Chapter 1

General Introduction

1

Biological nutrients removal, difficulties in conventional biological nutrients removal process, and novel biological wastewater treatment using real-time control strategy and granular sludge technology

Summary

Nutrients such as nitrogen and phosphorus cause eutrophication, leading to a significant negative impact to aqueous environment. Therefore, nutrients removal from municipal and industrial wastewater has recently been strongly required. Although biological nutrients removal (BNR) processes are cost-effective and widely adopted in the world, stable nutrients removal is often difficult because biochemical reaction is strongly affected by many parameters such as pH and temperature. In addition, quality of wastewater is often fluctuated, which also make it difficult to attain stable treatment performance. Meanwhile, conventional simultaneous nitrogen and phosphorus removal processes are very complicated, and difficult to be applied to small wastewater treatment plants. To solve these problems in conventional BNR processes, use of novel real-time control strategy and granular sludge technology is proposed in this chapter.

1.1 Necessity of nutrients removal

Enrichment of nutrients such as nitrogen and phosphorus in water bodies is one of the important factors affecting aqueous environment. Discharging wastewater with high level of nitrogen and phosphorus can result in eutrophication of surface waters, particularly lakes and slow moving rivers (Sundblad et al., 1994; Danalewich et al., 1998). This leads to high production of phytoplankton biomass and turbid water. The former includes appearance of toxic cyanobacteria in fresh water bodies, and it is often dominated by *Microcystis aeruginosa* (Orr et al., 2001; Park et al., 2001). *M. aeruginosa* and some other freshwater cyanobacteria often produce hepatotoxic cyclic heptapeptides microcystins (Kaya, 1996). The ingestion of intact *M. aeruginosa* cells or microcystins is reported to be toxic to aquatic organisms, domestic animals, wildlife and humans (Miura et al., 1989; Žegura et al., 2003). Besides, eutrophication often causes other undesired biological changes that include loss of biodiversity, disappearance of submerged macrophytes, fish stock changes, and decreasing top–down control by zooplankton on phytoplankton (Andersen and Ring, 1999; Boyle et al., 1999; Scheren et al., 2000; Xie and Xie, 2002; Søndergaard et al., 2003). In addition, eutrophication often leads to a reduction in the supply of ecosystem services (Carpenter et al., 1999; Mäler, 2000). For instance, it may affect recreation and fisheries (Hein, 2006).

Meanwhile, inorganic nitrogenous compounds (NH_4^+, NH_3, NO_2^-, HNO_2, NO_3^-) themselves are toxic for aquatic animals that can take up them directly from the ambient water. Among them, unionized ammonia is the most toxic, while ammonium and nitrate ions are the least toxic (Camargo and Alonso, 2006). In addition, nitrite and nitrate are toxic for humans. Ingested nitrites and nitrates from polluted drinking waters can induce methemoglobinemia in humans, particularly in young infants, by blocking the oxygen-carrying capacity of hemoglobin. Typical symptoms of methemoglobinemia are cyanosis, headache, stupor, fatigue, tachycardia, coma, convulsions, asphyxia and ultimately death (Craun et al., 1981; Nash, 1993; Ayebo et al., 1997; Knobeloch et al., 2000; Fwetrell, 2004; Greer and Shannon, 2005). Since 1945 more than 3000 cases of methemoglobinemia have been reported worldwide, most of which were associated with private wells with high nitrate concentrations (10 g/m^3 as NO_3–N basis) (Nash, 1993; World Health Organization, 1996; Ayebo et al., 1997; Knobeloch et al., 2000; Wolfe and Patz, 2002). Ingested nitrates and nitrites also have a potential role in developing cancers of the digestive tract through their contribution to the bacterial formation of *N*-nitroso compounds (i.e., nitrosamines), which are among the most potent of the known carcinogens in mammals (Nash, 1993; Knobeloch et al., 2000; Wolfe and Patz, 2002; Fwetrell, 2004). In addition, some scientific evidences suggest that ingested nitrates and nitrites might result in mutagenicity, teratogenicity and birth defects (Dorsch et al., 1984; Luca et al., 1987), contribute to the risks of non-Hodgkin's lymphoma (Ward et al., 1996), coronary heart disease (Cerhan et al., 2001), and bladder and ovarian cancers (Weyer et al., 2001), or play a role in the etiology of insulin-dependent diabetes mellitus (Virtanen et al., 1994; Parslow et al., 1997; Van Maanen et al., 2000) and in the development of thyroid hypertrophy (Van Maanen et al., 1994). Long-term consumption of drinking water with nitrate concentrations even below the maximum contaminant level of 10 g/m^3 as NO_3–N basis has been linked to higher risks for non-Hodgkin's lymphoma (Ward et al., 1996) and for bladder and ovarian cancers (Weyer et al., 2001). Indirect health hazards can also occur as a consequence of algal toxins, causing nausea, vomiting, diarrhoea, pneumonia, gastroenteritis, hepatoenteritis, muscular cramps, and several poisoning syndromes (paralytic shellfish poisoning, neurotoxic shellfish poisoning, amnesic shellfish poisoning) (Camargo and Alonso, 2006). Other indirect health hazards can also come from the potential relationship between inorganic nitrogen pollution and human infectious diseases because increasing nutrient availability may often favor disease-causing organisms (Townsend et al., 2003).

Thus, nutrients such as nitrogen and phosphorus cause eutrophication, leading to a significant negative impact to aqueous environment. In addition, inorganic nitrogenous compounds themselves are toxic for aquatic animals and humans. Therefore, nutrients removal from domestic and industrial wastewater has recently been strongly required.

1.2 Biological nitrogen and phosphorus removal

Nitrogen and phosphorus can be removed from wastewater by a variety of physicochemical and biological processes. Since biological processes are cost-effective (Osee Muyima et al., 1997) and less harmful to water environment (Terada, 2006), they have been widely adopted.

1.2.1 biological nitrogen removal

In general, the biological elimination of nitrogen from wastewater requires a two-step process, involving nitrification followed by denitrification as shown in Fig. 1.1.

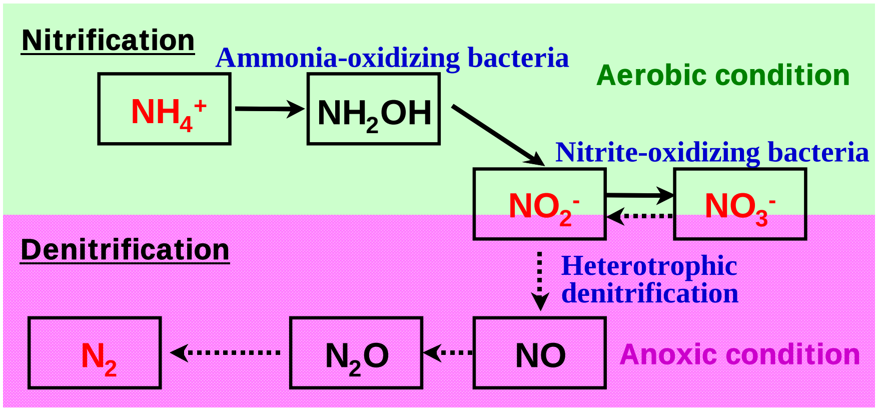

Figure 1.1 Schematic diagram of biological nitrogen removal pathway.

