest phenol removal percentage among algae was found (75%) by Desmodesmus sp. after 25 days at 25 mg/L, while the highest phenol removal percentage among fungi was found (86%) by Rhizopus sp. after 25 days at 100 mg/L. Bioremoval of phenol by the consortium (Desmodesmus sp. and Rhizopus sp.) was found to be 95% at the phenol concentration 25 mg/L.

Index Terms: Biodegradation, Bioremoval, Desmodesmus sp., Fungi, Green microalgal, Phenol.

1 INTRODUCTION

Over the last century, increasing population growth and industrialization, with the consequent release of pollutants, have resulted in the deterioration of various ecosystems on which human life relies on [1]. Phenol and phenolic compounds are widely distributed as environmental pollutants due to their common presence in the effluents of several industrial processes, including oil refineries, ceramic plants, coal conversion process, phenolic resins, pharmaceutical and food industries [2]. Due to the toxicity and frequent release in nature, they represent a serious ecological problem. Phenols can be removed from the environment and industrial effluents by physicochemical methods such as ozonation, activated carbon, adsorption, chemical oxidation, Fenton's reagent, and UV or hydrogen peroxide [3]. However, some of the aforementioned methods are restricted because of their complexity and high cost, production of hazardous end-products [3]. Biodegradation represent a better alternative method for phenol cleaning because organism-based method usually lead to mineralization of the starting pollutants to simpler compounds like CO_2, H_2O, NO_3 and other inorganic compounds [4]. The purpose of this study is to isolate green microalgae and fungi from local dry environment to investigate their capacity of phenol bioremoval, and the effect of several physiochemical factors (the initial phenol concentration, contact time, and inoculums size) on the bioremoval process. Additionally, the synergistic effect (Desmodesmus sp. and Rhizopus sp.) on phenol bioremoval was also investigated.

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2 Materials And Methods

2.1 Sampling Method

Samples of algae-containing water were collected from different local environments in Al-Mafraq city, Jordan during June-December, 2012. The samples were collected in clean and sterile glass bottles and transferred to the laboratory for isolation. Fungi were isolated from different local environments in Al-Mafraq city during the same period. The samples include soil and dead parts of plants. Samples were collected in clean plastic bags and transported to the laboratory for isolation.

2.2 Isolation and Identification of Algae

Algal species were grown in 3L flasks containing l000 mL of Bold's basal medium (BBM) [5]. Cultures were incubated in a growth chamber at 25±2°C under 1500 Lux (20.25 µmol photon.m^{-2}.S^{-1}) with a cycle of 12 h light and 12 h dark. Cells were transferred to fresh sterile medium every 14 days to maintain cultures in the healthy exponential growth phase. Isolated algae samples were then sent to the University of Texas Culture Collection, Utex CC, USA for identification.

2.3 Isolation and Identification of Fungi

All Fungal samples were isolated with potato dextrose agar (PDA). Pure cultures were obtained after several transfers. Pure cultures were identified Fungal species were grown in 250 mL flasks containing 85 mL of PDA slants. Flasks were maintained at room temperature (28±2 °C) for 7 days until sporulation. Tween 80 (0.2%) was used to prepare spore suspension. Fungal spore concentration was determined by haemocytometer.

2.4 Preparation of Phenol Solutions

A stock solution was prepared by dissolving 0.5 g of phenol in 100 mL of distilled water. The phenol solutions for the bioremoval experiments were prepared by diluting the stock solution to yield different concentrations: 25, 50 and 100 mg/L.

2.5 Effects of Initial Phenol Concentration and Contact Time on Bioremoval Efficiency

Algae were cultured at room temperature (25± 2°C) in 100 mL flasks containing 50 mL phenol solution of different phenol concentrations (25, 50 and 100 mg/L). Control cultures (without inoculum) were carried out under the same experimental conditions. Inoculated flasks and control were kept for a period of 25 days. Samples were then clarified by centrifugation at 5000 rpm for 10 min, and the residual phenol in the supernatant was analyzed spectrophotometrically at wavelength of 660 nm. Fungi were also cultivated in 50 mL of phenol solutions varying in concentrations (25, 50 and 100 mg/L) in 100 mL flasks. Cultures of fungi were incubated at room temperature (25 ± 2°C). Control cultures (without inoculum) were carried out under the same experimental conditions. Inoculated flasks and control were kept for a period of 25 days. Samples were then clarified by centrifugation at 5000 rpm for 30 min, and the residual phenol in the supernatant was analyzed spectrophotometrically as mentioned previously.

