1871

BIO-TREATMENT OF WASTEWATER USING MIXED ALGAL CULTURES

[149]

Olfat Salem[1*], Hammad[2] A., Ismail[3] S. and El-Gindy[4] A.

1- Botany and Microbiology Dept., Fac. of Sci., Helwan Univ., Cairo, Egypt
2- Agric. Microbiology Dept., Fac. of Agric., Minia Univ., Minia, Egypt
3- Sanitary & Environmental Engineering, Public Works Dept., Fac. of Engineering, Ain Shams Univ., Cairo, Egypt
4- Agric. Engineering Dept., Fac. of Agric., Ain Shams Univ., P.O. Box 68, Hadayek Shoubra 11241, Cairo, Egypt

*Corresponding author: olfatabdelhamed@yahoo.com

ABSTRACT

This study was carried out to evaluate the efficiency of mixed algal cultures for wastewater treatment. Free and alginate-immobilized forms of mixed algal culture were used. The highest removal percentages of biological oxygen demand (BOD) were 86.4% and 71.2 % after 32 hrs and 48 hrs. in case of free and immobilized cells, respectively. Moreover, the highest values of chemical oxygen demand (COD) removal were 83.74% and 59.71% after 32 hrs and 48 hrs for free and immobilized cells, respectively. Treatment with free cells showed removal values for total dissolved salts (TDS), phosphorus and NH3-N were 20.5%, 34.6% and 43.8% respectively after 32 hours. While in case of immobilized cells the highest removal values were18.4%, 34% and 58.5%, for TDS, Phosphorus and NH3-N, respectively recorded after 48 hrs. Generally, concentration of heavy metals decreased due to treatment with algal free and immobilized cells. As a result of treatment with algal free and immobilized cells, 100% and 95.9% of Cu wastewater content was removed after 8 hrs and 48 hrs, respectively. Moreover, 96.2% and 98.1% of Fe was removed after 32 and 48 hours due to application of free and immobilized cells, respectively. Furthermore, high removal percentages for Mn, Pb and Zn were recorded due to application of algae. Accordingly, treatment of wastewater with mixed algal free or immobilized cells is a fruitful method to produce an effluent of high quality to be used for irrigation. Whereas, the algal free cells were found to be more efficient than the immobilized ones.

1- INTRODUCTION

Water pollution is the cause of uncontrolled release of domestic, industrial and agriculture refuse (wastewater) into the environment. Microalgae cultures offer an environmentally friend solution for wastewater treatment due to the ability of microalgae to use inorganic nitrogen and phosphorus for their growth and also, for their capacity to remove heavy metals, as well as some toxic organic compounds. Microalgae show advantages in many applications such as renewable energy and biopharmaceutical industries, it is renewable, sustainable, and economical sources of biofuels, bioactive medicinal products, and food ingredients **(Khan et al 2018). Palmer 1969** listed the algae in the order of their tolerance to organic pollutants as reported by 165 authors. The list was compiled for 60 genera and 80 species. The most tolerant eight genera were found to be, *Oscillatoria*, *Chlamydomonas*, *Euglena*, *Scenedesmus*, *Chlorella*, *Nitzschia*, *Navicula* and *Stigeoclonium*.

The microalgae immobilization on Ca-alginate are commonly used in treatments of wastewater for removal of nitrogen, phosphate, organic carbons, pharmaceutical compounds, toxic textile dye compounds and heavy metals **(Moreno Garrido, 2008; El-Sheekh et al 2016; Wang et al 2016 and Salam et al 2017)**. Microorganism immobilization is a common approach using consortia of bacteria and algae to purification of hazardous contaminants. In this purification system, microalgae could produce oxygen that would be benefit for bacteria

to biodegrade hazardous pollutants **(Muñoz and Guieysse, 2006)**. Immobilized cells are characterized by its stability because the cells are entrapped in the matrix and protected from toxic compounds in wastewater; therefore, they have a higher efficiency for the removal of nutrients **(Hernandez et al 2006 and El-Sheekh et al 2016),** dye **(Huang et al 2000 and Revathi et al 2017)**, and heavy metals **(Tam et al 1998; El-Sheekh and Mahmoud, 2017)** than free cells. Many benefits support the potential of microalgae-based treatments, such as low operating costs, ability to reduce atmospheric CO_2 level and/or capture of CO_2 from industrial flue gases, and production of valuable end bio-products **(Oilgae, 2010)**. Free and immobilized cultures of *Spirulina maxima* were used for swine waste treatment and the study of the best dilution of the wastewater for maximum biomass production and for removal of (COD), ammonia and phosphorous to the microalgae has been studied by **Canizares et al 1993**.

This study was carried out to evaluate the efficiency of mixed algal cultures with different forms such as free cells and alginate immobilized cells of this mixture as a bio - treatment and bio-sorbent of heavy metals from wastewater, the use of these microorganisms for wastewater treatment as alternative for chemical and physical treatments is highly recommended, because it is less expensive and environmentally friendly.

2- MATERIAL AND METHODS

2.1- Collection of wastewaters

Source of sample

Wastewater samples were collected after primary treatment from Sarpium forest site-Ismailia Governorate, Ismailia, Egypt (October 2017). It is the site where the sustainable forestry in desert lands of Egypt using treated sewage water project (Implemented by Ain Shams Univ. and funded by Science and Technology development fund-STDF (18660) is conducted. The sample was transferred to the central lab. of wastewater and industrial wastes, water and waste water company canal provinces for analyses of chemical and physical parameters.

2.2- Analytical Methods

The wastewater was analyzed for pH, biological oxygen demand (BOD), chemical oxygen demand (COD), phosphate and nitrate before and after algal treatments using the standard techniques **(APHA, 1998)**.