Nitrification implies a chemolithoautotrophic oxidation of ammonia to nitrate under strict aerobic conditions and is conducted in two sequential oxidative stages: ammonia to nitrite (ammonia oxidation) and nitrite to nitrate (nitrite oxidation). Each stage is performed by different bacterial genera that use ammonia or nitrite as an energy source and molecular oxygen as an electron acceptor, while carbon dioxide is used as a carbon source. The most commonly recognized genus of bacteria that carries out ammonia oxidation is *Nitrosomonas*; however, *Nitrosococcus*, *Nitrosopira*, *Nitrosovibrio* and

Nitrosolobus are also able to oxidize ammonium to nitrite. These ammonium oxidizers are genetically diverse, but related to each other in the beta subdivision of the *Proteobacteria*. In the nitrite oxidation stage, several genera such as *Nitrospira*, *Nitrospina*, *Nitrococcus*, and *Nitrocystis* are known to be involved. However, the most famous nitrite oxidizer genus is *Nitrobacter*, which is closely related genetically within the alpha subdivision of the *Proteobacteria* (Teske et al., 1994; Rittmann and McCarty, 2001). Equations for synthetic-oxidation using a representative measurement of yield and oxygen consumption for *Nitrosomonas* and *Nitrobacter* are as follows (U.S. Environmental Protection Agency, 1975).

Ammonia oxidation (nitritation)

$$55NH_4^+ + 76O_2 + 109HCO_3^- \rightarrow C_5H_7NO_2 + 54NO_2^- + 57H_2O + 104H_2CO_3 \qquad (1)$$

Nitrite oxidation (nitratation)

$$400NO_2^- + NH_4^+ + 4H_2CO_3 + HCO_3^- + 195O_2 \rightarrow C_5H_7NO_2 + 3H_2O + 400NO_3^- \qquad (2)$$

Using Eqs. (1) and (1), the overall synthesis and oxidation reaction in nitrification can be represented as follows:

$$NH_4^+ + 1.83O_2 + 1.98HCO_3^- \rightarrow 0.021\ C_5H_7NO_2 + 0.98\ NO_3^- + 1.041\ H_2O + 1.88\ H_2CO_3 \qquad (3)$$

In these equations, yields for *Nitrosomonas* and *Nitrobacter* are 0.15 mg cells/mg NH_4-N oxidized and 0.02 mg cells/mg NO_2-N oxidized, respectively. Oxygen consumption ratios in the equations are 3.16 mg O_2/mg NH_4-N oxidized and 1.11 mg O_2/mg NO_2-N oxidized, respectively. Also, it can be calculated that 7.07 mg alkalinity as $CaCO_3$ is required per mg ammonia nitrogen oxidized. Severe pH depression can occur when the alkalinity in the wastewater approaches depletion by the acid produced in the nitrification process. The significance of pH depression in the nitrification process is that the reaction rates are rapidly depressed as the pH is reduced below 7.0. Therefore, in cases where the alkalinity of the wastewater will be depleted by the acid produced by nitrification, the proper alkalinity must be supplemented by a chemical addition, such as lime (U.S. Environmental Protection Agency, 1975).

As the second step, denitrification is generally performed by a heterotrophic bioconversion process under anoxic conditions. The oxidized nitrogen compounds (NO_2^- and NO_3^-) are reduced to gaseous dinitrogen (N_2) by heterotrophic microorganisms that use nitrite and/or nitrate instead of oxygen as electron acceptors and organic matter as carbon and energy source. Denitrifiers are common among the Gram-negative alpha and beta classes of the *Proteobacteria*, such as *Pseudomonas*, *Alcaligenes*, *Paracoccus*, and *Thiobacillus*. Some Gram-positive bacteria (such as *Bacillus*) and a few halophilic Archaea (such as *Halobacterium*) are also able to denitrify (Zumft, 1992). The process in environmental biotechnology is accomplished with a variety of electron donors and carbon sources such as: methanol, acetate, glucose, ethanol, and a few others (Table 1.1) (Ahn, 2006). Because methanol (CH_3OH) was relatively inexpensive, it gained widespread use (Rittmann and McCarty, 2001). Combined dissimilation-synthesis equations for denitrification using methanol as an electron donor are as follows (U.S. Environmental Protection Agency, 1975):

$$NO_3^- + 1.08CH_3OH + 0.24H_2CO_3 \rightarrow 0.056C_5H_7O_2N + 0.47N_2 + 1.68H_2O + HCO_3^- \quad (4)$$

In this equation, the theoretical methanol requirement for nitrate is 2.47 mg CH_3OH per mg NO_3-N. Neglecting synthesis, the requirement is decreased to 1.9.

Table 1.1 Organic requirements in heterotrophic denitrification (Ahn, 2006)

	Carbon sources	Organic requirements (g COD/g N)	References
NO_2-N	Acetic acid	1.56	Abeling and Seyfried, 1992
	Acetic acid	2.0	Akunna and Bizeau, 1993
	Lactic acid	2.8	Akunna and Bizeau, 1993
	Methanol	2.3	U.S. EPA, 1975
	Methanol	2.1-2.6	Ho and Choi, 2000
NO_3-N	Acetic acid	2.08	Abeling and Seyfried, 1992
	Acetic acid	3.7	Akunna and Bizeau, 1993
	Lactic acid	4.1	Akunna and Bizeau, 1993
	Methanol	3.75-4.5	U.S. EPA, 1975
	Methanol	7.35	Nyberg et al., 1992
	Raw sewage	5.2	Rogalla et al., 1992
	Piggery waste	8.44	Bae et al., 2001
NOx-N	Acetate	2.07	Narkis et al., 1979
	Methanol	4.2	Narkis et al., 1979
	Piggery waste	6.42	Bae et al., 2001

Meanwhile, biological nitrogen removal processes have been reported as potential sources of N_2O by many researchers (Schulthess and Gujer, 1996; Thorn and Sorensson, 1996; Kishida et al., 2004). N_2O is emitted as by-products or intermediates through nitrification and denitrification. Emission control of N_2O is very important in biological nitrogen removal because the global warming potential of N_2O per one molecule is about 150-300 times greater than that of CO_2 (IPCC, 2001), and N_2O is the major source of stratospheric NO, which causes the depletion of ozone layer (Cicerone, 1989).

1.2.2 biological phosphorus removal

Enhanced biological phosphorus removal (EBPR) from wastewater is based on the enrichment of activated sludge with polyphosphate-accumulating organisms (PAOs) (Brdjanovic et al., 1998; Wagner and Loy, 2002). EBPR exploits the potential for microorganisms to accumulate phosphate (as intracellular polyphosphate) in excess of their normal metabolic requirements (Brdjanovic et al., 1998; Mino et al., 1998). The EBPR process is primarily characterized by circulation of activated sludge through anaerobic and aerobic phases, coupled with the introduction of influent wastewater into the anaerobic phase (Wagner and Loy, 2002). In the anaerobic phase, sufficient readily degradable carbon sources, such as volatile fatty acids (VFAs), must be available, which induces PAOs to uptake the acids and release phosphate into solution (Morse et al., 1998). In the aerobic phase, luxury P-uptake occurs, which results in overall phosphorus removal rates of as much as 80–90% (Morse et al., 1998). These anaerobic and aerobic metabolisms of PAOs are extensively investigated by Smolders et al. (1994a, b). These metabolisms are schematically shown in Fig. 1.2. A high P-removal efficiency can be achieved by wasting excess sludge with high P-content (Mino et al., 1998).

