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REMOVAL OF AMMONIACAL NITROGEN FROM FISH PROCESSING WASTEWATER USING BIOAUGMENTATION TECHNIQUE

S.S. SARNAIK*[1], V.V. PHALKE[2] AND P.P. KANEKAR[3]

[1]*Microbial Sciences Division, MACS' Agharkar Research Institute, G.G. Agarkar Road, Pune 411004, Maharashtra State, India*
[2]*Present address: Moving Academy of Medicine and Biomedicine, 13 Swastishree Society,* Karve *Nagar, Pune 411052. Maharashtra State, India*
[3]*Present Address: Department of Biotechnology, Modern College of Arts, Science and Commerce, Shivajinagar, Pune 411 005, Maharashtra State, India*

ABSTRACT

Fish are a rich source of proteins beneficial to heart and eyes. Being perishable, they are processed immediately after collection. Water consumption in fish-processing industry is very large and high strength wastewaters rich in salts, ammoniacal nitrogen (NH_3-N), organic load are generated which cannot be discharged without any treatment. The present investigation describes development of bioaugmentation process to reduce the concentration of NH_3-N upto the drinking water level from the wastewater arising from an Up flow Anaerobic Sludge Blanket Reactor. Culture of *Bacillus* sp. was efficient in lowering the NH_3-N content of the wastewater from initial 370 mg/L up to 4 mg/L within 5 days of incubation at ambient temperature (28±2 ºC). At bench scale level, the culture reduced the NH_3-N content upto 60 mg/L from initial 558 mg/L. Thus, *Bacillus* sp. can be used successfully for developing a bioaugmentation process for removing NH_3-N from wastewater generated in fish processing industry.

KEYWORDS : Bioaugmentation process; Fish processing industry; *Bacillus* sp.; Ammoniacal nitrogen (NH_3-N)

***Corresponding author**

S.S. SARNAIK
Microbial Sciences Division, MACS' Agharkar Research Institute, G.G. Agarkar Road, Pune 411004, Maharashtra State, India

INTRODUCTION

Fish are rich in proteins and beneficial to the heart and eyes. Since fish are highly perishable, they have to be processed immediately after collection. Sun drying, salting are the traditional methods of short term preservation of fish, however, freezing is the most commonly used methods. A variety of snack items can be prepared from fish. Water consumption in a fish-processing industry is very high. Therefore cautious use of fresh water is beneficial. Recirculation of wastewater seems to be a good option. High-strength wastewaters generated from fish processing industries, are rich in salts and ammoniacal nitrogen. High concentration of ammoniacal nitrogen is reported to be toxic to living animals causing several biological and physiological changes at cellular level especially in fish[1]. Therefore wastewaters thus generated cannot be discharged without any appropriate treatment. Some clean technologies including nanofiltration, ultrafiltration etc. are reported in the literature[2-5]. However, bioremediation is the method of choice. Aerobic treatment processes such as activated sludge process[6] are suitable for removal of organic matter. Researchers have developed an aerobic and anaerobic treatment processes using various microorganisms [7-14] and their enzyme preparation[15].In the present investigation, attempts are made to develop a microbiological bioaugmentation process for treatment of wastewater from UASB Reactor Outlet with special reference to removal of ammoniacal nitrogen up to ≤ 10 mg/L at bench scale as well as at full scale level at the site of fish processing unit.

MATERIALS AND METHODS

1. *Collection and characterization of wastewater*

Gadre Marine Exports Pvt. Ltd. (GME) Ratnagiri, Maharashtra State, India produces and exports Surimi from ice preserved fish. Large quantity of wastewater (Reactor Inlet - IN) is generated during Surimi production. An Upflow Anaerobic Sludge Blanket (UASB) Reactor is developed for anaerobic treatment of the raw wastewater, designated as Reactor Outlet (RO) which is discharged in aeration tank I (AT I) and aerated with the help of commercial pumps, then passed to another aeration tank (AT II) operated in series and finally discharged in the environment after passage through sand filter. The raw wastewater samples were preserved at -20 °C in the cold room facility developed by GME in their premises and sent to Agharkar Research Institute (ARI), Pune, as and when required. The wastewater was stored in a cold room at 8-10 °C at ARI for further studies. Wastewater samples were analyzed for physical parameters like color, odor, turbidity etc. and chemical parameters like pH, organic matter contents in terms of COD, nitrogen content in the form of nitrate, nitrite and ammoniacal nitrogen as described in Standard Methods[16].

