A Study On

“Bio-removal of Nickel from electroplating industry”

Dissertation Report submitted

In the partial fulfillment of the requirement for the Award of

“MASTER OF TECHNOLOGY”

By

PADMA SERAGADAM

(20600004)

Dept of Chemical Engg.

National Institute of Technology

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Under the esteemed guidance of

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CERTIFICATE

This is to certify that the thesis entitled "Bio-removal of Nickel from electroplating industry" is submitted by **Ms. PADMA SERAGADAM**, (Roll No.-20600004) to this institute in partial fulfillment of the requirements for the award of the degree of Master of Technology in Chemical Engineering (Spl: Biotechnology and Biochemical Engineering), is a bonafide record of the work carried out under my supervision and guidance. It is further certified that no part of this thesis is submitted for the award of any degree.

Dr. (Mrs).Susmita Mishra

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Padma Seragadam

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CONTENTS

Abstract

Ni (II) has been designated as a priority pollutant by the US Environmental Protection Agency (USEPA) due to its ability to cause mutations and cancer in humans. The risk associated with soil and groundwater contamination of nickel waste generated by many industries is high, and therefore Ni (II) remediation is of critical importance. It is discharged into the environment through the disposal of wastes from industries like Electroplating industry, metallurgical and metal finishing, textiles and ceramics, pigment and wood preservatives, photographic sensitizer manufacturing, etc. In the environment nickel occurs in divalent and tetravalent forms. Comparative to tetravalent, divalent nickel is more hazardous to the environment.

Electroplating industry which commonly use nickel metal for plating process, discharges the effluents into the environment containing Nickel in excess of the maximum permissible limits. According to W .H.O. standards the permissible limits for nickel in effluent is 3mg/l and in drinking water is 0.02 mg/l. *Staphylococcus sp.* has polyphosphate in its cell, which is responsible for sequestering nickel.

Using chemical and biological methods conjointly can decrease the cost of remediating contaminated sites. Microbial reduction of Ni(II), an important aspect of biological remediation, requires the knowledge of microorganisms capable of reducing Ni(II) and the mechanisms involved in the reduction processes. *Staphylococcus sp.* has polyphosphate in its cell, which is responsible for sequestering nickel.

The present study is an attempt to evaluate the bio-removal of nickel from wastewater, which is collected and characterized from an electroplating industry, Coimbatore, Tamilnadu,RSP ,Rourkela. *Staphylococcus* cultured in the lab is used for the removal of nickel.

The overall objective of this study was to investigate the effect of various parameters like pH, inoculum volume, sugar amount, initial nickel concentration on nickel reduction by indigenous soil isolated bacteria isolated from soil contaminated sites.

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Chapter 1

Introduction

Pollution, in one sense is the introduction of contaminants into an environment, of whatever predetermined or agreed upon proportions, that causes instability, disorder, and harm to the physical systems or living organisms therein [1] Pollution can be in the form of chemical substances, or energy such as noise, heat, metals, gases & light. Pollutants can be naturally occurring substances or energies, but are considered contaminants when in excess of natural levels. In another sense, pollution is a term for any substance introduced into an ecology that causes instability and breakdown of the life or reproductive forces of said system. A substance as common and generally healthy as water can become a "pollutant" at high enough concentrations, e.g. if a human were to drink excessive amounts, leading to a burden on physical systems, a breakdown of such systems, and potentially leading to death. The term heavy metal is often used to cover a diverse range of elements which constitute an important class of pollutants. Such pollutants have received the attention of researchers all over the world, mainly due to their harmful effects on living beings. Human biology is full of instances where heavy metal toxicity has led to mass deaths. Heavy metals enter into the environment mainly via three routes:

1) Deposition of atmospheric particulates
2) Disposal of metal enriched sewage sludges and sewage effluents and
3) By-products from metal mining processes.

1.1What is Nickel?

1.1.1: Properties

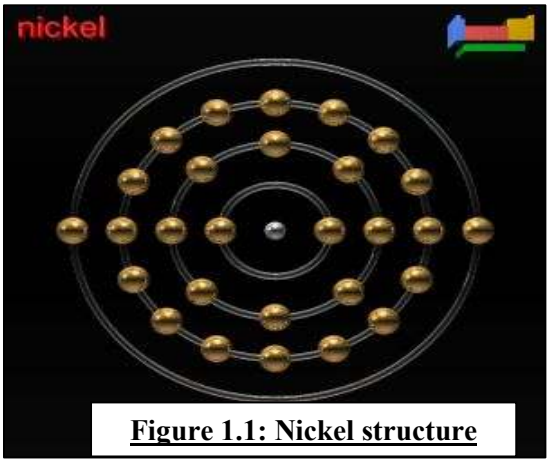

Figure 1.1: Nickel structure

Nickel (symbol Ni, atomic weight 58.71, atomic number 28) is a lustrous, silvery-white metal discovered in 1751 by Axel Fredrik Cronstedt. It is the 28th element on the periodic table [2]. It has a melting point of 1453° C, relatively low thermal and electrical conductivities, high resistance to corrosion and oxidation, excellent strength and toughness at elevated temperatures, and is capable of being magnetized. It is attractive and very durable as a pure metal, and alloys readily with many other metals. Reflecting these

qualities, nickel is widely used in over 300 000 products for consumer, industrial, military, transport/aerospace, and marine and architectural applications.

1.1.2: Uses

The public may recognize nickel in coins, as it is used for this purpose in pure or alloy forms by many countries, or as bright and durable electrolytic ally-applied coatings on steel (nickel plating). The biggest use, however, is as an alloying metal along with chromium and other metals in the production of stainless and heat-resisting steels [3]. The main uses of nickel and its compounds are in stainless steel, nickel containing alloys with anti-corrosion properties and electroplating. These are mostly used in industry and construction, but also for products in the home such as pots and pans, kitchen sinks, etc. Stainless steels are produced in a wide range of compositions to meet special industry requirements for corrosion and heat resistance, and also to facilitate a clean and hygienic surface for food and other processing. In fact, about 65 per cent of nickel is used to manufacture stainless steels, and 20 per cent in other steel and non-ferrous (including "super") alloys, often for highly specialized industrial, aerospace and military applications. About 9 per cent is used in plating and 6 per cent in other uses including coins and a variety of nickel chemicals. About 85% of this nickel is used in combination with other metals to make what are known as alloys.

1.1.3: Occurrence

Nickel is part of meteorites. Nickel occurs in nature principally as oxides, sulphides and silicates. Ores of nickel are mined in about 20 countries on all continents, and are smelted or refined in about 25 countries. Primary nickel is produced and used in the form of Ferro-nickel, nickel oxides and other chemicals, and as more or less pure nickel metal. Nickel is also readily recycled in many of its applications, and large tonnages of secondary or "scrap" nickel are used to supplement newly mined metal [3]. Only about 1 million tons of new or primary nickel are produced and consumed annually in the world, compared with over 10 million tons of copper and nearly 800 million tons of steel. Nickel makes up 0.008% of the Earth's crust. When the deeper core of the Earth is included, nickel becomes more abundant, ranking as the fifth most common element after iron, oxygen, silicon and magnesium. Nickel is economically extracted from deposits of nickel-bearing minerals in the Earth's crust, which, over geological time, have concentrated nickel into relatively small areas near to the surface in what are known as ore bodies. Ore minerals are combinations of nickel and other

elements, such as iron, sulphur, cobalt and oxygen that can be extracted economically. Nickel ores are found in many countries, but the

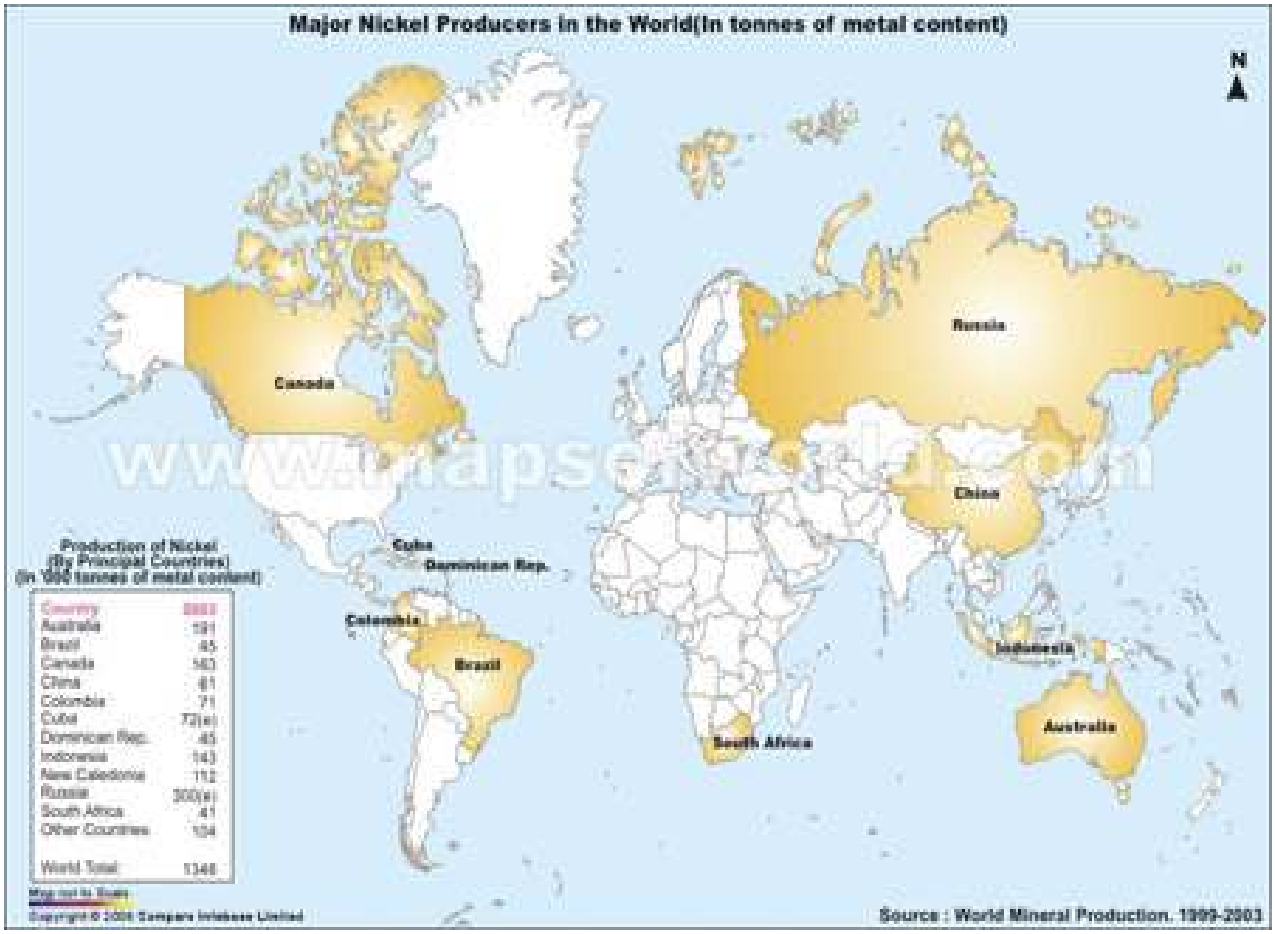

Figure 1.2: World-Nickel Producing countries

Principal nickel mining areas are Australia, Brazil, Canada, China, Colombia, Cuba, Greece, Indonesia, New Caledonia, Southern Africa and Russia [4]. Important nickel refineries also operate in Norway, Finland, France, Japan and the United Kingdom. The world's producers sell nickel in many forms, as sheets, powders, pellets, ingots, etc., to thousands of buyers. These substances have their own physical and mechanical properties which are distinct from those of their

