Australian Journal of Basic and Applied Sciences, 2(3): 685-700, 2008
ISSN 1991-8178

Effect of Yeast and Bacterial Recombinants on the Uptake of Heavy Metals from Wastewater

[1]Zaied, K.A., [3]H.N. Abd El - Mageed, [2]E.A. Fayzalla, [4]A.E. Sharief, [5]A.A. Zehry,

[1]Departments of Genetics, [2]Phytopathology, [3]Agric. Engineering, [4]Agronomy,
Faculty of Agriculture, Mansoura University, Egypt.
[5]Agric. Extention, Water, Soil and Ministry of Agriculture, Egypt.

Abstract: Water standards have been set and guidelines proposed by many countries and several intergovernmental organizations to determine the acceptable human exposure to certain environmental pollutants in drinking water. Ten bacterial strains and also seven *Saccharomyces cerevisiae* strains were used in this study . Bacterial strains were marking using 19 antibiotics to be use as a selectable markers in conjugation process. The available markers obtained were used to done 14 mating, 10 of them were success, two transcongugants from each conjugation were selected to be use in biosorption experiments. Two from *Saccharomyces cerevisiae* strains were mated and the hybrids were isolated to be use in uptake experiments. This leading us to developing biotechnology for use in pollution control of hazardous wastes. Modern ecological biotechnology attempts to solve the problems of pollution by screening and molecularly breeding microbial strains that are capable to biosorp heavy metals. This study aimed to improve the quality of wastewater and reduced the risk level associated with a major case of heavy metals pollution at the industrial area. The results appeared that *Saccharomyces cerevisiae* strain NRRL Y - 12632 was more efficient in heavy metals uptake than the other strains and hybrids resulted from the mating between two parental strains. Most of yeast hybrids appeared higher levels for all heavy metals uptake than the second parental one (*Saccharomyces cerevisiae* NRRL Y- 12632). Bacterial strains and their transconjugants were more efficient in uptake of Pb, Cd, Fe, Co and Cu (uptake percentage more than 70 % in relation to untreated control). However, lower percentage in uptake of heavy metals were achieved in Ni, Sb, Sr, V, Cr, Zn, Mo, Pt, Mn, As and Hg (uptake percentage more than 40 %).

Key words: bacteria, heavy metals uptake, recorebinants, Saccharomyces cereviesia

INTRODUCTION

Water is the most vital element among the natural resources, and is crucial for the survival of all living organisms including human, food production, and economic development. Today, nearly 40 percent of the world's food supply is grown under irrigation, and a wide variety of industrial processes depends on water (BCAS, 2000). Moreover, in Egypt, the environment, economic growth, and developments are all highly influenced by water - its regional and seasonal availability, and the quality of surface and groundwater. In terms of quality, the surface water of the country is vulnerable to pollution from untreated industrial effluents and municipal wastewater, runoff from chemical fertilizers and pesticides, and oil and tube spillage in the coastal area from the operation of sea and river ports. Water quality also depends on effluent types and discharge quantity from different type of industries, types of agrochemicals used in agriculture, and seasonal water flow and dilution capability by the river system (DHV, 1998).

Heavy-metal pollution represents an important environmental problem due to the toxic effects of metals, and their accumulation throughout the food chain leads to serious ecological and health problems. Among the biosorbents, there are marine algae, bacteria, yeasts, fungi and waste mycelia from the fermentation and food industry. Heavy meals are one of the more serious pollutants in our natural environment due to their toxicity, persistence and bioaccumulation problems (Tam and Wong, 2000). Trace metals in natural waters and their corresponding sediments have become a significant topic of concern for scientists and engineers in various fields associated with water quality, as well as a concern of the general public. Direct toxicity to man and aquatic life and indirect toxicity through accumulations of metals in the aquatic food chain are the focus of

Corresponding Author: Zaied, K.A Departments of Genetics Faculty of Agriculture, Mansoura University, Egypt.
E-mail: Khalazaid @ yahoo. com

this concern. The presence of trace metals in aquatic systems originates from the natural interactions between the water, sediments and atmosphere with which the water is in contact. The concentrations fluctuate as a result of natural hydrodynamic chemical and biological forces. Man, through industrialisation and technology, has developed the capacity to alter these natural interactions to the extent that the very waters and the aquatic life therein have been threatened to a devastating point.

There is now great awareness of the potential dangers of environmental pollution by heavy metal compounds which arise in waste waters from fertilizer industry. The removal of these pollutants from contaminated solutions by living or dead microbial biomass, and derived or excreted products, can provide an economically feasible and technically efficient means for element recovery and environmental protection .The removal and recovery of heavy metals from industrial effluents by genetically engineered microorganisms have several advantages related to their greater absorbing ability, rapidly in their metals accumulation and they are inexpensive.

Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. Heavy metals may enter the human body through food, water, air, or absorption through the skin when they come in contact with humans in agriculture and in manufacturing, pharmaceutical, industrial, or residential settings. Industrial exposure accounts for a common route of exposure for adults.

Bioremediation of industrial wastes containing heavy metals has been demonstrated by several biotechnology companies employing bioaccumulation (Macaskie and Dean. 1984). Biosorption, bioprecipitation, and uptake by purified biopolymers derived from microbial cells provide alternative and/or additive processes for conventional physical and chemical methods (Silver, S. 1991). Intact microbial cells, live or dead, and their products can be highly efficient bioaccumulators of both soluble and particulate forms of metals (Silver, 1991). The cell surfaces of all microorganisms are negatively charged owing to the presence of various anionic structures. This gives bacteria the ability to bind metal cations. Various microbial species, mainly Pseudomonas, have been shown to be relatively efficient in bioaccumulation of uranium, copper, lead, and other metal ions from polluted effluents, both as immobilized cells and in the mobilized state (Macaskie and Dean. 1984).

For these problems of pollution, bioremediation of industrial wastes containing heavy metals has been demonstrated by several biotechnology companies employing bioaccumulation (Lovely *et al* , 1991). Biosorption, bioprecipitation, and uptake by purified biopolymers derived from microbial cells provide alternative and/or additive processes for conventional physical and chemical methods (Silver, 1991). Intact microbial cells, live or dead, and their products can be highly efficient bioaccumulators of both soluble and particulate forms of metals (Niu *et al*, 1993). The cell surfaces of all microorganisms are negatively charged owing to the presence of various anionic structures. This gives bacteria the ability to bind metal cations. Various microbial species, mainly Pseudomonas, have been shown to be relatively efficient in bioaccumulation of uranium, copper, lead, and other metal ions from polluted effluents, both as immobilized cells and in the mobilized state (Lovely *et al* , 1991).

The level of public information and public concern ensures that high level public policy will be developed to manage these issues. There are parts in Egypt, where groundwater resources are used for human consumption. Nitrate leaching into these aquifers could represent a human health risk and would be an issue of high public concern should it occur. At this stage, current levels of concern are low.

The very direct relationship between fertilizers and the heavy metal impurities that they can contain means that the fertilizer industry had to deal effectively with this issue. The industry chose to work with Government to develop strategies that would manage the food safety risk and maintain internationally cost-competitive supplies of phosphate fertilizer products.

Under the National Cadmium Management Strategy, the industry and Government agreed on the lowest achievable maximum permissible concentrations of heavy metals in fertilizers, and a timetable for a two stage implementation. Through changes in phosphate rock sources and substitution of higher analysis fertilizers for single super phosphate, total inputs of cadmium in fertilizer has dropped by 75% since 1989.

The strategy recognizes that there are some specific combinations of crops and soil and water factors that may still result in unacceptable uptake of cadmium by food crops. The strategy has provided for additional measures including the manufacture of low cadmium superphosphate for high risk uses and education campaigns to ensure that cultural practices in these situations minimize the risk of uptake.

The history of success from the industry’s approach to heavy metal impurities was a significant factor in the willingness of the industry to adopt a similarly constructive approach to environmental issues.

In particular, water quality around fertilizer factories is so poor that water from the surrounding rivers can no longer be considered as a source of water supply for human consumption (DoE, 2001). This study aimed to improve heavy metal uptake from wastewater using recombinants of ycast and bacteria.

MATERIALS AND METHODS

Ten bacterial and seven *Saccharomyces cerevisiae* strains (Table 1) were used in this study, they are kindly obtained from National Center for Agriculture Utilization Research, USA. One of *Saccharomyces cerevisiae* strains (NBIMCC 82) was kindly providing from National Bank for Industrial Microorganisms and Cell Cultures, Bulgaria, Sofia. All strains used in this study are wild type strains.

