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EFFECT OF NICKEL STRESS ON *ESCHERICHIA COLI* AND *SACCHAROMYCES CEREVISIAE*

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Key words : Nickel Sulphate, *E. coli*, *S. cerevisia*e, metal-microbe interactions, Microbial growth.

ABSTRACT

This study is carried out to understand the tolerance level of microbes to nickel ions. *E. coli* (prokaryote) and *Saccharomyces cerevisiae* (eukaryote) has been taken for the study. The Minimum inhibitory concentrations of $NiSO_4$ for both the organisms were found to be 350 mg/L for *E. coli* and 3500 mg/L for *S. cerevisiae*. The growth of both of these organisms in different concentrations was studied. Generally, as the concentration of nickel sulphate in the media was increased, there was substantial decrease in growth of both the organisms. *E. coli* exhibited a delayed log phase at increasing concentrations of nickel sulphate whereas there was no such delayed log phase in the case of *S. cerevisiae*. In *E. coli*, at initial $NiSO_4$ concentration of 50 mg/L, the percentage of ions remaining in the supernatant was 81% whereas it was 94% corresponding to 300 mg/L initial concentration of $NiSO_4$. In *S. cerevisiae*, for initial $NiSO_4$ concentration of 1000 mg/L, the percentage of Ni ions remaining in the supernatant was 82% whereas at 3000 mg/L of initial $NiSO_4$ concentration, 98% Ni ions were remaining in the medium after 15 h. The amount of nickel ions removed from the medium at the end of 72 h was studied. In *E. coli*, the maximum removal at the end of 72 h was found to be around 33%. On the other hand, the maximum removal obtained in *S. cerevisiae* was around 77%. The cytosolic protein content was studied under nickel stress. In both the organisms, the high content of protein found corresponding to cultures treated with highest concentration of nickel sulphates. In *E. coli*, no significant difference was observed between the banding patterns of the control and treated samples except that far high molecular weight proteins were found to be over expressed in the nickel treated cultures. In *S. cerevisiae*, a protein corresponding to around 205 kDa was found to be down regulated in the 100 mg/L nickel sulphate treated culture. In addition, two new proteins corresponding to around 55 kDa and 80 kDa were over-expressed at higher concentrations of nickel sulphate.

INTRODUCTION

Heavy metals are metals with a density above 5 g/cm3. They are important as "trace elements" in complex biochemical reactions (Nies, 1999). Rapid development of various industries smelt in disposal metal containing waste water into the environment, especially in developing countries, this leading to serious environmental pollution and threatened of all organisms (Bishop, 2002; Volesky, 1990). Conventional methods for removal metal ions from aqueous solution have been studied in detail, such as chemical precipitation, ion exchange, electrochemical treatment of waste water, membrane filtration technologies, adsorption on activated carbon etc. Every method has its own advantages and disad-

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vantages besides being inefficient and expensive. Besides methods like chemical precipitation and electrochemical treatment are ineffective, especially when metal ion concentration in aqueous solution is as low as 1 to 100 mg/l. Ion exchange, membrane technologies and activated carbon adsorption process are expensive, especially when treating a large amount of water/wastewater containing heavy metal in low concentration, so they cannot be used at larger scale. Biosorption is an alternate and cost effective process that utilizes various organisms including bacteria, fungi, yeast, algae. These bio-sorbent possess metal sequestering properties and can decrease the concentration of heavy metal ions in solution from parts per trillion to parts per billion level. They can effectively and efficiently sequester dissolved metal ions out of dilute complex solutions. In last few decades, biosorption using microbial biomass has emerged as a potential alternative to the remediation of heavy metal contamination (Congeevaram et al., 2007).

Nickel, an inorganic metal occurs naturally in the soil and is often used in electroplating, stainless steel and alloy products. Nickel is discharged by mining, metal refineries, smelting, sewage sludge, combustion of fuel fossils and agricultural activities (Barceloux, 1999). It occurs constitutes a trace element in most living cells (Boyle and Robinson, 1988). Available literature reveals that nickel acts as a cofactor of several enzymes such as hydrogenase, methyl coenzyme M. reductase, CO dehydrogenase and urease in various organisms (Hausinger, 1987). However, higher concentrations of nickel have been reported to be toxic (Nies, 1999). The United States Environmental Protection Agency has fixed the drinking water standard for Ni (II) as 0.1 ppm and the industrial discharge limit in waste water as 2 ppm. The industrial effluent permissible discharge level of Ni (II) has been fixed as 3 ppm by ISI Bureau of Indian Standards (Congeevaram et al., 2007).