Table-1.1: ***World Resources of Nickel***

Country	**2001**	**2002**	**2003**
World: Total	1274	1293	1348
Australia	205	208	191
Brazil	45	45	45
Canada	194	189	163
China	51	54	61
Colombia	53	58	71
Cuba	73	72	72
Dominican Republic	33	37	45
Indonesia	102	123	143
New Caledonia	112	100	112
Russia	273	267	300
South Africa	36	39	41
Other Countries	97	101	104

(In '000 tonnes of metal content)

constituent elements. Alloys that contain nickel include hundreds of different grades of stainless steels, hundreds of different nickel alloys; many alloy steels, and a few copper-nickel alloys. This family of materials now consists of more than 100 separate grades offering a wide range of attractive properties, which, in turn, results in even greater diversity of use. As a result of new technology, the manufacture of stainless steel has become increasingly efficient and economical, rendering it competitive with alternative materials. The rise in the number of emerging applications of stainless steel, combined with its rapidly improving price competitiveness, largely accounts for a sustained underlying growth rate of some 5% to 6% per annum for this material. Many nickel salts are green in color. Nickel is obtained from minerals laterite, pentlandite and pyrhotite.

1.1.4: Effects

Nickel is designated by the U.S. Environmental Protection Agency (USEPA) as a priority pollutant due to its ability to cause genetic mutations and cancer. Nickel is an essential element for healthy animals and probably for humans. The most common adverse health effect of nickel in humans is an allergic reaction[3]. People can become sensitive to nickel when jewelry or other items containing nickel touch the skin. Major man-made sources of release of nickel are the combustion of coal and heavy fuel oil. Emissions from refineries and from refinery products (including road tar) are particularly important because of the large amount of refinery fuel oil and residues burnt which contain nickel from the original crude oil. Other sources include emissions from mining and refining operations, municipal waste incineration, and windblown dust. Excessive exposure to nickel may cause health effects on the blood, lung, nose, kidney, reproductive system, skin and the unborn child. In prolonged and direct contact with skin, nickel may cause an allergic reaction on nickel sensitized people. Chronic inhalation exposure to nickel fumes may cause cancer. Nickel is also toxic to aquatic life. However, hazards depend on the form and bioavailability of nickel. Very small amounts of nickel have been shown to be essential for normal growth and reproduction in some species of animals and plants. Once a person is sensitized to nickel, further contact with the metal will produce a reaction. The most common reaction is a skin rash at the site of contact. In some sensitized people, dermatitis may develop at a site away from the area of contact. For example, hand eczema is fairly common among people sensitized to nickel. Less frequently, some people who are sensitive to nickel have asthma attacks. Some sensitized individuals react when they eat nickel in food or water, or breathe dust containing nickel. Kidney and lung damage have been observed for large doses of nickel. Dust or fumes of nickel can be a human carcinogen. Nickel is unique among regulated toxic elements in the environment because different species of chromium, specifically Ni(ii) and Ni(iv), are regulated in different ways. Relying on the chemical, toxicological, and epidemiological evidence, regulation of Ni (ii) concentration is different from that of Ni(iv). Tetravalent Nickel is the nutritionally useful form, while the divalent form is toxic and mutagenic.

1.1.5: Environmental issues

All metals and metal compounds have a certain level of toxicity and may cause adverse effects on living organisms. Nickel in certain forms and under particular circumstances, may

generate detrimental environmental (including health and safety) effects, notwithstanding the fact that it is considered to be a vital element for public health by some scientist [2]. It appears to be extremely difficult to make a general assessment on the environmental consequences of nickel. However, growing concern on environmental matters worldwide stimulated several countries and/or international organizations to regulate metals uses, including nickel, according to their chemical and physical features and properties and possible adverse effects that their various applications may cause. Existing as well as new regulations are aimed at protecting workers and consumers (public health approach) whereas others focus on the protection of the environment (ecological approach). For instance regulations on occupational exposure limits intend to protect workers in various industries, while legislation on classification, packaging and labeling of products aims to inform the public on possible adverse health effects. To protect the environment, emission levels for various products have been introduced. INSG (International Nickel Study Group) is currently compiling information on regulatory issues affecting nickel to inform its member countries on existing and new legislations and the possible consequences for nickel production and consumption.

1.1.6: Permissible limits Of Nickel [5]

Table-1.2: Permissible limits

	Indian Standard(mg/l)	WHO
Surface Water	3	1
Marine coastal	50	----
Drinking water	0.02	0.02
Public Sewers	3	------

Chapter 2

Literature Review

Nickel is found maximum in the effluent coming from the electroplating industry. The amount of nickel can be measured in different ways like [6, 7]:

1) Spectrophotometric method: Reagent used is dimethylglyoxime,
2) X-Ray fluorescence Method
3) Particle-induced X-Ray emission spectrophotometer
4) Gas chromatography
5) HPLC
6) Atomic Emission Spectrophotometer
7) Atomic Absorption Spectrophotometer

Numbers of investigators have worked on the removal of heavy metals from wastewater or from aqueous solutions by using different methods. Initially they were using

2.1: Conventional methods [8]

2.1.1: Precipitation

It is the most common method for removing toxic heavy metals up to parts per million (ppm) levels from water [9, 10]. Since some metal salts are insoluble in water and which get precipitated when correct anion is added. The conventional precipitation induced by pH control, failed to achieve the required goals at sub-mg/l levels for chromium, lead, cadmium and nickel. In some cases, adsorption/co-precipitation with ferric chloride proved to be effective. This method is particularly applicable for treatment of low-volume industrial streams, where disposal of relatively large quantities of sludge generated [11].Nickel can be precipitated using chemicals like nickel hydrates or sodium hydroxide. Precipitations as metals oxide and probably as metals carbonate were two of the mechanisms that contributed to the removal of metals from their solution. The study has demonstrated that limestone was capable to remove more than 90% of heavy metal from a solution of 2 mg/L. Higher removal was achieved at a final pH of 8.5 with the quantity of limestone above 20 mL (equivalent with 56 g). This implies that limestone is an important media in the removal process [12, 13].

Disadvantages:

1) Lacks the specificity,
2) Ineffective in removal of the metal ions at low concentration, and

3) Requires addition of other chemicals, which finally leads to the generation of high water content sludge, the disposal of which is costly.

2.1.2: Reverse osmosis and Ion exchange

These are the most common methods for removing toxic heavy metals up to parts per million (ppm) levels from water [9, 10]. Since some metal salts are insoluble in water and which get precipitated when correct anion is added. An ion exchange is a solid capable of exchanging either cations or anions from the surrounding materials. Commonly used matrices for ion exchange are synthetic organic ion exchange resins. A very high-quality feed is required for efficient operation of RO units. Membrane elements in the RO unit can be fouled by colloidal matter and constituents in the feed stream. Thus, cartridge filters with a pore size of 5 to 10/~m and activated carbon have also been used to reduce residual suspended solids and other toxic matter. These units used before RO can contribute to better quality effluent for the product. It was shown in this study that the combination of RO and its PU sufficiently removed nickel and zinc ions from aqueous solutions. The metal rejections seem not to be greatly affected by different conductivity and pH. EDTA increased Zn +2 and Ni +2 removal, but the effluent conductivity also increased, especially in Zn +2 removal. The removal of nickel and zinc from metals that are toxic to humans and the environment by RO is extremely important to control these metals [14].

Disadvantages:

1) Does not effectively remove particles, pyrogens or bacteria,

2) Deionization beds can generate resin particles and culture bacteria, and

3) High operating cost.

2.1.3: Adsorption

It is a process that occurs when a gas or liquid solute accumulates on the surface of a solid or a liquid (adsorbent), forming a film of molecules or atoms. It has been reported that activated carbon prepared from coirpith is able to adsorb Ni (II) from aqueous solution. It was noted that a decrease in the carbon concentration with constant Ni concentration, resulted in a higher nickel uptake per unit weight of carbon. The adsorption capacity (Q_0) calculated from Langmuir isotherm was 62.5 mg Ni (II) g^{-1} at initial pH of 5.0 at 30°C for the particle size 250–500 μm [15] .

Disadvantages:

1. Desorption studies are required.
2. Maintance is difficult.
3. Not economical.

2.2: Microbial Processes used for Heavy Metal Remediation [16]

The estimation of metal input into environment from the two latter sources is relatively easy to measure .However atmospheric mixing of metal – emitting sources which contribute the overall atmospheric metal pool. Heavy metal contamination due to natural and anthropogenic sources is a global environmental 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. Non- biodegradability and sludge production are the two major constraints of metal treatment. Microbial metal bioremediation is an efficient strategy due to its low cost, high efficiency and eco-friendly nature. Recent advances have been made in understanding metal - microbe interaction and their application for metal accumulation/detoxification. The processes by which microorganisms interact with toxic metals are very diverse. However, in practice, there are three general categories of biotechnological process for treating soil containing toxic metals: biosorption (bioaccumulation), extracellular precipitation and uptake by purified biopolymers and other specific molecules derived from microbial cells.

