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Research Article – Bioremediation

Bioremediation of heavy metals from distilleries effluent using microbes

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Abstract

One of the major universal threats is water pollution. The untreated distilleries effluent discharge into the environment creates a serious problem to the living systems. It may contain carcinogenic aromatics amines, dyes, organic and inorganic chemical compounds. The physicochemical characterization of effluent was collected from Trichy Distilleries & Chemicals pvt Ltd, Tiruchirappalli, Tamilnadu, India and the chemical analyzed in Soil Testing Laboratory, Tiruchirappalli. The effluent was analyzed by different parameters. The physical parameters are BOD, COD, and DO, TDS, pH and electrical conductivity. Heavy metal analysis included Zinc, Lead, Manganese, Arsenic, Iron, Mercury, Copper, Chromium, Cadmium and Nickel. The soil sample was taken from Trichy Distilleries and Chemicals Ltd and analyzed by serial dilution technique. The three microbes such as *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas putida*, were isolated from soil source. The isolated organism was treated with the effluent for various concentrations in various hours. The metal accumulations were identified in soil test lab, Trichy. The hyper accumulations of metal in microbes were identified. The present study explores the effectiveness of bioremediation has a simultaneous removal of Zinc, Lead, Manganese, Arsenic, Iron, Mercury, Copper, Chromium, Cadmium and Nickel from the distilleries effluent. *Bacillus subtilis* bacterial strains effectively remove the heavy metal from the distilleries effluent.

Key words: Distilleries effluent, Bioremediation, Heavy metal, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas putida*.

Introduction

Distilleries industries are renowned in Trichy for chemical production. During this process the highly liquor is generated. Low pH value, high organic materials, depletion of oxygen content, bad aromas, it may leads to hepatic and renal system damages, mental retardation and degradation of basal ganglia of brain (FEPA 1991; Emongor et al., 2005) etc. hence it should not allowed to discharge into the water body. There are many conventional physicochemical methods are available for the removal of heavy methods. But all these methods are highly expensive and need skilled technicians. Hence bioremediation is proved as an effective eco-friendly affordable technology for the removal of heavy metal from the distillery effluent.

Bio-remediation can be defined as the process of using specific microorganisms to transform hazardous contaminants in soil/water to nonhazardous waste products. However, some definitions that give a broader outlook define bio-remediation as biological treatment systems to destroy, or reduce the concentration of hazardous waste from a contaminated site (Banat *et al*, 1996; Singh *et al*, 2004; Mendez – Paz *et al*, 2005; Pandey *et al*, 2007). The use of natural microorganisms found in soil, water, and sludge pioneered the field of bioremediation (Valls *et al*, 1998). Microorganisms have evolved coping strategies to either transform the element to a less-harmful form or bind the metal intra- or extracellular, thereby preventing any harmful interactions in the host cell. Plus, they are able to actively transport the metal out of the cell cytosol

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(Hamlett *et al*, 1992; Mowll and Gadd, 1984; White and Gadd, 1998).

At high concentrations, metal ions can either completely inhibit the microbial population by inhibiting their various metabolic activities or organisms can develop resistance or tolerance to the elevated levels of metals. Unlike many other pollutants, metals can undergo biodegradation and produce less toxic, less mobile and less bio-available products, heavy metals are difficult to be removed from contaminated environment. These metals cannot be degraded biologically, and are ultimately everlasting, though the speciation and bioavailability of metals may change with variation in the environmental factors. Some metals such as, Zinc, Copper, Nickel and Chromium are essential or beneficial micronutrients for plants, animals and microorganisms (Olson *et al*, 2001) while others (e.g., Cadmium, Mercury and Lead) have no known biological and/or physiological functions (Gadd, 1992). However, the higher concentration of these metals has great effects on the microbial communities in soils in several ways- it may lead to a reduction of total microbial biomass (Giller *et al* (1998). It decreases numbers of specific populations or it may change microbial community structure (Gray and Smith, 2005; Hugo Ribeiro *et al*, 2013). Thus, at high concentrations, metal ions can either completely inhibit the microbial population by inhibiting their various metabolic activities like protein denaturation, inhibition of cell division, cell membrane disruption etc. or organisms can develop resistance or tolerance to the elevated levels of metals. In this research work the various properties and metal analysis can be processed by using microorganism such as *E. coli, B. subtilis* and *P. putida.*

Materials and Methods

Effluent collection sites

The effluent was collected from Trichy Distilleries and Chemicals Ltd, Tiruchirappalli, Tamilnadu, India. The collected effluents were stored in plastic cane for our treatment process.

