Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(1): 1719 – 1726 (2024)
www.ejabf.journals.ekb.eg

Bio-removal of bisphenol A by cyanobacterium *Gloeocapsopsis crepidium*

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ARTICLE INFO
Article History:
Received: Jan. 5, 2024
Accepted: Feb. 9, 2024
Online: Feb. 23, 2024

Keywords:
Bisphenol A,
Cyanobacteria,
Bio-removal,
Gloeocapsopsis crepidium

ABSTRACT

Bisphenol A (BPA) has attracted worldwide attention as a toxic and endocrine-disrupting substance. It can be released into aquatic habitats through microplastic pollution, harming all aquatic life. To reduce its effects and toxicity, it has been necessary to use biological methods that occur naturally in water and are cheap and easy to obtain, such as algae. For this reason, the cyanobacterium *Gloeocapsopsis crepidium* was used to test its ability to biodegrade this toxic compound by exposing it to different concentrations of BPA (1, 5, 10, 20, 50, 75, 100) mg/l under controlled laboratory conditions at 25°C and a light intensity of 60 µmol $m^{-2}s^{-1}$. The results of the bioremoval of bisphenol A by the cyanobacterium *Gloeocapsopsis crepidium* exposed to the different concentrations of BPA (1, 5, 10, 20, 50, 75, 100) mg/l show higher removal efficiencies, the results showed that the highest removal efficiency reached 100% at 1mg/l BPA with residual value 0 mg/l. In contrast, the highest residual value reached 20.52 mg/l at 100 mg/l BPA with a removal percentage of 79.48%. These results are supported by statistical analysis with significant differences at $p \leq 0.05$.

INTRODUCTION

Bisphenol A is an organic molecule commonly used to make epoxy-coated polycarbonate products, including food and beverage cans and the hardness of the plastic. People typically consume food or drink that has come into contact with products made with BPA, exposing them to trace amounts of BPA (Greim, 2024). BPA is a toxic substance often classified as an endocrine disruptor. It affects reproduction, growth and the whole body. When BPA enters the body, it can cause immunological toxicity, neurotoxicity, low sex-specific neurodevelopment and disruption of cellular pathways (Ohore & Zhang, 2019). Microplastic particles, such as those made from low-density polyethene and polycarbonate, are persistent sources of bisphenol A release to aquatic environments. Microplastics can also act as sinks for hydrophobic organic pollutants in the environment. Bisphenol A can be an environmental concern and can be transported by plastic particles, it is can be released into the environment when plastics break down (Liu *et al*., 2019).

The widespread presence of bisphenol A and its derivatives in the aquatic environment, its endocrine disrupting and harmful effects on aquatic life are global concerns. BPA has a serious, harmful effect on aquatic life, inhibiting their ability to grow and develop (Czarny-Krzymińska *et al*., 2023). BPA is released when polycarbonate plastic breaks down, then enters domestic and industrial wastewater and eventually the aquatic

environment through the drainage system (Maturi *et al*., 2023). Due to the toxicity of bisphenols to aquatic life, biological techniques have been required to remove and reduce their toxicity. An effective method for the bioremediation of this compound is the use of algae, which are characterized by a general resistance to pollutants and a high abundance in the aquatic environment. This means that algae are a cheap, natural source that doesn't require chemicals or special equipment (Wu *et al*., 2022).

Many studies have been approved at the removal of bisphenols by algae. One such study was conducted by Ouada *et al* (2002) using two extremophilic Chlorophyta strains: an alkaliphilic strain of *Picocystis* and a thermophilic strain of *Graesiella*. Both species showed high BPA removal efficiencies, with *Graesiella* reaching 52.6% and Picocystis reaching 72% at 25 mg/L. The removal of BPA was mainly attributed to biodegradation by both species (Ben Ouada *et al*., 2018). Another study showed that a monoculture of *Chlorella sorokiniana* was able to partially remove BPA (Eio *et al*., 2015). Shimoda and Hamada (2009) reported that immobilized *Pavlova* cells outperformed free-suspended Pavlova cells in reducing and glycosylating bisphenol A to its glucoside product. This biotransformation by means of the immobilized form is useful for the chemical modification of BPA

The aim of this study is to use of a low-cost, effective method for the bio-removal of bisphenol A under laboratory conditions by the cyanobacterium *Gloeocapsopsis crepidium*.

MATERIALS AND METHODS

Cyanobacterial isolate

The isolate of the cyanobacterium *Gloeocapsopsis crepidium* (figure 1) was obtained from the University of Al-Qadisiyah, to confirm its purity, it was grown in nutrient medium at 37°C for 24 hours to check for the absence of bacteria and fungi (Andersen, 2005).

