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MICROBIOLOGICAL TREATMENT OF ALUM SLUDGE INDUSTRIAL EFFLUENT

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ABSTRACT

The microbial treatment of alum sludge industrial effluent was carried out. The chemical analysis of this waste was performed. The microbial treatment was conducted using two bacterial and two yeast strains These microbes namely *Bacillus megaterium*, 6SB, *Serratia marcescens*, 3STA, *Saccharomyces cerevisiae*, SC64 and *Saccharomyces cerevisiae*, SC66. The growth dynamic of these microbes was followed using four different dilutions of the tested waste i.e., 0.00%, 25%, 50% and 75 % (water: waste). The optimization for maximize management of this waste was examined by change of pH value and inoculums size % of microbe used. Monitoring of microbiological treatment was carried out by examining dry weight of obtained biomass, growth dynamics and Bioremoval of some trace metal ions. The observed result show that about 97.589 % of the total aluminium in the tested effluent was removed after the first 24 h. Furthermore, some of trace metal ions were removed in high percent such as cadmium in 100 % by *B.megaterium*, 6SB and *S. cerevisiae*,SC64; they are the most active strains. The highest value of Cr^{6+} removal is achieved by *S. cerevisiae*,SC66 and *S.cerevisiae*,SC64 have 95.46% and 92.05 % reduction,respectively.The two examined yeast strains exhibit maximum Pb removal with 100 %.Results of selenium showed that *B. megaterium*, 6SB gave the bighest value of 84.16% reduction. Kinetics of aluminium bioaccumulation, recovery of alum sludge aluminium and kinetics of selenium bioaccumulation were also carried out. Addition the microbial cells to the tested alum sludge reached the equilibrium in ten min.Observed rapid bioaccumulation indicated that this was not specific accumulation process.The eluted percent of Al^{3+} which adsorbed on the cell surface of the tested yeast strains, *S. cerevisiae*, SC64 and *S.cerevisiae*,SC66 using 0.2 M H_2SO_4 were 96, 96 and 82.67 % and 96.91, 88 and 80 % from Al^{3+}

doses (100, 200 and 300 ppm) used. The observed results of *B. megaterium*, 6SB confirm that the test biomass exhibited a rapid cation uptake from 0.913 to 0.275 mg /L at the first hour with more than 69.88 %, and with metal uptake value of 0.056 mg /g dry biosorbents.The observed results show that more than 75.36 % of equilibrium is reached within three hours. The yeast strain *S. cerevisiae*,SC66 gave Se removal of 72.62 % after the first hour of the incubation period. These results prove the possibility to recycle such waste as a part for taking a part of resolving of the environmental pollution problem.

Key words: Microbial treatment, alum sludge industrial effluent, *Bacillus megaterium*, 6SB, *Serratia marcescens*, 3STA, *Saccharomyces cerevisiae*, SC64 and *Saccharomyces cerevisiae*, SC66, bioaccumulation of Se and Al^3.

INTRODUCTION

Alum is used in the coagulation of raw water in almost all water treatment plants world-wide. The sludge produced from this process is usually thrown away. In Egypt, thousands of tons of alum are used annually in water treatment plants across the country, costing millions of dollars **[Andia & Martin (2002)]**.Alum recovery has recently gained more attention because many water utilities need to improve their sludge handling and disposal practices. As part of an overall sludge management program, alum recovery can reduce the amount of solids and allow for reuse of the recovered alum as a coagulant. It also has other potential uses such as phosphorus control at wastewater treatment plants. Although the exact nature of the biotransformed aluminum and the lipids associated with it have to await further delineation, it is not unlikely the latter moieties may have an important role in the detoxification of the metal. This aluminum evoked response may have interesting implications for aluminum biochemistry and the bacterial entrapment of toxic aluminum may have potential applications in waste management industries **[Appanna *et al.*, (1994)]**. Biosorption of aluminum by sulfate-reducing bacteria isolated from uranium mine tailings was examined. The mechanism of aluminum biosorption was found to be a passive one. Freezing and thawing of the cells resulted in higher sorption of aluminum, whereas heat treatment or the uncoupler carbonyl-cyanide- m-chlorophenylhydrazone (CCCP) had no effect. The pH value had significant influence on the aluminum ion adsorption, the most absorbance being at pH 3 and 5, and the lowest at pH 7.Addition of magnesium and the presence of iron sulfide precipitate decreased aluminum sorption.The relationship between biomass and Al^{3+}ions accumulated was linear.Use of the isolates in bioremediation processes for removing aluminum from water is considered **[Hard *et al.*, (1999)]**. Acid- and aluminum (Al)-tolerant microorganisms were isolated from tea fields, from which six strains were selected and identified as *Cryptococcus humicolal, Rhodotorula glutinis, Aspergillus flavus* Link, *Penicillium sp,Penicillium janthinellum* Biourge and *Trichoderma asperellum*. They were tolerant to Al up to 100-200 mM and could grow at low pH, 2.2-2.5. In a glucose medium (pH 3.5) the pH of the spent medium decreased to below 3.0. The toxic inorganic monomeric Al in the spent medium decreased with three strains (*A.flavus* P-6b, *Penicillium sp.* F-8b and *P.*

janthinellum F-13), but the total Al remained constant for all strains. In a soil extract medium (pH 3.5), the pH of the spent medium of all strains increased to around 6.0-7.2 and total Al in the spent medium was removed by precipitation due to pH increase.Thus,different tolerance mechanisms were suggested in glucose and soil extract media **[Kawai *et al.*, (2000)]**.

MATERIALS AND METHODS

Alum sludge effluent samples were obtained from water treatment plant of Sherbin city,Dakahlia Governorate **[summer (2003)]**. All samples were collected in 5 liter polyethylene bottles for the chemical analysis and one litre sterile glass bottle for microbiological analysis using the procedures recorded in the Standard Methods Examination of Water and Waste water **[APHA (1992)]**.

Chemical analysis:

All physicochemical parameters, organic constituents and trace metal ions determinations of industrial effluents samples were determined according to the standard methods mentioned in **[APHA (1992)]**.Aluminium,Al^{3+} (Eriochrome Cyanine R Method) and chromium (Cr^{6+}) (Diphenylcarbazide Method) were determined by measuring the absorbance (A) at λ max =535 nm and λ max =540 nm, with a light path of 1cm or longer using Spekol II spectrophotometer, respectively. A perkin-Elmer MHS-10 hydride generation system and 10 cm single slot burner attachments were used in Selenium determination as mentioned in **[APHA (1992)]**.

