Environ. Eng. Res. Vol. 10, No. 1, pp. 22~30, 2005
Korean Society of Environmental Engineers

BIOCHEMICAL MODEL AND MECHANISM FOR *ACINETOBACTER* NITRITE INHIBITION

Chan-Won Lee†, Seung-Yeon Weon*
Department of Environmental Engineering, Kyungnam University, Masan, 631-701, Korea
*Department of Environmental Engineering Sciences, University of Florida, Gainesville, FL 32611, USA
(received September 2004, accepted February 2005)

Abstract : Nitrite accumulation is not unusual in batch processes such as sequencing batch reactor (SBR) with high-strength of ammonium or nitrate wastewaters. A possible mechanism of nitrite inhibition on *Acinetobacter* was depicted in a biochemical model, which the protonated species, nitrous acid form of nitrite, affects proton relating transport at the proton-pumping site crossing the cell membrane under unlimited carbon and phosphorus conditions. This effect exerts inhibition of phosphorylation under aerobic condition and yields low APT/ADP ratio, consequently decrease poly-P synthesis and phosphorus uptake from outside the cell in the model.

Key Words : *Acinetobacter*, nitrite inhibition, biochemical model, ATP/ADP ratio

INTRODUCTION

More stringent phosphorus standards for treated wastewater discharged to sensitive areas are required because phosphorus loadings to natural water systems have accelerated eutrophication everywhere in the world. Phosphorus can be removed from wastewater by either chemical precipitation or biological phosphorus removal (BPR) process. Bacteria accumulate phosphorus in excess levels needed for cell synthesis and physiological functions in BPR process. BPR process provides a cost effective and important alternative to chemical precipitation. Biological phosphorus removal is accomplished by particular group of microorganisms that are able to take up and store phosphorus intracellularly in the form of inorganic polyphosphate chains. These microorganisms are known collectively as poly-P bacteria or phosphate accumulating organisms (PAO).

Fuhs and Chen[1] identified *Acinetobacter* sp. (poly-P bacteria) which is known to accumulate orthophosphate as intracellular polyphosphate under aerobic conditions and first described the morphological characteristics of PAO based on microscopic observations of PAO-enriched sludge. It was reported that several microorganisms (e.g., *Acinetobacter, Pseudomonas, Aerobacter, Klebsiella, Enterobacter, Moraxella, Microbacterium*) have ability to accumulate phosphorus in excess of the normal cell requirement (approximately 1~3% of the cell dry weight) by many researchers. For example, *Acinetobacter calcoaceticus* takes up phosphorus under aerobic conditions at a rate of 0.4~0.5 mmole/g dry cells per hour and release it under anaerobic conditions at a rate of 0.015 mmole/g dry cells per hour[2] Mino *et al.*[3] reviewed microbiological and biochemical aspects of the enhanced biological phosphorus removal (EBPR).

† Corresponding author
E-mail: water@kyungnam.ac.kr
Tel: +82-55-249-2988, Fax: +82-55-249-2994

There is a controversy on whether *Acinetobacter* is the predominant microorganism involved in EBPR. Mino *et al.*[3] concluded that *Acinetobacter sp.* are not the bacteria primarily responsible for EBPR reporting evidences as follows: (1) A fluorescence antibody staining technique for *Acinetobacter* revealed that *Acinetobacter* was less than 10% of total bacteria and could not count for the EBPR observed (2) *Acinetobacter* had a different respiratory quinone (Q-9) from PAO-enriched sludges (Q-8). (3) Auling *et al.*[4] used a polyamine, diaminopropane (DAP), as a biomarker for *Acinetobacter* and showed that EBPR plants eliminating phosphate very efficiently had nearly no DAP in polyamine pattern. (4) Many researchers utilized the classical culture-dependent methods that are strongly selective for *Acinetobacter sp.* Mino *et al.*[3] suggested a possibility that PAO could not grow as a single pure culture, but that some kinds of interspecies relations between different groups of microorganisms would be essential.