Nickel is of major environmental concern because of its larger usage in developing countries and it is non degradable. Microorganisms uptake metal, either actively (bioaccumulation) or passively (biosorption) (Hussein et al., 2003). Currently, microbial systems like fungi, bacteria and algae have been successfully used as adsorbing agents for removal of heavy metals (Munoz et al., 2006). Different species of Aspergillus, Pseudomonas, Sporophyticus, Bacillus, Phanerochaete, etc., have been reported as efficient nickel reducers (Yan and Viraraghavan, 2003). This study focuses on the comparative nickel uptake reducing capacity of prokaryote E. coli and eukaryote S. cerevisiae which will be useful for cost effective and efficient treatment process in the environment.

MATERIALS AND METHODS

Preparation of Nickel Solution : Stock (100 mg/mL) solution of Nickel sulphate ($NiSO_4.6H_2O$) salt (Sigma) was prepared, autoclaved and used for the studies.

Organism : *E. coli* strain was obtained from Bangalore genie (batch no. 121206). *S. cerevisiae* was isolated from Baker's yeast.

Growth Medium : *E. coli* was cultured in Luria Bertani medium containing 1% tryptone, 0.5% Yeast Extract and 1% Na Cl and pH made to 7.2. *S. cerevisiae* was cultured in YPD media containing Yeast extract, Peptone and Dextrose. 2 % agar was added to prepare solid media.

Susceptibility testing : Susceptibility tests were conducted in liquid medium. Precultures of *E. coli* were grown at 37° C with shaking in LB broth. Similarly *S. cerevisiae* were grown at 30° C with shaking in YPD media. These cultures were serially diluted 10-2 fold in the same medium supplemented with increasing amounts of $NiSO_4\ 6H_2O$ concentration. The minimal inhibitory concentration, defined as the lowest concentration of the metal that inhibited, with shaking, at optimum temperature. The cells were challenged with sub-inhibitory concentrations of the metal and the growth was measured using spectrophotometer.

E. coli : Five mL LB medium were inoculated with 1% of overnight grown *E. coli* culture. To these 50, 100, 150, 200, 250 and 300 mg/L concentration of $Ni\ SO_4\ 6H_2O$ was added. A culture without $Ni\ SO_4\ 6H_2O$ is used as a control (0 mg/L). These concentrations were determined after series of test experiments, concentration ranging from 50 mg/L to 500 mg/L of $NiSO_4.6H_2O$. The inoculated culture were grown in 150 rpm shaker at 37°C and harvested at 0h, 1h, 2h, 3h, 4h to plot the lag phase, and 6h, 8h, 10h, 15h, 18h, and 21 h cultures were harvested time intervals to plot log and subsequently for stationary phase. Harvested cultures were immediately measured for its growth based on its turbidity at 600 nm using UV-Visible spectrophotometer (Freeman *et al*. 2005). The values given in table are an average of five experimental values (Table 1). The growth curve of *E. coli* under different concentrations of $NiSO_4.6H_2O$ was constructed (Graph 1).

S. cerevisiae : Five mL YPD medium in boiling test

tubes were inoculated with 1% of inoculums. To these 1000, 1250, 1500, 1750, 2000, 2250, 2500, 2750 and 3000 mg/L concentration of $NiSO_4$ $6H_2O$ was added in individual test tubes. A culture without $NiSO_4$ $6H_2O$ was used as a control (0 mg/L). These concentrations were obtained after series of test experiments using 50 mg/L to 500 mg/L of $NiSO_4$ $6H_2O$. These cultures were grown in 150 rpm shaker at 37°C for given period of time and then harvested at 0h, 1h, 2h, 3h, 4h to plot the lag phase, and 6h, 8h, 10h, 15h, 18h, and 21 h cultures were harvested time intervals to plot log and subsequently to construct stationary phase. Harvested cultures were measured for its growth based on its turbidity at 540 nm using UV-Visible spectrophotometer (Nishimura *et al*. 1998) and tabulated (Table 2). Five sets of the above experiment were conducted in order to plot the growth curve (Graph 2).

Atomic Absorption Spectroscopy (AAS) : AAS is used to estimate the concentration of metal ions at very low concentrations in the given solution. The *E. coli* and *S. cerevisiae* cultures were treated with various concentrations of $Ni\,SO_4$ $6H_2O$ as mentioned earlier. The cultures were harvested after 15 h of growth and then centrifuged. The pellet and supernatant were processed separately for AAS analysis and the concentration of Ni ions was determined by AAS.

