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Screening and characterization of bacterial isolates for degradation of recalcitrant pollutants from River water

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ABSTRACT

The restoration of river ecosystems depends on the utilization of the most effective river water treatment techniques. Yamuna river water of Agra city is heavily contaminated with toxic pollutants including heavy metals and pesticides that causes severe damage to ecological and social aspects. At present, the direct use of river water for the purpose of drinking causes severe hazards due to anthropogenic activities causing environmental pollution in rivers. The four bacterial isolates (*Rhodopseudomonas palustris Bacillus subtilis, Bacillus fusiformis,* and *Rhodobacter sphaeroides*) were used in the form of monocultures and consortium for the bioremediation purposes. According to the results obtained, a significant reduction were observed in the physico chemical parameters including heavy metals. Hence, bioremediation is considered to be an attractive option for minimizing the pollution load from contaminated river water due to its high efficiency and economical impact than chemical remediation. Thus the current study clearly states that, application of bacterial consortia and monocultures can be used for the treatment of toxic effluents from Yamuna river water.

Key words: *Ecological, Social aspect, Restoration, Monoculture, Consortium, Economical, Bioremediation*

Introduction

One of the major environmental issues, particularly in developing and poor nations, is river water pollution. The water used for drinking, household, agricultural, commercial, industrial, and recreational purposes comes from rivers. However, in certain nations, river water pollution is so severe that it can barely be used. Additionally, it pollutes the air, releases strong, disagreeable odors, and contributes to the spread of water-borne diseases in many underdeveloped nations. The successful implementation of strict policy regulations for the discharge of solid waste, wastewater, storm water, and standards of treated or untreated wastewater is dependent on the management of water quality (Somlyody *et al.* 1995). As a result, in order to successfully remediate contaminated water, water management plans must take into account sustainable techniques and policies (Chen *et al.*, 2006).

A combination of biological and physicochemical processes are used to treat wastewater, and the treatment method used is mostly influenced by operational costs, the source and quality of influent wastewater, and the planned reuse of the effluent (Brar *et al.*, 2019). Although predictable methods for treating sewage and other wastewater have been shown to be efficient in reducing levels of heavy metals, toxic compounds, phosphorous, and nitrogen, these technologies have been shown to be ineffective in doing so, typically requiring more than one step to treat most of the compounds, and not being financially viable. Microbes extracting toxins from contaminated settings is one innovative therapy method. Utilizing local bacteria to change the organic environment is known as biological therapy for waterstuff. Microorganism- based bioremediation techniques provide a risk-free, affordable, flexible, and environmentally responsible treatment option. Bioremediation is the practice of employing living organisms, usually bacteria and fungi, to transform environmental contaminants into less dangerous forms. By transforming radioactive waste into living creatures, bioremediation improves the cleaning up of radioactive pollution. To eliminate or immobilize pollutants, low-harmful variants of certain contaminants are used. Utilizing naturally existing microorganisms that break down hazardous contaminants and give food for their growth is necessary for the procedures. As a result, only favorable conditions for microbial activity will allow for the success of bioremediation. The Yamuna river water of Agra city is heavily contaminated with toxic pollutants including heavy metals and pesticides that causes severe damage to ecological and social aspects. Hence, this study was proposed to evaluate the status of Yamuna river water of Agra and to remediate its pollutant biologically through bacterial monocultures and consortium.

Methodology

Site Characterization and Sample Collection

Yamuna river water samples were collected from different sites of Agra city, based upon the existence of industries and disposal of raw sewage which are responsible for main source of hazardous pollutant contamination into the river. The sampling sites were as follows:-S1- Kailash Ghat, S2- Poiyah Ghat and S3- Hathi Ghat. River water samples were collected in pre- sterilized bottles (5 L capacity) from River banks. Thereafter all the samples were brought to the laboratory for analysis of physico-chemical parameters and rest of the samples were preserved at 4 °C for further analysis.

Isolation and Identification of Bacteria from collected water samples

For isolation of bacteria from collected Yamuna river water samples, serial dilution technique was used (Aneja, 2001). In this technique, one ml of the sample water was transferred to the sterilized tube (marked as 10^{-1}) containing 9 ml distilled water and sample was mixed thoroughly by Vortex mixture to obtain a uniform suspension. This suspension was serially diluted up to 10^{-7} dilutions. From the solution (10^{-4} to 10^{-7} dilution), 0.5 ml suspension was pipetted onto the sterilized petridishes and then quickly melted nutrient agar medium was poured on each petridishes and was gently rotated (clockwise and anti-clockwise) for uniform mixing of medium and suspension. Control plates were kept uninoculated. Then all the petridishes were incubated at 37 °C for 24h. Each single colony appeared on plated medium was treated as one colony forming unit (cfu). The isolated bacterial cultures were identified through their physiological, morphological, and biochemical characteristic features by Bergey's Manual of Systematic Bacteriology (Claus and Berkley, 1986) and were later cross examined by BD-BBL Crystal Identification Autoreader (Becton Dickinson and Company, USA).

