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Removal of Pollutants from Waste Water Using *Dunaliella* Algae

HUDA A. QARI[1,2]* and IBRAHIM A. HASSAN[2,3]

[1]Faculty of Science, King Abdulaziz University, Jeddah, KSA.
[2]Centre of Excellence in Environmental Studies, King Abdulaziz University, Jeddah, KSA.
[3]Faculty of Science, Alexandria University, 21526 El Shatby, Alexandria. Egypt.
*Corresponding author: hagarai@kau.edu.sa, iagadallah@kau.edu.sa

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ABSTRACT

The toxicity and biosorption of heavy metals by the green unicellular flagellate *sp.* were investigated. We found that *Dunaliella* algae are able to biologically remove heavy metals from wastewater at concentration (about 85 mg/L). The *Dunaliella* cells were first immersed for seven days in wastewater samples collected from different sources in Jeddah, KSA, and their growth rates were monitored determined visibly at wavelength of 560 nm. It was observed that at the initial stage (0–12 hours) the adsorption rate was so rapid that 74% of the metal was biologically adsorbed. The maximum biosorption capacity of live *Dunaliella* was estimated to be 0.79 mg lead per 75 alga cells. Other elements were adsorbed at relatively lower rates Both toxicity and biosorption are very important in developing *Dunaliella* for the treatment of wastewater containing heavy metals. *Dunaliella* algae could be used as phytoemediators to decrease toxicity of heavy metal from polluted wastewater for human health.

Key words: Waste water; Heavy metal pollution; removal; adsorption; *Dunaliella* algae.

INTRODUCTION

The presence of heavy metals in the environment is of major concern because of their toxicity, bioaccumulating tendency, and threat to human life and the environment (Igwe & Abia, 2006). As human populations have expanded, Earth's atmosphere and natural waters have become dumps for agricultural and industrial wastes. Heavy metals are among the conservative pollutants that are nonbiodegradable (El-Nady & Atta, 1996; Walter *et al.*, 2011). As a result of this, their concentrations often exceed the permissible levels normally found in soil, water ways and sediments (Bhatnagar & Kumari, 2011). Hence, they find their way up the food pyramid. When they accumulate in the environment and in food chains, they can profoundly disrupt biological processes (Hassan & Basahi, 2013, Hassan *et al.*, 2013; 2014).

The chemistry and toxicology of these heavy metals are complex and interesting (Hassan *et al.*, 2014). Metals can be toxic to microbial population at sufficiently high concentrations. However, some metals such as silver, mercury, cadmium and copper are markedly more toxic even at very low levels (Puranik & Paknikar, 1997)

Remediation methods of the last half century have been largely unsuccessful[2]. Adsorptive removal of heavy metals from aqueous effluents which have received much attention in recent year is usually achieved by using activated carbon or activated alumina (Abdel-Raouf *et al*., 2012; Igwe & Abia, 2005, Igwe *et al*., 2005*a*;*b*; 2006) . Many other biosorbents of algal, fungal and bacteria biomass have been utilized (Mclean & Beveridge, 2001; Fedrickson *et al*., 2000; Vijayaraghan *et al*., 2005).

Bio-treatment with algae is particularly attractive because of their photosynthetic capabilities, converting solar energy into useful biomasses and incorporating nutrients such as nitrogen and phosphorus causing eutrophication (De la Nou & Basseres, 1989; De la Nou & De Pauw, 1988). Moreover, compared to physical and chemical treatment processes, algae based treatment can potentially achieve nutrient removal in a less expensive and ecologically safer way with the added benefits of resource recovery. Recently, Bhatnagar and Kumari (2013) stated that algae are significantly efficient in treating more than one problem at a time, which is not possible by conventional process of chemical treatment. The phycoremediation shows advantage over other chemical methods as the removal of algal mass from the treated effluents is easy and economic (De la Nou & De Pauw, 1988; Igwe & Abia, 2006; Bhatnagar & Kumari, 2013).

The aim of this study was to examine the possibility to biologically purify wastewater from heavy metals using the green unicellular flagellate *Dunaliella sp*.

MATERIALS AND METHODS

A pure culture of *Dunaliella sp*. was supplied by Department of Botany and Microbiology, Alexandria University. The algae were centrifuged (5000 rpm for 20 min) and then stored in liquid medium for 7 d at 20 °C under light (60 W white fluorescent lamp) (Vijayaraghan *et al*., 2005). Algae were grown in a standard growth medium (Ting *et al*., 1989, Chen 2005) with little modifications (Table 1).

Heavy Metal Sorption and Analysis

100 ml of wastewater was added to 250 ml flask containing 10 mg L^{-1}Dunaliella cells and were shaked at 25°C for 48 h. At the designed period of 12, 24, 36, 48, 60, 72, 84, 96, 108 and 120 h, 10 ml of the solution were collected for analysis.

Dunaliella in the solutions was removed by filtration and the filtrates were analyzed to determine the concentration of the remaining metal ions.

Concentrations of heavy metals (Cd, Pb, Ni, Cr, Zn and Cu) in water samples were determined before and after inoculating with algae using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) (Hassan & Basahi, 2013; Al- Dhaibani *et al*., 2013). All safety rules were applied (COST, 2008).

