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Optimization of a preselected bacterial consortium for Ni(II) uptake

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ABSTRACT

Nickel is a toxic heavy metal found in the environment as a result of various natural and industrial activities. Epidemiological studies have identified nickel as potentially carcinogenic and allergenic to humans. Bioreduction of nickel has not been documented however biosorption and bioaccumulation may be possible. Nickel tolerance in a preselected consortium was studied and found to be gradual. Aeration of preloaded consortium showed a rapid efflux of Ni(II). Uptake in Glucose minimal media was higher than the others tested. The accumulation of Nickel sulphate was higher than the other nickel salts tested. A cell density of 20% allowed greater accumulation of Ni(II) as demonstrated by Atomic absorption spectrophotometry. Uptake in whole cells was higher (65%) than immobilized cultures (48-51%). The optimum pH and temperature for nickel uptake with respect to this consortium was found to be 7.0 and 40°C respectively. The uptake with Nickel sulphate was found to be higher than other nickel salts tested. The uptake of nickel was reduced by over 78 % in distilled water and by 58% in phosphate buffer in presence of respiratory inhibitors like Sodium azide. Other metals such as Cu²⁺ and Mg²⁺ completely inhibited nickel uptake, while Zn²⁺ inhibited nickel uptake by 94.25% when compared to controls.

Keywords: Nickel uptake, consortium, biosorption, parameters, whole cells, immobilised cells.

INTRODUCTION

Heavy metals can create adverse effects on the environment and human health due to their bioavailability and toxicity in various environmental components [1]. Nickel is a toxic heavy metal found in the environment as a result of various natural and industrial activities. A large number of industries use nickel, mainly the electroplating industries. An elevated level of toxic heavy metal in the biosphere is a well documented phenomenon having notable biological and environmental implications[2]. Epidemiological studies have identified nickel as potentially carcinogenic and allergenic to humans[3,4]. Nickel binds to proteins and nucleic acids and frequently inhibits enzyme activity, DNA replication, transcription, and translation[5]. Nickel, however is an essential trace element for at least four enzymatic processes[6]. This study deals with the selection of Ni(II) remediating bacteria from diverse sources such as electroplating effluents, soil, sewage so as to constitute a consortium that may be useful in treatment of nickel containing effluents.

MATERIALS AND METHODS

Isolation and biochemical characterization of the cultures of the consortium:

Samples taken from above mentioned sources were inoculated in to Glucose minimal medium[7] containing 0.5mg/ml Nickel chloride. The enrichment was plated out using standard microbiological techniques on Ni(II)

containing Glucose minimal medium (GMM) plates. Isolates selected for the consortium were able to tolerate nickel to up to 2mg/ml of Ni(II) as observed from the MIC studies.

Individual cultures were suspended in 4ml of sterile normal saline and adjusted to A_{600} 0.5. 2ml of each suspension was pooled and vortexed briefly. 20% of the pooled suspension was transferred to 500 ml of glucose minimal media incorporated with 0.5mg/ml of Ni(II) and incubated at 35 °C for 24 h. Cell pellet obtained after centrifugation (REMI RM 24) at 10,000 g for 10 min was washed in sterile distilled water and suspended as required in different diluents. A_{600} of the consortium suspension was kept at 1.0 for all the experiments and a Ni(II) concentration of 1mg/ml unless otherwise mentioned.

Cleansing of glass wares.

Nickel contamination from glasswares was minimized by overnight soaking in 2 N HCl and then finally rinsing with deionized distilled water [8] particularly for recording nickel uptake in phosphate buffer or distilled water.

Analysis of nickel using atomic absorption spectrophotometer:

Intracellular accumulation of nickel was determined as per a standard procedure [9]. 10ml of bacterial culture grown in minimal media was centrifuged at 10,000 x g for 10 min. Supernatant was decanted and pellet was washed twice with deionized water and digested by adding 3 ml of concentrated HNO_3 at 100°C for 18 h. Acid digested samples were cooled and made up to 30 ml with sterilized distilled water[9].The content of Ni was determined by atomic absorption spectrophotometry (Perkin-Elmer AAnalyst 600) from calibration curves prepared with known concentrations of nickel.