2. *Isolation of microorganisms and their feasibility to reduce ammoniacal nitrogen content of the wastewater*

The bacterial cultures were isolated using garden soil sample from ARI campus, biomass developed by GME and the raw wastewater, by inoculating them on wastewater agar (wastewater solidified with 2 g% agar) Medium. Morphologically distinct isolates were maintained on wastewater agar medium for further studies.The efficiency of these isolates to remove ammoniacal nitrogen from the wastewater was studied by inoculating them in 100 ml unsterile wastewater and incubating under aerated culture condition upto 96 h at ambient temperature (28±2°C) along with uninoculated control. After an appropriate incubation period, the cells were removed by centrifugation at 8500 rpm using a refrigerated centrifuge (Kubota, Japan) and the cell free supernatant was used for estimation of ammoniacal nitrogen content by Nessler's method. The bacterial culture showing maximum removal of ammoniacal nitrogen was identified based on morphological and biochemical characterization[17, 18] and selected for carrying out further studies.

The effect of incubation period, cell density of inoculum, aeration etc. on removal of ammoniacal nitrogen of wastewater was studied in shake flasks (100 rpm) as described earlier, at neutral pH and ambient temperature (28±2°C) upto 72 h by changing one parameter at a time.

3. *Bioreactor studies using selected isolate* at ARI

Bioreactor was developed using a fish tank (glass container) having working volume of 2 L. pH of the wastewater under study was 7.2 and the experiment was carried out at ambient temperature (28±2°C) under aerated culture condition. Cell suspension of *Bacillus* sp. (10% v/v) was inoculated and samples were analyzed daily for estimation of nitrate, and nitrite as described earlier. Nitrification efficiency of the organism in the reactor was determined as described by Wittebolle *et al*[19].

4. Development of bench scale bioaugmentation process using Bacillus sp. in the laboratory at the site of GME

Bench scale experiment was carried out using 5 L plastic containers in the laboratory of GME. Culture of *Bacillus* sp. was grown in 3 L wastewater under aerated culture condition by maintaining cell density as 10^9cells/ml and added daily in AT I in one experiment and also in another aeration tank (AT II) operated in series, in another experiment. The experiments were carried out for one month (30 days). The samples were analyzed daily for ammoniacal nitrogen content as described earlier.

5. Studies on bioaugmentation using Bacillus sp. at full scale level at plant site of GME

In order to study the removal of ammoniacal nitrogen from the waste water at full scale level, at the site of plant of GME, the culture of *Bacillus* sp. was grown in 10,000 L wastewater under aerated culture condition by maintaining cell density as 10^9cells/ml and added in the aeration tank AT I in one experiment and in AT II in another experiment. The experiments were carried out for about a period of one month (34 days). The samples were analyzed daily for ammoniacal nitrogen content as described earlier.

RESULTS

1. *Characterization of the wastewater samples*

The wastewater samples were characterized for physical as well as chemical parameters as detailed in Table 1.

Table 1
Characterization of fish processing wastewater

Parameter under study	Raw Inlet (IN) wastewater	Reactor outlet (RO)
Physical parameters		
Colour	Off – white (cream color)	Whitish
Odour	Fishy	Slight fishy
Turbidity	Highly turbid	Slightly turbid
Chemical parameters		
pH	7.45	7.7
Total solids, mg/L	7600	2400
COD, mg/L	5000	173
Ammoniacal nitrogen, mg/L	668	509
Nitrate, mg/L	73.49	3.85
Nitrite, mg/L	-	0.027

The wastewater had fishy smell and was turbid in appearance with total solids as high as 7600 mg/L. Both the inlet and outlet wastewaters were neutral in nature. COD and ammoniacal nitrogen content of IN wastewater were around ~5000 and 668 mg/L respectively and that of RO wastewater were 173 and 509 mg/L respectively. The UASB treatment seems to be useful in reducing COD and total solid content from the wastewater, however it is not efficient in removing ammoniacal nitrogen content.

2. *Isolation of microorganisms and their feasibility to reduce ammoniacal nitrogen content of the wastewater*

In all, 10 isolates were able to grow luxuriously on the wastewater agar medium. *Bacillus* sp., were found to be more predominant among the isolates. Efficiency of these isolates to remove ammoniacal nitrogen from the wastewater is detailed in Table 2.