Metal accumulation experiments:

Batch type metal accumulation experiments were performed by using free cells. 5 mL of the cell suspension (3 g/L) were added to flasks containing 50 mL of sterilized tested industrial effluent. Samples of 2.5 mL were used for metal determination after 24, 48, 72 and 96 h, and acidified with 1% (v/v) of HNO_3 and stored at -20°C. The amount of tested metals (Al, Se and Cr) accumulated by the free microbial cells was determined by measuring the residual metal concentration in the supernatant after centrifugation at 6000 rpm for 20 min using VIO MED Export Centrifuge (USSR) and after filtrating using 0.2 µm (Ø 50 mm) membrane filter, ref No. 404114, Scheicher and Schüll, IDD, West Germany. Controls without cells are treated in the same manner. The initial and residual metal concentrations were determined by specific method as described above.

Desorption and Recovery of Al metal:

Sulphuric acid was used for Al metal desorbent in the examined free microbial cells. After metal accumulation for 24 hr at 30°C, cells were separated by centrifugation and the supernatant solutions were removed and filtered by 0.2 µm membrane filters, acidified with 1% (v/v) of $HN0_3$ and stored at -20 °C. The cell pellets were then washed with 25 ml of sterilized bidistilled water and, the supernatant obtained from the wash was acidified and stored as described above. Cell pellets were suspended in 10 ml of desorbent at 25 °C, centrifuged at 6000 rpm for 15 min and the supernatant solutions were acidified until measurement.

Microbiological examination:

The used microbes were *Bacillus megaterium*, 6SB, *Serratia marcescens*, 3STA, *Saccharomyces cerevisiae*, SC64 and *Saccharomyces cerevisiae*, SC66. These two yeast strains was kindly taken from Prof. Dr. S. A. El-Saied,Professor of Microbiology,Dept.Microbiol,Soil, Water and Environmental Res. Institute, Ministry of Agric, Giza, Egypt. These two yeast strains were recommended for its efficiencies of metal ion removal.

All the cultivation media used in this investigation were prepared as described in **[MERK (1994)]**. Standard inocula were prepared of each strain either bacteria or yeast by scrapping the 24 h old growth on slope agar with 5 mL sterilized water and transferred into 45 mL appropriate sterilized liquid TGY medium in 250 mL. Erlenmeyer flasks and then allowed to grow for 24 h to obtain abundant growth (5×10^5 cfu/mL) to be suitable for inoculating the experimental media for a required purpose. The experimental media used were the industrial effluents after filtration and dilution to appropriate ratio.

Optimization for microbial growth:

Effect of dilution ratio:

The prepared ratios of dilutions were ranged from 0.00 to 75 % for sugar beet (SB) industrial effluents. The bacterial propagation was carried out using 250 ml of Erlenmeyer flasks; each contains 50 ml of the tested industrial effluents inoculated with 1 ml inoculum of bacterial broth. The flasks were then incubated at 30^0C. The developed colonies were counted as colony forming units (cfu) per ml of the tested waste after plating on a nutrient agar (NA) medium.

Effect of initial pH:

This experiment was carried out to detect the ability of different bacterial strains to grow on the tested waste at different initial pH values. Therefore, the bacterial propagation was carried out using triplicate of 250 mL.Erlenmeyer flasks, each containing 50 ml of 50% concentration of sugar beet (SB) industrial effluent. The pH value was adjusted to 3, 5, 7 or 9, and then each flask was inoculated with 1 ml inoculum of bacterial broth. The flasks were then incubated at 30 ^{0}C and the developed colonies were counted as colony forming units (cfu) per ml of the tested waste after plating on NA medium.

Effect of inoculum size:

The microbial propagation was carried out using triplicate of 250 ml. Erlenmeyer flasks, each containing 50 ml of 50% concentration of sugar beet (SB) industrial effluent. Then the flasks were inoculated with different inoculum sizes (v/v) of 2, 4, 6, 8 or 10 % of bacterial broth. The flasks were then incubated at 30^0C and the developed colonies were counted as colony forming units (cfu) per ml of the tested waste after plating on NA medium.

Effect of yeast extract addition:

A weight of 0.2 % (w / v) of yeast extract was added to a one series of experimental flasks for the tested waste All the experimental results were subjected to statistical analysis. **[SAS (1989)].**

RESULTS AND DISCUSSION

Chemical analysis:

Alum sludge of SWT (clarifier sludge) is directly discharged to Al-Sahel canal. At the moment, the alum sludge is not listed as an industrial effluent in Egypt, while the Environmental Protection Agency (EPA) has classified alum sludge obtained from water treatment plants as an industrial wastewater **[Gruninger (1975)]**.These effluent samples are belonging to the article No. 61 of Law 48/1982.

As shown in **Table (1)**, alum sludge effluent samples have mean value of pH equal to 7.3 that nearly neutral. This value was within pH range of the article No.61 of the Egyptian Law 48/1982 being 6-9. The measured concentration of TS and TDS were 390 and 354 mg/L that were within the Egyptian standard (ES) level of the article No. 61 of Law 48/1982 (TDS > 800 mg/L). At the same time, the mean value of TSS concentration was 36 mg/L for alum sludge effluent samples, that exceeds the ES level of the article No. 61 of Law 48/1982 (TSS >30 mg/L).

Table (1): Physicochemical analysis of alum sludge effluent.

Physicochemical parameters (mg/L)								
TS	TDS	TSS	Hardness	Calcium	Chloride	Sulfate		
390	354	36	156	38.477	42.046	56.375		
TOC		TN		COD		BOD		
320.97		2.24		363.17		3.615		
Trace metal ions (mg/L)								
Al	Cd	Cr^{6+}	Cu	Fe	Mn	Pb	Se	Zn
9.547	0.176	0.88	1.9	25.278	0.095	0.125	0.913	2.23

Results recorded in **Table (1)** show that the hardness and Ca ion concentration were not high; their mean values equal to 156 and 38.477 mg/L, respectively. On the other hand, chloride and sulfate concentration equal to 42.046 and 56.375 mg/L, respectively. **[El-Fadaly *et al.*, (2000)]** observed that SWT, for the same sample, pH equal 8.26 which slightly basic where TDS, value of the hardness, calcium, chloride and sulfate concentrations were 191, 155.3, 37.8, 39.99 and 39.5 mg/L, respectively. These results show that TDS increased by 2.04 fold, but that the hardness and Calcium ion concentration were nearly the same. On the other hand, sulfate concentration was increased by 1.43 fold more than that obtained in year 2000. Regarding the organic constituents, the observed mean values of TOC, TN, COD and BOD were 320.97 mg/L, 2.24 mg/L, 363.17 and 3.615 mg/L, respectively. In this case,

COD level exceeds the ES (COD > 30 mg/L). On the other hand, BOD value was within the ES level by the article No.61, since the standard level must not exceed 20 mg/L.[**Makia (2000)**] indicated that BOD was 9.69 mg/L, which means that BOD decreased by 2.68 fold than that value obtained in year 2000. [**Sengupta & Shi (1992)**] found that the TOC concentration was 860 mg/L in samples of Allentown WTP.

