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Improvement of nitrogen-removal efficiency using immobilized microorganisms with oxidation–reduction potential monitoring

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The use of an immobilized-cell reactor for simultaneous carbon–nitrogen removal in wastewater with the monitoring of oxidation–reduction potential (ORP) in an intermittent aeration (IA) process was investigated. Under alternating aerated and nonaerated conditions, the ORP-time profile showed distinctive turning points that directly correlated with changes in the system chemistry and biological activity. The aeration ratio, defined as aeration time/cycle time, was optimum at 50% for obtaining the maximum efficiency of denitrification accompanied by sufficient nitrification. High simultaneous carbon–nitrogen removal efficiency could be achieved using the immobilized-cell reactor by applying the IA process. More than 90% of chemical oxygen demand (COD)-removal efficiency and over 80% of total-nitrogen-removal efficiency were obtained using three aerobic–anoxic cycles per day at an aeration ratio of 50% and with a hydraulic retention time of 10 h. *Journal of Industrial Microbiology & Biotechnology* (2000) **25,** 229–234.

Keywords: PVA immobilization; intermittent aeration process; oxidation–reduction potential; nitrification; denitrification

Introduction

Biological processes are frequently applied to remove carbonaceous and nitrogenous pollutant compounds in wastewater. However, conventional activated sludge processes cannot achieve a satisfactory efficiency in nitrogen removal due to washout of slow-growing autotrophic nitrifying bacteria from the reactor. Moreover, solid–liquid separation in the settling tank occasionally leads to difficulties in process operation. To resolve such difficulties, the feasibility of applying immobilization technology to wastewater treatment has received increasing attention [2,14,15]. The immobilized-cell treatment system allows for a high cell concentration in the reaction tank for increased efficiency, a reduction of excess sludge production [5,14], an easier solid–liquid separation in a settling tank and the elimination of problems associated with bulking [7,15]. In the early 1990s, a large-scale sewage treatment process was successfully constructed using immobilized nitrifying cells to improve the efficiency of nitrogen removal [13].

Biological nitrogen removal consists of aerobic nitrification and anaerobic denitrification. When using an immobilized-cell system, not only can nitrifiers at a high concentration be effectively retained in the surface layer of gel beads, but denitrifiers can also grow in the interior anoxic zone due to the diffusion resistance of oxygen, resulting in so-called "aerated denitrification" [5]. According to a related investigation, aerated denitrification occurred in the immobilized activated sludge process, which could promote the efficiency of nitrogen removal in municipal wastewater treatment [7]. However, the total-nitrogen-removal efficiency was still unacceptable when continuous and sufficient aeration was applied to the immobilized-cell reactor.

The intermittent aeration (IA) wastewater treatment process is operated in a repeated cyclic mode with the air on for a specific period followed by the air off for another specific period (the total duration for one cycle is called "cycle time"). The process incorporates an unaerated zone that offers many advantages such as the improvement of nitrogen removal, energy savings, less sludge production, and a more stable system pH due to denitrification. Therefore, IA operation mode has been applied to the sludge digestion and biological nutrient removal (BNR) processes [8,12,17]. However, there are few reports on the IA operation performance of an immobilized sludge reactor.

The oxidation–reduction potential (ORP) and the dissolved oxygen (DO) concentration measured *in situ* can continuously provide instantaneous and real-time information, which directly correlates with the chemical and/or biological states of the biosystem [6]. Peddie *et al.* [10] characterized a reproducible ORP profile that was associated with the cycled operation in a sludge digestion reactor. For each aeration–nonaeration cycle, the real-time ORP profile had a number of distinctive features directly related to changes in chemical and biological activities of the reactor system. Recent advances in BNR processes have indicated that the ORP measurement can be successfully used for on-line monitoring of the progress of the system and also as a useful real-time process control parameter. The use of the ORP breakpoints as an index to establish the strategy for real-time control of oxygen supply in wastewater treatment plants to achieve a high degree of nitrogen removal was proven to be feasible [11,18]. An aerobic–anoxic sludge digestion reactor operating in a real-time manner with 3 h of air-on and air-off time determined by computer detection of the nitrate breakpoint in the ORP-time profile was studied by Wareham *et al.* [16]. The use of ORP setpoint to control the aeration in an extended aeration wastewater treatment system was investigated by Lo *et al.* [9]. For a continuous-flow activated sludge reactor system, the breakpoints, setpoints and set-times on the ORP and pH–time curves were used to establish a real-time aeration control strategy, resulting in a better performance than

