Development and Use of Microbial Consortium for Treatment of Different Types of Industrial Waste Water

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Abstract: Global attention has been drawn on ways to sustain the environment using microorganism to remediate environmental pollutants because physical and chemical treatment are costly and can lead to production of toxic substance. Bioremediation involves the use of microorganism to reduce or remove the pollutants from contaminated area which may lead to restoration of the original natural substance without further disruption to the local environment. We developed an effective bio-augmentation treatment for different types of wastewater, which virtually eliminates excessive wastes within the system. The study was done on several important heavy metal contents against isolated microbial consortium and degradation was analyzed by qualitative analysis of each components. This unique microbial-based consortium containing both a liquid (vegetative state) and dry (spore and microencapsulated vegetative state) formulation. The formulated bacteria which can be highly efficient at producing hydrolytic enzymes to catalyze the hydrolysis of grease, fats, proteins and starches resulting in trouble free sewer lines at a minimum concentration of $1x10^9$ CFU per gram and extremely cost effective. This technology may also contribute to the full-scale treatment of various wastewater and marine sediment with high organic and hydro carbon content.

Keywords **- Bio-remediation, Microbial consortium, Industrial waste water, Heavy Metals.**

I. INTRODUCTION

With the rapid development of the economy, the displacement of industrial waste has increased significantly. Management or institutions involving in waste water treatment are under constant pressure to achieve the effluent quality requirements. In between environmental restrictions that are constraining allowable permit levels and management pressure to maintain environmental compliance; many waste water treatment plants are marginally capable of meeting these demands. The waste water treatment industry has challenges in meeting waste water compliance issues due to the complexity of the waste stream encounter (Luka et al., 2014). The wastes from industry are rich in protein and fat, making a herculean task to treat them than house hold or municipal wastes. In the conventional waste water treatment system, the removal of biological oxygen demand, suspended solids, nutrients, coliform bacteria and toxicity are the main goal for getting purified waste water. To achieve this removal many processing steps are being used. Also physiochemical methods to treat waste water treatment are widely accepted practice for many decades.

Finding a solution for the treatment and safe discharge of the wastewater is a difficult challenge because it entails integrated processes in which technical, economic and financial consideration come in play. The uniqueness of each situation makes it difficult to define a universal method for selecting the most adequate type of waste treatment plant. However, it is important to ensure that appropriate treatment standards are selected to meet local conditions, and alternative innovative technologies for treating wastewater are considered. This present study revealed that the bacterial isolates were able to tolerate different concentration of heavy metals recovered from the different industrial wastes and the isolates were resistant to the different antibiotics. Thus the present study defend that heavy metal resistance leads to antibiotic resistance, also these bacterial isolates can be used for the bioremediation of heavy metals.

II. METHODS AND MATERIAL

a. Sample collection

A total of three different industrial waste water samples were collected from various industries such as auto mobile industry, food industry and pharmaceutical industries metal dumping site located in District Krishnagiri, Hosur. Effluent samples were collected in dry, sterile polypropylene containers and transported immediately to Microbiology Research Laboratory at the Department of Microbiology, MGR college Hosur. These containers were maintained at 4ºC to ensure the minimal biological activity. Processing of samples for isolation of bacteria was carried out within 3h of sample collection.

b. Isolation of Bacteria

The bacterial species were isolated from the collected water samples with the help of conventional serial dilution technique8. For the pure culture of bacteria single colonies were picked and streaked on the nutrient agar plates containing different concentrations (200 to 2000 ppm) of different heavy metals (Aluminium nitrate ($Al(No_3)_3$), Calcium chloride ($CaCl_2$), Nickel sulphate ($NiSo_4$), Cobalt chloride ($CoCl_2$), Mercury chloride ($HgCl_2$), Potassium dichromate ($K_2Cr_2O_7$), Zinc sulfate ($ZnSo_4$) and Copper(II) sulfate ($CuSO_4$) (all from Sigma Aldrich, St. Louis, MO)) under sterile conditions. These concentrations were selected on the basis of previous studies reported in the literature. Pure cultures of strains which showed growth on plates containing 2000 ppm heavy metal concentration were grown on slants by stab and streak method for storage and subsequently for identification and biochemical characterization of bacterial isolates. These tests were used to identify the isolates referring to the Bergey's manual of systematic bacteriology (Harley et al, 1993) determinative bacteriology and probabilistic identification matrix.

c. Bio-Chemical Analysis

Biochemical tests were performed in order to establish the identities of different isolates such as indole test, citrate utilization test, nitrite reduction test, catalase test, MR–VP, urease test, sugar fermentation test (such mannose, sucrose, galactose glucose, lactose and fructose) of all collected samples were analyzed and also heavy metal degradation analysis was carried out in the laboratory (APHA, 1995).