Supernatant : Supernatant was taken and 1 mL of conc. HNO_3 was added and suspended the pellet. Sample were then boiled for 2 h and evaporated to 4 ml on a hot plate. Then, 1 mL of 12 N conc. $H_2\,SO_4$ was added and sample was boiled till brown fumes were evident. The sample was then cooled and diluted to 10 mL with double distilled water. The concentration of Ni ion in the sample was estimated by using AAS using appropriate standards.

Pellet : Pellet obtained in the above experiment was used for this experiment. Pellet was washed once with 50 mM EDTA and once then with water. The cell pellets were digested in 500 µL of 6M HNO3 at 100°C for 2 h. The resulting sample was diluted ten times with double distilled water. The concentration of Ni ion was analyzed in AAS.

DMG Assay : Nickel sulphate (1g) was weighed and made up to 250 mL with ddH_2O. This solution served as the stock. 5 mL of the stock was diluted to 100 mL to obtain the working standard. 20, 40, 60, 80 and 100 µL of the working standard were taken in test tubes. The volume was made up to 100 µL with double distilled water, 200 µL of 4% bromine water was added followed by 200 µL of concentrated ammonia. Then 100 µL of 1% Dimethyl glyoxime (DMG) was added to all the tubes. A tube without Nickel is a blank. The unknown solution of known volume and reagents were added in the same order and made up to 100 µL. The absorbance of these solutions was measured at 440 nm using a UV visible spectrophotometer. A graph was constructed by plotting the absorbance value against concentration. This served as the standard curve. The amount of Nickel in the unknown sample was calculated from the curve (Snell and Snell, 1949).

Protein estimation: One mL of culture was taken, centrifuged and pellet was washed in 1 ml double distilled H_2O and then resuspended in 0.3 mL double distilled H_2O. Then equal volume of 0.3 M Na OH was added. The sample was incubated for 10 min. The supernatant was discarded after centrifugation. Sample buffer (70µL) was added to the pellet and cells were re-suspended. The sample was boiled for 3 minutes. The extracted protein sample was either loaded on to the gel or stored at -20°C. The total cell protein was estimated according to the method of Lowry *et al*. (1951).

Protein profile : The cell pellets were re-suspended in SDS sample loading buffer and boiled for 10 minutes at 100 °C hot water bath. The crude proteins were separated on a 10% SDS - PAGE and stained in Coomassie brilliant blue (R 250).

RESULTS

1. Susceptibility test

The minimum inhibitory concentration of $NiSO_4$ $6H_2O$ was found to be 350 mg/L for *E. coli* and 3250 mg/L for *S. cerevisiae*. Based on test experimental values, a set of sub inhibitory concentrations were chosen for each organism. The concentrations of nickel sulphate for: *E. coli* are 50 mg/L, 100 mg/L, 150 mg/L, 200 mg/L, 250 mg/L and 300 mg/L and for *S. cerevisiae* : 1000 mg/L, 1250 mg/L, 1500 mg/L, 2000 mg/L, 2250 mg/L, 2500 mg/L, 2750 mg/L and 3000 mg/L.

2. Growth Studies

The growth of *E. coli* under different nickel concentrations were measured based on its turbidity, the OD600 values obtained were plotted against time. The growth curve for the control is normal and sigmoid curve. As the concentration of $Ni\,SO_4$ $6H_2O$ in

the media was increased, there is a significant and gradual decrease in the growth rate accordingly. The curves corresponding to cultures treated with 50 mg/L and 100 mg/L of $NiSO_4.6H_2O$ exhibited a growth pattern similar to that of the control. On the other hand, the cultures treated with nickel sulphate concentrations above 100 mg/L showed a delayed and slow log phase. The delay in growth is due to the time taken by the organism to adapt to high concentrations of the metal ion. The culture treated with 300 mg/L of the salt showed a shorter log phase (Table 1 and Graph 1) whereas in *S. cerevisiae*, growth curves corresponding to different concentrations were obtained. It was observed that there was shorter lag phase among cultures. The growth curves showed that log phase followed by a stationary phase. The growth patterns of the control and treated cultures were not found to differ much although there were differences in the growth rates. The curve corresponding to control showed a longer log phase compared to the treated samples. As the concentration of nickel sulphate in the media was increased, there was a corresponding decrease in the growth rate. The final cell densities for the different samples were also found to vary in the control having the highest density and lowest density corresponding to highest nickel sulphate concentration. (Table 2 and Graph 2).