2.3: Biosorption

Biosorption is a physico-chemical process of metal binding to microorganisms and bioaccumulation is an active process using natural or recombinant microbial biomass to absorb metal ions. Among them, the biosorption (bioaccumulation) has been one of the most active processes. The aim of this study was to determine if a bacterial biosorption and bioaccumulation systems could be used to remove metals from polluted soil and whether the organic acids – compounds produced by plants (e.g. carboxylic acids) have effect on these processes. The soil heavy metals-resistant isolates defined as *Pseudomonas* sp. and *Arthrobacter* sp. Have the capacity to remove large quantities of Ni, Pb and Cd from Minimal Medium. There was a direct correlation between the chain length of organic acids

and the extent of bioaccumulation of Ni and Cd. Generally organic acids with smaller molecular weights were more effective than the organic acids which have a long chain. Such correlation was not observed in case of Pb. The smaller effect on bioaccumulation was observed for *Arthrobacter* sp. in the presence of organic acids than for *Pseudomonas* sp.

Biosorption is a property of certain types of inactive, non-living microbial biomass to bind and concentrate heavy metals from even very dilute aqueous solution. Biomass exhibits this property, acting just as chemical substance, as an ion exchange of biological origin. It is particularly the cell wall structure of certain algae, fungi and bacteria, which was found responsible for this phenomenon (17). Till now, research in the area of biosorption suggests it an ideal alternative for decontamination of metal containing effluents.

2.3.1: Biosorption Mechanisms [8]

The complex structure of microorganisms implies that there are many ways for the metal to be taken up by the microbial cell. The biosorption mechanisms are various and are not fully understood. They may be classified according to various criteria. According to the dependence on the cell's metabolism, biosorption mechanisms can be divided into:

1. Metabolism dependent
2. Non -metabolism dependent.

According to the location where the metal removed from solution is found, biosorption can be classified as:

1. Extra cellular accumulation/ precipitation
2. Cell surface sorption/ Precipitation and
3. Intracellular accumulation.

2.4: The biological role of nickel

Nickel belongs to the so called “essential” metals [18, 19]. Up to date, nickel has been identified as a component in a number of enzymes, participating in important metabolic reactions, such as: ureolysis, hydrogen metabolism, methane biogenesis and acitogenesis . Nickel has also been identified as a component of a superoxide dismutase protein. A number of studies have identified nickel as trace element in various biological systems, while some microorganisms (like the cyanobacterium *Oscillatoria* sp. [20]) have demonstrated an absolute metabolic requirement for nickel. Nickel has also been identified as an

indispensable element for the chemilithotropic growth of a number of microorganisms, like *Pseudomonas flava* [21].

2.5: Role of Microorganism in the Uptake of metal [22]

Bacteria may carry determinations of resistance to a number of heavy metals, including Ag, Bi, Cd, Co, Cu, Hg, Pb, Ni, Tl, or Zn cat ions and oxyanions of As, Cr, Sb, Or W. Resistance is specific to one or few metals and the mechanisms of resistance include efflux of the metal, modification of the specification of the metal, sequestration of the metal, or a combination of these mechanisms. The anionic nature of bacterial surface enables them to bind metal cat ions through the electrostatic interactions. Because of their thickness and anionic nature, which is mainly due to peptidoglycan, teiochoic acid and teichuronic acids, the cell wall of Gram positive bacteria has a high capacity for metal binding and peptidoglycan is the major component responsible for the metal binding.

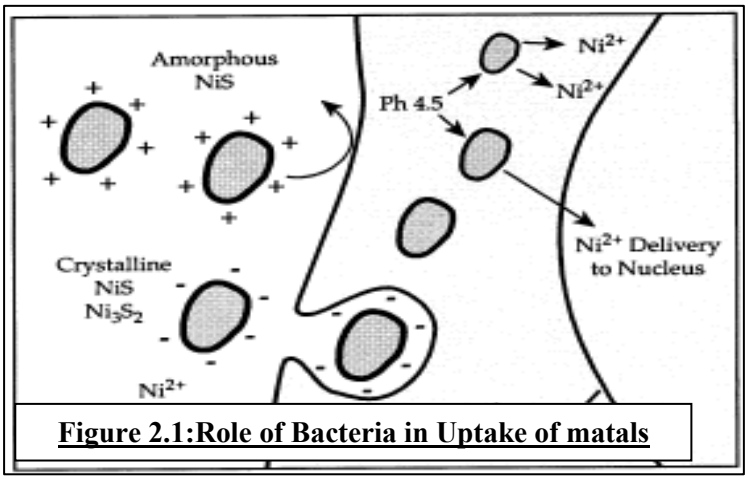

Figure 2.1:Role of Bacteria in Uptake of matals

2.5.1: Nickel Tolerant Microorganisms

The bacteria isolated from areas of high metal loading are resistant to that metals, this is because of adaptive response to excessive metal exposure.[23] Nickel (Ni)-tolerant microorganisms, including species of Cupriavidus (Alcaligenes), Burkholderia, Arthrobacter, Rhodococcus, and Streptomyces, have been isolated from naturally Ni-rich soils associated with Ni deposition & contamination. Two of the most-studied Ni-resistant microorganisms, Cupriavidus metallidurans CH34 and 31A, were isolated from a decantation tank at a zinc factory and a metal-contaminated industrial site [23]. Nickel tolerant microorganisms have been isolated from nickel polluted sites, or from natural sites lying close to nickel mineral deposits. [24], a bacterial strain may be characterized as nickel tolerant, if it is able to grow at Ni (II) concentrations higher than **100mg/L.** Wastewater treatment plants, treating industrial effluents from the metal processing industry, are places where nickel tolerant

species may be encountered, [25] have isolated a bacterial strain from such a plant, capable of growing at NiCl2 concentrations up to 1174 mg /L. [26] have experimentally estimated the minimal inhibition concentration of Ni (II) on 49 strains of *Arcobacter butzleri* to lie just below 236mg/L, while for one strain it was found to be just below472mg/L). The MIC of heterotrophic ally grown *A. eutrophus* CH34 has been experimentally estimated to be 147mg/L [27]. The wild strains of the fungus *Aspergillus niger* can tolerate nickel at concentrations up to 11.7–93.9mg/L); however, an isolate from metal contaminated soil was able to grow at 381.5mg/L) [28]. A nickel-tolerant strain of *E. coli* (strain V48) has been isolated from the municipal wastewater treatment plan of Vilnius [29]. The MIC for the above strain was measured as 5mM Ni (II) (293.5mg (Ni (II)) L−1), which, according to the authors, is 50 times greater than the MIC of the nickel-non-tolerant strain of *E. coli* JM101. Kaur et al. [30] have isolated, from an anaerobic digester, the aerobic microorganism *Alcaligenes denitrificans* strain (4a-2), which is able to grow heterotrophic ally at Ni(II) concentrations up to 20mM (1174mg L−1). Natural ecosystems rich in nickel, like serpentine soils, are often home to exceptionally nickel tolerant microorganisms, as these microorganisms have been acclimatized to grow at high nickel concentrations for centuries or more. [31] bacteria isolated from nickel-rich serpentine soil, from the Andaman islands, *Pseudomonas* sp. with MIC greater than 400mg (Ni(II))/L. *Pseudomonas* sp., from serpentine soils of central Italy, able to grow at 10 mM (Ni(II)) (=587 mg(Ni(II)) L−1) have also been isolated [32]. Nickel-tolerant strains, isolated from the vicinity of the roots of nickel-accumulating plants, in serpentine soils from New Caledonia, are able to grow at the presence of 20 mM(Ni(II)) (=1174mg (Ni(II)) L−1) [33]. Research [34] indicated that a strain of the fungus *Fusarium solani* (isolated from Saudi Arabian soil), was able to grow at the presence of up to 300 mg (Ni(II)/L.. Finally, [35] it is shown that reduction of biomass concentration for *Aspergillus* and *Micrococcus* species with the increase of Ni (II) concentration; while both species were able to grow even at the presence of 500mg (Ni(II)/L). Although microorganisms were isolated from naturally acidic sediments (e.g., pH 5.0–5.5), more colonies formed on pH 6 and 7 plates compared with pH 5, regardless of the presence or absence of metals. This could result from a number of reasons. For example, carbon sources not provided by 4M may be required for these isolates to grow at pH 5 [36]. Alternatively, microorganisms may occupy microenvironments having higher pH values than

the bulk sediment. The pH of sediment cores from Steed Pond varied with depth and sampling site (data not shown). Additionally, differences in pH may result in altered growth behavior.

Shankar Congeevran and Sridevi Dhanarani [35] showed that Microorganisms play a significant role in bioremediation of heavy metal contaminated soil and wastewater. In this study, heavy metal resistant fungi and bacteria were isolated from the soil samples of an electroplating industry, and the bioaccumulations of Cr (VI) and Ni (II) was examined. The optimal pH for fungal isolates was lower (5–5.2) than that for bacterial isolates (7). Experimental results indicated that expanded SRTs (stationary phase) can be recommended while using the fungal and bacterial Cr-resistant isolates for removing chromium. In the case of Ni-resistant bacteria isolate, a non-expanded SRT was recommended for designing continuous-flow completely stirred (CFCS) bioreactor so that a mid-log phase of cellular growth can be kept during the bioaccumulation process. Result indicates the applicability of the isolated *Micrococcus* sp. and *Aspergillus* sp. for the removal of chromium and nickel from industrial wastewater.

Reeta goel et al.[37] Showed nickel accumulation in pellet 2.0874 mM/g and in the supernatant 0.407mM/g at pH-9.At pH-7 pellet accumulation 1,444 mM/g and supernatant 0.966 mM/g. They showed metal accumulation increases the cell morphology.

Oguz Bayraktar in [38] in his study investigates the possibility of reusing metal-contaminated equilibrium fluid catalytic cracking (FCC) catalyst after bioleaching. Through bioleaching he could achieve 32% nickel removal whereas through chemical leaching only 21% nickel removal from catalyst particles was observed. The enhanced nickel removal from the catalysts in the presence of *A. niger* culture was attributed to the biosorption ability of the fungal mycelium and to the higher local concentration of citric acid on the catalyst surface. It was found that 9% of solubilized nickel in the liquid medium was biosorbed to fungal biomass.