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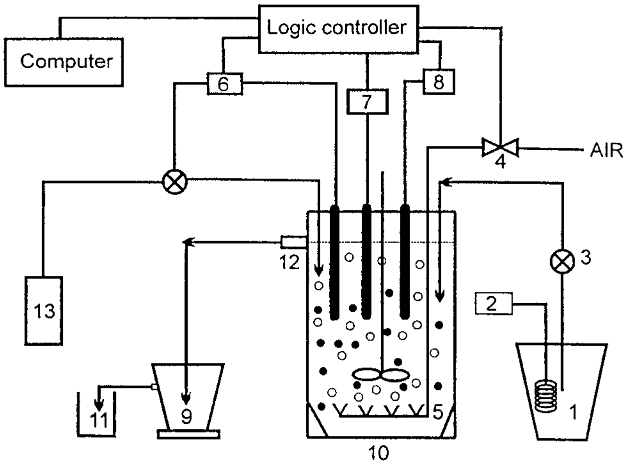

Figure 1 Reactor system for IA process using immobilized-cell beads. 1, synthetic wastewater tank; 2, cooler; 3, peristaltic pump; 4, air solenoid valve; 5, sparger; 6, pH controller; 7, ORP meter; 8, DO meter; 9, settling tank; 10, reaction vessel; 11, treated wastewater tank; 12, sieve; 13, reservoir for $NaHCO_3$.

fixed-time operation in the reduction of aeration energy and the promotion of the nitrogen and phosphorus removal [19].

Polyvinyl alcohol (PVA) gel is nontoxic and its production at an industrial scale is economically feasible. Our previous work developed a novel cell immobilization method based on the use of phosphorylated PVA [4]. The phosphorylated PVA has been successfully applied to the immobilization of denitrifying sludge in the denitrification process during a long period of operation for a continuous stirred tank reactor [4]. In this study, the IA process was applied to a PVA immobilized-cell reactor to promote the efficiency of total nitrogen removal. The real-time ORP values were monitored during the operation to elucidate the biological features of the reactor system. The effects of aeration ratio, which was defined as aeration time/cycle time, and hydraulic retention time (HRT) on the removal efficiencies of total nitrogen (TN) and COD were also investigated.

Materials and methods

Chemicals

PVA with a grade of 99.92% saponification and 2000 degree of polymerization was purchased from Chang Chun Petrochemical Co. (Miaoli, Taiwan). All other chemicals were reagent grade.

Sludge immobilization

The sludge used for immobilization was obtained from a sewage-treatment plant in Taipei City and was acclimated for more than 3 months. The sludge was immobilized in phosphorylated PVA gel beads according to the method described earlier [4]. A mixture containing sludge (55 g VSS/l) was thoroughly mixed with an equal volume of PVA (20% w/v). Next, this PVA–sludge mixture was dropped into a saturated boric acid solution and gently stirred for 0.5–1 h to form spherical beads. The formed labile beads were then transferred to a 0.5 M sodium phosphate solution for 0.5–1 h for gel hardening. The subsequent beads of 3- to 4-mm diameter were washed with tap water. The mechanical strength of the immobilized beads was measured according to our previous work [4].

