CULTIVATION OF *CHLORELLA VULGARIS* MICROALGAE IN WASTEWATER

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Abstract. In this work, the potential of Chlorella vulgaris microalgae for wastewater treatment was investigated. Parameters, such as pH, conductivity, COD, BOD, TOC and metals content were measured before and after the treatment of wastewater with *Chlorella vulgaris* microalgae. Chlorophyll a, chlorophyll b and the total amounts of carotenoids in microalgae biomass were determined using methanol as extraction solvent. A decreased trend of photosynthetic pigments was observed in the biomass after wastewater treatment, compared with the control biomass. Removal efficiencies up to 68.1% for Pb, 60.1% for Al, 53.6% for Zn, 51.7% for Cr, 51.6% for Cu and 40% for Cd were obtained. The BOD removal efficiency was 78.9%, while total organic carbon removal efficiency was 42% and COD removal efficiency was only 13.6%.

Keywords: biomass, heavy metals, microalgae, removal efficiency

INTRODUCTION

Environmental pollution is one of the major problems facing humanity, causing serious and irreparable damage to the natural ecosystems and human society. This issue has mainly been attributed to high concentrations of harmful substances that produce negative impacts to the natural habitats or for human health (Ukaogo et al., 2020). Anthropogenic activities, such as industrial, agricultural and domestic, generate large amounts of pollutants that are released into water, air and soil. Water pollution refers to contamination of water with organic and inorganic compounds (Wollmann et al., 2019). Thus, high concentrations of nitrates, phosphates, carbon compounds, lead, mercury and other heavy metals, microplastics and xenobiotics from agricultural runoff or industrial discharges are considered water pollutants (Sousa et al., 2018).

Reduction or removal of pollutants from wastewater often involves difficulties due to technical and economic considerations. Variables, such as types of contaminants or local conditions are also important in choosing the wastewater treatment technique (Arashiro et al., 2018, Wollmann et al., 2019). With conventional treatment technologies, biochemical oxygen demand, suspended solids, nutrients, coliform bacteria, are removed from wastewater (Abdel-Raouf et al., 2012). The treatment is carried out in several stages, but is not environmentally sustainable and requires considerable space and investment. The biological wastewater treatment process can be used as an alternative to conventional processes. Thus, by using microalgae for example, it is possible to obtain the reduction of organic material and removal of ammonium, nitrates and phosphate, a very expensive process with conventional technology. By using microalgae for wastewater treatment, pollution and toxicity will be reduced using natural, sustainable and economic resources (Sharma et al., 2020). Moreover, replacement of synthetic media, often expensive, with wastewaters, as a cheap resource, allows reducing the algal cultivation cost (Sharma et al., 2020). Regarding the uptake process of nutrients or metals by microalgae, various factors such as wastewater composition, environmental conditions, pH and presence of co-cations or algal species can have a significant influence (Shanab, et al., 2012).

Numerous studies have been reported on the use of algae for treating various types of wastewaters including those from municipal, industrial, agro-industrial or livestock sources (dairies, piggeries, wineries, slaughterhouses) (Higgins et al., 2018; Dourou et al., 2018). Thus, *Scenedesmus* sp. was found to be highly efficient for the removal of metals, such Pb, Cr, Zn and Cu from industrial wastewater (Ajayan and Kumar, 2015). Wang et al. reported a highly removal efficiency for the phosphorus (90%) and nitrogen (83%) in sewage wastewater by *Chlorella* sp. (Wang et al., 2010). Recently, was pointed out that *Leptolyngbya*- *Ochromonas* mixotrophic culture have the ability to remove organic and inorganic compounds from agro-industrial wastes and wastewaters (i.e. with removal rate of organic load of 94.91) simultaneously with a high lipid content obtained (Tsolcha et al., 2018).

In the present study, wastewater was tested as a growing medium for the freshwater microalgae *Chlorella vulgaris*. The main objectives were to investigate: the adaptation of the species to the wastewater medium and its capacity to reduce the metals concentration and other organic contaminants.

MATERIAL AND METHODS

1. Algal strain and growth media

Chlorella vulgaris was initially cultivated in 200 mL Erlenmeyer flasks for seven days using BG-11 medium containing 0.04 g/L K_2HPO_4, 0.075 g/L $MgSO_4{\cdot}7H_2O$, 0.036 g/L $CaCl_2{\cdot}2H_2O$, 0.006 g/L $C_6H_8O_7$, 0.006 g/L $(NH_4)_5Fe(C_6H_4O_7)_2$, 0.001 g/L EDTA, 0.02 g/L Na_2CO_3 and 1 mL/L of trace metal solution (2.86 g/L H_3BO_3, 1.81 g/L $MnCl_2{\cdot}4H_2O$, 0.222 g/L $ZnSO4{\cdot}7H_2O$, 0.39 g/L $NaMoO_4{\cdot}2H_2O$, 0.079 g/L $CuSO_4{\cdot}5H_2O$, 0.0494 g/L $Co(NO_3)_2{\cdot}6H_2O$) (Sharma et al., 2016). The cultures were continuously aerated and grown under artificial illumination (12/12 h day/night cycle) at intensity of 1200 lux at 25°C ± 1°C.

