Recirculating Aquaculture System: Microbial Treatment of Aquaculture Waste Water for Plant Irrigation

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Abstract

Aquaculture has been considered as an option to cope with the world food demand and India is the 2nd largest producer of aquatic animals globally. Though the Indian fisheries and the aquaculture serves as a major sector in food production it also has its own negative impact on the fishes and the environment. One of the environmental impacts that receives the most attention is the issue of nutrient depletion and effluent buildup. Ammonia is one of the major compound present in the effluent which results in toxicity. For every ton of fish, aquaculture operations produce between 42 and 66 kilograms of nitrogen waste. In the present study, the recirculating of aquaculture wastewater is done by converting the ammonia (NH_3^+) content to harmless nitrogen gas (via nitrite)with the aid of Nitrifying and Denitrifying bacteria which was isolated from the rhizosphere soil. Both the cell free supernatant and cells has the ability of reducing ammonia. The wastewater was collected from the aquaculture industry, Madurai, which contains 17µg/mL of ammonia, BOD –42 mg/L and COD – 96 mg/L. The ability of nitrifying and denitrifying bacteria on the simulated aquaculture waste water was analyzed. For nitrification, the cell free supernatant reduces 96.35% ammonia at 90th min. On the other hand, the cells reduce 90.98% ammonia at 210th min. In case of denitrification process, the cells reduce 30% nitrate content at 90th min whereas the cell free supernatant reduces 67.6% nitrate content at 180th min. The ability of immobilized crude enzyme and the cells were studied periodically. The aquaculture wastewater was treated and the characteristics of the treated simulated water were analyzed. The treated water can be used for irrigation purposes.

Keyword - *Aquaculture waste water, Recirculating aquaculture system, nitrification, denitrification, bacteria.*

I. INTRODUCTION

Aquaculture is growing more rapidly than all other animal food producing sectors and India is the 2nd largest producer of aquatic animals globally and secure 3rd position in fisheries. Aquaculture has been considered as an option to cope with the world food demand. Though the Indian fisheries and the aquaculture serve as a major sector in food production it also has its own negative impact on the fishes and the environment. In order to meet the future demand using traditional technologies, the industry will have to utilize more land and water. The lack of space for expansion, limited fresh water availability, and concerns over pollution are the key obstacles faced by the industry (Badiola, M et al, 2012)

The key to the success of the aquaculture relies on the maintenance of water quality. One of the environmental impacts caused by the aquaculture industry that receives the most attention is the issue of nutrient depletion and effluent buildup. Ammonia is one of the major compound present in the effluent which results in toxicity. If there is an increase in the ammonia concentration, the fish will be lethargic, fall into coma and eventually die. For every ton of fish, aquaculture operations produce between 42 and 66 kilograms of nitrogen waste (Knoll S et al, 2001).

The strategy of recirculation can tackle the problem faced by the industries. There are two process involved in converting ammonia into a harmless compound – Nitrification and Denitrification. In the Nitrification process, ammonia is oxidized to nitrites whereas the denitrification process involves in converting the nitrites and nitrates into harmless N_2 gas.

Nitrifying bacteria	Denitrifying bacteria

NH_4 ----------> NO_2/NO_3----------> N_2

This process is done with the aid of nitrifying and the denitrifying bacteria. In the present study, both the nitrifying and denitrifying bacteria were isolated from the soil which has the capability of reducing the ammonia content present in the aquaculture waste water. After treatment, the aquaculture water is used for the irrigation purpose as well as for the cultivation of fishes.

II. MATERIALS AND METHODOLOGY

A. Winogradsky media

Mineral salt media (MSM) which consists as follows: NaCl, 0.3 g.; $MgS0_4.7H_20$, 0.14 g.; $FeS0_4.7H_20$, 0.03 g.; $(NH_4)_2S0_4$, 0.66 g.; dissolved in 90 ml. glass-distilled water. KH_2 PO4 (0.1M), 10 ml. (previously boiled); 'A-Z' trace element mixture, 1 ml., total volume, 101 ml (Meiklejohn, J. (1950))

B. Ammonia Estimation

K-Na Tartarate: (Rochelle salt solution) - 50gram KNa -Tartarte in 100 ml dH_2O (free ammonia) you can boil this solution to expel ammonia Nessler Reagent: A-(100 gram Hg I_2+70 gram KI) dilute in small quantity of NH_4^+ free water (about 50 ml) B-(160 gram NaOH dilute to 50 ml (NH_4^+ free) water. Add (A) to (B) stir gently, dilute the final volume to 1 liter, Store in borosilicate bottle and out of sunlight-it will stay about 1 year

Procedure:

To the sample water, add 1 ml of KNa Tartarate (Filter before use (KNa Tartarate). Add 1 ml of Nesslers reagent, wait for 5 minutes. Read at 425 nm

C. Nitrate Estimation

Salicylic acid-H_2SO_4 Dissolve 5g of salicylic acid in 100 mL of conc H_2SO_4. The salicylic acid-H_2SO_4 reagent should be made fresh every week and stored in a brown bottle. Nitrate standards should be stored at 4C.

