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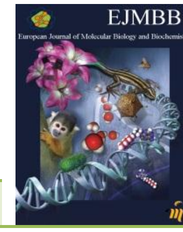

BIOREMEDIATION OF HEAVY METALS LIKE CHROMIUM AND NICKEL FROM ELECTROPLATING INDUSTRIAL WASTE

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ABSTRACT

Today indiscriminate and uncontrolled discharge of metal contaminated industrial effluents into the environment has become an issue of major concern. Release of heavy metal without proper treatment poses a significant threat to public health because of its persistence biomagnifications and accumulation in food chain. To reduce metal pollution problems many processes have been developed for the treatment and disposal of metal containing wastes. The major shortcomings of the conventional treatments & low efficiency at low concentration of heavy metals, expensive handling and safe disposal of toxic sludge. Microbial metal bioremediation is an efficient strategy due to its low cost, high efficiency and ecofriendly nature moreover it results in the partial or complete biotransformation of wastes to microbial biomass and stable end products. In present work, coloration, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) , turbidity, TS, TDS, TSS, Optimization of effluent and heavy metal (Nickel, Chromium) removal from electroplating industrial effluent by microorganisms such as *Bacillus sp, Micrococcus sp and Microbacterium sp* were studied and analysis suggested that three strains are better microbial tool for bioremediation of heavy metal. Hence possibility can be explored to remove heavy metal load, present even in low concentration, in waste water of electroplating industrial effluent by using microorganisms.

INTRODUCTION

The growing industrialization and modern agricultural practices that have spread worldwide are adversely affected the ecosystem. These practices have leave persistent toxic metals and organic pollutants in the surroundings which tend to accumulate and deteriorate the environment [1]. Contamination of heavy metals in the environment is a major global concern because of their toxicity and threat to human life and environment. Much research work has been carried out on heavy metal contamination in soils from various anthropogenic sources such as industrial wastes, automobile emissions, mining activity and agricultural practices. Bioremediation has evolved as the most promising one because of its economical, safety and environmental features. Since organic contaminants become actually transfer and some of them are fully mineralized [2]. This method is at least six times cheaper than incineration and three times cheaper than confinement. An Incorporation of Nickel in 4 microbial enzymes involved in Ureolysis, Hydrogen metabolism, methane synthesis and acetogenesis [3]. Chromium is useful in Tanning, Paints, Pigment and fungicide industry and effect of Nephritis, cancer and ulceration [4]. Microbes for metal remediation mechanisms by which metal ions bind to the cell surface include electrostatic interactions, Van der Waals forces, covalent bonding, redox interactions and extracellular precipitation (or) a combination of these processes [5].

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MATERIALS AND METHODS

Sampling site and isolation of microorganisms from the effluent:

The bacteria were isolated from electroplating industry. The samples were collected in the plastic cans and brought to the analytical laboratory for bioremediation studies. The efficient sample 1ml was suspended in 99ml of the sterile saline solution and was thoroughly mixed with a vortex after separating of 100 ml of each suspension on nutrient agar (NA). The sample was aerobically incubated for 3 day at 37^0C. After incubation , colonies were selected for isolation. *Bacillus sp, Micrococcus sp and Microbacterium sp* isolates were picked up and purified by repeated streaking on.

Identification of isolates:

For the identification of the isolates morphological and some biochemical properties of the isolates were determined by carrying out the gram staining, catalase test in accordance with Bergey's manual of systemic bacteriology [6].

Acclimatization of the culture

The isolated bacterial species were acclimatized in $NiSO_4$ and $K_2Cr_2O_7$ salt. Then allowed to grow at 1g/10ml concentration of Ni, Cr in broth for 24 hrs.

Estimation of Initial metal level in the effluent:

1ml of effluent was taken as a sample and the amount of nickel and chromium was estimated by using the standard method and the initial amount of metals were calculated by calibrating the concentration against the standard graph.

Analysis of Sample:

pH was recorded and further analyzed in the laboratory for their physiochemical characteristics like coloration, turbidity, TSS, BOD, COD by standard methods of APHA [7].

Optimization of Media:

The medium were prepared according the Plackette – Burmann design [8] for each trail of appropriate high and low concentration. Through this (high and low) variants, the medium prepared and used for the Plackette – Burmann method optimization.

Statistical Analysis: Analysis of Varinace (ANNOVA)

ANNOVA is the systematic procedure for the analysis of variation. It consists of classifying and cross – classifying statistical results and testing whether the means of specific classification differ significantly. It is used to test the significance of the difference among sample means.