d. Identification and characterization of the bacterial isolate

Heavy metal resistant bacterium obtained was initially characterized in terms of colony morphology and basic microscopic observations. Further confirmative identification of the isolated species was done on the basis of (1) Final counts of CFU (2) Minimum Inhibitory Concentration (MIC) of the metals which they can resist, and (3) Antibiotic susceptibility test as per our earlier publication (Mahilarasi et al 2018).

e. Screening

Bacteria which can able to survive and utilize the selective components source are screened by 7 subsequent transfer in selective mineral salt medium (sMSM) at 100 PPM Concentration. Isolates exhibiting distinct colonial morphologies were isolated by repeated streaking on the same sMSM agar medium.

f. Bio degradation under higher concentration

Samples (Consortium) are incubated for 72 h on a rotary shaker (120 rpm) and maintained at 30±1°C. The growth of the isolates was examined under highest concentration (200 to 2000 ppm) at different days of incubation and checked the viability and growth after 10, 20 and 30 days.

g. Consortium Selection:

All the potential isolates and strains were screened according to non- pathogenicity for human/animals and their higher biodegradable efficiency under below mentioned test parameters. Based on the analysis, the particular strains were selected in consortium formulation.

h. Analysis of Biodegradable Potential of Microbial Consortium

The parameters were analyzed as per APHA standard methods (2005) for the examination of water and waste water degradation level of different group of compounds: (A) Organics Compounds – A determination of the concentration of carbon-based (i.e., organic) compounds aimed at establishing the relative "strength" of wastewater were analysed for Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD), (B) Solids – A measurement of the concentration of particulate solids that can dissolve or suspend in wastewater (i.e., inorganic) were analysed for Total Solids (TS), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Total Volatile Solids (TVS), and Total Fixed Solids (TFS), (C) Other parameter such as pH, Sulphate, Chloride, and heavy metal analysis for Copper, Nickel, Zinc, Arsenic, Lead, Cadmium, Selenium, Mercury and Hexavalent chromium were analysed on day 0, 10, 20 and 30 after microbial consortium treatment on auto mobile industry, food industry and pharmaceutical industries waste water.

i. Formulation

Microbial-based consortium containing both a liquid (vegetative state) and dry (spore and microencapsulated vegetative state) formulation was designed and final formulated consort were tested under auto mobile industry, food industry and pharmaceutical industries waste water under environment climatic conditions, humidity and temperature to confirm the successful delivery.

III. RESULTS AND DISCUSSION

According to the fourth World Water Development Report, presently only 20% of globally produced wastewater receives proper treatment (UNESCO, 2012). Treatment capacity typically depends on the income level of the country, thus treatment capacity is 70% of the generated wastewater in high-income countries, compared to only 8% in low-income countries (Sato, 2013). In India, for example, nearly 40% of sewage treatment plants and pumping stations did not conform to operation and maintenance standards in 2012 (Hawkins, 2013). Many treatment plants have also been abandoned (or are not operational) because of lack of funds for operation and maintenance or lack of technical capacity to perform these tasks, especially at the local level and when operated by small water utilities. As per the latest estimate out of 22,900 Mld of wastewater generated in our country, only about 5900 Mld (26%) is treated before letting out, the rest i.e., 17000 Mld is disposed of untreated. Twenty-seven cities have only primary treatment facilities and forty-nine have primary and secondary treatment facilities. The level of treatment available in cities with existing treatment plant varies from 2.5% to 89% of the sewage generated (ENVIS, 2015).

A total of 27 isolates were recovered from metal dumping sites from Hosur region and 9 isolates were screened after heavy metal resistance experiment. The isolates were further identified on the basis of morphological characteristics such as size, color and texture. Also the microscopic examination of these isolates revealed that out of 9 isolates, 3 were gram positive cocci, 6 were gram negative bacilli. Finally, five best isolates are picked from samples which were tolerant to high concentrations of the heavy metals under study (C1, D1, D2, E1, and E2). All the five isolates were able to grow at the highest concentrations at 2000ppm of $Al(No_3)_3$ 18mm, $CaCl_2$ 19mm, $CoCl_2$ 25mm, $K_2Cr_2O_7$ 26mm, $HgCl_2$ 15mm, $NiSo_4$ 20mm, $ZnSo_4$ 25mm and $CuSO_4$ 26mm. The bacterial isolates were tested for their sensitivity to 19 different commonly used antibiotics. Almost all the bacterial isolates were resistant to most of the antibiotics. In the antibiotic cultural sensitivity assay, five isolates were found resistant to four or more different groups of antibiotics (Table 1). Such isolates were regarded as multidrug resistant. This number further increased when the intermediate resistance was also accounted for among resistant strains.