2.6 Synergistic Effect on Phenol Bioremoval

This experiment was carried out to investigate the synergetic effect of consortium (Desmodesmus sp. and Rhizopus sp.) on phenol bioremoval. Stock cultures of the consortium were prepared by inoculating 50 mL of phenol solutions varying in concentration (25 and 100 mg/L) with 4.0 g/100 mL of Desmodesmus sp. and $1.8x10^8$ spores/mL of Rhizopus sp. Inoculated flasks were kept at room temperature for 25 days. Then the cells were centrifuged and the supernatant was analyzed spectrophotometrically as mentioned before.

2.7 Determination of Phenol Concentration opyright Form

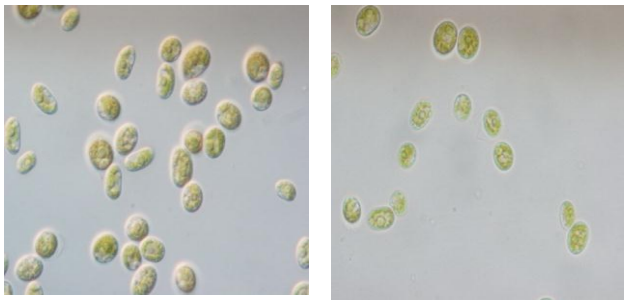
The ability of the isolates to degrade phenol was measured spectrophotometrically; 20µL of each supernatant were transferred into separate cuvettes from each flask after every 24 h for the first 5 days. After that, samples were removed from each flask after every 48 h for the rest 20 days, and to each cuvette. Distilled water (1.58 mL) and 100 µL of the Folin–Ciocalteu (FC) reagent were added. After 8 min, 300 µL of the sodium carbonate solution were added and mixed. The solutions were kept at 20 ºC for 2 h and the absorbance of each solution was measured at 765 nm against the blank (solution lacking phenol), then phenol concentration was determined. The bioremoval efficiency of the isolates was then calculate according to the following formula:

$$\% \text{ Phenol Removal Efficiency (PRE)} = \frac{(C_i - C_f)}{C_i} \times 100$$

Where C_i is the initial concentration of phenol (mg/L) and C_f is the final concentration of phenol. All experiments of phenol bioremoval were conducted in triplicates; the results were expressed as average ± standard deviation (SD).

3 RESULTS

Two algal species (AF1 and AF3) were isolated in this study. They represent different morphologies and cellular shapes base on macroscopic and microscopic examination. The species, AF1, and AF3, were identified as Desmodesmus sp. and Chlamydomonas sp., respectively (Figure 1), according to Utex culture collection, USA.



(a) (b)

Fig.1. Light microscope image of the isolated species AF1(Desmodesmus sp.) [a] and AF3 (Chlamydomonas sp.) [b] (magnification is about 1000x). Two fungal species (A1, and A2) were isolated in this work from dead plant parts and soil. The isolates were identified based on macroscopic and microscopic examination. The species, AF1 and AF2, were identified as Rhizopus sp. and Mucor sp., respectively. Initial phenol concentration plays an important role in the bioremoval process, since some contaminants, such as phenol are known to have inhibitory effect on the activity of the biomass. Experiments were carried out at different initial phenol concentrations ranging from 25 mg/L to 100 mg/L. Figure 2 shows the effect of phenol concentration on phenol bioremoval efficiency by Desmodesmus sp. and Chlamydomonas sp.. The highest phenol bioremoval efficiency was detected at 25 mg/L of initial phenol concentration by Desmodesmus sp., (70%) and Chlamydomonas sp. (56%). The minimum phenol bioremoval efficiency was detected at phenol initial concentration of 100 mg/L by Chlamydomonas sp. (7 %).