By the review of Jenkins and Tandoi[5], no single pure culture has been proved to be the predominant bacteria in the EBPR process. They also suggested that the anaerobic uptake and storage of CODsol, mediated by internally stored poly-P hydrolysis, appears to be a very low "gain" process occurring at rather low growth rates, only under anaerobic/aerobic cycling and possibly as a survival mechanism. Many other pure cultures as well as *Acinetobacter* were isolated from EBPR processes, but none of them have exhibited all the characteristics which EBPR sludge should possess. In many cases, there are lacking in acetate uptake and its conversion to PHA for storage coupled with hydrolysis of stored poly-P and consequent release of orthophosphate under anaerobic conditions.

However, Zafiri *et al.*[6] reported that the ability of *Acinetobacter sp.* to take up excess phosphorus under aerobic condition and to take up acetate with a simultaneous phosphorus release under anaerobic condition was verified with the measurement of acetate. *Acinetobacter sp.* cells presented a rapid adaptation ability to either aerobic or anaerobic conditions during shifting from one environment to the other. Nitrite accumulates during bacterial denitrification to a variable extent that depends not only on the organism but also on the growth conditions. Nitrite accumulation would be also triggered by discontinuous operation such as batch reactors. Wilderer *et al.*[7] observed nitrite accumulation in excess of 10 mg NO_2-N/L with either acetate or glucose as carbon source. Nitrite is also toxic to the denitrifying organisms themselves causing inhibition of denitrification and growth, subsequently leading to operational problems in nitrogen removal plants. Meinhold *et al.*[8] investigated the effect of nitrite on anoxic phosphate uptake with a series of batch experiments. Inhibition of phosphorus removal has been observed in swine wastewater treatment that has been shown to have nitrite build-up up to 80 mg/L (NO_2-N). This nitrite build-up resulted from high ammonium concentration in the influent and was suspected for inhibition of BPR. Nitrite would accumulate as an intermediate during nitrification or denitrification and poor phosphorus removal could be associated with nitrite build-up. Nitrite accumulation is of significance during phosphate release and phosphate uptake cycle since BPR processes generally include biological nitrogen removal.

Even though the nitrite build-up is known to cause severe problems in biological process in general, study concerning toxic effects of nitrite on biological phosphorus removal (BPR) bacteria was very limited. As we reviewed, there is no single bacterium sp. isolated from activated sludge systems, which is mostly responsible for EBPR.

Although several microorganisms have been involved in EBPR processes, *Acinetobacter* species would be one of microorganisms to be always detected in the activated sludge of EBPR plants. The purpose of this research was to study the mechanism of nitrite inhibition to *Acinetobater* under aerobic condition to get

better understandings for a relevant condition of nitrite build-up relating to biological phosphorus removal.

MATERIALS AND METHODS

Microorganism

Acinetobacter sp. (ATCC 11171) was used in this study.

Culture Media and Growth Conditions

The freeze-dried bacteria of *Acinetobacter sp.* were revived and cultured by the same procedures and culture media described by Weon *et al.*[9] Nitrite (as KNO_2) and phosphate (as K_2HPO_4) were added to the liquid medium a range of from 0~500 mg/L (NO_2-N) and 18.3 mg/L (PO_4-P), respectively. Ferric chloride and phosphate components were autoclaved separately. Also, the Tris-HCl buffer (50 mM) used in the experiment would not have been effective at controlling the pH values higher than 8 or lower than 6.

Pre-culture medium in 250 mL flask (125 mL liquid volume) was inoculated from agar plates and stirred at a constant rate of 300 rpm on a rotary shaker for two days at room temperature. Approximately 250 mL of the culture medium was added to a liter of liquid medium and allowed to mix to prevent any flocculation. The culture was then split into ten 100 mL volumes, transferred to each reactor simultaneously and diluted with liquid medium to an absorbance (550 nm) of 0.02~0.03 for use in experiments.

Growth Experiments

The experimental set-up is consisted of eight batch reactors. The reactors were autoclaved and covered with lid but were not air tight. Batch reactor working volume was 0.5 L. The reactors are equipped with an air supply and a sampling port. The air was filtered through air filter units (0.2 mm membrane filters) before entering the reactors. The DO concentration was maintained at 4.2~7.2 mg/L in experiments. Reactors were placed in shaker baths that maintained the cultures at a constant temperature (30±1°C) and provided agitation. To investigate the effects of nitrite and phosphate on cell growth, the concentrations of nitrite were varied in different experiments. Biomass in terms of absorbance, pH, and temperature were monitored throughout each experiment.

