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Full Length Research Paper

Resistance capability of microaerobic granular sludge for pentachlorophenol (PCP) degradation subjected to pH shock

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The resistance capability to pH shock of microaerobic granular sludge for pentachlorophenol (PCP) degradation was studied. The results show that it had little effect on the system under short-term shock, and the system returned to normal state immediately , while large adverse effect occurred under long-term shock, and the system required long time to recover. The content of extracellular polymers (ECPs) including proteins (PN) and polysaccharides (PS) decreased significantly, as well as the ratio of proteins and polysaccharides for 8 days pH shock, and there was an increase in sludge volumetric index (SVI) values of sludge and a decrease in settling velocity. Compared with the normal pH, it was found that the number of bacillus in microorganisms reduced for pH shock, and recession-type cells occurred by being observed with scanning electron microscope, especially at pH 6.0 and 9.0 there was a more serious decline in the treatment effect and deterioration in the physicochemical properties of granular sludge.

Key words: Resistance capability, microaerobic granular sludge, pentachlorophenol (PCP), pH shock.

INTRODUCTION

Pentachlorophenol (PCP) is a toxic and hardly biodegradable xenobiotic which can cause cancer, abnormality and mutation. It has been placed on the Priority List of Pollutants by the US. PCP is widely used as an herbicide, fungicide and wood preservative and a certain amount of PCP are produced from bleaching process with chlorine gas in pulp and paper, leather and spinning wastewater. Traditional biological technology is not the effective method for PCP treatment because of its biotoxicity. Aerobic granules has been cultured successfully and applied to wastewater treatment, which improves both aerobic and anaerobic micro-environments exist in aerobic granules that oxidative and reductive process can occur simultaneously. So it is an ideal technology to treat PCP waste water.Aerobic granular sludge process has been applied in nitrogen and phosphorous removal successfully and shown good treatment effect. Yang et al. (2003) reported the nitrogen removal with the aerobic granular sludge process by using self-made wastewater in which chemical oxygen demand (COD_{Cr}), ammonium (NH_4^+-N) and total phosphorus (TP) was 100 to 400,10 to 40 and 10mg/L respectively; the results showed that the removal efficiency of COD_{Cr} and ammonium (NH_4^+-N) was 83.6~92.8 and 82.3~98.5% respectively. The same experiments were carried out by Cassidya et al. (2005) which showed that the removal efficiency of COD_{Cr} and TP were both higher than 98% at higher concentration of COD_{Cr}, TKN and TP (2.6 $kg.m^{-3}.d^{-1}$, 1057 $mg.L^{-1}$ and 217 $mg.L^{-1}$ respectively) , and the TKN removal rate was higher than 97%, too. In the aerobic granules, the aerobic and facultative bacteria of the out layer used up the dissolved oxygen, so the anaerobic zone is formed in the inner layer in which no oxygen could arrive. Ammonium in

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Abbreviations: PCP, Pentachlorophenol; **ECPs,** extracellular polymers; **PN,** proteins; **PS,** polysaccharides.

Table 1. The reactor operating parameters

Hydraulic upward velocity ($m\cdot h^{-1}$)	Sludge retention time (day)	Volumetric loading rate (mgCOD/L.d)	Biomass concentration ($mg\cdot L^{-1}$)
4.58	15	2600	12000

the wastewater was oxidized to nitrite or nitrate by nitrifying bacteria in the aerobic zone, and then transferred to anaerobic zone in which nitrite or nitrate was converted to N_2 by denitrifying bacteria.

Influence of pH value on simultaneous nitrification and denitrification process of aerobic granular sludge was studied by Zhang et al. (2005) which showed that ammonia removal efficiency was up to more than 95% in the range of pH 8 to 9, and in the range of 6 to 9 the physicochemical properties of sludge were stable without disintegration. The research of Liu et al. (2008) proved that both the stabilities and the nitrogen and phosphorus removal abilities of aerobic granular sludge were good under higher organic loading.

Aerobic granular sludge has great advantages in the treatment of toxic and harmful wastewater. Phenol pollutants of wastewater were degraded successfully by Jiang et al. (2003) and Tay et al. (2004) using aerobic granular sludge process. In the research of Zhu et al. (2005), aniline wastewater was treated with aerobic granular sludge; the removal efficiency was stable to more than 99.9% under the aniline loading of $800 g.m^{-3}.d^{-1}$ after the granules were mature. Aerobic granular sludge for 2,4-dichlorophenol degradation was cultured successfully by Wang et al. (2007), the degradation rate was up to 39.6 mg $2,4\text{-DCP}.g^{-1}.VSS^{-1}.h^{-1}$ when the concentration of inlet dichlorophenol was 105 $mg.L^{-1}$.

