Research Article [Sarkar & Paul, 6(1): Jan., 2015:4110-4114]
CODEN (USA): IJPLCP ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES
(Int. J. of Pharm. Life Sci.)

Bioremediation of nickel ions from aqueous system by dry cells of *Pseudomonas aeruginosa DSGPM4* species

Diptendu Sarkar[1] and Goutam Paul[2*]
1, Department of Life Science, Biotechnology Division, Bangaluru, (Karnataka) - India
2, Dept of Physiology, Environmental Physiology Division, University of Kalyani, Nadia, (WB) - India

Abstract

The present research finding has shown that that the biomass of *Pseudomonas aeruginosa DSGPM4* sp is a suitable biosorbent agent for the removal of nickel from aqueous solution. The pollution of the environment with toxic heavy metals is spreading throughout the world along with industrial progress. The bioremediation of heavy metals using microorganisms has received a great deal of attention in recent years. The role of microorganisms in bioremediation is important because of their ability to degrade hazardous compounds into harmless ones. Nickel is known to be the most commonly heavy metals and wide spread contaminants of the environment. The removal of nickel ion from the aqueous solution under different experimental conditions by using dry cells of *Pseudomonas aeruginosa DSGPM4* sp [GenBank Accn No *JN245880*] was investigated in this study. The nickel uptake was dependent on initial pH and initial nickel concentration, with pH 5.0 being the optimum value. At various initial nickel concentrations (100-300 ppm), biosorption equilibrium was attended after 320 mint(s) incubation.The biomass concentration also influenced the biosorption efficiency. In this study biomass concentration was used 300mg/50ml to 2400mg/50ml. Maximum uptake was achieved by 2400mg/50ml of biomass concentration. It was also found that increase in the biomass concentration, removal percentage increases, but the specific uptake (q value) decreases. Other parameter also influenced the nickel biosorption efficiency, such as temperature. Optimum temperature was found in this study was 40^0C.

Key-Words: Biosorption, Heavy metal, Sorption equilibrium

Introduction

The pollution of the environment with toxic heavy metals is spreading throughout the world along with industrial progress. Heavy metals are stable and persistent contaminants since they cannot be degraded or destroyed (1). Therefore, they tend to accumulate in the soils and sediments. Environmental pollution with heavy metals is spreading throughout industrial progress. The role of microorganisms in bioremediation is important because of their ability to degrade hazardous compounds into harmless ones. Pseudomonas species are found ubiquitously in nature and abundantly in water, soil and plants. Many species of *Pseudomonas* are metabolically versatile and a large number of organic compounds can be used as unique carbon and energy sources. This versatility allows them to be present in many environments as natural autochthonous microflora with a high potential for bioremediation of pollutants (2).

*** Corresponding Author**
E.Mail: gpaul.kalyani@rediffmail.com;
diptendu81@gmail.com

The bioremediation of heavy metals using microorganisms has received a great deal of attention in recent years, not only as a scientific novelty but also for its potential application in industry(3).The role of microorganisms in bioremediation is important because of their ability to degrade hazardous compounds into harmless ones. This versatility allows them to be present in many environments as natural autochthonous microflora with a high potential for bioremediation of pollutants (4).

The present work report to optimizing the environmental variables for the removal of the nickel metals from the aqueous system by Gram negative *Pseudomonas aeruginos DSGPM4* sp[GenBank Accn No.*JN245880*] using chemically defined media.

Material and Methods

Sampling site and Isolation of microorganisms

Water samples were collected from a drainage system of Bangaluru, Karnataka, India. A quantity of 1ml of water from the collected sample was dissolved in 9ml sterile distilled waterand serial dilutions were made. Each dilution was plated on nutrient agar plate without antibiotics and incubated at 37oC. After the growth of

different microorganisms on the plate, each bacterial colony on the basis of its morphological characteristics was picked up and further purified by repeated streaking on nutrient agar plate . Each bacterial culture was then inoculated in nutrient broth, incubated and glycerol stocks were made and frozen at -70ºC (5).

