International Journal of Innovative Research and Advanced Studies (IJIRAS)
Volume 4 Issue 6, June 2017

ISSN: 2394-4404

Isolation And Characterization Of Heavy Metal Resistant Bacteria From Industrial Effluent, Influencing The Metal Degradation

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Abstract: ***Industrial development have lead to the recognition and increasing understanding of interrelation between pollution, public health and environment. Industries result in generation of industrial effluent, and if untreated result in water, sediment and soil pollution. Metal remediation through common modern instrumental techniques is expensive and unsuitable in case of voluminous effluents containing complexing organic matter and low metal contamination. Biotechnological technological approaches that are designed to cover such niches have dominated the literature and, subsequently, extensive reviews focusing on equilibrium and kinetics of metal biosorption have also come up. The purpose of this study deals with isolation of heavy metal resistant bacteria from industrial effluent which influences the metal degradation. Determination of pH, BOD, COD, is done to know how long contamination is present in the effluent. Degradation of certain toxic metal present in the industrial effluent eg. Copper, Nickel, Cadium, Lead, zinc etc, done by particular Bacillus spp, Pseudomonas spp, Klebsiella spp, Staphylococcus spp. These identification is done by biochemical characterization of various test. Recent studies shows that the isolation of heavy metal resistant bacteria from the industrial effluent, these strains are tolerant to various metal.***

Keywords: ***BOD, COD, Copper, Cadium, Nickel, Lead, pH, Zinc, Bacillus spp, Klebsiella spp, Pseudomonas spp, Staphylococcus spp.***

I. INTRODUCTION

Heavy metals from industrial process are of special concern because, they produce water of chronic poisoning in aquatic animals (Ellis., 1989). While some heavy metals are purely toxic with no cellular role (Shi et al., 2002), other metals are essential for life at low concentration but become toxic high concentration, high concentration of all heavy inhibits activity of sensitive enzymes (Koropatnick and Leibbrandt., 1995). Heavy metal can damage the cell membrane, alter enzyme specificity, distrupt cellular function and damage the structure of the DNA (Anushree Malik., 2004),. The toxic effect of Arsenic, Mercury and Lead were known to be ancient, but methodical studies of the toxicity of some heavy metal appear to date from only 1868 (Verma et al., 2001) in human, heavy metal poisoning is generally treated by the administration of chelating agents (Vinay kumar et al., 2013) (Shi W., Becker et al., 2006) some elements otherwise regarded as toxic metals are essential in small quantities (Edward Raja., 2000).

The toxic heavy metal are relatively dense metal or metalloid that is noted for its potential toxicity, especially in environmental context (Volesky B., 1987). The term has particular application to Cadium, Mercury, Lead, and Arsenic all of which appear in the world health organization examples include Manganese, Zinc, Selenium, Silver, Antimony and Thallium (Edward Raja et al., 2000) Uncontrolled discharges of large quantity of heavy metals containing waste create huge economic and health care burden (Sundaramoorthi et al., 2006) (Olukanni et al.,2015). Particularly for the people living near that area (Gadd., 1988). The toxic metal pollutant like Lead, Nickel and Cadium enter to the water bodies through industrial waste water (Fakayode., 2005). Among the heavy metal, Lead is a nonessential heavy metal and general toxicant (Merina Paul Das., and Neha Kumari., 2016),. The toxicity of these heavy metal occur through the displacement of essential

metal from their native binding site or through ligand interaction (Rajaram T., and Ashutost D., 2008) (Manisha Nanda et al 2011). The toxicity can occur as a alteration in the confirmational structure of the nucleic acid and protein and interference with oxidative phosphorylation and osmotic balance (Martin et al., 2006) (Holt et al 1994),. In the biosorption mechanisms, the complex structure of microorganisms implies that there many ways for the metal to be taken up by the microbial cell (Olukanni et al., 2015). The biosorption mechanisms are various, they may be classified according to the dependence on the cell metabolism, biosorption mechanism can be divided into: Metabolism dependent and non –metabolism dependent. According to the location where the metal removed from the solution is found biosorption can be classified as (1)Extra cellular accumulation /precipitation(2) Cell surface sorption /precipitation: and (3) intracellular accumulation (Narasimhulu et al 2009), Heavy metals are not biodegradable and tend to be accumulated in organism and because of numerous disease and disorder (Ruchita Dixit et al.,2015)

To survive under metal -stressed condition, bacteria have evolved several types of mechanism to tolerate the uptake of heavy metal ions (Ogunfowokan et al 2005) (Nieto et al., 1987). These metal mechanism include the efflux of ions outside the cell, accumulation and complexation of metal ions inside the cell (Olukanni et al., 2015). The objective of this work is to isolate the metal resistant bacteria from the industrial effluent by the determination of physio-chemical properties of the effluent and the biochemical characterization of the metal resistant bacteria.