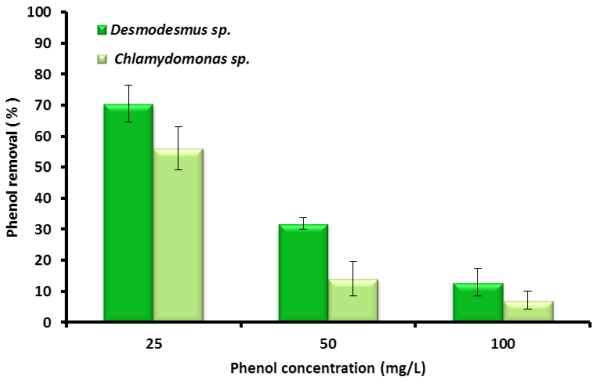


Fig. 2. *Effect of phenol concentration on phenol removal by Desmodesmus sp. and Chlamydomonas sp.. Incubation was carried out at room temperature after for 25 days.*

The effects of initial phenol concentration on phenol removal by the isolated fungal species Rhizopus sp. and Mucor sp. are shown in figure 3. The highest phenol removal was detected by Rhizopus sp. at 100 mg/L (84 %). However, Mucor sp. removed 82 % of phenol at the same initial concentration. The lower removal was detected at the lowest concentration (25 mg/L) by Rhizopus sp. (66 %) and Mucor sp. (58 %).

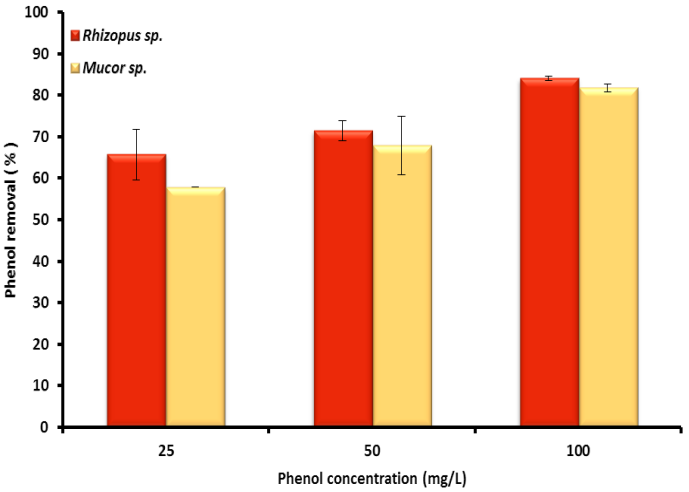


Fig. 3. *Effect of phenol concentration on phenol removal by Rhizopus sp. and Mucor sp. at room temperature after 25 days of incubation period.*

Figures (2 and 3) show that the highest phenol bioremoval was achieved at 25 mg/L, whereas the lowest phenol bioremoval was achieved at 100 mg/L when algae were used. In contrast, the phenol bioremoval increased as phenol concentration increased when fungi was used as bioremoval agent. Bioremoval experiments were carried out for 25 days and it was observed that, the amount of bioremoval of phenol increased with time at 25 mg/L. Figure 4 shows that the highest amount of phenol removed was 70 % by Desmodesmus sp. followed by Chlamydomonas sp., which removed 56 % after 25 days.

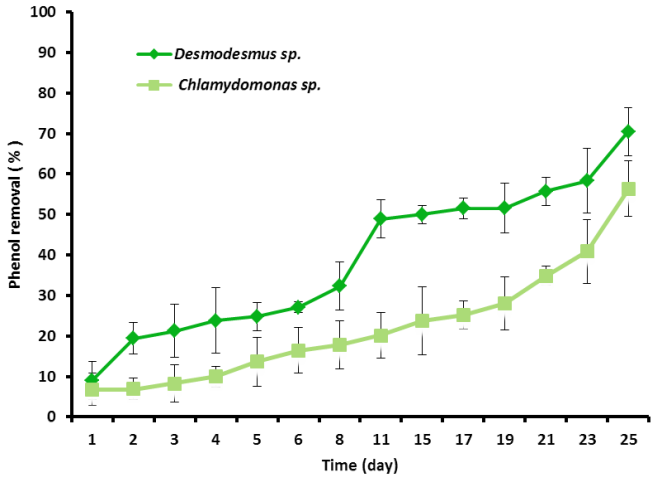


Fig. 4. *Effect of contact time on phenol removal by Desmodesmus sp. and Chlamydomonas sp. at 25 mg/L phenol concentration at room temperature after 25 days of incubation period.*

Contact time is an important parameter to determine the optimum time of bioremoval process. The experimental results for determining the optimum contact time by fungi is given in Figure 5 which obviously show that bioremoval capacity is proportional to contact time. Phenol removal by fungi after the first three days was significantly increased until day 18. After that, removal percentage varied slightly. The highest removal of phenol was (84 %) at 100 mg/L by Rhizopus sp. after 25 days. The phenol removal capacity increased with increasing contact time in both algae and fungi, but the highest phenol removal percentage was 84 % in case of Rhizopus sp. after 25 days of incubation.

