ISSN: 2277-9655
Impact Factor: 3.785

IJESRT

INTERNATIONAL JOURNAL OF ENGINEERING SCIENCES & RESEARCH TECHNOLOGY

BIOREMEDIATION OF INDUSTRIAL AND MUNICIPAL WASTE WATER USING BACTERIAL ISOLATES

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DOI: 10.5281/zenodo.51015

ABSTRACT

Bioremediation is a treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or non toxic substances. The microbes are effective in control of pollution due to waste water. The industrial and municipal waste water is analyzed for different Physico-Chemical parameters such as pH, Temperature, TDS, BOD, COD, Total Alkalinity, Chlorides. The collected waste water samples were serially diluted and pour plated on Nutrient Agar medium and incubated at 37˚c for 24 hours. Among 12 bacterial isolates, 4 isolates namely Bacillus subtilis,Bacillus cereus, Escherichia coli and Staphylococcus aureus showed high reduction in BOD&COD in selected waste water sample.The results shows that the COD is removed maximum by Bacillus cereus in textile and sugar mill waste water about 62.88% an 51.7% respectively and 74.21 % removal by Escherichia coli in municipal waste water. The maximum removal of BOD is 82.83% in municipal waste water by Bacillus subtilis. Escherichia coli and Bacillus cereus removed 80.83% and 77.77 % respectively.

KEYWORD: Bioremediation, Industrial and Municipal waste water, Bacterial isolates, Pour plate.

INTRODUCTION

The world is faced with problems related to the management of wastewater due to extensive increasing population density, industrialization and a highly urbanized society. Along with this increase in population and industrialization, the waste are produced and released into the environment, either in the form of solid waste, liquid or gas. The quality of wastewater effluents is responsible for the degradation of receiving water bodies, such as lakes, rivers, streams. The problem of environmental pollution on the industrial effluents and sewage sludge becomes more common. The volume of municipal wastewater increased by 5 million meter cubic per year, with an increase in the average content of 50%.Industrial and municipal discharges pollute water bodies imparting a high BOD and COD which could be due to the presence of organic substrates.

The two main treatment processes for the removal of impurities from wastewater are chemical and biological methods. Compared to chemical methods, biological treatment is attractive as it is economical and eco-friendly. Many new approaches are being investigated for understanding the metabolic pathways and enzymes involved in biodegradation processes. Bioremediation has been reported in some bacteria where toxic compounds are converted to less toxic compound.

In biological treatment, the activated sludge is utilized which is the combination of different microbial combinations. Formulated microbial consortia represent a broad selection of microbial population. This composition degrades organic matter present a wide range of substrates in a reproducible manner and also reduces the time required to carry out the same.

The present research is carried out to develop the formulated microbial consortia for reducing the BOD load as well as COD of municipal and industrial waste water. The textile and sugar mill effluent samples were taken for

industrial waste water for differentiation. As the municipal waste water possess similar characteristic in all places, one station sample is collected. The microorganisms were formulated randomly on the basis of their morphology, colour, size, shape etc.

MATERIALS AND METHODS

Sampling and Isolation of bacterial strains

The industrial and municipal waste water samples are collected in 10 L sterile cans and are refrigerated. The bacterial isolates were isolated by serial dilution agar plate technique. For this, the suspension of each sample was prepared by vortexing 1.0 ml of water sample in 9 ml sterilized distilled water and so prepared suspension of this dilution was aseptically spread on Petri plate having nutrient agar medium. The Petri plates were incubated at 37^0C± 2^0C for 48 hours. The bacterial colonies were isolated by streaking them on fresh plates containing nutrient agar medium. To avoid repeated isolation of same bacterial isolates, the colonies showing similar cultural characteristics, were obtained by repeated streaking of diluted suspension prepared from isolated bacterial colonies. The purified bacterial isolates were preserved on nutrient agar slants at 4˚C. All bacterial isolates were sub cultured after a time interval of 30 days.

Identification

The promising bacterial isolates were identified on the basis of their morphological, physiological and biochemical characteristic features (Bergey's Manual).

Waste water treatment

The waste water samples (500 ml) are taken in a 1L container was inoculated with 5% bacterial culture. The samples are divided into 4 portions in 250 ml conical flask. The conical flasks are covered air tight by cotton plug (fig 1). All the cultures were kept in shaking incubator at 37^0 C for 48 hrs. BOD and COD values of untreated water sample and treated samples were determined by tritimetric method (APHA, 1998 & Aneja, 2001).

Fig 1: Image of Bacterial cultures kept in shaking incubator

RESULTS AND DISCUSSION

In the present study, 12 bacterial isolates were isolated and cultured individually on Nutrient agar medium. Four isolates namely *Bacillus cereus,Bacillus subtilis, Staphylococcus aureus* and *E.coli* which showed greater capability in degradation of toxic organic compounds. The bacterial isolates were identified by various morphological, biochemical tests showed in Table 1.

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Physico-chemical analysis of waste water sample

The industrial (textile and sugar mill effluent) and municipal waste water is analysed for its physico-chemical parameters as per Indian standards immediately after the sample collection which is shown in Table 2.