II. MATERIALS AND METHODS

A. SAMPLE COLLECTION

Sample were collected from the zone of industry in Tamil nadu, from different places from the industrial effluent were in the untreated form. Samples were collected in 500ml watercan and stored in refridgerator. Samples were named as RX1, RX2, RX3, RX4, RX5 for identification Parameters have to be checked within 24 hours.

B. DETEMINATION OF pH OF THE INDUSTRIAL EFFLUENT SAMPLE

pH of the sample were checked using pH meter to know at what pH the sample is present and the metal resistant bacteria survive.

C. DETEMINATION OF BOD OF THE INDUSTRIAL EFFLUENT SAMPLE

BOD -Biological oxygen demand is a measurement of the amount of demand oxygen that is used by aerobic micro organisms when decomposing organic matter in water

MEASUREMENT OF BOD

Sample is first unanalysed and conditioned to ensure favourable growth condition for the bacteria, which include adjustment of pH, neutralization of residual chlorine and reduction of DO in supersaturated samples.

Sample is then diluted and the appropriate amount of seed bacteria added.

Initial dissolved oxygen content is recorded and the sample is then incubated for 5 days at 20°C

After the 5 days period. sample is removed from the incubator and the final dissolved oxygen reading is taken.

BOD is calculated from the DO depletion and the volume of sample used follows the formula

BOD_5 = BOD mg/l=[(IDO-DO_5)-Seed correction]x dilution factor

D. DETERMINATION OF COD OF THE INDUSTRIAL EFFLUENT SAMPLE

COD-chemical oxygen demand analysis, this method involve using a strong oxidizing chemical, potassium dichromate, to oxidize organic matter in solution to carbon dioxide and water under acidic condition.

Often, the test also involve a silver compound to encourage oxidation of certain organic compound and mercury to reduce the interference from oxidation of chloride ions.

The sample is then digested for approximately 2 hours at 150 °C.

The amount of oxygen required is calculated from the quantity of chemical oxidant consumed.

E. PREPARATION OF NUTRIENT AGAR PLATE FOR THE ISOLATION OF METAL RESISTANT BACTERIA

Samples collected from the industry were serially diluted upto 10^{-8} dilution Nutrient agar plates with metal consistency that help in the growth of metal resistant bacteria.

F. INOCULATION AND INCUBATION

Dilution of theSamples from RX1-RX5 were inoculated into nutrient agar plates by streak plate methods

Plates were kept for incubation at 37°C for 24 hours in an inverted position.

After incubation the plate was observed, the colonies were picked and purified by streaking method. The colonies were named as ST1,ST2,ST3,ST4,ST5

G. PREPARATION OF PURE CULTURE

Freshly prepared nutrient agar plates were inoculated with the colonies ST1,ST2,ST3,ST4,ST5

The inoculated plates were kept for incubation at 37°C for 24 hours.

The plates were labelled properly.

H. BIOCHEMICAL CHARACTERISTICS OF METAL RESISTANT BACTERIA

Indole test, MR test, VP test, Citrate test, Nitrate reduction test, Urease test, Catalase test, Oxidase test were performed to know the characterization of particular bacteria.

III. RESULT AND DISCUSSION

PHYSIO-CHEMICAL PARAMETERS OF EFFLUENT SAMPLE

DETERMINATION OF PH OF THE INDUSTRIAL EFFLUENT SAMPLE

ph of the effluent sample were checked using pH meter and it was observed, the values was shown in Table.1 and Figure.1. pH of the sample which has been checked it was ranged between 6.3-7.4

S. No	Sample	Ph
1	RX1	6.3
2	RX2	7.1
3	RX3	6.8
4	RX4	6.5
5	RX5	7.4

Table 1: pH of the industrial effluent sample

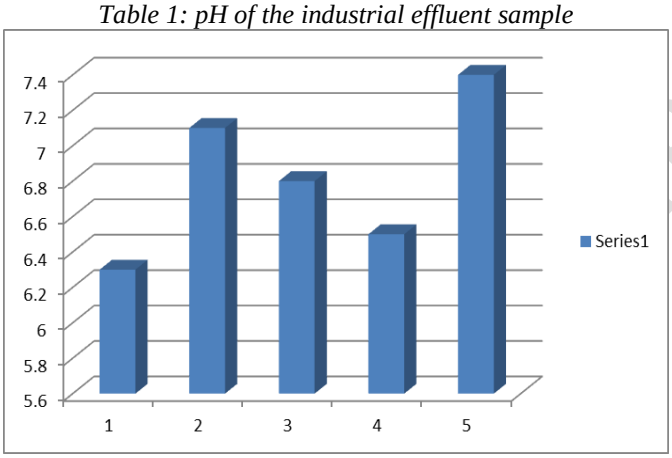

Figure1: pH of the industrial effluent sample

DETERMINATION OF BOD OF THE INDUSTRIAL EFFLUENT SAMPLE

BOD of the sample was taken and it was determined and the values was shown in Table.3 and Figure.3