The main disadvantage in the application of aerobic granular sludge was the requirement for high dissolved oxygen concentration which not only increased power consumption, but also formed smaller anaerobic zone in the granule to influence the anaerobic treatment effects of some pollutants, such as PCP, and its intermediate products of dechlorination. If there were volatile organic compounds or degradation products in the wastewater treatments, they might be blown off to produce secondary pollutions.

Lan et al. (2005) successfully cultivated granular sludge to degrade PCP under the micro-aerobic conditions, which solved the problems of aerobic granules and studied the related factors. The results showed that all the factors of pH, oxidation-reduction potential (ORP), dissolved oxygen (DO) concentration and PCP loading had important effects on the treatment efficiency. Based on this, the author of the paper further investigated the capability of pH shock resistance of micro-aerobic granular sludge system hoping to provide useful information for practical application of the technology.

MATERIALS AND METHODS

A schematic diagram of the setup

The experimental apparatus used in this study was self-made microaerophlic Upflow Sludge Bed (MUSB), the sketch was described by Lan et al. (2005). The MUSB mainly included the aeration vessel with a diameter of 30 mm and height of 700 mm and the reactor with a diameter of 50 mm and height of 1000 mm both were made of organic glass. The effective volumes of the aeration vessel and the reactor were 0.5 and 1.0 L, respectively. The reactor was operated as a sequencing batch reactor. Synthetic wastewater was first fed into the aeration vessel in which continuous aeration was carried out via a porous air diffuser ball, and then pumped into the reactor in which granular sludge existed from the bottom. Wastewater recycled into the aeration vessel from the top of the reactor, and pumped into the reactor again, at last discharged through the effluent valve. pH was adjusted by 6 M NaOH or 6 M H_2SO_4. The dissolved oxygen (DO) concentration was 0.6 $mg\cdot L^{-1}$, the hydraulic upward velocity was 4.58 $m\cdot h^{-1}$ and the treatment cycle was 24 h. The other operating parameters are listed in Table1.

Wastewater composition

The composition of the nutrient medium was glucose 2400 $mg.L^{-1}$, $(NH_4)_2SO_4$ 554 $mg.L^{-1}$, KH_2PO_4 210 $mg.L^{-1}$, $MgSO_4$ 10 $mg.L^{-1}$, $CaCl_2$ 20 $mg.L^{-1}$, $NaHCO_3$ 1500 $mg.L^{-1}$ and 1 ml trace solution. The composition of the trace solution was as follows: $FeCl_3\cdot 6H_2O$ 1.5 $g\cdot L^{-1}$, H_3BO_3 0.15 $g\cdot L^{-1}$, $CuSO_4\cdot 5H_2O$ 0.03 $g\cdot L^{-1}$, KI 0.03$g\cdot L^{-1}$, $MnSO_4\cdot H_2O$ 0.10 $g\cdot L^{-1}$, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ 0.065 $g\cdot L^{-1}$, $ZnCl_2$ 0.057 $g\cdot L^{-1}$, $CoCl_2\cdot 6H_2O$ 0.15 $g\cdot L^{-1}$, $Ni(NO_3)_2$ 0.15 $g\cdot L^{-1}$.The stock solution of PCP was prepared at a concentration of 2,000 mg L^{-1} in 2 M NaOH solution. Thirty milliliter (30 mL) PCP mother solution was drawn into the reactor making PCP concentration as 20 mg L^{-1}.

Analytical methods

The pH was measured by pH analyzer of Sension 6(HACH, U.S.); PCP was analyzed by purge and trap concentrator that was coupled with gas chromatography (AutosystemXL-Tekmar3100, made by PE-Tekmar company, U.S.); COD_{Cr} was measured by portable water quality analyzer (DR2700, made by HACH company, U.S).

Morphology and surface structure of granules were observed with an SEM (LEO 1530VP, German). The granule samples were fixed in 2% (w/v) glutaraldehyde for 2 h, and then marinated in 0.1 M phosphate buffer solution for 40 min after which the above operation was repeated by replacing the buffer solution. The fixed granules were dehydrated through a graded series of 30, 50, 70 and 90% ethanol solutions, each being treated for 15 min. Samples were then dehydrated with 100% ethanol for 10 min for three times. The dehydrated granules were subjected to a CO_2 Critical Point Dryer, and were observed by SEM finally.