Table 2
***Efficiency of the isolates in removing ammoniacal nitrogen from wastewater* after 96 h**

Isolate under study	Concentration of ammoniacal nitrogen, mg/L	% removal
Initial	370	
Uninoculated control	330	11
VA	93	75
VB	33	91
VC	4	99
VD	252	32
VE	100	73
VF	22	94
VG	96	74
VH	211	43
VI	111	70
VJ	233	37

It was observed that all the bacterial isolates could remove ammoniacal nitrogen from the wastewater in the range between 32-99 %, maximum reduction was exhibited by the isolate VC followed by the isolates VF and VB. The indigenous flora of the wastewater also contributed 11 % reduction.

The culture VC was found to be Gram positive spore bearing rod and was identified as Bacillus sp. using conventional approach of studying morphological, physiological and biochemical characteristics. The culture was facultative in nature growing under both aerobic as well anaerobic

conditions, able to grow in the temperature range between 10°C – 45 °C and could tolerate the salt concentration upto 2 g% NaCl. This culture was selected for further studies.

3. *Flask level studies using the selected culture of Bacillus sp*

Results on the effect of incubation period, cell density of inoculum, aeration etc. on removal of ammoniacal nitrogen of wastewater are detailed in Tables 3, 4 and 5 respectively.

Table 3
Removal of ammoniacal nitrogen at different incubation period

Incubation period, h	Ammoniacal nitrogen, mg/L	% removal
0	380	
24	95.5	75
48	36.4	90
72	12	97

The results showed that the culture of Bacillus sp. could reduce ammoniacal nitrogen upto ***12 mg/L from the initial 380 mg/L within 72 h*** which ***was nearer to the desired value of 10 mg/L.***

Table 4
Effect of cell density on removal of ammoniacal nitrogen

Cell density of inoculum, cells/mL	Ammoniacal nitrogen, mg/L						
	0 h	24 h	% removal	48 h	% removal	72 h	% removal
1×10^9	385	101	74	53	86	11	97
1×10^8	385	167	57	97	75	31	92
1×10^7	385	198	49	124	68	74	81
1×10^6	385	266	31	116	70	88	77

The results showed that the removal of ammoniacal nitrogen increases as the cell number increases. With cell number of $1x10^9$ cells/ml, maximum removal was observed, from initial 385 mg/L to 11 mg/L which was nearer to the desired value of 10 mg/L.

Table 5
Effect of aeration on removal of ammoniacal nitrogen

Incubation period, h	With Aeration		Without Aeration	
	Ammoniacal nitrogen, mg/L	% removal	Ammoniacal nitrogen, mg/L	% removal
0	380		380	
24	196	48	342	10
48	101	73	329	13
72	9	98	295	22

Aeration enhanced removal of ammoniacal nitrogen from the wastewater. After 72 h, the residual ammoniacal nitrogen content was only 9 mg/L from initial 380 mg/L which attained the required value of 10 mg/L. However, in absence of aeration, there was no appreciable removal indicating necessity of ***aeration.***

4 . Bench scale Bioreactor studies

The results on removal of ammoniacal nitrogen from the wastewater in bioreactor and oxidation of ammonia to form nitrate through nitrite are elaborated in Figure 1.