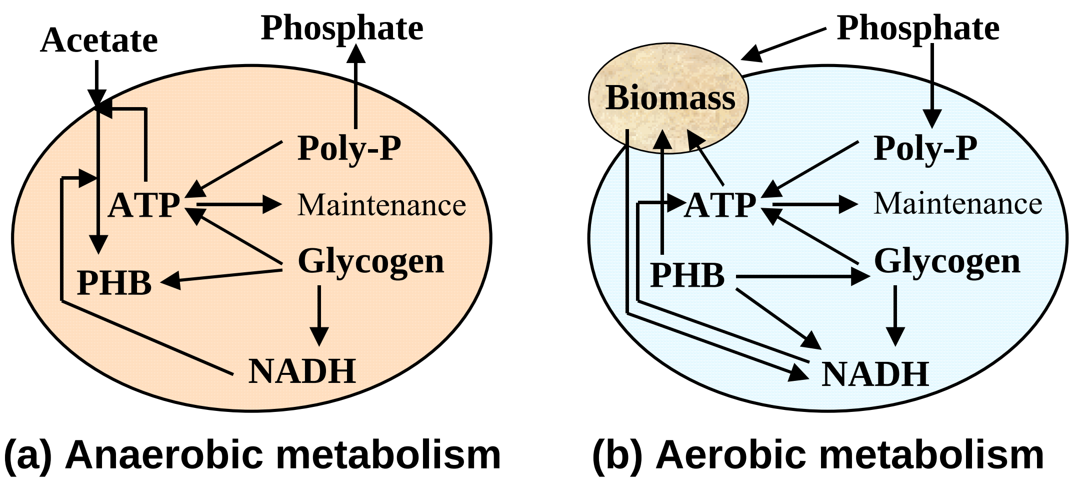

Figure 1.2 Schematic diagram of metabolisms of PAOs. (De Kreuk, 2006).

Meanwhile, a part of PAOs have been found to accumulate polyphosphate using nitrate as an electron acceptor (Kuba et al., 1993, 1996; Bortone et al., 1999). These organisms (referred to as denitrifying polyphosphate-accumulating organisms, DNPAOs) have metabolic characteristics similar to those of PAOs, based on the metabolic transformations responsible for EBPR. DNPAOs are 40% less efficient in generating energy and thus have a 20–30% lower cell yield (Murnleitner et al., 1997). Consequently, the utilization of DNPAOs affords many advantages in BNR.

Classical cultivation techniques were used for a long time to isolate and grow pure cultures of bacterial strains responsible for EBPR. *Acinetobacter* spp., which were the most common isolates, were thought to be important PAOs in EBPR process (Fuhs and Chen, 1975; Buchan, 1983). However, their pure-culture phenotype has never matched that of EBPR, and over the years, a series of carefully executed studies by different researchers has demonstrated that *Acinetobacter* spp. are not PAOs (Auling et al. 1991; Wagner et al. 1994; Kämpfer et al. 1996). On the other hand, Hesselmann et al. (1999) reported the definitive phylogenetic placement of the *betaproteobacteria*-2 subgroup PAO as a close relative of *Rhodocyclus* spp. for the first time and called the organism "*Candidatus* Accumulibacter phosphates" (henceforth called *Accumulibacter*). Crocetti et al. (2000) supported this finding and extended the knowledge by using fluorescence *in situ* hybridization (FISH) and post-FISH chemical staining to demonstrate that the *Accumulibacter* cells cycled poly-P according to EBPR. In recent years, *Accumulibacter* has been believed to be main PAOs by many researchers. However, identity of PAOs is still unknown despite much effort by many researchers because *Accumulibacter* has never isolated.

1.2.3 Difficulties in biological nutrients removal

Biological nutrients removal (BNR) are widely adopted because it is cost-effective and less harmful to water environment as mentioned above. However, they have some difficulties.

Firstly, it is known that stable treatment is often difficult because biological nutrients removing organisms are very sensitive to external conditions. Reportedly, activity of biological nitrogen removal bacteria （i.e. nitrifying and denitrifying bacteria）is affected by a lot of parameters such as dissolved oxygen (DO) (Bernet, 2001), pH (U.S. Environmental Protection Agency, 1975; Burton and Prosser, 2001), concentrations of free ammonia and nitrous acid (Anthonisen *et al.*, 1976), temperature (Grunditz and Dalhammar, 2001), and sludge retention time (SRT) (Police et al., 2002). Furthermore, it is known that activity of PAOs is more sensitive to external conditions than that of nitrifying and denitrifying bacteria. Biological phosphorus removal is also affected by many parameters such as chemical oxygen demand (COD) loading rate (Morgenroth and Wilderer, 1998), COD/P ratios of the influent (Chang et al, 1996), concentration of volatile fatty acids (Rustrian et al., 1996; Pitman, 1999), concentration of cations (Mg^{2+}, etc.) (Schönborn et al., 2001), temperature (Brdjanovic et al., 1997), DO (Shehab et al, 1996), pH (Smolders et al, 1994a) and SRT (Choi et al. 1996; Rodrigo et al., 1996). It is too difficult to control all parameters precisely. In particular, precise control is almost impossible in small wastewater treatment plants because general people normally maintain plants instead of engineers. In addition, characteristics of influent wastewater are often fluctuated, which also makes treatment performance unstable. Reportedly, BNR process is sensitive to disturbances, such as dilution of the wastewater, e.g. in times of heavy rainfall (Brdjanovic et al., 1998), with prolonged disturbances leading to recovery times of over 4 weeks (Okada et al., 1992). Thus, stable treatment is normally difficult in

BNR. Therefore, it is necessary to develop a novel BNR process that attains stable treatment performance.

Secondly, it is difficult to accomplish simultaneous nitrogen and phosphorus removal because PAOs compete with denitrifying bacteria for organic carbon in the influent wastewater. It is known that both denitrification and phosphate release require organic carbon. Therefore, the phosphorus removal efficiency often decreases when the available organic carbon content is low (Morling, 2001). To solve this problem, DNPAOs have recently received much attention. The use of DNPAOs can relieve the competition for organic carbon because they can treat nitrate/nitrite and phosphate using the same carbon sources (Mino et al., 1998; Shoji et al., 2003). In the same manner as PAOs, DNPAOs take up external carbon substrates and store them as polyhydroxyalkanoates in the cell under anaerobic conditions. However, they can utilize nitrite or nitrate instead of oxygen as an electron accepter to remove phosphorus under anoxic conditions (Ahn et al., 2002). Although external nitrification processes such as DEPHANOX and A_2N have already been developed for the effective use of DNPAOs (Kuba et al., 1996; Bortone et al., 1999; Shoji et al., 2003), they are very complicated processes that require many reactors and mixed liquor recycling streams as shown in Fig. 1.3. Therefore, it is necessary to develop an alternative simple BNR process that can utilize DNPAOs.

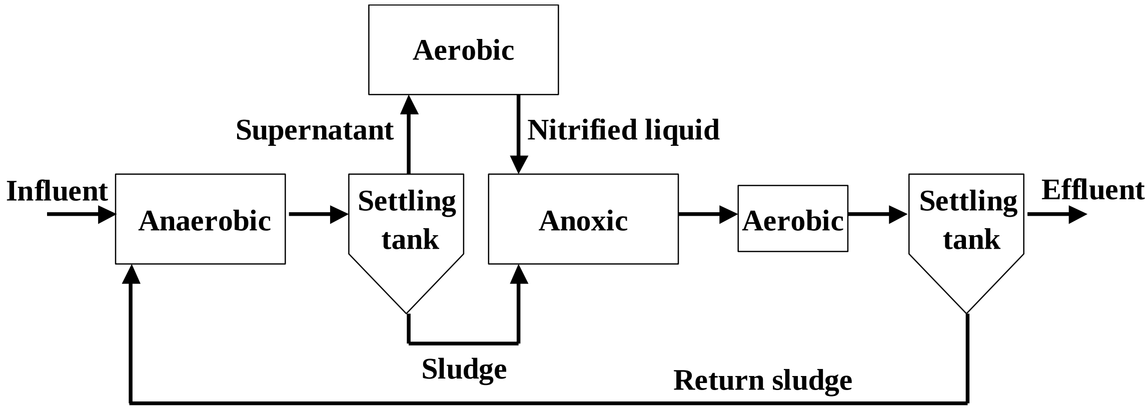

Figure 1.3 Schematic diagram of external nitrification (DEPHANOX) process.