Determination of the pattern of Ni(II) tolerance:

In order to observe the extent of growth taking place at different concentration of Ni(II), sterile Luria broth was incorporated with 0.2 - 2.0 mg/ml of presterilised nickel chloride, aseptically and inoculated with the consortium at 10% of the total volume.

Effect of inoculum size on nickel uptake:

The consortium was freshly harvested and transferred at either 10, 20 or 30% of the total volume in GMM along with Ni(II) and incubated for 24 h. Recording of the different results was done as before.

Effect of pH and temperature on nickel uptake:

Effect of pH on nickel uptake was noted at 1mg/ml using the procedure of Leung *et al.*(2000).(35°C / 24 h). The effect of temperature was noted in GMM incubated at 15, 20, 35, 40, and 50 °C for 24h. Growth was recorded as A_{600} colorimetrically (Elico159). Initial and residual nickel was estimated by AAS as before.

Effect of media composition on growth and Ni(II) uptake:

Nickel uptake at 1mg/ml nickel sulphate was observed in different media that have been employed in studies on heavy metal uptake. Media used were nutrient broth, glucose minimal medium, glucose mineral salts medium, medium by Goodhue *et al*, King's B medium ,Vogel Bonner medium, Tris gluconate medium ,CV medium [10,11]. Since growth and uptake of nickel in presence of nickel sulphate was better than other nickel salts tested , it was used as a source of Ni(II). Residual nickel in the supernatant , accumulated Ni(II) in the HEPES suspended cell pellet was determined using AAS. Initial and final cell proteins were estimated per the procedure of Lowry *et al.* [12].

Determination of time course for nickel uptake under optimized conditions:

The time required for nickel uptake at fixed cell density, pH and temperature was carried out in HEPES buffer (1mM).Growth and Ni(II) uptake was monitored for 4 days under static conditions.

Nickel uptake using different nickel salts:

Respective metal salts (Nickel sulphate, chloride, carbonate ,nitrate) were added at 1mg/ml in GMM. Nickel uptake was allowed for 24h and initial, residual or internalized nickel was determined as before.

Nickel uptake in immobilized consortium.

Nickel uptake in immobilized cells was studied following the procedure of cell entrapment using sodium alginate as described by Shide and co-workers[13]. The freshly harvested consortium (A_{600} 1.86) was used for entrapment.

Nickel uptake in immobilized cells and cell free alginate beads were recorded at 1mg/ml nickel chloride in distilled water. Initial nickel was recorded immediately after inoculation in clear supernatants after proper pretreatments by AAS. The sets prepared in duplicate were kept on a rotary shaker at room temperature for 24 h. after which residual nickel estimation was done using AAS.

Aeration of preloaded cells.

Nickel efflux if any brought about by aeration was noted in preloaded cells using the procedure of [14]. Ni(II) (1mg/ml) uptake was initially allowed for 6h in distilled water. The preloaded cell suspension was aerated in the same medium for an hour using an aquarium aerator [14]. Nickel concentration was determined as before.

Effect of respiratory inhibitor, sodium azide on Ni uptake:

In order to study the effect of sodium azide on nickel uptake, the consortium was precultured in presence of azide and then transferred at predetermined levels in a buffer for recording Ni (II) uptake.

Sterile sodium azide (0.05M) was incorporated at a final concentration of 0.0005M, 0.0015M and 0.0025M in minimal medium with a freshly harvested consortium and allowed to grow for 24h. along with appropriate controls. After 24h. the cell pellet was harvested and washed in sterile phosphate buffer pH 6.8. The respective azide treated consortia were adjusted to A_{600} 1.0 using phosphate buffer pH 6.8 and further inoculated in sterile phosphate buffer pH 6.8 as well as in sterile distilled water. Ni(II) provided as nickel chloride was kept in the range of 1.0 to 2.5mg/ml of nickel. Uptake was noted in distilled water and phosphate buffer. The tubes were vortexed for 30 sec. and incubated for 24 h in static conditions. The residual nickel was estimated by AAS in clear supernatants after due sample pretreatments.

41) Effect of other metal ions and salinity on nickel uptake:

Nickel uptake at 500µg/ml nickel chloride was observed in presence of different metal ions .Metal ions (Zn^{+2} , Cu^{+2} and Mg^{+2}) were added at 500µg/ml in distilled water[15] to which freshly harvested consortium was added at 10% level. Flasks were incubated at room temperature for 24-72h and residual nickel was estimated at 24h. interval using AAS.