Analytical Methods

Every 30 minutes liquid sample were taken. Samples were withdrawn from the reactor using a syringe inserted into a plastic tube attached to a pipette that extended through the cap to the bottom of the reactor. The sample line was initially flushed, then approximately 10 mL of culture was withdrawn. Biomass absorbance was measured with Milton Roy Spectronic 21D spectrometer at 550 nm. Cultures were sampled intermittently to determine NO_2-N and PO_4-P concentrations. Samples of 10 mL volume were withdrawn and filtered through a 0.45 mm pore size membrane filter (Gelman GN-6) with a 1.2 mm pore size glass fiber filter (Whatman GF/C) as a prefilter. The concentration of nitrite (NO_2-N) and ortho-phosphate (PO_4-P) were determined using the Colorimetric (HACH NitriVer 2) and Ascorbic Acid methods, respectively[10]. All analysis was in duplicate. Measurement of pH was by a combination electrode and analyzer (720A, Orion, Cambridge, Mass., U.S.A.). The electrode was calibrated using pH 7.0 and 10.0 buffers.

RESULT AND DISCUSSION

The Accumulation of poly-P and PHB under Aerobic Conditions

Acinetobacetr sp. (ATCC 11171) was selected as a test organism because Zafiri *et al.*[6] verified that this bacterium were able to take up more phosphorus than in normally needed for cell growth and store it intracellularly as polyphosphate. In addition, a sufficiently accurate kinetic model, describing the behavior of the bacterium under controlled conditions was developed with relating kinetic parameter values.

In a growth of batch pre-culture under aerobic condition, we obtained a growth profile under 30°C of temperature, 7.2 (±0.05) of initial pH and 18.3 mg/L of PO_4-P as shown in Figure 1. Acetate was added in excess amount of 1.25 g/L for unlimited carbon source. The biomass concentration measurements were made by spectrophotometer absorbance at a wavelength of 550 nm and were converted to cell biomass concentration using the cell biomass - spectrophotometer absorbance relationship. The experimental data has been well acceptably described with a linear model exhibiting a correlation coefficient of 0.99.

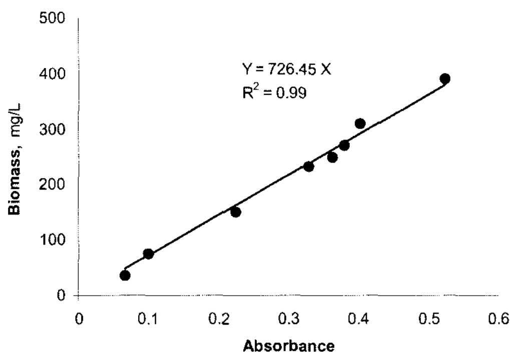

Figure 1. Relationships between absorbance and cell biomass dry weight.

A biochemical model (Figure 2) has been constituted to explain this experimental condition based on metabolic behavior of *Acinetobacter sp.* under aerobic phase in enhanced removal of phosphorus. The concentration of PO_4-P, Biomass, COD, DO and pH were monitored during the growth of *Acinetobacter sp.* under carbon and phosphate unlimited condition for subsequent nitrite inhibition experiments. The maximum dry cell mass concentration of 0.512 g/L was obtained in 48 hr with spectrophotometer absorbance of 0.705.

Specific growth rate (μ) of cells under such conditions in this experiment was 0.66 hr^{-1} and growth yield (Y) was 0.61~0.68 gVSS/gCOD. Zafiri *et al.*[6] used acetate in excess (1,573 mg COD/L initial concentration) in their experiment in order to avoid growth limitation by carbon throughout the batch experiment and measured the acetate concentration as 240 mg COD/L at the end of cell growth for confirmation of phosphorus growth limiting. Under aerobic conditions, the energy derived from the metabolism of external carbon source in the presence of oxygen is used for the accumulation of polyphosphates inside the cells (Figure 2). In our experiment, the cell accumulated up to 0.076 mg P/mg cell dry wt after reach at a maximum growth. This value was well consistent with 0.055 mg P/mg cell dry wt obtain by Zafiri *et al.*[6]

