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Experimental approach for improving the removal efficiency of a metal pollutant using the Activated Sludge as a wastewater treatment process.

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#### ABSTRACT

The objective of this study was to investigate the feasibility of developing improved activated sludge cultures to remove a heavy metal salt (CuSO<sub>4</sub>,5H<sub>2</sub>O) present in high concentrations. Two types of bioreactors were employed to develop and maintain the aerobic cultures used to conduct the heavy metal-uptake studies. These were continuously stirredtank batch reactors (CSTBR's) that used to sustain the aerobic cultures at selected specific growth rates beside detecting metal tolerant species and completely mixed batch reactors used to conduct metal uptake studies with cultures derived from the CSTBR's. Copper was selected for this application. The aerobic continuously stirred batch reactor (ACSBR) of high growth rate (HGR) exhibited good metabolic activities as indicated by the values of P<sup>H</sup>, dissolved oxygen, TSS, VSS, , BOD and COD belonging to the mixed liquor provide an evidence for the presence of metabolically active organisms. The data obtained in the present study established that the copper uptake by the test sludge in the different unit reactors at the first four hours ranged from 15.63% to 27.21%. The present study proved that the maximum removal percent of copper salt achieved 79.6% to 81.9% of the total percent of copper uptake, while those obtained by the control experimental sludge ranged from 0.20% to 2.10%. This means that there was a big difference among the averages of metal uptake.

## **INTRODUCTION**

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Due to a greater awareness of the ecological effects of toxic metals released into the environment, a number of studies on metal accumulation, metal recovery and removal from solution have been launched. Different conventional methods for removing metals from processing solutions may be ineffective or extremely expensive, especially when the metals are in solutions containing from 1 to 100 mg/l of dissolved metals (Brierly & Davidsan; 1989 and Brierly; 1991). It was suggested that biological methods could be as effective or even superior to chemical processes in some cases (Ghosh and Bupp;1991). Various species of fresh water and marine bacteria, algae, duckweed and other plants have the capacity to pick up heavy metal ions from solution. Fortunately, there is some evidence to suggest that heterogeneous cultures could be more effective in removing heavy metal ions, in some cases, than

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pure cultures. Alternative metal removal and/or recovery methods are being considered which are based on metal-isolating properties of certain natural materials of biological origin, where certain types of microbial biomass can retain relatively high quantities of metal ions by "passive" sorption and/or complexation which is commonly known as biosorptron. (Brierly & Davidsan; 1989 and Hany et al.; 2004). Studying interactions between heavy metals and microorganisms has increased in the last few decades concerning the different metal resistance mechanisms, especially those used by bacteria and fungi (Gadd and White, 1993 and Lovley, 2000). Ciliated protozoa are believed to be important grazers of bacteria and other microorganisms and this leads to stimulate the rates of carbon and nitrogen cycling in soils (Fenchel, 1987 and Finlay et al., 2000). It was proved that in activated sludge wastewater treatment plants, ciliated protozoa improved significantly the effluent quality (Curds, 1982 and Nicolau et al., 2001). The efficiency of wastewater treatment by activated sludge is related to bacterial and protozoan populations. Different species were found and listed by various authors such as Curds and Cockburn (1970a) and Richard (1991) under normal conditions where bacterial numerical densities ranged between  $(10)^6 - (10)^7$  and their abundance is larger than protozoa/l (~  $10^5/l$ ) which is corresponding to high effluent quality. On the contrary, lower densities of both organisms are indicative of the low efficiency of the plant (Drakides, 1978). According to Madoni (1994a). protozoa represented between 0.17 and 0.44 % of the sludge biomass during the colonization phase but illustrated up to 9 % at steady state. Galal (1997), studied the effect of various bacterial densities on the growth rates of certain ciliates. He found that within the first 40 hours of culturing the growth rates of bacteria and each of the ciliates populations showed high-significance increase from the statistical point of view and influenced with both temperature and bacterial densities. He also concluded that, food intake efficiency belonging *Colpidium* was more efficient in ingesting bacteria than paramecium. In addition, Galal (1996), estimated that *Cinetochilum* was the most resistant ciliate followed by *Paramecium* and then Cyclidium. His statistical analysis recommended that Cyclidium could be used as a bio-indicator in case of mercury and zinc compounds, while Paramecium and *Cinetochilum* could be used as indicator in case of lead and copper compounds respectively. The accumulation of heavy metals on the cell wall of microorganisms was explained by William et al. (2008). Diaz Silvia et al., (2006) carried out Laboratory acute cytotoxicity tests using Cd, Zn and Cu in five different strains of very common soil ciliate species (Colpoda steinii, Colpoda inflata and Cyrtolophosis elongata). Soil ciliates are quite resistant to heavy metals pollution with regard to ciliates from other habitats and the toxicity sequence was Cd>Cu>> Zn. Momba et al. (2008) studied the nutrient removal rate of wastewater protozoan isolates in a laboratory-scale batch reactor. The results showed phosphates removal rate ranging from 0.04 to 0.52 mg/L/h that of nitrates ranged from 0.08 to 0.16 mg/L /h, while those of nitrites and ammonia ranged between 0.022 and 0.087 mg/L /h. beside 0.05 and 0.16 mg/L /h respectively.