Effect of salinity on nickel uptake was also investigated at 1% salinity at nickel chloride concentration of 500µg/ml.(24-72h). Residual nickel was estimated using AAS as before.

RESULTS AND DISCUSSION

The isolates were selected on the basis of highest nickel uptake and were identified biochemically as in table 1.

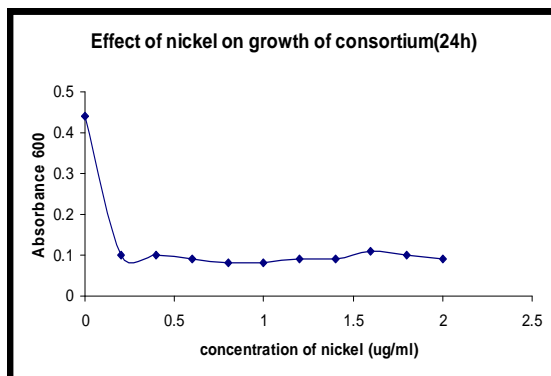
Table 1: Isolation and biochemical characterization of the cultures of the consortium:

Sr.no	Tentative culture designation	Identification based on biochemical tests.
1	NI1	<i>Bacillus pumilis</i>
2	NS1	<i>Bacillus brevis</i>
3	NC2	<i>Micrococcus luteus</i>
4	NI2	<i>Bacillus coagulans</i>
5	NC1	<i>Sporosarcina ureae</i>
6	S3N2	<i>Micrococcus varians</i>
7	NS3	<i>Staphylococcus sciuri</i> (coagulase negative)
8	S3N1	<i>Sporolactobacillus sp</i>
9	NiCh2	<i>Bacillus azotoformans</i>
10	BDNi4	<i>Kurthia spp</i>
11	PNi3	<i>Cellulomonas sp.</i>

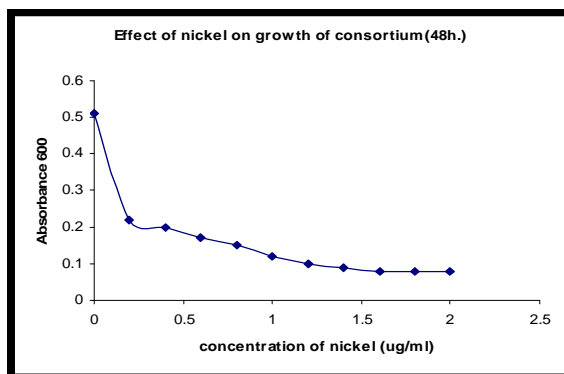
Effect of nickel on growth of the consortium:

Cell growth was inhibited for all the concentrations of nickel tested. The cell density was not more than 25% after 24h. when compared to nickel free controls. This inhibition was substantially reduced after 48 and 72h. of growth. Inhibition after 72h. was between 41.67% to 88.34% when compared with growth in absence of Ni(II). It was further observed that with a higher initial density and longer period of incubation (48h.), the inhibition was 22-42% at highest and lowest recorded cell density in presence of different concentrations of Ni(II). (Fig.1,2,3).

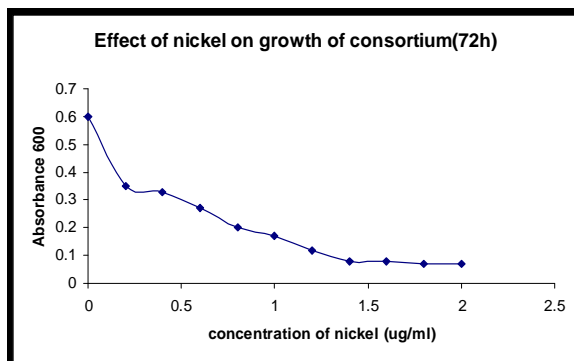
Figures 1: Growth of the consortium in presence of nickel in 24h.



Figures 2: Growth of the consortium in presence of nickel in 48h.



Figures 3: Growth of the consortium in presence of nickel in 72h.