Total aluminium concentration was detected as 9.547 mg/L. In the WTP in Dakahlia Governorate, approximately 17.423 tonnes of aluminium- alum sludge are directly discharged into Al-Sahel canal every year. The importance of aluminium in sludge and mass discharged into surface water without treatment was discussed by many workers. Few researches have been carried out on the periodical discharge of sludge into the surface waters. In the Volta Redonda WTP, approximately 19 tonnes of sludge are discharged in the main stream every 75 days while in the Guandu WTP almost 4 tonnes of sludge are discharged daily. This represents approximately 1.77 tonnes/year of aluminium discharged in the Paraiba River [**Azcue *et al.*, (1994)**]. The mean concentration of aluminium in the edible portion and intestines of fish was varied from 14 to 1350 mg/kg wet weight, respectively. Meanwhile, the guideline level recommended by the [**WHO (1993)**] is 200µg/L. So, there is, however, information regarding the toxic effects of alum sludge discharge in water on other aquatic organisms such as benthic invertebrates.

Heavy metal ions such as Cd, Cr^{6+}, Cu, Fe, Mn, Pb and Zn. were also detected. Concentrations of Cd, Cr^{6+} and Cu were equal to 0.176, 0.88 and 1.9 mg/L, respectively. Levels of these heavy metals exceeded the ES (Cd < 0.01, Cr^{6+} < 0.05 and Cu < 1mg/L). Fe, Mn, Pb and Zn Concentrations values were 25.278, 0.095, 0.125 and 2.23 mg/L, respectively. The levels of Fe and Pb were higher than the ES since the standard level must not exceed 1 and 0.05 mg/l, respectively. Conversely, Mn and Zn levels were within the ES (Mn < 0.5 mg/L and Zn < 1 mg/L). Selenium concentration was 0.913 mg/L.[**Sengupta & Shi (1992)**] pointed that the total aluminium was 5.60 mg/L, pH = 7.1, while TSS and Ca values of clarifier alum sludge from Allentown WTP were 112 and 30 mg/L, respectively. Also, their results showed that Fe, Mn, Zn and Cu concentrations were 1.7, 85, 23 and 3.2 mg/L, respectively. They added that copper, lead, cadmium, and other heavy metal concentration in the alum sludge was normally low, but it may warrant concern if recycled through recovered alum. The values of these metals measured by [**Makia (2000)**] were found to be 0.635, 0.087, 0.064, 0.101, and 0. 442, 0.191, and 0.590 mg/L for, Cd, Cr^{6+}, Cu, Fe, Mn, Pb, and Zn, respectively. This mean that Cd, Cr^{6+}, Cu , Fe, Pb and Zn metal ion concentrations increased from year 2000 by 2.77, 10.12, 29.69, 250.28, 0.65 and 3.779 fold, respectively. Conversely, Mn ion concentration was decreased by 4.65 fold.

Optimization of microbial growth:
Dilution ratio:

Four dilutions (0.00, 25, 50 and 75%) of the tested effluent samples investigated as shown in **Fig (1)**, the TMC of *B.megaterium*, 6SB that gave about 0.60, 0.166, 0.138 and 0.138 x 10^8 cfu/mL after 24 h, respectively. This mean that TMC was increased by 1.84 fold for 75 and 50%, while 2.213 and 8 fold for dilutions of 25 % and 0.00 %, respectively. At incubation period of 48 h, the treatment of 75% dilution was reached its maximum of the total count that equal to 5.245 x10^8cfu/mL, while 50 %

dilution sample achieved the maximum value of the TMC equal to 3.461 x10^8 cfu/ mL. On the other hand, 50 % dilutions exhibited the maximum value of the TMC being 4.51 x10^8cfu/mL after 72 h, respectively. All values were decreased in all treatment after 96 h incubation.

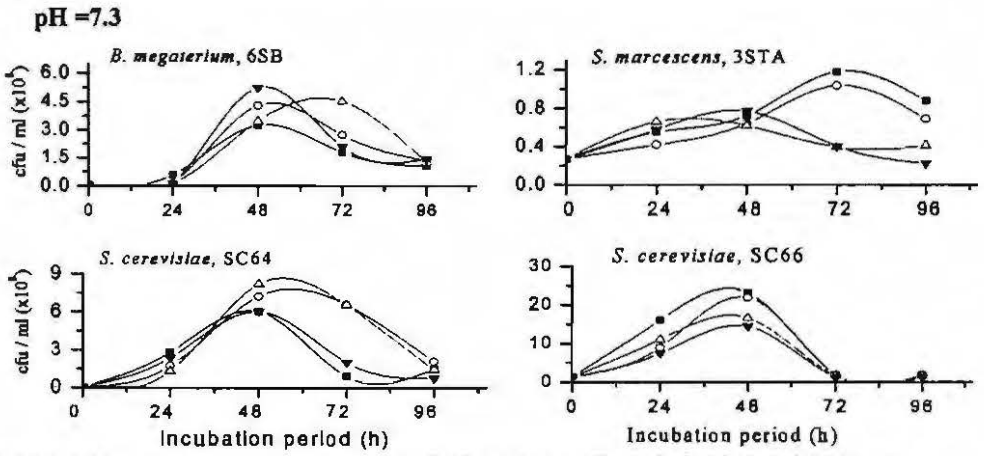

Fig (1): Growth dynamic the tested microbial strains on alum sludge industrial effluent

plotted results **Fig (1)** for *S. marcescens*, 3STA showed that all dilutions exhibited the TMC values more or less close to each other, being 0.549 ± 0.069, 0.416 ±0.035, 0.658 ± 0.048 and 0.576 ± 0.109 x10^8 cfu/mL , and 0.722 ± 0.111, 0.640±0.088, 0.617± 0.069 and 0.768±0.119 x10^8 cfu/mL after 24 and 48 h, respectively. The growth of *S. marcescens*, 3STA in the tested waste (dilutions 50% and 75%) were decreased to 0.393±0.032x10^8 cfu/mL, 0.402±0.069x10^8 cfu/mL and 0.407±0.021x10^8 cfu/mL and 0.2195±0.027 x10^8 cfu/mL, after 72 and 96 h, respectively. On the other hand, the microbial growth was still increased to 1.179± 0.167 x10^8 cfu/mL and 1.033± 0.088 x10^8 cfu/mL in case of 0.00 and 25 % dilutions of the alum sludge effluent after 72 h, respectively. The bacteria then showed a decrease after 96 h to 0.878±0.073x10^8 cfu/mL and 0.686 ± 0.069x10^8 cfu/mL for the last dilutions, respectively.

