Research Article

Bioremediation of Seafood Processing Plant Effluents Using Indigenous Bacterial Isolates

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ABSTRACT

Bioremediation of seafood processing plant effluent by microorganism serves as an effective method to substitute the conventional recovery and removal process of effluent treatment. Waste water samples were collected from five different seafood processing plants in and around Thoothukudi. 40 bacterial strains were isolated from five different seafood plant effluents, in in and around Thoothukudi. These isolates were subject to bioremediation trials with pond water and 9 species were selected based on the morphological, biochemical and biodegradation efficiency. These 9 isolates were the subjected to bioremediation experiments with sea food processing unit effluents and 5 isolates were found to be efficient in bioremediation of seafood processing unit effluents. Among the 5 isolates two isolates were *Bacillus cereus* and one isolate was *Aeromonas veronii*, and one each could be identified belonging to the groups *Bacillus* sp. and *Aeromonas* sp, The identification was based on DNA extraction and PCR amplification of 16s rRNA and sequencing protocol. The identified bacterial were assessed by measuring their percentage of reduction potentials, change in parameters such as BOD, COD, ammonia, nitrate and total nitrogen. Maximum degrading potential has been observed in the *Bacillus cereus* and *Bacillus* sp. compared to other isolates. This study indicates that these microorganisms can be further used for the bioremediation of seafood processing plant effluents.

Keywords: *Bioremediation, Biodegradation, Seafood effluents, Bioremediators*

[I] INTRODUCTION

India has a large network of seafood industry with a total of 465 registered seafood processing industries in all the coastal states of which 42 are located in Tamil Nadu and 33 are located in Tuticorin coast [13]. Wastewaters from seafood processing operations have high Biochemical Oxygen Demand (BOD), fat, oil and grease (FOG), and nitrogen content. Globally, in a year, on an average about 1,110,522 million liters of effluent containing soluble, colloidal and particulate forms are disposed from fish and shrimp processing plants [3] and [17]. The organic content may be as high as 1200-6000 mg/l of BOD_5 and 3000-10000mg/l of COD (eg. Herring processing) or even low, 700mg/l of BOD_5 and 1600mg/l of COD (eg. Tuna

processing) depending on the composition of raw materials and operation processes. Nitrogen and phosphorus are present in minor quantities but the suspended solids are quite high (2000–3000 mg/l) in the wastewater from fish processing plants [22]. The biological treatment of wastewater is one of the most important task that microorganism can perform under human direction. The role of microorganisms present in sewage can be seen in aerobic bacteria bringing about the stabilization of organic matter where protozoa brings about clarification by engulfing bacteria and other suspended matter content [16].

Bioremediation is the term applied to the technologies that accelerate natural processes for degrading or detoxifying harmful chemicals in soil, ground water and waste water. Microorganisms used to perform the function of bioremediation are known as bioremediators. The use of microorganisms mainly bacteria for bioremediation or transform hazardous contaminants is not a new idea because they have been in use since 600 B.C. by the Romans. In fact, bioremediation has been in use commercially for almost 30 years [19]. The first commercial use of bioremediation system was initiated in 1972 to clean up a Sun oil pipeline spill in Ambler, Pennsylvania [14]. Since then bioremediation has become a well-developed tool for cleaning up different contaminants. A successful, cost effective, microbial bioremediation program is dependent on hydro-geologic conditions, the nature of contaminants, microbial ecology and other spatial and temporal environmental factors that vary widely. In a bioremediation process microorganisms use the contaminants as nutrients or energy sources [21]. Bioremediation activity through microbes were stimulated by supplementing nutrients (nitrogen and phosphorus), electron acceptor (oxygen), and substrates (methane, phenol and toluene) or by introducing microorganisms with desired catalytic capabilities [12] and [1]. Some common

microorganism used in the process of remediation are species of *Acromobacter, Alcaligenes, Arthrobacter, Aeromonas, Bacillus, Cinetobacter, Corneybacterium, Flavobacterium, Micrococcus, Mycobacterium, Nocardia, Pseudomonas, Vibrio, Rhodococcus, Sphingomonas* etc.[5], [8], [9]. As compared to other methods it is a more promising and less expensive way for cleaning up contaminated soil and water [4].