**Effect of pH and temperature on nickel uptake:**

The optimum pH for Ni(II) uptake was in the range of 6.0 to 7.0. Highest uptake was at pH 7.0 at which higher total proteins were also detected. The highest specific uptake (% uptake/ total proteins) [16] was at pH 6.0 which was 0.14% / μg of total proteins produced. It was also observed that the A_{600} between pH 5.0-8.0 differed by 4-8% when compared with the highest A_{600} at pH 7.5. Metal uptake was substantially reduced at pH 8.0, although cell density was not affected.(Fig4). The optimum temperature for metal ion uptake was generally dependent on temperature of growth. Biosorption is however largely unaffected by temperature changes. In the case of this consortium it was found that the temperature at which higher cell density and total proteins was obtained was at 40 $^{\circ}\text{C}$. However, the highest uptake was at 35 $^{\circ}\text{C}$. The highest specific uptake (% uptake/total proteins) [16] was also at 35 $^{\circ}\text{C}$ which was 0.086% / μg of total proteins. It was also observed that the A_{600} and total proteins at temperatures 15 and 20 $^{\circ}\text{C}$ were reduced by over 50% than that observed at 40 $^{\circ}\text{C}$.(Fig5).

It was reported that an increase in pH reduced the toxicity of nickel to bacteria as well as an actinomycete, a yeast, and filamentous fungus.[17] It has been also reported that biosorption capacities were found to be dependent on solution pH [18]. An increase in cation uptake has been noted with increasing pH values[19]. The consortium was able to grow over a wide pH of 5.0 to 8.0. With respect to this consortium, the highest uptake was recorded at pH 7.0 along with higher total proteins. Although the specific uptake was higher at pH 6.0, higher total proteins are desirable when the process of uptake has to be sustained over a longer period of time. Uptake was comparatively lower at acidic pH which indicated that surface adsorption which was more likely at this pH did not occur.

Fig4: Determination of optimum pH for Ni(II) uptake.

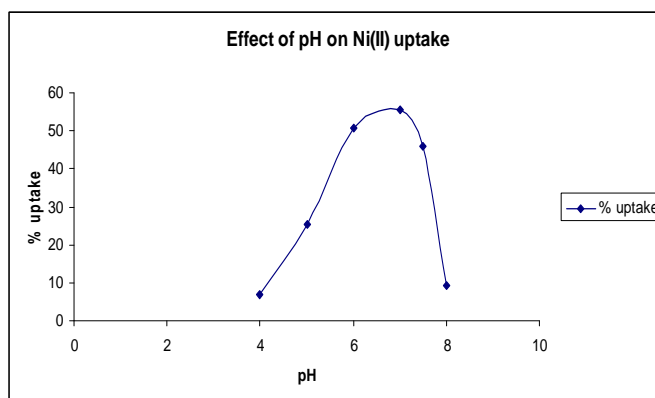
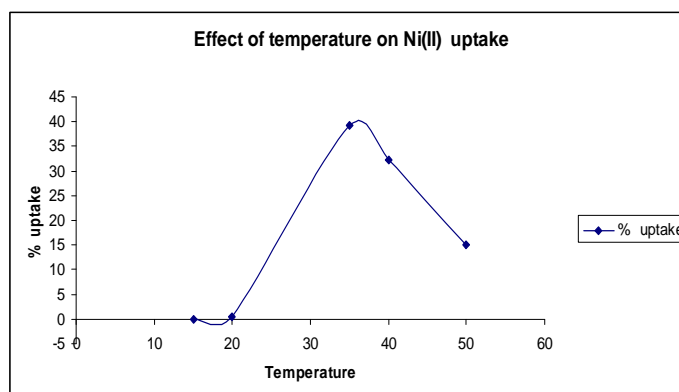


Fig5: Determination of optimum temperature for Ni(II) uptake.



The optimum temperature for Ni(II) uptake in a genetically engineered *E.coli* was found to be 37⁰C [4]. *K. ascorbata* SUD165 investigated for Ni(II) uptake was able to grow in nutrient broth within the temperature range 5 to 37⁰C, with an optimal growth temperature at 20⁰C [20]. Biosorption is largely unaffected by changes in temperature in the range of 20-35⁰C [21]. Since there was a difference in uptake noted, it may be said that nickel uptake may not have occurred only through cell surface sorption. Nickel uptake was dependent on optimal growth temperature. Therefore uptake was significant in the range of 35-40⁰C.