2. Experimental design

A volume of 40 mL of *Chlorella vulgaris* inoculum from primary culture was added to 200 mL of wastewater. Cultures were grown in wastewater for 10 days at 25 ± 1°C under an artificial illumination of 1200 lux for 12/12 h day/night cycle. The experiments were carried out in three replicates with control cultures (40 mL of *Chlorella vulgaris* inoculum was added to 200 mL of BG-11 medium).

3. Chemical analysis

In order to evaluate the ability of microalgae for pollutants removal, chemical analyses were performed: pH, conductivity, biochemical oxygen demand (BOD), total organic carbon (TOC), chemical oxygen demand (COD), metals content, chlorophyll a, b and carotenoids content. For these determinations, standardized methods and properly calibrated measuring instruments were used, before and after treatment with *Chlorella vulgaris.*

3.1 Determination of pH and conductivity (χ)

The pH and conductivity were determined at 20 ± 1°C using a Seven Excellence multiparameter, Mettler Toledo, Switzerland.

3.2 Determination of COD

COD was applied to determine the quantity of the oxygen needed to oxidize the organic matter from the wastewater. 0.4 g of mercury sulphate and 10 ml of 0.25 N potassium dichromate were added to 20 ml of wastewater sample. 30 ml of concentrated sulphuric acid was slowly added to the mixture. The mixture was heated at 150°C for 2 h into a reactor (Velp Scientifica). After cooling, the excess dichromate was titrated with 0.125 N ferrous ammonium sulfate.

3.3 Determination of BOD

BOD was determined in order to measure the amount of oxygen consumption, required for the biological decomposition of organic matter under aerobic condition in the aquatic environment at standardized time (5 day) and temperature (20°C). The wastewater was diluted with distilled aerated dilution water containing added nutrient salts in order to prevent the bacteria from depleting all the oxygen in the sample during 5 days of incubation. The samples were incubated at 20°C in the dark, in order to ensure that no additional oxygen is produced during photosynthesis. The sample's dissolved oxygen is measured before and after the incubation period using an oxygen meter WTW, INOLAB 740 electrode with StirrOxG sensor.

3.4 Determination of TOC

TOC analysis was conducted in order to measure the natural organic matter in the wastewater before and after the *Chlorella vulgaris* cultivation. TOC is a widely used parameter in order to characterize organic pollution in water bodies and to assess the environmental quality of aquatic systems. The TOC values were determined using a multi N/C 2100S Analytik Jena analyzer.

3.5 Determination of metal content

For metal content determination, a volume of 40 mL of wastewater was digested with 21 mL of 37% HCl and 7 mL of 69% HNO_3 and diluted to 50 mL. The metal concentrations of wastewater before and after treatment with *Chlorella vulgaris* were determined using ICP-MS spectrometry (ELAN DRC II, SCIEX, PerkinElmer, Toronto, Canada). The metal removal efficiency (%) was calculated using the following equation (1):

$$E(\%) = \frac{(C_o - C_e)}{C_0} . 100 \quad (1)$$

where, C_o and C_e are the initial and final heavy metal content (µg/L) (Mudyawabikwa et al., 2017).

3.6 Determination of chlorophyll and carotenoid content

The chlorophyll *a* (µg/mL), chlorophyll *b* (µg/mL) and carotenoid (µg/mL) concentration were determined using a volume of 5 mL of sample. The extractions were performed with methanol. The samples were heated to 70°C using a water bath (Memmert WNB 14) and centrifuged at 4500 rpm for ten min (Touloupakis et al., 2016). The absorbance (*A*) was measured at 470, 652.4 and 665.2 nm using Lambda 25 Perkin-Elmer UV/VIS spectrophotometer and the chlorophyll a, chlorophyll b and carotenoid concentration were calculated (Sumanta et al., 2014).