Reactor system

The immobilized-cell reactor was established in an acrylic vessel (21.5 cm×15 cm×50 cm) with a working volume of 12 l (Figure 1). The composition of the synthetic municipal wastewater was (in mg/l) 203 peptone, 136 beef extract, 15 NaCl, 7 KCl, 5 $MgSO_4 \cdot 7H_2O$, 50 Na_2HPO_4 and 7 $CaCl_2 \cdot 2H_2O$, which corresponded to 360 mg/l COD, 48 mg/l total Kjeldahl nitrogen (TKN), 240 mg/l BOD and 150 mg/l TOC. Synthetic wastewater was fed using a peristaltic pump. The reservoir of feed solution was maintained at 4°C using a cooler to reduce microbial contamination. The volume fraction of immobilized-cell beads in the vessel was 25% (v/v). The reaction system was automatically controlled at pH 7.5 by adding 0.1 M $NaHCO_3$. The reactor was operated at ambient temperature without control. The effluent passed through a steel sieve to prevent wash-out of the gel beads and was collected in a settling vessel. The quality of the effluent solution was monitored at regular intervals during the operation.

Air was used for aeration and mixing in the reactor. An agitator provided additional mixing to the IA reactor during the nonaeration period. Air passed through an air solenoid valve (on/off regulation controlled by computer) and then flowed through a sparger before entering the reactor. The IA was operated by an air solenoid valve connected to the data acquisition and control (DAC) system. The DAC system consisted of an IBM PC compatible computer with 586 microprocessor linked to a programmable logic controller (PLC). The PLC was installed inside together with a terminal panel, an interface card, a CPU 301, and user interface software. Sensors/meters to measure ORP (PT480, Mettler Toledo (formerly Ingold), Greifensee, Switzerland), DO (Microprocessor Oximeter ox; 1196, Wissenschaftlich-Technische Werksstatten, Weilheim, Germany) and pH (PC310, Suntex, Taipei, Taiwan), were connected to the terminal panel, which was subsequently joined to the interface card. Analog signals from the sensors/meters were converted to digital data and logged to the PC. Finally, a solenoid valve was used to regulate aeration, which was controlled by the solid state relay based on signals from the PC.

Reactor operation

The immobilized-cell reactor was operated under continuous aeration as a comparison with the IA operation for nitrogen-removal efficiency. The IA process of the reactor operation was then conducted in a repeated cyclic mode with the air on for a specific period, followed by the air off for another specific period. This "aeration–nonaeration" period was defined as a cycle. The cycle time was referred to as the period of one cycle, e.g., the IA process with a cycle time of 8 h has three cycles per day. Cycle timing was used to characterize the treatment system and optimize the aerobic/anoxic process. The aeration ratio was defined as the ratio of the period of aeration to the cycle time, and expressed as a percentage. In start-up, the aerobic phase was allowed to proceed until the bioactivity of cell-beads reached a stable maximum state, then cycling was initiated.

During the progression of the IA process, in addition to the continuous process monitoring of ORP and DO, samples were routinely analyzed for COD, TKN, NO_3^-, NO_2^- and biomass (estimated by cell protein). The accumulation of nitrogen oxides (nitrate and nitrite) was expressed in terms of NO_x^--N. The sample analysis procedures and estimation methods for factors such

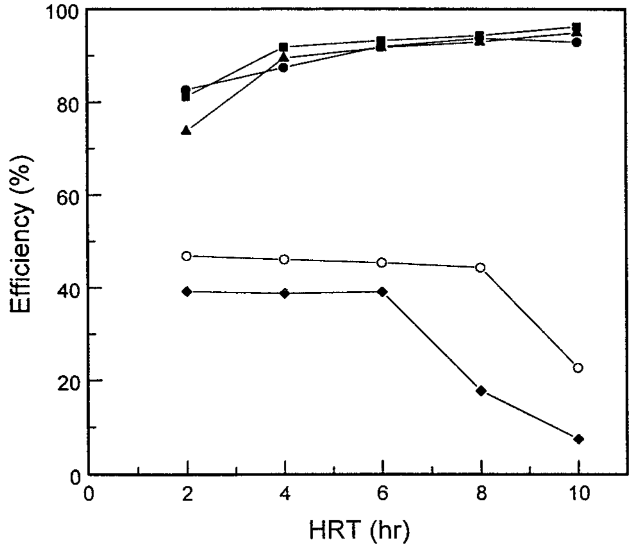

Figure 2 Simultaneous carbon–nitrogen removal efficiency of the continuous aeration reactor. (●) COD removal; (○) TN removal; (■) TKN removal; (▲) nitrification efficiency; (◆) denitrification efficiency.