The growth of yeast strain *S. cerevisiae*, SC64 reached 2.800 x10^8 cfu/mL for 0.00 % waste dilution, after 24h. The TMC values were found to be 6.00±0.400, 7.2±1.058, 8.167±2.673 and 6.00±0.909 x10^8 cfu/mL with 15.33 as a value of CV % for 0.00, 25, 50 and 75% dilution, after 48 h of incubation, respectively. Then the growth was gradually decreased after intervals of time of 72 and 96 h. *S.cerevisiae*, SC66 growth dynamics indicated clearly as can be seen in the same figure. **Fig. (1)** shows that 0.00 % dilution has the highest TMC value equal to 16.01±0.96 with 11.60 fold increase after 24 h.The maximum growth levels were observed for all dilutions after 48 h.The growth was then decreased at time intervals of 72 and 96 h.

The results obtained by **[Kawai *et al.*, (2000)]** using *Rhodotorula glutinis* (yeast) and *Cryptococcus humicola* (G^+ bacteria) showed that these microbes were tolerant to Al up to 100-200 mM. The same trend of these results was obtained by **[Fischer *et al.*, (2002)]** since *Acidiphilium cryptum* grew in glucose mineral medium containing 300 mM aluminium sulphate.

Initial pH:

Illustrated results **Fig (2)** confirmed that the highest growth was obtained at pH = 7.9 which represent the normal pH value of the alum sludge effluent giving 4.012 ± 0.047 x 10^8 cfu /ml, followed by pH = 7 at which the growth density equal to 3.593± 0.023 x10^8 cfu/ml after 24 h. On the other hand, pH 3 exhibited the lowest growth density being 0.977± 0.047 x x10^8 cfu/mL. interestingly, the pH 7.9 showed also the highest growth density at the end of all incubation time intervals.

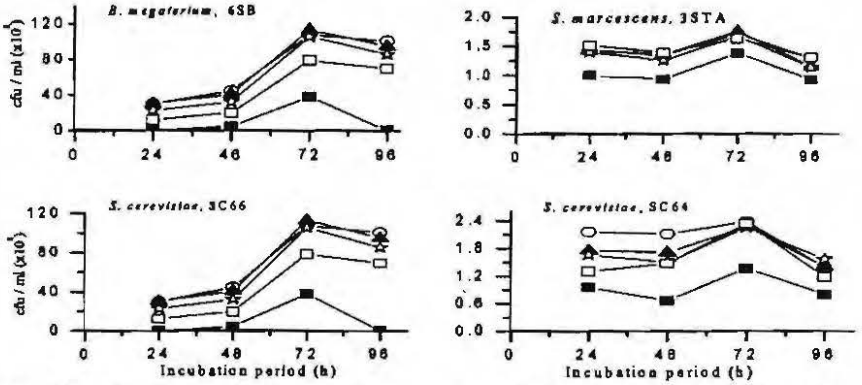

Fig (2): Effect of initial pH value on the microbial growth in alum sludge industrial effluent

The obtained values for *S marcescens,* 3STA **Fig (2)** showed that, after 24 h incubation, the values of growth density were 1.436± 0.00, 1.422 ± 0.014, 1.394± 0.014 and 1.513± 0.007 x10^8 cfu/ml at pH 5, 7, .9, and 9, respectively. The treatment of pH 3 clearly showed the lowest values of growth at all the time intervals of incubation.
Fig (2) also indicated that the highest growth of *S. cerevisiae*, SC64, was occurred at pH 5 with TMC of 2.158 ± 0.02 x10^8cfu/ml. *S. cerevisiae*, SC64 reaches its maximum growth after 72 h, where TMC at pH 5 was 2.373± 0.02 x10^8 cfu/ml which represented the highest growth value. The value of TMC for *Saccharomyces cerevisiae*, SC66 were 30.2 ± 0.95, 30.2±0.95, 22.6± 0.95 and 12.1 ± 0.95 x10^8 cfu/mL for pH 5, 7, 7.9 (normal pH of the tested waste) and 9, respectively. As observed in Fig (2), *S. cerevisiae*, SC66 reaches to its maximum growth after 72 h, since the TMC value at pH 5 was equal to113.1 ± 0.00x10^8cfu/ml. The growth values of all treatment are decreased after 96 h. Initial pH 3 again showed the lowest growth values at all the intervals time of incubation for the two tested yeast strains. **[Kawai *et al.*, (2000)]** isolated microorganisms that could grow at low pH of 2.5 -2.2. They added that when the pH value increased to around 6.0, 7.2, the total Al in the medium was recovered by precipitation due to increase.of pH.

Inoculum size:

The importance of inoculum size (%) in the determination of optimum growth has been clearly observed when 2, 4, 6, 8 and 10 % of living free cells were used.As shown in **Table (2)** when the inoculum size increased from 2 to 10 %, the value TMC was gradually increased for the all tested organisms after 24 h incubation. For bacterial strains, *B. megaterium*, 6SB and *S. marcescens*, 3STA the TMC value increased from 0.12±0.001 to 1.035±.023 (352.7 %) x10^8 cfu/ml and from 0.812 ±0.007 to 1.403±0.008 x10^8 cfu/ml which equal to 120.6 %, with CV % values of 67.38 and 21.82 % for 2 % and 10 %, respectively. Also the values of TMC increased for the two yeast strains, *S. cerevisiae*, SC64 *and S. cerevisiae*, SC66 from 0 .670±0.011 to 1.034±.011 x10^8 cfu/ml, and from 18.13± 0..550 to 27.02±0.550 x10^8 cfu/ml, with CV % of 18.83 and 17.06 % for 2 % and 10 % respectively.

Table (2): Effect of inoculum size (%) on the tested microbial strains growth on alum sludge industrial effluent after 24 hrs.

Inoculum size (%)	cfu x 10^8 / ml after h incubation			
	B. megaterium, 6SB	*S. marcescens*, 3STA	*S. cerevisiae*, SC64	*S. cerevisiae*, SC66
2	0.120 ± 0.001	0.812 ± 0.007	0.670 ± 0.011	18.13 ± 0.550
4	0.422 ± 0.013	0.983 ± 0.008	0.756 ± 0.011	19.24 ± 3.610
6	0.632 ± 0.013	1.177 ± 0.007	0.852 ± 0.021	19.24 ± 0.480
8	0.876 ± 0.007	1.359 ± 0.007	1.034 ± 0.011	20.67 ± 0.960
10	1.035 ± 0.023	1.403 ± 0.008	1.034 ±0 .011	27.02 ± 0.550
mean	*0. 62*	*1.15*	*0.87*	*20.86*
SD	*•,36*	*0.25*	*0.16*	*3.56*
CV %	*58.83*	*21.82*	*18.83*	*17.06*

Monitoring of microbiological treatment:

Dry weight:

Biomass yield was represented by measuring the dry weight of the microbial cells harvested after 72 h incubation period as shown in **Fig (3).** Firstly for *B. megaterium*, 6SB dry weight values were 1, 1.214 and 1.714 mg/L with 27.99 as a CV % for 50, 25 and 0.00%, respectively. This means that 0.00 % dilution of the alum sludge effluent exhibited the highest dry weight value but with a little difference from the other two dilutions. Also, dry weight mean values of *S. marcescens*, 3STA were 1.474, 1.36 and 1.263 mg/L with 7.73 CV % for the aforementioned dilutions. On the other hand *S.cerevisiae*, SC64 and *S. cerevisiae*, SC66, exhibited dry biomass values of 2.133, 1.923 and 2.133 mg/L with 5.88 as a CV % and 1.357, 1.4 and 1.2 mg/L with 7.98 as CV % for 50, 25 and 0.00% diluted waste, respectively.