1.3 Real-time control strategy for stable biological nutrients removal

Although BNR is an effective treatment method, there is a problem associated with instability of treatment as mentioned above. To solve this problem, the author focuses on real-time control strategy in this thesis.

In recent years, on-line monitoring of oxidation-reduction potential (ORP), pH and DO has been proven to be useful for process monitoring, particularly when applied to a biological nitrogen removal using a sequencing batch reactor (SBR) (Yu et al., 2000). Several investigators have identified the “nitrate knee” in ORP profiles and the “nitrate apex” in pH profiles, which indicate the end of denitrification (Koch and Oldham, 1985; Al-Ghusain and Hao, 1995). In this case, the “nitrate knee” is a break point related to the drastic change of the slope on ORP profiles, and the “nitrate apex” is a point of maximum in pH profiles. These points can be used for anoxic cycle control in a biological nitrogen removal process. It has also been found that the “nitrogen break point” or the “DO elbow” and the “ammonia valley”, which indicate the end of nitrification, appear on ORP and pH profiles, respectively (Ra et al., 1999; Wareham et al., 1994; Al-Ghusain et al., 1994). The “nitrogen break point” and the “DO elbow” are break points on ORP profiles, and the “ammonia valley” is a point of minimum in pH profiles. These points can also be used for oxic cycle control in a biological nitrogen removal process. Therefore, activity of biological nitrogen removal organisms can be monitored and controlled for stable treatment using these control points as shown in Fig. 1.4. In addition, real-time control strategy would be also used in the EBPR process because it was reported that time-course of phosphate is correlated with variations of electric conductivity (Maurer and Gujer). This real-time control strategy is useful for not only stable treatment but also cost reduction because mixing and aeration time can be optimized.

Although real-time control strategy is considered to be useful for stable treatment in BNR, there are some problems. Operational conditions for successful control and effectiveness of control parameters such as ORP and pH has not been well investigated. In addition, synthetic wastewater was used in most previous studies. Therefore, applicability of this technology to real wastewater treatment is still unknown.

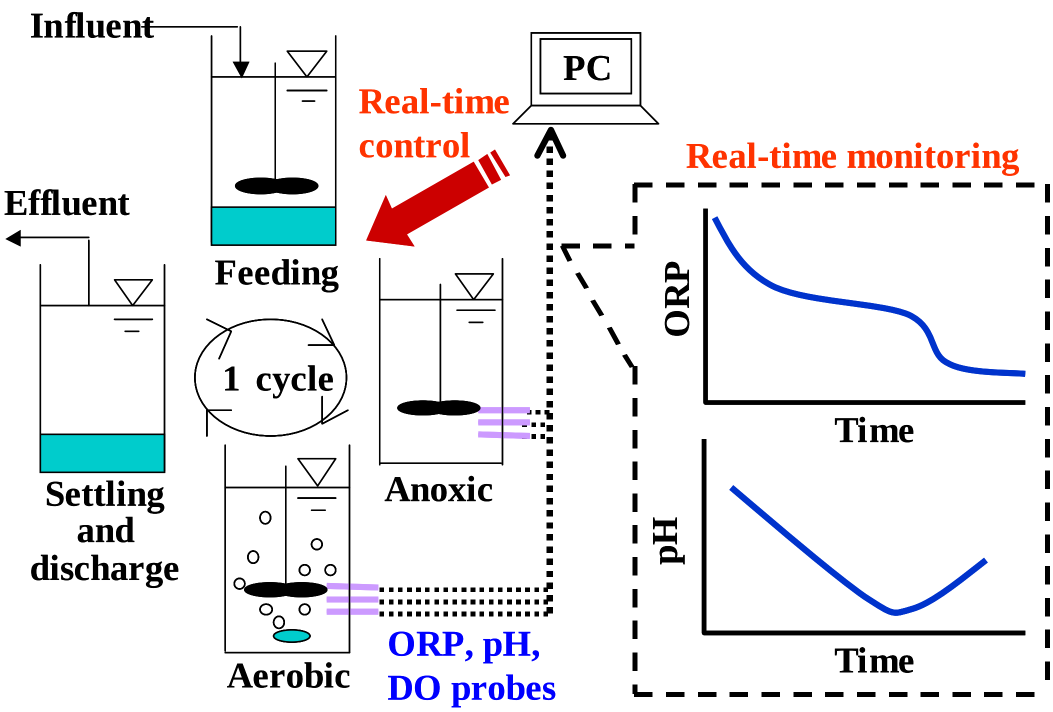

Figure 1.4 Schematic diagram of real-time control strategy in BNR.

1.4 Granular sludge technology

1.4.1 General information on granular sludge

Granular sludge can be regarded as compact and dense microbial aggregates with a spherical outer shape. Microbial granulation is quite fundamental in biology, and cell aggregation can be defined as gathering together of cells to form a fairly stable, contiguous and multi-cellular association under physiological conditions (Hoshino, 2004). Granular sludge technology is useful for improving efficiency of a wastewater treatment process because granular sludge has some advantages over conventional bioflocs, such as excellent settleability, high biomass retention and so on (Liu et al., 2004). In addition, this technology is cost-effective since microbial granules can be formed without any carrier materials such as activated carbon. In the past, microbial granulation was considered as a phenomenon unique to an anaerobic biological treatment process. Although anaerobic granular sludge is useful for high rate organic compounds degradation, it cannot be applied to nutrients removal because aerobic biological reaction is necessary to remove nitrogen and phosphorus.

Meanwhile, at the end of 1990's, formation of aerobic granular sludge using an SBR was reported for the first time (Morgenroth et al., 1997). Since then, aerobic granular sludge formation has been actively studied around the world. There are not only oxic zones but also anoxic zones in aerobic granular sludge because the oxygen penetration depth inside the granular sludge is limited. Hence, granular sludge technology became applicable to nutrients (nitrogen and phosphorus) removal because both aerobic and anaerobic microorganisms can exist in a single granule. Characteristics of aerobic and anaerobic granular sludge were summarized in Table 1.2.

Table 1.2 Characteristics of aerobic and anaerobic granular sludge

	Aerobic granular sludge	Anaerobic granular sludge
Main formation reactor	SBR*[1]	UASB*[2]
Aeration	Necessary	Unnecessary
Warming	Unnecessary	Necessary (> 30^{o}C)
Nitrogen and phosphorus removal	Applicable	Inapplicable
Low strength wastewater treatment	Applicable	Unsuitable
Organic compounds degradation rate	Relatively high	Extremely high
Recovery of valuable gasses	Incapable	Capable (CH_4 and H_2)
Sludge production	Relatively high	Low
History of studies	Short (Since 1990's)	Long (Since 1970's)
Examples of suitable application	*High rate treatment of low strength organic wastewater *Nutrients removal	*High rate treatment of high strength organic wastewater *Recovery of CH_4 and H_2 from wastewater

*1 Sequencing batch reactor; *2 Upflow anaerobic sludge blanket

1.4.2 Aerobic granular sludge

Despite long-term application of anaerobic granular sludge in wastewater treatment, aerobic granular sludge is a new observation. Therefore, a definition of aerobic granular sludge has been discussed in recent years. During the first aerobic granular sludge workshop (Munich, 2004), aerobic granular sludge was defined as follows (de Kreuk et al., 2005):

Granules making up aerobic granular activated sludge are to be understood as aggregates of microbial origin, which do not coagulate under reduced hydrodynamic shear, and which settle significantly faster than activated sludge flocs.