# MATERIALS AND METHODS

#### Physical examination and chemical determinations.

Certain parameters as temperature; PH; Dissolved Oxygen; Settleable solids; settled sludge volume; Total and Volatile Suspensed Solilds (TSS&VSS); Total Soilds ; Total Volatile solids (TS&TVS); and Total Dissolved Solids (TDS), organic constituens which include chemical oxygen demand (COD) and Biochemical oxygen

demand (BOD) were measured using the procedures adopted by (APHA, 1989). Copper metal was determined by absorption spectrometry SHIMADZU, Model AA660.

#### **Biological examination.**

Water samples were collected from the different units of the bench scale. Micro-scopical examination was carried out using Carl Zeiss Transmitted-light Inverted microscope within four hours of collection. The number of each organism per replicate was recorded and expressed as occurrence percentages. Protozoan organisms were identified according to Bick (1972) beside Patterson and Hedley, (1992).

MacConkey's bile salts-agar medium (Difco Manual, 1977) was used to determine the total bacterial counts. Triplicate plates incubated with 1.0 ml of the desired dilutions were poured with MacConky's medium and incubated at 35  $^{\circ}$ C for 24 hours for counting total coliform bacteria (red, pink or nearly colorless with a pink center colonies were only counted.

#### **Instruments:**

Two types of bioreactors were employed to develop and maintain the aerobic cultures that used to conduct the heavy metal-uptake studies. These were continuously stirred-tank batch reactor (CSTBR,s) and completely mixed batch reactors. The CSTBR,s were used to sustain the aerobic cultures at selected specific growth rates and to select metal tolerant species. The completely mixed batch reactors were used to conduct metal uptake studies with cultures derived from the CSTBR's.

One aerobic CSTBR of a heavy plastic material hasting a culture volume of ten liters was used . The sewage culture was collected from the mixed-liquors of Zenein Wastewater Treatment Plant, this plant is located at Zenein Distrect, Boulak-Eldakrour, Giza. The aerobic CSTBR was continuously aerated by a small air pump; where the air pressure was adjusted through a stopcock. The reactor was fed once a day with 4.95 liters of primary effluent sewage collected from the primary effluent treatment unit of Zenein WWTP, plus 4.95 liters of synthetic minerals for the test and distilled water for the control. The chemical composition of the synthetic growth media are presented in Table (1).

Nutrient salt	concentration	Table 1:
Glucose , C6H12O6	320 gm/m3	Growth
Ammonium chloride, NH4Cl	81.5 gm/m3	medium for
Potassium phosphate, KH2PO4	14.1 "	aerobic culture.
Sodium phosphate, Na2HPO4	14.7 "	culture.
Ammonium sulfate, Na2HPO4	6.2 "	
Magnesium chloride, Mg Cl2.6H2O	6.7 "	
Calcium chloride anhydrous, Ca Cl2	0.165 gm/5m3	
Ferric chloride, Fe Cl3.6H2O	0.029 "	
Manganese sulfate monohydrate Mn SO4.H2O	0.0041 "	
Zinc chloride, Zn Cl2	0.0001 "	
Ammonium molybdate, (NH4) Mo7O24.4H2O	0.0004 "	
Cobalt chloride, Co Cl2 6H2O	0.00018 "	

The CSTBR had a stopcock at a level above 10 liters to withdraw the supernatant or reactor effluent after each period of operation to maintain a fixed volume of the culture medium. Figures (1a) and (1b) are schematic representation of the aerobic continuously stirred tank batch reactor and completely mixed batch reactor used in this study.