[II] MATERIALS AND METHODS
2.1. Collection, Isolation and Identification of bacterial strains
The effluent samples, from five seafood processing plants in and around Thoothukudi, were collected in sterile containers and transported to the lab in an ice box. Initial physico-chemical parameters were analysed. These samples were used for isolation of bacteria and identification for their potential use as bioremediators. Serially diluted samples were plated onto nutrient agar plates and then incubated for 48 h at $30^{o}C$. Discrete bacterial colonies that grew on agar plates were grouped on the basis of the morphological biochemical and molecular characters [7]. The biochemical tests viz., Gram staining, Catalase test, Indole production test, Starch hydrolysis test, Triple Sugar Iron agar (TSI) test, Methyl Red (MR) test, Voges-Proskaeur (VP) test, Nitrate reduction test, Citrate utilization test, Oxidative-Fermentation (OF) test and Motility test were performed for the identification of the isolates [14]. The strain of the genome sequence was analyzed by 16SrDNA identification methods. Gene sequences were compared with those in the GenBank database using BLAST for arrived at the species of the isolates.

2.2. Experimental Design for Degradation studies
In the present study, ammonia (NH_3-N), nitrate (NO_3-N), total nitrogen, BOD and COD

parameters were chosen to understand the biodegradation efficiency.

These parameters indicate the organic load in any effluent. Degradation studies were carried out by estimating the BOD for 5 days incubation period and ammonia (NH_3-N), nitrate (NO_3-N), total nitrogen, COD for every 48 h. All the degradation experiments were conducted using a flocculator. 500ml with a capacity of beakers were taken and in each beaker 250ml of seafood plant effluent was added. 10% of the microbial isolate was added in to each beaker and kept in a flocculator.

Each day sampling was done and the bioremediation effect of selected colonies on the effluent was determined by estimating the change in levels of ammonia (NH_3-N), nitrate (NO_3-N), total nitrogen, COD and BOD_5 values from first to fifth day.

[III] RESULTS AND DISCUSSION
3.1. Quality of seafood effluents

The quality of seafood plant effluents were analysed based on the parameters like ammonia (NH_3-N), nitrite (NO_2-N), nitrate (NO_3-N), phosphate (PO_4-P), chemical oxygen demand and biochemical oxygen demand. The seafood plant effluents showed average ammonia (NH_3-N) level of 8.96 mg/l. On an average nitrite (NO_2-N) level of 85.42 mg/l and nitrate (NO_3-N) level of 0.17 mg/l was recorded.

Similarly the phosphate (PO_4-P), COD and BOD value of 11.21, 10.4 and 158 mg/l was recorded respectively. The average values recorded from five different seafood plant effluents are shown in Fig.1. The total viable plate count is one of the important parameters for determining water quality.

The total viable bacterial counts of different sampling units ranged from 1.4×10^7 cfu/ml to 1.0×10^9 cfu/ml.
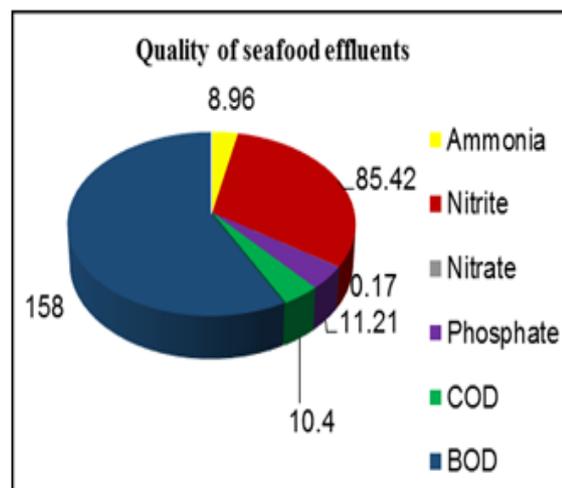

Fig.1 Average physico-chemical value (mg/l) recorded from five different

3.2 Isolation, Screening and Identification of indigenous adapted bacterial strains from seafood processing plant effluents

Forty bacterial strains were isolated from seafood processing plant effluents. These isolates were subject to bioremediation trials with pond water and 9 isolates were selected based on the morphological, biochemical and biodegradation efficiency.