Factory Effluents:

The present study was undertaken using the wastewaters resulted from ammonia unit of Fertilizer Factory (FF). Polluted water was collected from the main pipe of the factory before being mixed with water in the river. This collection was done on October 2007. A specific problem associated with heavy metals in the environment is accumulation in the food chain and persistence in the environment.

Table 1: Bacterial strains used in this study.

No.	Strains	Designation	Origin
1	*Citrobacter amalonaticus*	NRRL B-41228	USA
2	*Citrobacter freundii*	NRRL B-2643	USA
3	*Bacillus subtilis* var *niger*	NRRL NRS-213	USA
4	*Bacillus subtilis*	NRRL B-642	USA
5	*Bacillus licheniformis*	NRRL B-571	USA
6	*Bacillus licheniformis*	NRRL B-1584	USA
7	*Bacillus licheniformis*	NRRL NRS-1264	USA
8	*Bacillus licheniformis*	NRRL B-358	USA
9	*Micrococcus luteus*	NRRL B-287	USA
10	*Kocuria rhizophila*	NRRL B-4375	USA
11	*Saccharomyces cerevisiae*	NRRL Y - 12632	USA
12	*Saccharomyces cerevisiae*	NRRL Y - 11562	USA
13	*Saccharomyces cerevisiae*	NBIMCC 82	Bulgaria (National Bank for industrial microorganisms and cell cultures), sofia
14	*Saccharomyces cerevisiae*	NRRL Y - 12619	USA
15	*Saccharomyces cerevisiae*	NRRL Y - 136	USA
16	*Saccharomyces cerevisiae*	NRRL Y - 137	USA
17	*Saccharomyces cerevisiae*	NRRL Y - 1370	USA

Media:

Bacterial strains were grown as described previously by Horikoshi *et al.* (1981). However, yeast strains were grown on yeast extract peptone dextrose (YEPD) medium.

I I. Methodology:

Antibiotic Susceptibility Assays:

Antibiotic susceptibility was measured by plate diffusion method, according to Collins and Lyne (1985) with cultures grown to logarithmic growth phase in nutrient agar medium for each microbe. All antibiotics were used at a concentration of 100 mg/ml, according to Roth and Sonti (1989). The selectable markers were identified as antibiotic resistance and or sensitive genes as listed in Table 3 about conjugation. Antibiotic designation was listed in Table 2.

Table 2: Antibiotics and their abbreviations used for genetic marking against different bacterial strains.

No	Antibiotics	Designation
1	Flucamox	*flu*
2	Streptomycin	*Str*
3	Tetracycline	*Tc*
4	Neomycinsulphate	*Nm*
5	Ampicillin	*Ap*
6	Erythromycin	*Erth*
7	Amoxycillin and flucloxacillin	*Am-Fluc*
8	Rifampicillin	*Rf*
9	Ibiamox	*Ibim*
10	Amoxycillin	*Amoxy*
11	Ibidroxil	*Ibid*
12	Haiconcil	*Hico*
13	Velosef	*Velo*
14	Epicocillin	*Epico*
15	Nystatin	*Nyst*
16	Epicocillin	*Epico*
17	Erythrocin	*Ery*
18	Duricef	*Duri*
19	Pencillin	*pen*

Conjugation:

Nutrient broth cultures, in the late-exponential growth phase were used. Quantitative spot mating of conjugal transfer was carried out according to Lessel *et al*. (1993) by inoculating 10 ml samples of the donor culture onto the surface of selective medium, previously seeded with 100 ml of the recipient culture. A single colony of transconjugants was picked up and transferred to slant nutrient agar medium. Conjugation was carried out between strains carrying the opposite genetic markers as shown in Table 3. From each mating, two different isolates were selected to be used in pollutants uptake experiments.

Uptake Experiments:

In the heavy metals uptake test, precultured cells were suspended in 250 ml conical flasks containing 150 ml minimal medium and another using nutrient broth (1.5 g peptone and 0.5 g beef extract per litre) for bacteria and YEPD (0.5 g yeast extract 1.0 g peptone and 1.0 g glucose per litre) medium for yeast, each supplemented with factory effluents and incubated under a static conditions at 30°C for 48 h. Thereafter, the cells were collected by filtration on membrane filter (pore size 0.45 mm). Amounts of metals taken up by the cells were determined according to Nakajima and Sakaguchi (1986).

In the next step, overnight cultures yeast and bacteria were harvested, washed twice with distilled water, and resuspended in 150 ml factory effluents supplemented with 0.15 % g peptone and 0.05 % beef extract. After 48 hours of incubation at 30°C the cells were removed and the filtrate was used to determine the amount of heavy metals using atomic absorption spectrophotometry.

This indicated that bacteria were growning on modified nutrient browth (CM) of factory effluents without adjusting pH which reached up to 9.2 . It was consists of 0.15 % g peptone and 0.05 % beef extract) and incubated at 30°C for 48 hours.

Metal Biosorption:

Metal biosorption experiments were carried out in a 250 ml flask at 30 °C without shaking . The flask was filled with 150 ml of previously prepared media containing factory effluents without any dilution. Each experiment was conducted for 48 h, which was enough time to achieve steady state biosorption. The pH was uncontrolled throughout the experiment.

Dry Cell Weight:

Dry cell weight measurements were carried out by passing a volume of 50 ml cell culture through a previously weighted Millipore filters (Watman No. 1). Cell pellets were also washed twice with filtered deionized/distilled water to remove non-biomass ash. Filtered and collected cells were dried in an oven set at temperature 110 ◦C and weight for every 24 h until constant weight was obtained.

Determination of Heavy Metals Concentration:

The samples were collected and filtered using Millipore filters of 0.22 -m. The filtrate was collected for heavy metals analysis. The concentration of heavy metals in solution was determined using atomic absorption spectrophotometer (Pantech Instruments, Victoria, Australia) at the Atomic Absorption Unit, Department of Chemistry, Faculty of Science, Mansoura University. Heavy metals determined in this study were as follows; Lead, Cadmium, Nickel, Platinum, Copper, Cobalt, Iron, Manganese, Molybdenum, Vanadium, strontium, Zinc, Chromium, Antimony, Mercury and Arsenic.

Data Evaluation (Langmuir Isotherms):

The uptake of the metals (in mg of metal/g of dry cell weight) was calculated according to (Liu *et al*. 2004) using the following formula :

$Q = v(C_i - C_f)/m$

Where Q is the metal uptake (mg metal per g biosorbent), v the liquid sample volume (ml), C_i the initial concentration of the metal in the solution (mg/L), C_f the final (equilibrium) concentration of the metal in the solution (mg/L) and m the amount of the added biosorbent on the dry basis (mg).

RESULTS AND DISCUSSION

Bacterial conjugation was carried out in this study to obtain recombinants may having higher uptake of pollutants (Table 3), it is the transfer of genetic material between bacteria through direct cell-to-cell contact (*Holmes and Jobling 1996)*. Conjugation was discovered in 1946 by Joshua Lederberg and Edward Tatum, as a mechanism of horizontal gene transfer - as are transformation and transduction - although these mechanisms do not involve cell-to-cell contact (*Griffiths et al 1999)*.

The genetic information transferred is often beneficial to the recipient cell. Benefits may include; antibiotic resistance, heavy metals uptake, other xenobiotic tolerance, or the ability to utilize a new metabolite (*Holmes and Jobling 1996)*. Such beneficial plasmids may be considered bacterial endosymbionts. Some conjugative elements may also be viewed as genetic parasites on the bacterium, and conjugation as a mechanism was evolved by the mobile element to spread itself into new hosts. Five single colonies from that appeared in each conjugation were picked up and transferring to a nutrient agar slant, each colony may significantly differ than other ones on the same plate resulted from the same mating in harboring genetic background. This because these are recombinations, each recombination resulted from the mating between two bacterial cells.

Uptake of Heavy Metals by Saccharomyces Cerevisiae Using YEPD Medium Supplemented with Wastewaters:

Heavy metals are major pollutants in marine, ground, industrial, and even treated waters. Sorption of heavy metals onto live or dead biological materials (biosorption) is a potential method of removing toxic metals. Different bacterial and yeast strains, as well as their hybrids were evaluated in this study. An interesting and simple experiment was developed to show the potential of hybrids induced in this study in biosorption efficiency.