Effect of media composition on Ni(II) uptake:

The highest accumulation was in TY medium followed by Vogel-Bonner medium (VB) modified by Bopp *et al*, and GMM.VB medium, medium by Goodhue *et al*, Glucose mineral salts medium and King's B showed some precipitation after 18h, therefore these reduced the metal availability and a large part of the reported uptake was abiotic (Table2).

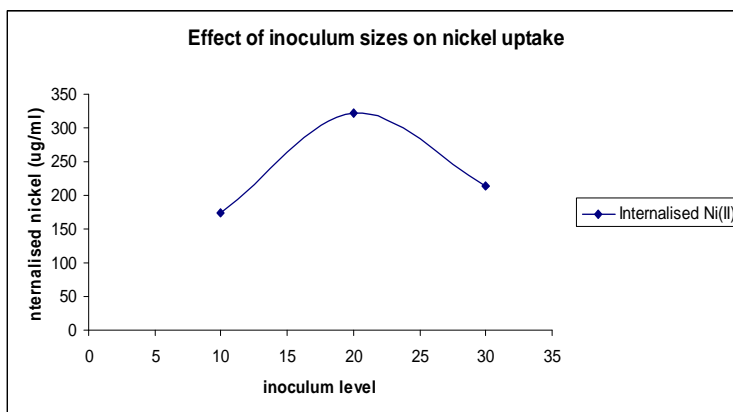
Table2: Nickel uptake in different media at 1mg/ml nickel.

Media used	% nickel accumulated.	Total proteins $\mu\text{g/ml}$
Luria broth	78.52	43.71
Goodhue	65.88	106.07
Glucose mineral salts medium	78.45	26.22
Kings B	68.71	21.31
Vogel-Bonner medium	54.37	39.34
Nutrient broth	59.05	36.64
GMM	57.89	65.48
Tris gluconate	78	80.74
TY medium	30	102.85
CVmedium	62	76.09

Higher amounts of glucose and sucrose in the medium encouraged exopolysaccharide formation and hence favoured biosorption. Organic media components (as in nutrient broth, Luria broth, CV and TY medium) have the ability to form organometallic complexes in presence of metal ions at concentrations mentioned that form precipitates leading to a lower initial nickel in the medium. Hence GMM was the medium of choice that recorded a specific nickel accumulation of 0.64% per μg of cell proteins (Table 2). The lowest abiotic uptake of Ni(II) was observed in GMM. (data not presented).

Effect of inoculum level on Ni(II) uptake:

The inoculum level at which maximum uptake occurred was 20% beyond which the uptake decreased. The uptake in HEPES wash was in the range of 228-252 $\mu\text{g/ml}$. i.e. 28, 26 and 23% nickel could be further traced out. HEPES being a non complexing buffer, the values obtained indicated that nickel that was biosorbed and subsequently released in HEPES wash. Increase in inoculum size beyond 20% increase nickel uptake. At inoculum levels of 40-50% there was a drastic reduction in nickel uptake. (Fig6).

Fig6: Determination of optimum inoculation levels for Ni(II) uptake.

It has been reported that an increase in biomass concentration may not necessarily result in increased uptake [22]. Since redox reactions for detoxification of nickel are not possible, therefore it is imperative that remediation will be largely through biosorption, more so as nickel is a cation. This would require a sufficient higher concentration of biomass. It has been reported that lower values of biomass concentration can lead to an increase in specific uptake [23]. It has been noted that metal ion uptake per gram of biosorbent increased as long as the biosorbent is not saturated [24]. An increase in biomass concentration leads to interference between the binding sites [25]. Similarly an increase in biomass results in a shortage of metal concentration in solution thereby causing a decrease in specific uptake [23].

It may also be reported that turbidity caused by inanimate particles cannot be readily distinguished from cells; Cells of different size and shape have different ratios of cell number; Absorbance caused by suspended constituents cannot be distinguished from turbidity. The A_{600} not only recorded turbidity as a result of suspended cells but was

also influenced by the copious amounts of exopolymer (mainly an exopolysaccharide) produced under metal stress, and therefore is not always a correct reflection of the actual cell number. Thus in some of the cases two identical A_{600} values have not yielded similar total proteins, or higher A_{600} may not always yield a proportionately higher total proteins.