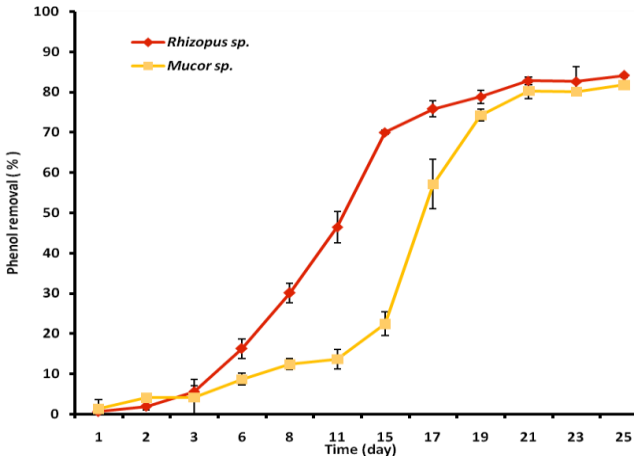


Fig. 5. *Effect of contact time on phenol removal by Rhizopus sp. and Mucor sp. at 100 mg/L phenol concentration at room temperature after 25 days of incubation period.*

The result presented in figure 6 shows phenol removal percentage using a consortium of Desmodesmus sp. and Rhizopus sp. at 25 mg/L after 25 days of incubation period at room temperature. The highest bioremoval of phenol was 95 % when using a consortium culture, compared to 70 % and 66 % of phenol bioremoval when axenic culture of Desmodesmus sp. or Rhizopus sp were used respectively.

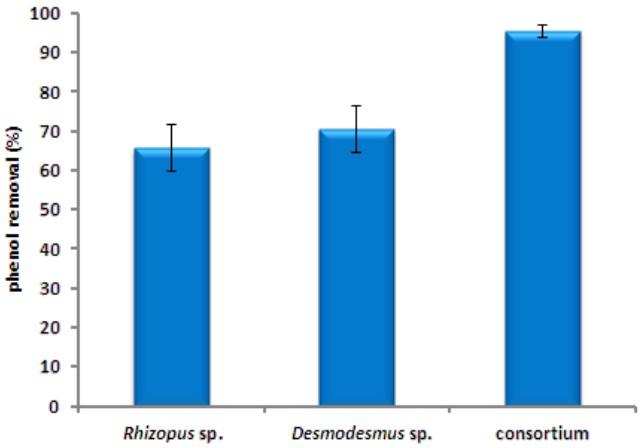


Fig. 6. *Phenol removal percentage using consortium compared with monoculture of Rhizopus sp. and monoculture of Desmodesmus sp. at 25 mg/L phenol concentration at room temperature after 25 days of incubation period*

Bioremoval of phenol is depicted in figure 7, which shows phenol bioremoval using a consortium of Desmodesmus sp. and Rhizopus sp. at 100 mg/L after 25 days of incubation period at room temperature. The highest bioremoval of phenol was 84 % when using an axenic culture of Rhizopus sp., compared to 20 % of phenol bioremoval when using consortium culture and 13 % of phenol bioremoval percentage when using axenic culture of Desmodesmus sp. culture.

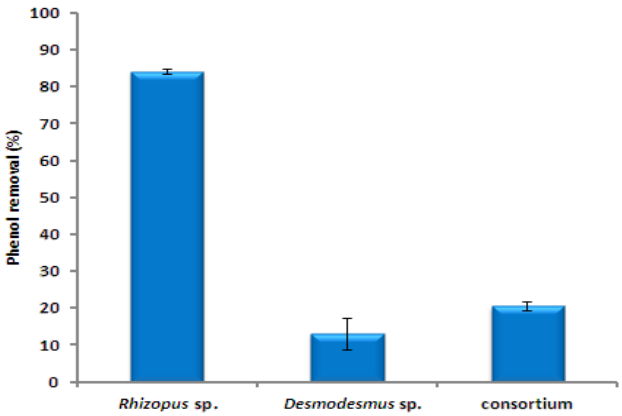


Fig. 7. *Phenol removal percentage using consortium compared with monoculture of Rhizopus sp., and monoculture of Desmodesmus sp. at 100 mg/L phenol concentration at room temperature after 25 days of incubation period.*