Procedure:

To the sample, mix thoroughly with 0.8 mL of 5% (w/v) salicylic acid in conc. H_2SO_4. After 20 minutes at room temperature, add 19 mL of 2 N NaOH to raise the pH above 12 Cool samples to room temperature. Measure absorbance at 410 nm

III.ISOLATION OF NITRIFYING AND DENITRIFYING BACTERIA

For Nitrification, rhizosphere soil was collected from the nearby the roots of the leguminous plant. It was then inoculated into the Winogradsky media or Mineral salt media (MSM) which consists as follows: NaCl, 0.3 g.; $MgS0_4.7H_20$, 0.14 g.; $FeS0_4.7H_20$, 0.03 g.; $(NH_4)_2S0_4$, 0.66 g.; dissolved in 90 ml. glass-distilled water. KH_2 PO4 (0.1M), 10 ml. (previously boiled); 'A-Z' trace element mixture, 1 ml., total volume, 101 ml (Meiklejohn, J. (1950)). The media was sterilized by autoclaving for 15 min. at 15 lb. After serial dilution and continuous subculture, the colonies are streaked on the agar plate and single type of colonies were observed. Gram's staining was performed for the confirmation which results in a positive way – Gram negative and rod shaped bacteria.

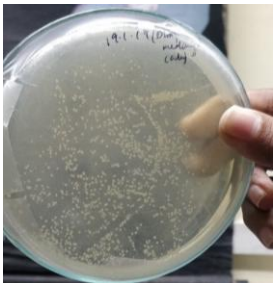

Fig:1.a Single colonies Were observed in 24 hr Culture

Fig.1.b Gram negative and rod shaped bacteria was observed in 100X magnification.

For denitrification, *Pseudomonas auerogenosa AMB AS7* was isolated from the soil which was obtained from our senior research group.

IV. EFFICIENCY OF AMMONIA REDUCTION:

For nitrification, the nitrifying media culture was centrifuged at 9000 rpm for 5 minutes. Both the cells and cell free supernatant was inoculated in the ammonium chloride to verify its efficiency of ammonia reduction. Ammonia estimation was done at regular interval of time. The cell free supernatant reduces 96.35% ammonia at 90th min. On the other hand, the cells reduce 90.98% ammonia at 210th min.

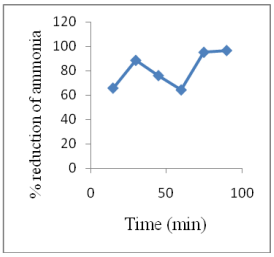

Fig.2.a % of reduction of ammonia by cell free supernatant of nitrifying bacteria.

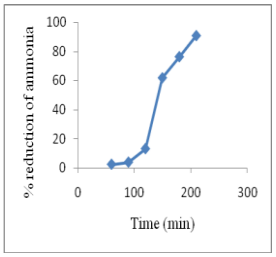

Fig.2.b % of reduction of ammonia by cells of nitrifying bacteria.

For denitrification, Sodium nitrate solution was used and the cells reduce 30% nitrate content at 90th min whereas the cell free supernatant reduces 67.6% nitrate content at 180th min.

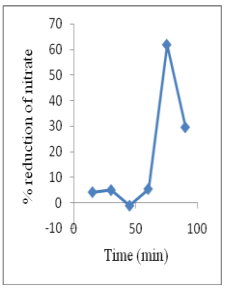

Fig 3.a. % reduction of nitrate by cell free supernatant of Pseudomonas

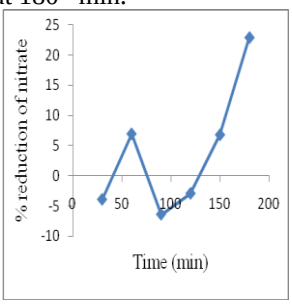

Fig.3.b % reduction of nitrate by the cells of Pseudomonas

From this observation, we can inferred that both the cells and the supernatant has the ability of reducing ammonia, but the culture supernatant is more efficient in reducing ammonia content.

V. TREATMENT OF AQUACULTURE WASTEWATER

The wastewater was collected from the aquaculture industry, Madurai and the characterization was done

TABLE I
Characterization of aquaculture wastewater

Parameters	Result	Permissible limit
pH	9.04	6.5-8.5
Turbidity	88.78% (transmittance)	-
Ammonia content	17mg/L	0.1 mg/L
BOD	42mg/L	<6ppm
COD	96mg/L	<50 ppm

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Permissible limit - Water quality guidelines for the management of pond fish culture (Anita Bhatnagar et al, 2013)

The wastewater was treated with both the cells and the supernatant. The nitrifying bacteria's cell free supernatant reduces 51.99% of ammonia at 315th minute and the cells reduce 84.04% of ammonia at 270th minute.