Microbial communities were isolated from soil contaminated with Ni and U. Four gram positive bacteria are isolated Arthrobacter oxydans NR-1, Streptomyces galbus NR-2, Streptomyces aureofaciens NR-3 and Kitasatosparacystarginea NR-4.These are well grown at pH-6 with higher concentration of nickel. Rizosphere microorganisms harboring nickel hyper accumulators. Soil at Andaman Islands, India, was screened for their tolerance and

accumulation of nickel. Bacteria is more tolerant than fungi. Viable cells of selected nickel tolerant and bacterial isolates (MIC-13.6-28.9 mM ni).Cupriavidus were capable of accumulating Ni 209.5-224.4 um Ni from aqueous solution. Km and Umax-1.5 mM Ni and 636.9um Ni/g protein. The Ni-hyperaccumulators in combination with these Ni-Resistant bacteria could be an ideal tool for Nickel bioremediation [39].

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2.6: Objectives

The main objective of my study is to reduce Nickel from electroplating wastewater using an microorganism isolated from soil contaminated with metals.

The specific objectives are:

1) To assimilate the organism in different concentrations of nickel environment.

2) To optimize growth conditions of the nickel resistant organism for maximum degradation of nickel.

3) To study the effect of other metal present in their electroplating wastewater.

4) To study potential of the organism in the electroplating waste water.

5) To verify the data using different model equations.

Chapter 3

Materials and Methods

3.1: Materials

3.1.1: Chemicals Required

Nickel Sulphate, Phosphoric acid, Tartaric acid, Shulphuric acid, Sodium Hydroxide, Bromine water, DimethylGlyoxime, Lead sulphate, Zinc sulphate, Potassium Dichromate, Tin etc. Composition of nutrient medium-10 Gms. Peptone, 10gms.-beef, 5gms.-Nacl and 15 Gms. – Agar in 1 liter of distilled water.

3.1.2: Equipments Required

Autoclave (Vertical), Centrifuge, Deep Freezer, Laminar air flow cabinet, pH meter, shaker, milliQ water from Millipore, UV-Vis spectrophotometer.

3.1.3: Accessories and miscellaneous

Aluminum foil, What man filter paper, cotton, paper towel, spirit lamp, thermometer, conical flask.

3.1.4: Preparation of Stock Solution

Nickel Sulphate is used for the preparation of synthetic nickel water. Dissolve 4.848 gm of Nickel sulphate in water and then make it upto 1 liter this is the stock solution Ni 1000 mg/l. Then diluting this stock solution we prepared different concentrations like 100,200,400 mg/l. This stock solution is preserved at 4 degree.

3.1.5: Bacteria Culture

Organism was available in the lab which was isolated from soil and identified. It is a gram positive bacterium, identified by gram stain test which indicates that it has thick peptidoglycan layer. The thicker peptidoglyacan layer suggests that it is a good adsorbent for heavy metal adsorption. It is also confirmed by SEM analysis of staphylococcus aureus that Poly phosphate bodies are the primary cellular site for sequestering nickel. No nickel was detected in the cell wall or cytoplasm areas [40].

3.2: Experiments

3.2.1: Assimilation of organism for heavy metal tolerance

Assimilation is done to make the bacteria to become accustomed to that metal environment. So that it will be resistant to that metal. Agar plates are prepared at different nickel concentrations using nickel sulfate and nutrient agar (1g of peptone, 1g of beef and 0.5g of

NaCl in 100 ml of water). Initially bacteria is grown on 50 mg/l Ni (II) then Ni (II) conc is increased to 1000 mg/l (50,100,200,400,800 and 1000mg/l). The time is noted as per the complete growth is seen on the plate (Streaking technique is used).This assimilated organism at higher concentration is used for the kinetics study.

3.2.2: Selection of medium

Every organism has different metabolic activities, depending on that they take particular composition of medium. To check it agar plates with varying compositions of nutrients are prepared .One is PYE (Peptone, Yeast extract),second is PBE(Peptone ,beef extract) and third is Fermentor medium i.e. (Beef + Peptone and Dextrose).In different medium the organism is inoculated and incubated and observed for 24 hours and noted down % cfu.

3.2.3: Minimum inhibitory concentration (**MIC**) [31]

In microbiology the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. An MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. Nickel tolerance of isolated bacterial was determined by evaluating the growth efficiency in Ni amended nutrient broth. Stock solution (1.0 M) of nickel (as Nickel sulphate) was sterilized separately and added to the media (20 ml/ 100 ml Erlenmeyer flask) before inoculating with 0.2 ml of overnight grown bacterial culture. Growth was measured by estimating dry weight of the biomass after 2–5 days of incubation at 28–30°C. The minimum inhibitory concentration (MIC) of Ni was determined by broth dilution method (W) and the lowest concentration of metal ion, which inhibited growth of the organism, was considered as the MIC. All experiments were performed in triplicates and average result was recorded.

3.2.4: Heavy metal assay and biomass quantification

Ni (II) concentration was estimated using di-methylglyoxime (DMG) at 366 nm. Estimation of Ni (II) using DMG is a very sensitive method and it can estimate as low as 1/1000 mg. Bacterial biomass was quantified using spectrophotometer at 595 nm. The initial and the final concentration of heavy metals used in batch mode studies were calculated by estimating the concentration of metals spectrophotometrically. From the difference in concentration the

removal efficiencies of the microorganism has been calculated [41, 7].For substrate measurement estimation of protein is done using Biurete Method [42].

3.2.5: Optimization of pH on heavy metal removal

Certain organisms have ionic groups on their active sites, and these ionic groups must be in a suitable form (acid or base) to function. Variations in the pH medium result in changes in the ionic form of the active site and changes in the activity of the organisms and hence the reaction rate. Bacterial isolates were inoculated into a series of 250 ml conical flask containing100 mg L^{-1} of nickel. Initially the pH of the nickel in the medium was varied from 5 to 8 (5, 6, 6.5, 7, and 8). The pH of the medium was adjusted using dilute HCl or NaOH. The cultures were shaken in a rotary shaker (150 rpm) in a temperature controlled water bath. After reaching the equilibrium incubation time, heavy metal removal and biomass were measured. Based upon the heavy metal removal and biomass data, the optimal pH was determined.

Figure 3.1:Incubator Shaker

3.2.6: Measurement of the kinetics of broth cellular growth and heavy metal removal

To know the optimized time at where there is more cell growth and high efficiency of nickel removal this is done. Bacterial isolates were added into a 250 ml flask containing 100 mg L^{-1} of Ni (II).The flasks were mixed in a rotary shaker (150 rpm) at optimum pH and fixed temperature of 28^0C for 24 h. During the incubation period, heavy metal concentration and biomass were monitored for every 4 hours interval until heavy metal removal attains a saturation level.

To explore the tolerance of the isolates to the heavy metals, optimal culture conditions were used with varying initial heavy metal concentrations. To each freshly prepared growth medium, Ni (II) using nickel sulphate Ni (II) concentrations between (100 - 400 mg L−1). After every 4 hrs incubation, the biomass was measured. The extent of tolerance was compared i.e., biomass at each heavy metal concentration per biomass using a control.

3.2.7: Effect of inoculum volume

In order to determine the optimum volume, 6 conical flasks of 150 ml capacity containing 100 ml of media were prepared with inoculums volume of 0.5, 1, 1.5, 2, 2.5,3 ml/100ml was used to inoculate in different flasks in a incubator shaker at 150 rpm. Samples were drawn after 12 hrs of incubation period and biomass and final concentration of nickel is noted.

3.2.8: Influence of other heavy metals on the growth of bacteria

This organism is also tested whether it is growing in the other metals like, zinc, chromium, iron, Sn, Pb, etc. So nutrient agar Petri plates are prepared with different metals by adding Zinc sulphate, Potassium chromate, Lead sulfate, ferrous sulphate of 100 mg/l each . One more plate is prepared using effluent from electroplating industry. And these plates were monitored for 24 hrs and after incubation period no colonies were counted using colony counter.

3.2.9: Kinetic study of nickel degradation with secondary metabolite

As in the early experiments we observed adding secondary metabolite the removal Ni is faster compared to the degradation in the nutrient medium. So here secondary metabolite is dextrose. Take 250 ml conical flask 100 ml of Ni solution (100 mg/l) is added with peptone; beef and dextrose added 2 ml of 24 hrs culture and placed it in the shaker of 150 rpm. Samples are collected every 2 hrs and corresponding biomass and nickel degradation readings are noted. After this we will optimize the dextrose amount. Take for conical flasks of 100 ml capacity add dextrose ranging 0.25-1 ml/50 ml of solution of Ni aqueous solution in these conical 1 ml of fresh culture.

3.2.10: Degradation kinetics of Ni in the fermenter at its optimized condition

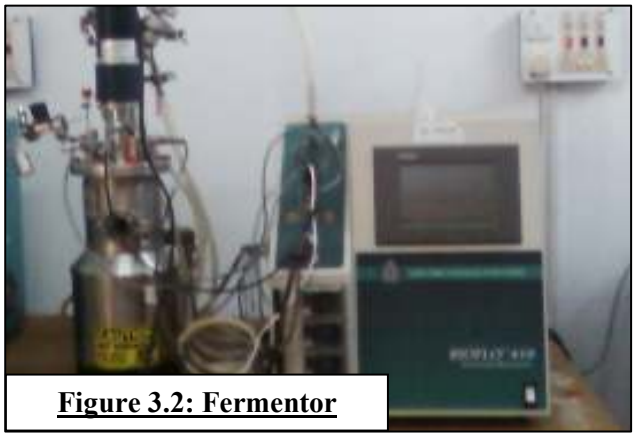

Figure 3.2: Fermentor

Ni with varying concentration 100 mg/land industrial effluent after precipitation method are studied by maintain all the optimized parameters,pH-7,2ml of inoculum,24 hrs fresh culture in 100 ml of solution in the medium 0.5 gm of beef+ 1 gm of dextrose. After every 3 hrs readings are

taken. The degradation of Nickel from Industrial effluent from electroplating industry which is collected after pretreatment is studied in the fermenter and compared with other experiments.

3.2.11: Scanning electron microscope and EDAX analysis [43]

Assessment of Morphological changes in response to nickel accumulated in bacterial strain, and quantification of chromium within bacterial strains was performed by SEM analysis of bacteria was shown at 24 hrs incubation without nickel exposure. In EDX analysis we can analysis the metal accumulation the metal and morphology of the cell.