as cell protein, oxygen uptake rates, removal efficiencies of nitrification and denitrification were according to those in Standard Methods [1] and our previous work [5].

Results and discussion

The concentration of the activated sludge used for PVA immobilization was estimated to be 55 g VSS/l. Around 3 kg of immobilized-cell beads (wet weight) was prepared from 2.1 l of activated sludge. The respiration activity of the sludge before and after immobilization was measured to be 14.8 mg O_2/g VSS per hour and 0.314 mg O_2/g gel per hour, respectively. Thus, the initial activity yield of the sludge immobilization was around 54%. The prepared gel beads were incubated in the medium, the composition of which was the same as that of the synthetic municipal wastewater, before reactor operation. The biomass density after reactivation was estimated to be 0.019 g VSS/g gel by the cell protein assay method [5]. The specific gravity of the beads was approximately 1.05. The beads' mechanical strength was around 0.9 kg/cm^2.

Reactor performance in continuous aeration condition

The immobilized-cell reactor system was initially aerated continually. A fixed concentration of COD (360 mg/l) and TKN (48 mg/l) was continuously fed into the reactor. Once the reactor system reached steady state, the experiments were manipulated by varying the HRT. Figure 2 depicts the relationships between HRT and the simultaneous carbon–nitrogen removal efficiency of the system. Estimation of process efficiencies was done following the methods proposed in our earlier work [5]. COD-removal efficiency increased with increasing HRT. A removal efficiency exceeding 90% was obtained when the HRT was higher than 4 h. Even when the reactor was operated at an HRT of 2 h, the removal efficiency was over 80%. Over 90% of nitrification efficiency and TKN-removal efficiency was achieved when HRT was higher than 4 h. The denitrification and TN-removal efficiencies were lower than 45%, even if a considerable degree of aerated denitrification occurred inside the gel beads.

Characteristics of ORP-time profile in IA process

The characteristics of ORP-time profiles obtained from sludge digestion and BNR processes undergoing IA operation provide substantial information, which correlate with meaningful chemical/biological events of the reaction system [10]. In this study, ORP, DO and process parameters (COD, TKN, NO_3^- and NO_2^-) were closely monitored over a number of complete aerobic–anoxic cycles to understand process dynamics more thoroughly. Figure 3 depicts the typical behavior patterns of ORP and the main process parameters in the IA immobilized-cell reactor for a wastewater treatment run at HRT of 8 h and an aeration ratio of 50%; i.e., with repeated cycles of 4 h aeration followed by 4 h without air. The trend of DO change correlated well with the ORP curve. According to concepts proposed by Peddie *et al.* [10], the characteristics of ORP-time profile appearing in Figure 3 are described as follows. An inflection point (point E) appeared on the ORP curve following the cessation of aeration. This point was referred to hereinafter as the "oxygen knee." At this time, the nitrogen oxides (NO_x^--N) were still at maximum value, indicating that denitrification had not yet started. Then, the DO decreased to zero (point A) owing to consumption by nitrifiers. The point of zero DO was labeled "DO breakpoint" and corresponded to the depletion of measurable DO. The "oxygen knee" referred to the abrupt change in slope of the ORP curve, resulting in a strong negative peak on the rate of change curve [d(ORP)/d*t*]. The "DO breakpoint"

