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Research Article

Impact of Various Concentration of Phenol and *p*-Chlorophenol to the Microalgal Population in Wastewater

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Abstract

Microalgae based biological treatment aiding in nutrient reduction in wastewater has attained growing interest in the wastewater remediation industry. This study focuses on the degradation of phenol and *p*-chlorophenol by microalgae, NH_4^+-N reduction, biomass residue, and flocculation efficiency of the microalgae. The microalgae culture was utilized for testing with a phenol compound with several concentrations, i.e., 10, 30, 50, 75, and 100 mg/L. To measure the degradation of concentration of the phenolic compound concentration in each bioreactor, the UV-VIS spectrometer is used. Consequently, NH_4^+-N, biomass residue, and flocculation efficiency were measured by means of titrimetric method and UV-VIS spectroscopy. These testing were conducted within 0 hour to 192 hours corresponding to eight days.

Keywords: Microalgae; Phenol; *p*-Chlorophenol; Biomass; Flocculation.

Introduction

According to the United Nations, 22% of wastewater consumption of global water utilization comes from industrial activities, where developed country was found to contribute the highest wastewater consumption. Moreover, first world countries treat about 70% of municipal and industrial wastewater they manufacture. Middle-income and low-income countries treat less wastewater compared to high-income countries because advanced wastewater treatment is expensive and requires substantial upkeep. This technology is far more affordable in high-income countries than in low-income countries[1,2].

Wastewater typically consists of 99% water, and 1% of suspended colloidal and dissolved solids[3]. The impacts of release unprocessed or raw wastewater can be categorized into three; firstly, it has a dangerous impact on human health. Secondly, it has bad environmental impacts, and lastly, it can be affected by the environment and economic activities[4]. Sustainable and green technology has been an alternative way to produce or implement a cost-saving, secure, and safe system. Hence, biological treatment of wastewater was regarded to be the best alternative method, given that it does not generate non-toxic by-products and operates at a lower cost than conventional treatment.In this regard, microalgae have been utilized as a candidate for wastewater treatment. The vast majority of microalgae have been extensively utilized for nutrient removal in wastewater and as an energy source for bio-fuel production.

The phenolic compound such as phenol and *p*-chlorophenol were found to exert significant toxicity effects towards the environment. Discharging phenol into water bodies can cause significant declines in aquatic life due to themolecule's high toxicity to these creatures. Although *p*-chlorophenol is toxic to both terrestrial and aquatic creatures, it is far more harmful to the former. When released in large quantities, it can potentially have a devastating effect on the soil and vegetation. Furthermore, both of these compounds can remain in the environment for extended periods of time and accumulate in living species, where they are able to build up in the tissues of animals and plants, and prolonged exposure may cause serious health consequences.

In recent years, treatment of phenolic compound posed significant issuesas the phenolic compound is resistant to biodegradation due to having a complex structure[5]. The most common method of removing phenol and p-chlorophenol from water is through activated carbon filtration. This process involves passing water through a bed of activated carbon, which will adsorb the contaminants. The major disadvantage of this method is that the carbon must be replaced periodically, which can be expensive. Additionally, due to the potential for bioaccumulation of phenol and p-chlorophenol, this method may not be effective in removing all of the contaminants from the water[6].Oxidation is another excellent method to mineralize phenol and p-chlorophenol in water. The introduction of oxygen, either as an oxidizing agent or as air, is necessary for oxidation reactions to take place. Oxidation reactions are used to break down organic compounds like phenol and p-chlorophenol into smaller molecules that can be more easily removed from the water. However, the oxidation of phenol and p-chlorophenol results may result in the formation of formaldehyde, chloroform, and other chlorinated chemicals, all of which have the potential to be dangerous to human health. Furthermore, oxidation reactions can be time-consuming, energy-intensive, and wasteful due to the volume of sludge they generate[7].