These 9 isolates were taken up for bioremediation experiments with seafood processing unit effluents and 5 isolates were found to be efficient in bioremediation of seafood processing unit effluents. These 5 among the 40 colonies showed better reduction of COD, ammonia, nitrate, BOD_5 and total nitrogen, and were bacterial taken up for identification process based on 16s rRNA gene sequencing and the results have been submitted to the gene bank and accession numbers obtained.

The isolates were identified as *Bacillus cereus* ($SFEB_1$ and B_2), *Bacillus* sp. ($SFEB_3$), *Aeromonas veronii* ($SFEB_4$) and *Aeromonas* sp. ($SFEB_5$). The performance of each of these bacterial strains with respect to bioremediation is given in table1.

Table 1. Biochemical test and bacterial accession number of each bacterial strains

Colony No./ Tests		*Bacillus cereus* (SFEB₁)	*Bacillus cereus* (SFEB₂)	*Bacillus* sp. (SFEB₃)	*Aeromonas veronii* (SFEB₄)	*Aeromonas sp* (SFEB₅)
Colony morphology		Creamy white flat round colony	White circular filamentous colony	Large irregular white colony	Creamy white, convex large circular colony	White large, irregular, lobate colony
Gram staining		-	-	+	-	+
Citrate		-	+	+	-	-
Catalase		+	+	+	+	+
Nitrate reduction		+	-	+	+	+
Indole		-	-	-	+	+
MR		+	+	+	-	+
VP		+	+	+	+	-
Starch hydrolysis		+	+	+	+	-
Oxidative	A	-	-	-	-	-
	N	-	-	-	-	-
Fermentative	A	-	-	+	+	+
	N	-	-	+	+	+
TSIA		A/A	A/A	A/A	A/A	A/A
Motility		+	+	+	-	-
Accession number		KT698866	KT698868	KT698867	KT698865	KT698864

Note:

SFEB	-	Sea Food Effluent Bacteria
MR	-	Methyl Red
VP	-	Voges Proskaeur
A	-	Aerobia
N	-	Anarobic
TSIA	-	Triple Sugar Iron Agar
A/A	-	Acid/Acid

In the present study and *Bacillus cereus* (SFEB₁ & SFEB₂) and *Bacillus sp*. (SFEB₃) showed better reduction of BOD, COD, Ammonia, and Total nitrogen than *Aeromonas veronii* (SFEB₄) and *Aeromonas sp*. (SFEB₅). The studies on bioremediation capabilities of each microorganism reveals that, BOD, COD,

Ammonia and Total nitrogen was reduced by 68.4, 40, 86.4 and 45.16% respectively by *Bacillus cereus* (SFEB1) and 68.40, 20, 91.9 and 68.40 % by *Bacillus* sp.(SFEB3). Another isolate *Bacillus cereus* (SFEB2) showed 91.98, 31.25 and 81.04% reduction of BOD, COD and ammonia. *Bacillus* sp exhibited COD reduction of 13.5 % in sugar mill effluents [2]. In the present study *Aermonas veronii* and *Aeromonas* sp showed BOD reduction of 52.12 and 53.3% respectively. Greater than 50% reduction of BOD using individual isolates like *Bacillus* sp., *Pseudamonas* sp., *Actinomycetes* sp. and *Staphylococcus* sp. in complex (Industrial, Municipal and Agricultural) waste water within a period of 3 days incubation [6].

