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BIOREMEDIATION OF AQUACULTURE WASTEWATER USING PHOTOSYNTHETIC BACTERIA

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ABSTRACT

Aquaculture plays a significant role in the provision of food to ever growing population. But high amount of toxic compounds like ammonium produced as a bye-product of fish metabolism are detrimental to both the cultured fish and surrounding environment. In this view, photosynthetic bacteria were isolated and their ability to remediate aquaculture wastewater was assessed. The aquaculture wastewater was characterized and all wastewater parameters were found to be above the standard discharge limit provided the environmental protection act. The photosynthesis activity of the isolates were determined by detecting the presence of bacteriochlorophy*a* and carotenoid pigments at 800 and 865 nm respectively. The isolated bacteria were found to remove ammonium, nitrite and nitrate from the wastewater. One of the isolate was characterized by molecular technique using 16S rRNA analysis and Identified as *Rhodobacterspheroides* ADZ101. Hence this group of bacteria can provide cheap alternative means of removing toxic nitrogenous compounds from aquaculture wastewater.

Keywords: Bacteriochlorophyll, carotenoid, nitrogenous compounds, *Rhodobacterspheroides* ADZ101

INTRODUCTION

Aquaculture industries have received enormous development in recent years due to the increase in demand for food to meet the world population growth. It is estimated that fish produced from aquaculture accounts for more than one-quarter of all fish consumed by man. It is also vital in the preservation of certain fish species and reducing the rate of their extinction by relieving pressure from wild fish [1]. Furthermore aquaculture has been shown to play a significant role in carbon sequestration. The annual rate of carbon burial in aquaculture is estimated to be higher than that of natural lakes and inland seas but lower than that of large rivers and small agriculturally-eutrophic impoundments [2].

Despite the above mentioned roles of aquaculture, its rapid development recently increases its impact to the environment. Aquaculture wastewater exerts two major impacts to the environment. It affects the production process itself by generating toxic compounds that are detrimental to the growth and survival of the cultured fish and polluting the surrounding environment when discharges are not treated. This impact is similar to industrial and sewage wastewaters discharge without any form of treatment, causing oxygen depletion, saltation, eutrophication [3] and other forms of pollutions to the environment. Its high level of nutrients due to the excess feeds may likely encourage the growth of pathogenic microorganisms in the surrounding water bodies leading to the possibility of an outbreak of contagious disease.

Furthermore, most of the aquaculture industries do not have any wastewater treatment plant before discharging to the nearby water bodies. Some only provide sedimentation ponds

where discharges are retained for days before releasing into the nearby water body. This form of treatment is only effective in reducing suspended particles while other forms of pollutants that are detrimental to the environment are released to the nearby water bodies. Therefore for the sustainability of both aquaculture industries and the surrounding environment, an effective and cheap wastewater management system must be established and maintained. Biological treatment offers a better solution to the conventional treatment methods in terms of cost and *in-situ* application. Nevertheless, the successful application of bioremediation process is very dependent on the microorganism exploited in the systems. For that reason, this research is intended to discover and to manipulate photosynthetic bacteria that presence in aquaculture environment for treatment of toxic nitrogenous compounds contains in aquaculture wastewater.

MATERIALS AND METHODS

Sampling sites

Wastewater samples were collected from an aquaculture shrimp farm from two different ponds (name withheld for ethical reasons). The first pond contained fresh wastewater from a single pond while the final pond contained wastewater stored for several days from various ponds. *In situ* temperature and pH of the first pond were recorded at 32 °C and 6.74, respectively. All samples were stored in 4 litre containers and transported in ice to the laboratory and later refrigerated at 4°C.

Wastewater analysis

All wastewater analysis was conducted based on the standard method for the examination of water and wastewater

provided by the American Public Health Association (APHA) [4]. COD was determined by open reflux method, Total Nitrogen by persulfate digestion method, Ammoniac Nitrogen by Nessler method, Nitrate by cadmium reduction method and Nitrite by ferrous sulfate method.