RESULT AND DISCUSSION

The removal of toxic metals such as Chromium and Nickel from the dilute solutions resulting from electroplating industry. The industrial effluents cause soil and groundwater pollution besides causing a number of adverse effect on plants, animal and human health. Due to awareness of the adverse ecological effects of toxic metals, several investigation advocate appropriate techniques for the different techniques implied in the treatment of the industrial wastes before being discharged out is the use of bacteria for the removal of metal ions from effluent which offers great potential. *Bacillus sp, Micrococcus sp* and *Microbacterium sp* plays a vital role in bioremediation studies. They easily acclimatize to any environment and shows their maximum growth efficiency from the effluents microbiological analysis was subjected and the isolates were *Bacillus sp., Micrococcus sp., Microbacterium sp.*

Then, the physiochemical character (BOD, COD, TDS, TSS, pH, Temperature) of the effluents were done by Dr. Rajan method. These physiochemical characters were measured to know the effect of the effluent. The effect of the effluent was calculated and denoted in Table. 1.

The biodegradation of chromium and nickel is run by minimal salt medium inoculated with *Bacillus sp*, *Micrococcus sp* and *Microbacterium sp* of various concentration and time intervals of each tubes respectively. The level of biodegradation of chromium and nickel were analysed in 6 days intervals. The highly biodegradable strains were *Bacillus sp, Micrococcus sp* and *Microbacterium sp* in chromium upto 95%, 93% and 95% at 5^{th} day (Table 2, figure – 1)

Then, these highly biodegradable strains were *Bacillus sp, Micrococcus sp and Microbacterium sp* in nickel upto 94%, 94% and 97% at 5^{th} day.

The highly biodegradable strains were *Bacillus sp, Micrococcus sp and Microbacterium sp* were selected for the optimization studies. Medium optimization was done by Plackette – burmann design. The effective degradation of nickel and chromium was done at trails 1 to trails 8, with the high and low amount of pH, temperature, glucose and yeast extract were shown in Table 3.

The highly degradation of chromium was done at trial 4 in *Bacillus sp,* trail 5 in *Micrococcus sp,* trail 8 in *Microbacterium sp* and percentage are 98%, 98%, 97%. (Table 4-9, Fig 2-7).

For statistical analysis, the respective totals were calculated between and within sample. Grand total was arrived by summing the total of different metal degradable microbes.

Table 1. Characteristics of the Effluent

S.NO	Characteristics	Sample 1	Sample 2
1	pH	5.80	2.92
2	Temperature	28°C	30°C
3	Color	Brown	Yellow
4	Odour	Irony	Irony
5	Toxic metals present	Chromium, Nickel	Chromium, Nickel
6	Total solids	16.6	2.6
7	Total Dissolved solids	17	2
8	Total Suspended solids	0.4	0.6
9	BOD	5	5
10	COD	1760	1440

Table 2. Estimation of Chromium (540 nm)

S.NO	Days	*Bacillus sp*		*Microcococcus sp*		*Microbacterium sp*	
		Optical Density	Percentage (%)	Optical Density	Percentage (%)	Optical Density	Percentage (%)
1	0 day	0.512	0	0.512	0	0.512	0
2	1 day	0.460	7	0.472	20	0.453	12
3	2 day	0.304	42	0.351	32	0.320	37
4	3 day	0.182	63	0.270	46	0.243	54
5	4 day	0.050	90	0.132	73	0.126	76
6	5 day	0.021	95	0.030	93	0.028	95

Table 3. Optimization Conditions (plackette burmann design)

Trials	pH	Temperature(°c)	Glucose	Yeast Extract
1	9	45	4	0.3
2	5	45	4	3
3	5	25	4	3
4	9	25	0.5	3
5	5	45	0.5	0.3
6	9	25	4	0.3
7	9	45	0.5	3
8	5	25	0.5	0.3

Table 4. Media Optimization for Microbial degradation of chromium in *Bacillus sp*