Therefore, this study intends to employ bioremediation approach by using microalgae to degrade the phenolic compounds[6,8,9]. Microalgae have been shown to be effective at removing phenolic pollutants from water sources. The removal of these pollutants is accomplished through both adsorption and biodegradation. Adsorption occurs when the pollutants attach to the surface of the microalgae cells, and biodegradation occurs when the microalgae use enzymes to break down the pollutants into harmless by-products[10]. In addition, some species of microalgae can also reduce the toxicity of these substances by converting them into non-toxic metabolites. However, the process of eradicating these toxic contaminants using microalgae is similarly intricate due to inconsistent wastewater compositions that surpasses the tolerant threshold levels of microalgae. Furthermore, no specific amount of phenol and *p*-chlorophenol concentration was established till date to indicate the tolerance level of microalgae. As such, this research gives prominence on the determination of phenol and *p*-chlorophenol concentration and its degradation profile in the culture medium of microalgae, while evaluating the impact of NH_4^+-N reduction concentration in the medium and determination of biomass residue and flocculation efficiency of microalgae culture.

Experimental

Microalgae stock

For the preparation of the microalgal stock solution, a 5 L bioreactor was employed where the *Chlorella Vulgaris*sp. microalgae stock was collected from the depository owned by the Centre for Biofuel and Biochemical research (CBBR) Universiti Teknologi PETRONAS. Nutrient used in this study was simulated synthetic wastewater with the following components: sucrose, $FeCl_3.6H_2O$, $CaCl_2$, $(NH_4)_2SO_4$, NaH_2PO_4, Na_2HPO_4, $MgSO_4$, and $NaHCO_3$[11].These nutrients were mixed into a volumetric flask 1000 mL and diluted with distilled water. 500 mL of the nutrient formerly prepared was then mixed with 4.5L of distilled water with the initial pH adjusted to the range of 6.9 to 7.1. The bioreactor was illuminated with cool-white, fluorescent lamp with an intensity of 60-70 µmol/m^2s and aerated with compressed air. The microalgae stock was monitored every two days by adjusting the pH to 6.9 to 7.1 for 14 days, at which point the culture had stabilised[12].

Photobioreactor setup

Five Erlenmeyer flask with 1 L capacity were used to cultivate the microalgae using phenolic compound as part of their medium. The nutrient used was similar to that of the mother stock microalgae such as sucrose, $FeCl_3.6H_2O$, $CaCl_2$, $(NH_4)_2SO_4$, NaH_2PO_4, Na_2HPO_4, $MgSO_4$, and $NaHCO_3$as simulated synthetic wastewater. 10% of microalgae was introduced to each bioreactor containing the microalgae stock. Phenolic compounds used in this experiment are phenol and *p*-chlorophenol. Upon the homogenization of microalgal culture, phenol or *p*-chlorophenol was introduced into the solution according to the concentration of 10 mg/L, 30 mg/L, 50 mg/L, 75 mg/L and 100 mg/L. Similar concentrations were also repeated using *p*-chlorophenol in another setup[13]. The initial pH of the solution was then adjusted to the range of 6.9 - 7.1. All the bioreactors were put at the shelves with

illuminate it the cool-white, fluorescent lamp with an intensity of 60-70 µmol/m^2s and aerated with compressed air as shown in the figure and analysed the parameter of each bioreactor for day 0 to day 9.