3.3 Theoretical Considerations for the estimation of cell growth kinetics in a batch system [40].

3.3.1Cell growth kinetics in Batch system

The rate of biomass production and substrate degradation can be determined by the differential technique as follows:

$$\frac{dX}{dT} = \frac{X_{n+1} - X_{n-1}}{t_{n+1} - t_{n-1}} \qquad \frac{dS}{dt} = \frac{S_{n+1} - S_{n-1}}{t_{n+1} - t_{n-1}} \quad \text{..... (3.1)}$$

Where X=Cell mass concentration(g/l)

S=substrate concentration (g/l)

n=sampling number.

The specific growth rate (μ) of biomass may be written as:

$$\mu = \frac{1}{X}\frac{dX}{Dt} \quad \text{.....(3.2)}$$

Where; $\frac{dX}{dt}$ and μ are calculated for different experimental data

Monod kinetics for biomass growth is given by

$$\mu = \frac{\mu_{\max} S}{k_s + S} \quad \text{..... (3.3)}$$

μ=Specific growth rate (h^{-1})

μ_{max}=maximum specific growth rate (h^{-1})

k_s=saturation constant (g/l).

3.3.2 Calculation of Kinetic parameters k_s and μ_{max}

Now equ. (3.3) is modified and written as Line weaver –Burk (LB) equation:

$$\frac{1}{\mu} = \frac{k_s}{\mu_{\max} S} + \frac{1}{\mu_{\max}}$$

Line weaver-Burk plot 1/μ vs. 1/S is drawn which is a straight line.

Intercept on Y-axis= $\frac{1}{\mu_{\max}}$, Slope of the line = $\frac{k_s}{\mu_{\max}}$

Therefore, from the above, values of kinetic parameters ks and μmax are found out in a batch system.

Chapter 4

Results and discussion

4.1: Characteristics of Wastewater from different electroplating industry

Table-4.1: Characteristics of Nickel electroplating – Source Tamil Nadu (Coimbatore):

parameter	Value Before Treatment	After Treatment(using precipitation technique)
pH	1.26	6.9
TDS (mg/l)	10,266	543
Ni(mg/l)	900	214
Fe(mg/l)	32	------

Table-4.2: Characteristics of Typical Zinc electroplating (RSP, Rourkela)

Parameter	Value Before Treatment	After Treatment(using precipitation technique)
pH	1.8	6.7
Fe (mg/l)	12.6	----
Zn(mg/l)	1500	489
Cu(mg/l)	25	58
Ni(mg/l)	600	234
Cr(mg/l)	50	43

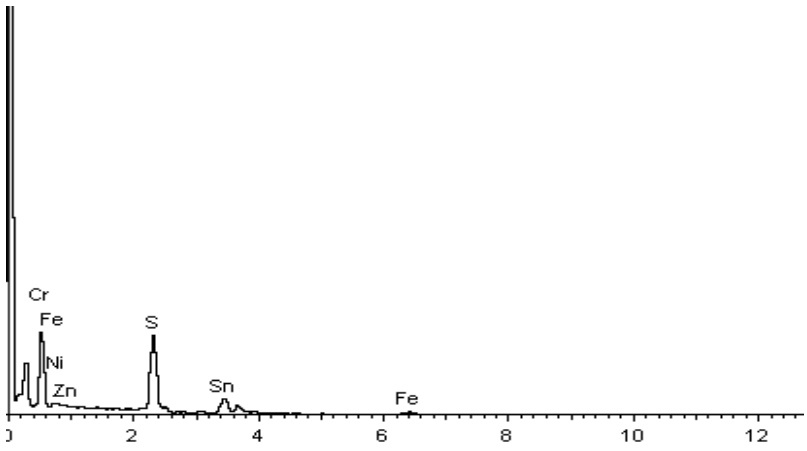

Figure 4.1: EDX analysis of electroplating waste water from RSP, Rourkela

Two different effluents are collected from different electroplating industries one is Nickel electroplating near Tamil Nadu state and other is Zinc electroplating from RSP, Rourkela in Orissa state. These are characterized and the parameters are listed above. It has been observed that Nickel concentration is very high with respect to other metals. RSP effluent also shows a significant content of Ni along with other metals. Due to lack of resistance of the organism to such high concentrations of Ni, the effluent was initially treated with alkali solution (NaOH) to precipitate some amount of Nickel and adjust the pH 6.8+/- 7. With initial treatment of the RSP effluent, the Ni concentration was reduced to 234 mg/l. It is also observed that Ni electroplating industry showed better reduction of Ni than Zinc electroplating effluent by initial precipitation technique. It could be attributed to the influence of other heavy metals such as Cr (VI) and Zn in the RSP effluent on metabolic activity of the organism. Presence of other metals such as Tin, Zinc, Chromium, Iron in the RSP effluent is also evident from EDX report [Fig4.1].

4.2: Selection of medium:

Using three different medium PBE, PYE and PBE with dextrose it is observed that Nutrient medium with dextrose is more effective for the growth of bacteria. So for further experiments the PBE with dextrose is used as the culture medium.

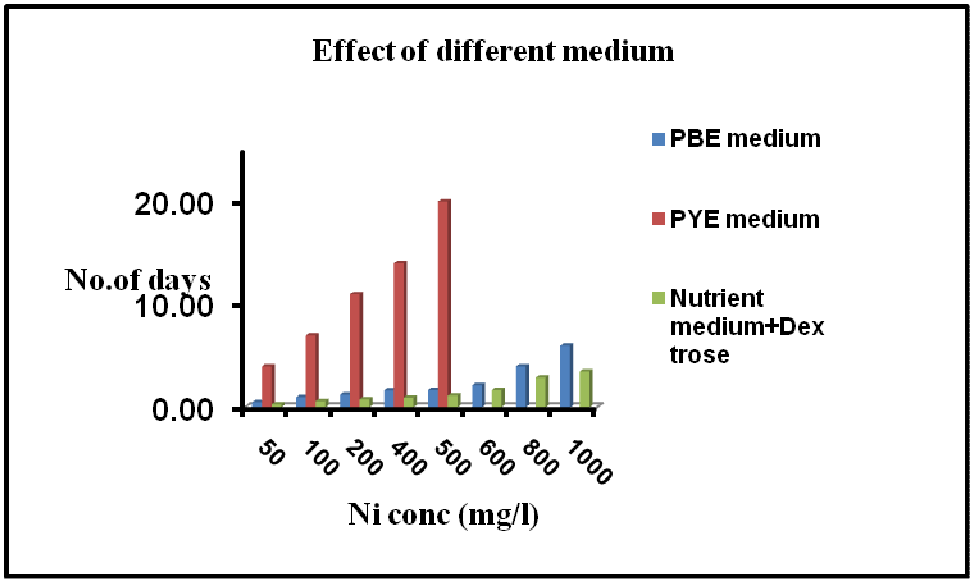

Figure 4.2: Effect of different medium on the bacteria growth.

4.3: Minimum inhibitory Concentration:

Fig 4.3 indicates that the bacteria can't grow at more than 1500 mg/l Ni concentration. It is seen that the organisms like E-Coli, A. Niger, A. Eutrophus and pseudomonas sp. have minimum inhibitory concentration 293.5,100,147,400 mg/l of nickel respectively [44]. However the present study shows that this bacterium has higher minimum inhibitory concentration than others. Hence it is understood that the organism can tolerate higher Nickel concentration.

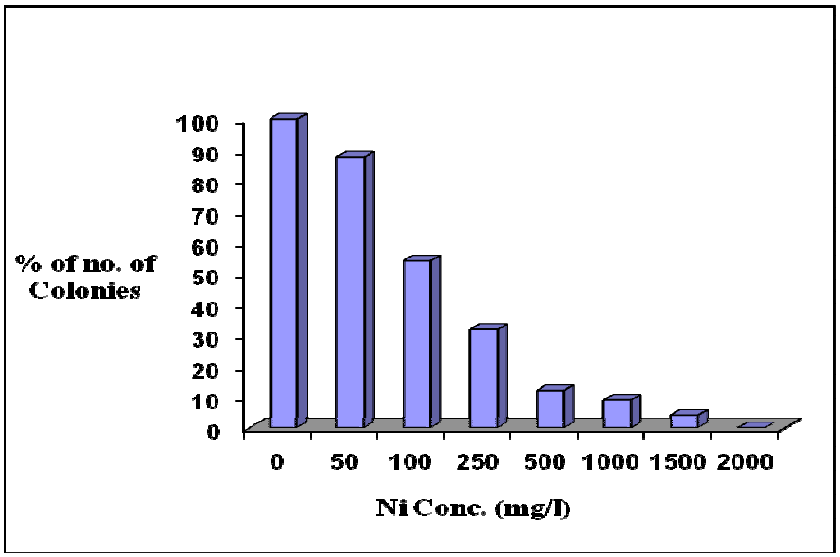

Figure 4.3: Minimum Inhibitory concentration of Ni on microbial growth.

4.4: Effect of Heavy Metal:

The promising isolates were tested for their tolerance to other heavy metals. The isolated Sp. showed high degree of resistance to different heavy metals in the order Zn>Ni> Cr>Cu>Pb>Co>Mn>Hg. It indicates that the bacterial sp. can be used to degrade the following

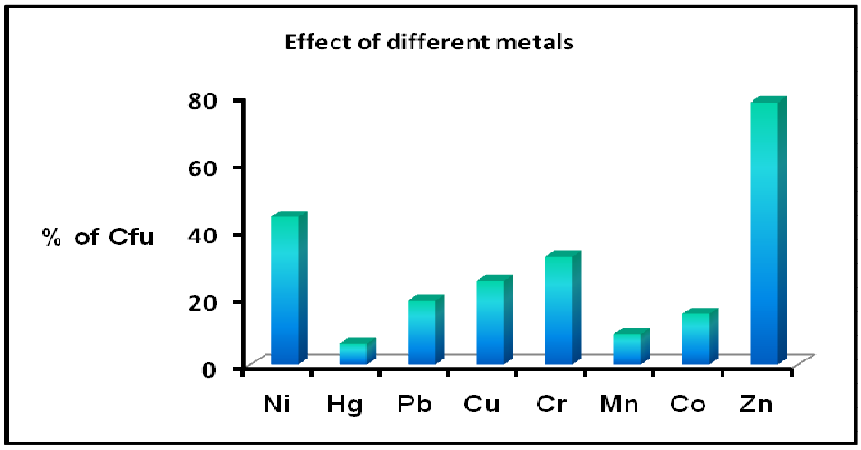

Figure 4.4: Effect on bacteria growth in different metal environments, monitored by % colony forming units Vs different metals.

metals at their optimized conditions. Zinc is a nutrient metal, hence higher resistance to Zn is observed.