2.3- Source of mixed algal cultures

The mixed algal cultures was used in this study were kindly supplied by algae lab –Faculty of Science-Helwan University, Helwan-Egypt.

2.4- Culturing characterization

The different 4 algal species used in this study fall under two algal divisions Chlorophyta and Cyanophyta (Commercial product, under national registration) each type was grown in BG11 medium under (16 h light \ 8h dark) at 28±2°C and 30 μmol photon m^2 s^{-1} light intensity. The different algal species were harvested at their exponential phase of growth which is 15th day and the mixed algal cultures were prepared.

2.5- Experimental design

2.5.1- Preparation of mixed algal cultures in free cells inoculums

All of different algal species was grown separately in liquid BG11 medium for 15 days under the optimum growth conditions. Then liquid cultures were mixed in equal proportions.

2.5.2-Preparation of mixed algal cultures in alginate immobilized inoculums

One hundred ml of algal liquid cultures mixture were added to an equal volume of a sterile solution of sodium alginate (2% w/v). The mixture was added drop-wise into 200 ml of 2% $CaCl_2$ sterile solution using a sterilized Pasteur pipette. Beads of approximately 2 mm in diameter were obtained and hardened in 2% $CaCl_2$ solution for 2 hrs. The beads were then rinsed with sterilized water and maintained at 4°C. All steps were conducted under aseptic conditions **(Marei and Elmaghraby, 2016)**.

2.5.3-Experimental design and treatments

The un-inoculated wastewater (control) compare with the treatments treated in photo-bioreactor of 60 L volume capacity and made of Perspex column with a thermostat and an air diffuser. The temperature of the reactor was adjusted at 25 ± 2°C in each treatment.

2.5.3.1- First photo-bioreactor cycle

In the treatments inoculated with free cells(normal mobilized cells), 1400 ml of the prepared mixed algal cultures in free cells inocula were added to 28 liters of wastewater. The experi-

ments were carried out in photo-bioreactor (it has a working volume of 30 liters and it consisted of a glass column illuminated with fluorescent light of 2400 Lux).The experiment was conducted under controlled conditions (Temp 25± 2º C) for 72 hours and the samples were collected (three replicates)at intervals of 4 hrs. up to the end of the cycle. Three for each treatment were involved. Algal cells were removed from the samples by filtration throw filter membrane. The filtrated samples were kept at 4ºC,

2.5.3.2- Second photo-bioreactor cycle

In the second cycle of this study fixed weight of immobilized mixed algal cells was added to the sewage wastewater in the photo-bioreactor. The duration was 72 hrs. Samples were collected at intervals of 4 hrs. Algal beads were removed from the samples by filtration. The filtrated samples were kept as previously mentioned at 4ºC.

The filtrates from first and second cycles subjected to physico-chemical analyses using standard methods of Biochemical analysis (APHA, 1998) at the Central Lab. of Wastewater and Industrial Wastes, Water and Wastewater Company Canal Provinces

2.6- Wastewater characteristics

According to APHA, 1998, The wastewater collected from Sarpium forest site-Ismailia Governorate, was filtered and subjected to physico-chemical analyses using standard methods Biochemical analysis and the parameters measured are listed in the following **Table (1)**.

2.7- Statistical analysis

Data were statistically analyzed according to Gomez and Gomez, 1976 using Honesty Significant Difference (H.S.D) at 5%. This test was equal Tukey test for mean separation using two ways analysis of variance with interaction.

3- RESULTS AND DISCUSSION

3.1-Physical and chemical pollutants

Data presented in **(Table 2)** and illustrated by **Fig. (1 and 2)** indicated that due to inoculation of wastewater with mixed algal cultures in free cells form the, BOD value reduced from 273 mg/l to 36.77 mg/l after 32 hours. This reduction represents the highest removal percentage (86.4%)

along the experimental period using free cells inoculum. Whereas, when using mixed algal cultures in immobilized cells, the highest removal percentage (71.2 %) was achieved after 48 hours, since, the immobilized cells reduced BOD from 273 mg/l to 77.8 mg/l after 48 hours. These results agree with those obtained by El-Bestawy (2008) who recorded 89.29% removal of BOD by using mixture of *Anabaena variabilis*, *Anabaena oryzae* and *Tolypothrix ceylonica*in. On the other side, the obtained BOD removal values in this study via either algal free or immobilized cells inocula were higher than those obtained by **Ganapathy et al (2011)** who recorded 53.5% reduction in BOD from distilleries effluent in 30 days by *Nostoc muscorum*. *Phormidium tenue* removed 17.6% of BOD from paper mill effluent in 20 days.**(Nagasathya and Thajuddin, 2008)** Moreover, the highest removal percentage (83.74%) of COD from wastewater by mixed algal cultures in free cells inoculum was recorded after 32 hours. **(Table 2)**, *i.e.* application of algal free cells mixture inoculum to wastewater resulted in reduction of COD from 351.67 mg/l to 57.17 mg/l after 32 hours. Similar results 73.68% COD removal percentage with *Anabaena oryzae* **(El-Bestawy, 2008)**. Furthermore, the removal efficiency of COD was 20-57.1% obtained by using cyanobacteria (*N. muscorum* or *A. subcylindrica* and mixed culture of both) **(El Sheekh et al 2014)**.

The highest removal percentage of TDS (20.5%) in wastewater treated with mixed algal cultures in free cells form was achieved after 32 hours, while in case of treatment with immobilized cells the highest removal percentage 18.4% was recorded after 48 hours. The removal percentage of TDS from sewage water ranged between 4.4-23.3% due to using (*N. muscorum* or *A. subcylindrica* and mixed culture of both) **(El-Sheekh et al 2014)**. The turbidity of wastewater also decreased with treatment by mixed algal cultures in free cells from 21 to 19.3 after 72 hours, in case of mixed algal cultures in immobilized cells after 8 hours it decreased from21 to18.23 these results are lower than 40% to 96.4% obtained by *N.muscorum* or *A. subcylindrica* and mixed culture of both used for wastewater treatment **(El-Sheekh et al 2014)**.