Determination of concentration of phenolic compounds using UV-VIS Spectroscopy

0.5 N of NH_4OH, 35 mL of NH_4OH stock solution was dissolved in distilled water before being diluted to 1 L. For phosphate buffer solution, 104.5 g of K_2HPO_4 and 72.3 g of KH_2PO_4 were dissolved with distilled water before being diluted to 1 L. The pH of this reagent is 6.8. For 4-amino antipyrine solution, 0.5 g of 4-amino antipyrine was dissolved with distilled water before being diluted to 25 mL. This reagent was prepared daily before starting the determination of phenol and *p*-chlorophenol concentration. For $K_3Fe(CN)_6$ solution, 2.0 g of $K_3Fe(CN)_6$ was dissolved with distilled water before being diluted to 25 mL. Phenol and *p*-chlorophenol were tested by using a UV-VIS spectrophotometer to measure the concentration. A standard curve for phenol and *p*-chlorophenol was prepared prior to the experiment for concentration determination. The preparation was initiated with sample collection of 10 mL in each reactor and diluted 25 mL with distilled water, followed by 25 mL of distilled water as blank (control). Next, 0.625 mL of 0.5 N standard NH_4OH was added to each solution and adjust the pH 7.9 ± 0.1 with phosphate buffer. Later, the $K_3Fe(CN)_6$ and 4-amino antipyrine were added with 0.25 mL to each solution and mixed well. After 15 minutes, the mixtures were transferred into the cell and ready to read the absorbance against the blank at 500 nm by using a spectrophotometer (Shimadzu UV-2600)[14].

Determination of $NH4^+$-N concentration using a titrimetric method

Prior to the determination of NH_4^+-N concentration, the reagents were prepared. Borate buffer solution was prepared using 9.5 g of $Na_2B_4O_7.10H_2O$ dissolved in distilled water, added with 88 mL of 0.1 N NaOH before being diluted to 1 L. To prepare the indicating boric acid solution, 200 mg of methyl red indicator was dissolved in 100 mL of 95% ethyl alcohol while 100 mg of methylene blue was dissolved in 50 mL of 95% ethyl alcohol. Both solutions were then combined. For indicating boric acid solution, 20 g of H_3BO_3 was dissolved in distilled water and added with 10 mL of mixed indicator solution before being diluted to 1 L. For 6 N NaOH solution, 240 g of NaOH was dissolved in distilled water and diluted to 1 L. For determination of NH_4^+-N concentration, 5 mL of filtered sample was transferred to the distillation flask and added with 2.5 mL of borate buffer solution and 3 to 5 drops of 6 N NaOH. The distillation flask was then placed into the distillation unit with the tip of the delivery tube situated below the surface of 10 mL of boric acid indicator solution in a 100-Ml Erlenmeyer flask. Under a hot alkaline condition, the NH_4^+-N species in the sample was distilled and trapped in the boric acid solution. The distillation rate was set at 30 mL/min, which enable 90 mL of distillate to be collected in 3 min, reaching a total volume of 100 mL in the Erlenmeyer flask. The concentration of NH_4^+-N (in mg/L) in the sample was determined by titrating with 0.008 N standard H_2SO_4 until the colour of the indicator turned from green to pale lavender in the Erlenmeyer flask and calculated as below:

$$NH^{4+}\text{-}N=\frac{(Vc\text{-}Vd)\times N\times 14\times 1000}{Ve}$$

Where the V_c is the volume of standard H_2SO_4 titrant titrated for sample (in mL), V_d is the volume of standard H_2SO_4 titrant titrated for blank (in mL), Ve is the filtered sample volume (in mL) which was 5 mL, and N is the normality of standard H_2SO_4 titrant[15].

Determination of biomass concentration

The determination of biomass concentration is analysed by dry cell weight which was obtained by calculating the total suspended solid concentration in the culture medium. The microalgae biomass determined by using the gravimetric method where the samples have dried the oven at 105°C for 24 hours and then the weight of biomass measure using analytical weighing balance[16].

$$\text{Biomass concentration(mg/L)}=\frac{\text{weight of weighing pan after (g)}\ \text{-weight of weighing pan before(g)}}{\text{volume of sample (mL)}}$$

Determination of flocculation efficiency (%) of microalgae using UV-VIS spectroscopy

The sample was homogenized via gentle stirring and measured instantly using spectrophotometer (Shimadzu UV-2600) absorbance at 670 nm. The sample was left agitated for 20 minutes, and the aliquot of the sample was then gently extracted at a depth of 2.5 cm below the sample's surface to measure for its optical density 670 nm again. The flocculation efficiency (%) of biomass was finally calculated based on the equation below:

$$\text{Flocculation efficiency}(\%)=\left(1\text{-}\frac{F}{I}\right)\times 100\%$$

Where the F is the optical density of the unagitated sample, and I is the optical density of the homogenizing sample[17].