Some aerobic granulation has been reported in continuously-fed biofilm airlift suspension reactors that have carrier material added (Kwok et al., 1998). However, almost all spontaneous aerobic granules has been formed in a SBR although it was reported that aerobic granular sludge can be formed using a continuously-fed reactor when completely inorganic wastewater mainly composed of ammonium sulfate was used (Tsuneda et al., 2003). Until now, research on the necessary factors for granule formation has focused on the effect of settling-time, shear force, selection of slow-growing organisms and feeding pattern (feast-famine regime) and so on. A short settling time has been utilized to select for fast settling flocs and granules, forcing the washout of less dense flocs and filamentous organisms as shown in Fig. 1.5 (Beun et al., 2002). Tay et al. (2001) operated four parallel SBRs with different aeration rates (superficial gas velocity: 0.3-3.6 cm/s), showing that granules formed only in reactors with a superficial gas velocity greater than 1.2 cm/s. De kreuk and van Loosdrecht (2004) and Liu et al. (2004) showed selection of slow-growing organisms such as nitrifying bacteria and PAOs is useful for stable granular sludge formation. These results are corresponded with experimental results in previous biofilm studies (Tijhuis et al., 1995; Liu, 1997).

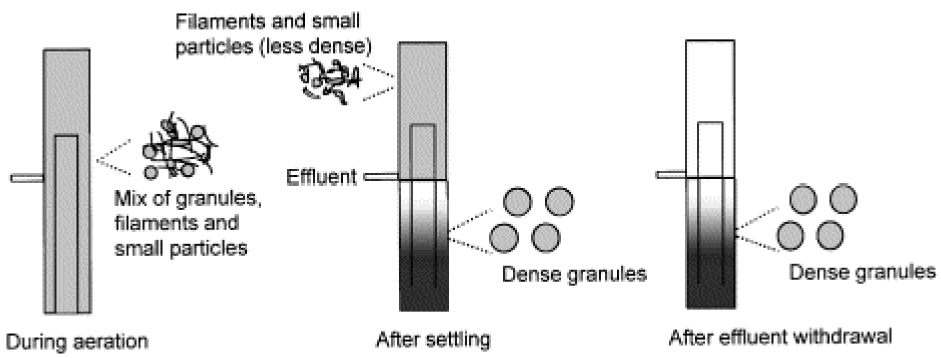

Figure 1.5 Selection of well-settling, dense granules by setting a short settling time (Beun et al., 2002).

Mcswain et al. (2004) operated three parallel SBRs with different feeding conditions, showing importance of period of high load followed by starvation (often referred to as feast-famine) for formation of aerobic granular sludge. Although other factors such as concentration of cations and formation of extra-cellular polymeric substances (EPS) are considered to be involved in formation of aerobic granular sludge, the role of these factors has not been well understood yet.

1.4.3 Development of an efficient simultaneous nitrogen and phosphors removal process using granular sludge

Although BNR is an effective treatment method, there is a problem associated with competition of denitrifying bacteria and PAOs for organic carbon. The use of DNPAOs can relieve the competition for organic carbon because they can treat nitrate/nitrite and phosphate using the same carbon sources. Although external nitrification processes such as DEPHANOX and A_2N have already been developed for the effective use of DNPAOs, they are very complicated processes that require many reactors and mixed liquor recycling streams. Therefore, it is necessary to develop a simple BNR process that can utilize DNPAOs. To simplify the process, the use of an SBR is one of the effective methods because an SBR makes it possible to remove nutrient in a single reactor without mixed liquor recycling streams. Moreover, it has been verified, in full-scale studies, that this BNR process of using an SBR is cost-effective as compared with continuous flow processes (Peters et al., 2004). Although an anaerobic/oxic (with low dissolved oxygen) process and an anaerobic/oxic/anoxic (AOA) process have been proposed for the utilization of DNPAOs in SBRs (Tsuneda et al., 2006; Zeng et al., 2004), these processes have some disadvantages. In the former, denitrification is mainly responsible for denitrifying glycogen-accumulating organisms (DNGAOs), competitors of PAOs. In the latter, a large amount of external carbon must be added at the beginning of the oxic phase to prevent aerobic uptake of phosphate by PAOs. The common problem in these processes is that DNPAOs are exposed to oxygen. Although necessary for nitrification, aeration creates hostile conditions for DNPAOs. To solve this problem and enable the use of DNPAOs, the author proposes use of granular sludge technology in this thesis.

Under an aeration condition, there are not only oxic zones but also anoxic zones in the granular sludge because the oxygen penetration depth inside the granular sludge is limited. Therefore, if the reactor is operated under alternate anaerobic/oxic conditions, it is expected that anaerobic/anoxic conditions suitable for the cultivation of DNPAOs (Ng et al., 2001) can be created inside the granular sludge, and simultaneous nitrogen and phosphorus removal can be accomplished using a single reactor if granular sludge is formed in the reactor. Hence, an efficient nitrogen and phosphorus removal process can be constructed using granular sludge.

1.4 Objective of this study

The final objective of this study is to develop novel efficient BNR processes using real-time control strategy and granular sludge.

For developing the BNR process using real-time control strategy, firstly, effectiveness of control parameters such as ORP and pH was experimentally demonstrated (Chapter 2). Based on these results, real-time control strategy was established, and applied to synthetic (low C/N) wastewater treatment (Chapter 3). Finally, effectiveness of this process was investigated by applying to real wastewater treatment. Stability of treatment performance was demonstrated in long-term experiments (Chapter 4).

For developing the BNR process using granular sludge, the granular sludge was firstly formed using an SBR, and possibility of simultaneous nitrogen and phosphorus removal was investigated. Inside of the granular sludge was also inspected by molecular and microsensor techniques in order to investigate nutrients removal mechanism. Additionally, applicability of real-time control strategy when granular sludge is formed in the reactor was investigated (Chapter 5). Finally, effectiveness of this process was investigated by applying to real wastewater treatment (Chapter 6).

References

Abeling, U., Seyfried, C.F., 1992. Anaerobic-aerobic treatment of high strength ammonium wastewater nitrogen removal via nitrite. Water Sci. Technol., 26, 1007–1015.

Ahn, J., Daidou, T., Tsuneda, S., Hirata, A., 2002. Transformation of phosphorus and relevant intracellular compounds by a phosphorus-accumulating enrichment culture in the presence of both the electron acceptor and electron donor. Biotechnol. Bioeng., 79, 83–93.

Ahn, Y.H., 2006. Sustainable nitrogen elimination biotechnologies: A review. Process Biochem., 41, 1709-1721.

Akunna, J.C., Bizeau, C., Moletta, R., 1993. Nitrate and nitrite reductions with anaerobic sludge using carbon sources: glucose, glycerol, acetic acid, lactic and methanol. Water Res., 27, 1303–1312.

Al-Ghusain, I.A., Huang, J., Hao, O.J., Lim, B.S., 1994. Using pH as a real time control parameter for wastewater treatment and sludge digestion process. Water Sci. Technol., 30(4), 159–168.

Al-Ghusain, I.A., Hao, O.J., 1995. Use of pH as control parameter for aerobic/anoxic sludge digestion. J. Environ. Eng., 121, 225–235.

Andersen, F., Ring, P., 1999. Comparison of phosphorus release from littoral and profundal sediments in a shallow, eutrophic lake. Hydrobiologia, 408/409, 175–183.