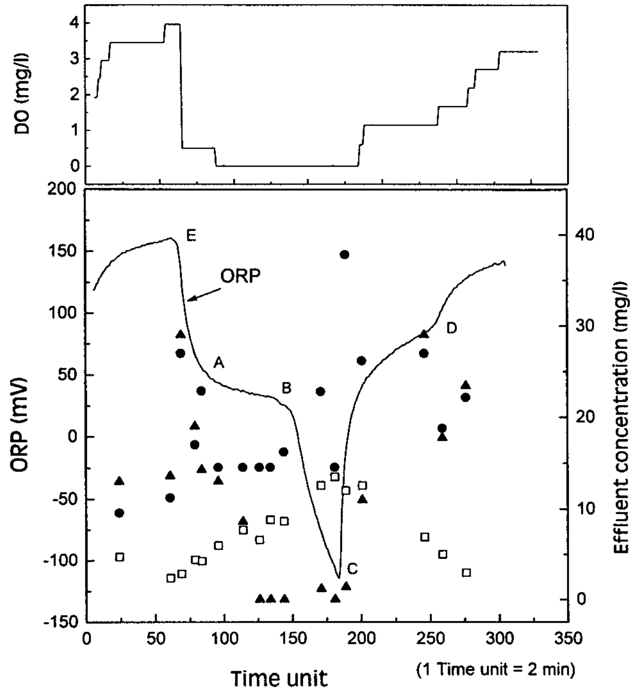

Figure 3 Typical ORP-time profile in an IA wastewater treatment system using immobilized-cell beads at HRT = 8 h, cycles per day = 3, aeration ratio = 50%. (●) COD; (□) TKN; (▲) NO_x^--N. Point A: DO breakpoint; Point B: nitrate breakpoint; Point C: onset of aeration; Point D: elbow; Point E: stopping of aeration.

defined the end of the aerobic state and the beginning of anoxic period. When entering the anoxic condition, the TKN increased with time due to the cessation of nitrification. A second inflection appeared at point B, following a second knee referred to hereinafter as the "nitrate knee" or "nitrate breakpoint." This point denoted the complete disappearance of nitrates through denitrification during anoxia. The "nitrate breakpoint" corresponded to a transition of a respiratory activity into a nonrespiratory state such as fermentation. After the nitrate breakpoint, the ORP value dropped sharply, as attributed to the anoxic endogenous respiration or fermentation. Point C was the rapid response of the ORP to the onset of aeration. The ORP values rose rapidly until a discontinuity (point D) appeared. Meanwhile, the responses of DO concentration jumped to 1 mg/l instantaneously and remained at this level until point D appeared in the ORP-time curve. During this period, oxygen, which was transferred from air into the bulk solution, was entirely consumed by rapid nitrification of the ammoniacal nitrogen, which had built up over the unaerated duration. After point D, the values of ORP and DO gradually rose and reached a plateau. Thus, point D, referred to hereinafter as the "elbow," denoted the end of the rapid nitrification of the ammoniacal nitrogen. However, in another study we observed no response of the DO probe until the elbow point appeared in the ORP-time profile, which was obtained in a sludge digestion system [10]. After the start of aeration, a sudden rise of the DO value to 1 mg/l observed in our work might be attributed to the diffusion resistance of the immobilized-cell beads. Owing to the fact that an immobilized-cell system is unlike a free-cell process, so at the start of aeration, the occurrence of nitrification and COD oxidization cannot associate with oxygen transfer instantaneously due to diffusion resistance in the cell beads.