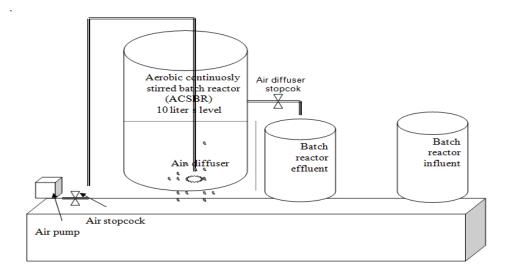


Fig. 1a: Schematic diagram of aerobic continuously stirred-tank batch reactor (CSTBR)

### **CULTURE DEVELOPMENT**

Aerobic culture was developed by inoculating synthetic mineral-salts media with feed sewage collected from the primary effluent at Zenein WWTP. In case of the control test the synthetic media were replaced by distilled water .

The CSTBR culture was maintained at high specific growth rates by appropriately adjusting the reactor flow rate. Aerobic culture was grown at flow rates of 0.42 per hour (i.e., at \*hydraulic retention times of 24 hours.)

Detention time (h) = tank volume (m<sup>3</sup>) X (24 h) / flow rate (m<sup>3</sup>/d)

Tank volume = 10 liters = 0.01 m<sup>3</sup>

Detention time = 24 hours

Then flow rate =  $0.01 \text{ m}^3/\text{d} = 10 \text{ liters/day}$ 

Aerobic reactor performance was monitored by measuring pH, temperature, dissolved oxygen (DO) concentration during the operation time of the culture. Grab samples were taken off where the settle-meter test, total suspended solids (TSS), volatile suspended solids (VSS), total solids (TS), total volatile solids (TVS) were measured. Total bacterial counts, and protozoan examinations were also performed to the culture samples three times per week. After 24 hours of operation, the air pump turned off and the reactor set aside for one hour, then supernatant was siphoned and PH, total suspended solids (TSS), volatile suspended solids (VSS), total solids (TSS), tota

### METAL UPTAKE STUDIES

#### **Instruments:**

A high-speed six-paddle stirrer unit (Philps & Bird stirrer, Model 7790-402) was used as completely mixed batch reactors (Fig. 1b). It consists of six paddles, 2.5 cm wide X 7.6 cm long driven by 1/30 hp. with variable speed motor up to 300 rpm.

Paddle depths of 30 cm provide a good mixing and aeration for the contents of the rectangular jar bath which is made of clear glass, with capacity of 2 liters; 10 cm long X 10 cm wide X 20 cm height.

## Metal Uptake Reactos Operation:

Control six reactor of aerobic continuously stirred batch reactor (ACSBR) started at 13 March till 11 April; while the test reactor was performed on a period extending between 28 April and 26 May 2007.

Metal uptake studies were initiated by transferring two liters of the ACSBR culture to each of the four completely mixed batch reactors, and adding different doses of copper sulfate, The doses of the four reactors are 0.284, 0.23, 0.184 and 0 gm of  $CuSO_4.5H_2O$  per 2liters. The instrument was adjusted at speed of 200 rpm for both control and the test units. Samples of the four batch reactors were collected at the 1-st, 2-ed, 3-ed, 4-th and 24-th hour of operation.

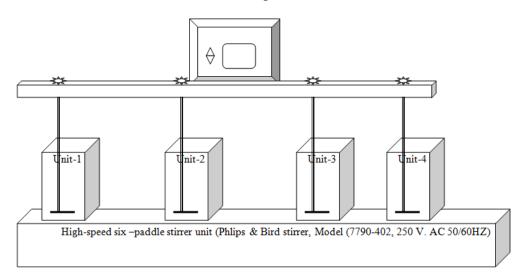


Fig. (1b): Schematic diagram of completely mixed-tank batch-reactor 1/30 hp variable speed motor

#### **RESULTS AND DISCUSSION**

## **CULTURE DEVELOPMENT ANALYSIS:-**

Monitoring data which presented in Tables (2a & 2b) collected for the copper removal control and the test reactors showed that the culture medium ranged from neutral to very slightly alkaline where the PH values ranged from 7.01 to 7.56 and from 6.13 to 7.06 unit respectively. The temperature variations throughout the operation period of aerobic culture medium ranged from 21.50 to 25.8 °C and from 23.10 to 28.3 °C for control and test culture media respectively. On the other hand, Dissolved oxygen of both control and test reactors varied from 1.70 to 6.65 mg/l and from 4.59 to 7.10 mg/l respectively.

The total suspended solids during the period of control operation unit was slightly higher than that of the test unit where their average values were 1417 and 1196 mg/l respectively. The total solid content proved the same behavior of the suspended solids with an average 1737 mg/l of the control unit which is slightly

higher than that of test unit (1564 mg/l). On the other hand, the average dissolved solid contents were 320 mg/l and 369 mg/l for both reactors respectively.