As shown from the results presents in Table 4 the parental *Saccharomyces cerevisiae* strain NRRL Y - 12632 was more efficient in heavy metals uptake than the second one and than all hybrids resulted from the mating between two parental strains. The hybrids resulted appeared moderate uptake levels in relation to the parental yeast strains (NRRL Y - 12632 x NRRL Y - 11562). Most of yeast hybrids appeared higher levels for all heavy metals uptake than the second parental one (*Saccharomyces cerevisiae* NRRL Y - 12632).

Table 3: Mating between bacterial strains that having the opposite genetic markers.

No. of mating	Mating	Revelant geneotype of mating
1	NRRL B-571 X NRRL B-1584	*Erth^{+} , Ap^{+}, Ibim^{+}, Amoxy^{+}, Hico+, Epico^{+},Cp^{-}* X *Erth^{-}, Ap^{+}, Ibim^{-}, Amoxy^{-}, Hico^{-},Epico^{-},Cp+*
2	NRRL B-571 X NRRL B-358	*Erth^{+}, flu^{+}, Hico+ Epico^{+},Cp^{-}* X *Erth^{-}, Flu^{-}, Hico^{-},Epico^{-},Cp+*
3	NRRL B-571 X NRRL B-2643	*Erth^{+} , flu^{+}, Epico^{+}, Velo^{-} , Duri^{-}, Cp^{-}, Ibid^{-}* X *Erth^{-} , flu^{-}Epico^{-}, Velo^{+}* , *Duri^{+}, Cp^{+}, Ibid^{+}*
4	NRRL B-571 X NRRL B-41228	*Erth , flu^{+}, Ap^{+}, Epico^{+},Cp^{-}* X *Erth^{-}, flu^{-}, Ap^{-}, Epico^{-},Cp$^{+}_{+}$*
5	NRRL B-1584 X NRRL B-41228	*Ap^{+}, Ibid^{-}, Amoxy^{-}, Ibim^{-}* X *Ap^{-}, Ibid^{+}, Amoxy^{+}, Ibim^{+}*
6	NRRL B-1584 X NRRL B-642	*Ap^{+}, Cp^{+}, Am-Fluc^{+}, pen^{+}, Hico^{-}, Epico^{-}* X *Ap^{-}, Cp^{-}, Am-Fluc^{-}, pen^{-}, Hico^{+}, Epico^{+}*
7	NRRL B-1584 X NRRL NRS-213	*Ap^{+}, Cp^{+}, Am-Fluc^{+}, pen^{+}, Amoxy^{-}* X *Ap^{-}, Cp^{-}, Am-Fluc^{-},pen^{-}, Amoxy^{+}*
8	NRRL NRS-1264 X NRRL B-2643	*Erth^{+}, Tc^{+}, Ibim^{+}, flu^{+}, Ibid^{-}, Velo^{-} , Duri^{-} X Erth^{-}, Tc^{-},Ibim^{-}, flu^{-}, Ibid^{+},Velo^{+},Duri^{+}*
9	NRRL B-358 X NRRL B-642	*Ap^{+}, Cp^{+}, Am-Fluc^{+}, pen^{+}, Ibim^{+}, Amoxy^{+}, Hico^{-}, Epico^{-}* X *Ap^{-}, Cp^{-}, Am-Fluc-, pen , Ibim^{-}, Amoxy^{-} Hico^{+}, Epico$^{+}_{-}$*
10	NRRL B-2643 X NRRL B-642	*Ap^{+}, Cp^{+}, Am-Fluc^{+}, pen^{+}, Ibim^{+}, Amoxy^{+}, Ibid^{+}, Velo^{+} , Duri^{+} , Epico^{-} X Ap^{-}, Cp^{-}, Am-Fluc^{-}, pen^{-}, Ibim^{-}, Amoxy^{-}, Ibid^{-}, Velo^{-} , Duri^{-},Epico^{+}*
11	NRRL B-41228 X NRRL B-642	*Cp^{+}, Am-Fluc^{+}, pen^{+}, Ibim^{+}, Amoxy^{+}, Epico^{-} X Cp^{-}, Am-Fluc^{-}, pen^{-}, Ibim^{-}, Amoxy^{-} Epico^{+}*
12	NRRL B-642 X NRRL B-4375	*Hico+, Epico^{+}, Am-Fluc^{-}, pen^{-}* X *Hico-, Epico^{-}, Am-Fluc^{+}, pen^{+}*
13	NRRL B-642 X NRRL NRS-213	*Hico+, Epico^{+}, Amoxy^{-} X Hico-, Epico^{-}, Amoxy^{+}*
14	NRRL B-4375 X NRRL NRS-213	*Am-Fluc^{+}, pen^{+}, Amoxy^{-}* x *Am-Fluc^{-}, pen^{-}, Amoxy^{+}*

Table 4: Heavy metals uptake (mg metal per g biosorbent) by parental strains of *Saccharomyces cerevisiae* and their hybrids growing on YEPD medium supplemented with wastewater .

Biocontrol agents	ppm				
	Cu	Co	Fe	Cd	Pb
Saccharomyces cerevisiae NRRL Y - 2632	14666	5083	5833	35250	9416
Saccharomyces cerevisiae NRRL Y -11562	1952	730	958	5065	1185
M.P.	8309	2906	3395	20157	5300
Hybrid No. 1	2737	836	4426	7000	1950
Hybrid No. 2	2356	767	821	5863	1630
Hybrid No. 3	1205	447	649	3054	837
Hybrid No. 4	2223	783	3496	5902	1384
Hybrid No. 5	1127	413	524	2806	649

Table 4: Continued

Biocontrol agents	ppb		Ppm		
	Hg	As	Mn	Pt	Mo
Saccharomyces cerevisiae NRRL Y - 12632	1500	6083	10250	9416	12750
Saccharomyces cerevisiae NRRL Y - 11562	47	706	1305	1185	1664
M.P.	773	3394	5777	5300	7207
Hybrid No. 1	393	1295	2114	1950	2606
Hybrid No. 2	328	1082	1767	1630	2178
Hybrid No. 3	151	548	909	837	1126
Hybrid No. 4	55	825	1524	1384	1944
Hybrid No. 5	26	386	714	649	911

Table 4: Continued

Biocontrol agents	ppb					
	Zn	Cr	V	Sr	Sb	Ni
Saccharomyces cerevisiae NRRL Y- 12632	31916	14416	15250	12750	16916	10250
Saccharomyces cerevisiae NRRL Y -11562	4419	1904	2024	1664	2263	1305
M.P.	18167	8160	8637	7207	9589	5777
Hybrid No. 1	6377	2934	3098	2606	3426	2114
Hybrid No. 2	5328	2452	2589	2178	2863	1767
Hybrid No. 3	2787	1270	1343	1126	1487	909
Hybrid No. 4	5160	2223	2363	1944	2643	1524
Hybrid No. 5	2419	1042	1108	911	1239	714

The activity of trace metals in aquatic systems and their impact on aquatic life vary depending upon the metal species. Of major importance in this regard is the ability of metals to associate with other dissolved and suspended components. Most significant among these associations is the interaction between metals and organic compounds in water and sediment. These organic species, which may originate naturally from process such as vegetative decay or result from pollution through organic discharge from municipal and industrial sources, have a remarkable affinity and capacity to bind to metals. This phenomenon would naturally alter the reactivity of metals in the aquatic environment. (Signer, 1974).

Many human activities (e.g.; mining, overuse of chemicals, industrial waste from ports and refineries) have a negative impact on several biological processes and there is no doubt that these will continue to affect the functioning of highly productive coastal ecosystems. Contamination caused by trace metals affects both ocean waters, those of the continental shelf and the coastal zone where, besides having a longer.

Trace metals, including those defined as “heavy”, arising from industrial and mining activities are discharged into coastal waters and estuaries at many sites. The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic, highly toxic or poisonous at low concentrations. These anthropogically derived inputs can accumulate in local sediments (up to five orders of magnitude above the overlying water, Bryan and Langston, 1992) and invertebrates living on or in food, and the rate of accumulation carries widely between species and heavy metal concentration found in “clean” conditions. Less is known of the uptake of these metals by ingestion with food or from close contact with contaminated sediments. For some time, there has been serious concern about the simultaneous input of unwanted trace elements, present in these mineral fertilisers, like Cd or Cr. These trace metals are much more likely available to biota than those amounts bound to the soil (Sager, 1997). Approximately 80% of total chromium from mineral fertilizers emanates from basic slag and basic slag potash. Regional differences in application rates and crops lead to differences in trace element loads per farmed area up to 6-fold. Further on, inputs from fertilizers have been compared with input by atmospheric deposition. As a source of lead and cadmium, long-range transport via the atmosphere supersedes the input from mineral fertilizers, whereas in case of chromium it is reverse. It is widely recognised that marine ecosystems can become contaminated by trace metals from numerous and diverse sources. However, anthropogenic activities, such as mining and industrial processing of ores and metals, still remain the principal cause of the increased amount of heavy metals which have been dumped into the oceans (DeGregori *et al.*, 1996). According to Mateu *et al.* (1996) trace metal levels can be indicators of the concentrations of other pollutants to which they are potentially related.