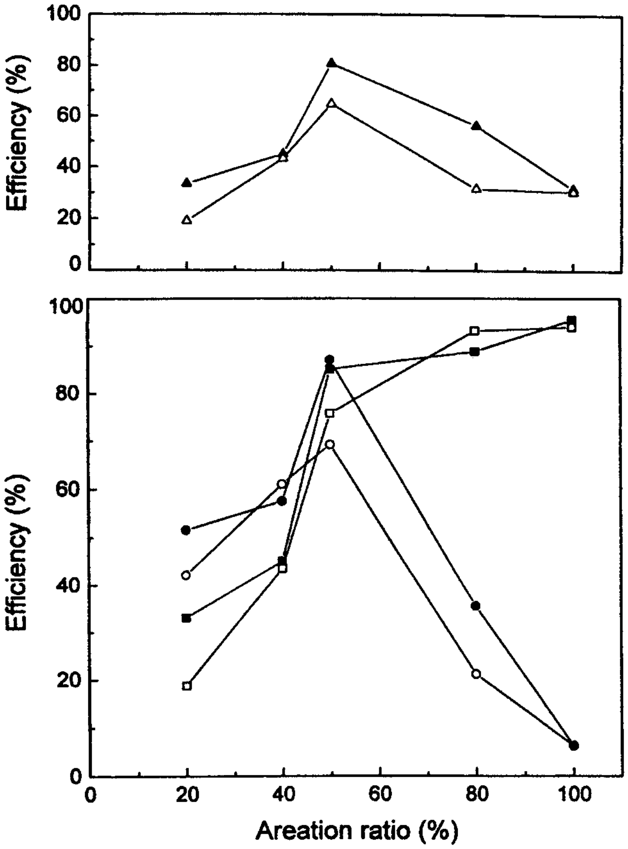

Figure 4 Effect of aeration ratio on nitrification, denitrification and TN removal at cycles per day = 3 or 4. Cycles per day = 3: (■) nitrification efficiency; (●) denitrification efficiency; (▲) TN removal efficiency. Cycles per day=4: (□) nitrification efficiency; (○) denitrification efficiency; (△) TN removal efficiency.

Effect of aeration ratio

Figure 4 presents the effect of aeration ratio on the efficiencies of denitrification and nitrification using the immobilized-cell reactor under IA operation. The experiments were run at three or four cycles per day with various aeration ratios at an HRT of 10 h. The results revealed that, as expected, the efficiency of nitrification increased with increasing aeration ratio. Nitrification efficiency of the bioreactor system reached a plateau of greater than 80% at an aeration ratio of 50% for cycle numbers of either three or four per day. Subsequently, only slight increases in efficiency were observed even when the aeration ratio was considerably raised. Additionally, the aeration ratio of 50% yielded the maximum efficiency of denitrification. The cycled reactor run at three cycles per day yielded better results than that at four cycles. Figure 4 also summarizes the experimental results regarding the removal efficiency of total nitrogen. The IA reactor run at three cycles per day at 50% of aeration ratio yielded an optimal efficiency (higher than 80%) for total nitrogen removal. The aerobic state at a high aeration ratio prevented the progression of denitrification, whereas the anoxic state at low aeration ratio markedly decreased formation of NO_x^--N owing to inhibition of nitrification [3]. Combining these two opposing factors resulted in an optimal aeration ratio of 50% in this IA process.

Effect of hydraulic retention time (HRT)

The immobilized-cell reactor was operated as an IA process at three cycles per day and an aeration ratio of 50% for various HRT. Figure 5 depicts ORP-time profiles obtained for HRTs of 3, 4.5, 6 and 8 h. The shapes of ORP-time profiles resembled those obtained in IA sludge digestion systems [16]. The runs operated under a shorter HRT (a higher COD loading rate) caused the system to be in the anoxic state and showed an absence of a nitrate breakpoint in the ORP-time profile, particularly for HRT of 3 h. Our previous work [5] demonstrated that under the conditions of high COD loading rate, faster growth of heterotrophic BOD oxidizers might occur in the surface layer of cell beads. Moreover, the IA (at only 50% of aeration ratio) can not supply sufficient air to the system. Depletion of DO due to faster growth of heterotrophic BOD oxidizers accompanied with endogenous respiration resulted in anoxia in the system with an HRT of less than 4.5 h. When the HRT was increased to 6 h, the ORP-time profiles were characterized by the clear appearance of nitrate breakpoint. The detection of nitrate breakpoint denotes the moment of completion of denitrification in the cyclic process. The appearance of a nitrate breakpoint allows the system to favor denitrification, resulting in a higher efficiency of total nitrogen removal.