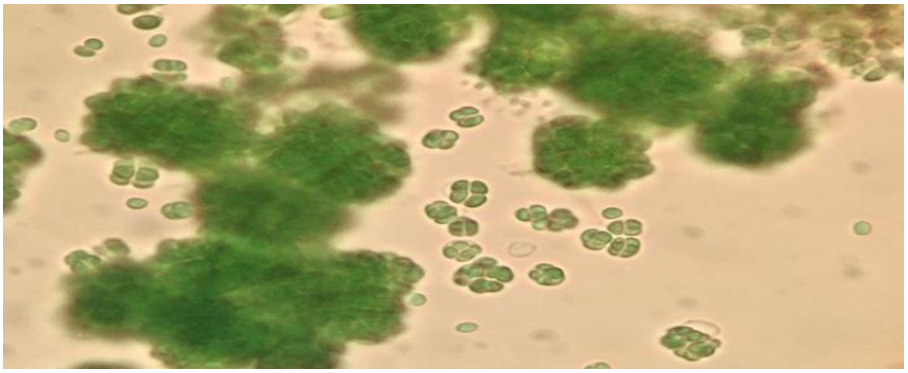

Figure 1. *Gloeocapsopsis crepidium* seen under the light microscope, magnified 40X

Bisphenol A treatment

Cyanobacterial isolate *Gloeocapsopsis crepidium* was grown in BG-11 medium under the influence of different concentration (1, 5, 10, 20, 50, 75, 100) mg/l under controlled laboratory conditions at 25°C and a light intensity of 60 µmol m^{-2} s^{-1}, pH 7.2, and a light period of 16:8. Darkness: Light.

Growth curve estimation

It was carried out by chlorophyll-a determination by taking 5 ml from the culture and centrifuging at 5000 rpm for 5 minutes, discarding the supernatant and taking the algal cell precipitate, then put in a shaking water bath at 25 °C for one hour, then centrifuging at 6000 rpm for 10 minutes and taking only the supernatant. The optical density of the supernatant is measured using a spectrophotometer at a wavelength of 664 nm and the concentration of chlorophyll a is calculated using the equation (Ritchie, 2006).

$$\text{Chl-a [µg/ml]} = 11.4062*A664$$

Bisphenol A estimated using HPLC

Bisphenol A was detected and quantified on a column (C18 PAH, 3 µm, 250 x 4.6 mm) (Agilent, Germany) using the method of Aristiawan *et al*. (2015). Mobile phase isocratic water-acetonitrile (40:60 v: v), flow rate 1.0 ml/min at room temperature, chromatograms recorded at 210 nm. Bisphenol was detected by comparison of retention time and absorbance spectrum of standards purchased from SIGMA-ALDRICH (239658-50G). Quantitation of the sample was calculated by measuring the integrated peak area and the content was calculated using a calibration curve by plotting the peak area against the respective standard sample concentration (Figure 2).

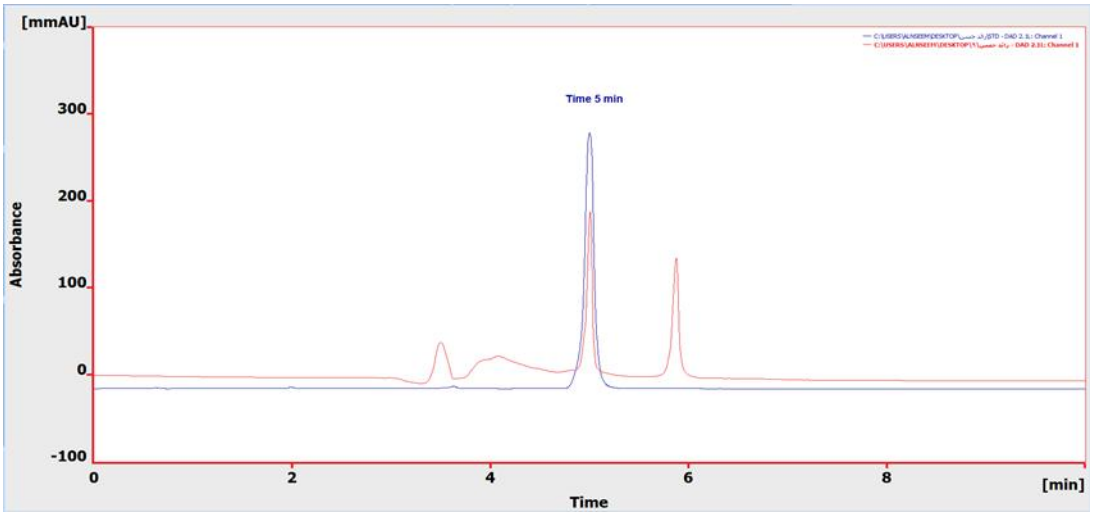

Figure 2. Determination of BPA after biodegradation by *Gloeocapsopsis crepidium* with red color matched to BPA standard with blue color at RT:5 min by HPLC

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) with least significant differences (LSD) to compare the concentrations of bisphenol used for bio-removal by the cyanobacterium studied, all treatments were performed in triplicate.