Anthonisen, A.C., Loehr, R.C., Prakasam, T.B.S., Srinath, E.G.,. 1976. Inhibition of nitrification by ammonia and nitrous acid. J. Water Poll. Contr. Fed., 48, 835–852.

Auling, G., Pilz, F., Busse, H.J., Karrasch, S., Streichan, M., Schön, G., 1991. 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, 3585–3592.

Ayebo, A., Kross, B., Vlad, M., Sinca, A., 1997. Infant methemoglobinemia in the Transylvania region in Romania. Int. J. Occup. Env. Health, 3, 20–29.

Bae, U.K., Ko, G.B., Lee, Y.W., Lee, C.H., 2001. Piggery wastewater treatment using short-cut nitrogen removal technology. In: Proceedings of the symposium of current issue in Korea: the technologies and policy for the livestock wastewater management, pp. 39

Bernet, N., Dangcong, P., Delgène, P., Steyer, J.P., Moletta, R., 2001. Nitrification at alow oxygen concentration in biofilm reactor. J. Environ. Eng., 127, 266–271.

Beun, J.J., van Loosdrecht, M.C.M., Heijnen, J.J., 2002. Aerobic granulation in a sequencing batch airlift reactor. Water Res., 36, 702–712.

Bortone, G., Libelli, S.M., Tilche, A., Wanner, J., 1999. Anoxic phosphate uptake in the DEPHANOX process. Water Sci. Technol., 40(4–5), 177–185.

Boyle, J.F., Rose, N.L., Bennion, H., Yang, H., Appleby, P.G., 1999. Environmental impacts in the jianghan plain: evidence from lake sediments. Water Air Soil Pollut., 112, 21–40.

Brdjanovic, D., van Loosdrecht, M.C.M., Hooijmans, C.M., Alaerts, G.J., Heijnen, J.J., 1997. Temperature effects on physiology of biological phosphorus removal. J. Environ. Eng., 123, 144–153.

Brdjanovic, D., Slamet, A., van Loosdrecht, M.C.M., Hooijmans, C.M., Alaerts, G.J., Heijnen, J.J., 1998. Impact of excessive aeration on biological phosphorus removal from wastewater. Water Res., 32, 200–208.

Buchan, L., 1983. Possible biological mechanism of phosphorus removal. Water Sci.

Technol., 15, 87–103.

Burton, S.A.Q., Prosser. J.I., 2001. Autotrophic ammonia oxidation at low pH through urea hydrolysis. Appl. Environ. Microbiol., 67, 2952–2957.

Camargo, J.A., Alonso, A., 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. Environ. Int., 32, 831–849.

Carpenter, R.S., Ludwig, D., Brock, W.A., 1999. Management of eutrophication for lakes subject to potentially irreversible change. Ecol. Appl., 9, 751–771.

Cerhan, J.R., Weyer, P.J., Janney, C.A., Lynch, C.F., Folsom, A.R., 2001. Association of nitrate levels in municipal drinking water and diet with risk of coronary heart disease mortality: the Iowa Women's Health Study. Epidemiology, 12, 457.

Chang, W.C., Chiou, R.J., Ouyang, C.F., 1996. The effect of residual substrate utilization on sludge settling in an enhanced biological phosphorus removal process. Water Sci. Technol., 34, 425–430.

Choi, Y., Shin, E.B., Lee, Y.D., 1996. Biological phosphorus removal from wastewater in a single reactor combining anaerobic and aerobic conditions. Water Sci. Technol., 34(1–2), 179–186.

Ciceron, R.J.,1989. Analysis of sources and sinks of atmospheric nitrous oxide (N_2O). J. Geophys. Res., 94, 18265-18271.

Craun, G.F., Greathouse, D.G., Gunderson, D.H., 1981. Methemoglobin levels in young children consuming high nitrate well water in the United States. Int. J. Epidemiol., 10, 309–317.

Crocetti, G.R., Hugenholtz, P., Bond, P.L., Schuler, A., Keller, J., Jenkins, D., Blackall, L.L., 2000. Identification of polyphosphate-accumulating organisms and design of 16S rRNA-directed probes for their detection and quantitation. Appl. Environ.

Microbiol., 66, 1175–1182.

Danalewich, J.R., Papagiannis, T.G., Belyea, R.L., Tumbleson, M.E., Raskin, L., 1998. Characterisation of dairy waste streams, current treatment practices, and potential for biological nutrient removal. Water Res., 32, 3555–3568.

De Kreuk, M.K., van Loosdrecht, M.C.M., 2004. Selection of slow growing organisms as a means for improving aerobic granular sludge stability. Water Sci. Technol., 49(11-12), 9–17.

De Kreuk, M.K., McSwain, B.S., Bathe, S., Tay, S.T.L., Schwarzenbeck, N., Wilderer, P.A., 2005. Discussion outcomes. In: Bathe, S., De Kreuk, M.K., McSwain, B.S., Schwarzenbeck N. (Editors), Aerobic Granular Sludge. London: IWA publishing, pp. 153–169.

De Kreuk, M.K., 2006. Aerobic granular sludge–Scaling-up a new technology [Ph.D. thesis]. Delft: Delft University of Technology, pp.8.

Dorsch, M.M., Scragg, R.K.R., McMichael, A.J., Baghurst, P.A., Dyer, K.F., 1984. Congenital malformations and maternal drinking water supply in rural south Australia: a case-control study. Am. J. Epidemiol., 119, 473–486.

Fuhs, G.W., Chen, M., 1975. Microbiological basis of phosphate removal in the activated sludge process for the treatment of wastewater. Microbiol. Ecology, 2, 119–138.

Fwetrell, L., 2004. Drinking-water nitrate, methemoglobinemia, and global burden of disease: a discussion. Environ. Health Persp.,112, 1371–1374.

Greer, F.R., Shannon, M., 2005. Infant methemoglobinemia: the role of dietary nitrate in food and water. Pediatrics, 116, 784–786.

Grunditz, C., Dalhammar, G.., 2001. Development of nitrification inhibition assays using pure cultures of *Nitrosomonas* and *Nitrobacter*. Water Res., 35, 433–440.

Hein, L., 2006. Cost-efficient eutrophication control in a shallow lake ecosystem subject to two steady states. Ecol. Econ., 59, 429–439.

Hesselmann, R.P.X., Werlen, C., Hahn, D., van der Meer, J.R., Zehnder, A.J.B., 1999. Enrichment, phylogenetic analysis and detection of a bacterium that performs enhanced biological phosphate removal in activated sludge. Syst. Appl. Microbiol., 22, 454–465.

Ho, J.H., Choi, E., 2000. Nitrogen removal from piggery waste by nitrite nitrification. In: Proceedings of the WEFTEC 2000 73rd annual conference and exposition on water quality and wastewater treatment. Session 30.

Hoshino, T., 2004. Development of nitrogen removal process using nitrifying granules and in situ detection of bacterial functional gene [Ph.D. thesis]. Tokyo: Waseda University, pp.7.

IPCC, 2001. Climate Change 2001: The Scientific Basis, Cambridge: Cambridge University Press.

Kämpfer, P., Erhart, R., Beimfohr, C., Bohringer, J.,Wagner, M., Amann, R., 1996. Characterization of bacterial communities from activated sludge – culture-dependent numerical identification versus in situ identification using group- and genus-specific rRNA-targeted oligonucleotide probes. Microb. Ecol., 32, 101–121.

Kaya, K., 1996. Toxicology of microcystins. In: Watanabe, M.F., Harada, H., Carmichael, W.W., Fujiki H. (editors.), Toxic Microcystis. Boca Raton, Fla: CRC Press. Inc., pp. 175–202.