A phenol-sulfuric acid method was used to quantify polysaccharides (PS) and proteins (PN) were measured by Folin spectrophotometry (Chen et al., 2010). Sludge volumetric index

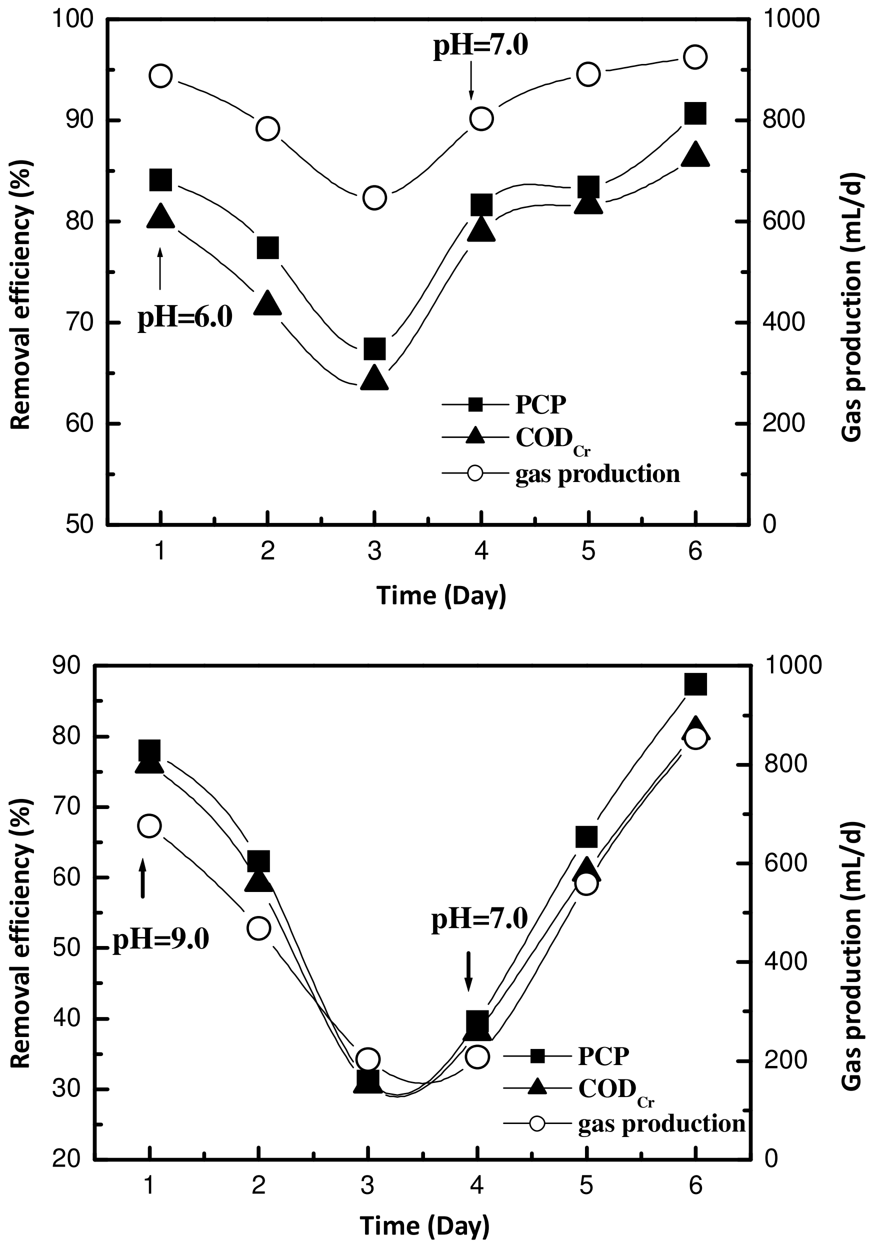

Figure 1. Effect of short time pH shock on reactor operation.

(SVI) was measured using standard methods (EPA, 2002). Settling velocity of sludge was measured by taking and by individual granule sample randomly from the reactor to settle at a certain height in a settling cylinder recording the time.

RESULTS AND DISCUSSION

Capability of resisting pH shock of microaerobic granular sludge

The variation of pH is vital to anaerobic bacteria in the microaerobic granules. Under normal operating conditions, pH was controlled at 6.8-7.5. Capability of shock resistance of microaerobic granular sludge system was researched when pH was beyond the normal value. In the shocking experiments, pH was adjusted to 6.0 and 9.0, respectively.

The result of short-term pH shock resisting experiment is shown in Figure 1. The recovery experiment was carried out by adjusting the pH at 7.0 after three days of pH shock.