From this study it is concluded that native bacteria present in the waste water have the property of heavy metal resistance and ability to accumulate heavy metal environment through the process of biosorption. *Acinetobacter pittii, Escherichia coli, Fictibacillus nanhaiensis, Lysinibacillus xylanilyticus* and *Planococcus maritimus* are efficient in removal of heavy metals (Cr, Cd, Cu, Zn, Pb, Ni) from the environment. Further, results obtained for analysis of the metal binding protein and alkaline phosphatase suggest that this native bacterium are potential bio tools for bioremediation of metal pollutants in the environment. The microbial consortium was clustered into two groups: gram negative bacterium *Acinetobacter pittii* and *Escherichia coli* was a grouped into first consortium formulation and the gram positive bacterium such as *Fictibacillus nanhaiensis, Lysinibacillus xylanilyticus* and *Planococcus maritimus* were formulated in the second group consortium.

Both first group and second group microbial consortium for the treatment of auto mobile industry waste water, food industrial waste water and pharmaceutical industrial waste water were dramatically decreased from the raw water values respectively (Table 2 to 4). Similarly, both groups consortium was actively reduced the heavy metal components efficiently. To conclude that the present study revealed that the bacterial isolates were able to tolerate of the discussed different concentration of heavy metals recovered from the contaminated sites. Furthermore, the isolates were resistant to the different antibiotics which were used against the isolates. Thus, the present study justify that heavy metal resistance leads to antibiotic resistance, also these bacterial isolates can be used for the bioremediation of heavy metals.

Table 1. Antibiotic susceptibility of heavy metal resistant bacteria isolates

Antibiotic disc name	A1	A2	B1	B2	C1	D1	D2	E1	E2
Cefepime [CPM] 30 mg		21 (R)		18 (I)		14 (R)	12 (R)		
Tobramycin [TOB] 10 mg		10 (R)				10 (R)	24 (R)		
Chloramphenicol [C] 30 mg	10(R)	14 (I)		17 (R)		17 (R)	11 (R)	24 (R)	
Piperacillin [PC] 100mg		14 (R)		11 (R)		<10(R)	10 (R)		
Ciftazidine [CA] 30 mg		11 (R)		11 (R)		<10(R)	11 (R)		
Ampicillin [A] 10 mg	<10(R)	10 (R)		<10(R)		<10(R)	<10(R)	<10(R)	
Cefixime/ clavulanic acid [CMC] 5/100 mg		26 (R)		14 (R)		10 (R)	16 (R)		
Ampicillin/ sulbactum [AS] 10 mg		14 (I)		10 (R)		<10(R)	10 (R)		
Penicillin-G [P] 30 mg			<10(R)		<10(R)				<10(R)
Amoxicillin [AC] 10 mg		12 (R)				<10(R)	<10(R)		
Bacitracin [B] 30 mg			<10(R)		<10(R)				<10(R)
Novobiocin [NV] 30 mg			<10(R)		<10(R)				<10(R)
Erythromycin [E] 15 mg	<10(R)		<10(R)		<10(R)			<10(R)	<10(R)
Methicillin [M] 30 mg			<10(R)		<10(R)				<10(R)

Note: All numerical values of diameter in mm and represents Resistant (R), Sensitive (S), Intermediate (I)

Table 2: Microbial Consortium for Treatment of Auto Mobile Industrial Waste Water

Test	Parameters	Unit	Day 0 (Before Treatment)	Consortium Group 1			Consortium Group 2		
				Day 10	Day 20	Day 30	Day 10	Day 20	Day 30
1	P^H	-	6.90@25 ^{0}C	7.39@25^0 C	8.06@25^0 C	7.59@25^0 C	8.03@25^0 C	8.04@25^0 C	7.64@25^0 C
2	Sulphate as SO_4	Mg/l	577.35	199.66	168.75	147.28	194.75	156.36	127.35
3	Chloride as cl	Mg/l	869.73	774.85	559.82	474.85	789.75	699.78	599.81
4	Chemical oxygen demand	Mg/l	490	160	120	100	252	112	88
5	Copper as cu	Mg/l	22	0.56	0.41	0.14	0.43	0.08	0.06
6	Nickel as Ni	Mg/l	0.54	0.39	0.12	0.11	0.17	BDL (<0.05)	BDL (<0.05)
7	Zinc as Zn	Mg/l	0.74	0.72	0.15	BDL (<0.05)	0.65	0.41	0.36
8	Arsenic as As	Mg/l	0.32	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	0.26	BDL (<0.05)	BDL (<0.05)
9	Lead as pb	Mg/l	17.02	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	14.87	BDL (<0.05)	BDL (<0.05)
10	Cadmium as cd	Mg/l	3.2	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)
11	Selenium as Se	Mg/l	2.7	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)
12	Mercury as Hg	Mg/l	0.06	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)
13	Hexavalent chromium	Mg/l	0.08	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)

