Chapter 1

GENERAL INTRODUCTION

Sludge reduction, Phosphorus recovery and Enhanced biological phosphorus removal

1.1 INTRODUCTION AND OBJECTIVES

In enclosed water bodies such as lakes and inland seas, water bloom and red tide have occurred with the increase in nutrient salts such as nitrogen and phosphorus. These nutrients cause the growth of toxic algae and affect the suitability of the water for a drinking use. Recently, the achieved levels for total nitrogen and phosphorus that are important causes of eutrophication were not yet satisfactory in lakes, marshes and coastal waters. Clearly, a result of slow progress in efforts to improve the state of pollution of lakes and marshes, inland harbors, inland seas, and other closed bodies of water as well as rivers in cities that receive discharged domestic wastewater has caused water pollution in Japan. Lakes and marshes are in extremely closed condition, nitrogen, phosphorus, and other nutrient salts accumulate, encouraging the propagation of primary producers such as algae etc. and creating blue tide and other eutrophication. Therefore, advanced wastewater treatment plants (advanced WWTPs), which attain both the nitrogen and phosphorus removal, are very important.

In Japan, the adoption rate of advanced wastewater treatment plants (advanced WWTPs), which have high nutrients (including nitrogen and phosphorus) removal capability, is quite lower (13% in 2005) than that in other advanced countries although the adoption rate of WWTPs, which have only carbon (and some nitrogen) removal capability, is high (68% in 2005) (Ministry of Land, Infrastructure and Transport). The increase in the introduction of WWTPs (including advanced WWTPs) causes the increase in the amount of excess sludge (Japan Sewage Works Association). In 2005, the amount of excess sludge retrieved form WWTPs was 75MT and it accounted for about 20% of the total industrial wastes (Ministry of the Environment). Ministry of Land, Infrastructure and Transport Government of Japan promotes the reduction and reuse of the excess sludge and it cause the reduction of the amount of landfill excess sludge (0.7 MT) (Ministry of Land, Infrastructure and Transport). However, the shortage of the dump yard is also the pressing problem and it is expected that the residual yard will be filled within 6 years

(Ministry of the Environment). Considering these pressing social problems, the sludge reduction processes should be introduced with the introduction or improvement of advanced WWTPs. There are two types of sludge reduction methods: one is the reduction of produced excess sludge by physicochemical techniques (e.g. ozonation, chlorination) and the other is the reduction of excess sludge production potential microbiologically (e.g. predation, extension of sludge age) (details are shown in 1.2)

On the other hand, the phosphorus recovery from wastewater is also important. It was suggested that within a time horizon of some 60-70 years about half the world currently economic phosphate resources will have been used up. In Japan, all phosphate rock is imported and Teduka et al. (2002) reported that 138 kt of phosphate (20% of the imported phosphate) was discharged to water bodies in 1998. The discharged phosphorus is contained in the municipal/ industrial wastewater. The phosphorus is removed by converting the phosphorus ions in wastewater into a solid fraction. This fraction can be an insoluble salt precipitate, a microbial mass in an activated sludge, or a plant biomass in constructed wetlands. These approaches do not recycle phosphorus as a truly sustainable product because it is removed with various other waste products, some of which are toxic. The non-solubilized phosphates are either buried at landfills after incineration of the organic matter or used as sludge fertilizer, if the treatment facility eliminates human pathogens and toxic compounds (details are shown in 1.3).

To date, denitrifying polyphosphate accumulating organisms (DNPAOs) have been adopted in some processes to achieve the reduction of the excess sludge reduction (Ahn *et al.*, 2002a, 2002b; Kishida *et al.*, 2006; Kuba *et al.*, 1996, 1997; Soejima *et al.*, 2006; Suzuki et al., 2006; Tsuneda *et al.*, 2006). Tsuneda *et al.* (2006) proposed Anaerobic/Oxic/Anoxic (AOA) process, however, external organic carbons source is required for the inhibition of oxic phosphorus uptake by polyphosphate-accumulation organisms (PAOs) (details are shown in 1.4). Suzuki *et al.* (2006) required this external organic carbon of excess sludge, in which high amount of organic carbon source is contained. Suzuki *et al.* (2006) combined A/O/A process with the ozonation process for sludge reduction and the phosphorus adsorption process for phosphorus recovery to meet these increasingly stringent requirements. Both effective sludge reduction and phosphors recovery were achieved in their study. However, the ozonation process produced slow-biodegradable organic carbon, and it caused the failure in the oxic phosphorus uptake inhibition and the increase of TOC concentration in the effluent.