Kishida, N., Kim, J.H., Kimochi, Y., Nishimura, O., Sasaki, H., Sudo, R., 2004. Effect of C/N ratio on nitrous oxide emission from swine wastewater treatment process. Water Sci. Technol., 49(5-6), 359–365.

Knobeloch, L., Salna, B., Hogan, A., Postle, J., Anderson, H., 2000. Blue babies and nitratecontaminated well water. Environ. Health Persp., 108, 675–678.

Koch, F.A., Oldham, W.K., 1985. Oxidation-reduction potential – a tool for monitoring, control and optimization of biological nutrient removal systems. Water Sci. Technol., 17(11/12), 259–281.

Kuba, T., Smolders, G., van Loosdrecht, M.C.M., Heijnen, J.J., 1993. Biological phosphorus removal from wastewater by anaerobic-anoxic sequencing batch reactor. Water Sci. Technol., 27(5–6), 241–252.

Kuba, T., van Loosdrecht, M.C.M., Heijnen, J.J., 1996. Phosphorus and nitrogen removal with minimal COD requirement by integration of denitrifying dephosphatation and nitrification in a two-sludge system. Water Res., 30, 1702–1710.

Kwok, W.K., Picioreanu, C., Ong, S.L., van Loosdrecht, M.C.M., Ng, W.J., Heijnen, J.J., 1998. Influence of biomass production and detachment forces on biofilm structures in a biofilm airlift suspension reactor. Biotechnol. Bioeng., 58, 400–407.

Liu, Y., 1997. Estimating minimum fixed biomass concentration and active thickness of nitrifying biofilm. J. Environ. Eng.-ASCE, 123, 198–202.

Liu, Y., Yang, S.F., Tay, J.H., 2004. Improved stability of aerobic granules by selecting slow-growing nitrifying bacteria. J. Biotechnol., 108, 161–169.

Luca, D., Luca, V., Cotor, F., Rileanu, L., 1987. In vivo and in vitro cytogenetic damage induced by sodium nitrite. Mutat. Res., 189, 333–340.

Mäler, K.G., 2000. Development, ecological resources and their management: a study of complex dynamic systems. Eur. Econ. Rev., 44, 645–655.

Maurer, M., Gujer, W., 1995. Monitoring of microbial phosphorus release in batch experiments using electric conductivity. Water Res., 29, 2613–2617.

McSwain, B.S., Irvine, R.L., Wilderer, P.A., 2004. Effect of intermittent feeding on aerobic granule structure. Water Sci. Technol., 49(11-12), 19–25.

Mino, T., van Loosdrecht, M.C.M., Heijnen, J.J., 1998. Microbiology and biochemistry of the enhanced biological phosphate removal process. Water Res., 32, 3193–3207.

Miura,G.A., Robinson,N.A.,Geisbert, T.W., Bostian,K.A.,White, J.D., Pace, J.G., 1989. Comparison of in vivo and in vitro toxic effects of microcystin-LR in fasted rats. Toxicon, 11, 1229–1240.

Morgenroth, E., Sherden, T., van Loosdrecht, M.C.M., Heijnen, J.J., Wilderer, P.A., 1997. Aerobic granular sludge in a sequencing batch reactor. Water Res., 31, 3191–3194.

Morgenroth, E., Wilderer, P.A., 1998. Modeling of enhanced biological phosphorus removal in a sequencing batch biofilm reactor. Water Sci. Technol., 37(4–5), 583–587.

Morling, S., 2001. Performance of an SBR-plant for advanced nutrient removal, using septic sludge as a carbon source. Water Sci. Technol., 43(3), 131–138.

Murnleitner, E., Kuba, T., van Loosdrecht, M.C.M., Heijnen, J.J., 1997. An integrated metabolic model for the aerobic and denitrifying biological phosphorus removal. Biotechnol. Bioeng., 54, 434–450.

Narkis, N., Rebhun, M., Sheindorf, C.H., 1979. Denitrification at various carbon to nitrogen ratios. Water Res., 13, 93–98.

Nash, L., 1993. Water quality and health. In: Gleik, P.H. (editor), Water in crisis: a guide to the world's fresh water resources. New York: Oxford University Press, pp. 25–39.

Ng, W.J., Ong, S.L., Hu, J.Y., 2001. Denitrifying phosphorus removal by anaerobic/anoxic sequencing batch reactor. Water Sci. Technol. 43(3), 139-146.

Nyberg, U., Aspegren, H., Andersson, B., Jansen, J.C., Villadsen, I.S., 1992. Full-scale application of nitrogen removal with methanol as carbon source. Water Sci. Technol., 26, 1077–1086.

Okada, M., Lin, C.K., Katayama, Y., Murakami, A., 1992. Stability of phosphorus removal and population of bio-P-bacteria under short term disturbances in sequencing batch reactor activated sludge process. Water Sci. Technol., 26(3–4), 483–491.

Orr, P.T., Jones, G.T., Hunter, R.A., Berger, K., De Paoli, D.A., Orr, C.L.A., 2001. Ingestion of toxic *Microcystis aeruginosa* by dairy cattle and the implications for microcystin contamination of milk. Toxicon, 39, 1847–1854.

Osee Muyima, N.Y., Momba, M.N.B., Cloete, T.E.,1997. Biological methods for the treatment of wastewaters. In: Cloete, T.E., Moyima, N.Y.O. (editors), Microbial community analysis: the key to the design of biological wastewater treatment systems. London: IAWQ Publishers, pp. 1– 24.

Park, H.D., Sasaki, Y., Maruyama, T., Yanagisawa, E., Hiraishi, A., Kato, K., 2001. Degradation of the cyanobacterial hepatotoxin microcystin by a new bacterium isolated from a hypertropic lake. Environ. Toxicol., 16, 337–343.

Parslow, R.C., McKinney, P.A., Law, G.R., Staines, A., Williams, R., Bodansky, H,J., 1997. Incidence of childhood diabetes mellitus in Yorkshire, northern England, is associated with nitrate in drinking water: an ecological analysis. Diabetologia, 40, 550–556.

Peters, M., Newland, M., Seviour, T., Broom, T., Bridle, T., 2004. Demonstration of enhanced nutrient removal at two full-scale SBR plants. Water Sci. Technol., 50(10), 115–120.

Pitman AR., 1999. Management of biological nutrient removal plant sludges—change the paradigms? Water Res., 33, 1141–1146.

Pollice, A., Tandoi, V., Lestingi, C., 2002. Influence of aeration and sludge retention time on ammonium oxidation to nitrite and nitrate. Water Res., 36, 2541–2546.

Ra, C.S., Lo, K.V., Mavinic, D.S., 1999. Control of a swine manure treatment process using a specific feature of oxidation-reduction potential. Bioresour. Technol., 70, 117–127.

Rittmann, B.E., McCarty, P.L., 2001. Environmental biotechnology: Principles and applications. New York: McGraw-Hill Book Co.

Rodrigo, M.A., Seco, A., Penya-roja, J.M., Ferrer, J., 1996. Influence of sludge age on enhanced phosphorus removal in biological systems. Water Sci. Technol., 34(1–2), 41–48.

Rogalla, F., Badard, M., Hansen, F., Dansholm, P., 1992. Upscaling a compact nitrogen removal process. Water Sci. Technol., 26, 1067–1076.

Rustrian, E., Delgenes, J.P., Moletta, R., 1996. Effect of the volatile fatty acids on phosphate uptake parameters by pure cultures of *Acinetobacter* sp. Lett. Appl. Microbiol., 23, 245–248.