Results and discussion

Phenolic compound degradation

Figure 1demonstrated that the phenol concentration in all reactors degraded after 56 hours. Phenol in samples A and B degraded quickly at 32 hours while samples C, D, and E degraded at 56 hours. From the observation and sample test, higher concentration of phenol has positively militated the degradation rate of phenol. Overall, the phenol concentration was completely reduced by 100% in less than 72 hours. This is because the microalgae could transform the phenol into its energy source to supplement its growth. Literatures stated that microalgae utilize autotrophic metabolism to convert phenol into their energy source. During photosynthesis, microalgae take light energy and convert it into ATP which functions as the molecular energy[18]. This ATP is subsequently used for cellular respiration, allowing the microalgae to degrade phenol into simpler molecules such as carbon dioxide and water. The microalgae utilize these simpler molecules as a source of energy to support its growth and development[19].Similar studies conducted by Hirooka et al., (2003) had corroborated that utilization of *Chlorella Fusca* strain for complete phenol degradation took place on the 5th day[20].Also,another study had mentioned the maximum degradation of phenol to take place using *Scenedesmus* sp. was observed on 6th day[21]. Our study had outperformed most of the studies by showing complete degradation in only 4 days, proving the efficacy of *Chlorella Vulgaris* in mineralizing the phenol. Meanwhile, Figure2displayed the degradation of *p*-chlorophenol by microalgae from 0 hr to 192 hrs. From the result obtained, the concentration o*f p*-chlorophenol was found to inversely affect the degradation rate. This can be further justified on the basis that the microalgae have consumed additional time to acclimatize the chlorophenol, which has higher toxicity compared to phenol due formation of electrophilic metabolite. Thus, the microalgal performance in degrading the *p*-chlorophenol is inferior to phenol. Researchers suggested that this is due to the structural complexity of *p*-chlorophenol which justifies its higher molecular weight. Phenol is a hydroxyl-bonded aromatic hydrocarbon while p-chlorophenol are organic compounds with a phenol group (aromatic hydrocarbon linked to hydroxyl) coupled with one or more chlorine atoms. This could pose more resistance to degradation by microalgae, due to the electron-withdrawing properties of the chlorine atom, which reduces the availability of electrons for microbial metabolism. As a result, it takes longer for p-chlorophenol to degrade compared to phenol[22,23].The degradation rate of *p*-chlorophenol in sample A and B achieved 43% even though it is relatively less significant compared to phenol while sample C, D, and sample E demonstrated reduction below 20%.However, it is noteworthy to mention that similar research reported at least 10 days for complete degradation of *p*-chlorophenol using different microalgal strain, *Tetraselmis marina*[24]. Similar research had reported congruent results with current study where complete degradation of *p*-chlorophenol took place in 8 days[25].