Figure 6 depicts the simultaneous carbon–nitrogen removal efficiency of the IA process for various HRTs at three cycles per day and an aeration ratio of 50%. The removal efficiency of COD slightly increased with increasing HRT, i.e., decrease of COD loading rate. Meanwhile, TN-removal efficiency markedly increased with an increase in HRT. As HRT changed from 3 to

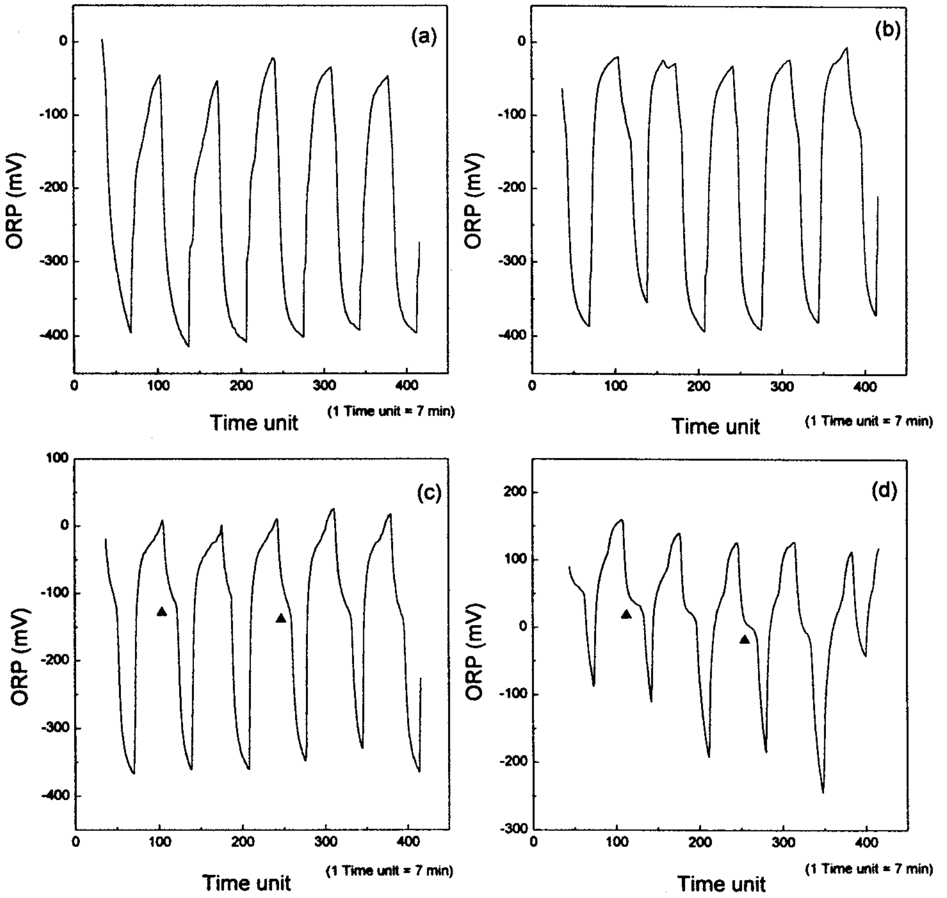

Figure 5 ORP-time profiles of the cycled reactor for various HRT at cycles per day = 3, aeration ratio = 50%. (a) HRT = 3 h, (b) HRT = 4.5 h, (c) HRT = 6 h, (d) HRT = 8 h, (▲) Nitrate breakpoint.

