Korean J. Chem. Eng., **25**(4), 787-792 (2008)

SHORT COMMUNICATION

Bio-denitrification of the nitrate waste solution from the lagoon sludge in a continuous bio-reduction process

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(*Received 3 September 2007 • accepted 4 October 2007*)

Abstract−The treatment of lagoon sludge is a serious task during the decommissioning of a uranium conversion plant. The main component of the sludge is ammonium nitrate, which is a very explosive material. Therefore, biological removal of the ammonium nitrate would be an attractive process. In this work, the bio-denitrification of the nitrate solution from lagoon sludge with a continuous fermentation process was studied. The optimal conditions for removal of nitrate by *Pseudomonas halodenitrificans* in the continuous bio-reduction process were a C/N ratio and pH of 3.1 and 7.5, respectively, with CO_2 gas control, and five times the microelements as used in a batch culture, at 30 °C. It was concluded that bio-denitrification could be applicable to lagoon sludge waste, but with some limitations.

Key words: Nitrate, Denitrification, *Pseudomonas halodenitrificans*, Continuous Culture

INTRODUCTION

In early 1992, KAERI (Korea Atomic Energy Research Institute) decided that the uranium conversion plant operation should be stopped, and in 2000 the decision to decommission the plant was finalized, and a program launched. The treatment of the sludge waste generated during the operation of the plant, as well as that stored in the lagoon, was one of the most important tasks in the plant decommissioning program. The uranium content in the deposits at the bottom of the lagoon was very high; therefore, the sludge could not be treated as a simple industrial waste. The sludge would have to be containerized in 200 liter drums and managed as a low-level radioactive waste. Because the sludge contained a large number of nitrate compounds, which are easily dissolved in water and deliquescent, it was not permissible for the sludge to be directly drummed after simply drying. Therefore, the sludge of the lagoon had to be economically and safely treated to reduce the volume and remove the nitrate salts.

A proper process needed to be developed by considering the characteristics of the sludge. The main component of lagoon sludge is ammonium nitrate, which is a very explosive material. Therefore, bio-denitrification is more attractive process than other thermal treatments for the removal of nitrate. Bacterial denitrification is a dissimilatory nitrate reduction, which involves four metabolic steps, where nitrate is sequentially reduced to nitrogen via nitrite, nitric oxide and nitrous oxide [3,8]. Many microorganisms have developed unique properties to aid their survival under extreme conditions. Halophiles are an example of such organisms, which are found in an environment that resembles those of the earliest periods on Earth, during the Archaic Period [7]. The best-characterized heterotrophic bacteria from the Pseudomonas denitrificans are able to achieve complete nitrate removal; other species of Pseudomonas have also been reported to be capable of the reduction of nitrate and nitrite [1,2,4].

In this study, a newly isolated bacterial strain, *Pseudomonas halodenitrificans*, from France, used for the bio-denitrification of nitrate waste solution from lagoon sludge in a continuous bio-reduction is described.

1. Characteristics of the Lagoon Sludge

There are two lagoons at KAERI, with dimensions of 3(H)×10(W)×40(L) m and 2.7×9×40 m, respectively. When the uranium conversion plant was constructed, only one lagoon was made. After the start of its practical operation, in 1988, the capacity of the lagoon was insufficient; therefore, a second lagoon was constructed. After settlement of the particulates, the solution in lagoon 1 was neutralized with calcium hydroxide. During this step, the heavy metals, including uranium, were precipitated. After filtration to remove the solid particles, the solution was transferred to lagoon 2 for storage.

The sludge from lagoons 1 and 2 each consisted of three different layers. The upper layer was a saturated solution; the middle layer, crystalline; and the bottom layer, particulate material deposits. The compositions of the lagoons were very complicated, with the main compounds being ammonium nitrate, sodium nitrate, calcium nitrate, calcium carbonate and uranium [5]. The chemical composition and ratio of each layer are shown in Table 1.