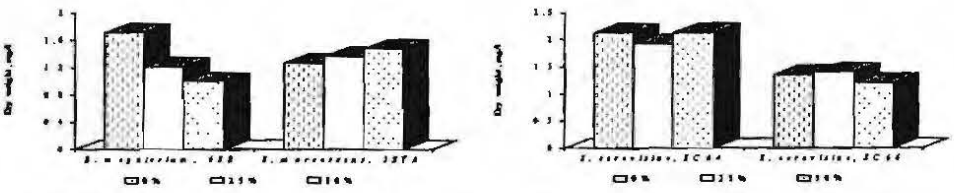

Fig (3): Microbial dry weight obtained by the tested microorganisms from examined alum sludge industrial effluent.

Microbial growth dynamics:

B. megaterium, 6SB, *S. marcescens*, 3STA, *S. cerevisiae*, SC64 and S. *cerevisiae*, SC66 were grown at different dilutions of alum sludge industrial effluent 0.00, 25 and 50 % and their efficiencies for aluminum removal, at intervals time were studied.

The value of CV % was 40.62, 44.99 and 125.46 % of *B. megaterium*, 6SB, after 24, 48 and 72 h, respectively **Fig (4).** The highest TMC value at 0.00% dilution was recorded to be $2.163x10^8$cfu/mL after 72 h. Results showed that the highest growth after 48 h observed for dilution of 25 % being $3.429x10^8$cfu/mL. At the same time aluminum removal % for alum sludge effluent dilutions 0.00, 25, and 50 % were 90.140, 92.197 and 94.287 % with CV % value of 2.25, with small differences between them after 24 h incubation period. These results mean that about 90.140 % of the total aluminum in the tested effluent was removed rapidly at the first day. The removed % of aluminum was almost the same after 48 and 72 h as seen in **Fig (4).** A little difference in value not exceeded 2.5 % or less was ranged from 93.302 to 95.401 % as a mean value after 48 h. Aluminum metal uptake (Q) after 24 h at 0.00, 25 and 50 % values were 0.251, 0.183 and 0.117 mg Al^{3+}/ g dry biosorbent with CV % value of 36.30. This means that the highest Q value was observed with dilution 0.00 %. *B. megaterium*, 6SB did not show any observed differences in metal accumulation at 48 and 72 h which showed the same values to be 0.252, 0.85 and 0.126 mg/g biosorbent with CV % value of 33.85 with the three mentioned dilutions.

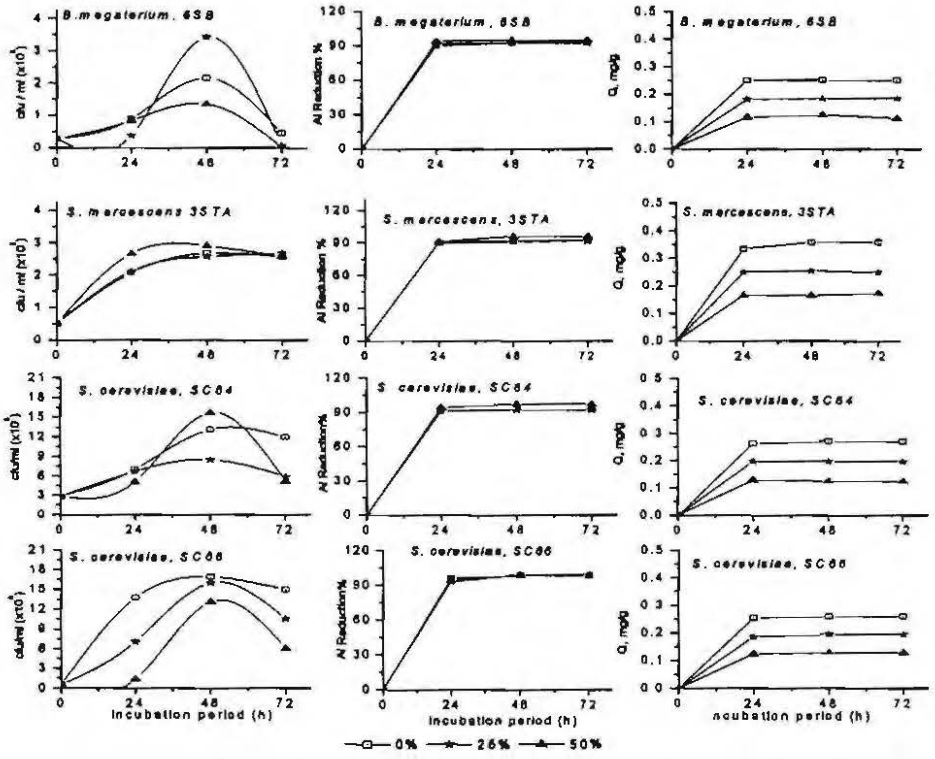

Fig 4. Growth dynamic, bioreduction percent of Al and metal uptake (Q, mg/g) occurred by microbial strains on alum sludge industrial effluent

Results in **Fig (4)** showed the total microbial count (TMC) values, aluminum reduction % and Al^{3+} accumulation (Q) by *S. marcescens*, 3STA at the three dilutions 0.00, 25 and 50 %. These values were 2.071, 2.123 and 2.669 $x10^8$ cfu/mL , 90.551, 90.888 and 91.19 3 % and 0.334, 0.250 and 0.167 mg /g biosorbent after 24 h, respectively. These results indicated that the differences of the TMC of S. *marcescens*, 3STA in the three dilutions was still low and did not exceeded 14.48 as a value of CV %. The Al^{3+} reduction % corresponding to the mentioned TMC values were 90.551, 90.888 and 91.19 3 %, respectively. This also means that the values of Al^{3+} reduction % were very close to each other with a little difference between them,. that is to say that about 90.551% of the initial aluminum was uptake by the microorganism at the first 24 h. Furthermore, metal (Al) accumulation was equal to 0.334 mg/g biosorbent when the total count was 2.071 $x10^8$ cfu/ml at 0.00 % dilution,. that was considered to be the highest value of Q. Results showed also the negative relation between TMC value and corresponding value of Q as can see in **Fig (4).**