Strain characterization

Gram's staining and 16s rRNA sequencing: Gram's staining was performed to confirm a Gram positive/ Gram negative bacterial strain .16s rRNA sequencing from genomic DNA with universal primers was performed . To know the identity of organism, obtained sequences were compared with nucleotide databases like GenBank, EMBL and the Ribosomal Database Project (RDP) through BLAST programme (6,7).

Nucleotide sequence accession: The sequence was submitted to Gen Bank and accession numbers obtained.

Nickel (Ni^{+2}) Tolerance study: Metal salt of $NiCl_2$ ware used for tolerance study. Stock solution was prepared on the basis of the molar concentration as per formulae (M= moles of solute/ volume of solution).From an overnight grown culture of a single colony 1% (V/V) was transferred to 30 ml of media supplemented with Ni^{+2}. The initial metal concentration was taken as 1 millimolar. The tolerance was measured on the basis of growth curve observed (turbidimetry method) within 12 to 48 hr. If growth was observed inoculums was added to the media with increase in the concentration of Ni^{+2} (1mM, 2mM, 10mM, 100mM and so on). This step was repeated till Minimum Inhibitory Concentration (MIC) was obtained as visualized by cessation of growth (8).

Effect of pH on Ni^{+2} ^{2}Biosorption: 1.2 gm/ 50 ml of bacterial sample was taken in a 50 ml saline water containing 150 ppm Ni^{+2} ion in a Erlenmeyer flask separately. The temperature was properly maintained at 40ºC .pH of the reaction medium was varied from 4.0 to 7.0. Sample was collected at 20, 40, 80, 160, 320 and 640 mints interval and centrifuged at 8000 rpm for 10 min. The supernatant were taken for analysis of Ni^{+2} ion concentration. The biosorption saturation was reached after of 320 min. after of 640 min there was no sampling (7,8) .

Effect of Biomass on Ni^{+2} Biosorption: For optimum cell mass concentration studied, cell mass was changed from 300mg/50ml to 2400mg/50ml at constant pH 5.0 and temperature 40ºC. Required amount of cell mass was taken in 50ml 100 ppm (Ni^{+2}) solution in 250 ml flask . The reaction solution ware incubated in a BOD shaker incubator at 120 rpm. The reaction ware carried out up to saturation. Sample were collected at 20, 40, 80, 160, 320 and 640 mint interval and centrifuged at 8000 rpm for 10 mints. The supernatant ware taken for analysis of Ni^{+2} ion concentration (8,9) .

Effect of temperature on Ni^{+2} Biosorption: For this experiment the temperature was used in the range of 30-40ºC and the biomass concentration was 1.2gm/50ml solution. The pH of the reaction mixture ware adjusted to 5.0. Initial concentration of cadmium ion was 100 ppm. 50ml of normal saline containing 100 ppm and 200 ppm Ni^{+2} respectively with 1.2 gm bacterial cell were incubated at different temperature using rotary shaker at 120 rpm. At different time intervals samples were taken out and centrifuged at 8000 rpm for 10 mints. The supernatant ware taken for analysis of **Ni^{+2}** ion concentration (10) .

Effect of initial metal concentration on Ni^{+2} Biosorption: For this experiment the temperature was used in the range of 40ºC and the biomass concentration was 1.2gm/50ml solution. The pH of the reaction mixture ware adjusted to 6.0 for nickel. Initial concentration of metal ions was varied from 100ppm, 200ppm, 250ppm and 300 ppm. 50ml of normal saline containing 100 ppm, 200 ppm, 250 ppm and 300 ppm of Ni^{+2} with 1.2 gm bacterial cell were incubated at different temperature using rotary shaker at 120 rpm. At different time intervals samples were taken out and centrifuged at 8000 rpm for 10 mints. The supernatant ware taken for analysis of **Ni^{+2}** ion concentration respectively (8, 11).

Results and Discussion

Isolation and purification of the microorganisms

The bacterial strain was isolated from water sample, collected from Nandi hills area in Bangaluru, Karnataka. It was distinguished on the basis of their colony morphology on nutrient agar plates.