Determination of time course for Ni(II) uptake:

It was observed that Ni (II) uptake increased with time up to 72h. of growth. Thereafter the residual Ni (II) concentration increased which demonstrated that an efflux had taken place that had resulted in higher residual Ni (II) concentration. (Table3).

Table3: Determination of time course for nickel uptake under optimized conditions.

Time in h.	% total uptake
0	0
24	22.73
48	60
72	79.1
96	76.39

It was observed that the uptake of Ni(II) progressed with time. The efflux reported after 96h. was not dependent on energy as metal uptake was also possible in a non complexing buffer such as HEPES buffer. This could be desorption, as a result of saturation of the biomass [26].The increase in residual Ni(II) concentration between 72 h (0.46mg/ml) and 96h (0.52mg/ml) could be because of cell lysis. This is a characteristic finding for organisms carrying out only biosorption. Such an efflux has been rarely noted with respect to this consortium and further studies have indicated that uptake may also be metabolism dependent.

Nickel uptake using different nickel salts:

Nickel sulphate was well tolerated as shown by the higher cell density. The lowest uptake was with nickel carbonate followed by nitrate salt of nickel. Cell density was also low in presence of nickel nitrate. Nickel chloride was also well tolerated; however the uptake was 6.83% lower than the uptake recorded with nickel sulphate.(Table 4).

Table 4: Ni(II) uptake using different nickel salts.

Nickel salt 5mg/ml	Initial Ni(II) µg/ml	Intracellular nickel µg/ml	% uptake	A_{600} (initial)	A_{600} (final)
Nickel chloride	4578	2921	63.80	0.06	0.29
Nickel sulphate	4689	3312	70.63	0.06	0.31
Nickel nitrate	4998	2567	51.36	0.06	0.24
Nickel carbonate	4759	2348	49.33	0.06	0.27

There can be a large variation with respect to water solubility of different nickel salts. The salts in decreasing order of solubility are: nitrate > chloride > sulphate > fluoride > hydroxide, carbonate > oxide. Thus nickel uptake could be dependent on the solubility of the salt used .When the influence of four different cadmium salts : nitrate, chloride, sulphate and oxide was tested for resistance of different Gram-positive and Gram-negative species, cadmium chloride was the most toxic cadmium salt whereas cadmium nitrate was efficiently absorbed by some strains [27]. It was reported that exopolymeric substance production was higher in sulphate reducing biofilms [28].The presence of sulphate may have stimulated EPS production.

Table 5: Nickel uptake in free cell and immobilized consortium.

Preparation used	Initial nickel µg/ml	Residual nickel µg/ml	% uptake	Initial nickel µg/ml	Residual nickel µg/ml	% uptake
Immobilised cells.	876	321.05	63.36	877	321	63.4
Cell free alginate beads.	888	782.06	11.94	867	734	15.35
Free cells.	917	212	76.89	953	432	54.67

Effect of immobilization of cells on Ni(II) uptake:

The net uptake by immobilized cells was only 51.42 and 48.05 respectively as compared to free cells where the average uptake was 65.77%.(Table 5). There was some reduction in uptake on reuse of the beads.(data not given) and was higher in free cells as compared to immobilized cells. The uptake further improved when carried out in

GMM instead of distilled water.(data not presented).Phosphate buffer was not suitable for nickel uptake owing to the precipitation on addition of the beads.

It has been reported that nickel removal using immobilised cells was possible through optimization of the growth conditions for the bacterial cells such as the composition of the growth medium, incubation time, incubation temperature; and the operational parameters of the bioreactor such as retention time and pH of the Ni²⁺ containing solution, respectively [29].

It has been reported earlier that microbial biomass consists of small particles with low density, poor mechanical strength and little rigidity. The immobilization of the biomass in a solid structures creates a material with the right size, mechanical strength, rigidity and porosity necessary for metal accumulation. Immobilisation can yield beads and granules that can be stripped of metals, reactivated and reused in a manner similar to ion exchange resins or activated carbon. Immobilized cells help in detoxification of metal pollutants with far less toxicity associated complications as immobilization protects the cells from toxicity at sub lethal concentrations of metal ions. Immobilization also traps the microorganisms in inert gels such that their activity remains unaltered and allows the reuse of the immobilized beads too.