Results listed in Fig (4) further show that the two yeast strains *S. cerevisiae*, SC64 and S. *cerevisiae,* SC66 have the same behavior of that the two bacterial strains illustrated above. The highest TMC of *S. cerevisiae*, SC64 was 6.987 $x10^8$ cfu/mL at 0.00 % dilution (100% waste) after 24 h incubation period.The 15.629$x10^8$cfu/mL was achieved as the highest total count followed by 13.083$x10^8$cfu/mL for 50 and 0.00 %

dilution % with CV % value of 29.37,after 48 h, respectively.Al^{3+} reduction % was very close to each other since the values were 91.199, 92.487 and 94.469 %. Al^{3+} reduction % was showed the same values to be 92.451, 92.144 and 97.475 % at 0.00, 25 and 50% diluted waste, after 48 and 72 h, respectively. Metal uptake or metal (Al) accumulation values (Q) after 24 h at 0.00, 25 and 50 % were 0.263, 0.198 and 0.130 mg Al^{3+}/g dry biosorbent. This means that metal accumulation increased with decreasing dilution%. Metal accumulation increased with increasing of aluminum concentration since Q value was increased by 2.02 at 0.00 % dilution than 50%, and increased by 1.52 at 25 % than 50 % of dilution. Also, as in case of Al^{3+} reduction %, metal uptake maximum values were achieved at the first 24 h of the incubation period.The highest total count values of S. *cerevisiae*, SC66 at 0.00 % dilution with 13.695, 16.863 and 14.962 $x10^8$ cfu/ml after incubation periods of 24, 48 and 72 h, respectively. These values against Al^{3+} reduction % maximum values of 96.295, 99.850 and 98.850 % at incubation periods of 24, 48 and 72 h, respectively. Illustrated maximum metal uptake values for Al^{3+} being 0.255, 0.261 and 0.261 mg/g dry biosorbent for the same intervals time **Fig (4)**. The above results concluded that 0.00 % dilution of alum sludge (100 %) was the most suitable treatment for aluminum accumulation experiments.

Bioremoval of some trace metal ions:

Plotted results in **Fig.(5)** illustrates the values of some trace metals reduction (%) as a result of the tested microbes in alum sludge as a culture medium For Cd removal, *B. megaterium*, 6SB and *S. cerevisiae*, SC64 showed the most active strains since they gave 100% removal. While, *S. marcescens*, 3STA and *S. cerevisiae*, SC66 exhibited 88.333 % and 82.50, removal respectively. The highest reduction value of Cr^{6+} was achieved by *S. cerevisiae*, SC66 and *S.cerevisiae*, SC64 being 95.46 % and 92.05 %,respectively.The highest values of Cu removal were achieved by *S. cerevisiae*, SC64 and *S.cerevisiae*, SC66 *which* equal to 86.05 % and72.09 % with fold decrease of 7.166 and 3.583, respectively. At the same time results showed that *S. marcescens*,3STA was the most efficient in removing Fe followed by *S. cerevisiae*, SC66 being of 48.72 and 43.59 reduction %, respectively. *B. megaterium*, 6SB came first in case of Mn removal, giving 66.93% reduction followed by 33.86 % by S. *marcescens*, 3STA or *S. cerevisiae*, SC66.

As illustrated in **Fig. (5)**, the two examined yeast strains exhibited maximum Pb removal with 100 % reduction. Results of selenium removal showed that *B. megaterium*, 6SB gave the highest value of 84.16 reduction% which represented the most effective one followed by *S. cerevisiae*, SC64 and *S. cerevisiae*, SC66 giving 82.497 and 72.38 reduction %, respectively.*S. cerevisiae*, SC66 exhibited the maximum removal % of Zn of 93.27 with 14.86 fold decrease, while 79.81 % reduction (4.953 fold) was recorded by *S. cerevisiae*, SC64. This means that yeast strains exhibited the highest removal efficiency for zinc metal. *S. marcescens*, 3STA came second to show 61.31 reduction% (2.584 fold) of Zn while *B. megaterium*, 6SB gave the lowest removal % for Zn equal to 34.39 % with 1.524 fold decreases. These results are in agreement with those obtained by **[Hu & Boyer (1996)]** who decided that bacteria accumulate and immobilize heavy metals. They added that the cell wall of Gram positive bacteria have strong metal- binding properties.

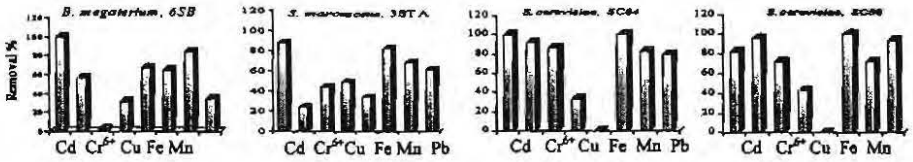

Fig (5): Bioreduction percent of some metal ions from alum sludge industrial effluent.

Kinetics of aluminium bioaccumulation:

The aluminium bioaccumulation was very rapid by free microbial cells the equilibrium.Addition of the microbial cells to the tested alum sludge reaches the equilibrium in ten min.This was approved by the measurement of Al^{3+} in the supernatant in which the Al^{3+} concentration dropped rapidly. Al^{3+} removal efficiency was 98.675±0.02, 98.88±0.02, and 87.28±1.26 and 86.64±0.02 percent for *B. megaterium*, 6SB, *S. marcescens*, 3STA, *S. cerevisiae*, SC64 and *S. cerevisiae*, SC66, respectively. Metal uptake with the dry weight of cells that 1.7, 1.3, 1.5 and 1.8 mg/L were0.824±0.002,1.064±0.001,0.820±0.001and 0.689±0.07 mg Al^{3+}/g dry cells (biosorbent).Observed rapid bioaccumulation indicated that this was not specific accumulation process. For *B. megaterium*, 6SB and *S. marcescens*, 3STA, aluminium concentration was directly decreased at the first ten minutes from 9.55±0.06 mg /L to 0.13 ±0.01 and 0.11±0.01 mg/L with a highly CV% values of 79.167 and 68.037%, respectively. That is to say that the metal accumulation were reached to their maximum values at the first ten min being 0.824±0.002 and 1.064±0.001mg/g cells of *B. megaterium*, 6SB and *S. marcescens*, 3STA, respectively. At the same time from the first ten min to the end of 48 h interval time, the metal reduction % were changed from 98.675±0.02 to 99.929±0.02 % with a little change in its uptake values from 0.8244±0.002 to 0.8256±0.002 mg/g cells with 2.37 and 2.38 as CV% values, respectively. This behavior was repeated with the other three microorganisms as shown in **Fig (6).**

The accumulation of aluminium by microorganisms is of great importance since the high dose in drinking water can cause many reported health effect although allegation of impaired mental function have not been substantiated **[Andia & Martin (2002)]**. Aluminium can also increase the acidity of water which causes the solution of Cu from pipes and Pb from solders **[Campbell (2002) and Appanna *et al.*, (1994)]**demonstrated that *Pseudomonas fluorescens* (G^- bacteria) survives in an aluminium rich environment by trapping the toxic metal as an insoluble phosphorus residue rich in lipids. In agreement with these results, **[Hu & Boyer (1996)]** reported that *Bacillus megaterium* (G^+ bacteria) had the ability to accumulate Al^{3+} and/or Fe^{3+} through forms complexes with siderophores. They added that the cell wall of G^+ bacteria have strong metal- binding properties. In addition **[Hard *et al.*, (1999)]** observed that the interaction between aluminium and sulfate-reducing bacteria is a passive one and occurs on the surface of the cells. It is independent on the physiological status of the cells, which can be alive or dead, intact or broken.