MATERIALS AND METHODS

1. Micro-organism

The *Pseudomonas halodenitrificans* strain used in this study was isolated from the Cadarache, France (protected bacterial strain *Pseu-*

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Table 1. Chemical compositions of the lagoon sludge

		wt%	NH_4NO_3 wt%	$NaNO_3$ wt%	$Ca(NO_3)_2$ wt%	$CaCO_3$ wt%	U ppm
Lagoon 1	Upper	19.62	61.86	28.43	2.07	-	10
	Middle	17.16	68.81	25.30	0.30	-	586
	Bottom	63.21	53.04	17.98	9.53	-	20300
Lagoon 2	Upper	15.38	79.84	13.82	1.77	-	<0.5
	Middle	47.19	90.31	0.31	0.93	-	8
	bottom	37.42	20.17	3.14	18.94	38.11	305

Total sludge=250 m^3/460 ton
Total NO_3^-=60 wt% (150 m^3/270 ton)

Table 2. 0.2 M NH_4NO_3 medium and micronutrient for the seed culture

	Component	Amount
Medium	NH_4NO_3	16 g (0.2 M)
	$MgSO_4$	0.967 g
	KH_2PO_4	0.853 g
	$FeSO_4$	2.667 mg
	$CuSO_4$	0.133 mg
	CH_3COOH	12 ml (0.2 M)
	pH	6-7
Micronutrient (dissolution in 1 *l* water and add 1 ml to medium)	H_3BO_3	100 mg
	$MnSO_4$	100 mg
	$ZnSO_4$	100 mg
	$Mo_7O_{24}(NH_4)_6$	1,000 mg
	$Co(NO_3)_2$	100 mg

Table 3. Chemical compositions of the original nitrate waste solution and diluted medium

	Original solution (mg/L)	Diluted medium to 0.2 M NO_3^- (mg/L)
NO_3^-	174,000	12,400
NH_4^+	34,000	2,423
Ca	1,600	114
Na	13,000	926
U	95	7

Ca and U: ICP-AES, Na: AAS, NO_3^-: IC, NH_4^+: UV

domonas halodenitrificans nº I-1563 at the Collection Nationale de Cultures de Microorganisms, Institut Pasteur-FRANCE (Budapest Treaty), belonging to the CEA, COMHUREX and INRA: FRANCE) [9,10]. The effect of *P. halodenitrificans* on denitrification was highly sensitive to the shearing force [6].

2. Culturing Conditions

The composition of the standard medium used in this study is detailed in Table 2. The denitrification medium was modified with the original nitrate waste solution (Lagoon sludge) and a diluted medium (Table 3) [9].

A seed culture was inoculated into 500 ml of standard medium in a 1,000 ml bioreactor, and the culture allowed to proceed for 24 h in a shaken culture. The bioreactor used in this study is described in Fig. 1 and 2. Cultivations were performed at 30 ºC and pH 7.0, with agitation by a magnetic stirrer. The volume of the seed inoculum was 10%, with an initial seed concentration of 0.5-0.8 at OD_{650}. As the denitrification process was advanced, medium in the reactor was discharged and the same volume of fresh nitrate substrate was put in to the reactor. The effects of the substrate injection rate, C/N

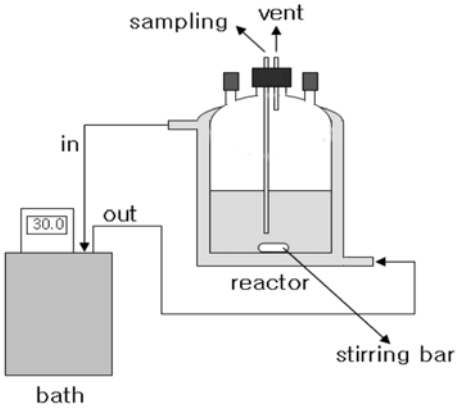

Fig. 1. Experimental set-up for bio-denitrification.