3. Estimation of Ni (II) by Atomic Absorption Spectroscopy

In *E. coli*, at initial Ni SO_4 $6H_2O$ concentration of 50 mg/L, the percentage of ions remaining in the supernatant was 81% whereas it was 94% corresponding to 300 mg/L initial concentration of $NiSO_4$. In *E. coli*, as the concentration of nickel ions in the media was increased, the concentration of ions in the pellet and supernatant were found to increase. (Table 3 and Graph 3 & 4) In *S. cerevisiae*, for initial Ni SO_4 $6H_2O$ concentration of 1000 mg/L, the percentage of Ni ions remaining in the supernatant was 82% whereas at 3000 mg/L of initial $NiSO_4$ concentration, 98% Ni ions were remaining in the medium after 15 h. (Table 4 and Graph 5 and 6) In *S. cerevisiae*, as the concentration of Ni ions in the media increases, the concentration of ions in the supernatant increased, whereas the concentration in the pellet was found to be less, indicating that no more adsorption take place beyond certain limit.

4. Dimethyl Glyoxime assay

Dimethyl Glyoxime (DMG) assay was used to estimate nickel ions in culture medium before and after growth. The cultures were treated with specific concentration of $NiSO_4.6H_2O$. The supernatant was collected every 12 h and the amount of nickel ions in the supernatant was determined by DMG assay. The percentage of nickel ion that has been removed from the supernatant has been monitored up to 72 h at a frequency of 12 h. Based on the difference in the initial and final concentration, the percentage decrease in the nickel ion concentration in the media was calculated. In E. coli, the maximum removal at the end of 72 hours was found to be around 33% (Graph 7). It has been observed that the maximum removal obtained in S. cerevisiae was around 77% (Graph 8). In *E. coli*, there was 16% reduction at the end of 48 hours after which the rate of removal from the medium has been found to increase. For S. cerevisiae, a steady rate of removal has been observed starting from the 12 h.

6. Protein content in the treated samples

The cytosolic proteins of both *E. coli* and *S. cerevisiae* were extracted and quantified after 15 h culture with $NiSO_4.6H_2O$ as per the observations in SDS PAGE profile. In *E. coli*, there was no significant difference in the protein content for the treated and untreated samples and also within different $NiSO_4.6H_2O$ treated samples. But the amount of protein in the culture treated with maximum concentration (300mg/L) was higher than in the untreated samples (Graph 9). This may be due to increased expression of stress related proteins at high ion concentration. In *S. cerevisiae*, there was no significant difference between the protein content of the treated culture and/or the cultures treated with relatively lower concentrations of $NiSO_4$ $6H_2O$. But, the protein content has increased significantly for cultures treated with nickel sulphate concentrations above 2000 mg/L, the maximum protein content corresponding to 3000 mg/L (Graph 10). The reason for this may be over-expressed some proteins to overcome/tolerate the increased metal stress.

6. SDS PAGE profile of treated and untreated samples

E. coli: Intracellular proteins were extracted from cultures treated with 50 mg/L and 300 mg/L of the $NiSO_4$ $6H_2O$ and the protein profiles were compared. No significant difference was observed between the banding patterns of the control and treated samples. However, some high molecular weight proteins (45-70 kDa) were found to be over expressed in the nickel treated cultures (Fig. 1).

S. cerevisiae: Intracellular protein profile of *S.*

cerevisiae cultures treated with two different concentrations of $NiSO_4.6H_2O$, i.e., 100 mg/L and 1000 mg/L were compared with control. It was observed that a protein corresponding to 205 kDa was down regulated suppressed in the culture treated with 100 mg/L of $NiSO_4.6H_2O$. It is also observed that two new proteins corresponding to 55 kDa and 80 kDa were expressed when treated with higher concentrations of $NiSO_4$ $6H_2O$. (Fig. 2)

DISCUSSION

Nickel is a known environmental pollutant that is frequently encountered in sewage and industrial waste water (Parameswari *et al*. 2009). The tolerance level of a Pseudomonas putida strain was reported to be 1mM (Lee *et al*. 2001). Patel *et al*. (2006) have isolated a nickel resistant bacteria isolate (NiRBI) with a tolerance level up to 2.5 mM. Hussein *et al*. (2005) have isolated a chromium resistant strain of *Pseudomonas fluorescens* and a strain of *Pseudomonas putida* resistant to copper, cadmium and nickel. Vieira and Volesky (2000) have reviewed that bacteria make excellent bio-sorbents because of their high surface-to-volume ratios and a high content of potentially active chemo-sorption sites such as on teichoic acid in their cell walls. Two Gram-negative strains *E. coli* (K-12) and *Pseudomonas aeruginosa* and a Gram-positive strain Micrococcus luteus have been used to demonstrate biosorption of Cu^{2+}, Cr^{3+}, Co^{2+} and Ni^{2+}. Their sorption binding constants suggested that *E. coli* cells were the most efficient at binding copper, chromium and nickel and M. luteus adsorbed cobalt most efficiently (Churchill *et al*. 1995).