Nickel uptake was significant in immobilized cells , however the uptake was higher with free cells. The slightly higher activity with the culture suspensions as compared to immobilized cells can be attributed to the presence of other bioactive substances in the filtrate which in turn stimulate the enzyme activity. Further, the polysaccharides of EPS may act as chellators[30].Conrtium produced EPS which was contributing to the higher uptake with free cells.The same has been reported by A Cyanobacterium , *Aulosira fertilissima* has been found to release organic substances that can chelate free metal ions [31]. Notable uptake in cell free alginate beads was noted on account of abundant free hydroxyl groups in alginate which bind to metal ions [32,33].

Efflux of preloaded cells on aeration:

The initial nickel recorded in controls of aerated cells was higher as compared to non aerated sets. The difference in uptake between aerated and static cultures was 1.54%. A₆₀₀ and total proteins were higher under aerated conditions when compared with non aerated growth. The uptake within 6h. was 23.55%. On aeration of preloaded cells , the uptake was reduced to 11.73%. There was an efflux observed when preloaded cells were aerated to the extent of 11.82 % from the recorded uptake of 23.55% before aeration.(Table 6).

Table 6 : Effect of aeration with respect to Ni(II) efflux in preloaded cells.

Sample	Initial nickel µg/ml	Residual nickel (µg/ml)	Residual nickel after 1h.aeration
Uninoculated control	998	--	990
Preloaded consortium	998	763	881

The percent uptake of nickel recorded with and without aeration was almost similar. The difference was however in cell density and total proteins thereof. Aeration allows efficient biological interactions with the substrate and other media components. The energy output under aerated conditions is higher and this results in increase in cell density and total proteins. Since results were observed within 10h. of incubation , EPS production will have occurred only marginally. However it should be noted that aeration procedure that was adopted led to evaporation to the extent of 50% which concentrates the metal ion also. It was observed the extent of retention of Ni(II) in preloaded cells by aerating the solution using aquarium pumps and observed an efflux after aeration with respect to *Alcaligenes eutrophus*, the wild type strain N9A and its transconjugant N9A-M243 [14].

This preloaded consortium on being aerated for an hour, resulted in 50% efflux resulting in an increase of residual nickel concentration.

Effect of respiratory inhibitors on Ni uptake:

Nickel uptake was partly inhibited in consortium pretreated with azide. Cells pregrown in presence of increasing amounts of sodium azide showed inhibition of nickel uptake at initial Ni(II) concentration ranging from 1 to 2.50mg/ml in phosphate buffer (pH 6.8) .Uptake of nickel was severely inhibited in distilled water when consortium was pregrown with 0.0015 and 0.0025M of sodium azide respectively. When cells were treated with 0.0025M

Sodium azide, the uptake of nickel was reduced by over 78 % in distilled water and by 58% in phosphate buffer calculated at lowest (in presence of azide) and highest uptake (in absence of azide) at 2.5mg/ml of Ni(II). (Table 7).

Table 7: Effect of respiratory inhibitors on Ni (II) uptake at 2.5mg/ml of Ni(II).

NaN ₃	Uptake in distilled water (mg/ml)		Uptake in buffer (mg/ml)		% uptake	
	Initial	Residual	Initial	Residual	Buffer	D/w
0.0005M	2.50	1.91	2.52	1.35	46.43	23.6
0.0015M	2.50	1.86	2.52	1.46	42.07	25.6
0.0025M	2.50	2.06	2.52	1.63	35.32	17.6
None	2.50	0.41	2.52	0.39	84.53	83.6

The Cytochrome c oxidase inhibitor, azide at concentration of 10mM was reported to have inhibited nickel uptake in totality in *B.japonicum* [34]. It has been reported that the respiratory inhibitor azide (10mM), significantly inhibited short term (5 min) Ni²⁺ uptake, showing that Ni²⁺ uptake in *B.japonicum*, strain JH was energy dependent [35].

