Effect of Some Chemical Compounds on Sedimentation Rate of Different Yeast Strains

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Abstract: Heavy metal pollution represents an increasing problem in industrialized as well as developing countries. Yeast cells are capable to accumulate these pollutant from different environments. In this investigation, eight baker's yeast strains were collected from different Egyptian markets. The source of these yeast strains were Misr Yeast, Alinson, Vahine professional, Fermipan, Hollandia Saf–instant, H.u.G and Pakamaya. These strains were grown on basal medium or in molasses medium to determine their efficiency in the bioaccumulation of some metals. The sedimentation measurement was carried out at different salt solutions and different times intervals. The results clearly indicated that SnCl2 followed in descending order by Pb (CH3CooH)2 and AgNO3 were the most effective compounds in increasing the rate of sedimentation of all the tested yeast strains. In contrast; the lowest Figures were recorded with KH2PO4 ,FeCl3 , NiSO4,Co(NO3)2,CaSO4,MgSO4 , Zn SO4, Al2(So)4 and Co CL 2. Other minerals showed a moderate sedimentation capability. It can be stated that yeast cells have a considerable capability to uptake Zinc and iron from the growing medium whereas, manganese showed moderate capability. The lowest values were observed in2the case of copper and lead. *Saccharomyces cerevisiae* can be used as a bioremediation agent for removing heavy metals from the surrounding environment due to its high uptake capacity, taking in consideration that it must be economically competitive with existing technologies.

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1. Introduction:

As early as 1000 BC, the Roman, Phoenicians and other early civilizations recovered copper from waters, which had passed through mining operations and ore bodies. In the 1700s, the Spanish at RioTinto applied leaching process to extract copper from copper-bearing minerals. Microbial metal recovery holds great promise for low cost treatment of metal-contaminated industrial effluents and economic recovery of valuable metals, Brierley *et al.*, (1985); Ross (1990).

Fungi and yeasts can accumulate heavy metals and radionuclides even from dilute external concentrations ,Gadd,(1989). Yeast cells are capable of carrying out biosorption (sorption can includeboth adsorption and absorption) and describes the movement of a component from one phase to be accumulated in another solid phase, Gadd, (1989), with various heavy metals. The biomass deriving from*S. cerevisiae* coming from brewing industries is a by-product that is possible to be used in the purification of water contaminated with these ions,Omar *et al*, (1996) showed that yeast biomass fromone of city's breweries can adsorb uranium efficiently, up to 204 mmol of this metal per gram of drybiomass. A study comparing the biosorption of Sr+2 by a laboratory strain and an industrial one of *S.cervisiae* has shown that the industrial strain was more efficient,Avery and Tobin(1992).

Spectrographic analyses have shown that yeasts contain various trace elements, e.g. silver, cobalt, chromium, copper, molybdenum, nickel, lead, tin, vanadium, and zinc, Suomalainen and Qura, (1971). In *Saccharomyces cerevisiae* and *Neurospora crassa*, cobalt is transported via the general3cation uptake system ,Fuhrmann and Rothstein (1968); Venkateswerlu and Sastry (1970). Uptake consists of an initial binding to the cell surface followed by active transport across the membrane. Two K+ are released for each Co +2 Taken up ,Norris and Kelly(1977). Copper and mercury-tolerant strains of *S. cerevisiae* produced more H2S than do their non tolerant parent strains, the metal being precipitated as insoluble sulphides (Ehrlich, and Fox 1967; Kikuchi, 1965, and Naiki, 1957). Electron micrographs have shown that the copper sulphide was chiefly deposited in and around the cell wall, Ashida (1976) ;Gadd and Griffiths (1978).

Precipitation within or on cell-walls may be particularly evident with radionuclides such as uranium and thorium. In *S. serevisiae*. Uranium was deposited as a layer of needle-like fibres on cell-walls reaching up to 50% of the biomass dry weight. That such a large amount was bound by the cells implied that additional uranium had crystallized on already bound molecules, Strandberg *et al*., (1981).The present study was undertaken to assess the ability different strains of *Saccharomyces cerevisiae* to adsorb different types

of heavy metals and remove them from the polluted area.