Nickel tolerant microorganisms including species of *Cupriavidus, Alcaligenes, Burkholderia, Arthrobacter, Rhodococcus and Streptomyces* have been isolated from naturally Ni- rich soils (Idris *et al*. (2004); Mengoni *et*

Table 1. Data shows microbial growth of *E. coli*. Liquid culture were grown in various nickel concentrations and harvested at various time intervals. 1mL of 5 mL culture has been used for measurement @ 600nm. (n=5).

Time (h)	0 mg/L	50 mg/L	100 mg/L	150mg/L	200 mg/L	250 mg/L	300 mg/L
0	0.168	0.194	0.188	0.187	0.191	0.193	0.186
1	0.336	0.222	0.193	0.169	0.147	0.132	0.125
2	1.031	0.459	0.257	0.197	0.159	0.132	0.125
3	1.501	0.929	0.477	0.256	0.184	0.147	0.099
4	1.745	1.391	0.906	0.27	0.216	0.136	0.128
6	2.021	1.639	1.335	0.341	0.309	0.159	0.131
8	2.147	1.875	1.655	0.571	0.314	0.202	0.119
10	2.267	2.062	1.905	0.817	0.488	0.249	0.168
15	2.327	2.228	2.125	1.837	0.907	0.347	0.182
18	2.396	2.175	2.117	2.034	1.415	0.745	0.302
21	2.426	2.207	2.123	2.027	1.486	0.808	0.384

Table 2. Table shows microbial growth of *S. cerevisiae*. Liquid culture grown in various nickel concentrations and harvested various time intervals. 1mL of 5 mL culture has been used for measurement @ 600 nm. (n=5).

Time (h)	Control	1000 mg/L	1250 mg/L	1500 mg/L	1750 mg/L	2000 mg/L	2250 mg/L	2500 mg/L	2750 mg/L	3000 mg/L
0	0.321	0.297	0.282	0.279	0.275	0.269	0.257	0.272	0.2665	0.256
1	0.643	0.608	0.528	0.468	0.461	0.46	0.409	0.356	0.321	0.32
2	1.019	0.864	0.765	0.664	0.538	0.5	0.514	0.457	0.356	0.348
3	1.369	1.009	0.885	0.737	0.665	0.571	0.56	0.413	0.395	0.376
6	2.56	1.355	1.173	0.986	0.895	0.786	0.689	0.555	0.432	0.417
9	2.666	1.813	1.429	1.294	1.222	1.08	0.792	0.742	0.549	0.512
12	2.742	2.144	1.746	1.531	1.49	1.298	1.061	0.855	0.673	0.598
15	2.859	2.19	1.903	1.678	1.6	1.497	1.306	1.034	0.967	0.849
18	2.873	2.287	1.946	1.811	1.677	1.588	1.324	1.147	1.009	0.961
21	2.897	2.375	2.059	1.8735	1.752	1.691	1.495	1.2	1.078	1.015
24	2.907	2.4395	2.19	1.902	1.838	1.702	1.596	1.3565	1.224	1.168
27	2.938	2.6595	2.3245	1.958	1.939	1.842	1.672	1.4595	1.316	1.1947

Table 3. AAS analysis for *E. coli* pellet

Conc. of $NiS0_4$ in media (mg/L)	Initial conc. of Ni (II) in media (mg/L)	Conc. of Ni(II) in pellet (mg/L)	%of Ni(II) in pellet (mg/L)	Conc. of Ni(II) supernatant (mg/L)	% of Ni(II) in supernatant (mg/L)
50	11.05	1.06	9.59	8.96	81.08
100	22.1	1.68	7.6	18.79	85.02
150	33.15	2.43	7.34	30	90.5
200	44.2	2.64	5.99	40.4	91.6
250	55.25	3.182	5.76	51.18	92.65
300	66.3	3.64	5.5	62.15	93.75