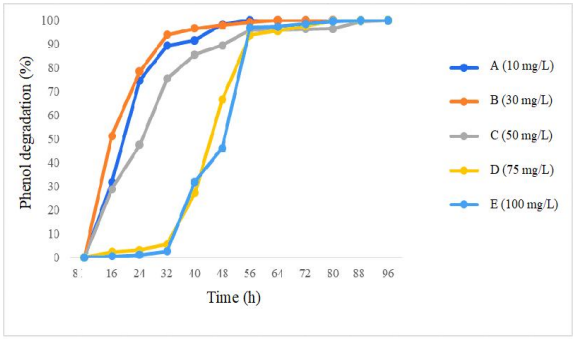

Figure 1: Degradation of phenol in relation to time

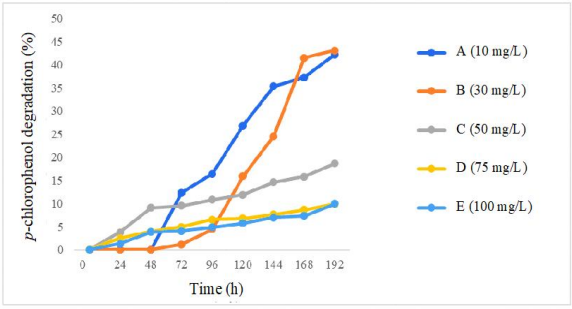

Figure 2: Degradation of p-chlorophenol in relation to time

Characterization of $NH4^+$-N concentration (mg/L)

Agricultural waste, sewage, and industrial effluent with nitrogen compounds could potentially contribute to ecological eutrophication[26]. Contrariwise, microalgae require this nitrogen alongside with CO_2 and light to facilitate the autotrophic growth[27]. Nitrogen metabolism is usually believed to be connected to carbon metabolism in algae because they share organic carbon and energy supplied directly via photosynthetic electron transport and CO_2 fixation, as well as from the metabolic pathway of organic carbon[28].Ammonium (NH_4^+-N) is a nitrogen-based components being evaluated on their degradation in this study. Figure 3revealed the ammonium degradation profile in all reactors with phenol from 0 hr to 240 hrs which is attributed to simultaneous nitrification and the assimilation of microalgae biomasses[29]. Sample A to E had completely degraded by 216 hrs. Nonetheless, sample 0 with no phenol in solution degraded in 240 hours, which is longer than the degradation duration of

former samples, attributing lesser microalgal performance.Furthermore, it can be observed that due to nitrification and nutrient obtained from ammonium ion, there was an increase in the microalgae growth rate and biomass(explained in next section). While for NH_4^+-N reduction with *p*-chlorophenol in Figure 4, it shows that the degradation of ammonium ion from 0 hr to 192 hrs in sample A to Dis higher compared to sample E because it has less concentration of *p*-chlorophenol. Due to the toxicity of the *p*-chlorophenol, it affects the microalgal performance in engulfing the ammonium ion. However, the microalgae are still capable of utilizing of the ammonium as a nutrient source to an extent. Microalgal confluency appear to be efficient with phenol since it demonstrated complete elimination of $NH4^+$-N in a shorter amount of time.