RESULTS AND DISCUSSION

Growth curve determination

Chlorophyll-a values were used to estimate the growth curve of *Gloeocapsopsis crepidium* (Figure 3). It was observed that the exponential phase started after day 4 as there was a steady increase in growth and continued until reaching stationary phase after day 10. However, the decline phase started after the 16th day and continued to decline until the death of the algae. The results were obtained at a light intensity of 60 µmol m^{-2} s^{-1}.

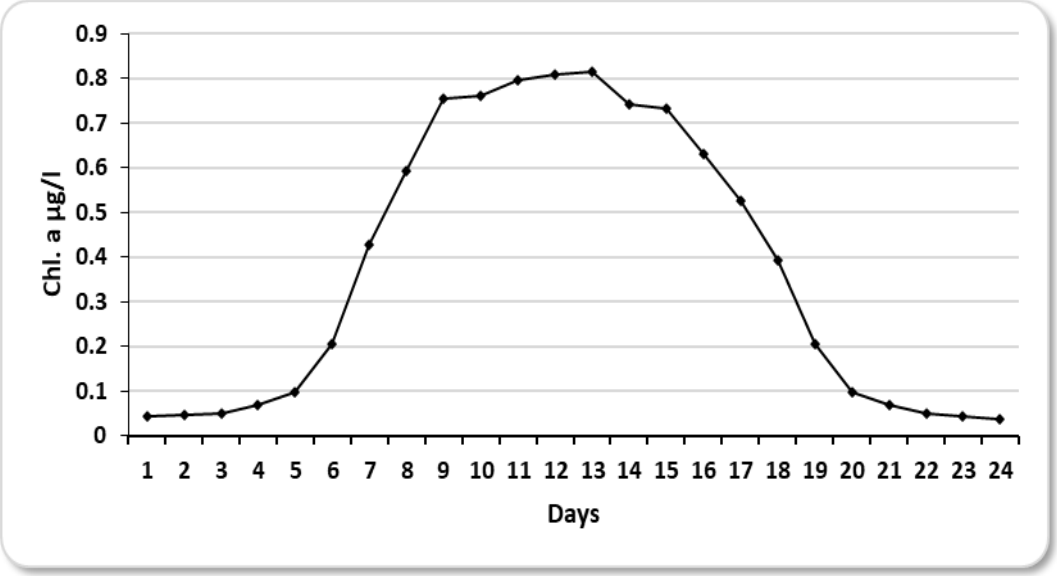

Figure 3. Growth curve of *Gloeocapsopsis crepidium* expressed as chlorophyll a values

Bisphenol Bioremoval

The results of the bioremoval of bisphenol A by the cyanobacterium *Gloeocapsopsis crepidium* exposed to the different concentrations of BPA (1, 5, 10, 20, 50, 75, 100) mg/l showed higher removal efficiency reached 100% at 1mg/l BPA with residual value 0 mg/l. In contrast, the highest residual value reached 20.52 at 100mg/l BPA with a removal percentage of 79.48% (Figure 4, Table 1). The results statistical analysis showed that significant differences at $p \leq 0.05$ (Table 1, Figure 4).

Table 1. Bioremoval and residual of BPA at different concentrations by *Gloeocapsopsis crepidium*

Bisphenol A mg/l	Residual Bisphenol A mg/l	Removal %
1	0±0 D	100
5	0.45±0.012 D	91
10	5.70±0.651 C	43
20	5.6±0.404 C	72
50	6.35±0.384 C	87.3
75	8.37±0.406 B	88.84
100	20.52±0.467 A	79.48
LSD	0.994	LSD