The results obtained in this study are in agreement with those obtained by Liu *et al* 2004, who found that indigenous *T. thiooxidans* is a microorganism that has extremely high capacity for Zn(II) biosorption (qm = 172.4mg of Zn(II)/g of dry biomass at 40 °C and pH 6.0) when pretreated with NaOH, whereas it shows relatively low capacity for Cu(II) uptake (qm = 39.84 mg of Cu(II)/g of dry biomass at 40 °C and pH 5.0). The initial pH value of the solution has a significant effect on the capacities for both Zn(II) and Cu(II) uptake, principally, due to the protonization that occurs at low values of pH and due to the effect it has on the chemistry of both the solution and the functional groups on the cell walls. Typically, the adsorption capacity increases with increasing pH in the ranges of 2.0 - 6.0 and 4.0 - 5.0 for Zn (II) and Cu (II), respectively. Also, an appropriate physical or chemical pretreatment of the biomass shows positive effects on its capacity for metal biosorption. Higher temperature increases the capacity of metal biosorption more significantly for Zn(II) than Cu(II).

The results presented in Table 5 appeared that some of yeast hybrids appeared higher increase in recovery of heavy metals above the mid parents and the better parent. This indicated that the yeast *Saccharomyces cerevisiae* has been used to remove heavy metals such as Cr(VI), Fe(III) , etc (Goyal *et al.* 2003), Pb(II) (Suh *et al.* 1998), Cu(II) (Jianlong, 2002), zinc and nickel (Zouboulis *et al.* 2001) from aqueous solutions. It can distinguish different metal species based on their toxicity, such as selenium, antimony and mercury. This kind of property makes *S. cerevisiae* useful not only for the bioremediation, removal or recovery of metal ions, but also for their analytical measurement (Wang and Chen, 2006).

Table 5: Percentage of heavy metals uptake by parental strains of *Saccharomyces cerevisiae* and their hybrids growing on YEPD medium supplemented with wastewater .

Biocontrol agents	ppm				
	Cu	Co	Fe	Cd	Pb
Saccharomyces cerevisiae NRRL Y - 12632	97	86	16	87	75
Saccharomyces cerevisiae NRRL Y - 11562	89	86	18	87	66
M.P.	93	86	17	87	71
Hybrid No. 1	91	72	61	90	79
Hybrid No. 2	95	79	14	91	79
Hybrid No. 3	91	87	20	87	77
Hybrid No. 4	86	79	57	86	66
Hybrid No. 5	95	89	18	91	66

Table 5: Continued

Biocontrol agents	ppb				
	Hg	As	Mn	Pt	Mo
Saccharomyces cerevisiae NRRL Y - 12632	44	96	48	69	56
Saccharomyces cerevisiae NRRL Y - 11562	33	95	63	60	47
M.P	39	96	55.5	64.5	51.5
Hybrid No. 1	55	97	58	55	37
Hybrid No. 2	62	96	79	65	56
Hybrid No. 3	65	97	73	42	37
Hybrid No. 4	31	95	92	60	56
Hybrid No. 5	85	95	71	93	47

Table 5: Continued

Biocontrol agents	ppb					
	Zn	Cr	V	Sr	Sb	Ni
Saccharomyces cerevisiae NRRL Y - 12632	90	73	68	94	21	81
Saccharomyces cerevisiae NRRL Y - 11562	86	33	41	92	25	83
M.P.	88	53	54.5	93	23	82
Hybrid No. 1	88	81	73	89	54	83
Hybrid No. 2	86	38	41	92	25	87
Hybrid No. 3	86	48	55	42	78	69
Hybrid No. 4	88	38	64	82	33	91
Hybrid No. 5	88	52	50	32	76	44

In multi-metal mixtures, heavy metal ions compete for a limited number of binding sites in the biomass. Depending on the composition of the solution and the form of biomass, this competitive ion exchange may severely reduce the efficiency of the metal-removal process (Kratochvil *et al* , 1998). Though the performance of the battery in multimetal ion solution was satisfactory, its efficiency in the case of industrial effluents will be affected by the presence of other elements and complex molecules in the solution (Ramelow *et al* , 1992).

Uptake of Heavy Metals by Bacterial Cells Using Modified Nutrient Broth Supplemented with Wastewaters :

As shown from the results presented in Table 6 many of bacterial strains used in this proposal giving higher removal of heavy metals in relation to their uptake. However, one of transconjugants (Tr 1) resulted from the mating between NRRL B-571(P1) X NRRL B-1584) (P2) revealed higher uptake of heavy metals for Pb, Cd, Fe, Co and Cu than the parental strains. This indicated that this is a superior isolate in heavy metals. These observations are consistent with the concept of homogeneity of the bacterial surface, which contains a variety of functional groups. These groups that serve as adsorption sites may differ both with respect to the strength of the metal sorptive bond and the rate of adsorption onto the sites. This will result in different rates of metal uptake by the biosorbent and in general can be classified into fast and slow uptake (Matheickal *et al*, 1999). The higher rates of Pb, Cd, Fe, Co and Cu uptake than other metal ions may be due to the inhibitory role of other ions on sorption process which can be well understood by comparing the metal uptake capacities of the biosorbent in the case of single and multi metal ion solutions. In multi-metal mixtures, heavy metal ions compete for a limited number of binding sites in the biomass. Reduction in metal uptake observed during the successive battery operations could be attributed to lowered metal / biosorbent ratio in solution. According to Fourest and Roux (1992), reduction in biomass concentration in the suspension at a given metal concentration enhanced the metal / biosorbent ratio, and thus increased the metal uptake per unit biosorbent as long as the latter was not saturated. Thus reducing the biomass concentration with reduction in the metal concentration can be suggested to improve metal uptake in the final stages of biobattery operations. Depending on the composition of the solution and the form of biomass, this competitive ion exchange may severely reduce the efficiency of the metal-removal process. Though the performance of the battery in multimetal ion solution was satisfactory, its efficiency in the case of industrial effluents will be affected by the presence of other elements and complex molecules in the solution (Ramelow, *et al* 1992).

As shown from the results presented in Table 7 that all bacterial strains and their transconjugants were more efficient in uptake of Pb, Cd, Fe, Co and Cu (uptake percentage more than 70 % in relation to untreated control). However, lower percentage in uptake of heavy metals were achieved in Ni, Sb, Sr, V, Cr, Zn, Mo, Pt, Mn, As and Hg (uptake percentage more than 40 %). These results are in accordance with those reported by Brady *et al.* (1994), who found that cadmium was accumulated to a greater extent than either cobalt by yeast. Recently, Winge *et al.*, 1989 reported that the yeast *Saccharomyces cerevisiae* is a good host for heterologous gene expression and protein secretion. It has been suggested that metallothioneins (MT, which are small cysteine-rich polypeptides that can bind essential metals, e.g. Cu and Zn, as well as, non-essential metals like Cd, have been recorded in all microbial groups such as bacteria and yeasts (Winge *et al.*, 1989) may be of potential in metal recovery since it can bind other metals besides Cu, e.g. Cd, Zn, Ag, Co and Au, although these metals do not generally induce MT synthesis (Butt and Ecker, 1987). From these results it can be seen that the amounts of pollutants absorbed by the parental cells differs markedly in different strains of bacteria and of their transconjugants . Of special interest to this discussion is the wide range in the effectiveness with which different species and genera of bacteria absorb pollutants. This suggests that the selective accumulation of heavy metal ions by bacterial strains and their transconjugants is determined by interionic competition (Nakajima and Sakaguchi, 1986). The present results are in accordance with those obtained by Nakajima and Sakaguchi (1986), who found that the relationship between the uptake of uranium and absorption of mercury is not the same in all groups of microorganisms and the total quantity of metal ions absorbed by microbial cells differed greatly from species to species. They also found that in bacteria and yeasts many species were found to accumulate mercury more abundantly than uranium. Our results showed that transconjugant cells had better and excellent adsorbing characteristics for some heavy metals. Successful biotechnological exploitation of microbial metal accumulation may depend on the ease of metal recovery and biosorbent regeneration for use in multiple biosorption-desorption cycles (Volesky, 1990). The mechanisms used for metal recovery from loaded biomass depends on the case of removal from the biomass and

Table 6: Heavy metals uptake (mg metal per g biosorbent) by parental strains of bacteria and their transconjugants growing on modified nutrient broth (1.5 g peptone and 0.5 g beef extract per litre) supplemented with wastewaters .