Table 4. AAS analysis for *S. cerevisiae* pellet

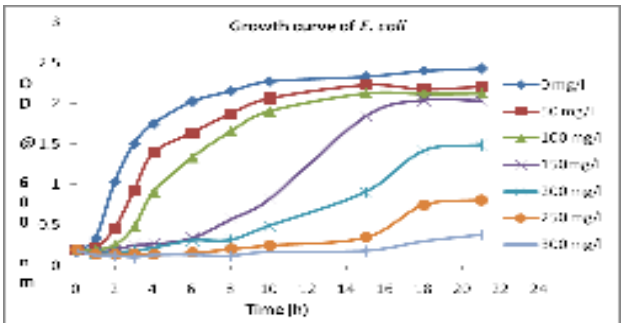
Conc. of $NiS0_4$ in media (mg/L)	Initial conc. of Ni (II) in media (mg/L)	Conc. of Ni (II) in pellet (mg/L)	%of Ni (II) in pellet (mg/L)	Conc. of Ni (II) supernatant (mg/L)	% of Ni (II) in supernatant (mg/L)
1000	221	15.82	7.15	181.6	82.17
1250	276.25	8.28	2.99	246.91	89.37
1500	331.5	7.72	2.32	304.13	91.74
1750	386.75	6.62	1.711	361.5	93.47
2000	442	5.9	1.334	420.44	95.12
2250	497	5.58	1.12	475.73	95.72
2500	552	4.7	0.85	531.98	96.37
2750	607	3.23	0.532	591.14	97.38
3000	663	2.07	0.312	651.28	98.23

al. (2001); Sclegel *et al*. (1991); Schmidt and Schlegel, (1994). Different species of *Aspergillus, Pseudomonas, Sporophyticus, Bacillus,* Phanerochaete, have also been reported as efficient nickel reducers (Yan and Viraraghavan, 2003). Four aerobic, Gram positive, Ni tolerant heterotrophic bacteria namely *Arthrobacter oxydans* NR-1, *Streptomyces galbus* NR-2, *Streptomyces aureofaciens* NR-3 and *Kitasatospora cystarginea* NR-4 have been isolated (Van Nostrand *et al*. 2007). Different studies have shown that actinomycetes are often isolated from sites with increased concentrations of Ni, either naturally occurring and/or from anthropogenic sources (Van Nostrand *et al*. 2007). Vivas *et al*. (2006) have isolated a Ni tolerant strain of *Brevibacillus brevis*.

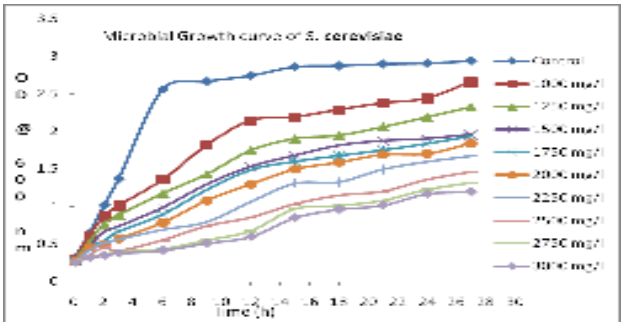
A. chrococcum, Bacillus sp. and *Pseudomonas fluorescens* were reported to exhibit growth/tolerance even at higher concentration of nickel. However, the biomass production was found to decrease with increase in metal concentration (Parameswari *et al.* 2009). Kanopka *et al*. (1999) have observed that the microbial biomass generation was decreased as the concentration of heavy metal increased. Hussein *et al*. (2003) have reported that the total amount of biomass production was decreased when the heavy metal concentration was increased. Vivas *et al*. (2006) have reported that the growth of *Brevibacillus* brevis decreased concomitantly as the concentration of Ni in the media was increased.

Removal efficiency of Ni by *Azotobacter chroococcum, Bacillus* sp. and *Pseudomonas fluorescens* isolated from sewage irrigated soils has been carried out by Parameswari *et al*. (2009). *A. chroococcum, Bacillus* sp. and *P. fluorescens* have been reported to remove 86.16%, 84.32% and 90.98% respectively, at the end of 72 h, at an initial metal concentration of 25 ppm. *Aspergillus* sp. and *Micrococcus* sp. treated with 50 mg/L of nickel sulphate were documented to exhibit a maximal nickel removal of 90% and 55% respectively (Congeevaram *et al*. 2007).