In fish processing effluents greater than 50% ammonia reduction was observed using *Bacillus* sp. within 5 days of incubation at ambient temperature (28±2 °C) [20]. In the present study, *Aeromonas veronii* increased the percentage change in nitrate concentration from 0.003 to 0.036 (mg/l) within 5 days of incubation. The possible way of nitrate input could be through oxidation of ammonia to nitrate [18]. Similar results have been observed in seafood processing factory [11]. The increase in nitrate levels indicate the efficient conversion of ammonia and other nitrogenous substances into nontoxic nitrates, which is a nutrient and is reflected as increased total nitrogen [10]. Removal of COD, BOD, Ammonia, Nitrate and Total nitrogen values are depicted in Fig.2-6. And these values are tabulated in Table 2 and these values
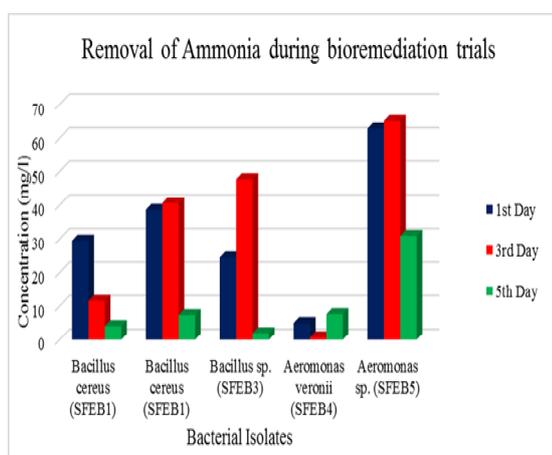
Fig 2. Removal of Ammonia (mg/l) during bioremediation trial for the period of 5 days
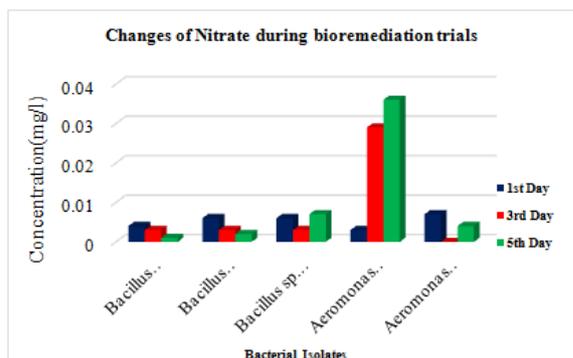
Fig 3. Changes in Nitrate (mg/l) during bioremediation trials for the period of 5 days
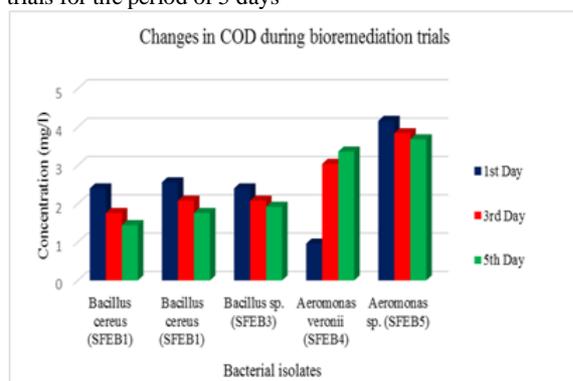

Fig 4. Changes in COD (mg/l) during bioremediation trials for the period of 5 days
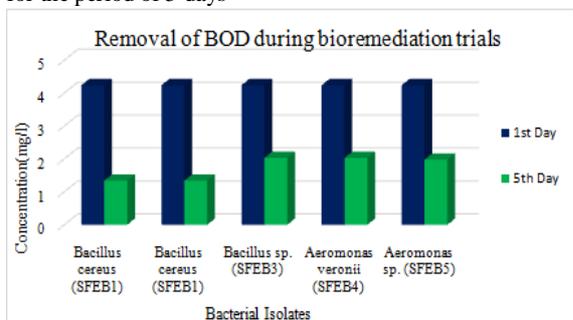