Phosphorus in untreated wastewater was found to be 3.47 mg/l, this value reduced to 2.27 mg/l after 32 hours which represents the highest removal percentage (34.6%) for mixed algal cultures in free cells form and 34% in case of mixed algal cultures in Immobilized cells form after 48 hours. Four freshwater green microalgae species, (*Chlamydomonas reinhardtii, Scenedesmus obliquus,*

Table 1. The biochemical and physical characters of the sewage wastewater sample without any treatments (control) represented by means ±SD

Parameters	Unit (mg/l. wastewater sample)	Parameters	Unit (mg/l. wastewater sample)
BOD	270±2.64	HCO_3	152.7±1.18
COD	351.67±2.12	Total alkaline	250.7±1.25
Turbidity (NTU)	21.0±0.36	Al	0.00
Ammonia Nitrogen	26.1±0.26	B	0.00
Phosphate	3.47±0.23	Cu	0.073
TDS	700±2.08	Fe	0.266
pH	7.5±0.17	Mn	0.366
Cl	191.3±1.7	Mo	0.003
Ca	85.77±0.76	Ni	0.001
Mg	17.02±0.22	Pb	0.073
Na	146.2±1.22	V	0.0
K	16.89±0.12	Zn	0.076
SO_4	149.5±1.41	Cd	0.00

Table 2. The concentration (mg/ l) of physical and chemical pollutants after treatment with mixed algal compound in free and Immobilized cells

Treatments Time (hrs)	BOD Free cells	BOD Immobilized cells	COD Free cells	COD Immobilized cells	P Free cells	P Immobilized cells	NH₄ Free cells	NH₄ Immobilized cells	TDS Free cells	TDS Immobilized cells	Turbidity Free cells	Turbidity Immobilized cells
0 (control)	270.00ᵃ	270.00ᵃ	351.67ᵃ	351.67ᵃ	3.47ᵃ	3.47ᵃ	26.10ᵃ	26.10ᵃ	700.00ᵃ	700.00ᵃ	21.00ᵃ	21.00ᵃ
4	55.00ᵉᶠ	98.67ᵇ	81.33ᶠᵍ	226.33ᵇ	2.63ᵃᵇᶜ	3.20ᵃᵇ	22.20ᶜᵈᵉᶠᵍ	24.13ᵇᶜ	608.00ᵇ	632.00ᵇ	20.33ᵃᵇ	18.57ᵃᵇ
8	47.23ᵉᶠ	95.67ᵇᶜ	76.67ᶠᵍ	190.67ᶜ	2.63ᵃᵇᶜ	3.13ᵃᵇᶜ	22.00ᵇᶜᵈᵉᶠ	23.90ᵇᶜᵈ	593.00ᵇᶜᵈᵉᶠ	607.67ᵇᶜᵈᵉ	20.33ᵃᵇ	**18.23ᵃᵇ**
12	45.27ᶠ	94.17ᵇ	76.00ᶠᵍ	178.67ᶜᵈ	2.53ᵃᵇ	3.10ᵃᵇᶜ	22.00ᵇᶜᵈᵉᶠ	23.63ᵇᶜᵈ	591.00ᵇᶜᵈᵉᶠ	600.00ᵇᶜᵈᵉᶠ	20.35ᵃᵇ	**18.23ᵃᵇ**
24	42.27ᶠ	93.47ᵇᶜ	74.33ᶠᵍ	161.00ᶜᵈᵉ	2.45ᵃᵇ	2.93ᵃᵇᶜ	20.03ᶜᵈᵉᶠᵍʰ	21.76ᵇᶜᵈᵉ	589.00ᵇᶜᵉᶠ	598.33ᵇᶜᵈᵉᶠ	20.37ᵃᵇ	18.33ᵃᵇ
28	40.57ᶠ	93.57ᵇᶜ	70.00ᵍ	145.67ᵉ	2.47ᵃᶜ	2.47ᵇᶜ	19.40ᶜᵈᵉᶠᵍʰ	21.60ᵇᶜᵈᵉ	581.67ᶜᵈᵉᶠ	598.00ᵇᶜᵈᵉᶠ	20.41ᵃᵇ	18.33ᵃᵇ
32	**36.77ᶠ**	91.40ᵇᶜ	**57.17ᵍ**	143.67ᵉ	**2.27ᶜ**	2.43ᵇᶜ	**14.67ʰⁱʲᵏ**	18.87ᶜᵈᵉᶠᵍʰʲ	**556.33ᶠ**	596.30ᵇᶜᵈᵉᶠ	20.68ᵃᵇ	18.37ᵃᵇ
36	38.47ᶠ	90.93ᵇᶜ	68.33ᵍ	142.33ᵉ	2.43ᵇᶜ	2.43ᵇᶜ	14.90ⁱᵏ	18.70ᵈᵉᶠᵍʰʲ	571.33ᵈᵉᶠ	596.00ᵇᶜᵈᵉᶠ	20.67ᵃᵇ	19.30ᵃᵇ
48	38.43ᶠ	**77.80ᶜᵈ**	69.61ᵍ	141.67ᵉ	2.45ᵃᵇ	2.29ᶜ	14.90ⁱᵏ	10.83ᵏ	574.67ᵈᵉᶠ	**571.00ᵈᵉᶠ**	20.44ᵃᵇ	24.10ᵇ
52	39.48ᶠ	83.63ᵇᶜᵈ	69.00ᵍ	143.33ᵉ	2.63ᵃᵇᶜ	2.70ᵃᵇᶜ	15.23ⁱʲᵏ	13.63ʲᵏ	598.00ᵇᶜᵈᵉᶠ	571.67ᵈᵉᶠ	19.97ᵃᵇ	23.51ᵃᵇ
56	40.43ᶠ	87.67ᵇᶜ	74.00ᶠᵍ	150.00ᵈᵉ	2.67ᵃᵇᶜ	2.70ᵃᵇᶜ	15.24ⁱʲᵏ	13.90ⁱᵏ	596.33ᵇᶜᵈᵉᶠ	593.00ᵇᶜᵈᵉᶠ	19.81ᵃᵇ	22.35ᵃᵇ
60	41.43ᶠ	95.67ᵇᶜ	78.0ᶠᵍ	165.67ᶜᵈᵉ	2.70ᵃᵇᶜ	2.87ᵃᵇᶜ	16.03ᵉᶠᵍʰⁱʲ	16.00ᶠᵍʰⁱʲᵏ	596.50ᵇᶜᵈᵉᶠ	624.00ᵇᶜ	19.33ᵃᵇ	19.95ᵃᵇ
72	65.28ᵈᵉ	95.33ᵇᶜ	104.33ᶠ	177.33ᶜᵈ	2.70ᵃᵇᶜ	2.93ᵃᵇᶜ	16.83ᵉᶠᵍʰⁱʲ	16.83ᵃ	610.33ᵇᶜᵈᵉ	630.67ᵇ	**19.30ᵃᵇ**	19.98ᵃᵇ