Isolation of bacteria

Three different enrichment media were used for the isolation of the bacteria. (a) purple non sulphur bacteria enrichment media consisted of 1.0g of NH_4Cl, 0.5g of $NaHPO_4$, 0.2g of $MgCl_2$, 0.2g of NaCl, 2.0g of Yeast extract and 6mL of 80% Sodium lactate at pH7[5]. (b) G5 medium consisted of 5g of Peptone, 5g of Yeast extract, 4g of L-glutamic acid, 3.2g of Malic acid, 0.12g of KH_2PO_4 and 0.18g of K_2HPO_4 at pH7. (c) Succinate medium consisted of 0.33g of K_2HPO_4, 0.33g of $MgSO_4.7H_2O$, 0.33g of NaCl, 0.5g 0f NH_4Cl. 0.5g of $CaCl_2.2H_2O$, 1.0g of Sodium succinate and 1.0g of Yeast extract at pH7[6]. The ingredients for each medium were weight appropriately and 1 L of distilled was added. The media were mixed thoroughly and pH adjusted before autoclaving at 121°C for 15 minutes. For the solid medium, 15g of agar was added and was allowed to cool down at room temperature before pouring onto the plate. Using 10% (v/v), the samples were inoculated into the three different broth media in universal bottles and incubated under facultative anaerobic condition with light intensity between 2000-3000 lux at room temperature. The samples were left for days until the media completely turn read in colour before it was harvested by serial dilution and spread plating followed by subsequent streaking.

Identification of the Isolates

The isolates were firstly subjected to nitrate reductase test following the protocol described by [7] and the photosynthetic pigment analysis was determined according to a method used by [8]. One of the isolate (PSD) was further identified using a molecular technique and reported somewhere else [9].The denitrification potential of PSD was further investigated by successful amplification of the reductase gene (napA and nirK) as reported [9]

RESULTS AND DISCUSSION

Waste Water Analysis

Water quality parameters such as COD, BOD, ammoniacal and total nitrogen were analysed in less than 12 h after sampling. It was observed that wastewater from the first pond appeared clearer and more homogenous due to the continuous mixing when compared to the final pond which appeared darker with many suspended particles. As a result of this homogeneity, the first pond was selected for the treatment. The amount of all wastewater parameters analysed, except pH were found to be higher compared to the required discharge limits. This justified the need for the treatment. Table 1 gives the amount of water quality parameters of the two ponds in comparison with standard effluent discharge limit as described by the environment protection act[10].

Isolation and Identification of the Isolates

Phototrophic bacteria are found in nearly all aquatic environments. They can be easily recognized by their ability to form a "bloom". Their diversity in aquatic environment is likely due to their versatile metabolic activity. They can photoassimilate variety of organic compounds hence their

presence depends on the extent to which the aquatic environment is polluted with organic matter[11]. Determination of photosynthetic pigment was the first criteria used for screening of photosynthetic bacteria in this study. Photosynthetic pigments are present in all photosynthetic organisms as light harvesting centre. However, these pigments differ in different organisms. While plants, green algae and cynobacteria possess chlorophyll used for absorption of light during photosynthesis, phototrophic bacteria use bacteriochlorophyll (s) instead for the same purpose. Bacteriochrophyll*a* with absorption peaks between 800 and 865 nm and carotenoid related to the spheroidene series with peaks ranging between 376-589 nm were obtained.

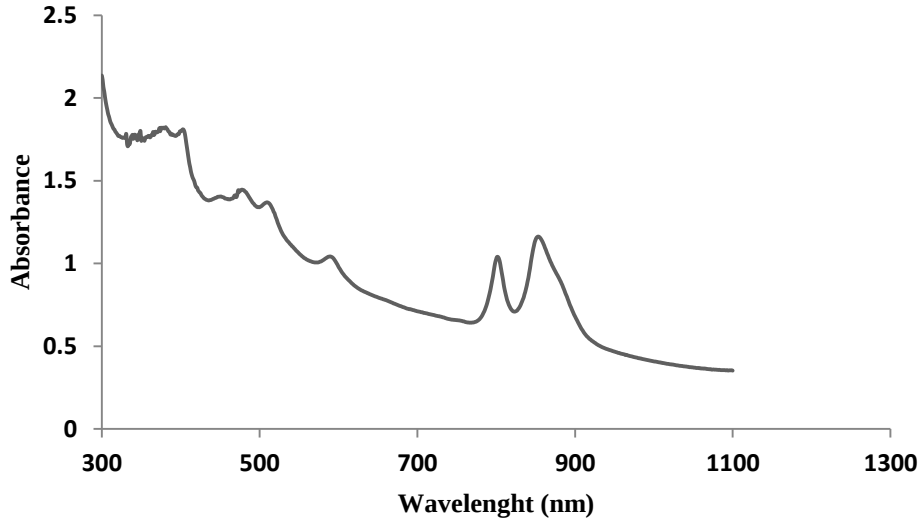

Fig. 1 Absorption spectrum showing photosynthetic pigments of sample PSC

Nitrogen removal from aquaculture wastewater

It was observed that all the screened bacteria were unable to grow on aquaculture wastewater without the addition of a carbon sources. For this reason, 1.0 g/L of sodium succinate was added to the wastewater as external carbon source.