Results and Discussion

Isolation and Identification of Culturable Bacteria from water samples

After collection of water samples from Yamuna River during July 2014 to December 2014, total 15 bacteria were isolated through serial dilution technique. In present study, from all six samples (July to December 2014) number of cfu's was found 16, 26, 18, 14, 11, and 9 respectively. Total 15 bacterial strains were isolated from 94 bacterial colonies and were purified at 37 °C on Nutrient Agar Media (suitable for massive growth) under sterilized conditions. All fifteen bacterial strains were screened for bioremediation studies. The present study is in accordance with the findings of Shrivastava *et al.* (2013) who isolated 31 bacterial strains from Yamuna River water of Agra out of which 8 bacterial isolates (*Rhodopseudomonas palustris, Rhodobacter spheroides, Bacillus subtilis, B. fusiformis, B. thurigiensis, B. cereus, Lactobacillus* sp. and *E.coli*) showed maximum reduction in BOD and COD.

Similarly Gupta *et al.* (2014) isolated 11 bacterial cultures from Yamuna River water and effluents. The result signifies that maximum diversity in the microflora (bacterial) of Yamuna River may be due to dilution of industrial effluents. The present study is also in the agreement with the work of Dubey *et al.* (2014), they reported that bioremediation is a promising approach for decontamination of Yamuna River through natural means, while studying microbial diversity of River water it was found that *Thermomicrobium*, *Shigella*, *Escherichia*, *Azoarcus*, *Pseudomonas*, *Nitrosomonas*, *Bacillus* and were the dominant genera. Sewage contamination in the River water is due to the presence of *Shigella* and *Escherichia* while the presence of *Bacillus* and *Pseudomonas* is capable to de-pollute the River water.

Preliminary Screening of effective bacterial isolates, Dual combination and consortium for bioremediation study

Total fifteen bacteria (BI-1, BI-2, BI-3, BI-4………BI-15) were isolated from Yamuna River water samples. Among these bacteria only four bacteria showed maximum reduction in physico-chemical parameters (DO, BOD, COD, TDS and Hardness) and heavy metals (Cd, Cr, Pb, Zn and Ni). In Yamuna River water sample Bacterial Isolate 2 (BI-2) showed 82.6%, 77.4%, 80.3%, 88.7%, 86.2%, 98.5%, 88.1%, 94.7%, 96.6% and 61.9%, Bacterial Isolate 7 (BI-7) showed 86.4%, 81.8%, 84.9%, 91.6%, 91.6%, 99.7%, 99.06%, 96.7%, 97.8% and 95.2%, Bacterial Isolate 8 (BI-8) showed 84.4%, 80.2%, 78.2%, 91.1%., 86.1%, 99.02%, 99.6%, 97.7%, 99.4% and 71.4%, Bacterial Isolate 14 (BI-14) showed 80.5%, 75.8%, 87.4%, 87.4%, 83.7%, 96.6%, 75.7%, 99.3%, 97.7%and 76.19% reduction in DO, BOD, COD, Hardness, TDS, Cd, Cr, Pb, Zn and Ni respectively. (Table 1, Fig. 1).

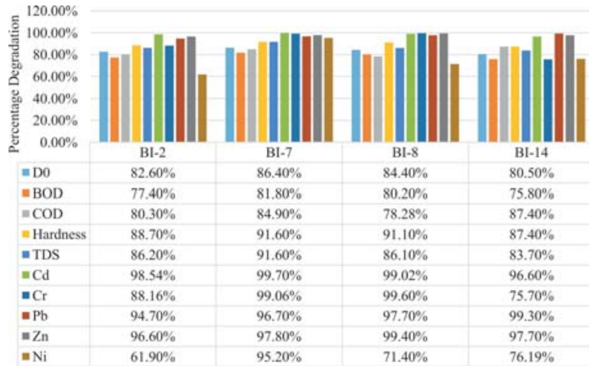

Fig. 1. Percentage reduction in physico-chemical parameters and heavy metals of Yamuna River water using effective bacterial isolates