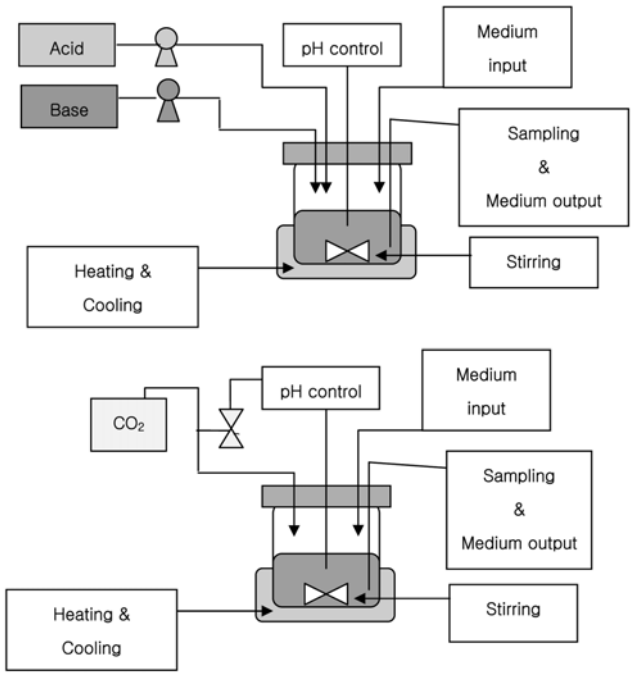

Fig. 2. Experimental set-up for bio-denitrification.

ratio and the initial pH on the denitrification were investigated by using *P. halodenitrificans*.

3. Analytic Methods

The cell growth was estimated photometrically by following the optical density of the broth at 650 nm with a spectrophotometer (Lambda 2S, Perkin Elmer). During the fermentation, the pH value was monitored by using a pH-meter (Orion. EA 940) with an inserted pH probe. The nitrate concentration was measured with a DR-4000 spectrophotometer (Hach Co.). Culture samples were centrifuged at 10,000 rpm for 10 min, with the supernatants obtained. 1 ml of the supernatants was put into test kits (Nitrate Nitrogen Standard Solution and NH_4-N Standard Solution, respectively), mixed with reagents and the nitrate concentration then analyzed [11].

RESULTS AND DISCUSSION

The influence of the substrate injection rate on the growth of the microorganisms and the nitrate removal is shown in Fig. 3. The nitrate removal trend of *P. halodenitrificans* was obtained by using acetic acid and ethanol as the sole carbon sources under continuous culture condition. The concentration of the substrate was 2.8 M, 14 times higher than in a batch culture. The feeding rate was allowed the same amount of nitrate in the initial medium per day. The results indicated that the carbon source and nitrate in the continuous culture affected the nitrate removal rate by this strain. In the course of the continuous culture with ethanol, the nitrate removal rate started to decrease after 62 hr; however, when acetic acid was used, the nitrate removal rate started to decrease after 94 hr. The C/N ratio was maintained close to 3.1.

To estimate the impact of the concentration of microelements on the continuous culture using *P. halodenitrificans*, the additions of 0, 5 and 10 times the levels as used in a batch culture [6] were applied, the results of which are shown in Fig. 4. On the injection of the same amount of microelements, the nitrate removal rate started to decrease after 129 hr. On the addition of 5 and 10 times the levels as in a batch culture, the nitrate removal rate was observed to decrease after 176 and 144 hr, respectively. Therefore, with 5 times the initial concentrations of microelements as used in a batch culture [6], strain *P. halodenitrificans* showed the complete capability of nitrate removal.

In generally, denitrification is affected by compound organisms. With a small C/N ratio, the addition of organisms for the removal of nitrate can cause a high level of denitrification [10]. The typical results for the removal of NO_3-N as a function of the C/N ratio under continuous bio-reduction are shown in Fig. 5. It can be seen that the concentration of NO_3-N in the effluent gradually decrease with a C/N ratio of 3.1. It was also observed that the nitrate removal and