Under this condition, the bacteria were able to grow and reduce the amount of nitrogenous compounds from the wastewater. The isolate PSC remove 18, 43, 45%, PSD remove 13, 57, 91%, PST1 remove 12, 71, 45% and PST2 remove 16, 57 and 45% of NH_4 –N, NO_2^- and NO_3^- respectively as described in Fig. 2. The growth curves and the removal patterns were described in Fig. 3,4, 5 and 6 for PSC, PSD, PST1 and PST2 respectively.

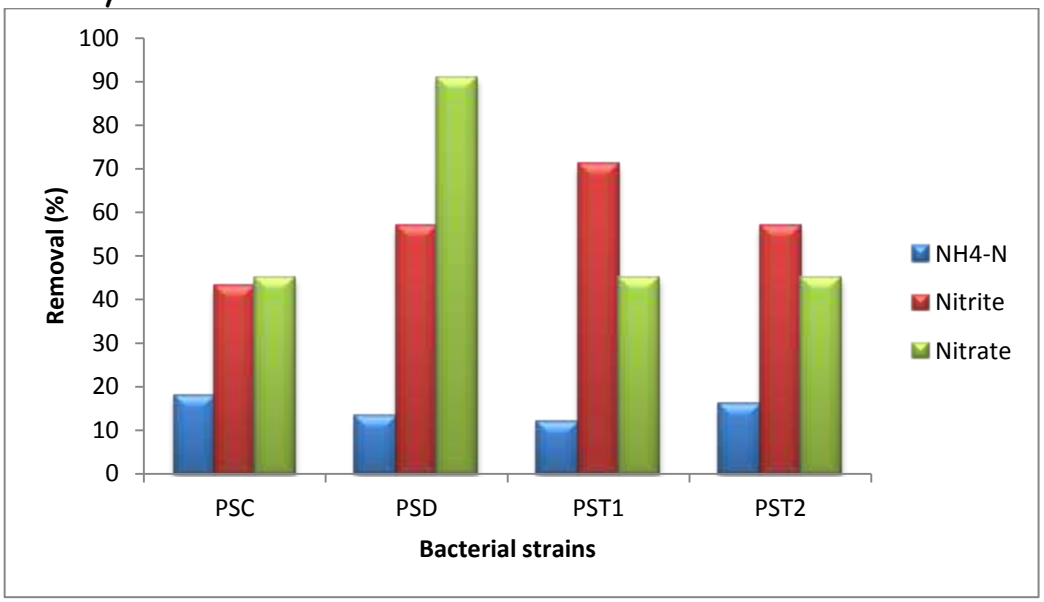

Fig. 2 Percentage removal of nitrogen from aquaculture wastewater

The results of nitrate removal obtained from this study were lower than those reported by [12] using immobilized and free cells of photosynthetic bacterium *R. capsulate*. However their study was carried out using synthetic wastewater as such possible inhibitory factors or mechanisms are excluded by the use of known wastewater content. In addition, the synthetic wastewater did not contain other forms of nitrogen like NH_4-N and NO_2-N, as such it had only NO_3^- as nitrogen source.