On the other hand, the phylogenetic affiliation and physiological characteristic of PAOs and DNPAOs are still unknown. Some major candidates had been reported by using culture-independent molecular techniques; however, other candidates are still unidentified (Bond *et al.*, 1999; Crocetti *et al.*, 2000; Hesselmann *et al.*, 1999; Kawaharasaki *et al.*, 1999; Kong *et al.*, 2004, 2005; Liu *et al.*, 2001; Mino *et al.*, 1998; Seviour *et al.*, 2003) (details are shown in 1.5). The relation among the process operations, the wastewater characteristics and the dominant PAOs species is important for the process operation and process improvement; however, no clear explanation has been made.

In this study, the A/O/A process combined with the sludge ozonation process and the phosphorus adsorption process was improved by using the fine-bubbled ozonation system, which can achieve both high sludge solubilization efficiency and high biodegradability. To understand the microbial function in the process, microbial community analysis was performed and discussed. The method for selective concentration of PAOs was also developed. Microautoradiography-fluorescence *in situ* hybridization (MAR-FISH) analysis was performed to determine *in situ* activity of organisms which play important roles for nutrient removal.

1.2 EXCESS SLUDGE REDUCTION PROCESSES

Current technologies for sludge reduction can be roughly classified into two major categories: microbiological and physicochemical methods. Microbiological methods mainly include enhanced hydrolysis of biomass in membrane bioreactor (MBR) or in extended aeration process, and use of protozoa and metazoa for decreasing sludge production in aerobic wastewater treatment. Endogenous respiration including PAOs and DNPAOs contributed to the reduction of sludge production potential. Ødetaard *et al.* (2003) listed the mechanisms involved in reduction of biomass reported by Van Loosdrecht and Henze (1999) (Table 1).

Table 1 Processes involved in reduction of biomass mass (van Loosdrecht and Henze, 1999)

Process	Description
Maintenance	Direct consumption of cell external or internal substrates for maintenance of cell integrity
Endogenous respiration	Respiration with oxygen or nitrate using cell internal components
Death-regeneration including	Decay followed by growth on the secondary substrate arising from
growth and lysis (cryptic growth)	the decay

On the other hand, recently, the feasibility of physical and chemical sludge reduction has been investigated and showed great potential in produces excess sludge reduction.

1.2.1 Physical sludge reduction

The stirred ball-mill consists of a cylindrical or conical tank, vertically or horizontally arranged. It has a disc mixer and 0.2–0.3 mm ball-shaped millstones inside. The sludge is mechanically crushed when passing through the mill. The balls are following the sludge out of the mill, separated from the sludge downstream and recycled to the mill (Ødetaard *et al.*, 2003).

The high-pressure homogenizer is a simple device consisting of two main components; a multistage high-pressure pump and a homogenizer valve. The high-pressure pump forces the sludge through the valve at a velocity of 300 m/sec and the static pressure in the valve reaches that of the vapor pressure of the liquid. The cavitation bubbles that result induce the forces that disintegrate the sludge (Ødetaard *et al.*, 2003).