BOD of the sample which has been checked it was ranged between 245-415 mg/l

S.no	Sample	BOD (mg/l)
1	RX1	325
2	RX2	245
3	RX3	415
4	RX4	335
5	RX5	364

Table 2: BOD of the industrial effluent sample

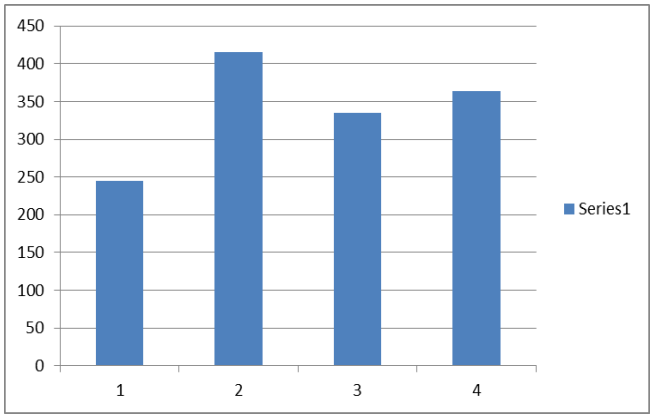

Figure 2: BOD of the industrial effluent sample

DETERMINATION OF COD OF THE INDUSTRIAL EFFLUENT SAMPLE

COD of the sample was taken and it was determined and the values was shown in Table.3 and Figure.3 COD of the sample which has been checked it was ranged between 556-850 mg/l.

S.no	Sample	COD (mg/l)
1	RX1	645
2	RX2	556
3	RX3	734
4	RX4	667
5	RX5	850

Table 3: COD of the industrial effluent sample

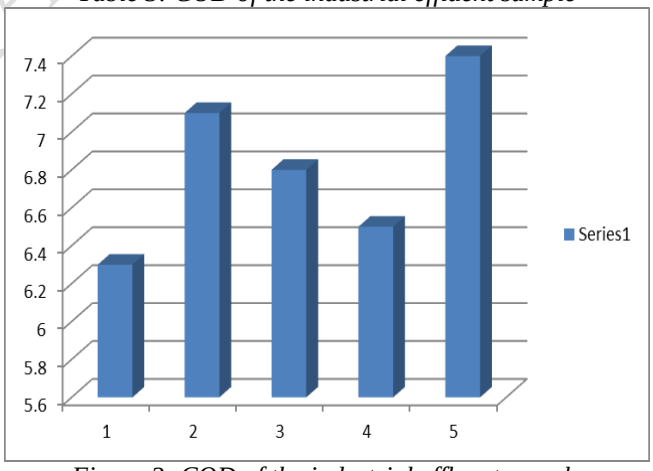

Figure 3: COD of the industrial effluent sample

BIOCHEMICAL CHARACTERISTICS OF THE METAL RESISTANT BACTERIA

Identification of metal resistant bacteria using biochemical characteristics colonies from the nutrient agar plates supplied with metal, after preparation of pure culture were characterized according to Bergy's manual of bacteriological classification. Identification of metal resistant bacteria using biochemical characterization is shown in Table.4

S. no	Indole Test	MR Test	VP Test	Citrate utilization Test	Urease Test	Nitrate test	Catalase Test	Oxidase test	Identified bacteria
1	–	–	+	+	–	+	+	+	*Pseudomonas spp.*

2	–	+	–	+	–	+	+	+	*Staphylococcus spp.*
3	+	+	–	–	+	+	–	–	*Bacillus spp.*
4	–	–	+	+	+	–	+	–	*Klebsiella spp.*

Table 4: Biochemical characteristic of metal resistant resistant bacteria present in the industrial effluent sample

IV. CONCLUSION

From the present study, five samples which have been taken from the industrial effluent were checked and analysed that *Pseudomonas spp, Staphylococcus spp, Bacillus spp, Klebsiella spp* were present in the industrial effluent sample. It was seem to live under metal environment and be resistant. It was concluded that based on the pH, BOD, COD analysis of the sample were taken. Ph of the sample which has been checked it was ranged between 6.3-7.4. BOD of the sample which has been checked it was ranged between 245-415 mg/l. COD of the sample which has been checked it was ranged between 556-850 mg/l. Biochemical characterization of isolated samples were done and further test concluded that *Pseudomonas spp, Staphylococcus spp, Bacillus spp, Klebsiella spp present* in the sample. This study further leads to removal of heavy metal by their metal resistant bacteria present in the industrial effluent.

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