The dual combination and consortium of four selected bacteria were tested for reduction in physico-chemical parameters (DO, BOD, COD, Hardness, TDS) and heavy metals (Cd, Cr, Pb, Zn, Ni). Maximum percentage reduction were observed by treatment with bacterial consortium i.e. 88.7%, 85.1%, 89.9%, 93.8%, 89.4%, 99.5%, 99.06%, 98.6%, 98.7% and 90.4% in DO, BOD, COD, Hardness, TDS, Cd, Cr, Pb, Zn and Ni respectively. Rest of the combinations showed degradation ranging between 81.5% to 86.6% in DO, 76.6% to 82.4% in BOD, 79.7% to 854%

Table 1. Treatment trials by different bacterial isolates

Bacterial Isolates	DO (3.2)	BOD (36.4)	COD (128)	Hardness (1843)	TDS (624.8)	Cd (0.82)	Cr (0.32)	Pb (1.17)	Zn (0.78)	Ni (0.021)
BI-1	7.6	20.2	70.2	765.2	523.4	0.721	0.231	0.982	0.537	0.017
BI-2	18.6	8.2	25.2	208	85.7	0.012	0.038	0.062	0.026	0.008
BI-3	6.4	24.3	83.5	643.4	302.8	0.813	0.242	1.024	0.645	0.015
BI-4	8.7	30.7	43.4	825.7	425.9	0.624	0.178	1.136	0.281	0.013
BI-5	4.24	15.8	103	1023.4	602.8	0.673	0.124	1.123	0.654	0.019
BI-6	6.2	18.6	94.3	1137.2	598.5	0.528	0.168	1.093	0.263	0.014
BI-7	23.8	6.6	19.3	153	52.3	0.002	0.003	0.038	0.017	0.001
BI-8	20.8	7.2	27.8	162.4	86.4	0.008	0.001	0.026	0.004	0.006
BI-9	10.2	14.7	65.6	986.2	203.7	0.613	0.155	0.128	0.386	0.011
BI-10	5.24	26.5	84.3	728.4	218.5	0.542	0.163	0.117	0.574	0.02
BI-11	8.43	18.7	63.4	628.7	305.7	0.321	0.183	0.114	0.625	0.015
BI-12	11.2	13.4	52.6	425.4	201.3	0.178	0.128	0.078	0.504	0.011
BI-13	5.13	28.6	73.5	1624.5	207.5	0.138	0.263	0.058	0.328	0.013
BI-14	16.6	8.8	15.8	232.2	101.7	0.028	0.078	0.008	0.018	0.005
BI-15	7.2	24.2	96.2	1040.5	436.3	0.275	0.128	0.083	0.413	0.017

*Highlighted values indicate initial values i.e. before treatment values, Highlighted values indicate bacterial isolates who showed maximum degradation, All values are expressed in mg/L in COD, 87.5% to 91.4% in Hardness, 84.2% to 88% in TDS, 97.2% to 99.1% in Cd, 80.0% to 95.4% in Cr, 90.3% to 98.4% in Pb, 96.9% to 98.4% in Zn and 71.4% to 85.7% in Ni (Table 2, Fig. 2).

For treatment trial different bacterial densities (0.5 NTU, 0.6 NTU, 0.7 NTU and 0.8 NTU) were fixed to check the maximum degradation capacity. Through the result obtained maximum degradation

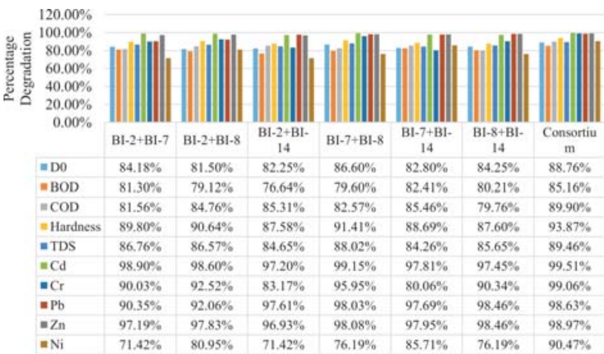

Fig. 2. Percentage degradation in physico-chemical parameters and heavy metals by bacterial dual combinations and consortium