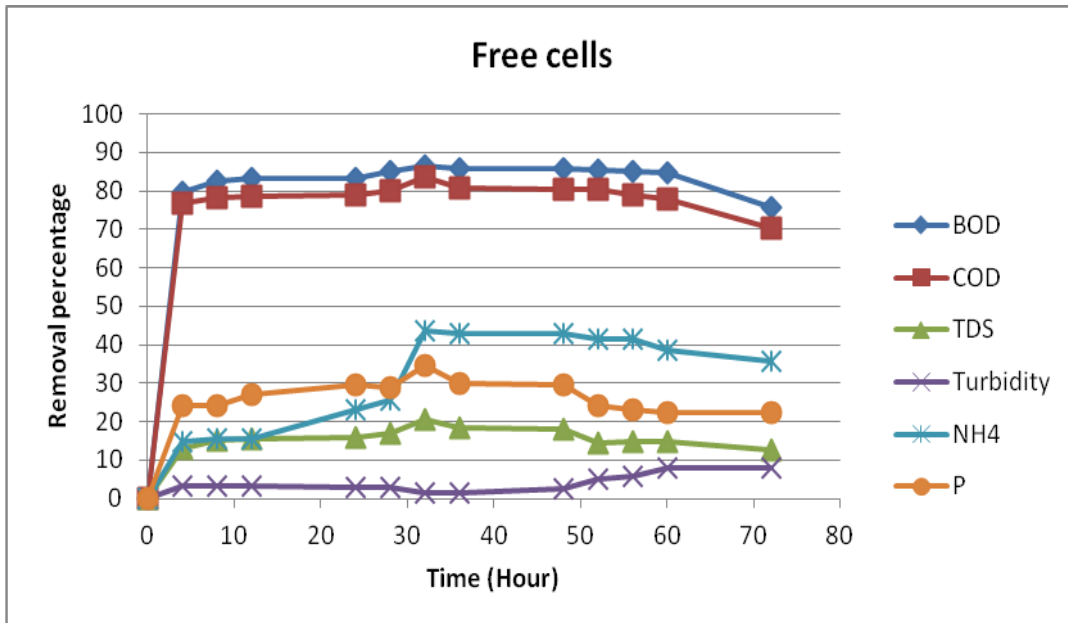The values having different letters are significantly different at p< 0.05

Fig. 1. Removal percentage of physical and chemical pollutants from wastewater treated with mixed algal cultures in free cells form
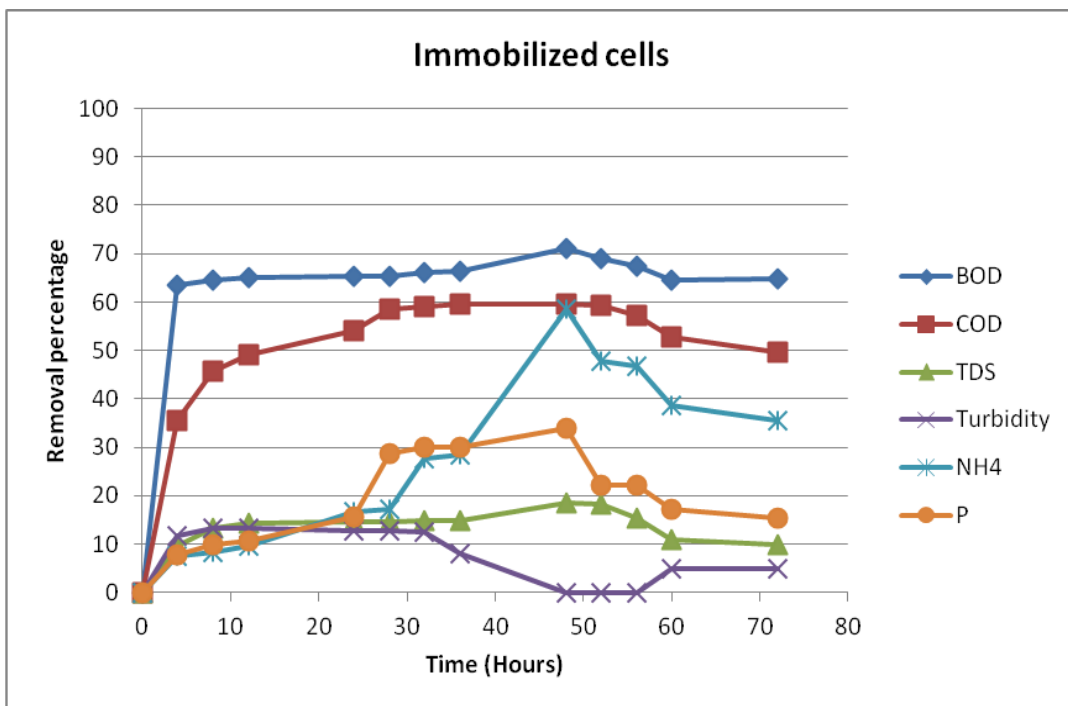