Nickel uptake with respect to this consortium was dependent on energy and hence the uptake was reduced in cells that were pretreated with azide, quite in accordance with several earlier reports. Inhibition in uptake was significant, however uptake that was still noted was on account of metabolism independent biosorption which is not directly affected by azide. However there is an indirect effect on such processes too. As growth or maintenance is dependent on energy and biosorption on available biomass, the inhibition of growth would result in reduction in the rates of biosorption too, albeit in an indirect manner. The extent of nickel uptake through biosorption was therefore lower compared to the uptake in absence of azide. Metabolism dependent accumulation and biosorption will be however completely affected depending on the concentration of the inhibitor used. Hence sublethal concentrations of azide have been used in this study.

The concentration at which the inhibitor is able to inhibit would differ from organism to organism. Since sodium azide is a proton inhibitor it may be therefore concluded that part of the nickel transport is energy dependent and hence nickel uptake was reduced when compared to controls.

Effect of other metal ions and salinity on nickel uptake:

It may be noted as per table 8 that nickel uptake was the highest in the absence of any other added metal ion. Cu²⁺ and Mg²⁺ had completely inhibited nickel uptake, while Zn²⁺ inhibited nickel uptake by 94.25% when compared to uptake in absence of Zn²⁺ after 48h. There was also no further uptake recorded after 72h. of inoculation in presence of Zn²⁺.

Table 8: Effect of metal ions and salinity on Ni(II) uptake.

Metal ion added	% nickel uptake		
	24h	48h	72h
Zinc, copper and nickel	5.34	6.17	0
Zinc and nickel	2.10	3.54	0
Copper and Nickel	0	0	0
Magnesium and nickel	0	0	0
Nickel only	25.61	61.54	79.49
Nickel only in presence of NaCl	21.88	0	0

It has been reported that in natural environment, metal-microbe interactions are very important, metal-metal interactions can also be of greater significance as metals generally occur in combinations [36]. Since the cellular metal binding sites are never entirely specific for a single metal, metals with similar structures and charge can often bind competitively. They can therefore interfere with the metabolism of related metals.

Competition of metal ions during biosorption was investigated in binary metal solutions [37]. It was observed in this case too, that nickel uptake was highest in the absence of any other added metal ion except nickel. In nickel accumulating strain, the accumulation was inhibited at higher salt concentrations [4]. Though there are a number of organic compounds that act as chelates, inorganic ligands, such as chloride and sulphate play an important role in metal uptake. These agents are able to form tight complexes with metals, which may decrease their bioavailability. Non bioavailable metals are referred to those that have precipitated, sorbed or have been immobilized [38]. At 1%

salt concentration there was no uptake recorded after 48h and a slight reduction in uptake was noted within 24h of inoculation. Chloride salts complex with metal ions thereby reducing its bioavailability and lowering the uptake.

Accumulation has been reported to have been inhibited by Mg(II) and other agents that form strong metal complexes [35]. It has been reported in case of *Bradyrhizobium japonicum* JH, that Ni²⁺ entry inside the cell was inhibited significantly by Mg²⁺ and other ions like Zn²⁺, Co²⁺ and Mn²⁺, which can be relieved by increasing the Ni²⁺ concentration. It has been noted that nickel uptake in the fission yeast *Saccharomyces pombe* resulted in a 20 fold decrease in nickel accumulation because of addition of magnesium salt to the medium. In the presence of 10 mM Mg²⁺, a strong effect was observed [39].

Nickel uptake in this consortium was completely inhibited in presence of magnesium. Zinc and copper have also been found to inhibit nickel uptake strongly as a result of competition. It has been reported that zinc and copper are effective competitors for nickel in *Bradyrhizobium japonicum* [34]. The observed results are therefore in accordance with earlier reports.

CONCLUSION

It may be noted that when the consortium was challenged with a dose of the metal ion, it was sequestered intracellularly, precipitated, sorbed extracellularly or reduced to an immobile state, the processes being contributed by one of the members, a few of them or all the isolates of the consortium. Therefore many of the available processes will be displayed by a consortium unlike a monoculture, although the dominating processes will be responsible for detoxification. Thus nickel bioremediation, through such a study can be a useful alternative to conventional systems for the removal of toxic metals from industrial effluents, particularly at low metal ion concentrations where other established procedures may not be able to deliver the desired results.

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