Data analysis

The sorption of metals onto microbial surface is described by the following model (Chen, 2005);

$$1/q = 1/(q_{max}\, bc) + 1/q_{max}$$

where c is the final metal concentration (mg L^{-1}), q is the metal uptake (mg/10^5 cells), b is the sorption binding constant (L/mg), q_{max} is the saturation capacity (mg/10^5 cells), from the slope and intercept of a $1/q$ vs $1/c$ linear plot such that q_{max} =intercept"1 and =intercept/slope.

Statistical analysis

Each treatment was made in 10 replicates to ensure statistical validity. One –way ANOVA was applied to log-transformed data (to ensure they were normally distributed) using STATGRAPHICS statistical Package (STAT. 4). The differences between means were analyzed by Student T- Test at the $P<0.05$ significance level.

RESULTS AND DISCUSSION

The analyses of heavy metal concentrations in wastewater samples are presented in Table 2.

Zinc (Zn) was found the most abundant element in wastewater (179.12 mg L^{-1}), while Ni was less abundant (19.14 mg L^{-1}). Other elements showed relatively high concentrations 34.87, 53.97, 62.17 and 80.25 mg L^{-1}, for Cr, Pb, Cu and Cd,

Table 1: Composition of growth medium

Constituents	Concentration (gL^{-1})
EDTA	0.10
K_2HPO_4	0.50
$NaNO_3$	3.10
NaCl	1.20
$NaHCO_3$	19.90
K_2SO_4	1.20
$FeSO_4 \cdot 7H_2O$	0.05
$MgSO_4 \cdot 7H_2O$	0.30
$CaCl_2 \cdot 2H_2O$	0.08

Table 2: Mean (+SD) heavy metal concentration ($mg\ L^{-1}$) in wastewater. (n = 10)

Element	Concentration
Cd	80.25 ± 6.76
Pb	53.97 ± 3.27
Ni	19.14 ±1.72
Cr	34.87 ± 4.98
Zn	179.12 ± 21.25
Cu	62.17 ± 7.04

respectively. The concentrations recorded are higher than those recommended by WHO[24]. Moreover levels recorded in our study were much higher that that recorded in wastewater in other semi-arid regions of the world such as Iran (Mansouri, & Ebrahimour, 2011; Qishlaqi *et al*., 2008), India (Shama *et al*., 2006; Vijayaraghan, 2005) and Jordan (Al-Khashman, O.A. (2013).

Figure 1 indicated that Dunaliella has removed 95% of Zn and Cd after 108 hours, and 90% of Cu after 60 hours of incubation. Moreover, 93% of Pb, Ni and Cr were removed after 36 hours of incubation. This indicates that biosorption efficiency towards Pb, Cr and Ni is higher than other elements (Puranik & Paknikar, 2011).

The dependence of heavy metals biosorption by Dunaliella on time is shown in Fig.2. Generally, it is reported that the uptake of metal ions can be divided into two stages: rapid and slow stage (Walterv *et al*., 2011). In the 'rapid' stage, the metal ions are adsorbed onto the surface of microorganism. In the 'slow' stage, the metal ions transport across the cell membrane into the cytoplasm.

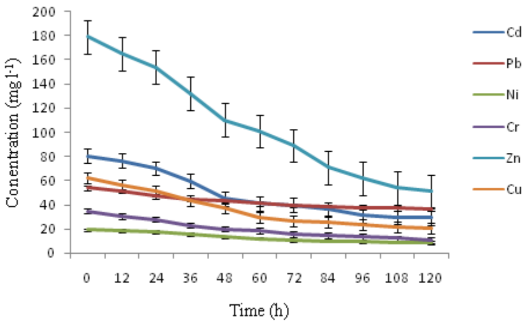

Fig. 1: The effect of *Dunaliella* on heavy removal (at 25 °C, n = 10 ± 3)

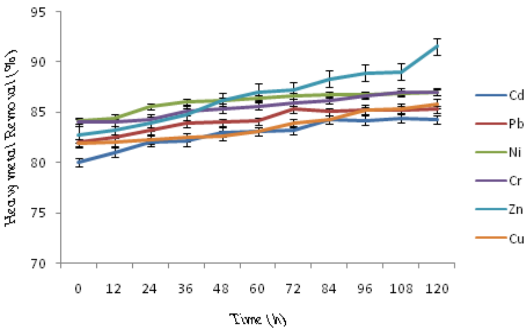

Fig. 2: Time-dependence of heavy metal biosorption (n = 10 ± 3)

It was reported that lead phosphate precipitated on the cell wall and inside the cell of cyanobacteria (*Anabaena cylindrical*). Their results confirmed a very fast uptake in the cell envelope and then a longer uptake period inside the cell envelope and then a longer uptake period inside the cell (Sa'idi, 2010).

In this study, rapid biosorption was observed at the beginning (0–12 min, with 74% of metal adsorbed), then reached the equilibrium within 24 h with 95% of metal ions adsorbed. Rangsayatorn *et al*. (2004) and Horikoshi *et al*. (1979) reported that cadmium was rapidly adsorbed by *S. platensis* during the first 5 min and by *C. regularis* within 6 min, respectively. Such rapid uptake of heavy metals by living cells is very significant when the cells are used in bioremediation process (De la Nou, 1989; WHO, 2006; Abdel-Raouf *et al*., 2012).

In conclusion, the results of the present study showed that *Dunaliella* has substantial for phytoremediation of heavy metals from the wastewater. However, further study is required before considering this plant Species for phytoremediation

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