Fig. 2. Removal percentage of physical and chemical pollutants from wastewater treated with mixed algal cultures in Immobilized cells form

Chlorella pyrenoidosa and *Chlorella vulgaris*) used for 7 days for wastewater treatment 67.5–82.2% of Total phosphorus was removed **(Guang et al 2014)**. Phosphorus removal from wastewater recorded 20.8-95% as a result of treatment with *N. muscorum* or *A. subcylindrica* and mixed culture of both **(El-Sheekh et al 2014)**.

The concentration of NH3-N in untreated wastewater was found to be 26.1 mg/l, this value reduced to 14.67 mg/l, after 32 hours which represents the highest removal percentage (43.8%) due to application of mixed algal cultures in free cells form and 58.5% in case of mixed algal cultures in Immobilized cells form after 48hrs. Ruiz-Marin et al. (2010) reported that the microalgae *C. vulgaris* and *S. obliquus* showed preferences for ammonium to any other form of nitrogen present in wastewater. Ammonia nitrogen elimination efficiency by cyanobacterial system was 20.9-96% **(El-Sheekh et al 2014)**.

3.2-Heavy metals

Data presented in **Table (3)** and illustrated by **Fig. (3) and (4)** indicated that the concentration of heavy metals in general decreased due to treatment with mixed algal cultures in free and immobilized cells forms. Concentration of Cu in untreated wastewater (control) was estimated to be 0.073 mg/l and decreased to zero mg/l as a result of treatment with mixed algal cultures in free cells after 8 hours and still zero up to 48 hours and increased again. The recorded increase of Cu after 48 hours may be due to the decay of algal cells and release their Cu contents. On the other hand the mixed algal cultures in immobilized cells decreased Cu concentration in wastewater from 0.073 mg/l to 0.003 mg/l (95.9% removal) after 48 hours. **Alison et al (2014)** showed that *Chlorella vulgaris, Spirulina maxima,* and a naturally growing algae sample found in the wastewater from a wastewater treatment plant (containing *Synechocystis* sp. (dominant) and *Chlorella* sp. (common) and a few cells of *Scenedesmus* sp.) removed up to 81.7% of the copper after 10 days.

The concentration of Fe in untreated wastewater (control) was found to be 0.266 mg/l, and 96.2% removal percentage was achieved after 32 hours due to application of mixed algal cultures

in free cells. Whereas, the mixed algal cultures in immobilized cells decreased Fe concentration and the highest percentage of removal (98.1%) was recorded after 48 hours. **Noor Maisara et al (2015)** used *Scendesmus* sp. in waste water treatment and the highest percentage of Fe removal was 65.76%.

Mn concentration in untreated wastewater (control) was 0.366 mg/l, as a result of inoculation with the mixed algal cultures in free cells, 99.4% of Mn content was removed after 32 hours. Whereas, in case of using the immobilized cells 97.8% of Mn content was removed after 48 hours.

Concentration of Pb in untreated wastewater (control) was found to be 0.073 mg/l, 98.6% and 89.0% of Pb content was removed after 8 and 48 hours using mixed algal cultures in free and immobilized cells, respectively. The removal percentages of Pb in this study were higher those recorded by Rajiv and Dinesh (2010) who stated that *Chlorella* sp. causes lead removal up to 66.3%.

Zn content in untreated wastewater (control) was estimated to be 0.076 mg/l, due to application of mixed algal cultures infree and immobilized cells, Zn content was removed by 84.2% and 97.37% after 32 and 48 hours, respectively. Rajiv and Dinesh (2010) showed that *Chlorella* sp. removed 60-70%, of Zn. Using microalgae would not only improve the overall wastewater treatment but would also generate biomass that has high energy value.

In our results Pb and Cu were removed faster than Zn, these results was compatible with Qari and Hassan (2014) which found 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.

Generally, concentrations of heavy metals in wastewater were decreased due to treatment with mixed algal cultures infree and immobilized cells. Whereas, in wastewater treatment the mixed algal cultures infree cells form were found to be more efficient than the immobilized one, this may be due to presence of mixture algal cells inside alginate beads which may reduce the transmitted light to the immobilized cells and hence growth and activity of algal cells could be affected negatively.