Table 2a: Certain physico-c	chemical paramete	ers and bacterial c	counts of the high g	rowth rate (HGR) of
aerobic continuously	stirred batch read	ctor (ACSBR) of t	the copper removal	control reactor.

parameter	28&29/4	2 & 3/5	4 & 5 / 5	8 & 9 /5	11&12/5	16&17/5	18&19/5	23&24/5	25&26/5
P <sup>H</sup> unit	7.05	5.54	6.13	6.77	7.06	7	6.86	6.87	6.91
Temp. C	23.8	24.3	26.1	24.7	24.4	24	23.1	24.5	28.3
DO mg/l	6.7	7.1	4.59	6.5	6.5	5.67	4.77	5.59	5.65
TSS mg/l	987	920	960	1113	1407	1240	1790	1253	1093
VSS mg/l	820	807	800	927	1147	973	64	953	833
TS mg/l	1430	1650	1330	1410	1670	1580	1.56	1600	1470
TVS mg/l	920	1120	820	1020	1120	1140	1260	1050	910
TDS mg/l	443	730	370	297	263	340	150	347	377
MII Ü	150	140	120	140	190	130	120	100	70
SVI	152	152.2	125	125.8	135	104.8	67	79.8	64.04
SDI	0.66	0.66	0.8	0.8	0.74	0.95	1.49	1.25	1.56
Bact.count (10 <sup>6</sup> /ml)	8.96	10.01	25.30	40.89	92.80	87.79	56.76	94.69	89.87

MI l = ml settled sludge index

SV I = sludge volume index

SD I = sludge density index

Table 2b: Certain physico-chemical parameters and bacterial counts of the high growth rate (HGR) of aerobic continuously stirred batch reactor (ACSBR) of the copper removal test reactors.

parameter	28&29/4	2 & 3/5	4 & 5 / 5	8 & 9 / 5	11&12/5	16&17/5	18&19/5	23&24/5	25&26/5
P <sup>H</sup> unit	7.05	5.54	6.13	6.77	7.06	7	6.86	6.87	6.91
Temp. C	23.8	24.3	26.1	24.7	24.4	24	23.1	24.5	28.3
DO mg/l	6.7	7.1	4.59	6.5	6.5	5.67	4.77	5.59	5.65
TSS mg/l	987	920	960	1113	1407	1240	1790	1253	1093
VSS mg/l	820	807	800	927	1147	973	64	953	833
TS mg/l	1430	1650	1330	1410	1670	1580	1.56	1600	1470
TVS mg/l	920	1120	820	1020	1120	1140	1260	1050	910
TDS mg/l	443	730	370	297	263	340	150	347	377
MI1 -	150	140	120	140	190	130	120	100	70
SVI	152	152.2	125	125.8	135	104.8	67	79.8	64.04
SDI	0.66	0.66	0.8	0.8	0.74	0.95	1.49	1.25	1.56
Bact.count	8.96	10.01	25.30	40.89	92.80	87.79	56.76	94.69	89.87
(10 <sup>6</sup> /ml)									

Having a glance to Table (3), it was proved that the control influent BOD ranged from 116 to 227 mg/l and those of COD are 245 - 353 mg/l, while those of the test reactor are 108–192 mg/l and 220-363 mg/l respectively. On the other hand, those of the effluent two parameters ranged from 10-56 mg/l, 35-170 mg/l for the control and from 4-48 mg/l and 19-96 mg/l in the test reactor.

Accordingly, the removal percentage of BOD and COD in the control ranged from 66.5 to 94.3 and from 22.7 to 88.6 within an average of 86.5 and 70.6 respectively, while that of the test unit varied between 73.3 and 97.6 and from 66.9 to 94.3 with average values of 92.3- 92.2% respectively. This means that the performance of the test reactor was much better than that of the control unit. The increase in efficiency of the test reactor especially after the first three days of the starting up the operation may due to the special nutrient added to the influent which enhanced the bio-faunal activity in the biological treatment.

It was demonstrated that microbiological communities in Zenein activated sludge treatment plant as a batch operation of the high growth rate (HGR) of aerobic continuously stirred batch reactor (ACSBR) were represented by bacteria and protozoa with smaller groups of other invertebrates as nematodes and rotifers which showed an agreement with Curds and Hawkes (1975) who reported that the

microbial communities in the different biological water treatment processes are usually illustrated by bacteria and protista as the most active organisms.