S.NO	Trials	O day		1st day		2nd day		3rd day		4th day		5th day	
		OD	%	OD	%	OD	%	OD	%	OD	%	OD	%
1	1	0.562	0	0.490	5	0.380	27	0.294	44	0.120	56	0.020	95
2	2	0.562	0	0.477	10	0.331	34	0.214	59	0.160	71	0.010	98
3	3	0.562	0	0.437	21	0.318	39	0.299	44	0.124	76	0.058	98
4	4	0.562	0	0.451	10	0.337	34	0.268	48	0.182	66	0.040	99
5	5	0.562	0	0.424	17	0.316	39	0.200	61	0.174	68	0.030	93
6	6	0.562	0	0.468	12	0.358	34	0.271	49	0.191	63	0.070	83
7	7	0.562	0	0.424	17	0.318	39	0.245	51	0.155	68	0.080	81
8	8	0.562	0	0.488	7	0.362	32	0.216	59	0.182	66	0.060	86

Table 5. Media Optimization for Microbial degradation of chromium in *Micrococcus sp*

S.NO	Trials	O day		1st day		2nd day		3rd day		4th day		5th day	
		OD	%	OD	%	OD	%	OD	%	OD	%	OD	%
1	1	0.509	0	0.443	12	0.355	34	0.236	46	0.127	76	0.077	83
2	2	0.509	0	0.492	5	0.383	27	0.259	54	0.185	66	0.083	81
3	3	0.509	0	0.429	17	0.354	30	0.221	56	0.198	63	0.047	90
4	4	0.509	0	0.447	12	0.353	30	0.233	54	0.176	68	0.083	81

5	5	0.509	0	0.465	12	0.366	32	0.287	46	0.154	68	0.030	93
6	6	0.509	0	0.455	14	0.364	32	0.240	51	0.120	76	0.090	78
7	7	0.509	0	0.470	10	0.381	27	0.238	54	0.180	66	0.048	90
8	8	0.509	0	0.481	7	0.362	32	0.258	49	0.190	63	0.060	86

Table 6. Media Optimization for Microbial degradation of Chromium in *Micro bacterium sp*

S.NO	Trials	oday		1st day		2nd day		3rd day		4th day		5th day	
		OD	%	OD	%	OD	%	OD	%	OD	%	OD	%
1	1	0.548	0	0.447	14	0.318	39	0.216	58	0.111	78	0.077	83
2	2	0.548	0	0.445	10	0.358	30	0.263	46	0.160	71	0.082	80
3	3	0.548	0	0.432	15	0.303	42	0.285	46	0.113	78	0.052	88
4	4	0.548	0	0.453	10	0.320	37	0.287	46	0.188	66	0.054	88
5	5	0.548	0	0.432	21	0.358	34	0.252	49	0.176	68	0.089	73
6	6	0.548	0	0.414	20	0.376	29	0.282	46	0.127	76	0.059	88
7	7	0.548	0	0.437	21	0.384	27	0.288	47	0.134	73	0.067	86
8	8	0.548	0	0.488	7	0.321	37	0.271	49	0.162	71	0.048	90

Table 7. Media Optimization for Microbial degradation of Nickel in *Bacillus sp*

S.NO	Trials	O day		1st day		2nd day		3rd day		4th day		5th day	
		OD	%	OD	%	OD	%	OD	%	OD	%	OD	%
1	1	0.552	0	0.461	29	0.320	51	0.229	66	0.180	92	0.07	96
2	2	0.552	0	0.476	26	0.389	42	0.200	69	0.168	76	0.010	98
3	3	0.552	0	0.420	36	0.318	52	0.226	66	0.150	77	0.038	95
4	4	0.552	0	0.498	25	0.320	51	0.250	62	0.192	71	0.021	97
5	5	0.552	0	0.408	39	0.330	49	0.231	65	0.164	76	0.082	88
6	6	0.552	0	0.487	26	0.381	43	0.224	66	0.142	78	0.050	92
7	7	0.552	0	0.443	32	0.312	52	0.279	58	0.100	85	0.020	97
8	8	0.552	0	0.415	37	0.331	49	0.264	68	0.176	74	0.062	91

Table 8. Media Optimization for Microbial degradation of Nickel in *Micrococcus sp*

S.NO	Trials	O day		1st day		2nd day		3rd day		4th day		5th day	
		OD	%	OD	%	OD	%	OD	%	OD	%	OD	%
1	1	0.576	0	0.490	25	0.370	43	0.294	55	0.180	72	0.05	92
2	2	0.576	0	0.479	12	0.380	42	0.262	60	0.152	77	0.038	95
3	3	0.576	0	0.442	32	0.352	46	0.210	68	0.120	81	0.070	89
4	4	0.576	0	0.465	29	0.380	42	0.247	63	0.192	71	0.081	88
5	5	0.576	0	0.460	29	0.368	45	0.237	65	0.200	69	0.101	85
6	6	0.576	0	0.450	31	0.364	45	0.210	68	0.111	83	0.020	97
7	7	0.576	0	0.448	32	0.312	52	0.240	63	0.180	72	0.060	91
8	8	0.576	0	0.467	39	0.381	42	0.218	68	0.120	83	0.010	98