Mating	Parental strains and resulted transconjugants	Heavy metals uptake (ppm)				
		Pb	Cd	Fe	Co	Cu
NRRL B-571(P1) X NRRL B-1584) (P2)	P1	7571	6762	7905	4714	5000
	P2	26000	22833	28333	16500	19167
	M.P	167855	147975	18119	10607	120835
	Tr1	278000	280000	3e+05	186000	240000
	Tr2	3438	3079	3730	1978	2360
NRRL B-571 (P1) X NRRL B-2643 (P2)	P1	7571	6762	7905	4714	5000
	P2	13238	13810	16952	8857	10952
	M.P	104045	10286	1e+05	67855	7976
	Tr1	3942	4116	4696	2870	3188
	Tr2	1505	1327	1664	972	981

Table 6: Continued.

NRRL B-571 (P1) X NRRL B-41228(P2)	P1	7571	6762	7905	4714	5000
	P2	631	586	730	408	437
	M.P	4101	3674	43175	2561	27185
	Tr1	3188	3021	3542	1938	2208
	Tr2	6000	5686	7137	3647	4510
NRRL B-1584(P1) X NRRL B-642(P2)	P1	26000	22833	28333	16500	19167
	P2	16000	16706	21059	11647	12941
	M.P	21000	197695	24696	140735	16054
	Tr1	1167	1246	1456	772	921
	Tr2	4500	4118	4765	2588	3118
NRRL B-1584 X NRRL NRS-213	P1	26000	22833	28333	16500	19167
	P2	15143	12762	16190	9905	11429
	M.P	205715	177975	2e+05	132025	15298
	Tr1	4076	3671	4405	2354	2658
	Tr2	3533	3156	4044	2200	2356
NRRL NRS-1264 X NRRL B-2643	P1	70500	72500	82000	46500	53000
	P2	13238	13810	16952	8857	10952
	M.P	41869	43155	49476	276785	31976
	Tr1	34000	36750	42500	23250	28750
	Tr2	4567	4179	1791	2776	2836
NRRL B-358 X NRRL B-642	P1	92667	94667	1e+05	62000	73333
	P2	16000	16706	21059	11647	12941
	M.P	543335	556865	63196	368235	43137
	Tr1	1920	1933	2373	1320	1413
	Tr2	16588	17294	21412	11647	12941
NRRL B-2643 X NRRL B-642)	P1	13238	13810	16952	8857	10952
	P2	16000	16706	21059	11647	12941
	M.P	14619	15258	2e+05	10252	119465
	Tr1	1571	1385	878	966	1024
	Tr2	3975	3506	4296	2296	2617
NRRL B-41228 X NRRL B-642	P1	631	586	730	408	437
	P2	16000	16706	21059	11647	12941
	M.P	83155	8646	1e+05	60275	6689
	Tr1	2623	2774	3170	1660	1698
	Tr2	8167	7778	8778	4889	6389
NRRL B-642 X NRRL B-4375)	P1	16000	16706	21059	11647	12941
	P2	66500	73500	85000	46500	55000
	M.P	41250	45103	5e+05	290735	339705
	Tr1	5000	5480	6920	3720	3400
	Tr2	24727	24545	31273	16909	22727
NRRL B-642 X NRRL NRS-213)	P1	16000	16706	21059	11647	12941
	P2	15143	12762	16190	9905	11429
	M.P	155715	14734	2e+05	10776	12185
	Tr1	9929	10500	12429	6643	7857
	Tr2	3495	3077	4000	2176	2330
NRRL B-4375 X NRRL NRS-213	P1	66500	73500	85000	46500	55000
	P2	15143	12762	16190	9905	11429
	M.P	408215	43131	50595	282025	332145
	Tr1	2879	2710	2486	1738	2056
	Tr2	859	883	1045	517	661
Micrococcus luteus (NRRL B-287)		12480	11600	12960	7040	8400
Mating	Parental strains and resulted transconjugants	Heavy metals uptake (ppm)				
		Mo	Pt	Mn	As	Hg
NRRL B-571(P1) X NRRL B-1584) (P2)	P1	4476	5857	3238	310	243
	P2	13667	19833	10667	967	817
	M.P	90715	12845	69525	6385	530
	Tr1	164000	178000	96000	12200	6800
	Tr2	2067	2202	989	155	108
NRRL B-571 (P1) X NRRL B-2643 (P2)	P1	4476	5857	3238	310	243
	P2	7524	11143	4952	467	371
	M.P	6000	8500	4095	3885	307
	Tr1	2290	2232	1507	177	107
	Tr2	654	776	579	75	50
NRRL B-571 (P1) X NRRL B-41228(P2)	P1	4476	5857	3238	310	243
	P2	346	507	264	16	19
	M.P	2411	3182	1751	163	131
	Tr1	2290	2232	1507	177	107
	Tr2	654	776	579	75	50

Table 6: Continued.

NRRL B-1584(P1) X NRRL B-642(P2)	P1	13667	19833	10667	967	817
	P2	9059	12235	5647	682	400
	M.P	11363	16034	8157	8245	6085
	Tr1	2228	2709	1418	203	134
	Tr2	1711	2089	1333	178	113
NRRL B-1584 X NRRL NRS-213	P1	13667	19833	10667	967	817
	P2	8857	11238	5714	286	448
	M.P	11262	155355	81905	6265	6325
	Tr1	2228	2709	1418	203	134
	Tr2	1711	2089	1333	178	113
NRRL NRS-1264 X NRRL B-2643	P1	44500	55000	33000	3500	2000
	P2	7524	11143	4952	467	371
	M.P	26012	330715	18976	19835	11855
	Tr1	22250	29250	16000	1625	700
	Tr2	2746	3433	1552	239	140
NRRL B-358 X NRRL B-642	P1	64667	83333	45333	4400	2400
	P2	9059	12235	5647	682	400
	M.P	36863	47784	25490	2541	1400
	Tr1	1053	1293	920	87	52
	Tr2	10235	12824	7529	918	447
NRRL B-2643 X NRRL B-642)	P1	7524	11143	4952	467	371
	P2	9059	12235	5647	682	400
	M.P	82915	11689	52995	5745	3855
	Tr1	780	1122	644	78	53
	Tr2	2840	2642	1630	198	131
NRRL B-41228 X NRRL B-642	P1	346	507	264	16	19
	P2	9059	12235	5647	682	400
	M.P	47025	6371	29555	349	2095
	Tr1	1472	1755	1358	123	74
	Tr2	4944	5944	3778	422	233
NRRL B-642 X NRRL B-4375)	P1	9059	12235	5647	682	400
	P2	52500	57000	31000	3100	1800
	M.P	307795	346175	2e+05	1891	1100
	Tr1	4600	4600	2880	232	116
	Tr2	17636	19636	12364	1091	618
NRRL B-642 X NRRL NRS-213)	P1	9059	12235	5647	682	400
	P2	8857	11238	5714	286	448
	M.P	8958	117365	56805	484	424
	Tr1	5929	7071	4857	500	271
	Tr2	1714	2549	1407	167	112
NRRL B-4375 X NRRL NRS-213	P1	52500	57000	31000	3100	1800
	P2	8857	11238	5714	286	448
	M.P	306785	34119	18357	1693	1124
	Tr1	1308	1664	1346	148	88
	Tr2	565	523	432	36	23
Micrococcus luteus(NRRL B-287)	Wild type	8560	9760	5440	560	392

Mating	Parental strains and resulted transconjugants	Heavy metals uptake (ppm)					
		Ni	Sb	Sr	V	Cr	Zn
NRRL B-571(P1) X NRRL B-1584) (P2)	P1	5714	4952	6429	4762	1429	8048
	P2	18333	11667	21333	19667	17167	31667
	M.P	120235	83095	13881	122145	9298	198575
	Tr1	232000	140000	254000	194000	1e+05	320000
	Tr2	3146	2247	1798	1573	1663	4382
NRRL B-571 (P1) X NRRL B-2643 (P2)	P1	5714	4952	6429	4762	1429	8048
	P2	9524	9048	7619	7619	8476	16571
	M.P	7619	7000	7024	61905	49525	123095
	Tr1	3623	2609	2899	2899	2580	4058
	Tr2	1121	963	1168	925	467	1701
NRRL B-571 (P1) X NRRL B-41228(P2)	P1	5714	4952	6429	4762	1429	8048
	P2	371	363	206	247	367	771
	M.P	30425	26575	33175	25045	898	44095
	Tr1	1667	1458	2500	2125	2458	3521
	Tr2	4471	2353	4314	4510	3490	6275
NRRL B-1584(P1) X NRRL B-642(P2)	P1	18333	11667	21333	19667	17167	31667
	P2	10588	10824	14118	10588	5882	18824
	M.P	144605	112455	177255	151275	1e+05	252455
	Tr1	1096	614	1044	956	904	1518
	Tr2	2941	2647	3647	3353	2618	4912

Table 6: Continued.

