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NITROGEN REMOVAL AND POLYHYDROXYALKANOATES ACCUMULATION VIA TWO-STAGE PROCESS

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ABSTRACT

The recovery of value added products is becoming an important aspect of wastewater treatment process. Hence this study exploits the recovery of microbial biomass during nitrogen removal and evaluates its potential application in PHA production via two stage processes. The concentration of nitrate, ammonium and PHA were determined by cadmium reduction method, Nessler method, and percentage respectively. The results showed that at the concentration of 92mg/L, 62% of NH_4^- N was removed and 2.7 g/L of DCW was obtained which accumulated 72% of PHA. At the concentration of 72mg/L, 74% of NO_3^- was removed and 3.4 g/L of DCW was obtained which accumulated 62% of PHA. The results also showed that nitrogen removal is related to concentration, DCW and PHA accumulations for both $NO_3^-$and NH_4^- N. Based on the study, the feasibility of the integrating nitrogen removal and PHA accumulation via two-stage was established.

Keywords: *Nitrogen, Polyhydroxyalkanoates, Ammonium, Photosynthetic bacteria*

INTRODUCTION

Photosynthetic bacteria have been shown to have a great potential in bioremediation and generation of value added products. Hence it is highly recommended for bioremediation[1]. The concept of incorporating wastewater treatment and generation of value added products is based on the philosophy of eco-efficiency, which promotes the idea of maximizing the production process with limited resource while minimizing waste and pollution. Its main strategy is to have more value with few natural resources and less generation of waste. It advocates the replacement or integration of wastewater treatment with resource recovery. Hence, apart from removing pollutants, the wastewater treatment processes should be able to generate value added products for sustainability and effective maintenance and operation. Therefore resource recovery becomes crucial for sustainable wastewater management. This concepts was applied in potato chips industrial waste water treatment, where starch, oil and grease were recovered as value added products [2]. In fish processing industry, apart from the removal of total nitrogen, COD, oil and grease, oxycarotenoids and biomass were also recovered as value added products by *Rubrivivaxgelatinosus* [3]. Under natural condition *Rhodobacter Spharoides* Z08 effectively removed 96% COD of soybean wastewater with the recovery of bacterial proteins and other short-chain organic acids[4].

Based on this concept, this study investigated the recovery of PHA during nitrogen removal via two stage processes. The two-stage fermentation processes under different experimental set up addressed this concept for ammonium and nitrate removal with PHA accumulation. The two-stage process of PHA production normally involve a growth phase, where bacteria are grown in the normal growth medium until a desired amount of biomass is achieved. This is followed by the accumulation phase, where PHA production is carried out using the fermentation medium usually growth-limited medium. During this phase the residual cell concentration (cell concentration without PHA) remain almost constant. Increase in cell concentration is only observed due to PHA accumulation.

MATERIALS AND METHODS

Isolation of Bacteria

The isolation and characterization of the bacteria used in this study was reported earlier.[5]

PHA Quantification

PHA was quantified in form of percentage as reported by [6] using the formula below.

$$PHA(\%) = \frac{Dry\ cell\ weight\ of extracted\ PHA}{Dry\ cell\ weight\ of\ the\ biomass} \times 100\%$$

Nitrogen Removal Methods

Ammoniacal Nitrogen (NH_4^- N) was estimated by Nessler method (APHA) [7]. Sample and blank were prepared in a separate 25 mL graduated cylinders. For blank, deionized water was used. In each of the cylinder, three drops of mineral stabilizer were added and inverted several times. Another 3 drops of polyvinyl alcohol dispersing agent were also added and inverted several times. Finally, 1 mL of Nessler reagent was also added to each graduated cylinder and inverted several times. One minute reaction was allowed to occur before 10 mL of the sample was transferred into the square cell cuvette and read after zeroing with blank. The readings were recorded at the wavelength of 425nm.Nitrate was determined by Cadmium Reduction Method (APHA) [7]. The square cell cuvette was filled with 10 ml of sample. The content of one NitraVer® 5 nitrate reagent powder pillow was added and shook vigorously for one minute. The sample was allowed for 5 min reaction to occur. The blank was prepared with 10 mL of sample. Readings were recorded at 500 nm within one minute after zeroing with the blank.