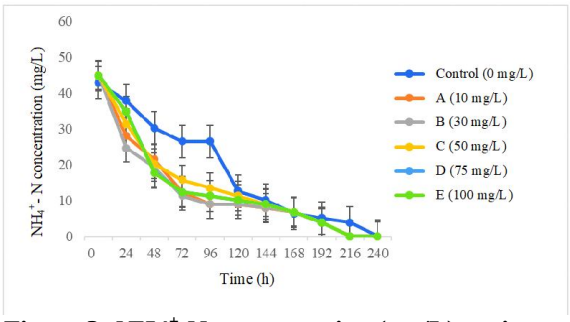

Figure 3: $NH4^+$-N concentration (mg/L) vs time with phenol

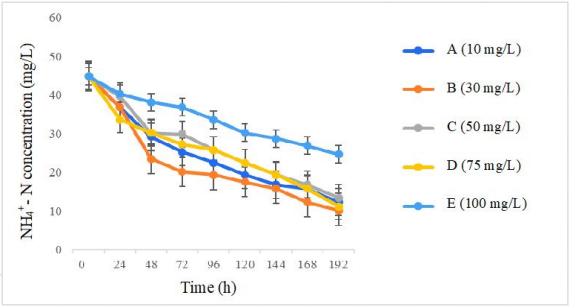

Figure 4: $NH4^+$-N concentration (mg/L) over time with *p*-chlorophenol

Biomass residue

The biomass residue was evaluated from 0 hr to 192 hrs. Based on Figure 5, all reactors exhibited increment of biomass residue in relation to time with phenol. In reactor E, the biomass residue was 480 mg/L, which was the highest value compared to A, B, C, D, and 0 reactor. This is because Reactor E has the largest concentration of phenol, which justifies maximum nutrient assimilation in promoting microalgal proliferation. As explained in the previous section, higher microalgal confluency observed in all reactors compared to control was due to the influence of N from ammonia. In the presence of light and oxygen, microalgae had utilized phenol as an energy and carbon source for growth. The microalgae then exploited the ammonia as a source of nitrogen to synthesize proteins, lipids, and other cellular components[30].This simply explains the relationship shared by carbon and nitrogen metabolism.This also shows that higher phenol concentration could still be tolerated by *Chlorella Vulgaris*as the tolerance threshold is not achieved as yet.As for microalgal performance with *p*-chlorophenol (Figure 6), Sample A and B yielded higher biomass residue while sample E conceded lower residue. However, their biomass residue was lower compared to phenol. This could be due to the structural complexity and toxicity of *p*-chlorophenol posing intolerance towards microalgal cell development thus inhibiting its growth. The microalgae were capable of degrading the phenolic compound and convert it into its energy source, only to an extent. At higher *p*-chlorophenol concentrations, the microalgal growth is supressed due to their interference with metabolic functions such as photosynthesis, respiration etc. Furthermore, higher concentration of this phenolic compound could result in oxidative stress, which could potentially impair cell function and jeopardize the cellular membranes, causing disruption cell proliferation[31].Petroutsos*et al*., (2017) have reported the efficacy of *p*-chlorophenol (maximum 20g/L) to boost the *Tetraselmismarina*microalgal growth up to 150mg/L under photoautotrophic conditions[24]. Meanwhile, another study had reported maximal microalgal growth of *Scendenamus* sp. achieved at 120 mg/Lusing chlorinated phenols over 5 days[32]. This simply shows the effectiveness of *Chlorella Vulgaris* strain in promulgating their cells under various harsh conditions.

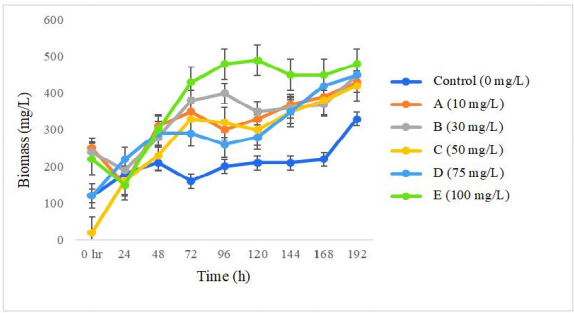

Figure 5: Biomass residue in relation to time with phenol

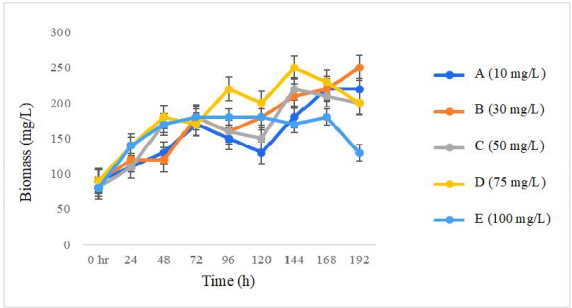

Figure 6: Biomass residue in relation to time with*p*-chlorophenol

Flocculation efficiency (%)