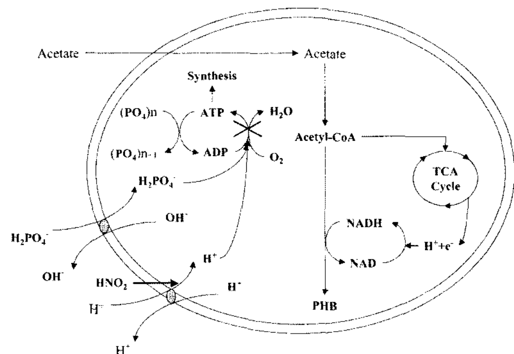

Figure 2. A biochemical model based on metabolic behavior of Acinetobactor sp. under aerobic condition.

Wentzel *et al.*[11] proposed a biochemical model of *Acinetobacter sp.* in enhanced biological phosphorus model for aerobic phase. The microorganisms were in an environment where organic substrate in the bulk liquid surrounding them is limited because available COD has been readily assimilated in the preceding anaerobic phase. However, external carbon source is available and cells do not possess stored PHB in their experiment. Wentzel *et al.*[11] discussed that a consequence of the presence of oxygen as an external electron acceptor is a reduction in NADH/NAD ratio and the decrease of NADH/NAD ratio stimulatse PHB degradation, the TCA cycle and associated glyoxylate pathway. In this experimental case, oxidative phosphorylation (ATP generation) takes place and the ATP/ADP ratio increases. A decrease in the NADH/NAD

ratio causes the removal of TCA cycle inhibition and further NADH generation by TCA cycle is possible with an electron sink. Thus, the high ATP/ADP ratio stimulates poly-P synthesis.

Lotter *et al.*[12]concluded that *Acinetobacter* strains isolated both from systems exhibiting excess phosphorus removal and from systems that do not, have the propensity to accumulate poly-P and PHB under aerobic culture conditions, with acetate and with glucose as substrate (by Enter-Doudoroff pathway which operates only with oxygen and/or nitrate present).

Kuba *et al.*[13] also reported that the organisms would accumulate the substrate as PHB when oxygen and an external substrate are simultaneously available. The accumulation of poly-P and PHB under the conditions of aerobic culture and external substrate available was depicted in the biochemical model (Figure 2). Thus, excess uptake of phosphorus could be accomplished by several different bacterial groups including *Acinetobacter* exerting a supplemental mechanism under man-made conditions. This would be a similar case to that involvement of yeast (e.g., *Saccaromycetes, Candida* etc.) into activated sludge system was sometimes very beneficial to improvement of treatment efficiency.

Nitrite Inhibition

Biological phosphorus removal (BPR) process is usually applied with simultaneous nitrogen removal in wastewater treatment process. This process may include one or more anoxic zones in which bacteria reduce nitrate and nitrite in absence of oxygen to accomplish nitrogen removal as well. The several investigators have been devoted to the study of denitrifying phosphorus removal bacteria which can uptake phosphorus under anoxic conditions during nitrate reduction[14~18] Biological nitrogen removal consists of nitrification and denitrification. Nitrification occurs under high dissolved oxygen (DO) and low organic matter concentrations. Denitrification dominates under high organic matter and low oxygen levels. Several studies have been published about factors inhibiting oxidation of NO_2^- to NO_3^- by *Nitrobacter*. The best known works are those of Anthonisen *et al.*[19] and Balmelle *et al.*[20] showing that high ammonium concentration and high pH can be responsible for a transient accumulation of nitrite. They found that *Nitrobacter* was inhibited at unionized or free nitrous acid (FNA) concentrations from 0.2 mg/L to 2.8 mg/L. However, studies of Hanaki *et al.*[21] showed that low DO level down to 0.5 mg/L could also cause nitrite build-up. Also, Nitrite is an intermediate of biological denitrification and can accumulate in solution in significant amounts during denitrification of nitrate as parameters including temperature, pH, nitrate concentration and salinity[22,5] Some investigators have suggested that instead of the nitrite ion (NO_2^-) concentration, undissociated nitrous acid (HNO_2) is the form that inhibits bacterial denitrification. Abeling and Seyfried[23] suggested that HNO_2 concentration might be the controlling factor in their own batch experiments using activated sludge, and an HNO_2 concentration of 0.13 mg/L (0.04 mg/L HNO_2-N) was proposed as the toxicity threshold for nitrite. More recently, Glass *et al.*[24] found that, at even near-neutral pH values of 6 and 7, nitrite concentrations of 30 and 250 mg/L NO_2-N, respectively, could inhibit denitrification. Sijbesma *et al.*[25] revealed by in vivo 31P-NMR study that nitrite had the inhibitory effect on denitrifying bacteria, Pseudomonas fluorescence acting as a protonophore (an uncoupler that increase the proton permeability of membranes by a shuttling mechanism).