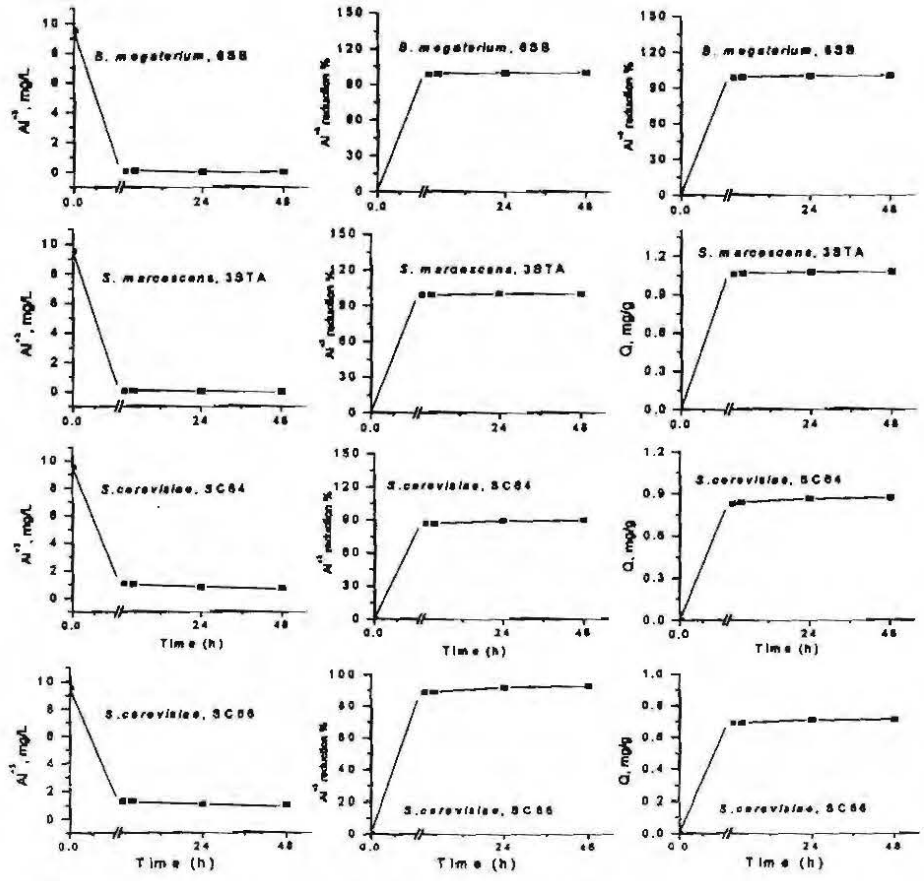

Fig (6): Growth dynamic, bioreduction percent and Q (mg/g) of aluminium metal occurred by the tested bacterial strains on alum sludge industrial effluent samples

Recovery of alum Sludge aluminium:

Hoping to save money and to reduce solid waste, water utilities are evaluating various alternatives for the disposal of alum sludge industrial effluent (mainly clarifier sludge) containing 30 to 50 percent hydrated aluminium hydroxide. Water Treatment Plant showed that alum can be recovered. Alum that is recovered can be caused as a coagulant, there by reducing operating costs **[Sengupta & Shi (1992)]**. Both *S. cerevisiae*, SC64 and *S. cerevisiae*, SC66, as detected in **Fig (7)**, were represented excellent viable biomass for aluminium bioaccumulation. The residual percent from 100, 200 and 300 mg/L aluminium doses were 5.6, 13.2 and 4.97 %, and 22.49, 7.81 and 5.57 % for *S. cerevisiae*, SC64 and *S. cerevisiae*, SC66, respectively. The eluted

percent of Al^{3+} which adsorbed on the cell surface of the tested yeast strains *S. cerevisiae*, SC64 and *S. cerevisiae*, SC66 using 0.2 M H_2SO_4 were equal to 96, 96 and 82.67 %, and 96.91, 88 and 80% from used Al^{3+} doses of 100, 200 and 300 mg/L, respectively.

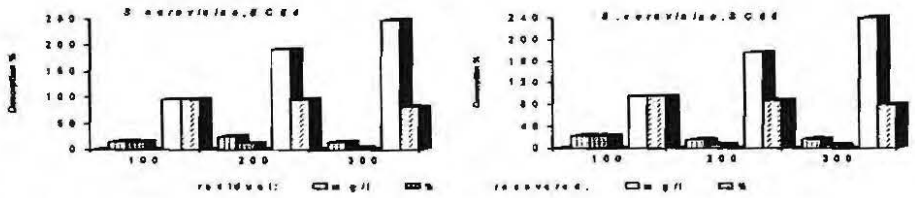

Fig (7): Aluminium desorption by yeast free cells after bioaccumulation using different Al^{3+} doses

Kinetics of selenium bioaccumulation:

Selenium bioaccumulation (metal removal) by living free microbial cells was done by using 1.714 g and 1.8 g dry biosorbents of *B. megaterium*, 6SB and *S. cerevisiae*, SC66. As shown in **Fig (8)**, obtained results of *B. megaterium*, 6SB confirmed that the test biomass exhibited a rapid cation uptake from 0.913 to 0.275 mg/L with CV % value of 102.54 at the first hour with more than 69.88 %, and with metal uptake value of 0.056 mg/g dry biosorbent. More than 75.36 % of equilibrium was reached within three hours with Q value of 0.060 mg/g dry biosorbent. At the end of 48 h, the maximum selenium uptake was equal to 0.075 mg /g dry biosorbent which represented 91.79 reduction percent. At the same time the residual selenium concentration remains in the solution was reached to 0.073 mg/L. The yeast strain *S. cerevisiae*, SC66 gave Se removal by 72.62 % and Q value was equal to 0.055 mg/g at the first hour of the incubation period. At the end of 48 h, the Se reduction % reached to 97.26 % with Q value of 0.074 mg/g dry biosorbent.