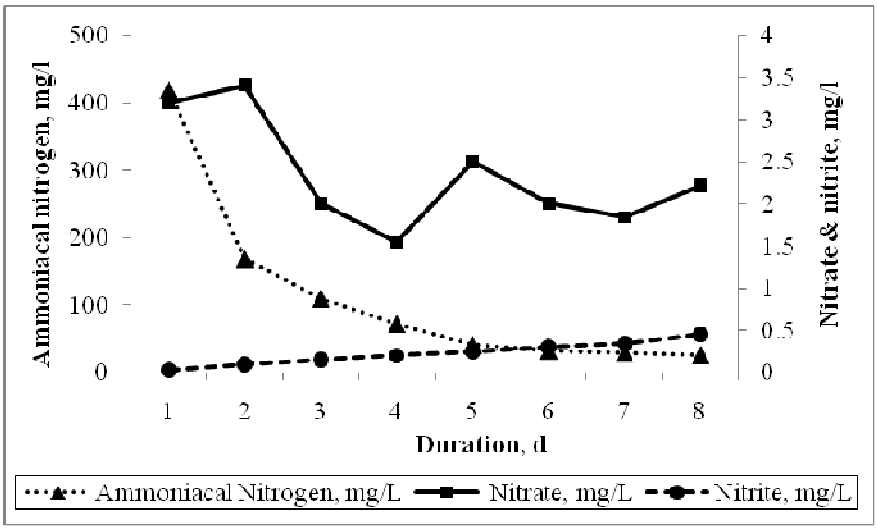

Figure 1
Nitrification *efficiency of isolate Bacillus sp. in bioreactor*

The results showed that there is fast removal of ammoniacal nitrogen from the wastewater upto 96 h, then the rate was slowed down and was almost constant upto 168 h. Nitrification efficiency of Bioreactor using culture of *Bacillus* sp. is 94 % which seems to be quite high, indicating good performance of the culture of *Bacillus* sp. in the bioreactor.

5 Bioaugmentation process using Bacillus sp. at bench scale level in the laboratory at site of GME

The results on removal of ammoniacal nitrogen from the wastewater after addition of *Bacillus* sp. in aeration tanks AT I and AT II in bench scale reactor at site are detailed in Figure 2.

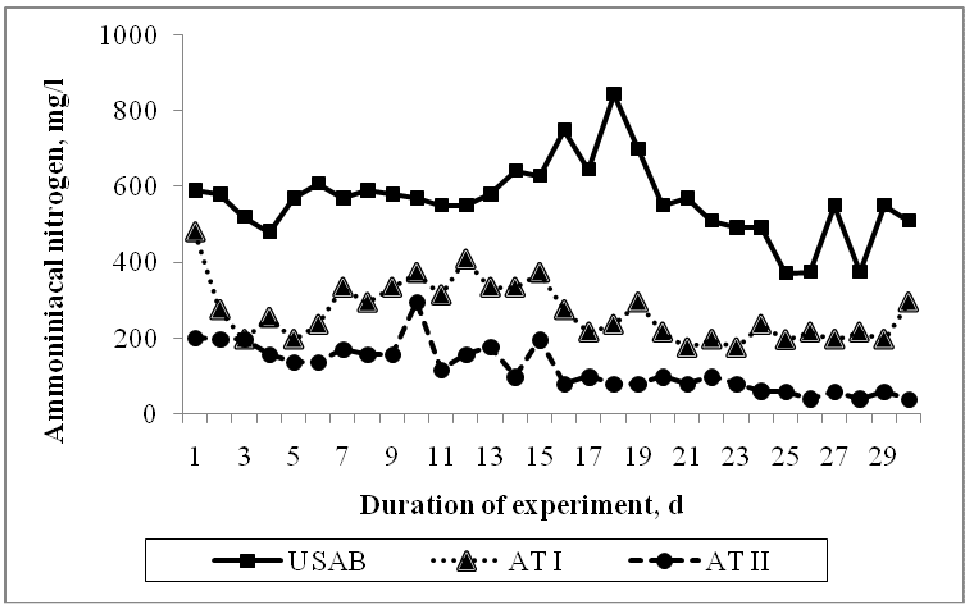

Figure 2
Removal of ammoniacal nitrogen by Bacillus sp. bioaugmented at bench scale level in the laboratory at GME

It can be seen that the ammoniacal nitrogen content was reduced from initial 558 mg/L to 267 mg/L i.e. ~52% when the culture of *Bacillus* sp. was added in AT I. After addition of culture in AT II operated in series, the ammoniacal nitrogen content was reduced further upto 114 mg/L thus resulting in further reduction of 57% thus resulting in a total reduction of ~80%. In case of AT II, the residual ammoniacal nitrogen content was >100 mg/L i.e. in the range 295 – 118 mg/L with the average of 167 mg/L for initial 17 days and afterwards the residual ammoniacal nitrogen content was <100 mg/L i.e. in the range between 88 – 39 mg/L with the average of 60 mg/L. This indicated the establishment of culture in the tank AT II. The value is nearby the limit (50 mg/L) laid down by Central Pollution Control Board (CPCB) for discharge of wastewater in the environment.

6 Bioaugmentation using Bacillus sp. in full scale plant at the site of GME

The results on removal of ammoniacal nitrogen from the wastewater after addition of culture of *Bacillus* sp. in aeration tanks AT I and AT II at site at full scale level are detailed in Figure 3.