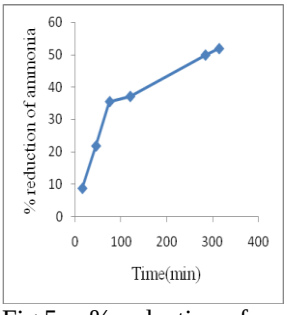

Fig.5.a. % reduction of ammonia by the cell free supernatant of nitrifying bacteria

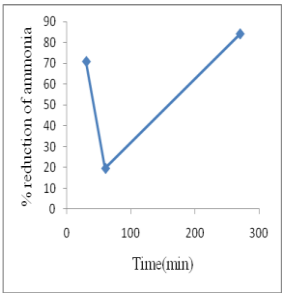

Fig.5.b. %reduction of ammonia by the cells of nitrifying bacteria

The wastewater was taken and the culture was inoculated in three different ways.

- Treated water I- Nitrifying bacteria supernatant + Pseudomonas cells
- Treated water II- Nitrifying bacteria cells+ Pseudomonas supernatant
- Treated water III- Nitrifying bacteria cells + Pseudomonas cells

The water was then kept incubated at room temperature for overnight and the ammonia estimation was done on the next day

TABLE III
Ammonia reduction estimation of wastewater
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Sample	% of reduction of ammonia
Treated water I	50.11%
Treated water II	30.68%
Treated water III	5.81%

VI. EFFECT OF TREATED WASTEWATER IN PLANTS

Wastewater was taken in a three separate flask and the three combinations of culture was added.

- Treated water I- Nitrifying bacteria supernatant + Pseudomonas cells
- Treated water II- Nitrifying bacteria cells+ Pseudomonas supernatant
- Treated water III- Nitrifying bacteria cells + Pseudomonas cells

It was then kept for overnight incubation and then the effect of treated water is analyzed by spraying it on the *Vigna radiata* (green gram). Six different petri plates were taken and marked as- control(tap water), untreated water, treated water I, treated water II, treated water III. Cheese cloth was wrapped around the cotton and placed on the petriplate. 5 seeds were taken and placed on the cheesecloth and 5 mL of water is sprayed. It was then kept undisturbed for overnight. The germination of the green gram was observed on the next day.

A. Germination percentage estimation

Seed germination percentage was calculated for each plate based on the primary root emergence from the seeds and the results were expressed in percentage based on the following equation. (Huy, V.,et al, 2018)

$$\text{Germination Percentage} = \frac{\text{Total number of seed germinated}}{\text{Total number of seed treated}} \times 100$$

B. Seeding length estimation

The root length was determined by the primary seed radical. Seeding length results were expressed as centimeter.

TABLE IIIII

Observation of germination of green gram

Sample	Germination percentage	Root Length (cm)
Control	100%	0.8,0.6,0.4,0.3,0.5
Untreated	60%	0.3, 0.7, 0.5
Treated water I	60%	0.6, 0.3, 0.5
Treated water II	40%	0.1,0.2
Treated water III	60%	0.4,0.5,0.9

VII. RESULT AND DISCUSSION

The overall aim of this study is to reduce the ammonia content present in the aquaculture wastewater which can be reused for plant irrigation purpose as well as for the cultivation of fishes. To achieve the objective, nitrifying bacteria and the denitrifying bacteria were isolated from the soil. Its efficiency of ammonia reduction is estimated using ammonium chloride solution of concentration 10mg/L which results in the positive way– for nitrification, the cell free supernatant reduces 96.35% ammonia at 90^{th} min. On the other hand, the cells reduce 90.98% ammonia at 210^{th} min. In case of denitrification process, the cells reduce 30% nitrate content at 90^{th} min whereas the cell free supernatant reduces 67.6% nitrate content at 180^{th} min. Then the aquaculture wastewater was treated-the nitrifying bacteria's cell free supernatant reduces 51.99% of ammonia at 315^{th} minute and the cells reduce 84.04% of ammonia at 270^{th} minute. To understand the better efficient composition wastewater was treated in three different way- treated water I, treated water II, treated water III and the treated samples was further studied on the green gram to observe the germination and root length. On comaparison, the combination of nitrifying bacteria supernatant (crude enzyme) and the denitrifying bacteria cells produce better results in reducing ammonia content and in germination. The plant irrigation studies will be extended further and the immobilization techniques will be adopted for efficient recycle of aquaculture wastewater

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