On the other hand, **[Tinggi (2003)]** showed that Se deficiency causes health implications in humans and animals. It is also very toxic in high concentrations that can cause Se poisoning which called selenosis in humans and animals. Deficiency of Se has caused health problems to livestock; however, the problems were eliminated after adding Se supplementation. Any how, the major source of Se is diet, and the levels of Se in the soils generally reflect the Se status in human population. The bioavailability and toxicity of Se depend on its chemical forms. Generally, organic forms of Se are more bioavailable and less toxic than the inorganic forms (selenites, selenates) **[Tinggi (2003)]**. Further more, the dermatologic effects after exposure to high levels of environmental selenium was also considered **[Vinceti *et al.*, (2001)]**.

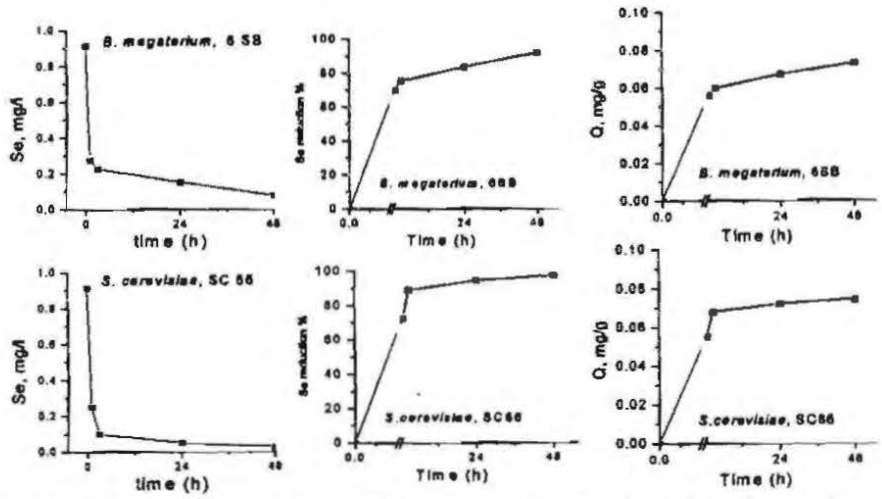

Fig (8): Selenium reduction concentration (mg /L), percent and metal uptake, mg /g From alum sludge industrial effluent samples.

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الملخص العربى

المعالجة الميكروبيولوجية للمخلفات الصناعية السائلة الناتجة عن محطات تنقية مياه الشرب

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أجريت التحاليل الفيزيائية والكيميائية لعينات المخلفات الصناعية السائلة الناتجة عن محطات تنقية مياه الشرب. وقد إشتملت هذه القياسات على تقدير الأملاح الكلية ، الأملاح الكلية الذائبة، المواد الصلبة العالقة، العسر الكلى ، الكالسيوم ، الكلوريدات ، والكبريتات. وكذلك إشتملت الدراسة على تقدير بعض المكونات العضوية لهذه العينات مثل الكربون العضوى الكلى، الأكسجين الحيوى الممتص، الأكسجين الكيميائى المستهلك، والنيتروجين الكلى. وأيضا عنصرى الألومنيوم والسيلينيوم ، بالإضافة إلى بعض العناصر الثقيلة مثل الكادميوم، الكروم السداسى، النحاس، الحديد، المنجنيز، الرصاص، والزنك. وأظهرت هذه الدراسة مدى نجاح المعالجة الميكروبيولوجية للمخلفات الصناعية السائلة الناتجة عن محطات تنقية مياه الشرب باستخدام أجناس البكتيريا *B. megaterium*, 6SB ، *S. marcescens*, 3STA ، وأجناس الخميرة *S. cerevisiae*, SC64 و *S. cerevisiae*, SC66 فى إزالة الملوثات العضوية والمعدنية السامة. وإشتملت الدراسة على العوامل المثلى للنمو الميكروبى ، وهى نسبة التخفيف للمخلف (٠,٠٠ ، ٢٥ ، ٥٠ و ٧٥ %)، والرقم الهيدروجينى، وحجم اللقاح المستخدم. وأجريت متابعة نتائج المعاملة الميكروبيولوجية بدراسة كل من الوزن الجاف للكتلة الحية الناتجة، ديناميكية النمو الميكروبى، و إزالة بعض العناصر الثقيلة . وأوضحت النتائج أنه قد تمت إزالة ٩٧,٥٨٩ %، و٨١,٤٧ % من عنصر الألومنيوم الكلى بالمخلف السائل باستخدام الخلايا الحرة لبكتيريا *B.megaterium*, 6SB ، وبكتيريا *S.marcescens*, 3STA فى ٢٤ ساعة الأولى من فترات التحضين. أما خميرة *S. cerevisiae*, SC64 ، و *S. cerevisiae*, SC66 فقد كانت أعلى القيم المتراكمة من عنصر الألومنيوم تساوى ٠,٢٦٣، و٠,٢٥٥ ملجم/ل، بعد ٢٤ساعة تحضين باستخدام المخلف السائل بدون تخفيف. وأوضحت نتائج الدراسة أن أعلى نسبة إزالة لعناصرالكادميوم، الكروم السداسى، النحاس، الحديد، المنجنيز، الزنك، والسيلينيوم كانت ١٠٠% ، ٩٥,٤٦ % ، ٨٦,٠٥ %، ٤٨,٧٢ % ، ٦٦,٩٣ % ، ١٠٠ %، و ٨٤,١٦ % على الترتيب. وأيضا تمت دراسة حركيات الإزالة الميكروبية و إسترجاع عنصر الألومنيوم ، وحركيات الإزالة الميكروبية لعنصر السيلينيوم. وبرهنت التجارب على أن إزالة عنصر الألومنيوم من المخلف السائل الناتج من محطات تنقية مياه الشرب تمت خلال ١٠ دقائق الأولى من التجربة. فقد وصلت نسبة

الإزالة إلى حوالى ٨٥ – ٩٩% باستخدام الميكروبات الأربعة . وتم إسترجاعه من الخلايا الميكروبية *S. cerevisiae*, SC64 و*S. cerevisiae*, SC66 بنسبة تصل إلى (٩٦ ،٩٦ و٨٢,٦٧ %) و (٩٦,٩١ ، ٨٨ ، ٨٠ %) بعد إمتصاصها لهذا العنصر من المخلف الذى يحتوى على تركيزات ١٠٠ ،٢٠٠ ، و٣٠٠ ملجم/ل من عنصر الألومنيوم، على الترتيب. ودلت النتائج على نجاح إزالة عنصر السيليكون بواسطة بكتيريا *B. megaterium*, 6SB وخميرة *S. cerevisiae*, SC66 بنسبة وصلت إلى ٦٩,٨٨% و ٧٢,٦٢ % خلال الساعة الأولى من التجربة والتى تعادل ٠,٠٥٦ و٠,٠٧٤ ملجم سيليكون لكل جرام من الخلايا الجافة على الترتيب.