BDL: Below Detectable limits [DL-0.05]

Table 3: Microbial Consortium for Treatment of food Industrial Waste Water

Test	Parameters	Unit	Day 0 (Before Treatment)	Consortium Group 1			Consortium Group 2		
				Day 10	Day 20	Day 30	Day 10	Day 20	Day 30
1	P^H	-	6.70@25° C	7.39@25^0 C	8.03@25^0 C	8.64@25^0 C	7.35@25^0 C	7.85@25^0 C	6.94@25^0 C
2	Sulphate as SO_4	Mg/l	292.84	227.44	199.66	130.74	202.25	188.74	82.97
3	Chloride as cl	Mg/l	1444	1099.65	574.85	434.86	1049.67	662.48	449.86
4	Chemical oxygen demand	Mg/l	22400	16000	4400	3200	22100	12000	5300
5	Copper as cu	Mg/l	58	6.54	2.53	0.08	2.55	0.31	0.23
6	Nickel as Ni	Mg/l	0.55	0.18	0.11	0.06	0.21	0.19	0.09
7	Zinc as Zn	Mg/l	0.98	0.7	0.46	BDL (<0.05)	0.54	0.52	0.42
8	Arsenic as As	Mg/l	2.81	BDL (<0.05)	BDL (<0.05)	1.28	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)
9	Lead as pb	Mg/l	2.46	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	1.77	BDL (<0.05)	BDL (<0.05)
10	Cadmium as cd	Mg/l	0.08	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)
11	Selenium as Se	Mg/l	1.23	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)
12	Mercury as Hg	Mg/l	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)
13	Hexavalent chromium	Mg/l	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)

BDL: Below Detectable limits [DL-0.05]

Table 4: Microbial Consortium for Treatment of Pharmaceutical Waste Water

Test	Parameters	Unit	Day 0 (Before Treatment)	Consortium Group 1			Consortium Group 2		
				Day 10	Day 20	Day 30	Day 10	Day 20	Day 30
1	P^H	-	6.90@25 ^{0}C	7.99@25^0 C	7.49@25^0 C	6.94@25^0 C	7.93@25^0 C	7.46@25^0 C	6.94@25^0 C
2	Sulphate as SO_4	Mg/l	368.26	316.75	92.08	82.97	306.58	93.65	64.35
3	Chloride as cl	Mg/l	1749.45	1444	1184.63	1089.66	1679.47	1069.35	964.32
4	Chemical oxygen demand	Mg/l	412	396.23	352	208	369.25	258.15	192
5	Copper as cu	Mg/l	1.16	0.67	0.55	BDL (<0.05)	0.98	0.11	0.06
6	Nickel as Ni	Mg/l	7.21	0.37	0.19	BDL (<0.05)	0.24	BDL (<0.05)	BDL (<0.05)
7	Zinc as Zn	Mg/l	1.52	0.59	0.38	0.17	0.65	0.52	0.18
8	Arsenic as As	Mg/l	0.07	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)
9	Lead as pb	Mg/l	0.09	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)
10	Cadmium as cd	Mg/l	1.2	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)
11	Selenium as Se	Mg/l	0.08	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)
12	Mercury as Hg	Mg/l	0.65	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)

13	Hexavalent chromium	Mg/l	0.84	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)	BDL (<0.05)

BDL: Below Detectable limits [DL-0.05]

I. CONCLUSION

Microbial cultures are the only method that can eliminate the waste problem in economic way. The living organisms that continually adapt and grow in the system they consume the waste, chemicals and medicals; many strains of bacteria will not degrade grease, fats, oil, protein, phosphates, nitrates, sulfates or other different chemicals under any circumstances. The only effective way of handling pollutants is to degrade all the human / dairy/ industrial waste uniquely by certain microbes. This requires a carefully selected formula. Preliminary lab test of these non-pathogenic bacterial consort has the efficiency to degrade certain waste materials. Still more intensive research is needed to standardize concentration of microbes, rate of enzyme production and their sustainability in varied environment will unravel the full potential of these microbes. This ecological approach will lead to economically and environmentally safe waste water treatment system and reducing the burden of waste water management.

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