2.Materials and Methods:

2.1.Materials:

2.1.1.Yeast strains:

Yeast samples (baker's yeast) were rehydrated twice in malt broth medium (Seifert, 1990)) and left to grow for 2 days. Streak method has been used for isolation. The developing colonies on the malt agar plates (incubated at 30°C for 2 days) were picked up under aseptic conditions, purified. Stock culture slants were maintained at 5°C on malt medium after incubation at 25-30°C for 24-28 hrs. Different baker's yeasts were collected from different market in Cairo, Egypt.They included different baker'syeast produced from different countries being Misr yeast, Alinson, Vahine', Fermipan, Hollandia,H.U.G, Saf-instant and Pakmaya.

2.1.2.Standard inoculum

It was prepared by double activation of yeast cells in Erlenmeyer flasks (250 ml in volume) containing 100 ml of basal medium with 1 ml of yeast broth culture 24 hr old to make sure that yeast cells in log phase. The culture should be contained approximately 5X 107Cells/ml (Becker et al., 1990). Baker's yeast strains Were grown on basal medium consists of: 5g Ammonium Sulphate, 10g Bacto-dextrose, 1g KH_2PO_4, 0.5g $MgSO_4.7H_2O$, 0.5-1g yeast extract /L (pH 3.9)on shaker at 150 rpm for 48 hr at 300C.

2.1.3. Salt solutions:

Five percent salt solutions were prepared from CuSO4.5H2O (extra pure Merck), MnSO4.H2O (typeanalysis, Riedel de Haen)MgSO4.7H2O (extra pure Merck), Na3PO4.12H2O (GR Merck), (NH4)2Cr2O7 (pure Koch Light), CaCl2 (anhydrous, Prolabo France), NiCl2(anhydrous for synthesis Merck), KH2PO4(extra pureMerck), Co(NO3)2.6H2 (GR Merck), FeCl3.6H2O (pure Merck),FeSO4 7H2O(GPR Winlab), Al2(SO4)3. 18H2O (extra pure Merck),(Zn SO4 7H2) (analytical grade Nentech,CsCl (ultra pure optical grade BRL),CdSo4.8H2O (type analysis, Riedel de Haen) ,molybdic acid about 90% MoO3(containing ammonium molybdate)(Prolabo France), Pb(CH3COO)2 .3H2O(type analysis, Riedel deHaen),AgNO3,(ACS, Fisher), NiSO4. 6H2O(May and Baker) and K 3 As O4 (analytical grade, Germany).

2.2.Methods:

1% VO SO4. 5H2O(research grade, serva)-2.5% SnCl2. 2H2O (analytical grade, Prolabo France). The pellets were dissolved in HNO3 (Analar , BDH) heated at 60°C until water evaporated and 5 g of the generated salt dissolved in distilled water up to 100 ml.

Four ml of liquid culture was added to five replicates of 25ml volumetric cylinder with stopper. And one ml of tested salt solution was added using a syringe dispenser, (in the case of SnCl2 .2H2O and VO SO4.5H2O 2ml and 2.5 were added respectively instead of one ml) to each cylinder then shake well and let to sediment.

Sedimentation rate (ml/h) was determined after a time range of 1-4 h from the 5 beginning of experiment to evaluate the capability of different chemical compounds and heavy metal solutions to flocculate yeast cells of different strains.

The selected yeast strains which showed the highest efficiency in the bioaccumulation of metals were grown on molasses medium (Abdel-Hafez, 1981) for 6 hours, at 30 C on 150 rpm shaker and the heavy metal were determined using the atomic absorption

3.Results:

3.1.Sedimentation Rates of yeast cells :