Table 3. The concentration (mg/l) of Heavy metals after treatment with mixed algal cultures in free and Im-mobilized cells

Treatments Time (hrs)	Cu		Fe		Mn		Pb		Zn	
	Free cells	Immobilized cells	Free cells	Immobilized cells	Free cells	Immobilized cells	Free cells	Immobilized cells	Free cells	Immobilized cells
0 control	0.073^b	0.073^b	0.266^a	0.266^a	0.366^a	0.366^a	0.073^a	0.073^a	0.076^{bcdef}	0.076^{bcdef}
4	0.001^c	0.010^c	0.046^b	0.046^b	0.217^{abcd}	0.333^{ab}	0.007^a	0.073^a	0.063^{bcdef}	0.063^{bcdef}
8	0.000^c	0.006^c	0.023^b	0.040^b	0.167^{cdefg}	0.233^{abc}	0.001^a	0.050^a	0.058^{bcdef}	0.033^{bcdef}
12	0.000^c	0.006^c	0.023^b	0.033^b	0.133^{cdefg}	0.190^{bcde}	0.001	0.050^a	0.16^{ef}	0.023^{cdef}
24	0.000^c	0.006^c	0.030^b	0.020^b	0.120^{cdef}	0.073^{defgh}	0.001^a	0.030^a	0.013^{ef}	0.020^{def}
28	0.000^c	0.005^c	0.020^b	0.017^b	0.010^h	0.027^{fgh}	0.001^a	0.015^a	0.013^{ef}	0.17^{ef}
32	0.000^c	0.005^c	0.010^b	0.007^b	0.002^h	0.027^{fgh}	0.001^a	0.020^a	0.012^{ef}	0.005^f
36	0.000^c	0.005^c	0.020^b	0.008^b	0.003^h	0.018^h	0.001^a	0.030^a	0.061^{bcdef}	0.005^f
48	0.000^c	0.003^c	0.023^b	0.005^b	0.004^h	0.008^h	0.001^a	0.008^a	0.087^{bcde}	0.002^f
52	0.010^c	0.006^c	0.020^b	0.007^b	0.005^h	0.009^h	0.001^a	0.009^a	0.093^{abcd}	0.014^{ef}
56	0.020^c	0.007^c	0.020^b	0.013^b	0.008^h	0.018^h	0.001^a	0.035^a	0.096^{abc}	0.033^{bcdef}
60	0.040^c	0.025^c	0.023^b	0.016^b	0.010^h	0.027^{fgh}	0.001^a	0.037^a	0.100^{ab}	0.047^{bcdef}
72	0.043^c	0.035^c	0.023^b	0.030^b	0.047^{efgh}	0.029^{fgh}	0.001^a	0.041^a	0.167^a	0.063^{bcdef}

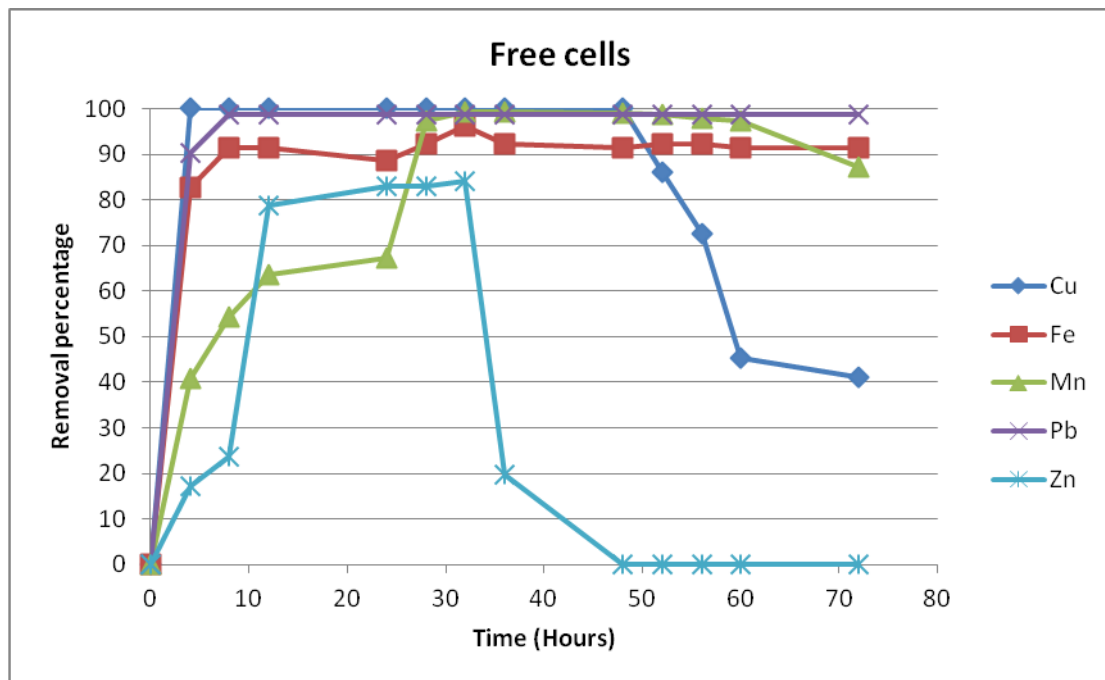The values having different letters are significantly different at $p < 0.05$