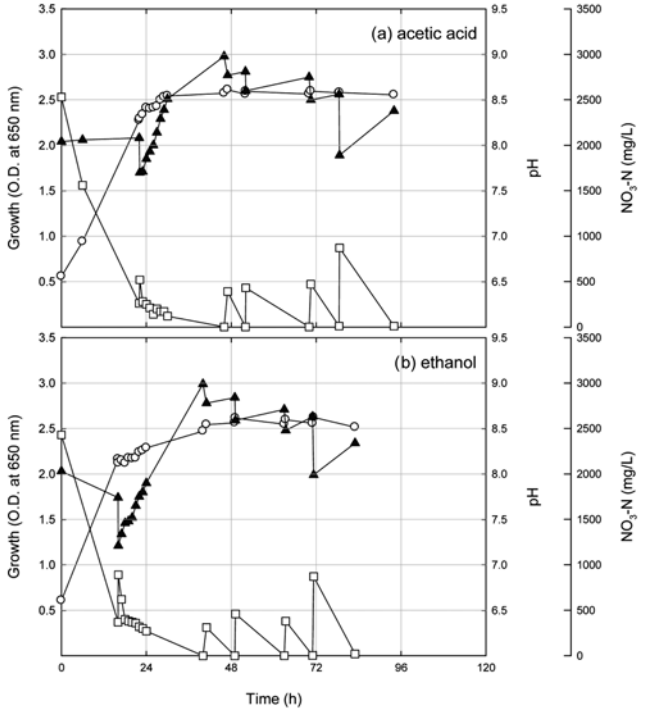

Fig. 3. Continuous bio-reduction on the denitrification by *P. halodenitrificans* with different electron donors (temperature= 30 °C, nitrate=0.2 M, seeding=10%, ○; O.D., ▲; pH, □; nitrate conc.).

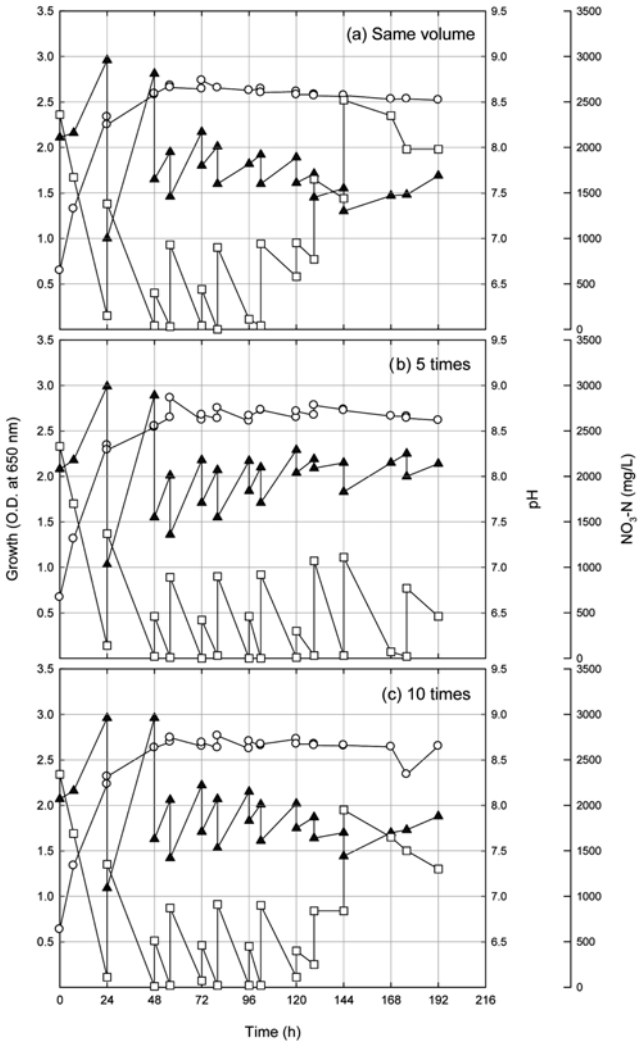

Fig. 4. Continuous bio-reduction on the denitrification by *P. halodenitrificans* with different mineral solution concentrations (temperature=30 °C, nitrate=0.2 M, seeding=10%, C/N=3.1, ○; O.D., ▲; pH, □; nitrate conc.).