It was found that SnCl2 followed in descending order by Pb(CH3COO)2 and AgNO3 are the most efficient compounds in enhancing yeast cells sedimentation. Where the sedimentation rate were 35, 30 and 15 ml/h respectively after 2-3 hours. These yeast strains were the most active baker's yeast in the sedimentation studies as compared with other yeast strains. On the contrary, other heavy metals did not show any effect on yeast cells. The obtained results clearly indicate that solution (2.5%) of Sn Cl2 followed in descending order by solutions (5%) of Pb(CH3COOH)2 and AgNO3, are the most effective compounds in increasing the rate of sedimentation of all tested yeast strains giving >36, >30 and >15 ml/h after 1 hour respectively (Figs.1a, 1b, 1c and 1d). In contrast, the lowest sedimentation rates were obtained with KH2PO4, FeCl3,NiSO4, Co (NO3)2, CaSO4, MgSO4, ZnSO4, Al2 (SO4)3 and CsCl when they gave rates of sedimentation less than 1.0 ml/h. Other compounds are considered to be of a moderate sedimentation rates around 1.0 ml/h.(Fig.2)

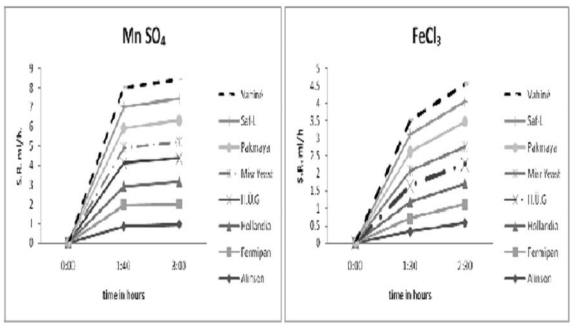

Fig.(1a): Effect of some salt solutions on the sedimentation rate of the different yeast strains

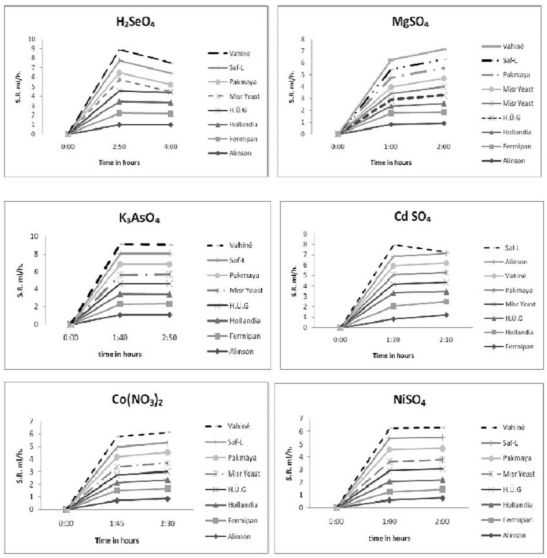

Fig.(1b): Effect of some salt solutions on the sedimentation rate of the different yeast strains.

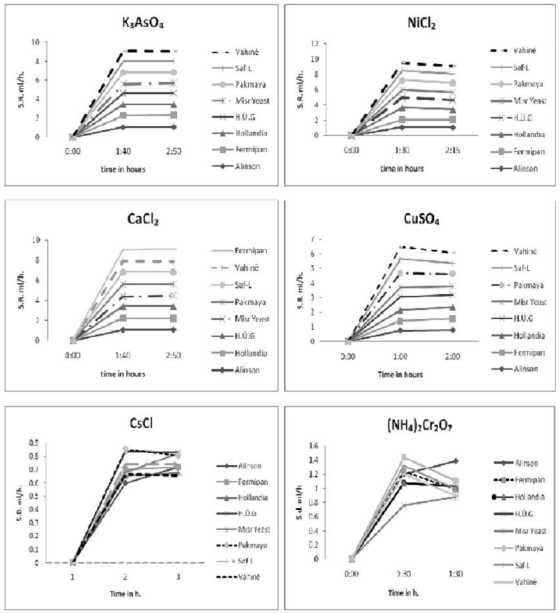

Fig.(1c): Effect of some salt solutions on the sedimentation rate of the different yeast strains.