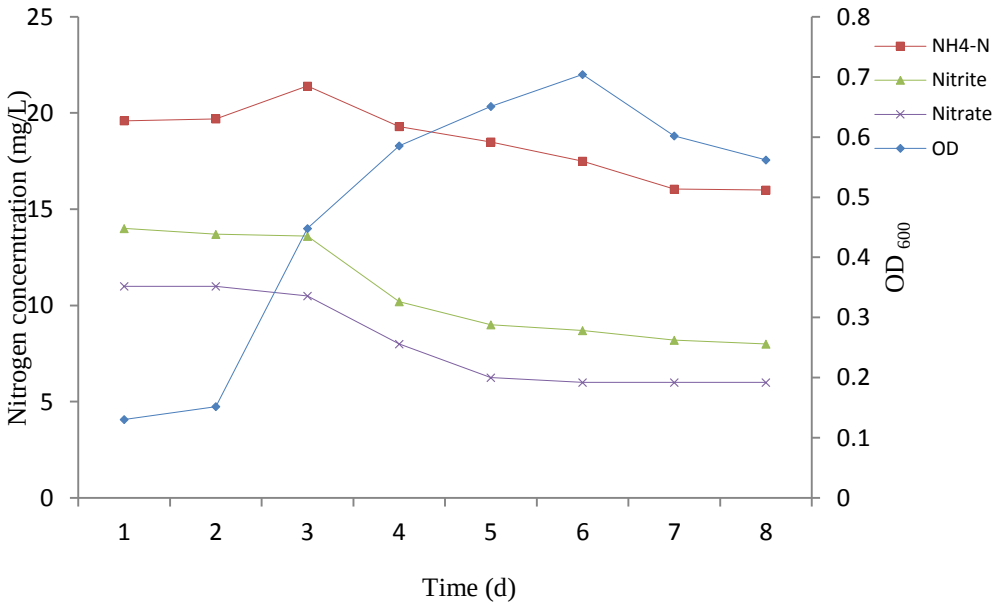

Fig. 3 Growth and removal pattern of nitrogen by PSC

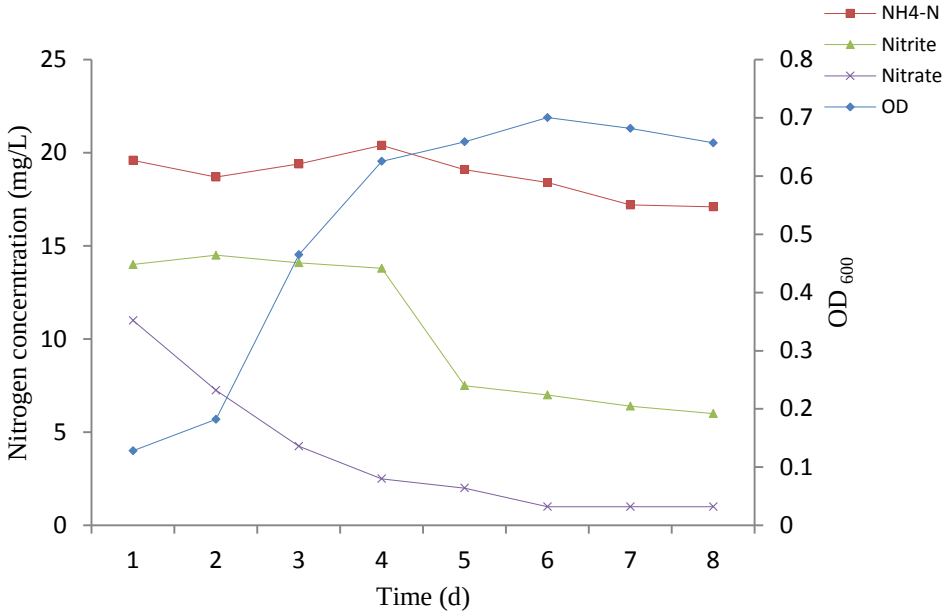

Fig. 4 Growth and removal pattern of nitrogen by PSD

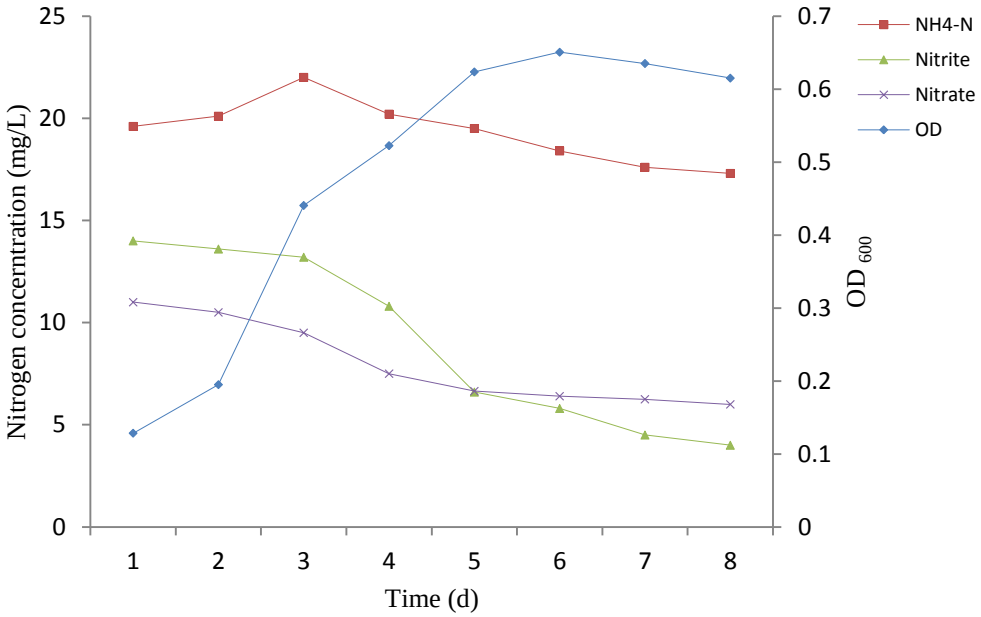

Fig. 5 Growth and removal pattern of nitrogen by PST1

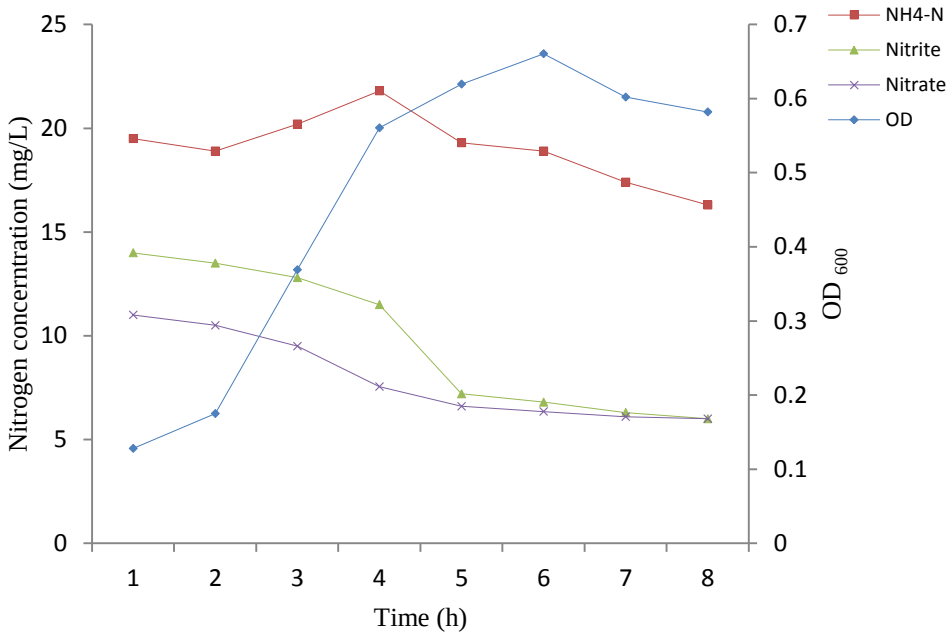

Fig.6. Growth and removal pattern of nitrogen by PST2

CONCLUSION

Different bacteria were isolated and screened for their photosynthetic and nitrogen removal ability. Photosynthetic activities were determined by detecting the photosynthetic pigments (bacteriochloroplyll and carotenoid) and denitrification capability were determined by nitrogen reductase test. The isolates were found to remove toxic nitrogenous removal from aquaculture wastewater. Molecular characterization of one the isolate revealed to be *Rhodobactersphaeroides* ADZ101.

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Table 1 Aquaculture wastewater characterization

S/N	Wastewater parameters	Pond First (mg/L)	Final (mg/L)	Effluent discharge standard (mg/L)
1	COD	898	857	120
2	BOD_5	-	6840	40
3	Total nitrogen	27	45	25
4	NH_4-N	19.5	28	1.0
5	Nitrate	12	11	10
6	Nitrite	10	10	1.0
7	TSS	730	1790	35
8	ADMI	940	1640	-
9	pH	7.06	6.96	5-9

References to this paper should be made as follows Ahmad Idi (2019), Bioremediation of aquaculture Wastewater using photosynthetic bacteria. *J. of Agriculture and Veterinary Sciences,* Vol. 11, No. 1, Pp. 28-40