Effluent analysis

The effluent physiochemical parameters were analyzed in Soil Testing Laboratory, Tiruchirappalli. The effluent were analyzed by two different parameters, the physical parameters are BOD, COD, DO, TDS, pH and Electrical conductivity and the heavy metal analysis are Zinc, Copper, Chromium, Cadmium and Nickel. And the sample was analyzed under different parameters there are FTIR, UV-Visible Spectrum.

Isolation of bacteria from Effluent soil

The soil samples are collected from the different areas. The soil sample were collected in the bottle and tightly locked. The microbial populations from the effluent were isolated by viable plate count method (Serial dilution method).

Serial dilution technique

One gram of effluent was taken and mixed with 9ml of sterile distilled water (10^{-1}). With a sterile pipette, 1ml of suspension was transferred to the second tube. Mix the tube well and with the help of pipette 1ml sample from the first dilution tube was mixed to the next tube to make the dilution to be 10^{-2}. This was continued for up to 10^{-4} dilutions. 1ml of 10^{-3} to 10^{-4} dilutions was taken using sterile pipettes and delivered to petridishes containing Nutrient agar medium (Harley Prescott, 2002). Through this technique we isolate the pure culture. Using biochemical parameters we identified the microbes from theses test. We select three microbial species for our studies namely *Escherichia coli, Bacillus subtilis and Pseudomonas putida.*

Removal of heavy metals from distilleries effluent

Degradation of heavy metal experiments were carried out in 250 ml of separated bottles containing 100 ml of nutrient broth and 5ml of raw distilleries effluent. The pH was adjusted to 7 ± 0.2. Then the bottles were autoclaved at 121 °C for 15 minutes and the strains were inoculated to each bottles and kept for 5days, 10day and 15days

Heavy metal analysis

The effluent heavy metals were analyzed in Soil Testing Laboratory, Tiruchirappalli. The heavy metal analysis are Zinc, iron Copper, manganese, lead, Chromium, Cadmium, arsenic and Nickel. And the sample was analyzed under different parameters there are FTIR, atomic absorption spectroscopy and UV-Visible Spectrum. Heavy metal assay using atomic absorption spectroscopy as per standard method

Table 1: Physiochemical parameter of raw effluent and overall percentage reduction of distilleries effluent. Result of Treatment for *Escherichia coli, Pseudomonas putida, and Bacillus subtilis*

Sl. No.	Parameter	Raw	*Escherichia coli*			*Pseudomonas putida*			*Bacillus subtilis*		
			5 days	10 days	15 days	5 days	10 days	15 days	5 days	10 days	15 days
1.	Colour	< 1hue	< 1hue	< 1hue	< 1hue	< 1hue	< 1hue	< 1hue	< 1hue	< 1hue	< 1hue
2.	Odour	Dis. Agreeable	Dis. Agreeable	Dis. Agreeable	Dis. Agreeable	Dis. Agreeable	Dis. Agreeable	Dis. Agreeable	Dis. Agreeable	Dis. Agreeable	Dis. Agreeable
3.	Turbidity (NTU)	3500	2150	1850	1625	2450	1850	1450	1500	750	250
4.	Total Dissolved Solids (mg/L)	2918	2016	1568	1472	1997	1574	826	1395	973	557
5.	pH	8.26	8.06	7.65	7.32	7.86	7.52	7.16	7.82	7.62	7.42
6.	EC (dsm^{-1})	4.56	3.15	2.45	2.23	3.12	2.46	1.29	2.18	1.52	0.87
7.	BOD	4500	2950	1870	1530	2750	1875	1450	2450	1850	1420
8.	COD	2200	1560	1250	950	1560	1260	1100	850	725	620
	Heavy Metal										
9.	Zinc (mg/L)	1.56	1.20	1.06	0.92	0.98	0.82	0.56	1.26	1.09	0.82
10.	Copper (mg/L)	0.85	0.62	0.48	0.38	0.56	0.42	0.26	0.45	0.35	0.22
11.	Iron (mg/L)	5.69	4.82	3.72	2.54	3.65	3.12	2.16	3.25	2.18	1.16
12.	Manganese (mg/L)	2.28	1.89	1.57	1.40	1.82	1.52	1.06	1.25	1.05	0.85
13.	Chromium (mg/L)	2.58	1.85	1.63	1.24	2.05	1.86	1.74	1.06	0.75	0.62
14.	Lead(mg/L)	0.39	0.28	0.20	0.19	0.28	0.22	0.16	0.09	0.02	0.02
15.	Cadmium (mg/L)	0.06	0.05	0.03	0.02	0.02	0.02	0.01	0.01	0.01	NIL
16.	Nickel (mg/L)	0.12	0.09	0.04	0.04	0.08	0.05	0.02	0.02	NIL	NIL
17.	Arsenic (mg/L)	0.002	Nil	Nil	Nil	Nil	Nil	Nil	NIL	NIL	NIL
18.	Mercury (mg/L)	0.002	Nil	Nil	Nil	Nil	Nil	Nil	NIL	NIL	NIL