Flocculation is the destabilized particles that are induced to coagulate and make contact with the form of larger agglomerates with a high sedimentation rate[33]. This technique is considered as an efficient, convenient, and preferable process for the harvesting of microalgae biomass[34]. Flocculation efficiency can be positively correlated to biomass growth. Based on Figure 7, all the reactors have shown increment in the flocculation efficiency (%) of the microalgae with phenol. Flocculation efficiency in reactor A rendered highest value compared to other reactors. Based on Figure 8, the flocculation efficiency microalgae with *p*-chlorophenol had also shown significant increment over time. Sample Biwas found to give the highest flocculation efficiency, followed by sample A due to the lower concentration of *p*-chlorophenol, which is 10 mg/L and 30 mg/L respectively. However, sample E presented the lowest efficiency due to the higher concentration of *p*-chlorophenol. The trend of flocculation efficiency in p-chlorophenol was found to be in tandem with the microalgal growth. The trend was followed by the series of sampleB> A>C >D>E. Also, another correlation was observed between the concentration of *p*-chlorophenol and the flocculation efficiency of microalgae. Literature had suggested that the cytotoxicity of *p*-chlorophenol is directly proportional to its concentration[35]. Therefore, the higher the concentration, the greater is the lethality towards microalgal growth. Due to this, the efficiency of the microalgae is jeopardized as a corollary. Since flocculation efficiency is positively correlated to microalgal growth in this study, the negative effect hailing from the cytotoxicity of *p*-chlorophenol were found to supress the flocculation efficiency altogether. Overall,phenol-induced microalgal growth was found to be highly effective than compared to *p*-chlorophenol due to less toxicity and high tolerance threshold of microalgae. Nevertheless, the microalgae could still acclimatize the *p*-chlorophenol but with longer time compared to phenol, influencing the overall efficiency of the microalgae.

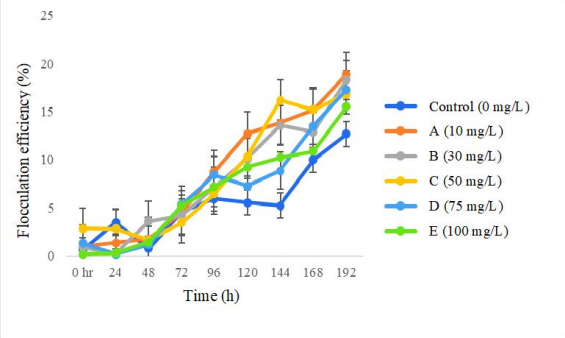

Figure 7: Flocculation efficiency over time with phenol

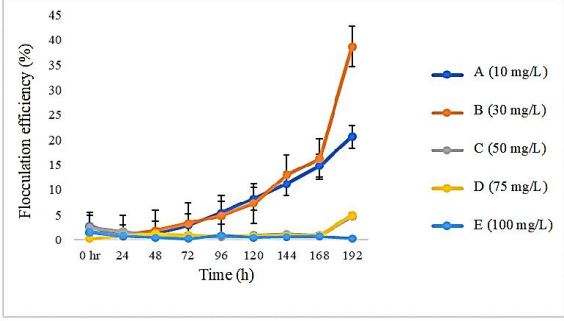

Figure 8: Flocculation efficiency over time with *p*-chlorophenol

Conclusions

This study has elucidated that microalgae could degrade phenolic compounds, with a certain concentration threshold. This study had affirmed total phenol degradation in less than 72 hours, while the *p*-chlorophenol concentration was reduced by less than 45% in 192 hours. Current study had proven that phenol degrades faster than *p*-chlorophenol. This is due to the fact that*p*-chlorophenol has higher toxicity and complex structure than phenol. The reduction of $NH4^{+}$-N concentration with phenol was larger than that with *p*-chlorophenol, due to the increased assimilation of microalgal biomass into the medium, which reduces its toxicity. The microalgae medium containing phenol yielded higher concentration of biomass than the microalgae medium containing *p*-chlorophenol due to the former triggers. The toxicity of *p*-chlorophenol influences the growth and adaptability of microalgae biomass. In conclusion, the microalgae medium containing phenol produced a greater biomass residue and a higher degradation rate than *p*-chlorophenol.

Conflict of Interest

All the authors declare no potential conflicts of interest.

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