Fig. 3. Removal percentage of heavy metals from wastewater treated with mixed algal cultures in free cells form
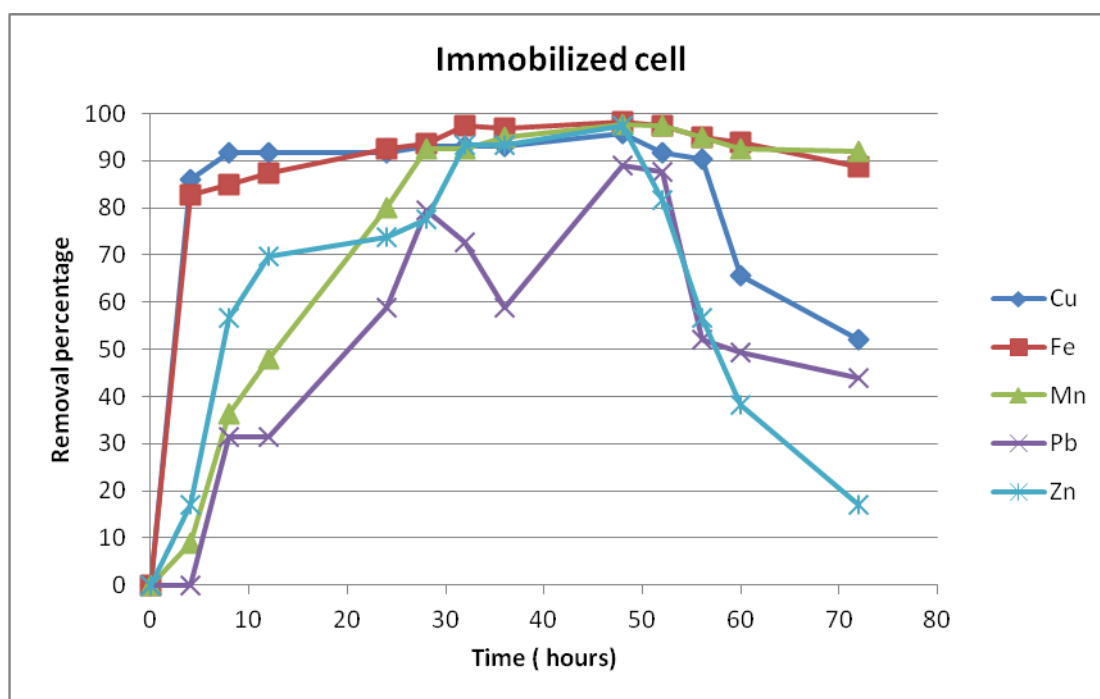
Fig. 4. Removal percentage of heavy metals from wastewater treated with mixed algal cultures in immobilized cells form

4- CONCLUSION

Algae can be used in wastewater treatment for a range of purposes, including; reduction of BOD, removal of N and P and removal of heavy metals. On the basis of the obtained results it can be concluded that the mixed algal cultures were found to be of high efficiency in wastewater as a bio- treatment and bio-sorbent of heavy metals from wastewater .The immobilized systems could facilitate the separation of the biomass from the treated wastewater but the free mixture of algal cells is highly recommended due to its efficiency in wastewater treatment by produce an effluent of high quality to be used for irrigation.

5- ACKNOWLEDGEMENT

REFERENCES

Alison C., Hamidreza S. and McBean E.D. 2014. Heavy metal removal (Copper and Zinc) in secondary effluent from wastewater treatment plants by microalgae. **ACS Sustainable Chem. Eng., 2(2), 130-137.**

American Public Health Association 1998. Standard method for examination of water and waste water, 20th Ed., American Public Health Association, Washington DC., USA, **pp. 773-774**.

Canizares R.O., Dominguez A.R., Rivas L., Montes M.C. and Travieso L. 1993. Free and immobilized cultures of *spirulina maxima* for swine waste treatment. **Biotechnol. Lett. 15, 321-325.**

El-Bestawy E. 2008. Treatment of mixed domestic-industrial wastewater using cyanobacteria. **J. Ind. Microbiol. Biotechnol. 35, 1503-1516.**

El-Sheekh M.M., El-Shouny W.A., Osman M.E., and El-Gammal E.W. 2014. Treatment of Sewage and Industrial Wastewater Effluents by the Cyanobacteria *Nostoc muscorum* and

Anabaena cylinderica, **J. Water Chemistry and Technology. 36(4), 190-197.**

El-Sheekh M.M., Farghl A., Gala H.R. and Bayoumi H.S. 2016. Bioremediation of different types of polluted water using microalgae. **Rend Fis Acc Lincei. 27(2), 401-410.**

El-Sheekh M.M. and Mahmoud YA.G. 2017. Technological Approach of Bioremediation using Microbial Tools: bacteria, fungi, and algae. In: Handbook of Research on inventive Bioremediation Techniques. Bhakta J.N. (Ed.) IGI Global, USA, **pp. 134-154.**

Ganapathy S.G., Baskaran R. and Mohan P.M. 2011. Microbial diversity and bioremediation of distilleries effluent. **J. Res. Biol. 3, 153-162.**

Gomez K.A. and Gomez A.A. 1976. Statistical Procedures for Agricultural Research with Emphasis on Rice, The international Rice Research Institute, Los-Banos, Manila, Philippines., **pp. 75-88.**

Guang-Jie Zhou, Guang-Guo Y., Shan Liu, Li-Jun Zhou,Zhi-Feng Chen and Fu-Qiang P., 2014. Simultaneous removal of inorganic and organic compounds in wastewater by freshwater green microalgae. **Environ. Sci., Processes Impacts, 16, 2018-2027.**

Hernandez J.P., de-Bashan L.E. and Bashan Y., 2006. Starvation enhances phosphorus removal from wastewater by the microalgae *Chlorella* sp. co-immobilized with *Azospirillumbrasilense*. **Enzyme Microbial Technol. 38,190-198.**

Huang G.L., Sun H.W. and Cong L.L. 2000. Study on the physiology and degradation of dye with immobilized algae. **Artif. Cells Blood Substitutes Immobil. Biotechnol. 28, 347-363.**

Khan M.I., Shin J.H. and Kim J.D. 2018. The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. MICROB CELL FACT, **pp.17-36.**

Marei E.M. and Elmaghraby, I. 2016. Protection of *Bacillus subtilis* **Against Bacteriophage Attack. Curr. Res. Bacteriol. 9, 1-8.**

Moreno-Garrido I. 2008. Microalgae immobilization: current techniques and uses. **Bioresour Technol. 99, 3949-3964.**

Mun˜oz, R. and Guieysse B. 2006. Algal–bacterial processes for the treatment of hazardous contaminants a review. **Water Res. 40, 2799-2815.**

Nagasathya A. and Thajuddin N. 2008. Decolourization of paper mill effluent using hypersaline cyanobacterium. **Res. J. Environ. Sci., 2, 408-414.**