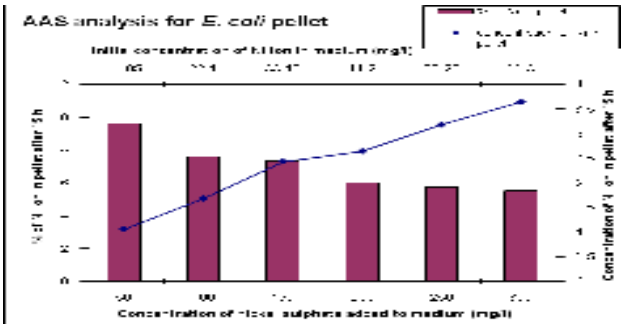
Salzano *et al.* (2007) identified different proteins that significantly vary in their abundance in the nickel treated versus the control samples of hyperthermo acidophilic archaeon Sulfolobus solfataricus. Proteins disappearing after Ni treatment were a NAD dependent malic enzyme involved in energy uptake and a group of four hypothetical proteins of unknown function made up of three CO dehydrogenase subunits (large chain cut A-6 and small chain cut C-1) and acetyl-coenzyme A synthetase.



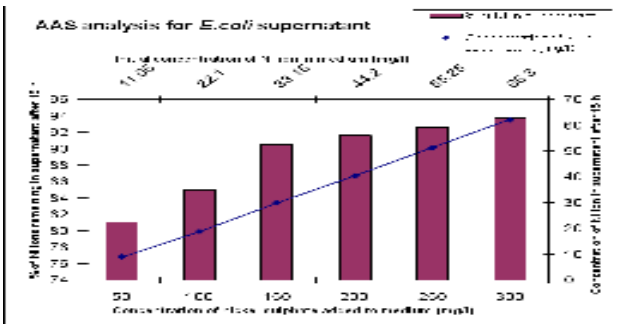
Graph 1. Microbial growth curve of *E. coli*. The data shown in table 1 is used to plot this curve. (X axis 1 U = 5 h; Y axis 1 U = 0.2 OD). (n=5).



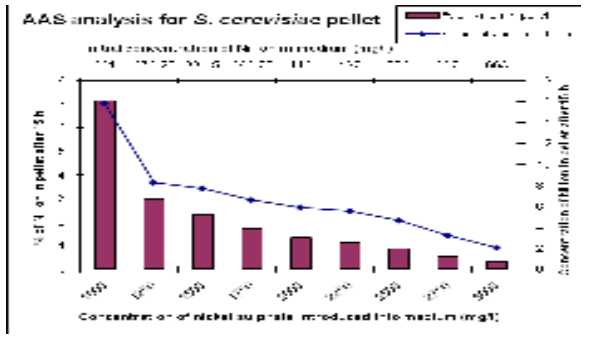
Graph 2. Microbial growth curve of *S. cerevisiae*. The data shown in Table 2 is used to plot this curve. (X axis 1 U = 2 h; Y axis 1 U = 0.5 OD). (n=5).



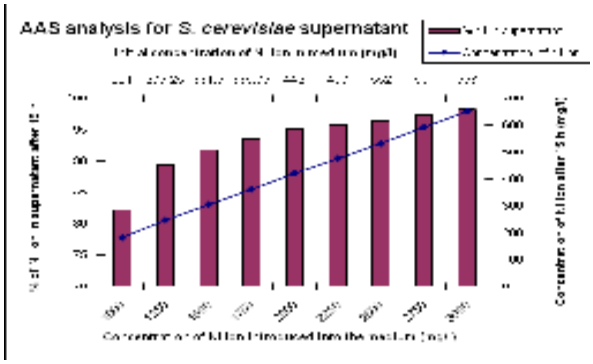
Graph 3. AAS analysis shows the concentration of Ni in *E. coli* pellet



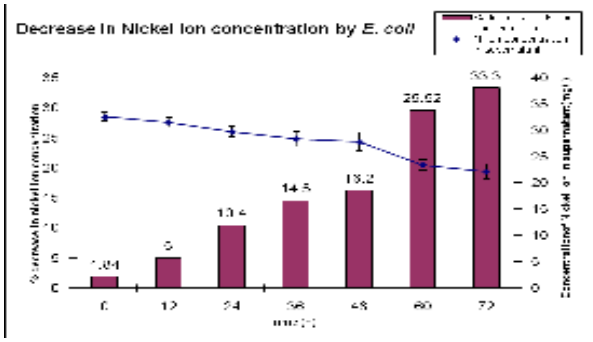
Graph 4. AAS analysis shows the concentration of Ni in *E. coli* supernatant