I- Control reactors					II- T	est reac	tors	<u>.</u>
	Influent Effluent			Influent	Effluent		_	
Date	BOD	COD	BOD	COD	BOD	COD	BOD	COD
28&29/4/2001	227	245	14	66	108	293	12	29
2&3/5/2001	180	259	11	56	192	344	48	78
4&5/5/2001	188	288	14	35	152	308	14	96
9&10/5/2001	116	250	12	70	110	299	7	72
11&12/5/2001	162	261	15	69	127	298	7	69
16&17/5/2001	165	259	10	88	153	281	4	29
18&19/5/2001	166	269	11	39	120	290	11	43
23&24/5/2001	152	336	40	167	184	363	7	19
25&/26/5/2001	162	353	56	170	167	220	10	67
average	151	300	20	84	146	280	13	56

 Table 3: Average values of BOD and COD of influent and effluent water during the copper removal test.

The protozoan organisms are illustrated by three main classes in both reactors in the following order; Ciliophora, Mastigophora and Sarcodina at Zenein WWTP in Giza province. As shown in Table (4), it was proved that ciliates are represented by 60% and 72%, flagellates by 29% and 15%, while sarcodines by 11% and 13% for control and test reactors respectively. Ciliophorans of the control reactor comprised four subclasses; Holotrichia (41.7%), Peritrichia (20%), Spirotrichia (30%) and Suctoria (8.3%), while those of the test unit are exhibited by 45%, 17%, 33% and 5% respectively.

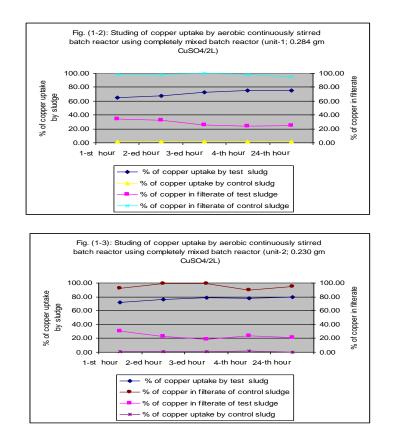
Living organisms	Control	Test
Bacterial N <sup>o</sup> (10 <sup>6</sup> /ml)	34.26	56.34
Flagellates/Protozoa	(29%)	15%
Sarcodines/Protozoa	(11%)	(13%)
Ciliates/Protozoa	(60%)	(72%)
1- Holotrichs/ciliates	42%	45%
2- Peritrichs/ ciliates	20%	17%
3- Spirotrichs/ ciliates	30%	33%
4- Suctoria/ ciliates	8%	5%

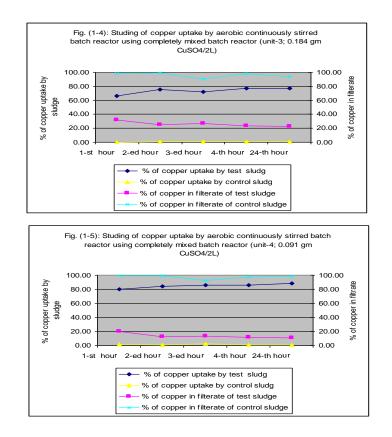
Table 4: Average occurrence percentages of various protozoan organisms and bacterial populations of the aerobic continuously batch reactor (ACSBR) of the copper removal experiment.

Accordingly, the protozoan prevalence of holotrich and peritrich ciliates are higher in the test than those of the control units, while those of spirotrichs and suctorians illustrated an antagonistic pattern. This might be correlated with the higher efficiency performence in the test reactor than the control one as holotrich and peritrich ciliates are mostly bacterial feeders, while spirotrichs and suctorians are carnivorous and/or algivorous protozoans. This is confirmed by Toni Glymph (2006) who concluded that the presence of particular types of protozoa in the activated sludge treatment process is related to effluent quality and plant performance. He reported also that amoebae can only multiply more frequently when there is an abundance of nutrients (small organic particles) in the aeration tank. Their presence may also indicate that there is a low D.O situation in the aeration basin. Flagellates and bacteria both feed on organic nutrients in the sewage so the nutrient level declines. He mentioned also that ciliates feed on bacteria not on dissolved organics, while bacteria and flagellates compete for dissolved nutrients, ciliates compete with other ciliates and rotifers for bacteria. The presence of ciliates indicate a good sludge, because they dominate after the floc has been formed. According to Curds and Hawkes (1975), the free-swimming ciliates appear as the flagellates begin to disappear. After most of the organic nutrients have been removed, a lot of dispersed bacteria is available for feeding and free-swimming ciliates begin to dominate and feed on the increased bacterial numbers and when the floc particles enlarge and stabilize, crawling ciliates begin to graze on food inside these particles. Stalked ciliates appear in the mature sludge where both crawling and stalked ciliates compete for dominance. Most protozoans can survive and reproduce in a temperature range at which activated sludge processes are carried out where growth achieved best results in ambient temperatures between 15 and 25 °C (Galal;1993) and they are more sensitive to pH than floc-forming bacteria where their optimal pH range is 7.2-7.4. Shortage of oxygen will severely limit protozoa from both qualitative and quantitative points of view, keeping in mind that most municipal wastewater treatment plants have sufficient nutrients to support most of the protozoan organisms associated with wastewater.