In pre-culture, *Acinetobacter* reaches the stationary phase in its growth cycle within 18h and then begins the declining phase. These observation are consistent with the results obtained from Kim *et al.*[26] that *Acinetobacter* grown in batch mode on acetate medium at 25°C reaches maximum dry cell mass concentration within 16hr. The maximum *Acinetobacter* cell absorbance was 0.716 (550 nm). It was of interest to know what concentration of the nitrite would inhibit *Acinetobacter* growth in aerobic conditions. The cell growth in a typical

batch culture experiment of *Acinetobacter* with various nitrite concentrations is shown in Figure 3. Initial pH values were 7.2 (±0.05) and nitrite concentration in the reactors varied from 0 to 500 mg NO_2-N/L. Initial cell absorbance in all reactors was approximately 0.025~0.027. *Acinetobacter* was growing exponentially, however, as nitrite concentration was increased over about 400 mg/L, cell growth was significantly inhibited. It appears clearly that cell growth decreases as nitrite concentration increases.

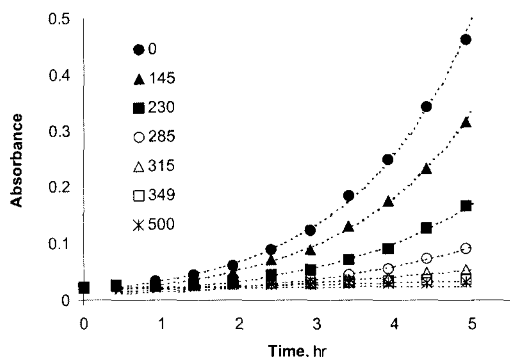

Figure 3. Growth of Acinetobacter sp. in relation to nitrite concentration with 18.3 mg/L of PO_4-P. Legend indicates initial nitrite concentration in mg/L.

In spite of the buffering of the nutrient solution, the pH value increased in some cultures up to 7.55. When the initial nitrite was in the range of 0~500 mg/L, nitrite concentration was constant in whole experimental periods (Figure 4). These results demonstrate that *Acinetobacter* is not able to take up nitrite as electron accepter in aerobic conditions. Even though *Acinetobacter* is able to utilize nitrite, bacteria prefer oxygen to nitrite in aerobic conditions. During the batch experiments involving activated sludge obtained from a pilot scale BPR plant, Meinhold *et al.*[8] reported that nitrite low concentrations (up to 4 or 5 mg NO_2-N/L) is not detrimental to anoxic biological phosphate uptake. However, at higher concentrations (above 8 mg NO_2-N/L) nitrite interferes with PAO metabolism, so that the anoxic phosphate uptake was totally inhibited over 8 mg/L of nitrite concentrations. Comeau *et al.*[27] also reported that anoxic phosphate uptake did not occur with over 10 mg/L of nitrite as electron acceptor.

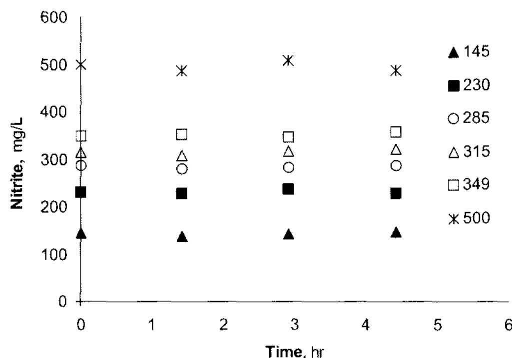

Figure 4. Change of nitrite concentration during experimental periods under the same conditions as Figure 3.