4 DISCUSSION

Phenol is one of the major aromatic pollutants, which enters the environment due to domestic and industrial activities. Petroleum refineries, pesticide industries, insecticides, herbicides, metallurgical, plastic and paint industries contribute to phenolic liquid waste. The presence of phenol in water imparts carbolic odor to receiving water bodies and can cause toxic effects on aquatic flora and fauna . It is also known to be toxic to terrestrial life including human beings [6]; [7]). In this study, two species of green microalgae (Desmodesmus sp., Chlamydomonas sp.) and two species of fungi (Rhizopus sp., and Mucor sp.) were isolated and described. , The ability of each isolated species to remove phenol from aqueous solutions was examined. Phenol removal was dependent on several factors including the initial phenol concentration, contact time, and inoculums size. Algal samples were identified as new species of Desmodesmus and Chlamydomonas based on the identification carried out at the University of Texas Culture Collection, Utex CC, USA. The two species are unicellular green algae (Chlorophyta). Green algae (Chlorophyta) are naturally found in fresh water, with a few members inhabiting brackish habitats, soils, and crusts [8]. Generally, cell wall of green algae is made of amorphous mucilaginous material composed of polysaccharides, lipids, proteins and encrusting substances such as silica and calcium carbonate. All cell walls of known green algae usually have cellulose as the main structural polysaccharide. The cellulosic composition of the algal cell wall provides binding sites such as hydroxyl, carbonyl and carboxyl groups [9]; [10]. Fungal samples were Identified as Rhizopus and Mucor species. Rhizopus and Mucor are genera of fungi within the Zygomycetes class. They are mostly terrestrial fungi, living in soil, a variety of food stuffs, decaying plant and in animal material. Some are parasites of plants, insects, and small animals, while others form symbiotic relationships with plants. Zygomycota possess a cell wall of chitin. They grow primarily as mycelia or filaments of long cells (hyphae), most Zygomycota hyphae are generally coenocytic because they lack cross walls or septa [11]. The highest bioremoval capacity was observed at initial phenol concentration of 25 mg/L for both species of algae Desmodesmus sp. and Chlamydomonas sp., even though a higher concentration (100 mg/L) was tested. One possible explanation is that all binding sites became occupied on the algal biomass and higher concentrations exerted elevated toxicity on the algal biomass. This may indicates that the microbial metabolism was inhibited at higher concentration of phenol. It may also be attributed to the inhibition of cell growth resulting from changes at cellular and genetic levels [10]; [12]. It worth indicating that very little is known about the bioremoval of phenol with algae as compared to fungi and bacteria. In case of the two species of fungi, Rhizopus sp., and Mucor sp., the highest bioremoval capacity was observed at initial phenol concentration of 100 mg/L for both species. The bioremoval capacity was found to be proportional to phenol concentration. Because most fungi have the ability to produce extracellular enzymes for the metabolism of complex carbohydrates, the degradation of a wide range of pollutants such as phenol could be possible [13]. For instance, the enzyme laccase produced by was found to be able to catalyze the oxidation of some phenols [14]. There are reports on many microorganisms (bacteria and fungi) capable of degrading phenol through the action of variety of enzymes. These enzymes may include oxygenases, hydroxylases, peroxidases, tyrosinases and oxidase. Aerobically, phenol is first converted to catechol which is degraded via ortho or meta fission to intermediates of central metabolism. The initial ring fission is catalyzed by an ortho cleaving enzyme, catechol 1, 2-dioxygenase or by a meta cleaving enzyme catechol 2, 3-dioxygenase, where the product of ring fission is a cis-muconic acid for the former and 2-hydro cismuconic semi aldehyde for the latter [15]. Phenol removal at lower concentrations (in this experiments 25 mg/L) is attributed to the presence of more available sites on the adsorbent (fungi biomass) than the number of phenol ions which are available in solution. The bioremoval in our study was proportional to the concentration. This agrees with the results of [16], who showed that the yeast Saccharomyces cerevisiae used phenol as the sole energy and carbon source and tolerated up to 120 mg/L phenol. However, our results disagree with the results of [17], which showed an inverse correlation between phenol concentration and bioremoval by Aspergillus fumigatus. Most studies on phenol bioremoval and degradation have been carried out with bacteria, mainly from Pseudomonas genus. A great number of yeast and filamentous fungi strains have a phenol degrading capacity. Among the yeast strains, Candida tropicalis has been the most studied. C. tropicalis is a hydrocarbonoclastic yeast able to degrade phenol, phenol derivatives and aliphatic compounds, at relatively high phenol concentrations. However, as in many other microorganisms, phenol inhibits the C. tropicalis growth and can also cause cellular lysis [18]. The obtained results showed that phenol was removed by the biological treatment. The phenol removal was found to be increased gradually with time from the beginning of bioremoval by the two species of algae, Desmodesmus sp., and Chlamydomonas sp., at 25 mg/L after 25 days. Maximum phenol removal capacity was obtained after 25 days of incubation. Higher removal capacity with increasing time can be explained by increase in the number of dead cells, which lead to increase in total surface area of algae, which leads to biosorption process and enter the phenol to the cells. Also, phenol bioremoval was found to be increased with increasing contact time by the two species of fungi, Rhizopus sp. and Mucor sp. at 100 mg/L after 25 days. Phenol removal by the two species of fungi at the first three days slightly differed. This can be explained by the long acclimation period, which was carried out for the organism to start potential growth with