**Bio-accumulation of Heavy Metals:** The aerobic continuously stirred batch reactor (ACSBR) of high growth rate (HGR) exhibited good metabolic activities as indicated by a mixed-liquor of  $P^H$  5.54-7.06, a dissolved oxygen concentration of about 5-7 mg/l and total suspended solid of 920-1790 and volatile suspended ranged from 800-1470mg/l. The observed BOD reduction ranged from 73.3-97.6 % while the COD reduction was 66.7-94.3 % provided further evidence of the presence of metabolically active organisms.

Efficiencies of Heavy Metals uptake by unacclimated cultures: Data of batch uptake of copper by fast-growing chemostat cultures maintained at specific growth rates of  $1 \text{ day}^{-1}$  are shown in Figures (1-2 to 1-5).





According to Ghosh and Bupp (1991), heavy metal uptake was related to the protein mass because proteins are primarily responsible for removal of these cations. It was proved that copper was removed maximally and very rapidly from solution with more than 80% of the total uptake within the first one hour of batch incubation.

Data of copper uptake in the test sludge in the different unit reactors at the first four hours ranged from 15.63% to 27.21%, while the maximum percentages of copper removal were about 79.6% to 81.9% of the total percent of copper uptake.

Having a glance to Figures (1-2 to 1-5), it was obvious that there was a big difference among the averages of metal uptake which were 69.40%, 75.91%; 73.14% and 83.69% for the reactors 1, 2, 3 and 4 respectively. Accordingly, it was concluded that organisms at the low specific growth rate removed more heavy metal ions than those at the exponential growth phase indicating that biopolymers synthesized at low growth rates were better metal removers. Simultaneously, it was concluded that microorganisms used to conduct the batch runs began to die and lyse shortly after exposing to the heavy metals.

At low concentrations, the viability of the organisms and the BOD removal efficiency of the process are not significantly impacted (Chang *et al.*, 1984). Other authors as Rudd *et al.* (1984); Ghosh and Bupp (1992), stated that removal of heavy

metals including copper by activated sludge system is well documented, however copper removal down to possible National Pollutant Discharge Elimination System

(NPDES) permit levels of 6-25 ppm would not be achieved readily by municipalities unless they were to employ carefully executed methodologies. It was

proved also that a sludge storage time of 18- 26 h was better than longer or shorter times; non-aerated or recently anaerobic storage of sludge was favorable as compared with well aerated. A PH range of 6.2- 8.5 was better than higher or lower. A mass blending ratio between stored sludge and fresh sludge of 1:1 was as effective as higher ratios and more effective than lower ratio.

Heavy metal ions can be entrapped in the cellular structure and subsequently biosorbed onto the binding sites present in the cellular structure (biosorption or passive uptake). Also, these heavy metal ions can also pass through the cell membrane in the process of cell metabolic cycle (active uptake). Both active and passive modes of metal uptake are termed as bioaccumulation (Hosoya et al., 2004). Ciliates have many advantages as a test organism for investigating environmental pollution (Madoni et al., 1992 and 1996; Tanaka et al., 2005; Salvado et al., 2001 and Gutierrez et al., 2003). Data on the bioaccumulation of heavy metals by invertebrates are available for lead/cadmium in marine protozoan communities (Fernandez-leborans and Olalla, 2000) and for lead/cadmium/copper/zinc in terrestrial invertebrates by Heikens et al. (2001). However, data for chromium accumulation by ciliates have been reported by Golam Mortuza et. al. (2009) who studied the cell growth of five strains of P. bursaria in a lettuce medium supplemented with different concentrations of potassium dichromate. Although the toxic effects of chromium on microorganisms and invertebrates have been a topic for researchers over the past few decades (Stasinakis et al., 2002; Madoni et al., 1996; Hadjispyrou et al., 2001; Yap et al., 2004) less information using P. bursaria is available. In the present study, it was concluded that some heavy metals might be removed or uptaken from the treated sewage by a conventional activated sludge method after conditioning the biofauna of the activated sludge using special nutrients.