Table 9. Media Optimization for Microbial degradation of Nickel in *Micro bacterium sp*

S.NO	Trials	O day		1st day		2nd day		3rd day		4th day		5th day	
		OD	%	OD	%	OD	%	OD	%	OD	%	OD	%
1	1	0.596	0	0.479	28	0.380	42	0.242	63	0.178	74	0.076	89
2	2	0.596	0	0.437	34	0.349	48	0.289	57	0.200	69	0.198	71
3	3	0.596	0	0.442	32	0.362	45	0.258	62	0.159	77	0.029	97
4	4	0.596	0	0.425	36	0.321	51	0.226	66	0.124	81	0.082	88
5	5	0.596	0	0.493	25	0.366	45	0.242	63	0.144	78	0.033	95

6	6	0.596	0	0.485	26	0.308	54	0.249	63	0.133	80	0.071	89
7	7	0.596	0	0.421	36	0.357	46	0.251	62	0.128	81	0.050	92
8	8	0.596	0	0.487	26	0.362	45	0.287	57	0.137	80	0.092	86

Figure 1. Estimation of Chromium in *Bacillus sp, Micrococcus sp, Micrococcus sp*

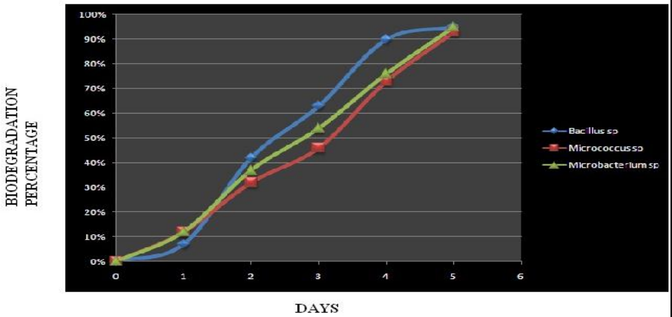


Figure 2. Media Optimization for Microbial degradation of Chromium in *Bacillus sp*

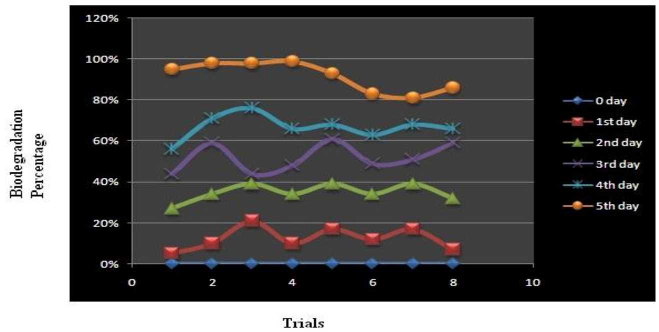


Figure 3. Media Optimization for Microbial degradation of Chromium in *Micrococcus sp*

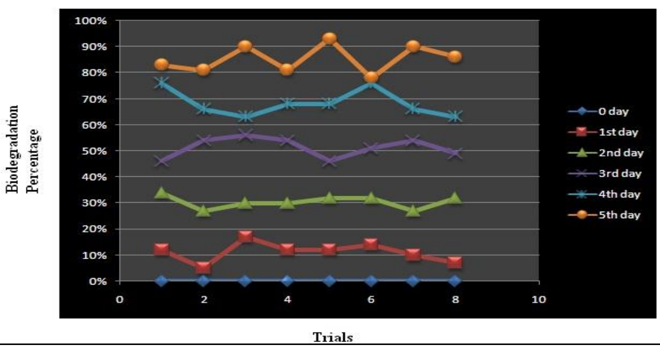


Figure 4. Media Optimization for Microbial degradation of Chromium in *Micro bacterium sp*

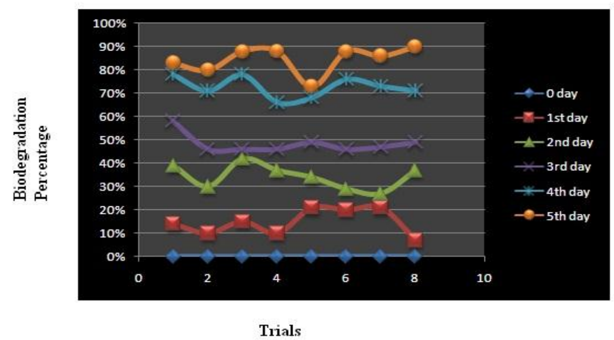


Figure 5. Media Optimization for Microbial degradation of Nickel in *Bacillus sp*