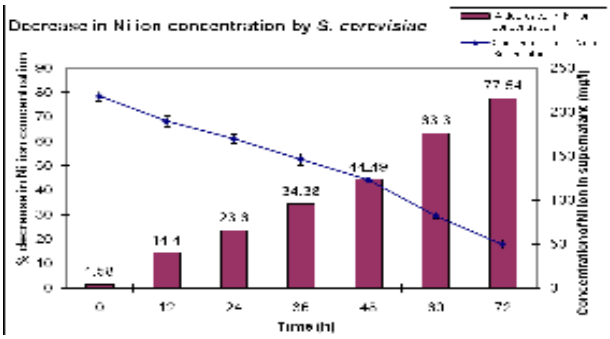
Graph 5. AAS analysis shows the concentration of Ni in *S. cerevisiae* pellet



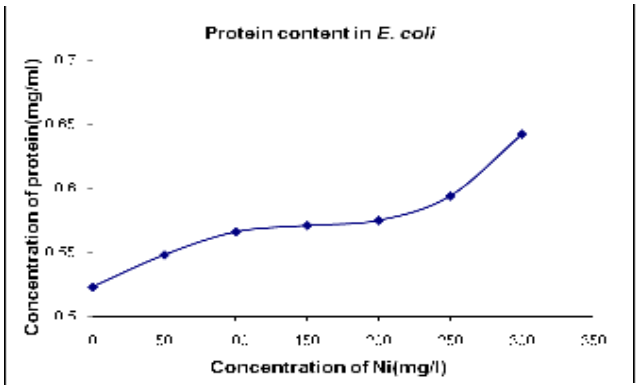
Graph 6. AAS analysis shows the concentration of Ni in *S. cerevisiae* supernatant



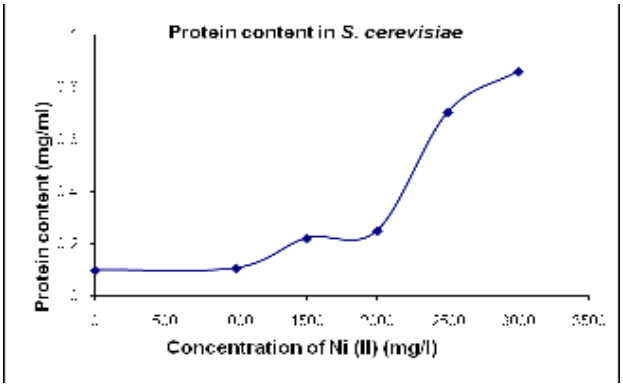
Graph 7. Percentage reduction of Nickel ions in the supernatant of *E.coli* up to 72 hours of growth.



Graph 8. Reduction of Nickel ions in the supernatant of *S. cerevisiae* up to 72 h of growth.



Graph 9. Protein content in *E. coli*



Graph 10. Protein content in *S. cerevisiae*

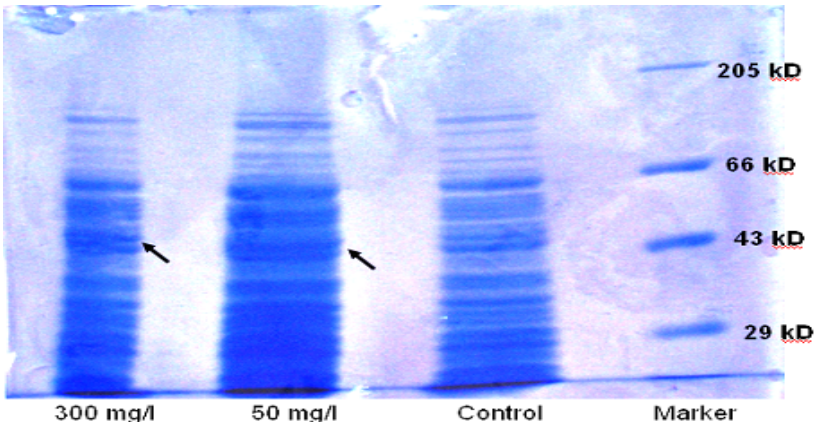


Fig. 1 SDS PAGE profile of *E .coli*

Fig. 2 SDS PAGE profile of *S. cerevisiae*

It has been observed that metal binding by bacteria was profoundly influenced by the concentration of metal. Also, as the incubation period increased, the percentage of biosorption of Cr and Ni by the bacterial cultures also increased. At higher initial concentration of the metal, the percentage removal of Ni was lower in all the organisms (Parameswari *et al*. 2009). Increasing the concentration of Ni (II) upto 4 mmol/l greatly decreased the accumulation of Ni into the bacterial biomass (Hussein *et al*. 2005).

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