were shown by fixing 0.6 NTU bacterial density i.e. 87.8%, 77.2%, 85.7% 89.2%, 86.1%, 92.1%, 89.0%, 97.8%, 76.1% and 73.5% of DO, BOD, COD, hardness, TDS, Cd, Cr, Pb, Zn and Ni respectively while other bacterial densities showed degradation in the range between 24.5% to 63.9% in DO, 15.6% to 55.3% in BOD, 24.7% to 57.3% in COD, 30.1% to 58.5% in hardness, 39.7% to 57.9% in TDS, 12.5% to 36.4% in Cd, 1.6% to 41.8% in Cr, 40.6% to 76.4% in Pb, 14.2% to 42.8% in Zn, 5.8% to 17.6% in Ni. (Table 3, Fig.3.) Ramasany *et al.* (2012) supported the present findings, in their study two fungal species (*Aspergillus* sp. and *Penicillium* sp.) and three bacterial species (*Bacillus* sp., *Pseudomonas* sp., and *Alcaligenes* sp.) were screened from 35 fungal isolates and 265 bacterial isolates respectively, different microbial combination degrades COD by 75%, Total Solids 90%, TDS 69% and color by 50% from textile waste water.

Ayyasamy *et al.* (2002) observed highest reduction of BOD and COD through aerobic microbial consortium (*Bacillus* sp., *Micrococcus* sp., *Alcaligenes*

Table 2. Treatment trials by different bacterial combinations and consortium

Combinations	DO (3.2)	BOD (36.4)	COD (128)	Hardness (1843)	TDS (624.8)	Cd (0.82)	Cr (0.32)	Pb (1.17)	Zn (0.78)	Ni (0.021)
BI-2+BI-7	20.4	6.8	23.6	186.2	82.7	0.009	0.032	0.113	0.022	0.006
BI-2+BI-8	17.5	7.6	19.5	172.4	83.9	0.011	0.024	0.093	0.017	0.004
BI-2+BI-14	18.2	8.5	18.8	228.8	95.9	0.023	0.054	0.028	0.024	0.006
BI-7+BI-8	24.2	7.4	22.3	158.3	74.8	0.007	0.013	0.023	0.015	0.005
BI-7+BI-14	18.7	6.4	18.6	208.3	98.3	0.018	0.064	0.027	0.016	0.003
BI-8+BI-14	20.5	7.2	25.9	228.4	89.6	0.021	0.031	0.018	0.012	0.005
Consortium	28.7	5.4	12.9	112.8	65.8	0.004	0.003	0.016	0.008	0.002

*Highlighted values indicate initial values i.e. before treatment values, Highlighted values showed maximum degradation capacity by bacterial consortium, All values are expressed in mg/L

Table 3. Treatment trials at different bacterial densities

Parameters	0.5 NTU	0.6 NTU	0.7 NTU	0.8 NTU
DO (3.2)	8.97	26.54	6.24	4.28
BOD (36.4)	16.24	8.29	28.3	30.7
COD (128)	54.63	18.28	78.27	96.3
Hardness (1843)	764.2	198.24	1032.2	1287.2
TDS (624.8)	262.5	86.4	289.7	376.5
Cd (0.82)	0.524	0.065	0.628	0.721
Cr (0.32)	0.984	0.128	1.123	1.153
Pb (1.17)	0.185	0.017	0.243	0.465
Zn (0.78)	0.012	0.005	0.017	0.018
Ni (0.021)	0.028	0.009	0.032	0.032

*Highlighted values indicate initial values i.e. before treatment values, Highlighted values showed maximum degradation capacity by 0.6 NTU bacterial densities All values are expressed in mg/l, NTU= Nephelometric Tubidity Units sp., *Corynebacterium* sp. and *Pseudomonas* sp) isolated from sago factory effluent. Our studies agrees with the findings of Ugoji and Aboaba (2004) who reported maximum degree of reduction in BOD and COD through bacterial consortium (*Micrococcus* sp., *Enterobacter* sp., *Alcaligenes* sp, *Bacillus* sp and *Acinetobacte*r sp.) isolated from industrial effluent. The present data is in accordance to the research work of Singh and Vaishya (2016) who studied the degradation of heavy metals through three defined consortia and seven isolates. Results revealed that

	0.5	0.6	0.7	0.8
DO	63.99%	87.82%	48.23%	24.53%
BOD	55.38%	77.22%	22.25%	15.65%
COD	57.32%	85.71%	38.85%	24.76%
Hardness	58.53%	89.24%	43.99%	30.15%
TDS	57.98%	86.17%	53.63%	39.74%
Cd	36.40%	92.11%	23.78%	12.50%
Cr	16.04%	89.07%	41.80%	1.62%
Pb	76.40%	97.83%	69.00%	40.68%
Zn	42.85%	76.19%	19.04%	14.28%
Ni	17.64%	73.52%	5.88%	5.88%