### REFERENCES

- American Public Health Association (APHA) (1989). Standard Methods for the Examination of Water and Wastewater, En., Clesceri, L.S., Greenberg and R. R. Trussell. 17<sup>th</sup> Edition,.
- Bick, H. (1972). Ciliated Protozoa: An illustrated guide to the species used as biological indicator in freshwater biology. W.H.O. Geneva, Switzerland.
- Brierly, C.L. (1991). Bioremediation of metal-contaminated surface and ground waters. Geomicrobial. J., 8: 201-223.
- Brierly, C.L., and Davidson (1989). Applied microbial processes for metals recovery and removal from wastewater, in metal ions and Bacteria, Beveridge, T.J. and Doyle, R., Eds., John Wiley & Sons, New York, 359.
- Chang, S.Y., Huang, Z.C. and Liu, Y.C. (1984). Effects of Cd and Cu on a biofilm treatment system proceedings 39<sup>th</sup> Purdue Industrial Waste Conference, 305-312.
- Curds, C.R. (1982). The ecology and role of tha ciliated protozoa in the activatedsludge process, Ann. Rev. Microbiol, 36: 27-46.
- Curds C.R. and Cockburn A. (1970a). Protozoa in biological sewage treatment process-1. A survey of the protozoan fauna of British Percolating Filters and activated-sludge
- Curds, C.R and Hawkes, H.A, (1975). Ecological Aspects of Used-water Treatment, Volume 1: The organisms and their ecology. Academic Press Inc. London LTD.

- Diaz Silvia; Martin-Gonzalez Ana; Gutierrez Juan Carlos (2006). Evaluation of heavy metal acute toxicity and bioaccumulation in soil ciliated protozoa, Environment international ISSN 0160-4120 Coden Envid, 32(6): 711-717.
- Difco Manual of Dehydrated culture. (1977). Media and Reagents of Microbiological Laboratory Procedures. Ninth Edition. P 134.
- Drakides C. (1978). L'Observation Microscopique des Boues Activee's Appliquee a'la surveillance des installations d'epurations: Technique d'etude et Interpretation, TSM. L'EAU, 2, 85-98.
- Fenchel,T. (1987). Ecology of protozoa, Sci. Tech.,Springer-Verlag, Berlin/Madison. Fernandez-leborans G, Olalla YH (2000). Toxicity and bioaccumulation of lead and cadmium in marine protozoan communities. Ecotox.. Environ. Saf. 47: 266-276.
- Finlay, B.Z.; Black, S. Brown, K. J. Clark, G.F. Esteban, R.M.; Hinde, J.L.; Olmo, A.; Rollett, K.; Vickerman, (2000). Estimating the growth potential of the soil protozoan community. Protist 151: 69-80.
- Gadd, G.M.; White, C.; (1993). Microbial treatment of metal pollution a working biotechnology; TTB TECH: 353-359.
- Galal, M. (1993). The effect of temperature on the growth rates and cell sizes of some freshwater ciliates. J. Egyp. Ger. Soc. Zool., 10 (D) : 359 367.
- Galal, M. (1996). Effects of some heavy metals compound on densities and growth rates of certain freshwater ciliates. J. Union Arab Biot. Cairo, Vol. 6 (A), 9-20, 1996.
- Galal, M. (1997). Effect of various bacterial densities on growth rates of some fresh water ciliated protozoa in the Laboratory. J. Egypt. Ger. Sec. Zool., 24 (D) 37-50.
- Ghosh, S and Bupp S. (1991). Uptake of transition heavy metals by unacclimated aerobic cultures. The conference on hazardous waste Research Kansas State University May 29 &30,1991.
- Ghosh, S. and Bupp, S. (1992): Stimulation of biological uptake of heavy metals; Wat. Sci. Tech., 26 (1-2): 227-236.
- Golam Mortuza M., Toshiyuki Takahashi2, Tatsuya Ueki, Toshikazu Kosaka, Hitoshi Michibata and Hiroshi Hosoya (2009). Comparison of hexavalent chromium bioaccumulation in five strains of paramecium, *Paramecium bursaria*; Journal of Cell and Animal Biology, 3(4): 062-066.
- Gutierrez JC, Martin-Gonzalez A, Diaz S, Ortega R (2003). Ciliates as potential source of cellular and molecular biomarkers/biosensors for heavy metal pollution. Europ. J. Protistol. 39: 461-467.
- Hadjispyrou S, Kungolos A, Anagnostopoulos A (2001). Toxicity, bioaccumulation and interactive effects of organotin, cadmium and chromium on *Artemia franciscana*. Ecotoxicol. Environ. Saf. 49: 179-186.
- Hany, H.; Soha, F.,I.; Kamal, K; Hassan, M. (2004). Biosorption of heavy metals from waste water using Pseudomonas sp; Microbial Biote-chnology.
- Heikens, A. Peijnenburg, WJ, Hendriks GM. (2001). Bioaccumulation of heavy metals in terrestrial invertebrates. Environ. Pollut. 113: 385-393.
- Hosoya H, Kimura K, Matsuda S, Kitaura M, Takahashi T, Iyer A, Mody K, Jha B (2004). Accumulation of hexavalent chromium by an exopolysaccharide producing marine *Enterobacter cloaceae*. Mar. Pollut. Bull. 49: 974-977.
- Lovley, D.R. (2000). Environmental Microbe-metal interaction, ASM Press, Washington, DC.