Scheren, P.A.G.M., Zanting, H.A., Lemmens, A.M.C., 2000. Estimation of water pollution sources in Lake Victoria, East Africa: application and elaboration of the rapid assessment. methodology. J. Environ. Manage., 58, 235–248.

Schulthess, R.V. and Gujer, W., 1996. Release of nitrous oxide (N_2O) from denitrifying activated sludge: verification and application of a mathematical model. Water Res., 30, 521-530.

Schönborn, C., Bauer, H.D., Röske, I., 2001. Stability of enhanced biological phosphorus removal and composition of polyphosphate granules. Water Res., 35, 3190–3196.

Shehab, O., Deininger, R., Porta, F., Wojewski, T., 1996. Optimizing phosphorus removal at the Ann Arbor wastewater treatment plant. Water Sci. Technol., 1996, 34(1–2), 493–499.

Shoji, T., Satoh, H., Mino, T., 2003. Quantitative estimation of the role of denitrifying phosphate accumulating organisms in nutrient removal. Water Sci. Technol., 47(11), 23–29.

Smolders, G.J.F., van der Meij, J., van Loosdrecht, M.C.M., Heijnen, J.J., 1994a. Model of the anaerobic metabolism of the biological phosphorus removal process: Stoichiometry and pH influence. Biotechnol. Bioeng., 43, 461–470.

Smolders, G.J.F., van der Meij, J., van Loosdrecht, M.C.M., Heijnen, J.J., 1994b. Stoichiometric model of the aerobic metabolism of the biological phosphorus removal process. Biotechnol. Bioeng., 44, 837–848.

Sundblad, K., Tonderski, A., Rulewski, J., 1994 Nitrogen and phosphorus in the Vistula river, Poland—changes from source to mouth. Water Sci. Technol., 30 (5), 177–186.

Tay, J.H., Liu, Q.S., Liu, Y., 2001. The effects of shear force on the formation, structure and metabolism of aerobic granules. Appl. Microbiol. Biot., 57, 227–233.

Terada, T., 2006. Design of a material surface for rapid biofilm formation and application to a membrane-aerated biofilm reactor for simultaneous nitrification and denitrification [Ph.D. thesis]. Tokyo: Waseda University, pp.4.

Teske, A., Alm, E., Regan, J.M., Toze, S., Rittmann, B.E., Stahl, D.A., 1994. Evolutionary relationship among ammonia- and nitrite-oxidizing bacteria. J. Bacteriol., 176, 6623–6630.

Thorn, M. and Sorensson, F., 1996. Variation of nitrous oxide formation in the denitrification basin in a wastewater treatment plant with nitrogen removal. Water Res., 30, 1543–1547.

Tijhuis, L., van Loosdrecht, M.C.M., Heijnen, J.J., 1995. Dynamics of biofilm detachment in biofilm airlift suspension reactors. Biotechnol. Bioeng., 45, 481–487.

Townsend, A.R., Howarth, R.W., Bazzaz, F.A., Booth, M.S., Cleveland, C.C., Collinge, S.K., Dobson, A.P., Epstein, P.R., Keeney, D.R., Mallin, M.A., Rogers, C.A., Wayne, P., Wolfe, A.H., 2003. Human health effects of a changing global nitrogen cycle. Front. Ecol. Environ., 1, 240–246.

Tsuneda, S., Nagano, T., Hoshino, T., Ejiri, Y., Noda, N., Hirata, A., 2003. Characterization of nitrifying granules produced in an aerobic upflow fluidized bed reactor, Water Res., 37, 4965–4973.

Tsuneda, S., Ohno, T., Soejima, K., Hirata, A., 2006. Simultaneous nitrogen and phosphorus removal using denitrifying phosphate-accumulating organisms in a sequencing batch reactor. Biochem. Eng. J., 27, 191–196.

U.S. Environmental Protection Agency, 1975. Process design manual for nitrogen control. Washington, DC: EPA Technology Transfer.

Van Maanen, J.M.S., van Dijk, A., Mulder, K., de Baets, M.H., Menheere, P.C.A., van der Heide, D., Mertens, P.L.J.M., Kleinjans, J.C.S., 1994. Consumption of drinking waters with high nitrate levels causes hypertrophy of the thyroid. Toxicol. Lett., 72, 365–374.

Van Maanen, J.M.S., Albering, H.J., de Kok, T.M.C.M., van Breda, S.G.J., Curfs, D.M.J., Vermeer, I.T.M., Ambergen, A.W., Wolffenbuttel, B.H.R., Kleinjans, J.C.S., Reeser, H.M., 2000. Does the risk of childhood diabetes mellitus require revision of the guideline values for nitrate in drinking water? Environ. Health Persp., 2000, 108, 457–461.

Virtanen, S.M., Jaakkola, L., Rasanen, L., Ylonen, K., Aro, A., Lounamaa, R., et al., 1994. Nitrate and nitrite intake and the risk for Type 1 diabetes in Finnish children. Diabetic Med., 11, 656–662.

Wagner, M., Erhart, R., Manz, W., Amann, R., Lemmer, H., Wedi, D., Schleifer, K.H., 1994. Development of an rRNA-targeted oligonucleotide probe specific for the genus Acinetobacter and its application for in situ monitoring in activated sludge. Appl. Environ. Microbiol., 60, 792–800.

Wagner, M., Loy, A., 2002. Bacterial community composition and function in sewage treatment systems. Curr. Opin. Biotech., 2002, 13, 218–227.

Ward, M.H., Mark, S.D., Cantor, K.P., Weisenburger, D.D., Correa-Villasenor, A., Zahm, S.H., 1996. Drinking water nitrate and the risk of non-Hodgkin's lymphoma. Epidemiology, 7, 465–471.

Wareham, D.G., Marvinic, D.S., Hall, K.J., 1994. Sludge digestion using ORP-regulated aerobic anoxic cycles. Water Res., 28, 373–384.

Weyer, P.J., Cerhan, J., Kross, B.C., Hallberg, G.R., Kantamneni, J., Breuer, G., Jones, M.P., Zheng, W., Lynch, C.F., 2001. Municipal drinking water nitrate level and cancer risk in older women: the Iowa Women's Health Study. Epidemiology, 12, 327–338.

Wolfe, A.H., Patz, J.A., 2002. Reactive nitrogen and human health: acute and long-term implications. Ambio, 31, 120–125.

World Health Organization., 1996. Guidelines for drinking-water quality. Health criteria and other supporting information 2nd edition. Geneva: WHO.

Xie, L., Xie, P., 2002. Long-term (1956–1999) dynamics of phosphorus in a shallow, subtropical Chinese lake with the possible effects of cyanobacterial blooms. Water Res., 36, 343–349.

Yu, R.F., Liaw, S.L., Cheng, W.Y., Chang, C.N., 2000. Performance enhancement of SBR applying real-time control. J. Environ. Eng., 126, 943–948.

Žegura, B., Sedmak, B., Filipič, M., 2003. Microcystin-LR induces oxidative DNA damage in human hepatoma cell line HepG2. Toxicon, 41, 41–48.

Zeng, R.J., Lemaire, R., Yuan, Z., Keller, J., 2004. A novel wastewater treatment process: simultaneous nitrification, denitrification and phosphorus removal. Water Sci. Technol., 50(10), 163–170.

Zumft, W.G., 1992. The denitrifying prokaryotes. In: Balows, A., Truper, H.G., Dworkin, M., Harder, W., Schleifer, K.H. (editors), The prokaryotes A handbook on the biology of bacteria: ecophysiology isolation identification applications, vol. 1, 2nd ed. New York: Springer-Verlag, pp.554–582.