Acinetobacter could utilize oxygen as electron acceptor due to aerobic growth conditions in this experiment. Thus, cell growth inhibition by nitrite was lower than those of anoxic conditions. The specific growth rate (μ) was determined from a liner regression of a semi-logarithmic plot of the exponential biomass growth as a function of time using Monod growth model.

To determine the values of specific growth rate (μ), inhibition tests were performed with various nitrite concentrations and the same initial phosphate concentration of 18.3 mg/L, and the data of ln Xt/Xo was plotted against time for various nitrite concentrations. A typical specific growth rate (() of *Acinetobacter* for various nitrite and FNA concentration is shown in Figure 5. The result showed that the specific growth rate of *Acinetobacter* without nitrite was 0.67 hr^{-1}.

IC_{50} values of *Acinetobacter* growth in this experiment were 306 mg/L as nitrite and 0.103 mg/L as FNA in Figure 5. Antonisen *et al.*[19] reported that the inhibition of nitrifying organisms was initiated at concentrations of FNA between 0.22 and 2.8 mg/L. We have found that nitrite would have more inhibitory effects on the growth of *Acinetobacter* than that

of nitrifying organisms.

When nitrite appears as an intermediate in the nitrification and denitrification steps from enhanced biological phosphorus removal systems, *Acinetobacter* would be affected more seriously than nitrifying and denitrifying organisms.

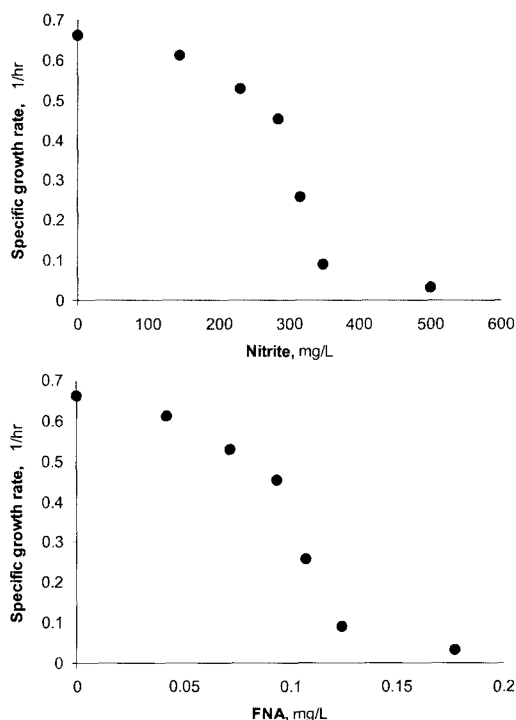

Figure 5. Specific growth rates as function of nitrite concentration and free nitrous acid.

Nitrite Inhibition Characteristics

A characteristic of *Acinetobacter* is that they utilize sugars exclusively via the Entner-Doudoroff pathway-a pathway that is inoperative under anaerobic conditions. As a consequence they cannot produce energy from fermentation due to the absence of the glycolytic or similar metabolic pathways- they are able to utilize sugars only under aerobic conditions[11] Furthermore, the genus is capable of storing phosphorus as poly-P and organic carbon as PHB. Lotter *et al.*[12] reported that *Acinetobacterr* strains isolated both from systems exhibiting excess phosphorus removal and from systems that do not, have the propensity to accumulate poly-P and PHB under aerobic culture conditions, with acetate as substrate. In case that oxygen and an external substrate are simultaneously available the organisms will accumulate the substrate as PHB[13] Thus, the organism used in this study seems to accumulate phosphate and to synthesize the PHB under aerobic and acetate available conditions. Sijbesma *et al.*[25] studied uncoupling effect of nitrite during denitrification by Pseudomonas fluorescens. Yarbrough *et al.*[28] reported that nitrite inhibited growth and energy generation in wide range of physiological types of bacteria. They also clearly discussed and concluded that one inhibitory action of nitrite is exerted at the bacterial cell membrane level and nitrite inhibits bacteria by several different means. A possible mechanism of nitrite inhibition on *Acinetobacter* is that nitrous acid form of nitrite affects proton-relating transport at the proton-pumping site crossing the cell membrane. As nitrite concentration increases, phosphorus uptake decreases with unlimited phosphorus concentration (18.3 mg/L as PO_4-P). This phenomina were well observed by the measurement of phosphorus uptake for nitrite inhibition of poly-P bacteria. As several other studies have demonstrated a great pH dependence on the extent of toxicity exerted by nitrite and led to the proposal that the toxic effect of nitrite is due to the protonated species, HNO_2, which is capable of crossing the membrane [25].