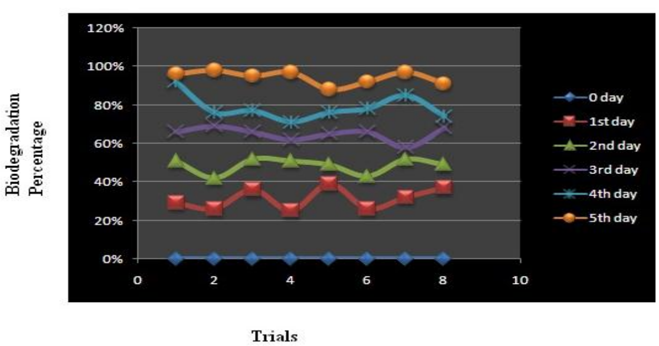


Figure 6. Media Optimization for Microbial degradation of Nickel in *Micrococcus sp*

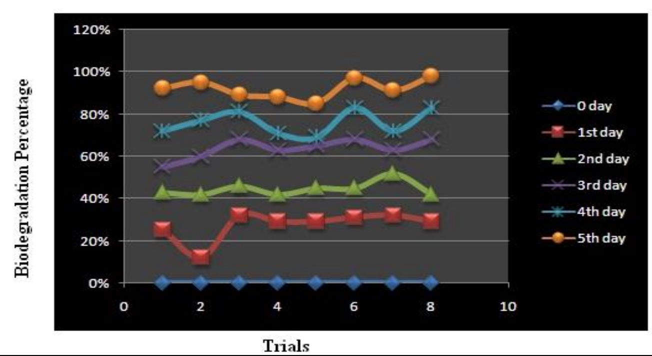
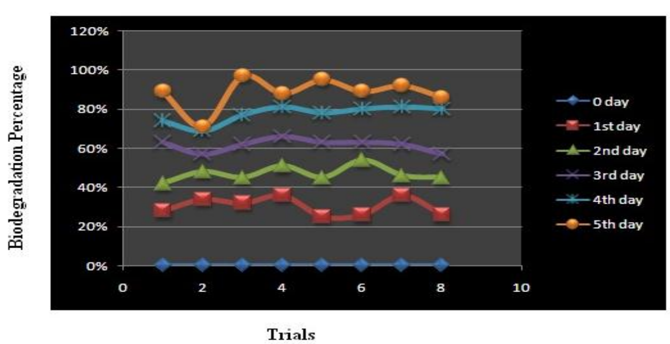


Figure 7. Media Optimization for Microbial degradation of Nickel in *Micro bacterium sp*



CONCLUSION

Remediation of metal contaminated soil and water is a necessity in order to have a safe and healthy environment that will in turn results in healthy style across the globe. Biological remediation of metal contaminated soil and water offers a better and more eco- friendly technique that if properly and thoroughly explored can bring our environment into a better place for both plant and animals well being to its enormous advantages over other treatment methods.

Future research and development will requires focus on the use of cheap, eco- friendly and widely available nutrients that can be used to enhance the microbial and plant activities in mineralizing hydrocarbons and heavy metals in soil and water environment.

Metal degrading bacterial strains were isolated from waste disposable site. That microbial strain were subjected and it was identified as the bacterial strains were identified as the bacterial strains were *Bacillus sp*, *Micrococcus sp* and *Microbacterium sp* confirmed family by Bergey's manual of systemic bacteriology. The BOD, COD, TS, TDS, TSS, pH, Temperature, Color, Turbidity and metals of the effluents were identified. The biodegradation of metal done by minimal salt medium which was supplement with microbial strains were *Bacillus sp*, *Micrococcus sp*, and *Microbacterium sp* were effectively degraded Chromium and Nickel.

Then, the optimization studies were followed by the Plackette RL and Burmann JP design and 3 different strains of *Bacillus sp*, *Micrococcus sp* and *Microbacterium sp* were Chromium and Nickel.

ANNOVA results confirm that 3 different strains of *Bacillus sp*, *Micrococcus sp* and *Microbacterium sp* were degraded the Chromium and Nickel metals.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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