PRELIMINARY STUDY ON THE EFFECTS OF ANAERORIC MICROORGANISM ON THE AMMONIUM OXIDATION OF BIOMASS CULTURED FROM PIG FARM SLUDGE

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ABSTRACT

The study was conducted to implement applications of less common nitrogen bio-transformation process. Previous work with batch systems under autotrophic anaerobic conditions, revealed conversions of nitrogenous compounds that could not be explained by traditional nitrification and denitrification. In this study, continuous flow treatment with biomass carriers was investigated. This treatment allowed the development and retention of extremely slowly growing organisms. Biomass containing micro-organisms was isolated from Go Sao pig farm sludge. Tow bioreactors seeded with acclimated sludge were fed with ammonium and nitrite as electron donor and acceptor, respectively, under anoxic conditions. Ammonium and nitrite disappeared, while nitrate was produced at low level with stoichiometric ratios. At a total nitrogen loading rate of 7 mg-N/L/hr (hydraulic retention time, 27 hrs), removal efficiency of about 60% was demonstrated. A distinct growth of red biomass, a typical developed in bioreactors, The micro-organisms were identified to be of planctomycete responsible for the bioreaction.

Keywords: Bioreactor, Go Sao pig farm sludge, U.V spectrophotometer

INTRODUCTION

Aerobic, autotrophic nitrification and anoxic, heterotrophic denitrification were, with increasing frequency, being investigated as they were considered as only means for effective removal of soluble N-NH_4 in biological systems. Non-conventional microbial conversions of nitrogen have demonstrated significant roles in nature and in unit processes (1). The mechanisms under observed transformations, however, seemed to be difficult to verify, and the bioreaction rates are often too slow for useful treatment applications. Furthermore, nitrogenuos gases produced under stressed conditions may be of an unsafe form, e.g, greenhouse gases. Recently, a potential useful microbial process consisting of anaerobic ammonium oxidation (Anammox) and utilizing nitrite (N-NO_2) as an electron acceptor with production of nitrogen gas (N_2) has been discovered by Van de Graaf, et al. (2). The existence of lithotrophic bacteria capable of such an energetic, favorable and exergonic reaction was predicted by Broda (3), the species responsible for the observed conversion was identified as a deep branching planctomycete (4). The stoichiometry of the reaction was determined by Stous et al. (5) as followed:

$$NH_4^+ + 1.32NO_2 + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O$$

This study presents the effect of anaerobic micro - organism on the ammonium oxidation of biomass cultured from pig farm sludge.

MATERIALS AND METHODS

Two bioreactors were designed and constructed from plastic cylinders. Bioreactor I had a volume of 25 L (diameter of 200 mm and height of 800 mm). Bioreactor II has a volume of 10 L (diameter of 100 mm and height of 1000 mm). The influent medium was added at 10 L/day and 25 L/day into bioreactors I and II, respectively, by a peristaltic pump. The influent medium composition for the culture of anammox consisted of N-NH_4 and N-NO_2 (10 - 60) mg-N/L, EDTA 5 mg/L, $KHCO_3$ 100 mg/L, KH_2PO_4 30 mg/L, $FeSO_4.7H_2O$ 10mg/L. The bioreactors were operated at a room temperature of 25-30 °C, in the dark and under an anaerobic condition.

Sludge in anaerobic tank derived from a pig farm in Go Sao, located in district 12 of Ho Chi Minh city, was used as seed for the bioreactor I. 500g sludge was seeded into the bioreactor I. This sludge was adapted under anoxic conditions with $N\text{-}NH_4$ and $N\text{-}NO_2$ as substrates. 200g adapted sludge was seeded into the bioreactor II. Under these conditions, the pumped influent into the bioreactor I was stopped after three months and into the bioreactor II after six months. During this period, changes of biomass solids were appreciable. Adapted, sludge could be stored at the room temperature in the dark for several months without further activity. During the operation, the control of influent oxygen regularly was done with $Na_2S.9H_2O$ (50-125 mg/l). The bioreactors I and II were operated totally in nine months. Effluent of each bioreactor was taken for analysis for every ten days.

ANALYSIS

$N\text{-}NH_4$, $N\text{-}NO_3$, $N\text{-}NO_2$ and COD were determined by using a UV spectrophotometer based on standard methods for applied the Examination of Water and Wastewater.