Strain characterization

Different staining methods

The isolated bacterial strain was subjected to Gram's staining. Based on the result of Gram's staining, it was found Gramnegative rod shape bacteria. The isolate bacterial strain was used for the further biochemical characterization [Table 1].

Table 1: Morphological and biochemical characteristics of *Pseudomonas aeruginosa* DSGPM4

Morphological, Cultural, Physiological and Biochemical characteristics	*Results*
Cell shape	Rod
Size	5.25 µm
Gram's reaction	Gram negative
Capsule	Present

Spore	spore forming
Growth	Aerobic
Motility	Motile
Growth in NaCl (20%)	+
Growth temperature (°C)	35-37
Catalase	+
Oxidase	-
Glucose	Acid
Sucrose	Acid/Gas
Lactose	Acid
Fructose	Acid
Arabinose	Acid/Gas
Galactose	Acid/Gas
Mannitol	Acid
Raffinose	Acid/Gas
Xylose	Acid/Gas
Methyl red	-
VP-test	+
Indole	-
Citrate	-
Nitrate	+
Urease	+
Casein Hydrolysis	+
Starch	+
Gelatin	+
Identification	**Above characteristics indicates that the isolate belongs to the genus *Pseudomonas aeruginisa(GenBank Accn NoJN245880)***

Nickel (Ni^{+2}) Tolerance: The bacterial strain was found to grow in presence of heavy metal lead (II) ion. The MIC value was found 1000µg/ml. The growth in presence of metal can be due to two conditions: metabolism dependent metal uptake inside the cell or because of energy independent process of biosorption to the membrane (9,12).

Effect of pH on Ni^{+2} Biosorption: The pH dependence of metal uptake is due to the various functional groups on the bacterial cell wall. In this study, optimum pH was found to be 5.0. Since, a significant amount of lead (II) ion concentration precipitated at pH 6.0-7.0. At the beginning of the biosorption, both Ni^{+2} and protons are absorbed, but when the Ni^{+2} concentration is increased, a partial desorption of protons occurs allowing the absorption of Ni^{+2} onto the sites left by protons at the surface of the biomass. According to this it may be predicted that there was a clear competition for the biomass absorption sites, between the Ni^{+2} ions and protons. It is also cleared that ion exchange is the main sorption mechanis (4, 6,13) [Table 2].

Effect of Biomass on Ni^{+2} Biosorption: From the obtained result it is clear that Ni^{+2} absorption efficiency depends on the biomass concentration [Table 3]. It was found that 2400mg/50 ml biomass concentration gives optimum Ni^{+2} biosorption. About 99% of Ni^{+2} absorption were found by using 2400 mg dry bacterial cell. With increase in biomass concentration, percentage of Ni^{+2} removal increases, but the q value decreases. It may be attributed to reduce total surface area of the biosorbent(8,10). It is because of the aggregation of metal ions during biosorption and or to modification of the biomass surface which is also depending on the experimental conditions such as pH, ionic strength, temperature (14) .The Ni^{+2} biosorption reached at equilibrium when the biomass concentration gives no such biosorption efficiency.

Effect of temperature on Ni^{+2} Biosorption: From the experimental values [Table 4A, B] it was found that the maximum Ni^{+2} ion uptake observed at 40^0 C (About 95% of Ni^{+2} biosorption was observed). But at 30^0 C about 76% of lead biosorption was observed up to the saturation. Probable reason may be that, biological membrane generally stays in gel state in a specific temperature. This temperature ranges around optimum temperature, membrane fluidity changes. So the mobility of the absorbent membrane molecules may changes its normal orientation as well as their metal absorption affinity (12,14).