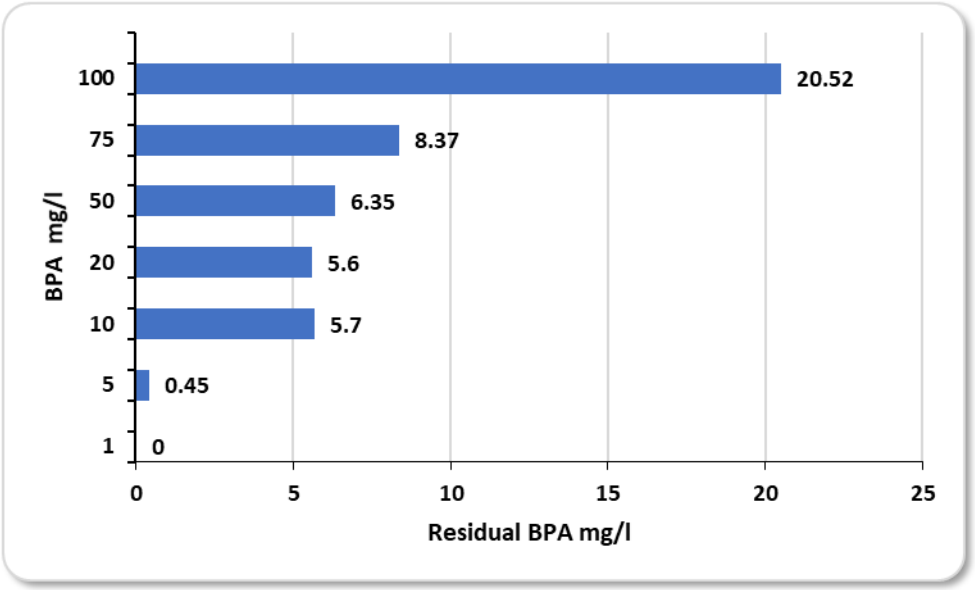

Figure 4. Bioremoval and residual of BPA at different concentrations *Gloeocapsopsis crepidium*

The results of this research were consistent with the findings of Zhang *et al*. (2019), who investigated the ability of algae to remove bisphenol from the aquatic environment using *Ulva prolifera* for phycoremediation of bisphenol in coastal waters during green tide bloom. The results showed that approximately 94.3% of the BPA could be rapidly removed by the live *U. prolifera*, while 2.5% of the BPA was removed by the dead biomass. Similar results were found in another study, when *Chlorella vulgaris* was used to bioremove BPA under both light and dark culture conditions, light promotes the growth of *Chlorella vulgaris* by increasing cell density, so the removal of BPA under light conditions was clearly more effective than under dark conditions (Wang *et al*., 2017). Likewise, *Picocystis sp*. was employed for the efficient removal of bisphenol A. The results showed a maximum BPA removal of 91.36%, with optimal culture conditions achieved at an initial BPA concentration of 10 mg/l (Ben Ali *et al*., 2021).

The results of the present study indicate that cyanobacterium *Gloeocapsopsis crepidium* have a high bisphenol removal rate from culture media, with a percentage of 100% at 1 mg/L, and that this removal rate tends to decrease with increasing BPA concentration, which may be due to the fact that algae can remove BPA from the medium in several ways, including binding to surface layers, accumulating internally, and metabolizing or transforming into other substances (Azizullah *et al*., 2022). Nakajima *et al*. (2007) mentioned that algae can metabolize bisphenols into glycosides and accumulate them. Algae may be able to remove bisphenol due to their enzymatic properties. It has also been proposed that algae may assist in the degradation of BPA by producing extracellular enzymes that aid in the degradation of BPA or by producing certain oxygen species that may cause BPA to photodegrade (Baghour, 2019; Otto *et al*., 2015).

Ji *et al*. (2014) also noted the ability of *Chlorella vulgaris* and *C. mexicana* to accumulate significantly higher amounts of BPA at concentrations of 25 and 50 mg/l compared to 1, 5 and 10 mg/L, suggesting that bioaccumulation of bisphenols in algae increases with increasing concentration. This was also confirmed by Guo *et al*. (2017) through the use of C^{14} labelled radioactive BPA, which decreased in the medium, while it increased in the cells of *Chlorella pyrenoidosa* over time. This is consistent with the results of the current study, as a high amount of bisphenol was removed and accumulated inside the cyanobacterium, especially at the higher concentrations of 50, 75 and 100 mg/l.

CONCLUSION

The results of the current study showed that algae are the ideal choice for removing pollutants from aquatic environments and demonstrated the ability of the cyanobacterium *Gloeocapsopsis crepidium* to remove bisphenol A with a high removal efficiency that reached 100% at 1mg/l, and the removal rate decreased with increasing concentration of bisphenol. This indicates that the algae were able to degrade or metabolize this compound and accumulate it in their bodies.

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