The results obtained in this study indicates that nitrite accumulate and pH is very important for biological phosphorus removal, specially when treating high strength of ammonium wastewater, because of nitrite inhibitory effect on poly-P bacteria and pH dependency of nitrite inhibitory form.

CONCLUSION

1. The mechanism of nitrite inhibition to *Acinetobater* under aerobic condition relating to nitrite build-up in biological phosphorus removal was explained in a biochemical

model.

2. Intracellular pH decreased by nitrous acid (HNO_2) crossing the membrane and shuttling protons, and ATP synthesis inhibited as shown in the biochemical model.
3. Poly-P synthesis pathway identified in a variety of organisms is via phosphorylation of the poly-P by ATP. Regulation of this pathway is via ATP/ADP ratio, therefore, inhibition of ATP synthesis yields low ATP/ADP ratio, consequently decrease poly-P synthesis and phosphorus uptake as appeared in the model.
4. Thus, the results obtained in this study indicates that nitrite accumulate and pH is very important for biological phosphorus removal, specially when treating high strength of ammonium wastewater, because of nitrite inhibitory effect on poly-P bacteria and pH dependency of nitrite inhibitory form.

ACKNOWLEDGMENTS

The present research was carried out at the Academic Interface Laboratory of the University of Florida Water Reclamation Facility. The author was supported by a study grant from Kyungnam University (2004). The authors gratefully acknowledge the cooperation of Charles Fender, plant superintendent, and Richard Smith, chemist, in the experiments.

REFERENCES

1. Fuhs, G. W. and Chen, M., "Microbiological basis of phosphate removal in the activated sludge process for the treatment of wastewater," *Microb. Ecol.,* **2**, 119-138 (1975).
2. Ohtake, H., Takahashi, K., Tsuzuki, Y., and Toda, K., "Uptake and release of phosphate by a pure culture of *Acinetobacter calcoaceticus,*" *Water Res.*, **19**, 1587-1594 (1985).
3. Mino, T., van Loosdrecht, M. C. M., and Heijnen, J. J., "Microbiology and biochemistry of the enhanced biological phosphate removal process," *Water Res.*, **32**, 3193-3207 (1998).
4. Auling, G., Pilz, F., Busse, H.-J., Karrasch, S., Streichan, M., and Schon, G., "Analysis of the polyphosphate-accumulating microflora in phosphorus eliminating,anaerobic-aerobic activated sludge systems by using diaminopropane as a biomarker for rapid estimation of acinetobacter spp," *Appl. Environ. Microbiol.,* **57**, 3685-3692 **(1991)**.
5. Jenkins, D. and Tandoi, V., "The applied microbiology of enhanced biological phosphate removal accomplishment and needs," *Water Res.,* **25**, 1471-1478 (1991).
6. Zafiri, C., Kornaros, M., and Lyberatos, G., "Kinetic modeling of biological phosphorus removal with a pure culture of *acinetobacter* sp. under aerobic, anaerobic and transient operating conditions," *Water Res.*, **33**, 2769-2788 (1999).
7. Wilderer, P. A, Jones, W. L., and Dau, U., "Competition in denitrification systems affecting reduction rate and accumulation of nitrite," *Water Res.*, **21**, 239-245 (1987).
8. Meinhold, J., Arnold, E., and Issaacs, S., "Effect of nitrite on anoxic phosphate uptake in biological phosphorus removal activated sludge," *Water Res.,* **33**, 1871-1883 (1999).
9. Weon, S.-Y., Lee, C.-W., Lee, S.-I., and Koopman, B., "Nitrite inhibition of aerobic growth of *Acinetobacter* sp," *Water Res.,* **36**, 4471-4476 (2002).
10. American Public Health Association; American Water Works Association; and Water Environment Federation., *Standard Methods for the Examination of Water and Wastewater.* 19th Ed., Washington, D.C (1995).
11. Wentzel, M. C., Lötter, L. H., Loewenthal, R. E., Marais, G. v. R., "Metabolic behavior of *Acinetobacter* spp. In enhanced biological phosphorus removal: a biochemical model," *Water SA.,* **12**, 209-224 (1986).
12. Lötter, L. H., "The role of bacterial phosphate metabolism in enhanced phosphorus removal from the activated sludge process," *Water Sci. Technol.,* **17**, 127-138 (1984).