Noor MaisarabteJais, Radin Maya Saphira bteRadinMohamed, Wan Asma Wan Mohamad Apandi, Hazel Monica Matias Peralta, 2015. Removal of Nutrients and Selected Heavy Metals in Wet Market Wastewater by Using Microalgae *Scenedesmus* sp. Appl. Mech. Mater., **pp. 1210-1214.**

Oilgae 2010. Oilgae guide to algae-based wastewater treatment: A sample report. Retrieved from http:// repository.uobabylon.edu.iq/2010_2011/4_6558_416.pdf

Palmer C.M. 1969. Composite rating of algae tolerating organic pollution. **J. Phycology. 5, 78-82.**

Qari H.A. and Hassan I.A. 2014. Removal of pollutants from waste water using *Dunaliella* algae. **Biomed. Pharmacol. J. 7(1), 147-151.**

Rajiv K. and Dinesh G. 2010. Waste water treatment and metal (Pb^{2+}, Zn^{2+}) removal by microalgal based stabilization pond system. **Indian. J. Microbiol. 50(1), 34-40.**

Revathi S., Kumar S.M., Santhanam P., Kumar S.D., Son D. and Kim M.K. 2017. Bioremoval of the indigo blue dye by immobilized microalga *Chlorella vulgaris* (PSBDU06). **J. Sci. Ind. Res. 76(1), 50-56.**

Ruiz-Marin A., Mendoza-Espinosa L.G., Stephenson T. 2010. Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. **Bioresour. Technol. 101, 58-64.**

Salam E., Kurade M.B., Abou-Shanab R., El-Dalatony M.M., Yang I.S. Min B., Jeon B.H., 2017. Recent progress in microalgal biomass production coupled with wastewater treatment for biofuel generation. **Renew Sustain Energy Rev. 79, 1189-1211.**

Tam N.F.Y., Wong Y.S. and Simpson C.G., 1998. Repeated removal of copper by alginate beads and the enhancement by microalgae. **Biotechnol. Tech. 12, 187-190.**

Wang Y., Ho S.H., Cheng C.L., Guo W.Q., Nagarajan D., Ren N.Q., Lee D.L. and Chang J.S. 2016. Perspectives on the feasibility of using microalgae for industrial wastewater treatment. **Bioresour Technol. 222, 485-497.**

المعالجة الحيوية لمياه الصرف بإستخدام خليط من الطحالب فى مستنبتاته

[149]

ألفت سالم[1]* – عادل حماد[2] – سيد إسماعيل[3] – عبد الغنى الجندي[4]

1- قسم النبات والميكروبيولوجى – كلية العلوم – جامعة حلوان – القاهرة – مصر

2- قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة المنيا – المنيا – مصر

3- الهندسة الصحية والبيئية – قسم الأشغال العامة – كلية الهندسة – جامعة عين شمس – القاهرة – مصر

4- قسم الهندسة الزراعية – كلية الزراعة – جامعة عين شمس – ص.ب 68– حدائق شبرا 11241 – القاهرة – مصر

*Corresponding author: olfatabdelhamed@yahoo.com

الموجـــــــز

أجريت هذه الدراسة لتقييم كفاءة خليط من الطحالب تمت تنميته فى مستنبتاته لمعالجة مياه الصرف الصحي. وقد تم استخدام الطحالب على صورتين : طحالب حرة وأخرى مرتبطة بالالجينات (المثبتة). وكانت أعلى نسب إزالة للطلب على الأكسجين البيولوجى (BOD) هى 86.4% و 71.2% بعد 32 ساعة و 48 ساعة فى حالة الخلايا الحرة والمثبتة، على التوالي. علاوة على ذلك، فإن أعلى قيم لإزالة الطلب على الأكسجين الكيميائى كانت 83.74% و59.71% بعد 32 ساعة و 48 ساعة للخلايا الحرة والمثبتة بالالجينات على التوالي. وقد أظهرت المعالجة بإستخدام الخلايا الحرة قيم إزالة لمجموع الأملاح الذائبة (TDS)، والفوسفور و NH3–N كانت 20.5%، 34.6% و 43.8% على التوالي بعد 32 ساعة. بينما في حالة الخلايا المرتبطة بالالجينات، كانت أعلى قيم الإزالة 18.4%، 34 % و 58.5%، لـ TDS، الفوسفور و NH3–N، سجلت على التوالى بعد 48

ساعة. بشكل عام، انخفض تركيز المعادن الثقيلة بسبب العلاج بخلايا الطحالب الحرة والمثبتة. كنتيجة للمعالجة بإستخدام الطحالب على صورتيها الحرة والمثبتة فقد تمت إزالة 100% و 95.9% من محتوى مياه الصرف الصحي من النحاس بعد 8 ساعـــــات و 48 ساعة على التوالي. وعلاوة على ذلك، تمت إزالة 96.2% و 98.1% من الحديد بعد 32 و 48 ساعة على التوالي. علاوة على ذلك، تم تسجيل النسب المئوية العالية للإزالة بالنسبة للعناصر الثقيلة مثل Mn و Pb و Zn بسبب تطبيق الطحالب. بناءً على ذلك، فإن معالجة مياه الصرف الصحي بالخلايا المختلطة من الطحالب الحرة أو المثبتة بإستخدام الالجينات هى طريقة مثمرة لإنتاج مياه صرف ذات جودة عالية لإستخدامها في الري. بينما تعتبر الخلايا الحرة من الطحالب أكثر كفاءة فى عملية المعالجة من الخلايا التي تم تجميدها.

الكلمات الدالة: المعالجة الحيوية، ميكروبيولوجي، الخلايا المتجمدة، مياه الصرف