Table 2: Effect of pH on Ni^{+2} absorption

Biosorption at pH 4.0

Incubation time (minute)	Final concentration (ppm)	% of nickel removal	q Value (mg/gm)
20	64.58	56.95	17.79
40	53.75	64.17	20.05
80	45.41	69.73	21.79
160	37.77	74.82	23.38
320	35.41	76.39	23.87
640	35.39	76.41	23.88

Biosorption at pH 5.0

Incubation time (minute)	Final concentration (ppm)	% of nickel removal	q Value (mg/gm)
20	47.50	68.33	21.35
40	29.10	80.60	25.19
80	21.25	85.83	26.82
160	13.33	91.11	28.47
320	9..91	93.39	29.19
640	9..79	93.47	29.21

Biosorption at pH 6.0

Incubation time (minute)	Final concentration (ppm)	% of nickel removal	q Value (mg/gm)
20	48.33	67.78	21.18
40	42.08	71.95	22.48
80	22.50	85.00	26.56
160	28.33	81.11	25.35
320	17.70	88.20	27.56
640	17.56	88.29	27.59

Biosorption at pH 7.0

Incubation time (minute)	Final concentration (ppm)	% of nickel removal	q Value (mg/gm)
20	65.41	56.39	17.62
40	59.16	60.56	19.93
80	37.91	73.39	23.35
160	35.41	76.39	23.87
320	24.19	83.87	26.21
640	24.05	83.97	26.24

Table 3: Effect of Biomass on Ni^{+2} absorption

Biomass (mg)	Incubation time(min)	Final concentration of Ni+2 (ppm)	% of nickel removal	q Value (mg/gm)
300	20	68.83	31.17	25.98
	40	68.33	31.17	26.56
	80	57.08	42.92	35.77
	160	52.50	47.50	39.58
	320	52.08	47.92	39.93
	640	52.02	47.8	39.98
600	20	26.80	73.20	30.50
	40	25.16	74.84	31.18
	80	15.83	84.17	35.07
	160	10.75	89.25	37.19
	320	10.33	89.67	37.36
	640	10.31	89.69	37.37
1200	20	28.75	71.25	20.23
	40	21.66	78.34	16.32
	80	12.58	87.42	4.371
	160	9.16	90.84	18.93
	320	8.12	91.88	19.14
	640	8.10	91.90	19.15
2400	20	14.25	85.75	8.93
	40	6.16	93.84	9.78
	80	3.66	96.34	10.03
	160	1.83	98.17	10.23
	320	1.46	98.54	10.26
	640	1.42	98.58	10.27

Table 4: Effect of Temperature on Ni^{+2} absorption

A. Initial Ni^{+2} ion concentration 100 ppm

Temperature (^{0}C)	Incubation time (minute)	Final concentration of Ni+2 (ppm	% of removal	q Value (mg/gm)
30^{0}C	20	48.33	51.67	10.76
	40	46.66	53.23	11.11
	80	34.16	65.84	13.72
	160	23.33	76.67	15.97
	320	23.64	76.36	15.91
	640	18.75	81.25	16.93
40^{0}C	20	21.66	78.34	16.32
	40	8.58	91.42	19.05
	80	6.16	93.84	19.55
	160	4.13	95.87	19.97
	320	4.09	95.91	19.98
	640	3.85	96.15	20.03

B. Initial Ni^{+2} ion concentration 200 ppm

Temperature (^{0}C)	Incubation time (minute)	Final concentration of Pb+2 (ppm	% of removal	q Value (mg/gm)
30^{0}C	20	65.00	67.50	28.13
	40	61.80	69.10	28.79
	80	58.33	70.84	29.51
	160	52.50	73.75	30.73
	320	45.00	77.50	32.29
	640	44.28	77.86	11.60
40^{0}C	20	57.50	71.25	71.25
	40	48.43	75.79	33.58
	80	39.55	80.23	33.43
	160	26.16	86.92	36.22
	320	20.36	89.82	37.43
	640	20.30	89.86	37.44

Conclusion

From our experimental result it is become cleared that the biomass of *Pseudomonas aeruginosa DSGPM4* sp is a suitable biosorbent agent for the removal of nickel from aqueous solution.

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How to cite this article

Sarkar D. and Paul G. (2015). Bioremediation of nickel ions from aqueous system by dry cells of *Pseudomonas aeruginosa DSGPM4* species. *Int. J. Pharm. Life Sci.*, 6(1):4110-4114.

Source of Support: Nil; Conflict of Interest: None declared

Received: 10.12.14; Revised: 15.12.14; Accepted:06.01.15