13. Kuba, T, W., van Loosdrecht, M.C.M., and Heijnen J. J., "Effect of nitrate on phosphorus release in biological phosphorus removal systems," *Water Sci. Technol.*, **30**, 263-269 (1994).
14. Barker, P. S. and Dold P. L., "Denitrification behavior in biological excess phosphorus removal activated sludge systems," *Water Res.*, **30**, 769-780 (1996).
15. Comeau, Y., Hall, K. J., Hancock, R. E. W., and Oldham, W. K., "Biochemical model for enhanced biological phosphorus removal," *Water Res.*, **20**, 1511-1521 (1986).
16. Kern-Jespersen, J. P. and Henze, M., "Biological phosphorus uptake under anoxic and aerobic conditions," *Water Res.*, **27**, 617-624 (1993).
17. Kuba, T., van Loosdrecht, M. C. M., and Heijnen, J. J., "Effect of cyclic oxygen exposure on the activity of denitrifying phosphorus removing bacteria," *Water Sci. Technol.*, **34**, 33-40 (1996).
18. Vlekke, G. J. K., Comeau, Y., and Oldham, W. K., "Biological phosphorus removal from wastewater with oxygen nitrate in sequencing batch reactors," Environmental Technology Letters 9, 791-796 (1988).
19. Anthonisen, A. C., Loehr, R. C., Prakasam, T. B. S., and Srinarh, E. G., "Inhibition of nitrification by ammonia and nitrous acid," *J. Water Pollut. Control Fed.*, **48**, 835-852 (1976).
20. Balmelle, B., Nguyen, K. M., Caodeville, B., Cornier, J. C., and Deguin, A., "Study of factors controlling nitrite build-up in biological process for water nitrification," *Water Sci. Technol.*, **26**, 1017-1025 (1992).
21. Hanaki, K., Wantawin, C., and Ohgaki, S., "Nitrification at low levels of dissolved oxygen with and without organic loading in a suspended-growth reactor," *Water Res.*, **24**, 297-302 (1990).
22. Bode, H., Seyfried, C., and Kraft, A., "High-rate denitrification of concentrated nitrate wastewater," *Water Sci. Technol.*, **19**, 163-174 (1987).
23. Abeling, U. and Seyfried, C., "Anaerobic-aerobic treatment of high-strength ammonium wastewater-nitrogen removal via nitrite," *Water Sci. Technol.*, **26**, 1007-1015 (1992).
24. Glass, C., Silverstein, J., and Oh, J., "Inhibition of denitrification in activated sludge by nitrite. *Water Environ. Res.*, **69**, 1086-1093 (1997).
25. Sijbesma, W. F. H., Almeida, J. S., Reis, M. A. M., and Santos, H., "Uncoupling effect of nitrite during denitrification by Pseudomonas fluorescens: An in Vivo P-NMR study," *Biotechnol. Bioeng.*, **52**, 176-182 (1996).
26. Kim, H. J. and Pagilla, K. R., "Competitive growth of *Nocardia* and *acinetobacter* under anaerobic/aerobic bath operation," *Water Res.*, **34**, 2667-2674 (2000).
27. Comeau, Y., Oldham, W. K., and Hall, K. J., "Dynamics of carbon reserves in biological dephosphatation of wastewater," Proceeding of an IAWPRC specialized conference in Rome on biological phosphate removal from wastewater. pp. 39-55 (1987).
28. Yarbrough, J. M., Rake, J. B., and Eagon, R. G., "Bacterial inhibition effects of nitrite : inhibition of active transport, but not of group translocation, and of intracellular enzymes," *Appl. Environ. Microbiol.*, **39**, 831-834 (1980).
29. Thomsen, J. K., Geest, T., and Cox, R. P., "Mass Spectrometric studies of the effect of pH on the accumulation of intermediates in denitrification by paracoccus denitrificans," *Appl. Environ. Mocrobiol.*, **60**, 2, 536 (1994).