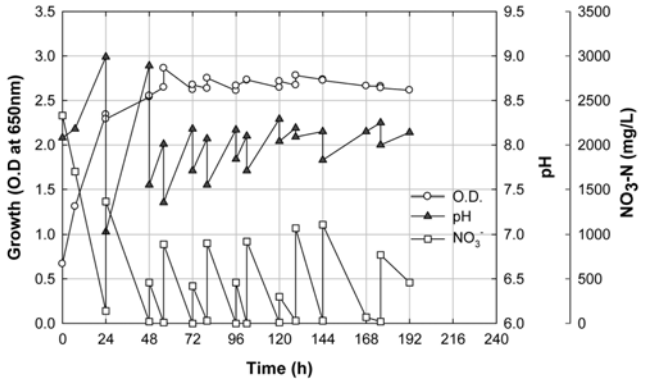

Fig. 5. Continuous bio-reduction on the denitrification by *P. halodenitrificans* (temperature=30 ºC, nitrate=0.2 M, seeding=10%, C/N=3.1, auto-control of pH, ○; O.D., ▲; pH, □; nitrate conc.).

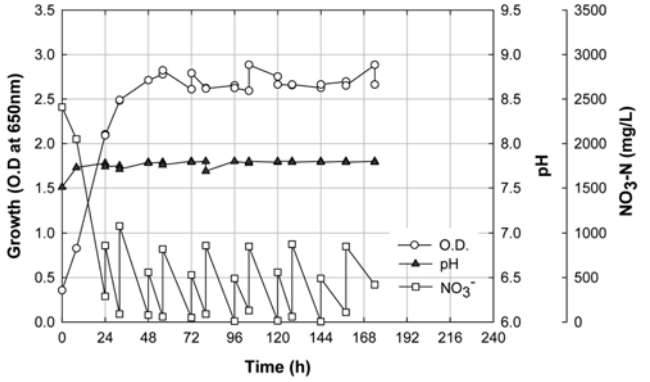

Fig. 6. Continuous bio-reduction on the denitrification by *P. halodenitrificans* with pH control using NaOH and HCl (temperature=30 ºC, nitrate=0.2 M, seeding=10%, C/N=3.1, NaOH and HCl-control of pH=7.5-8.0, ○; O.D., ▲; pH, □; nitrate conc.).

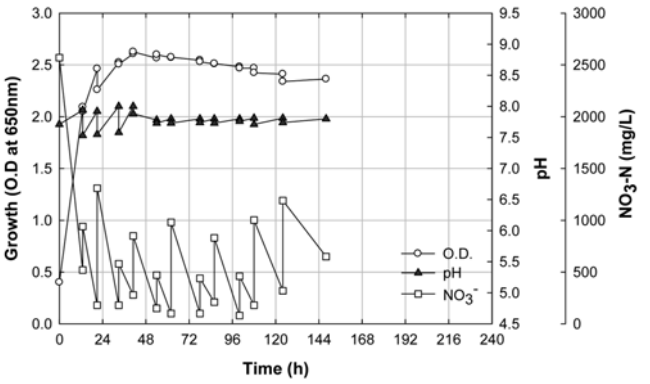

Fig. 7. Continuous bio-reduction on the denitrification by *P. halodenitrificans* with pH control using acetic acid (temperature=30 ºC, nitrate=0.2 M, seeding=10%, C/N=3.1, acetic acid-control of pH=7.5-8.0, ○; O.D., ▲; pH, □; nitrate conc.).