RESULTS

The bioreactor I, seeded with the pig farm sludge, demonstrated an erratic nitrogen removal efficiency from 0 up to 7% over three months prior to the termination. The concentrations of $N\text{-}NH_4$ and $N\text{-}NO_2$ of the bioreactor I influent were increased from 10 - 30 mg-N/L over time, and the hydraulic retention time was fixed at 24 hrs. In the effluent existed nitrate and COD (Table 1). The sludge changed the color from black to brown after three months and its volume deducted from 5 L to 3 L.

Table 1: Effect of micro-organisms on the nitrogen and COD concentrations in the bioreactor I during 3 months

First month							
$N\text{-}NH_4$ (mg/L)		$N\text{-}NO_2$(mg/L)		COD(mg/L)		$N\text{-}NO_3$(mg/L)	
Inf	eff	Inf	eff	Inf	eff	Inf	eff
10	10	10	8.2	0	426	0	1.5
10	10	10	8.7	0	288	0	1.5
10	9.8	10	8.7	0	225	0	1.2
Second month							
20	19.5	20	16.3	0	210	0	1.2
20	19.2	20	16.0	0	195	0	1.2
20	18.8	20	16.0	0	180	0	1.2
Third month							
30	28	30	23.6	0	180	0	1.0
30	27.6	30	23.6	0	172	0	1.0
30	27.6	30	23.3	0	168	0	1.0

The bioreactor II demonstrated an erratic nitrogen removal efficiency from 10 up to 30% over six months prior to the termination. The concentrations of $N\text{-}NH_4$ and $N\text{-}NO_2$ of the bioreactor II influent were increased from 30 - 60 mg-N/L over time, and the hydraulic retention time was fixed at 24 hrs. In the effluent existed nitrate and COD (Table 2). The sludge changed the color from brown to rosy-brown after six months and its volume deducted from 3 L to 1 L.

Table 2: Effect of micro-organisms on the nitrogen and COD concentrations in the bioreactor II during 6 months

First month							
$N\text{-}NH_4$(mg/L)		$N\text{-}NO_2$(mg/L)		COD(mg/L)		$N\text{-}NO_3$(mg/L)	
Inf	eff	Inf	eff	Inf	eff	Inf	eff
30	27.2	30	23.0	0	150	0	1.0
30	27.0	30	22.8	0	150	0	1.0
30	26.8	30	22.5	0	143	0	1.0
Second month							
40	35.5	40	29.5	0	146	0	1.0
40	35.3	40	29.2	0	142	0	1.0
40	35.3	40	28.1	0	135	0	1.0
Third month							
40	34.8	40	27.6	0	133	0	1.0
40	34.2	40	27.2	0	139	0	1.0
40	33.6	40	26.7	0	135	0	1.0
Fourth month							
50	41.2	50	32.5	0	128	0	1.0
50	39.5	50	30.2	0	132	0	1.0
50	39.5	50	30.0	0	131	0	1.0
Fifth month							
50	39.2	50	28.9	0	122	0	1.0
50	37.6	50	28.6	0	122	0	1.0
50	36.6	50	28.6	0	109	0	1.0
Sixth month							
60	43.2	60	33.5	0	98	0	1.0
60	42.4	60	33.5	0	99	0	1.0
60	42.4	60	33.2	0	86	0	1.0

CONCLUSION

- Biomass derived from red micro-organism was able to reduce $N\text{-}NH_4$ and $N\text{-}NO_2$, and produce $N\text{-}NO_3$ and N_2 as well.
- The cultured micro-organism was defined as one of Anammox

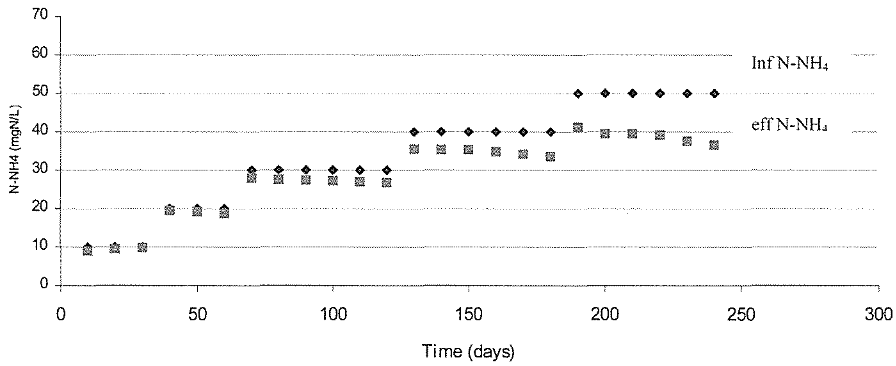

Figure 1: Time courses of the influent and effuent solubes $N\text{-}NH_4$ in bioreactors I and II