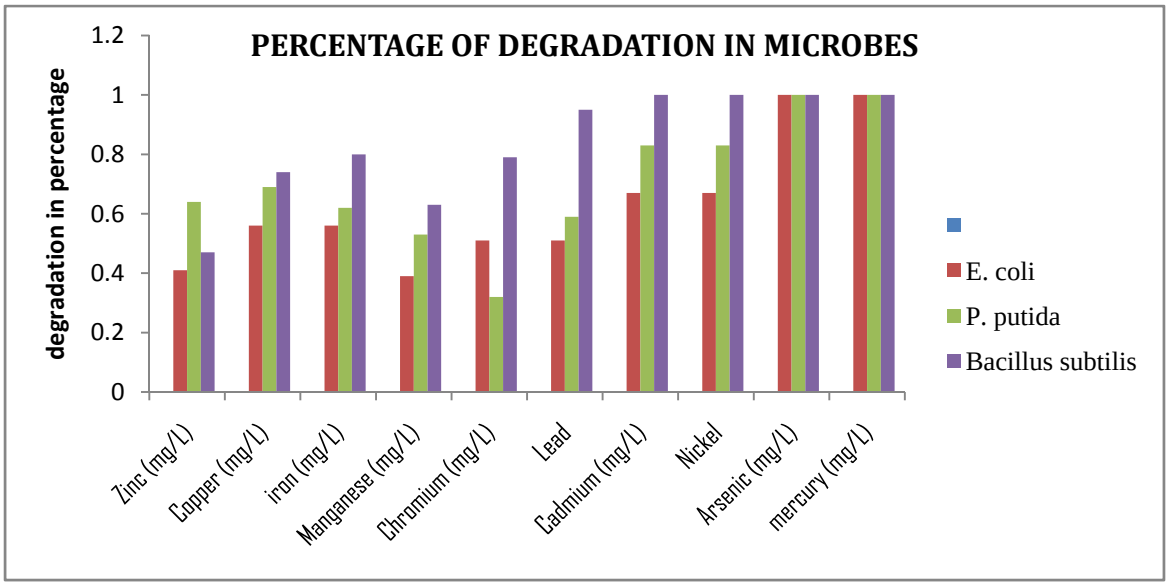
Table 2: Physio - chemical characterization of distilleries effluents in percentage

Microbs	Turbidity (NTU)	TDS (Total Dissolved Solids)(mg/L)	Electrical Conductivity ($dsm^{-1)}$	Biological oxygen Demand (mg/L)	Chemical Oxygen Demand (mg/L)
E. coli	54%	49%	51%	66%	56%
P. putida	59%	71%	71%	68%	50%
B. subtilis	92%	81%	81%	68%	72%

Table 3. Bio-Degradation of heavy metals in percentage

Microbes	Zinc (mg/L)	Copper (mg/L)	iron (mg/L)	Manganese (mg/L)	Chromium (mg/L)	Lead (mg/L)	Cadmium (mg/L)	Nickel (mg/L)	Arsenic (mg/L)	mercury (mg/L)
E. coli	41%	56%	56%	39%	51%	51%	67%	67%	100%	100%
P. putida	64%	69%	62%	53%	32%	59%	83%	83%	100%	100%
B.subtilis	47%	74%	80%	63%	79%	95%	100%	100%	100%	100%

Fig. 1 Microbial bioremediation of heavy metals from distilleries effluent using Bacterial strains.



recommended by ALPHA, 1998. The percentage of degradation was calculated from the following equation, % Degradation = Initial amount – final amount/ Initial amount x 100.

Results and Discussion

Isolation and identification of microbe from soil sample

The soil sample was taken from Trichy distillery effluent at bank of the river side where the Trichy distilleries effluent gets miscellaneous with river. The serial dilution technique applied for microbe's identification. Through this test it was identified that the microbes are *Escherichia coli, Bacillus subtilis and Pseudomonas putida*. The various tests were analyzed using biochemical test (IMVIC).