NRRL B-1584 X NRRL NRS-213	P1	18333	11667	21333	19667	17167	31667
	P2	11429	4762	9524	9333	8286	16095
	M.P	14881	82145	154285	14500	1e+05	23881
	Tr1	3038	2405	3519	3013	2608	4785
	Tr2	2667	1556	2644	2378	1978	3533
NRRL NRS-1264 X NRRL B-2643	P1	62000	45000	68500	52500	37000	80000
	P2	9524	9048	7619	7619	8476	16571
	M.P	35762	27024	380595	300595	22738	482855
	Tr1	31750	29750	34500	31000	21250	46250
	Tr2	3582	3642	4388	3552	3075	6030
NRRL B-358 X NRRL B-642	P1	66667	58667	80000	60000	49333	113333
	P2	10588	10824	14118	10588	5882	18824
	M.P	386275	347455	47059	35294	3e+05	660785
	Tr1	1627	1400	2027	1693	400	2453
	Tr2	11765	12353	14588	10588	8706	18588
NRRL B-2643 X NRRL B-642)	P1	9524	9048	7619	7619	8476	16571
	P2	10588	10824	14118	10588	5882	18824
	M.P	10056	9936	108685	91035	7179	176975
	Tr1	293	966	1346	1239	488	1737
	Tr2	2222	2222	3160	2469	494	4864
NRRL B-41228 X NRRL B-642	P1	371	363	206	247	367	771
	P2	10588	10824	14118	10588	5882	18824
	M.P	54795	55935	7162	54175	31245	97975
	Tr1	2264	1868	2264	2264	1679	3283
	Tr2	7778	6389	7667	6889	4111	10833
NRRL B-642 X NRRL B-4375)							
	P1	10588	10824	14118	10588	5882	18824
	P2	63500	46500	60000	40000	30000	84000
	M.P	37044	28662	37059	25294	17941	51412
	Tr1	3600	3560	5120	4760	4120	7160
	Tr2	23636	24545	26364	18545	10909	33455
NRRL B-642 X NRRL NRS-213)	P1	10588	10824	14118	10588	5882	18824
	P2	11429	4762	9524	9333	8286	16095
	M.P	110085	7793	11821	99605	7084	174595
	Tr1	9214	6429	9929	9571	5286	14071
	Tr2	2637	2637	2857	2791	2264	3692
NRRL B-4375 X NRRL NRS-213	P1	63500	46500	60000	40000	30000	84000
	P2	11429	4762	9524	9333	8286	16095
	M.P	374645	25631	34762	246665	19143	500475
	Tr1	2411	2019	2692	2224	1383	3271
	Tr2	703	553	829	601	444	961
Micrococcus luteus(NRRL B-287)		9600	7280	11120	8000	5760	14000

this can depend on the element involved and the mechanism of accumulation. Metabolism-independent biosorption is frequently reversible by nondestructive methods and may often be considered analogous to an ion exchange process. Metabolism-dependent accumulation and intracellular compartmentation or sequestration within organelles or by binding to induced proteins etc., is often irreversible requiring destructive recovery. If a cheap and plentiful supply of waste biomass is used to recover valuable metals then the economics of destruction may be satisfactory. This has been demonstrated that it may be possible to apply selective desorption of chosen elements from a biosorbent loaded with a number of different elements with an appropriate choice of eluant. Industrial application of biosorption depends on such factors as loading capacities, efficiencies and selectivity, ease of metal recovery and an equivalence at least to traditional physical and chemical treatments in performance (Brierley, 1990). In comparison with existing treatment methods, several biosorptive processes after advantages including high efficiency at low metal/radionuclide concentrations (Volesky, 1990). Furthermore, microbial biomass may be supplied as a fermentation by-product or specifically grown using cheap substrates. Many biosorbents appear competitive in cost with ion exchange resins while frequently exhibiting higher efficiencies (Volesky, 1990). This is in accordance with the results obtained by Nakajima and Sakaguchi (1986), who found that uranyl, mercury, lead and copper ions were more readily accumulated by cell of bacteria, yeasts, fungi and actinomycetes than the other ions in the medium, indeed, the quantities of zinc, manganese, cobalt, nickel and cadmium absorbed by almost all species of microorganisms were found to be extremely low. On the other hand, the authors also found that cobalt accumulation from a mixed solution containing nine different heavy metals was very poor, but all species tested by them accumulated large amounts of cobalt ion from a solution containing cobalt as the only metal ion. The results suggests that the selective accumulation of heavy metal ions by microorganisms is determined by interionic competition. This results are also in agreement with those

Table 7: Selective absorption of heavy metals by parental strains of bacteria and their transconjugants growing on nutrient broth (1.5 g peptone and 0.5 g beef extract per litre) supplemented with wastewaters .

Mating	Parental strains and resulted transconjugants	presentage of heavy metals uptake (%)				
		Pb	Cd	Fe	Co	Cu
NRRL B-571(P1) X NRRL B-1584) (P2)	P1	94	95	87	90	81
	P2	92	91	89	90	88
	M.P	93	93	88	90	85
	Tr1	82	93	89	85	92
	Tr2	90	91	87	80	81
NRRL B-571 (P1) X NRRL B-2643 (P2)	P1	94	95	87	90	81
	P2	82	97	94	85	88
	M.P	88	96	91	88	85
	Tr1	80	95	85	90	85
	Tr2	95	95	94	95	81
NRRL B-571 (P1) X NRRL B-41228(P2)	P1	94	95	87	90	81
	P2	90	95	93	90	82
	M.P	92	95	90	90	82
	Tr1	90	97	89	85	82
	Tr2	90	97	96	85	88
NRRL B-1584(P1) X NRRL B-642(P2)	P1	92	91	89	90	88
	P2	80	95	94	90	85
	M.P	86	93	92	90	87
	Tr1	78	95	87	80	81
	Tr2	90	93	85	80	82
NRRL B-1584 X NRRL NRS-213	P1	92	91	89	90	88
	P2	82	97	94	85	88
	M.P	87	94	92	88	88
	Tr1	95	97	92	85	81
	Tr2	94	95	96	90	82
NRRL NRS-1264 X NRRL B-2643	P1	83	97	86	85	82
	P2	82	97	94	85	88
	M.P	83	97	90	85	85
	Tr1	80	98	89	85	88
	Tr2	90	93	32	85	73
NRRL B-358 X NRRL B-642	P1	82	95	83	85	85
	P2	80	95	94	90	85
	M.P	81	95	89	88	85
	Tr1	85	97	94	90	82
	Tr2	83	98	96	90	85
NRRL B-2643 X NRRL B-642)	P1	82	97	94	85	88
	P2	80	95	94	90	85
	M.P	81	96	94	88	87
	Tr1	95	95	47	90	81
	Tr2	95	95	92	85	82
NRRL B-41228 X NRRL B-642	P1	90	95	93	90	82
	P2	80	95	94	90	85
	M.P	85	95	94	90	84
	Tr1	82	98	88	80	69
	Tr2	86	93	83	80	88
NRRL B-642 X NRRL B-4375)	P1	80	95	94	90	85
	P2	78	98	89	85	85
	M.P	79	97	92	88	85
	Tr1	74	91	91	85	65
	Tr2	80	90	91	85	96
NRRL B-642 X NRRL NRS-213)	P1	80	95	94	90	85
	P2	82	97	94	85	88
	M.P	81	96	94	88	87
	Tr1	82	98	92	85	85
	Tr2	94	93	96	90	82
NRRL B-4375 X NRRL NRS-213		P1	78	98	89	8585
	P2	82	97	94	85	88
	M.P	80	98	92	85	87
	Tr1	91	97	70	85	85
	Tr2	84	98	92	78	85
Micrococcus luteus(NRRL B-287)		92	97	85	80	81