Two Stage Fermentation Process

Rhodobacter sp. ADZ101 was grown separately in different concentrations of ammonium and nitrate. The concentrations of ammonium and nitrate removal were determined as described above. At the logarithm phase, 10% (v/v) of the accumulated biomass was transferred as inoculum into the optimized PHA production medium of 3.00 g/L acetate, 0.06 g/L NH_4Cl and pH 7 [8]. The amount of PHA accumulated was determined as described above. Similar procedure was also reported for two-stage fermentation process for PHA production elsewhere [9]

RESULTS AND DISCUSSION

NH_4^-N Removal and PHA Accumulation

The results of NH_4^-N removal and PHA accumulation are presented in Table 1. At initial concentration of 95 mg/L of NH_4^-N, 62% was removed and 2.76 g/L DCW was obtained which accumulated 72% of PHA. When 212 mg/L of NH4-N was used, 50% was removed and 2.55 g/L of DCW was obtained which accumulated 58% of PHA. When NH4-N concentration was increased to 320 mg/L, 21% was removed with corresponding DCW accumulation of 2.105 g/L that produced 45% of PHA. As the concentration further increased to 420 mg/L, 19% was removed and 1.421 g/L of DCW was obtained which yielded 40% of PHA. It can be deduced from the results that the initial concentration of NH_4^-N influences both its removal and accumulation of biomass as well as PHA production. Lower concentrations led to higher NH4-N removal, higher biomass accumulation and higher PHA recovery. It can also be deduced from the results that PHA accumulation is proportional to the biomass accumulation. At lower cell biomass, low amount of PHA was obtained while high cell biomass produced high amount of PHA. Hence high cell density is a requirement for improved PHA production. This is in conformity with assertion of [10] that high cell density is a significant strategy for improving PHA accumulation, because high cellular content is needed for intracellular PHA accumulation.

Table 1: NH_4^-N removal and PHA accumulation

	First stage (NH_4-N removal)		***Second stage (PHA production)***
Concentration (mg/L)	**% removal**	**DCW (g/L)**	**PHA (%)**
92	62	2.7	72
212	50	2.5	58
320	21	2.1	45
420	19	1.4	40

NO_3^- Removal and PHA Accumulation

The results of NO_3^- removal and PHA accumulation are presented in Table 2. It can be deduced that when the initial concentration of 72 mg/L of NO_3^- was used, 74% was removed while 3.45 g/L DCW was produced which yielded 62% of PHA. As the concentration increased to 108 mg/L, 60% was removed and 2.95 g/L DCW was obtained which accumulated 55% of PHA. When the concentration increased to 176 mg/L, 45% was removed and 2.55 g/L DCW was accumulated with the corresponding PHA accumulation of 42%. Finally, at the initial concentration of 212 mg/L, 36% was removed and 1.95 g/L DCW of biomass was accumulated with the corresponding PHA accumulation of 35%. This result showed a similar pattern to the two-stage process of ammonium removal and PHA production, where initial concentration of NO_3^- influenced its removal, the accumulation of biomass and PHA production. However, the biomass produced during NO_3^- removal were higher compared to ones obtained during NH_4-N removal. But the PHA accumulations were found to be higher with the biomass accumulated during NH_4-Nremoval. This may be due to the differences in experimental set up of the two processes. NO_3^- removal was carried out under photoheterotrophic growth condition while NH_4-N removal was conducted under aerobic condition which is also the same condition for PHA accumulation. As such the bacteria may require more time to acclimatize in a new environment before PHA accumulation began. While this acclimatization may not be required in the case of PHA accumulation with biomass obtained during NH4-N removal. It is important to note that the amount of carbon source consumed with time was not incorporated during the experimental design. As such PHA yield and productivity were not determined. This is because the study focused mainly on providing fundamental information about the possible application of *Rhodobacter sp*. ADZ101 in nitrate and ammonium removal with PHA accumulation rather than determining the yield and productivity of the two-stage processes. It shows that to maximize productivity, high cell density is required for the accumulation of PHA. This has been shown to be possible, when the biomass that was accumulated during ammonium and nitrate removal significantly increased the PHA production. This increment is attributed to high amount of biomass and PHA production without cell growth[11].

Table 2: NO_3^- removal and PHA accumulation

	First stage (NO3-removal)		***Second stage (PHA production)***
Concentration (mg/L)	**% removal**	**DCW (g/L)**	**PHA (%)**
72	74	3.4	62
108	60	2.9	55
176	45	2.5	42
212	36	1.9	35

CONCLUSION

This paper demonstrates the possibility of incorporating nitrogen removal with PHA accumulation via two stage process to achieve simultaneously, environmental and economic benefits. *Rhodobacter spheroidess* ADZ101 has been shown to remove high amount of both NH_4^-N and NO_3^-. The biomass recovered during the removal process can be use in the accumulation of PHA, thereby having dual benefits.

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