From Figure1, we could see the treatment efficiency of

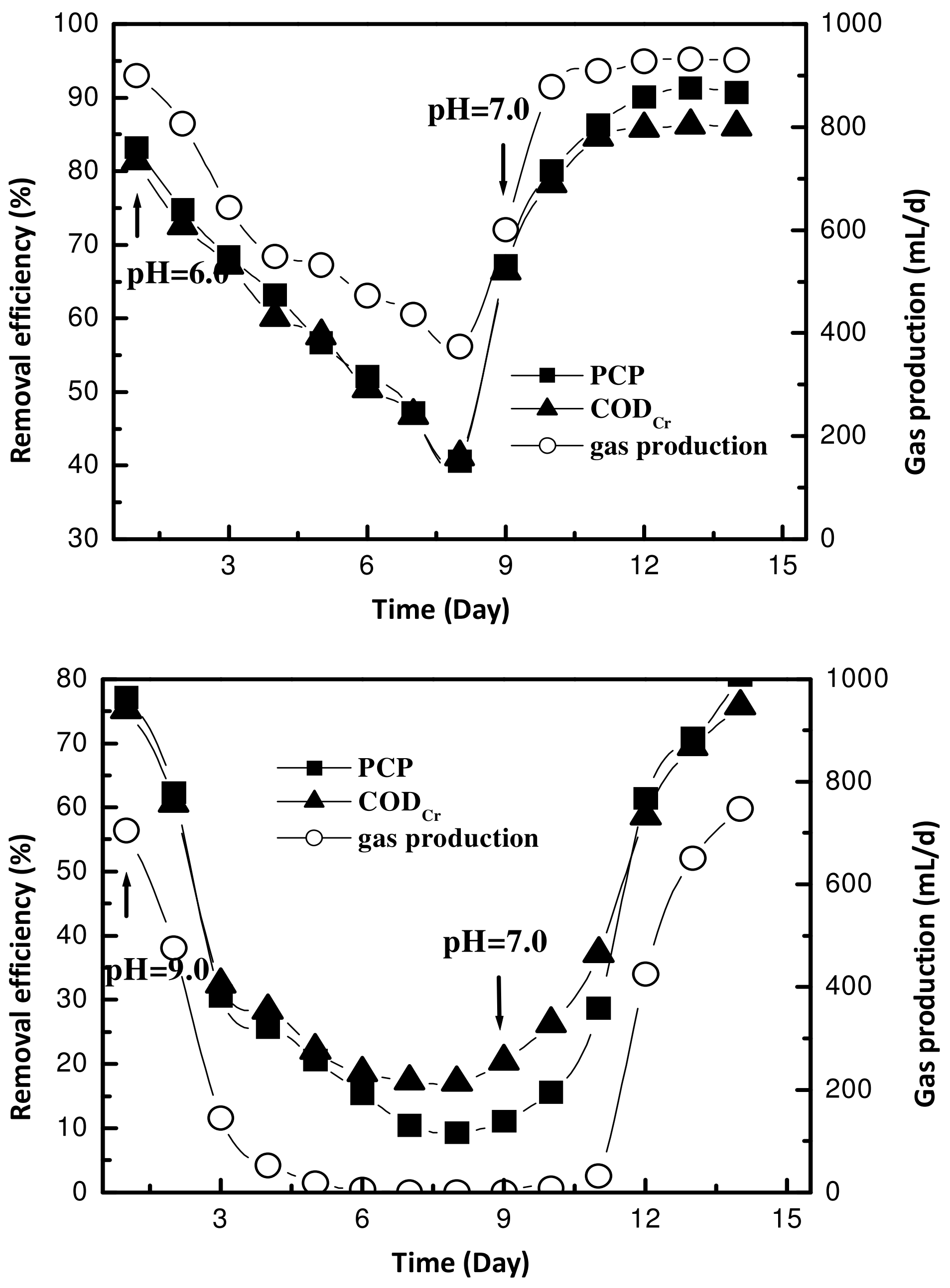

Figure 2. Effect of long time pH shock on the reactor operation.

the system decreased when pH was beyond normal scope. But obvious deterioration of the system working performance did not occur at 1st day pH shock because the system had cushioning capacity. The system buffering capacity was destroyed with continuous pH shock, so the process efficiency kept declining. Compared with pH = 6.0, PCP and COD_{Cr} removal rate decreased distinctly when pH was 9.0. Most of the bacteria except methanogenic bacteria could maintain their regular behavior when pH was 6.0, while hardly any bacteria could keep activity when pH was 9.0. During the recovery experiments, the operating condition of the system got right at once when pH was adjusted from 6.0 to 7.0, while the recovery was ongoing step by step when pH was adjusted from 9.0 to 7.0, but the system could also get right for 3 days normal operation.