1.2.2 Chemical sludge reduction (reduction by chemical oxidant)

Ozone is a strong chemical oxidant and has been commonly used. Ozonated sludge reduction process is based on the idea that part of activated sludge is mineralized to carbon dioxide and water, while part of sludge is solubilized to biodegradable organics that can be biologically treated. Many studies have been conducted with respect to the ozonated sludge reduction process (Yasui and Shibata, 1994; Sakai *et al.*, 1997; Saktaywin *et al.*, 2005; Kamiya and Hirotsuji, 1998; Deleris *et al.*, 2000; Suzuki *et al.*, 2006). A combined activated sludge process and intermittent ozonation system had been successfully developed (Yasui and Shibata, 1994; Sakai et al., 1997; Kamiya and Hirotsuji, 1998). Ozone is a strong cell lysis agent. When sludge is contact with ozone, most microorganisms would be killed and oxidized to organic substances. Böhler and Siegrist (2004) have estimated the cost and energy consumption for ozone treatment. They found that the cost for operation and investment of sludge ozonation was compensated for by the decreasing operation cost for sludge treatment and disposal. They estimated the energy consumption for partial ozonation of the return sludge with an excess sludge reduction of 30% to about 15% of the total electrical energy consumption of a municipal WWTP.

As an alternative solution of sludge reduction, recently a chlorination-combined activated sludge process had been developed for minimizing excess sludge production (Saby et al., 2002). This chlorination-combined activated sludge process is similar to the ozonation. The sludge production could be reduced by 65% in the chlorination-activated sludge system. Since chlorine is a weak oxidant as compared to ozone, the dosage of chlorine used in the chlorination-activated sludge process is about 7–13 times higher than that of ozone applied in the ozonation. In the chlorination, the undesirable chlorinated by-products would be produced (e.g. trihalomethanes).

1.3 PHOSPHORUS RECOVERY PROCESSES

Phosphorus is removed by converting the phosphorus ions in wastewater into a solid fraction. This fraction can be an insoluble salt precipitate, a microbial mass in an activated sludge, or a plant biomass in constructed wetlands and adsorption by phosphorus-selective adsorbent.

1.3.1 Phosphorus precipitation by metals

Today, the main commercial processes for removing phosphorus from wastewater effluents are chemical precipitation with iron, alum, or lime (reviewed by de-Basahan and Bashan, 2004). Occasionally, auto-precipitation (like struvite, described later) occurs as an outcome of special conditions and composition of the wastewater (Van Der Houwen and Valsami-Jones, 2001). Although phosphorus precipitation is a common commercial practice, further refining, fine-tuning, optimization, using leftovers and new materials from other industrial processes, and a few other innovations were proposed in recent years.

1.3.2 Struvite

The most promising compound for recovery from wastewater plants is magnesium ammonium phosphate hexahydrate ($MgNH_4PO_4 \cdot 6H_2O$), commonly known as struvite, which precipitates spontaneously in some wastewater processes. If formation and collection are controlled and cost-effective, struvite might have potential in the fertilizer market. Small quantities of recovered struvite are currently being tested as fertilizer, mainly in Japan.

1.3.3 Phosphorus assimilation by organisms

Removing phosphate by biological can be accomplished by two independent mechanisms: the direct assimilation by growing cells (e.g. microalgae) and plants (e.g. plants in constructed wetlands) and polyphosphate accumulating organisms (PAOs) (reviewed by de-Basahan and Bashan, 2004).

1.3.4 Phosphorus adsorption by a zirconium ferrite adsorption

Phosphorus adsorption methods by a zirconium-ferrite adsorbent have been proposed recently (Suzuki *et al.*, 2006; Takai *et al.*, 2002). This adsorbent adsorbs orthophosphate (PO_4) high selectively and has adsorption capacity (Takai *et al.*, 2002). Using a zirconium-ferrite adsorbent would be cost-effective because the adsorbent can reuse many times by desorption of adsorbed phosphorus by 7% of sodium hydrate and reactivated by sulfuric acid.

1.4 ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL PROCESSES

To eliminate nitrogen and phosphorus biologically, anaerobic/anoxic/oxic (A_2O) processes have been used for domestic/industrial WWTPs. In this process, nitrogen is removed by nitrification in oxic tank and denitrification in anoxic tank by circulation of nitrate-rich liquid from the oxic tank. Phosphorus is removed by the anaerobic/oxic cyclic operation. In anaerobic tank, PAOs take up volatile fatty acids (VFAs) and polymerize them as polyhydroxyalkanoates (PHA) while releasing intercellular polyphosphate (PolyP) as orthophosphate (P_i) (Mino *et al.*, 1998; Seviour *et al.*, 2003). In the subsequent aerobic period, PAOs accumulate large amount of P_i in excess of released P_i as PolyP (Mino *et al.*, 1998; Seviour *et al.*, 2003). The A_2O process can achieve efficient nutrient removal when the C/N ratio and/or C/P ratio is enough for both denitrification and phosphorus removal. However, when these ratios become low, the competition for organic carbon uptake between heterotrophic denitrifying bacteria and PAOs deteriorate the nitrogen and/or phosphorus removal efficiency. To solve this problem, new processes had been developed by using DNPAOs.