4.5: Effect of pH:

pH range is studied from 5 – 8 in the interval of 0.5 . It is evident from Fig 3 that maximum removal of metal occurs at the neutral pH 7. The initial nickel concentration in the aqueous solution was maintained at 100 mg/L with varying initial pH of the solution from 5-9. The decrease in removal of Ni (II) above pH 7 is due to the formation of $Ni(OH)_2$. Substantial precipitation of nickel as nickel hydroxide occurs at high pH values. The formation of hydroxide precipitate reduces the amount of free nickel ions, which accumulates to the organism. Shankar Congeevaram et al. studied Nickel biosorption using micrococcus bacteria at initial Ni concentration 50 mg/l.and optimized maximum Ni uptake at pH 7 [35, 45]. Similar reports has been reported for Ni (II) removal using *S. cerevisiae*, where the outer cell wall consists of protein coat, which develops a charge by the dissociation of ionizable side groups of the constituent amino acids. The ionic state of ligands such as carboxyl, phosphate,

imidazole, and amino groups will promote reactions with the positively charged metal ions. At low pH, cell walls ligands were closely associated with the hydronium ions [H_3O^+] and restricted the approach of metal cat ions as a result of the repulsive forces. At low pH, some of the functional groups will be positive charged and may not interact with metal ions [46].

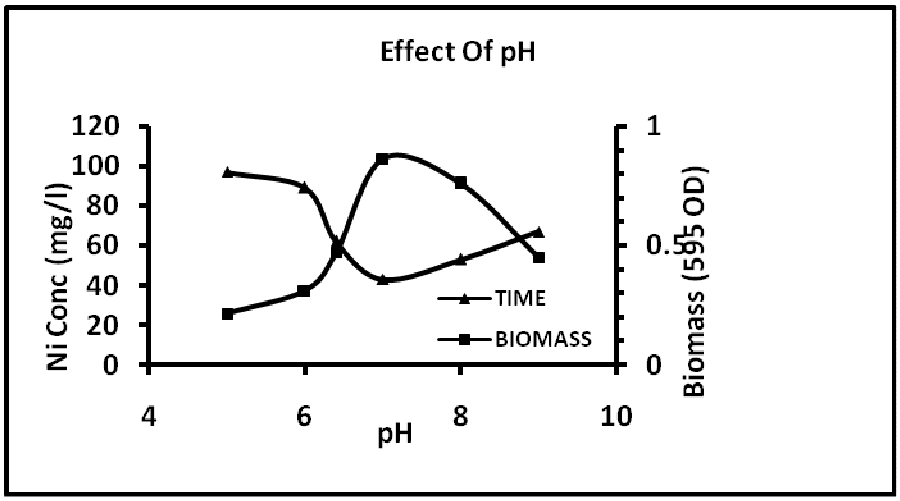

Figure 4.5: Influence of Cell growth and Ni(II) removal by bacteria at varying initial solution pH at temperature-35°C, innoculum volume 2 ml, incubation time- 12 h, concentration of Ni(II)-100 mg L⁻1, agitation 150 rpm.

4.6: Effect of Innoculum Volume:

The innoculum of the bacterial strains cultured overnight was used for this experiment. Culture flasks (150 ml) with a final volume of 100ml supplemented with (100mg/L) of Ni were inoculated with different innoculum volumes ranging from (.5- 3 ml/100 ml of feed) . After 12-hrs maximum degradation was seen with 2 ml of inoculums' volume. However no significant reduction of Nickel & cell growth by the organism was observed by increasing the volume of the innoculum. Hence the optimum volume of the innoculum was considered as 2 ml/100 ml solution. Several researchers also reported increase in Ni removal with increasing innoculum volume due to increase in sites for metal degradation. Each cell has a limit in the intake of metal, above which it can't resist with favorable environmental conditions. So by adding more cells to the effluent will cannot reduce nickel efficiently. Above certain limit as increase in inoculums size may affect other parameters like pH, temp etc which doesn't support the high efficiency of nickel reduction.

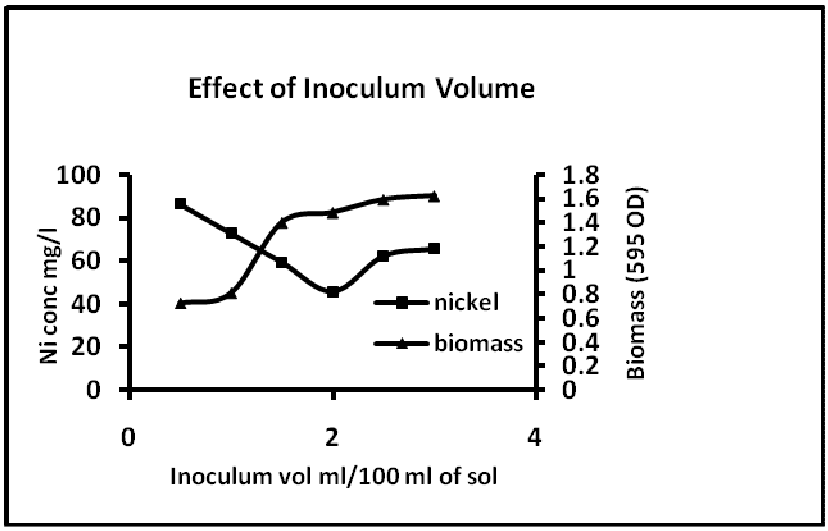

Figure 4.6: Influence of innoculum volume on Cell growth and Ni (II) removal by bacteria at temperature- 35◦C, incubation time-12 h, agitation 150 rpm ,concentration of Ni(II)-100 mg L⁻1.

4.7: Effect of varying nickel concentration on nickel degradation kinetics:

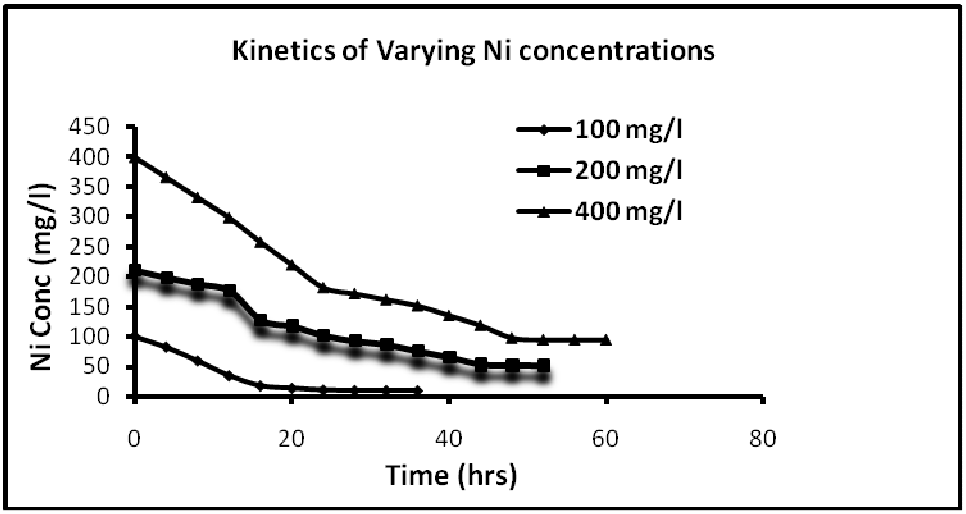

Figure 4.7: Kinetics of Ni(II) removal by bacteria at varying Ni(II) concentrations at pH 7, temperature- 30 ◦C, innoculum volume 2ml, agitation 150 rpm

The nickel Ni(II) reduction ability of the bacteria was growth dependent & the *Staphylococcus* sp. reduces nearly 400mg/L of Ni(II) during degradation experiment in 60-h [Fig 5]. It is evident from the experiment that the time required for Ni(II) degradation varies

with initial nickel concentration. The time required for nickel degradation increased with initial nickel concentration. But the rate of nickel removal showed little change at 200 mg/L nickel concentration. We can also find three different stages of degradation with initial rapid stage followed by slow rate and finally degrades at a till slower rate. Initial rapid degradation is observed within 20 hrs for varying nickel concentration. The next stage is negligible at lower metal concentration 50 mg/L. At lower nickel concentration equilibrium is achieved within 20 hours with 100% removal. The equilibrium values for initial nickel concentrations of 200 mg/L and 400 mg/L are 47 mg/L and 97 mg/L respectively. Hence we can see that maximum reduction is observed within 20 hrs at initial nickel concentration 400mg/L. This is attributed to high concentration gradient of nickel in the solution which is the main driving force for nickel degradation.

4.8: Effect of cell growth with different Initial Nickel concentration:

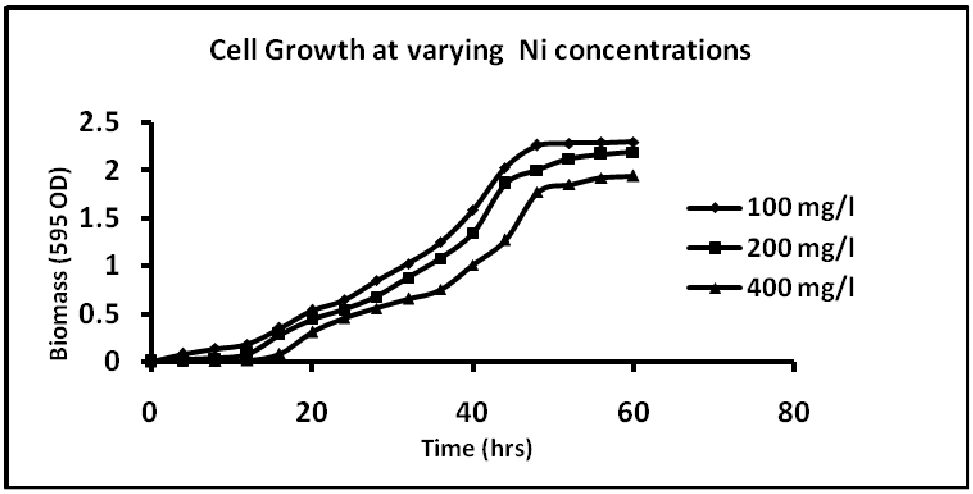

Figure 4.8: Kinetics of cell growth by bacterial sp. at varying Ni(II)concentration, pH 7, temperature-30 ◦C, agitation 150 rpm.