Table 1: Morphological and Biochemical tests of Bacterial isolates

SI.No	**Characters**	***Bacillus subtilis***	***Bacillus cereus***	***Escherichia coli***	***Staphylococcus aureus***
1	Gram staining	+	+	-	+
2	Shape	Rod	Rod	Rod	Cocci
3	Motility	Non motile	Non motile	Non motile	Non motile
4	Catalase	+	+	+	+
5	Oxidase	-	+	-	-
6	Indole test	-	-	+	-
7	Methyl red test	-	+	+	-
8	Voges proskauer test	+	-	-	+
9	Citrate test	+	-	-	+
10	Starch test	+	+	+	+
11	Urease test	-	-	-	-
12	Triple sugar ion agar test	-	+	+	+

Table 2: Physico-chemical parameters of Industrial and Municipal waste water

Parameter	**Municipal waste water**	**Industrial waste water**	
		Textile waste water	**Sugar mill waste water**
pH	8.61	7.83	4.2
Temperature	35°C	35°C	33ºC
Electrical conductivity (µS/cm)	1923	894	2.23
Colour	Greyish black	Rose	Light brown
Odour	Unpleasant	Odourless	Foul smell
Total dissolved solids(mg/l)	3000	1000	1480
Total suspended solids(mg/l)	5000	3000	4220
Total Solids (mg/l)	8000	4000	5700
Biochemical Oxygen Demand(mg/l)	60	84	181
Chemical oxygen demand(mg/l)	232	199.28	271
Dissolved oxygen(mg/l)	26	17	13
Total hardness(mg/l)	110	270	629
Chloride(mg/l)	512. 34	349.89	422
Ca Hardness(mg/l)	45	60	361
Mg hardness(mg/l)	65	210	268
Total Alkalinity	681	786	862

Analysis of Waste water treatment with bacterial isolates

The waste water is inoculated with the bacterial culture and are incubated. The municipal, textile and sugar mill waste water samples showed an average pH of 8.61,7.84 and 4.2 respectively. The municipal waste water showed average DO, COD of 5 mg/l and 157.28 mg/l respectively. The dissolved oxygen of textile and sugar mill waste water is 12 mg/l and 15.3 mg/l respectively. The COD of textile and sugar mill effluent is 199.28 mg/l and 185.7 mg/l respectively. After treating with individual bacteria , the toxic organic compounds reduced gradually. The maximum reduction in BOD (80.83 %) and COD (72.74%) were shown by *Bacillus subtilis.*The minimum reduction in both BOD and COD is shown in sugar mill effluent by all bacterial isolates. The comparison on reduction in BOD and COD in industrial and municipal waste water by bacterial isolates is shown in Table 3 and Table 4.

Table 3: Percentage removal of BOD in waste water

Species	Municipal waste water		Textile waste water		Sugar mill waste water	
	BOD (mg/l)	**% removal**	**BOD (mg/l)**	**% removal**	**BOD (mg/l)**	**% removal**
Bacillus subtilis	2.6	78.33	1.4	72	3.4	77.77
Bacillus cereus	4.8	60	0.9	82	6.1	60.13
E.coli	2.3	80.83	1.2	766	5.6	63.3
Staphylococcus aureus	2.9	75.83	1.1	78	5.3	65.35

Initial values:
Textile waste water: 12 mg/l
Municipal waste water : 5 mg/l
Sugar mill waste water : 15.3 mg/l

Table 4: Percentage removal of COD in waste water

Species	Municipal waste water		Textile waste water		Sugar mill waste water	
	COD (mg/l)	**% removal**	**COD (mg/l)**	**% removal**	**COD (mg/l)**	**% removal**
Bacillus subtilis	73.97	62.88	50.34	67.99	89.65	51.7
Bacillus cereus	102.19	48.72	43.34	72.44	100.85	45.69
E.coli	87.83	55.92	40.56	74.21	94.35	49.19
Staphylococcus aureus	118.97	40.30	62`43	60.30	130.43	29.76

Initial values:
Textile waste water: 199.28 mg/l
Municipal waste water : 157.28 mg/l
Sugar mill waste water : 185.7 mg/l

CONCLUSION

In this study, the individual bacterial cultures are used for the treatment of industrial and municipal waste water. The role *B.subtilis* in reduction of BOD and COD levels is greater compared to other bacterias. They showed reduction upto 80.83% in BOD reduction. The *Bacillus cereus* showed maximum BOD reduction upto 82 % in textile waste water. The need for economical, effective and safe method for disposal of pollutant in effluents has resulted in bioremediation. The bioremediation of municipal and industrial waste water showed in reduction in toxic compounds.Thus, the environmental pollutions can be reduced by adapting bioremediation all over.

ACKNOWLEDGEMENTS

My sincere thanks to our respected principal Mr. A. Rajesh M.Tech., Ph.D., for his continuous encouragement showed for the completion of our project work and for rendering infrastructure facilities.

I solemnly express my immense gratitude to Mr. T. Sankar M.Tech., Head of the Department, Department of Civil Engineering, CAHCET, Melvisharam for his encouragement throughout the project work.

I am very thankful to my project guide Mr. J. Sharpudin M.E., Assistant Professor, Department of Civil Engineering, CAHCET, Melvisharam for his valuable ideas and his support for the completion of my project.
I am very much thankful to our project head Mr. N. Babu M.E., Assistant Professor, Mr. K. Balakumar M.Tech., Assistant professor, Department of Civil Engineering, CAHCET, Melvisharam for their encouragement and support for completion of the project.

I also wish to express our sincere thanks to all teaching and Non-Teaching staff members, Department of Civil Engineering for their valuable guidance and advices throughout the course of my project work.

Finally, I express my sincere thanks and gratitude to my parents and friends for their support during the project work.

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