Fig 5. Changes in BOD (mg/l) during bioremediation trials for the period of 5 day
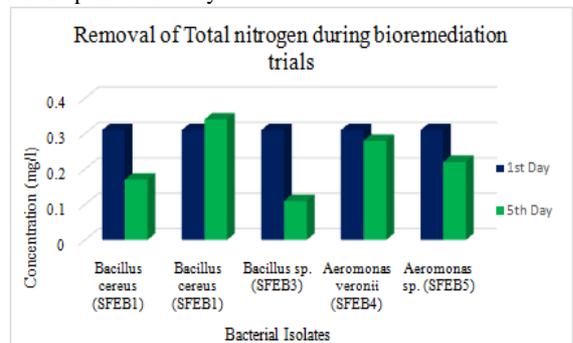

Fig 6. Changes in Total nitrogen (mg/l) during bioremediation trials for the period of 5 days

Colony name	Parameters (mg/l)	1st day (mg/l)	3rd day (mg/l)	% removal	5th day (mg/l)	% removal
Bacillus cereus (SFEB₁)	Ammonia	29.50	11.51	60.9	4.07	86.4
	Nitrate	0.004	0.003	25	0.001	75
	COD	2.40	1.76	26.67	1.44	40
	Total nitrogen	0.31	-	-	0.17	45.16
	BOD5	4.24	-	-	1.34	68.40
Bacillus cereus (SFEB₂)	Ammonia	38.61	40.60	-5.15	7.32	81.04
	Nitrate	0.006	0.003	50	0.002	66.66
	COD	2.56	2.08	18.75	1.76	31.25
	Total nitrogen	0.31	-	-	0.34	-9.67
	BOD5	4.24	-	-	0.34	91.98
Bacillus sp. (SFEB₃)	Ammonia	24.44	47.70	-95.17	1.96	91.9
	Nitrate	0.006	0.003	50	0.007	-16.66
	COD	2.40	2.08	13.33	1.92	20
	Total nitrogen	0.31	-	-	0.11	64.51
	BOD5	4.24	-	-	1.34	68.40
Aeromonas veronii (SFEB₄)	Ammonia	4.99	0.74	85.17	7.62	-52.71
	Nitrate	0.003	0.029	-866.66	0.036	-24.13
	COD	0.96	3.04	-216.67	3.36	-250
	Total nitrogen	0.31	-	-	0.28	9.67
	BOD5	4.24	-	-	2.03	52.12
Aeromonas sp. (SFEB₅)	Ammonia	62.79	65.03	-3.57	30.93	50.74
	Nitrate	0.007	0.009.	-28.57	0.004	42.85
	COD	4.16	3.84	7.69	3.68	11.54
	Total nitrogen	0.31	-	-	0.22	29.03
	BOD5	4.24	-	-	1.98	53.30

Table2. Removal of Ammonia, Nitrate, COD, Total nitrogen and BOD₅ for the period of 5 days

Note:
- Positive value indicates percentage of decrease and negative value indicates percentage of increase
- Total Nitrogen and BOD₅ – values are only for five days.

[V] CONCLUSION

Bioremediation is emerging as the most ideal alternative technology for removing pollutants from the environment, restoring contaminated sites and preventing further pollution. This environment friendly technology contains a range of organisms used for bioconversion, to clean up pollution and to degrade environmental pollutants. Fish processing waste water

management has been one of the serious problems having the greatest impact on the environment. Therefore, the present study was aimed at using bioremediation technology to remediate seafood processing waste water for better usage. Studies on the activities of biodegradation of effluents from seafood processing plant using microbes are in the initial stage, with a bright future application for bioremediation of wastewaters in seafood processing plants. The present study was carried out to identify bacterial strains for bioremediation of seafood processing plant effluent. In this study *Bacillus cereus* and *Bacillus* sp showed better reduction of BOD, COD, Ammonia, and Total nitrogen than *Aeromonas veronii* and *Aeromonas* sp. *Aeromonas veronii* increased the nitrate concentration than the other isolates. This preliminary study indicates that these microorganisms can be successfully employed bioremediation of seafood plant effluents. Further research in this field can result in the development of a viable product of individual or mixed bacterial consortium for the effective treatment of seafood processing plant effluents.

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