Table 7: Continued

Mating	Parental strains and resulted transconjugants	Heavy metals uptake presentage (ppm)				
		Mo	Pt	Mn	As	Hg
NRRL B-571(P1) X NRRL B-1584) (P2)	P1	59	72	85	71	85
	P2	51	70	80	64	82
	M.P	34	76	98	58	58
	Tr1	51	52	60	67	57
	Tr2	58	58	55	76	80
NRRL B-571 (P1) X NRRL B-2643 (P2)	P1	59	72	85	71	85
	P2	49	69	65	54	65
	M.P	34	76	98	58	58
	Tr1	49	45	65	67	62
	Tr2	44	49	78	88	88
NRRL B-571 (P1) X NRRL B-41228(P2)	P1	59	72	85	71	85
	P2	53	72	80	44	78
	M.P	34	76	98	58	58
	Tr1	54	62	85	86	77
	Tr2	59	58	83	88	78
NRRL B-1584(P1) X NRRL B-642(P2)	P1	51	70	80	64	82
	P2	48	61	60	64	57
	M.P	34	76	98	58	58
	Tr1	49	46	55	76	48
	Tr2	44	56	85	86	78
NRRL B-1584 X NRRL NRS-213	P1	51	70	80	64	82
	P2	58	69	75	33	78
	M.P	55	70	78	49	80
	Tr1	55	63	70	88	88
	Tr2	48	55	75	88	85
NRRL NRS-1264 X NRRL B-2643	P1	56	65	83	77	67
	P2	49	69	65	54	65
	M.P	34	76	98	58	58
	Tr1	56	69	80	71	47
	Tr2	58	68	65	88	78
NRRL B-358 X NRRL B-642	P1	61	74	85	73	60
	P2	48	61	60	64	57
	M.P	34	76	98	58	58
	Tr1	49	57	86	71	65
	Tr2	54	64	80	86	63
NRRL B-2643 X NRRL B-642)	P1	49	69	65	54	65
	P2	48	61	60	64	57
	M.P	34	76	98	58	58
	Tr1	50	68	83	88	90
	Tr2	72	63	83	88	88
NRRL B-41228 X NRRL B-642	P1	53	72	80	44	78
	P2	48	61	60	64	57
	M.P	34	76	98	58	58
	Tr1	49	55	90	71	65
	Tr2	56	63	85	84	70
NRRL B-642 X NRRL B-4375)	P1	48	61	60	64	57
	P2	66	67	78	68	60
	M.P	34	76	98	58	58
	Tr1	72	68	90	64	48
	Tr2	61	64	85	66	57
NRRL B-642 X NRRL NRS-213)	P1	48	61	60	64	57
	P2	58	69	75	33	78
	M.P	34	76	98	58	58
	Tr1	52	58	85	77	63
	Tr2	49	68	80	84	85
NRRL B-4375 X NRRL NRS-213	P1	66	67	78	68	60
	P2	58	69	75	33	78
	M.P	34	76	98	58	58
	Tr1	44	52	90	87	78
	Tr2	59	51	90	66	63
Micrococcus luteus(NRRL B-287)		67	72	85	77	82

Table 7: Continued

Mating	Parental strains and resulted transconjugants	Heavy metals uptake presentage (ppm)					
		Ni	Sb	Sr	V	Cr	Zn
NRRL B-571(P1) X NRRL B-1584) (P2)	P1	57	58	64	53	19	68
	P2	52	39	61	62	64	76
	M.P	55	49	63	58	42	72
	Tr1	55	39	60	51	46	64
	Tr2	67	56	38	37	46	78
NRRL B-571 (P1) X NRRL B-2643 (P2)	P1	57	58	64	53	19	68
	P2	48	53	38	42	56	70
	M.P	53	56	51	48	38	69
	Tr1	60	50	48	53	56	56
	Tr2	57	57	60	52	31	73
NRRL B-571 (P1) X NRRL B-41228(P2)	P1	57	58	64	53	19	68
	P2	43	49	24	32	56	75
	M.P	50	54	44	43	38	715
	Tr1	38	39	57	54	74	68
	Tr2	54	33	52	61	56	64
NRRL B-1584(P1) X NRRL B-642(P2)	P1	52	39	61	62	64	76
	P2	43	51	57	47	31	64
	M.P	48	45	59	55	48	70
	Tr1	60	39	57	57	64	69
	Tr2	48	50	59	60	56	67
NRRL B-1584 X NRRL NRS-213	P1	52	39	61	62	64	76
	P2	57	28	48	52	54	68
	M.P	55	34	55	57	59	72
	Tr1	57	53	66	63	64	76
	Tr2	57	39	57	56	56	64
NRRL NRS-1264 X NRRL B-2643	P1	59	50	65	55	46	64
	P2	48	53	38	42	56	70
	M.P	54	52	52	49	51	67
	Tr1	60	66	66	65	53	74
	Tr2	57	68	70	63	64	81
NRRL B-358 X NRRL B-642	P1	48	49	57	47	46	68
	P2	43	51	57	47	31	64
	M.P	46	50	57	47	39	66
	Tr1	58	58	72	67	19	74
	Tr2	48	58	59	47	46	63
NRRL B-2643 X NRRL B-642)	P1	48	53	38	42	56	70
	P2	43	51	57	47	31	64
	M.P	46	52	48	45	44	67
	Tr1	14	55	66	67	31	71
	Tr2	43	50	61	53	13	79
NRRL B-41228 X NRRL B-642	P1	43	49	24	32	56	75
	P2	43	51	57	47	31	64
	M.P	43	50	41	40	44	695
	Tr1	57	55	57	63	56	70
	Tr2	67	64	66	65	46	78
NRRL B-642 X NRRL B-4375)	P1	43	51	57	47	31	64
	P2	60	52	57	42	38	67
	M.P	52	52	57	45	35	655
	Tr1	43	49	61	63	64	72
	Tr2	62	75	69	54	38	74
NRRL B-642 X NRRL NRS-213)	P1	43	51	57	47	31	64
	P2	57	28	48	52	54	68
	M.P	50	40	53	50	43	66
	Tr1	61	50	66	71	46	79
	Tr2	57	67	62	67	64	67
NRRL B-4375 X NRRL NRS-213	P1	60	52	57	42	38	67
	P2	57	28	48	52	54	68
	M.P	59	40	53	47	46	675
	Tr1	61	60	69	63	46	70
	Tr2	56	51	66	53	46	64
Micrococcus luteus(NRRL B-287)		57	51	66	53	45	70

reported by Brierley (1990), who found that the granulated *Bacillus* preparation is non-selective and can remove many heavy metals from solution independent of differing initial concentrations, e.g. Cd, Cr, Cu, Hg, Ni, Pb, U and Zn, but does not bind Ca, Na, K or Mg. Single or mixed metals are generally loaded to > 10% of the dry weight giving a removal efficiency of > 99% and effluents with total metal concentrations around 10 - 50 ppb. Microbial transformations of arsenic and chromium species are also associated with a decrease in toxicity and may have relevance to wastewater treatment (Williams and Silver, 1984). On the other hand, microorganisms can transform heavy metal and metalloid species by reduction (Lovley *et al.*, 1991). Hansen *et al.* (1984) found that a continuous cultures of Hg^{2+} - resistant bacteria, can reduce Hg^{2+} to Hg^{o} with mercuric reductase, volatilized Hg^{o} from contaminated sewage at a rate of 2.5 mg L^{-1} h^{-1} (98% removal).

The results obtained in this proposal indicated that bioremediation of heavy metal pollution remains a major challenge in environmental biotechnology. Many bacterial polysacchrides have been shown to bind heavy metals with varying degrees of specificity and affinity.