The innoculum of the bacterial strains cultured overnight was used for this experiment. Culture flasks (150 ml) with a final volume of 100ml supplemented with (100-400mg/L) of Ni were inoculated with 2ml of inoculums for 24 hour. The growth kinetics of bacteria is characterized as initial lag phase, second exponential phase, stationary phase and death phase. In this experiment it is observed that lag phase is increasing with increased initial Ni

concentration [Fig 4.8]. It is basically due to inhibitory effect of higher Ni concentration on the growth of the organism. Each organism has a specific resistance at a specified growth condition. As the initial age of the innoculum was fixed at 24 hours the acclimatization period at varying Ni concentration will not remain same. Hence the following behavior is observed. The Ni-resistant bacteria isolate exhibited reduced bioaccumulation when cells were in stationary phase. At higher concentrations the growth of the bacteria is inhibited due to fixed amount of innoculum for all the different concentration of Ni considered in the experiment.

4.9: Effect of Secondary Metabolite on Nickel reduction:

Two different types of media were used for this experiment. The first one is composed of BPE medium (peptone beef extract) & the second one was composed of BPE with dextrose supplemented as a secondary metabolite. It is seen that the Nickel reduction was higher in medium containing secondary metabolite, which suggests that dextrose support rapid growth and high cell yields that enhances Ni degradation. The dextrose level optimized for this experiment was 1gm/100 ml of the media. There is no significant Ni reduction with further increased dextrose level. As the concentration of secondary metabolite increased,

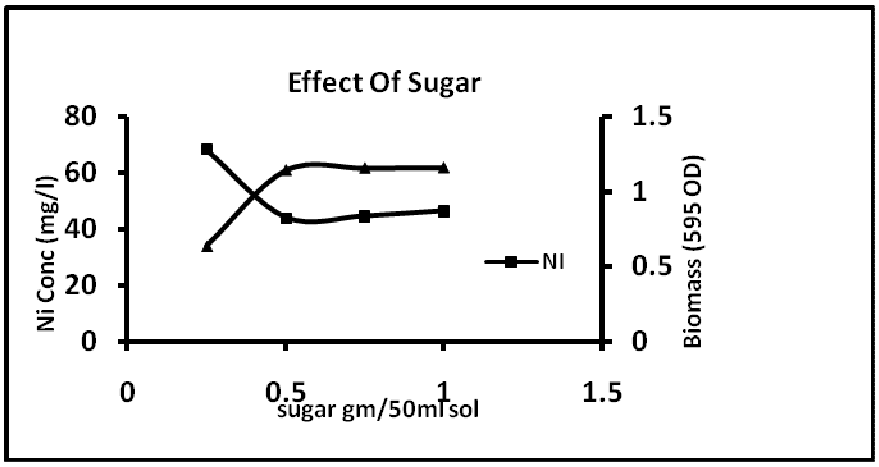

Figure 4.9: Influence of varying sugar amount on Cell growth and Ni(II) removal by Bacteria at temperature-35 ◦C, incubation time-12 h, agitation 150 rpm, concentration of Ni(II)-100 mg L¯1.

the effective Ni(II) reduction was also increased to certain extent afterwards it remained constant. There was no substrate inhibition observed. Initially the system followed 1st order kinetics with respect to substrate concentration gradually it turned to zero order as the

concentration of sugar increased. The same trend is seen in the work [47] with chromium reduction.

4.10: Fermenter experiments:

Two liters of medium were inoculated with fresh cultures of strain. The medium used in fermented was Dextrose (10 g/l) and beef extract (5 g/l).Cultivation was carried out in a fermenter (New Brunswick Scientific BIOFLO *410) at 30^0 C for 24 h under these following conditions: agitation 150 rpm and no control of pH. It is observed that the reduction was much faster in these optimized conditions compared to earlier experiments. Two different sample water one industrial effluent whose nickel concentration is reduced to 100 mg/l using precipitation technique and another 100 mg/l only Ni in its aqueous solution is considered. It is observed that there was less reduction of nickel due to inhibition of other metals in the RSP effluent,

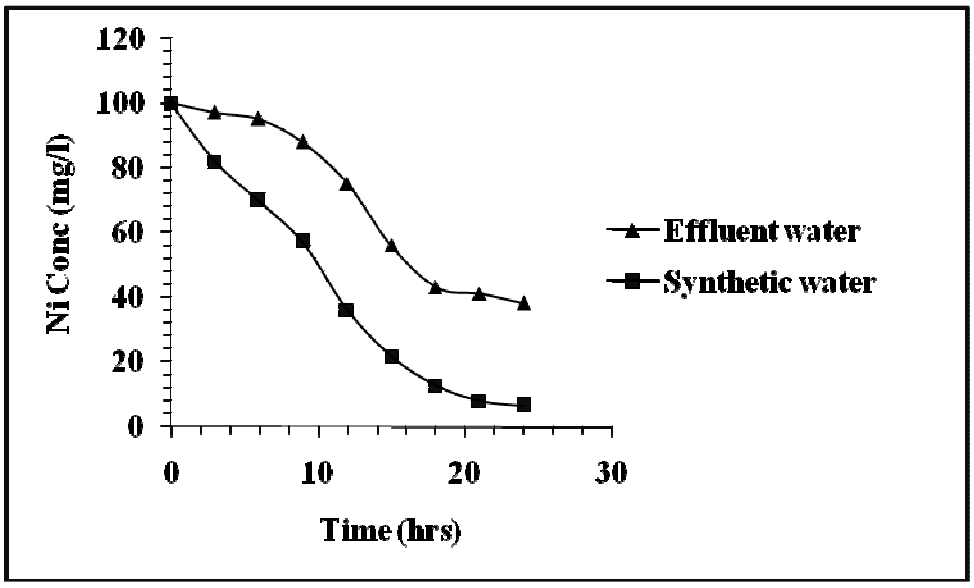

Figure 4.10: Kinetics with optimized conditions study of nickel water and industrial effluent.

Nickel in the effluent reduced to 37 mg/l in 24 hrs where as nickel degradation was faster in its aqueous solution as it degraded to 7 mg/l in 24 hours (Fig-4.10). So we used secondary treatment of the effluent in order to bring it to below permissible limit 3 mg/L.

As the industrial effluent did not reach below permissible limit so we used secondary treatment and transferred the effluent to another batch reactor under the same optimized

condition. The effluent containing Ni(II) in the second batch reactor was reduced from 38 mg/l to 3mg/l within 15 hours which is below permissible limit[fig-4.11].

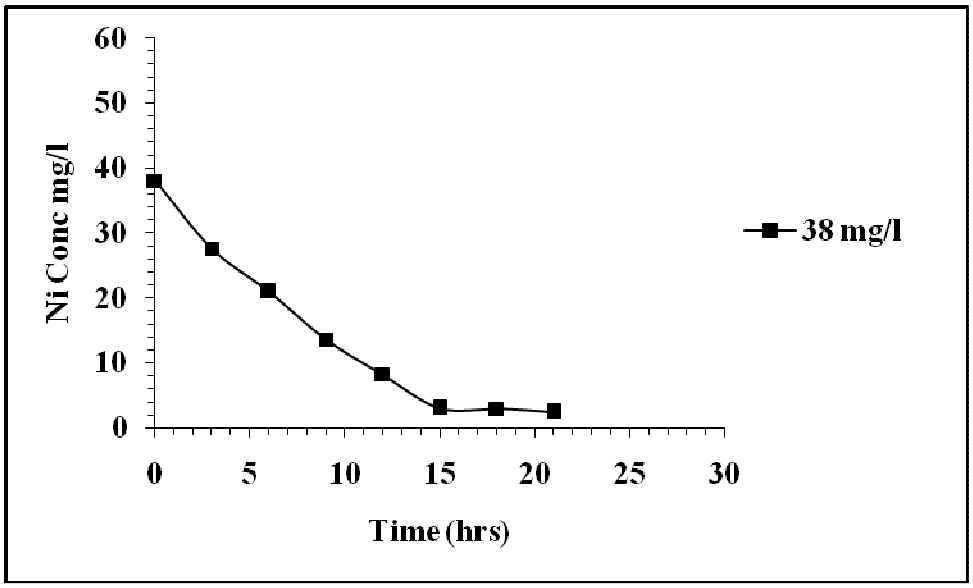

Figure 4.11: Secondary treatment for the industrial effluent at optimized conditions to bring down to below permissible limit.

EDAX Analysis:

Before Nickel treatment:

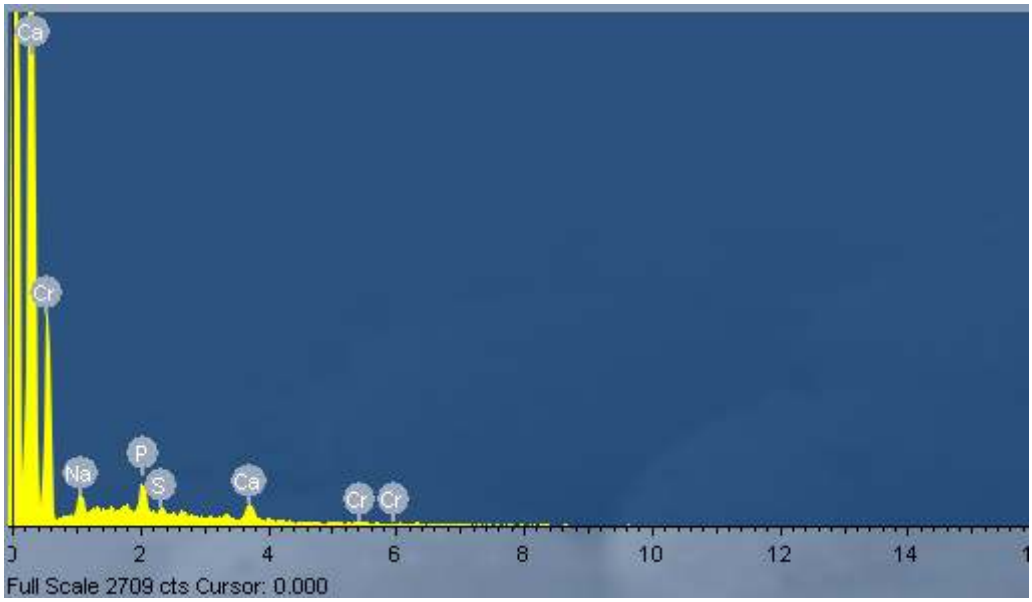

Figure 4.12: This is the metal analysis of bacteria before nickel treatment, so there is no nickel found.