The use of DNPAOs can relieve the competition for organic carbon because they can accumulate phosphorus by utilizing nitrate/nitrite as electron accepter instead of oxygen (Mino *et al.*, 1998; Seviour *et al.*, 2003). Moreover, DNPAOs are 50% less efficient in generating energy and it indicates employing DNPAOs decrease the sludge production efficiency (Kuba *et al.*, 1996). The two-sludge systems and single-sludge system were developed. The former is A_2N and DEPHANOX process (Kuba *et al.,* 1996; Bortone *et al.*, 1996). These processes can maintain high mount of nitrifying bacteria in external nitrification processes and prevent exposure of DNPAOS to oxygen. These processes also have an advantage to increase phosphorus content; however, they are very complicated processes because external nitrification tank and additional flows are required. On the other hand, single sludge systems are University of Cape Town (UCT) process, A/O/A SBR and A/O/A granular sludge (AOAGS) (Kishida *et al.*, 2006; Kuba *et al.*, 1996; Soejima *et al.*, 2006; Suzuki *et al.*, 2006; Tsuneda *et al.*, 2006). Although the common problem in these single-sludge systems is that DNPAOs are exposed to oxygen, SBRs have advantages for these complicated processes because an SBR consists of only one single reactor. A UCT process consists of anaerobic, anoxic and oxic tank same as A_2O but it has two mixed liquor circulations; anoxic to anaerobic and oxic to anoxic. Tsuneda *et al.* (2006) described an anaerobic/oxic/anoxic (AOA) system and succeeded in causing DNPAOs to take an active part in simultaneous nitrogen and phosphorus removal in an acetate-fed sequencing batch reactor by adding organic carbon at the start of oxic period. Suzuki *et al.* (2006) combined A/O/A process with the ozonation process for sludge reduction and the phosphorus adsorption process for phosphorus recovery to meet these increasingly stringent requirements. Both effective sludge reduction and phosphors recovery were achieved in their study. However, the ozonation process produced slow-biodegradable organic carbon, and it caused the failure in the oxic phosphorus uptake inhibition and the increase of TOC concentration in the effluent.

1.5 CHARACTERISTICS OF (DENITRIFYING) POLYPHOSPHATE-ACCUMULATING ORGANISMS

1.5.1 Identification and ecophysiology of Polyphosphate-Accumulating Organisms

In the past decade, culture-independent molecular techniques have identified some major bacteria playing important roles for phosphorus removal processes. A member of the phylogenetically defined *Rhodocyclus*-relatives in the β-*Proteobacteria* have been recognized as the dominant group in acetate-fed SBRs (Bond *et al.*, 1999; Crocetti *et al.*, 2000; Hesselmann *et al.*, 1999; Kong *et al.*, 2004; Mino *et al.*, 1998; Seviour *et al.*, 2003). These bacteria have been named *Candidatus* 'Accumulibacter phosphatis' or *Rhodocyclus*-related PAOs, RPAO (Crocetti *et al.*, 2000; Hesselmann *et al.*, 1999). Kong *et al.* (2004) reported that RPAO actually played important role for phosphorus removal and can accumulate phosphate utilizing nitrate/nitrite as electron accepter instead of oxygen. It emphasized RPAO is the important DNPAOs; however, recent study revealed that respiratory nitrate reductase appears to be absent from RPAO (Martin *et al.*, 2006).