The results are in agreement with those obtained by Volesky *et al* 2001, who examined cadmium uptake by nonliving and resting cells of *Saccharomyces cerevisiae* obtained from aerobic or anaerobic cultures from pure cadmium - bearing solutions. They found that the highest cadmium uptake exceeding 70 mg Cd / g was observed with aerobic baker`s yeast biomass from the exponential growth phase. Nearly linear sorption isotherms featured by higher sorbing resting cells together with metal deposits localized exclusively in vacuoles indicate the possibility of a different metal-sequestering mechanism when compared to dry nonliving yeasts which did not usually accumulate more than 20 mg Cd/g. The uptake of cadmium was relatively fast, 75% of the sorption completed in less than 5 min.

In conclusion, we can use the efficient recombinants resulted in this study in the merits of the biobattery designed to uptake heavy metals; the design of the biobattery is capable of adsorbing complex metal ions, industrial effluents can be treated for the control of metal pollution. The biosorbent is capable of adsorbing metals rapidly within the first 30 min, which will facilitate shorter adsorption columns. Operation of the battery is so simple that each catridge can be replaced without affecting the continuity of the process. Though the adsorbed metal can be retrieved using suitable elutants and the catridge can be reused. The biobattery can be considered as an ecofriendly and cost-effective technique for treating industrial effluents.

ACKNOWLEDGMENTS

We are very grateful to thanks and gratitude to Dr. Abdullah Al - Najar - President of ASTF and Mr. Eng . Amar Munir - Director of funding program , for their organizational support . We also wish to record our thanks and gratitude to senior officials of ASTF and to all ASTF members for their supporting this work and helping us very well during the conducting of this study . We are grateful to Dr. Noor Rays for answered about all my questions at the same time via the e-mail . This work was supported from ASTF , United Arab Emirates .

This manuscript was released with other three manuscripts from the project code number : WA062083, entitled : biocontrol of heavy metals pollution from wastewater , funded from ASTF . The authors are very grateful to thanks ASTF for kindly cooperation in supporting , funding and sponsorship this work very well

REFERENCES

Brady, D., A.D. Stoll, L. Starke and J.R. Duncan, 1994. Chemical and enzymatic extraction of heavy metal binding polymers from isolated cell walls of *Saccharomyces cerevisiae*. Biotechnology and Bioengineering, 44: 297-302.

Brierley, J.A., 1990. Production and application of a *Bacillus* - based product for use in metals biosorption. In Biosorption of Heavy Metals, ed. B. Volesky, pp. 305-311. Boca Raton: CRC Press.

Bryan, G.W., R.J. Langston, 1992. Bioavailability, accumulation and effects of heavy metals in sediments, with special reference to United Kingdom estuaries: a review. Environmental Pollution, 76: 89-131.

Butt, T.R. and D.J. Ecker, 1987. Yeast metallothionein and application in biotechnology. Microbiological Reviews, 51: 351-364.

Collins, C.H. and P.M. Lyne, 1985. Microbiological Methods. 5th ed. Butterworths, London, 167-181.

DeGregori, I., H. Pinochet, M. Arancibia, A. Vidal, 1996. Grain Size Effects on Trace Metals Distribution in Sediments from Two Coastal Areas of Chile. Bull. Envrin. Contam. Toxicol., 57: 163-170.

DHV, 1998. Meghna Estuary Study, Draft Master Plan, Volume 1, Main Report for BWDB, Dhaka, Bangladesh.

DoE, 2001. The General over view of pollution status of Rivers of Bangladesh, Department of Environment, Dhaka, Bangladesh.

Goyal, N., S.C. Jain and U.C. Banerjee, Comparative studies on the microbial adsorption of heavy metals. Advances in Environmental Research, January 2003, vol. 7, no. 2, pp. 311-319.

Griffiths A.J.F., *et al*., 1999. An Introduction to genetic analysis, 7th ed., San Francisco: W.H. Freeman. ISBN 0-7167-3520-2.

Hansen, C.L., G. Zwolinski, D. Martin and J.W. Williams, 1984. Bacterial removal of mercury from sewage. Biotechnology and Bioengineering, 26: 1330-1333.

Horikoshi, T., A. Nakajima and T. Sakaguchi, 1981. Studies on the accumulation of heavy metal elements in biological systems XIX. Accumulation of uranium by microorganisms. Eur. J. Appl. Microbiol. Biotechnol., 12: 90-96.

Holmes R.K., M.G. Jobling, 1996. Genetics: Conjugation. in: Baron's Medical Microbiology (Baron S *et al*, eds.), 4th ed., Univ of Texas Medical Branch. ISBN 0-9631172-1-1.

Kratochvil, David, Pimentel, Patricia and Volesky, Bohumil, 1998. Removal of trivalent and hexavalent chromium by seaweed biosorbent. Environmental Science and Technology, 32(18): 2693-2698.

Lederberg, J., E.L. Tatum, 1946. "Gene recombination in E. coli". Nature 158: 558.

Lederberg, J., 1952. "Cell genetics and hereditary symbiosis". Physiol. Rev. 32(4): 403-30.

Lessel, M., D. Balzer, K. Weyrauch and E. Lanka, 1993. The mating pair formation system of plasmid RP4 defined by RSF 1010 mobilization and donor-specific phage propagation. J. Bacteriol., 175(20): 6415-6425.

Lovely, D.R., E.J.P. Phillips, Y.A. Gorby and E.R. Landa, 1991. Microbial reduction of uranium. Nature, 350: 413-416.

Liu H.L., B.Y. Chen, Y.W. Lana, Y.C. Cheng, 2004. Biosorption of Zn(II) and Cu(II) by the indigenous *Thiobacillus thiooxidans.* Chemical Engineering Journal, 97: 195-201.

Macaskie, L.E. and A.C.R. Dean, 1984. Cadmium accumulation by a Citrobacter sp. J. Gen. Microbiol., 130: 53-62.

Mateu, J., M. Forteza, M. Colom-Altes, V. Cerda, 1996. Atmospheric background levels and transport of heavy metals in the Balearic Islands. Water, Air and Soil Pollution 86: 157-172.

Matheickal, J.T., Q. Yu, and G.M. Woodburn, 1999. Biosorption of cadmium(II) from aqueous solution by pre-treated biomass of marine algae Durvillaea potatorum. Water Res., 33: 335-343.

Nakajima, A. and T. Sakaguchi, 1986. Selective accumulation of heavy metals by microorganisms. Appl. Micro. Biotech., 24: 59-64.

Niu, H., X.S. Xu and J.H. Wang, 1993. Removal of lead from aqueous solutions by penicillin biomass. Biotechnol. and Bioeng., 42: 785-787.

Ramelow, G.J., D. Fralick and Y. Zhao, 1992. Factors affecting the uptake of aqueous metal ions by dried seaweed biomass. Microbios, 72L: 81-93.

Roth, J.R. and R.V. Sonti, 1989. Role of gene duplications in the adaptation of Salmonella typhimurium to growth on limiting carbon sources. Genetics, 123: 19-28.

Sager, M., 1997. Possible trace metal load from fertilizers. Die Bodenkultur, 48(4): 217-223.

Silver, S., 1991. Bacterial heavy metal resistance systems and possibility of bioremediation. In: Biotechnology, Bridging Research and Applications, 265-287. Kluwer, Academic Publishers, London.

Singer, P.C., 1974. Trace Meals and Metal-Organic Interactions in Natural Waters. Ann. Arbour. Science, USA.

Tam, N.F.Y., Y.S. Wong, 2000. Spatial variation of heavy metals in surface sediments of Hong Kong mangrove swamps. Environmental Pollution, 110: 195-205.

Volesky, B., (ed) 1990. Biosorption of heavy metals. Boca Raton: CRC Press.

Wang, Jianlong and Chen, Can, 2006. Biosorption of heavy metals by Saccharomyces cerevisiae*: A review.* Biotechnology Advances, September-October 2006, 24(5): 427-451.

Winge, D.R., R.N. Reese, R.K. Mehra, E.B. Tarbet, A.K. Hughes and C.T. Damerson, 1989. Structural aspects of metal-Y-glutamyl peptides. In Metal Ion Homeostasis: Molecular Biology and Chemistry, eds D.H. Hamer and D.R. Winge, pp. 301-311. New York: Alan R. Liss Inc.

Williams, J.W. and S. Silver, 1984. Bacterial resistance and detoxification of heavy metals. Enzyme and Microbial Technology, 6: 530-537.

Zouboulis, Anastasios I., A. Matis, Kostas and N.K. Lazaridis, 2001. Removal of metal ions from simulated wastewater by Saccharomyces yeast biomass: combining biosorption and flotation processes. Separation Science and Technology, 36(3): 349-365.