Fig. 3. Percentage degradation in physico-chemical parameters and heavy metals by different bacterial densities

consortia 3 showed maximum degradation i.e. 93.7% and 81.6% in zinc after 72h and 24h of incubation respectively. Reduction of lead and chromium was found to be 84.3% and 87.6% by consortia 1 and 2 respectively after 72h of incubation. Benazir *et al.* (2010) reported that the chromium content of the tannery effluent was approximately 770 mg/L before treatment but after remediation (*Bacillus subtilis*, *Pseudomonas aeruginosa* and *Saccharomyces cerevisiae*) it is reduced to 5.2-5.7 mg/L.

Identification of bacterial isolates

After preliminary screening, total four bacteria were selected on the basis of maximum degradation capacity. These bacterial isolates were further purified on medium plates (nutrient agar medium). The bacterial isolates were identified through morphological, physiological features and biochemically characterized by fermentation of different sugars, indole production, citrate utilization, methyl red (MR), Voges-Proskauer (VP) tests. The bacterial tests were also characterized by determining the action of catalase, amylase, oxidase and urease enzyme. (Table 4, 5 and 6) Afterwards biochemical tests, identified bacterial cultures were cross examined by the BD-BBL Crystal Identification Autoreader for the identification surety. Hence it identified the bacteria and

Table 4. Morphological and Physiological characteristics of bacterial isolates

Isolates	Colony Characteristics	Gram Reaction	pH	Temperature (°C)
BI-2	Red Color colonies, Ovoid and Small Rods	Negative	6.5 – 7.0	30 – 37°C
BI-7	White Color colonies, Rod shaped	Positive	6.5 – 7.0	30 – 37°C
BI-8	Waxy whitish colonies, Long Rod Shaped	Positive	6.0 – 9.5	17 – 37°C
BI-14	Creamy white slimy colony, Ovoid and Small Rods	Negative	6.5 – 7.0	30 – 37°C

Table 5. Sugar fermentation and IMViC test for identification of bacterial isolates

Biochemical Tests	Bacterial Isolates			
	BI-2	BI-7	BI-8	BI-14
Sugar Fermentation Test				
Glucose	+	+	-	+
Sucrose	+	+	-	+
Lactose	+	+	-	+
Mannitol	-	+	-	+
Fructose	+	+	-	+
IMViC Test				
Indole	-	-	-	-
Methyl Red (MR)	+	+	-	+
Voges Proskauer (VP)	-	-	-	-
Citrate Utilization	+	+	-	+

(*where + =Positive; - = negative)

Table 6. Activities of enzyme shown by selected bacterial isolates

Enzymes	Bacterial Isolates			
	BI-2	BI-7	BI-8	BI-14
Catalase	+	+	+	+
Amylase	+	+	+	+
Oxidase	-	+	+	+
Urease	-	-	-	-

*where + =Positive; - =Negative

showed its purity between 90 to 99%.

The bacteria were identified as- BI-2- *Rhodopseudomonas palustris*, BI-7- *Bacillus subtilis*, BI-8- *Bacillus fusiformis*, BI-14- *Rhodobacter sphaeroides*

Conclusion

Rivers have played a very important role in development of civilization, culture, settlement of urban area thus it plays a critical and crucial role in the prosperity of a nation effecting the different aspects of its economic status. River Yamuna is one of the most sacred still most polluted rivers of India. Therefore, management of river water comprises balancing of water quality and quantity through proper plan and policy instruments. The receiving river water gets polluted with nutrients, organic compounds, metals, and nanomaterials as a result of the random discharge of treated and untreated solid and liquid wastes into water. For the treatment of contaminated river water, several physical, chemical, biological, ecological, and engineering techniques are available. In the present study an attempt has been made to reduce the level of river pollutants through biological method i.e. Effective Microorganisms (EM) technology due to its cost effectiveness and eco-friendly nature. From the results and observation it can be concluded that the quality of Yamuna river water of Agra city is highly contaminated at all sampling sites that may be due to small and large scale industries, disposal of untreated sewage and agricultural sectors. The effective organisms present in EM decomposes the organic matter and converts it to carbon-dioxide and methane; necessary for growth and reproduction. EM technology is eco-friendly as well as moves a step further to protect the environment.

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