- Madoni P, Esteban G, Gorbi G (1992). Acute toxicity of cadmium, copper, mercury and zinc to ciliates from activated sludge plants.Bull. Environ. Contam. Toxicol. Environ., 49: 900-905.
- Madoni P. (1994a). Estimates of ciliated protozoa biomass in activated sludge and biofilm, Bioresource Technology, 48, 245-249 (1994a).
- Madoni, P.; Davoli, D.; Gorbi, G.; Vescovi, L. (1996). Toxic effect of heavy metals on the activated sludge protozoan community, Water Res. 30: 135-141.
- Momba M.N.B.; Akpor O.B.; Okonkwo J.O.; Coetzee M.A. (2008). Nutrient removal from activated sludge mixed liquor by wastewater protozoa in a laboratory scale batch reactor. Courtesy of International Journal of Environmental Science and Technology Sep. 1.
- Nicolau, A.; Dias, N.; Mota, M.; Lima, N. (2001). Trends in the use of protozoa in the assessment of wastewater. Res. Microbiol. 152: 621-630.
- Patterson, D.J. and Hedley, S. (1992). Free-living freshwater protozoa. A colour guide. Wolfe publishing Ltd. England.
- Richard M., (1991). Activated sludge microbiology, the Water Pollution Control Federation, 2<sup>nd</sup> edition, Virginia.
- Rudd, T., Sterritt, R.M., and Lester, J.N. (1984). Complexation of heavy metals by extracellular polymers in the activated sludge process. J. Water Pollution Control Fed., 56: 1260-1268.
- Salvado H, Mas M, Menendez S, Gracia MP (2001). Effects of shock loads of salts on the protozoan communities of activated sludge. Acta. Protozool. 40: 177-185.
- Stasinakis AS, Mamais D, Thomaidis NS, Lekkas TD (2002). Effect of chromium (VI) on bacterial kinetics of heterotrophic biomass of activated sludge. Wat. Res. 36: 3341-3349
- Tanaka M, Ishizaka Y, Tosuji H, Kunimoto M, Nishihara N, Kadono T, Kawano T, Kosaka T, Hosoya N, Hosoya H (2005). A new bioassay system for detection of chemical substances in environment using green Paramecia, Paramecium bursaria. In Environmental Chemistry (Eds., Lichtfouse, E., Schwarzbauuer J, Robert D.), Springer-Verlag, Berlin. pp.495-504,
- William Underwood and Shauna C. Somerville (2008). Focal accumulation of defences at sites of fungal pathogen attack. J. Exper. Bot., 59(13):3501-3508
- Yap CK, Ismail AH, Omar H, Tan S G. (2004). Toxicities and tolerance of Cd, Cu, Pb and Zn in a primary producer (Isochrysis galbana) and in a primary consumer (Perna viridis). Environ. Int., 29: 1097-1104.

## ARABIC SUMMARY

دراسة تجريبية لتحسين كفاءة ازالة احد الملوثات المعدنية باستخدام الحمأة النشطة لمعالجة مياه الصرف

## منصور جلال – أحمد عبد العزيز – رمضان عبد ربه – عاطف الطوخى

أجريت هذه الدراسة لتحسين معدل التخلص من أحد املاح النحاس باستخدام نموذج يحاكى محطات معالجة مياه الصرف (الحمأة النشطة) وذلك بمتابعة التغيرات للخواص الفيزيقية والكيميائية والبيولوجية أثناء تلك العملية فى وجود خلطة غذائية محفزة للنشاط البيولوجى. ولقد لوحظ أن معدلات التخلص من هذه المادة الملوثة تظهر اختلافات معنوية فى التركيزات المختلفة لأملاح هذا العنصر وذلك اعتماداً على معدلات النمو لكل من البكتيريا والكائنات الأولية (البروتوزوا).