Two *Actinobacterial* PAO (coccus-APAO and rod-APAO) had been also reported as important PAOs (Kong *et al*, 2005). Ecophysiology of APAO is different form that of RPAO and the biological model of PAOs. The PHA staining revealed that APAO do not form PHA during anaerobic organic carbons and accumulate phosphate by using unknown energy under aerobic conditions (Kong *et al*, 2005). Kong *et al.* (2005) also reported APAO could not denitrify and use nitrite as an electron accepter for phosphorus uptake, however, APAO could accumulate phosphate utilizing nitrate (Kong *et al*, 2005).

Some PAOs candidates in the α-*Proteobacteria*, the γ-*Proteobacteria* and *Actinobacteria* have been also reported existed in EBPR processes (Kawaharasaki *et al.*, 1999; Liu *et al.*, 2001).

1.5.2 Glycogen-Accumulating Organisms (GAOs) and G-bacteria

Although the anaerobic-aerobic cyclic operations provide the selective advantage for growth of PAOs, other organisms are also able to grow in these operational conditions (Fig.1). These organisms, named glycogen-accumulating organisms (GAOs) or G-bacteria, which have a morphotype of cocci usually arranged in tetrads and/or clusters, were assumed to compete with PAOs for organic carbon uptake and to cause the deterioration of EBPR (Beer *et al.*, 2004; Cech and Hartman, 1993; Crocetti *et al.*, 2002; Kong *et al.*, 2001, 2002a, 2002b, 2006; Liu *et al.*, 2001; Meyer *et al.*, 2006; Mino *et al.*, 1998; Nielsen et al., 1999; Seviour et al., 2000, 2003; Wong et al.,2004). Culture-independent molecular techniques defined that GAOs belong to members of the lineage GB or *Cand.* 'Competibacter phosphatis' in the γ-*Proteobacteria* (Crocetti *et al.*, 2002; Kong *et al.*, 2002b, 2006; Liu *et al.*, 2001; Nielsen et al., 1999). Members of the order *Sphingobacteriales* and genus *Defluvicoccus* belonging to α-*Proteobacteria* are also identified as G-bacteria (Beer *et al.*, 2004; Kong *et al.*, 2001, 2002a; Liu *et al.*, 2001; Meyer *et al.*, 2006; Wong et al.,2004).

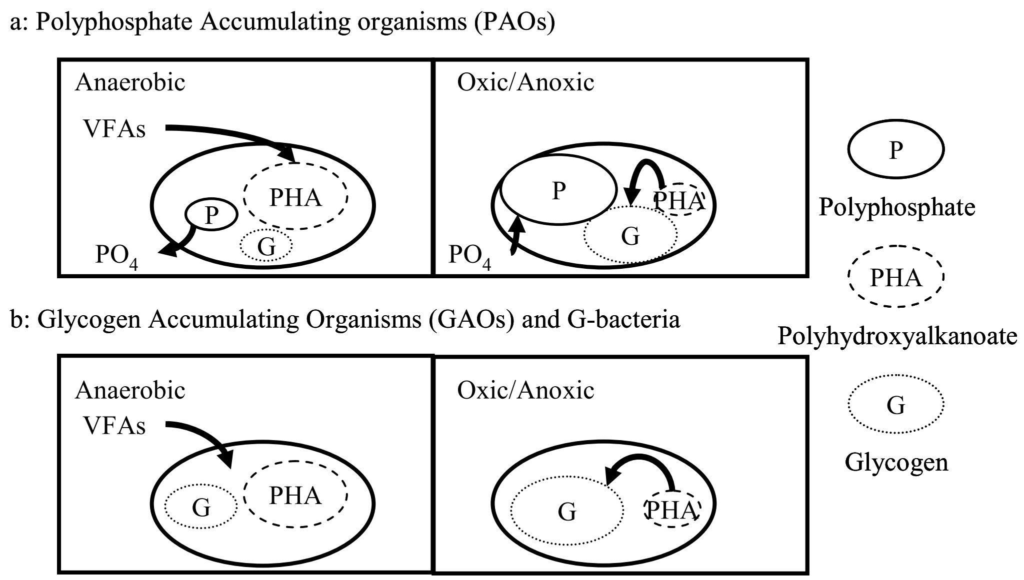

Fig.1 The biochemical model of polyphosphate-accumulating organisms (PAOs) and glycogen-accumulating organisms (GAOs)

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