RESULTS AND DISCUSSION

1. Characteristics of wastewater

The chemical characteristics of wastewater were determined in order to evaluate the performance of microalgae *Chlorella vulgaris* for pollutants removal. The following parameters were measured: pH, conductivity, COD, BOD, TOC and metals content. The composition of wastewater consisted of: pH 7.7, 701.1 µS/cm, 162 mg/L COD, 35 mg/L BOD, 58 mg/L TOC, 1151.1 µg/L Cd, 14.8 µg/L Cr, 5142.8 µg/L Cu, 44.3 µg/L Ni, 87.8 µg/L Pb, 610.7 µg/L Zn, 250.0 µg/L Al, 3191.3 µg/L Si, 2438.8 µg/L K, 8927.5 µg/L Ca, 487.5 µg/L Fe. The pH of the culture medium increased after ten days of growth up to 8.4. An increase of pH suggests that the *Chlorella vulgaris* adapted to the culture medium and started to grow. Also, the conductivity of the wastewater reached a value of 1161 µS/cm after treatment with *Chlorella vulgaris.* The concentration of COD in the wastewater before treatment was 162 mg/L and decreased to 140 mg/L after treatment (13.6% removal efficiency). The initial BOD concentration was 35 mg/L and decreased considerably after ten days of treatment at 7.4 mg/L. Thus, a BOD removal efficiency of 78.9% was obtained after treatment, while the TOC removal efficiency was 42%.

2. Removal of metals from wastewater

High removal efficiencies were observed in case of the Pb (68.1%), Al (60.1%), Zn (53.6%), Cr (51.7%), Cu (51.6%) and Cd (40%) (Figure 1). Various types of microalgae exhibit different levels of tolerance to heavy metals. The microalgal biomass ability to sequester non-essential metals from the wastewater in the cell walls can be explained as a protective mechanism for limiting the metal toxicity (Shanab et al., 2012; Tüzün et al., 2005).

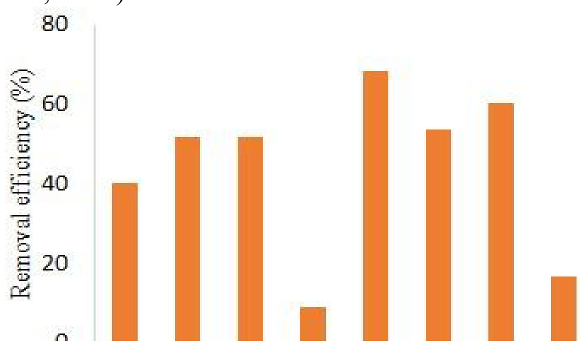

Fig. 1. Removal efficiency of metals after treatment with *Chlorella vulgaris*

The results showed that *Chlorella vulgaris* was able to remove a significant amount of a non-essential heavy metal, such as Pb and Cd (Figure 1). Fe, Si, Ni and Ca removal efficiencies were low, below 30%. The lowest removal efficiency of 0.5% was obtained in case of K. This can be explained by the organic matter content of the wastewater, which could have a strong influence on the adsorption of the metals on microalgal surfaces.

3. Determination of chlorophyll and carotenoid content

In order to evaluate the effect of wastewater composition on *Chlorella vulgaris* the content of photosynthetic pigments was determined. The obtained results are presented in Figure 2.

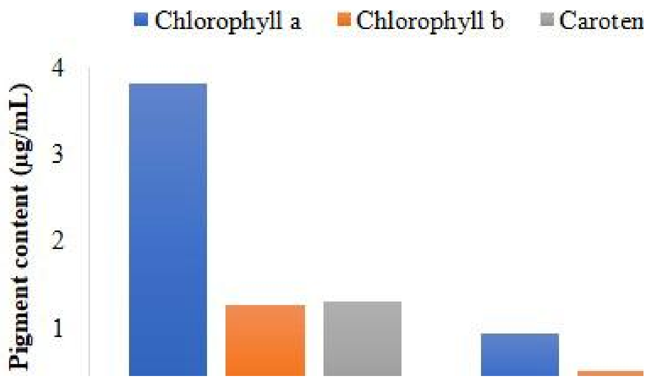

Fig. 2. Chlorophyll *a*, chlorophyll *b* and carotenoid content of *Chlorella vulgaris* biomass after wastewater treatment

Comparing the results between the control biomass and the one obtained after treatment, a decrease trend of photosynthetic pigments (chlorophyll *a*, *b* and carotenoid) was observed. The *Chlorella vulgaris* after the wastewater treatment reached a 4.1-fold smaller chlorophyll value compared to the control culture (Figure 2). In case of the chlorophyll *b* and total carotenoid, 2.5 and 3.6 -fold smaller values, respectively were obtained in comparison to that of control biomass.

CONCLUSIONS

The present study demonstrates the potential use of *Chlorella vulgaris* microalgae for wastewater treatment. The following series was depicted based on removal efficiencies of metals from wastewater after treatment with *Chlorella vulgaris*: Pb (68.1%) > Al (60.1%) > Zn (53.6%) > Cr (51.7%) > Cu (51.6%) > Cd (40%) > Fe (30.3%) > Si (16.7%) > Ni (8.7%) > Ca (7.1%) > K (0.5%). The results showed that the removal efficiency of the BOD was 78.9% while the COD was 13.6%. Further research will be carried out to find the optimum conditions for microalgae growth in wastewater.

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