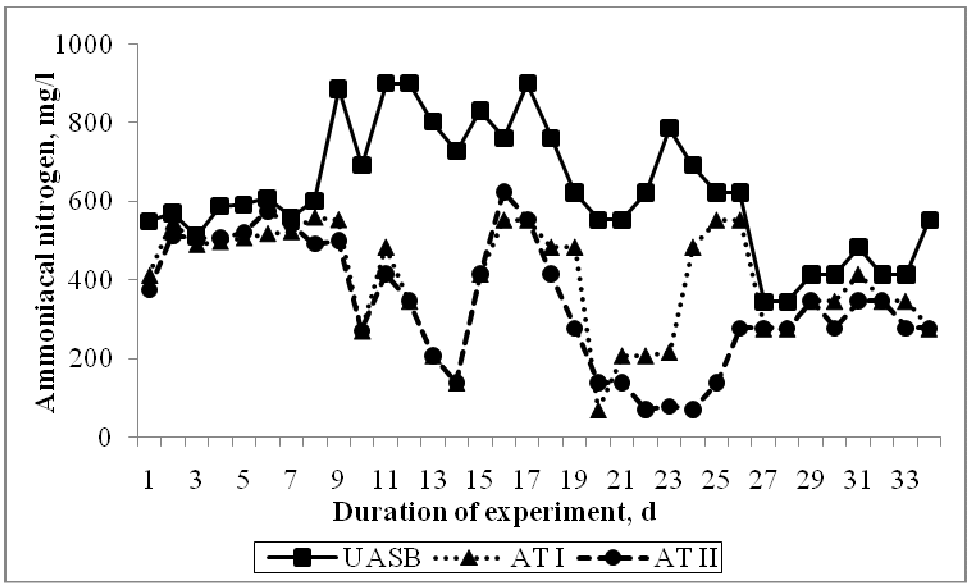

Figure 3
Removal of ammoniacal nitrogen by Bacillus sp. bioaugmented at full scale plant at the site of GME

The results showed that there was removal of ammoniacal nitrogen from initial 624 mg/L to 396 mg/L (37%) when the culture of *Bacillus* was added in tank AT I and further there was only 15% reduction when the culture was added in AT II. The results indicate that at the full scale level some more period is required for establishment of the culture in both the Aeration Tanks to attain the value below the permissible limit of CPCB.

DISCUSSION

Fish processing waste management, has been one of the problems having the greatest impact on the environment. Wastewater from fish processing industry**,** treated in UASB Reactor, reached the COD below the prescribed limit of Central Pollution Control Board (CPCB) for COD. The Indian standard specifications (IS 10500) have the limits for ammoniacal nitrogen as 50 mg/L. Since the fish processing is a batch process, the ammoniacal nitrogen content of the wastewater varies and its removal was low which could not attain the prescribed limits. In the present studies the ammoniacal nitrogen content of the RO wastewater was ~370 mg/L

which was high as compared to prescribed limits. Therefore, it was necessary to remove ammoniacal nitrogen from the wastewater. The microbial culture of *Bacillus* sp. was found to reduce ammoniacal nitrogen from the fish processing wastewater upto ≤ 10 mg/L from initial ~ 500 mg/l. Members of genus *Bacillus* are aerobic organisms requiring oxygen for their growth. Some species of *Bacillus* can utilize ammonia via both heterotrophic and chemotropic pathways[20]. This may be the reason for enhanced removal of ammoniacal nitrogen from wastewater only after addition of the culture of *Bacillus* sp. Reports are available on degradation of different wastes by *Bacillus* sp.[21,22]. Removal of ammoniacal nitrogen by microbial cultures including algae is reported[23-26].Modern technologies like nanofiltration, ultrafiltration are not economically feasible and cannot be applied by small scale fish processing units. Thus the biological process like anaerobic treatment followed by aerobic treatment involving bioaugmentation using the culture of *Bacillus* sp. seems to be a good and economic solution for treating the fish processing industry wastewater containing high ammoniacal nitrogen content.

CONCLUSION

Fish, a rich source of high grade proteins, are highly perishable. They have to be processed immediately after collection. Water consumption in fish-processing industry is very high, recirculation of wastewater seems to be a good option. High-strength wastewaters are generated from fish processing industries, rich in salts and ammoniacal nitrogen. *Bacillus* sp. can remove ammoniacal nitrogen from wastewaters generated in fish processing unit. In spite of modern clean technologies, the biological processes like anaerobic treatment followed by aerobic treatment seems to be a good and economic solution for treating the fish processing industry wastewater containing high ammoniacal nitrogen content.

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