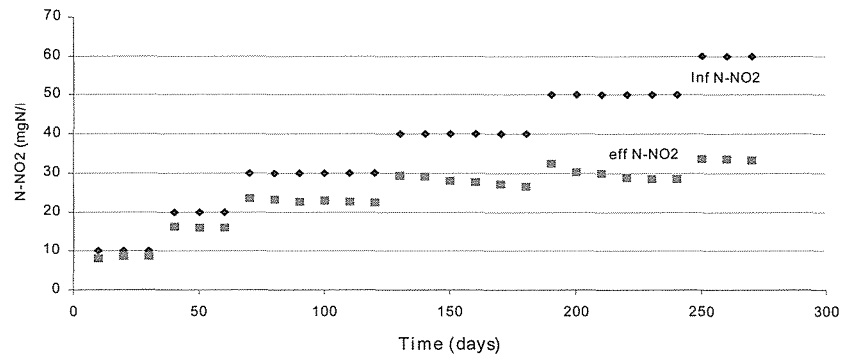

Figure 2: Time courses of the influent and effuent solubes N-NO_2 in bioreactors I and II

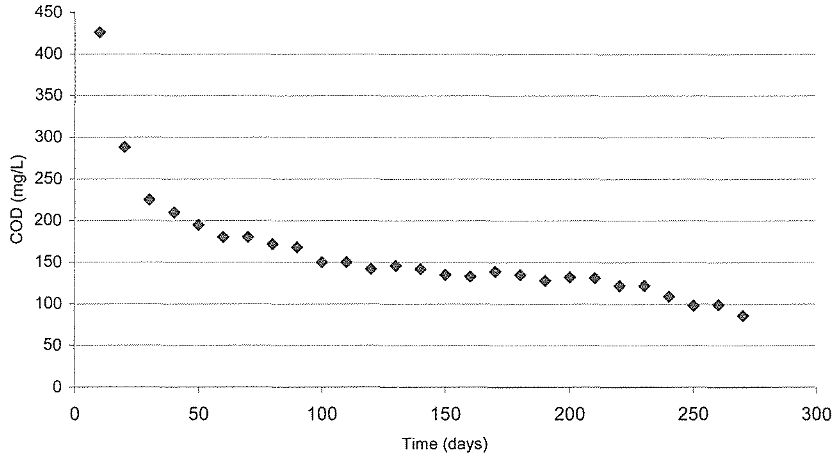

Figure 3: Change of effluent CODs

References

1. Furukawa K., Rouse J.D., Bhatti Z.I. & Imajo U. *Anaerobic Ammonium Oxidation (Anammox) in Continuous Flow Treatment With Non-Woven Biomass Carrier*. Proceedings of the Fifth international Symposium on Environmental Biotechnology, July 9-13, 2000, Kyoto, Japan.Kyoto University. P 1-4, 2000.
2. Van de Graaf A.A., De bruijn P., Robertson L.A., Jetten M.S.M. & Kuenen J.G. *Metabolic pathway of anaerobic ammonium oxidation on the basis of N-15 studies in a fluidized bed reactor*. Microbiology UK 143. 2415-2421. 1997.
3. Broda E. *Two kinds of lithotrophs missing in nature*. Z.All.Mikrobiol.17. 491-493. 1977
4. Fujii T., Sugino H., Furukawa K.& Rouse J.D. *Characterization of the Microbial Commumity in an Anaerobic Ammonium Oxidizing Biofilm Cultured on a Nonwoven Biomass Carrier*. J of Bios.Bioeng., Vol. 94, 5, 412-418. 2002.
5. Strous M et al. *Missing lithotroph indentified as new planctomycete*. Nature. 400. 446-449. 1999.
6. Kuypers M.M.M., Sliekers A.O., Lavik G., Schmid M., Jorgensen B.B., Kuenen J.G., DamsteJ.S.S., Strous M. & Jetten M.S.M. *Anaerobic Ammonium Oxidtion by Anammox Bacteria in the Black Sea*. Bremen, Germany, Netherlands. Nature, U 22, 608-611, 2003.
7. Van Dongen I.U., Jetten M.S.M. & Van Loosdrecht M.C.M. *The Sharon Anammox process for treatment of ammonium rich wastewater*. J. of Water Science & Technology. Vol. 44, 1, 153-160. 2001.