Physio - chemical characterization of distilleries effluents

The distilleries effluent indicates a high level of pollution compared with standard of NEOS, (2000). When compared to NEQS limits chart, (2000) the Biological Oxygen Demands and COD Level was very high in table -1. Table 2 represents the reduction rate of Turbidity, Total Dissolved Solids and Electrical Conductivity. The result finals the *Bacillus subtilis* has high reduction rate when compared to other organism and its comes to NEQS limits too.

Bio removal of heavy metal

Tables 3 represent the heavy metals degradation in percent. All three organisms show the degradation rate of heavy metals. The biochemical characterization of above isolated bacteria was given in Table 1. The distilleries effluent consists of heavy metals such as zinc 1.56 (mg/L), Copper 0.85 (mg/L), iron 5.69 , manganese 2.28 chromium 2.58, cadmium 0.06 mg/l, nickel 0.12, arsenic 0.002 mg, mercury 0.002 mg/l

Except zinc all other metals are degraded fast in the *Bacillus subtilis*. About 64% of Zinc was degraded in *Pseudomonas*; 74% of copper, 80% of iron, 63% of manganese, 95% of lead was degraded in *Bacillus subtilis* within 15 days. Cadmium, nickel, arsenic and mercury were degraded completely in Bacillus *subtilis*. Hence the reduction rate of heavy metals was high in *Bacillus subtilis.* The Biodegradation of heavy metals was shown in Table 3 and Graph 1.

Discussion

Water pollution due to heavy metals through distilleries effluent remains a serious environmental and universal problem in developing countries. Hence strict environmental legislations on the discharge of distilleries effluent without pretreatment make its necessary to

develop various innovative technologies for the removal of pollutants from the effluent. Hence various technologies were implementing for the treatment of effluent before released into the environment. In this study the effluent collected from the Trichy distilleries (India) chemical limited, Trichy, Tamil Nadu. The result indicated the reduction rate of physio - chemical character such as COD, BOD, pH and Total Dissolved Solids. Heavy metals were also higher than the permissible limit. Heavy metals such as Mercury, Cadmium and Chromium can bio accumulate and through the food chain to toxic levels in man (Howells, 1990). The results obtained after treatment indicate a very good correlation with reduction in the COD, TDS, PH and BOD. The COD, TDS, PH and BOD were reduced in 15days of treatment for permissible limits. Surprisingly, Arsenic and Mercury was completely disappeared after 5^{th} days of treatment and some metal such as cadmium and nickel was disappearing within the 15^{th} day treatment in the *Bacillus subtilis*. The result obtained from this present investigation showed that distilleries effluent is highly polluted in close agreement with the Randall and king (1980), (Kertell and Hill, 1982); (Sofianosheen and Khalil, 2000). The removal efficiency of the physicochemical parameters suggested that the *Bacillus subtilis, Pseudomonas putida* and *Escherichia coli* has heavy accumulation of distilleries effluent. When compare to other remediator the *Bacillus subtilis* accumulate Mercury, Arsenic, Cadmium and Nickel within 5 day and all the heavy metal was degraded high amount in the bacillus subtilis when compares to *Escherichia coli* and *Pseudomonas putida*. This study reveals that the *Bacillus subtilis* is a best remedier for the degradation of heavy metal in distillery effluent.

Conclusion

From this study it could be concluded that the microbes namely *Bacillus subtilis, Pseudomonas putida* and *Escherichia coli* do possess the bioremediation ability and colour reduction. The microbes are able to reduce the heavy metal pollutants of effluent sample maintained for 15 days. Arsenic and mercury was completely disappeared after 5^{th} days of treatment and some metals such as cadmium and nickel was disappear in the 15^{th} day treatment in the *Bacillus subtilis*. About 100% of heavy metals such as Cadmium, nickel, arsenic, mercury were completely remediated in the 15^{th} day. It is expected that the *Bacillus subtilis, Pseudomonas putida* and *Escherichia coli* 5^{th}, 10^{th} and 15^{th} days based study (dynamic) could lead to much better success towards expected development in treatment technology to find out minimum time interval required for maximum quantitative reduction of heavy metal by providing favorable conditions or altering other physicochemical parameters. Then it might be possible to have a significant reduction. Due to which pollutants from effluent can be reduced effectively heavy metal in minimum time period. The present study concluded that the best remedier to degrade the heavy metal of effluent treatment is *Bacillus subtilis* when compared to other organism.

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