After Nickel treatment:

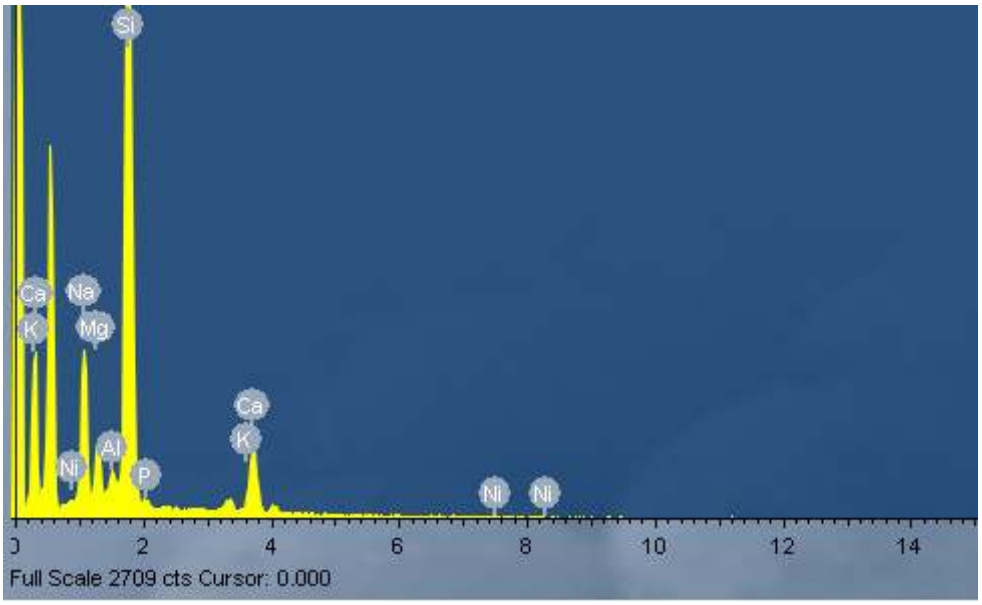

Figure 4.13: When the organism is grown in the nickel medium and analyzed.

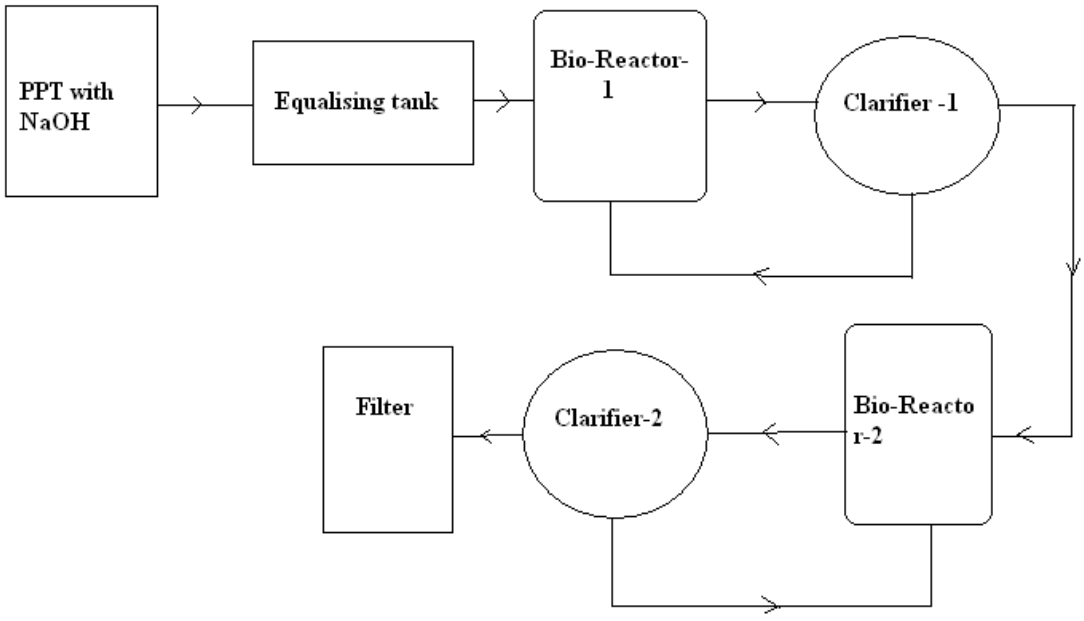

Figure 4.14: Flow chart of design of wastewater treatment

4.11: Growth kinetic constants:

Cell growth kinetics was calculated from Line-weaver-burk model equation using the experimental data.

Table-4.3: Evaluated values of maximum specific growth rate and product inhibition constant

Ni mg/l	k_s (mg/l)	μ_{max} (h^{-1})
50	0.0138	2.625
100	0.01047	2.6178
200	0.0144	2.88

Chapter 5

Conclusion

1. The bacterial species isolated in the lab has a potential to degrade Ni(II) in the aqueous solution.
2. Significant colonies of the pure bacterium could be observed using Beef extract with dextrose as secondary metabolite as the culture medium compared to PYE and PBE.
3. Minimum inhibitory concentration of the bacteria for Ni(II) was observed as 1500 mg/L
4. Maximum degradation took place at optimized condition such as solution pH 7, temperature 30± 2 ^{o}C, 2 ml innoculum volume within 12 hours retention time.
5. Ni(II) degradation improved with lower initial Ni(II) concentration that signifies the inhibitory effect of the organism to higher Ni(II) concentrations.
6. Rate of degradation was faster at higher initial Ni(II) concentration due to increase in the driving force which is the concentration gradient of Ni(II) in the solution.
7. Rate of Ni(II) degradation was lowered in the presence of other metals such as Cr(VI), Zn(II) etc.
8. The organism shows higher degree of resistance to Zn followed by Ni and Cr.
9. The organism exhibits potential to reduce Ni (II) from the electroplating waste water to its permissible limits under its optimized condition.
10. The data obtained at different Ni (II) concentration were verified using Linewever – Burk model equation and K_s and μ_{max} values were estimated.

Appendix

Tables

Table-7.1: Effect of medium:

Ni concentration mg/l	PYE	PBE	PBE+Dextrose
50	4	0.5	0.3
100	7	1	0.6
200	11	1.25	0.8
400	14	1.667	0.95
500	20	1.7	1.15
600		2.166	1.68
800		4	2.9
1000		6	3.5

Table-7.2: Minimum Inhibitory Concentration

Nickel Conc Mg/l	0	50	100	250	500	1000	1500	2000
% cfu	100	88	54	32	12	9	4	0

Table-7.3: Effect of Different metals:

Metal	Ni	Hg	Pb	Cu	Cr	Mn	Co	Zn
% cfu	44	6	19	25	32	9	15	78

Table-7.4: Effect of pH on Nickel degradation and Cell growth

pH	5	6	6.4	7	8	9
TIME	97	89	62	43	59	74
BIOMASS	0.216	0.3116	0.4708	0.8631	0.758	0.5123

Table-7.5: Effect of inoculums Volume for the degradation of nickel:

Inoculum size	Biomass (O.D-595)	Nickel conc. (mg/l)
0.5	0.7297	86.5
1	0.8130	73
1.5	1.4015	59.5
2	1.4847	46
2.5	1.5927	62.6
3	1.6233	65.8

Table-7.6: Kinetics of Nickel degradation at different initial concentration

	Nickel concentration diff conc			Biomass growth (OD 595)		
Time(hrs)	**100 mg/l**	**200 mg/l**	**400 mg/l**	**100 mg/l**	**200 mg/l**	**400 mg/l**
0	100	210	400	0	0	0
4	82	198	366.34	0.0783	0.0023	0.02
8	59	187.4	332.68	0.1346	0.0569	0.169
12	34	176	299	0.1813	0.1108	0.318
16	17	127	258	0.345	0.1621	0.4501
20	13	119	220	0.5316	0.307	0.8251
24	10	111	182	0.6436	0.4532	1.2016
28	9.2	109	172	0.8453	0.5532	1.3106
32	9.1	107	162	1.023	0.6532	1.4196
36	8.9	105	152	1.2463	0.7518	1.5303
40		103	135.5	1.589	1.01015	1.696
44		101	119	2.031	1.2685	1.8617
48		99	98	2.261	1.77	2.004
52		86	95	2.282	1.85	2.12
56			95	2.293	1.92	2.17
60			94.8	2.299	1.92	2.19

Table-7.7: Effect Of Dextrose on Nickel degradation

Sugar	0.25	0.5	0.75	1
Biomass	0.6335	1.1412	0.705	0.727
Ni conc. Mg/l	68	44	62.5	61.5

Table-7.8: Effect of Nickel degradation With Addition of secondary metabolite:

	Nickel concentration mg/l			Cell growth (OD 595)		
Time (hrs)	50	100 mg/l	200 mg/l	50	100 mg/l	200 mg/l
0	50	100	200	0	0	0
4	38.2	78.4	184.133	0.023	0.001	0.0003
8	32.02	54.6	168.267	0.3460	0.260	0.216
12	15.77	31.77	152.4	0.8672	0.6324	0.4364
16	11.76	12.02	104.8	1.2451	0.9462	0.851
20	4.88	8.43	97.3	1.3241	1.1321	0.9986
24	4.02	7.56	96.4	1.3632	1.2462	1.112

Table-7.9: μ_{max} and k_s calculations

Nickel 50 mg/l		Nickel 100 mg/l		Nickel 200 mg/l	
1/μ	1/S	1/μ	1/S	1/μ	1/S
0	0.3537	0	0.3578	0	0.3611
5.9988	0.4435	5.998	0.4154	5.0845	0.3879

9.98	0.5333	12.5	0.432	11.8802	0.43135
19.763	0.632	27.68	0.545	32.37	0.5372
30.45	0.7263	43.789	0.613	46.015	0.5991

Table -7.10: Fermentor Experiment:

Time	Industrial Effluent	100 mg/l	38 mg/l
0	100	100	38
3	97	81.76	27.6
6	95	69.67	21
9	88	57	13.6
12	75	36	8.2
15	56	21.6	3.02
18	43	12.48	2.89
21	41	7.867	2.43
24	38	6.45	

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