10 h, the TN-removal efficiency increased from 40% to more than 80%. The percentage of nitrogen removal by cell uptake and the denitrification were determined according to the methods proposed in our previous work [5]. Figure 6 reveals that around 20% of influent nitrogen was removed by cell uptake, whereas the nitrogen removed by denitrification increased markedly with an increase in HRT. These results correspond to the fact that the nitrate breakpoint appeared in the ORP-time profile when the HRT exceeded 6 h (Figure 5). As mentioned earlier, the appearance of the nitrate breakpoint constitutes a situation in which biological states enhance denitrification. However, after the appearance of nitrate breakpoint and until the next start of aeration, the biological system would transit into a nonrespiratory state, such as fermentation [18]. The length of this anoxic/anaerobic period might play an important role in the IA system. Therefore, real-time control based on the detection of nitrate breakpoint in the IA cycled reactor should be applied. Examination of the feasibility of IA processes with real-time control is currently underway in our laboratory.

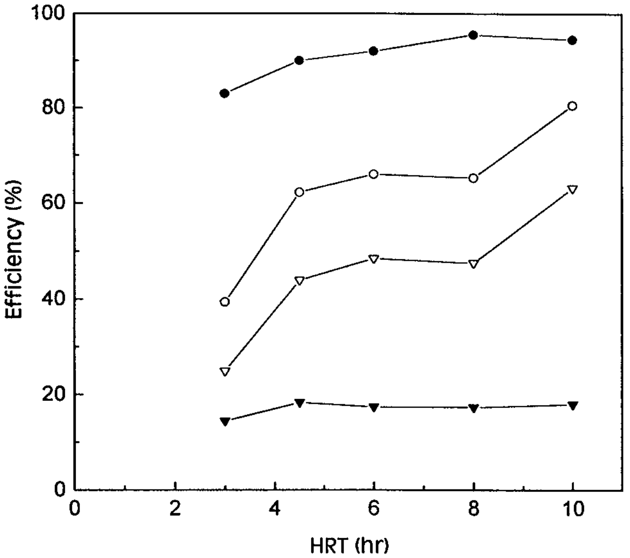

Figure 6 Simultaneous carbon–nitrogen removal efficiency of the cycled reactor at cycles per day = 3, aeration ratio = 50%. (●) COD removal; (○) TN removal; (▽) TN removal by denitrification; (▼) TN removal by sludge.

PVA-immobilized beads used throughout the investigation were all prepared at the same time. The duration of the entire experiment was roughly 1 year. The activity of the cell beads remained stable until the experiments were completed. The mechanical strength of the cell beads increased slightly (from 0.9 to 1.1 kg/cm^2) in long-term operation. Such an occurrence is primarily due to propagation of the biomass inside the beads. Increase of the cell density in the interior of the beads with the increase of incubation time is a general phenomenon in such an immobilized-cell system [7]. Moreover, the elastic property of the PVA would allow cell growth inside the beads to increase the cell density [5]. Less than 5% of the beads were broken during long-term operation in this study.

Conclusions

This work elucidates the operational features of a model municipal wastewater treatment system using PVA immobilized-cell beads under an IA process. In contrast to continuous aeration process, performance with an IA process yields a nitrification–denitrification cycle accompanied with BOD oxidization. The real-time ORP measurements during IA operation offer meaningful *in situ* information with biological and chemical significance. The ORP-time profile is characterized by three turning points. During the unaerated period, the "DO breakpoint" denotes the disappearance of DO and the "nitrate breakpoint" is linked to the disappearance of nitrate. After subsequent aeration onset, a point, called "elbow," always corresponds to the end of the rapid nitrification of ammoniacal nitrogen accumulated over the unaerated period. According to our results, the ratio of the duration of aeration to cycle time was an important factor affecting the efficiency of total nitrogen removal during IA operation, particularly in denitrification; a 50% aeration ratio appeared to be optimum. Moreover, at a 50% aeration ratio, 8 h cycle time and various HRTs from 3 to 10 h, COD-removal efficiency reached more than 90% as the HRT exceeded 4 h. Although these levels closely resemble those obtained in the continuous aeration process, the IA process clearly exhibits a better efficiency in total nitrogen removal. High simultaneous carbon–nitrogen removal efficiency, energy saving and easy retrofitting are highly promising for the application of IA process to wastewater treatment system using immobilized microorganisms.

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