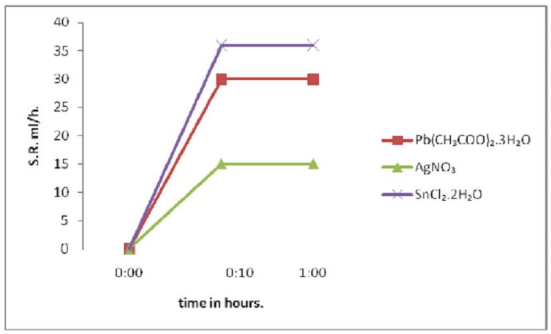

Fig.(2): The most effective salts on the sedimentation rate of all yeast strains.

3.2. Evaluating the capability of *S. cerevisiae strains* (Misr yeast and Fermipan) to uptake heavy metals:

It was found that both tested yeast strains have the ability to accumulate zinc and iron within their cells more than other tested heavy metals namely manganese, copper and lead (Figs.3 and 4). Fermipan yeast strain gave 100% uptake of iron and zinc after 6 h. at 300c, in comparison to only 35.8% uptake of lead. Misr yeast strain gave nearly similar results to those of Fermipan where it showed 100 and 98.6% accumulation of zinc and iron respectively after the same incubation period. Copper proved to be the lowest metal in its affinity to accumulate in the growing yeast cells. In the two yeast strains under investigation, manganese followed iron and zinc descendingly in its affinity to accumulation in both cases.

Fig.(3): The percentage of metal uptake in the yeast cells.

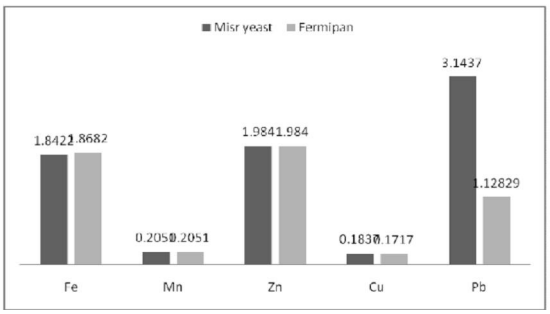

Fig.(4): The uptake of metals in yeast cells in ppm.

4.Discussion:

To elucidate the interaction between 8 yeast srains and 25 different chemical compounds (including some heavy metal ions) the edimentation rate was determined after different periods of time. Kuyucak and Volesky (1988);Mullen *et al*, 1989; McLean and Beveridge(1990) listed the factors affecting metal adsorption by different microorganisms in the following:

a) Composition of biomass.

b) Physical and chemical factors such as the presence of other anions and cations can also affect adsorption either by precipitation (phosphates-hydroxides) or by competition for adsorption sites.

c)Biomass concentration.

d) Living and dead cells.

Regarding the bioaccumulation of heavy metals yeast cells determind by using atomic absorption technique, the obtained results clearly showed that molasses content of zinc followed by iron have a high ability to accumulate within the growing cells of *Saccharomyces cerevisiae*, while others such as manganese has a moderate ability to accumulate in yeast cells. Brierley (1990); Brich & Bachofen (1990); McLean & Beveridge (1990); Volesky (1990), Avery *et al* (1992); McQuanttie *et al* (1992); Denny & Ridge (1995)); Ramirez *et al* (1996); Leyval *et al* (1997); Joner & Leyval (1997) and Childress et al7(1998) demonstrated that different microorganisms can accumulate heavy metals from their external environments.

The slight difference in the present study between the capabilities of different yeast stains to accumulate heavy metals occurred in molasses is previously interpreted by Somers (1963) who noticed that cell wall of fungi and yeast is vary considerably in their overall composition. He added that differences in uptake capacities may exist between different species, between cells of different ages, and even between different cell forms of the same organism e.g., *Penicillium italicum* spore walls take up more copper than vegetative cell walls did.

In the present study, obtained results show that growing yeast strains on molasses medium for only 6 hours gave the maximal metal accumulation. In the same direction, De Rome and Gadd (1987) observed that yeast cells accumulate more metal ions at low cell densities than at high cell densities.

On the light of the our obtained results, it can be generally concluded that *Saccharomyces cerevisiae* can be used as a bioremediation agent for removing heavy metals from the surrounding environment due to its high uptake capacity, taking in consideration that it must be economically competitive with existing technologies.

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