phenol and utilize it as sole carbon and energy source. Another reason for this trend may be attributed to sporulation stage which requires a period of time to enter mycelium stage. After the first three days, phenol removal by fungi was significantly increased until day 18. Then, bioremoval efficiency was slightly different. The detected increased removal capacity can be explained by the abundance of a carbon source which improves the fungi performances and growth and afterwards the depletion of carbon source in the solution which is reflected as decrease or an inhibition in the bioremoval process (mortality of the cells). Because there was no significant differences between the two species of algae, one species of algae (Desmodesmus sp.) was selected in the experiments, and one species of fungi (Rhizopus sp.) was selected to be used in the experiments for the same reason. Bioremoval processes may be carried out by pure culture or by a consortium of microbes [19]. When phenol bioremoval efficiency of the consortium (Desmodesmus sp., and Rhizopus sp.) is compared to axenic cultures of Desmodesmus sp., or Rhizopus sp. at 25 mg/L of phenol concentration, it can be concluded that more than 94% phenol bioremoval was achieved in the presence of the consortium. It seem that there is a both species (Desmodesmus sp. and Rhizopus sp.) tolerate phenol as a consortium better than do individual species [20]. Remarkably, at higher phenol concentration, it was found the phenol bioremoval by the consortium (Desmodesmus sp., and Rhizopus sp.) is lower than axenic cultures of either Desmodesmus sp. or Rhizopus sp. Lower phenol bioremoval at higher concentration of phenol by the consortium as compared to axenic cultures may be may be explained by the use of algal exudates to fulfill nutritional requirements by the fungus instead of phenol. Therefore, phenol bioremoval was decreased over time. Other possible explanation is that bioremoval capacity is a self-limiting process, i. e., in the mixed culture of the alga and the fungus, one of the microorganisms grows faster than the other microorganism. Subsequently, the faster microorganism will reach the stationary phase faster than the other microorganism. This is associated with the production of inhibitory secondary metabolites that may inhibit the growth of the other organism and finally lowers the phenol bioremoval. Acidic by-products which produced by Microbes which grow faster than the others will inhibit the growth of the other microorganisms present. So the degradation process will be decreased in mixed culture of microorganisms [21]. It worth motioning that although there have been some studies reported the isolation of phenol-and even polycyclic aromatic hydrocarbons-biodegrading algal species, none of the isolated algal strains in the present study showed a proof to degrade any of the tested pollutants. Similarly, many other studies reported that algal isolates lack the degradation capacities [22]. Bioremoval offers an economically feasible technology for pollution removal. Furthermore, to the best of our knowledge, this is the first report to compare between algae and fungi in phenol bioremoval. The isolated species have not been previously considered to be phenol bioremoval agents. In order to increase the feasibility of the algae and fungi isolates as possible commercial strains, future studies are needed to clarify the factors affecting the ability and phenol degradation efficiency by using immobilization techniques.

5 CONCLUSION

Two algal species (Desmodesmus sp., Chlamydomonas sp.) and two fungal species (Rhizopus sp. and Mucor sp.) were isolated from very dry environments and they were capable of bioremoving phenol. Bioremoval of phenol was found to be dependent on the initial phenol concentration, contact time, and inoculum size. In the present research, 75% of phenol was removed at initial phenol concentration of 25 mg/L after 25 days. Phenol removal by Rhizopus sp. was 86% at initial phenol concentration of 100 mg/L after 25 days of incubation. The highest bioremoval of phenol was 95% by the consortium (Desmodesmus sp., and Rhizopus sp.) at 25 mg/L of phenol concentration, which indicates that the consortium was more efficient than single species of isolates at low phenol concentration.

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