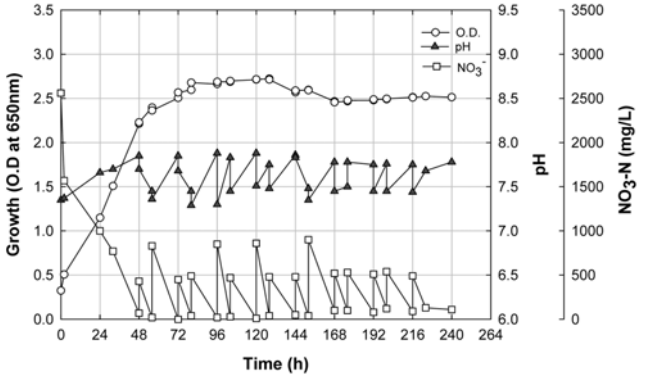

Fig. 8. Continuous bio-reduction on the denitrification by *P. halodentrificans* with pH control using CO_2 (temperature=30 ºC, nitrate=0.2 M, seeding=10%, ○; O.D., ▲; pH, □; nitrate conc.).

continuous culture reach a reasonable level with a C/N ratio of 3.1. The pH was also very sensitive, and had a tendency of decrease. The auto-control of the pH failed after 7 days. Finally, the denitrification rate started to decrease after 7 days.

The pH is also an important factor in biological denitrification. Fig. 6 illustrates the influence of pH on the NO_3-N removal at the same temperature, 30 ºC, with a C/N ratio of 3.1. The pH was controlled by the addition of NaOH to increase the pH of the mixed solution to 6, and with HCl solution to decrease to pH to 6. The C/N ratio was maintained close to 3.1, with the pH controlled close to 7.7. The denitrification rate started to decrease after 5 days, which was considered to be due to the increase in the Cl^- concentration in the medium; therefore, pH control using HCl solution was not preferable.

With the feeding rate of the substrate, the pH was controlled by using only acetic acid, which was very easy and stable to control (Fig. 7). The C/N ratio was gradually decreased, and thee denitrification rates finally started to decrease after 6 days. The amount of acetic acid added to control the pH was insufficient for the denitrification reaction.

According to the results, the method for the operational pH control was changed to CO_2 gas instead of HCl, the result of which is shown in Fig. 8. The denitrification rate was maintained for 10 days. The influence of CO_2 gas on a real lagoon sludge solution in a continuous culture with a C/N ratio of 3.1, pH value of 7.5, 5 times the microelements as in a batch culture and a temperature of 30 ºC is shown in Fig. 9. The pH control using CO_2 gas had a considerable effect on the removal of NO_3-N in a continuous culture (Fig. 10).

CONCLUSIONS

Continuous operation was possible and more effective for nitrate removal than batch culture. The reaction rate was increased by a factor of 2.5. Secondary waste liquid was decreased by a factor of 14. An increase in the nitrate concentration, with uniformity of the concentration in the mother liquor, did not really improve the decomposition rate, which could also be a reason for the increased nitrogen content in the final waste.

To improve the applicability of our process for the treatment of KAERI'S lagoon sludge, other variables should be evaluated.

It was concluded that bio-denitrification could be applicable to lagoon sludge waste, but with the following limitations:

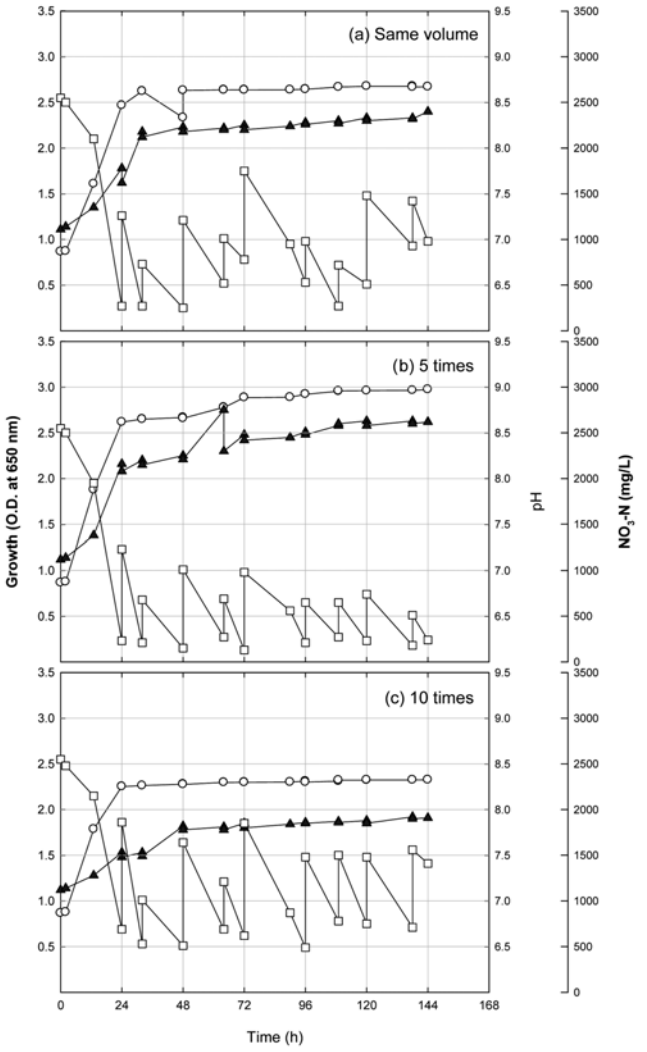

Fig. 9. Incubation of lagoon waste solution with different mineral solution concentrations (temperature=30 °C, nitrate=0.2 M, seeding=10%, ○; O.D., ▲; pH, □; nitrate conc.).

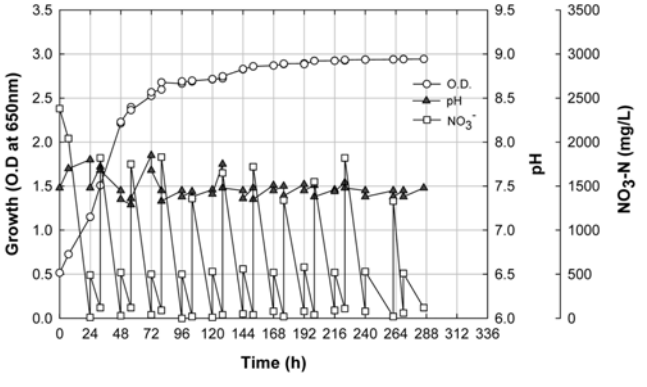

Fig. 10. Long-term incubation of lagoon waste solution with continuous bio-reduction (temperature=30 °C, nitrate=0.2 M, seeding=10%, CO_2 gas-control of pH=7.5, ○; O.D., ▲; pH, □; nitrate conc.).

1. The reactor size for the treatment of the lagoon sludge in our project was too large.

- The total amount of nitrate in the lagoon: 228 tons.
- The decomposition rate in the case of the batch reactor: 12.4 g/(liter * 2.5 days)=5 g/liter.d.
- reaction time: 600 days.
- size of the batch reactor=76 m^3 (even in the case of the continuous reactor; 30 m^3)
- for a general bio-reactor, this size is not large, but for our case was too large, as no other application is intended after this project, with the expected risk being too great for a scale-up without any pilot scale test.

2. Before our results for continuous reactor, there was too much secondary liquid waste.
- Expected amount of liquid waste: 18,000 m^3
- It would also be expected to be classified as a non-discardable waste, due to the high nitrate concentration compared to the allowable national discharge limits, and the contents of uranium and heavy metals, as well as the deactivated biological component.
- The secondary waste requires another treatment process (and plant), which is to be developed.

3. There was no clear economic advantage over that of a thermal process.
- Because of the very large reactor, much equipment and complex procedures required, as well as the installation of the plant would incur much greater costs.
- A much greater cost of chemicals, such as acetic acid, NaOH and additives and nutrients, would also be incurred.

4. There is no effective decomposition of ammonia.
- The main composition of our sludge was ammonium nitrate, with the ammonia also being a nitrogen-compound pollutant. Therefore, the ammonia should be decomposed.

Therefore, the decomposition rate and secondary liquid waste will have to be increased (by factors 4-5 and 5-10, respectively, from now) by using water recyclers.

ACKNOWLEDGMENT

This project was performed under the Nuclear R&D Program by MOST.

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