The result of long-term pH shock experiment was shown in Figure 2, including 8 days shocking experiments and 6 days recovery experiments. Deterioration of the system working performance occurred under long - term

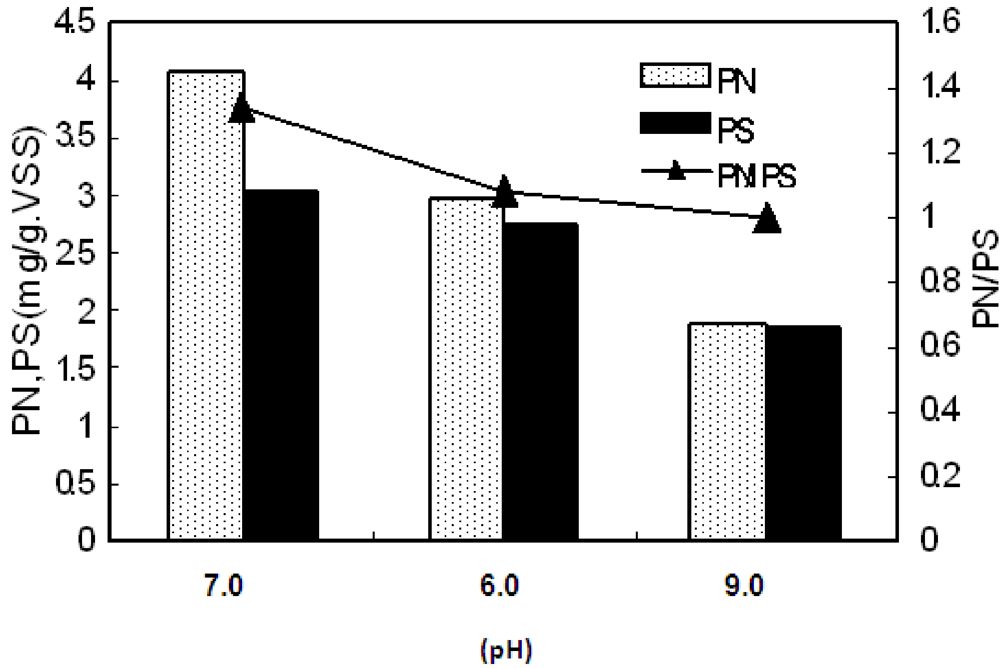

Figure 3. Effect of pH shock on extracellular polymers

shock, especially when pH was controlled at 9.0. PCP and COD_{Cr} removal efficiency decreased to below 20% for 6 days operation, the system deteriorated seriously. In the recovery experiments, the recovery of the system was a gradual process when pH was adjusted from 6.0 to 7.0, which was different from the situation of short-term shock. But after 3 days normal running, both PCP and COD_{Cr} removal efficiency reached above 80% and the system operated normally. The recovery process was very slow when pH was adjusted from 9.0 to 7.0, PCP and COD_{Cr} removal efficiency reached 80.6 and 75.8% respectively after 6days operation, and the system still did not recover to the normal level, which indicated that activity of the organisms was inhabited seriously for long time running under pH = 9.0.

The effect of pH on Methanogens was the most strict in the microorganisms of the micro-aerobic granules. Their adaptability was the worst with pH variation. The optimum pH of the methanogens was 6.7 to 7.5. The activity of methanogens was inhibited when the environmental pH was lower than 6.5. In addition, PCP was an optional ionized phenol whose pKa was 4.8. Variation of pH caused different state of PCP, so the toxic effect would change. PCP existed as molecular state under low pH. Under this state, PCP had stronger lipotropy, and caused high concentration in the organism, so the high toxicity to the microorganisms occurred which resulted in inactivation of the microorganisms and decreasing of PCP and COD_{Cr} removal efficient (Fisher et al., 1999). Although, it was beyond the optimum pH of methanogens when subjecting to the shock of pH 6.0 which deflected a relative smaller extent compared to the normal pH, the aerobic bacteria and the other anaerobic bacteria, example acid-forming bacteria and sulfate reducing bacteria were still active that offered the protection to the methanogens in inner layer of the microaerobic granules and lightened the inhibition extent of the methanogens. So the microorganism's activity and the system recovered at once when the pH adjusted to the normal value. The ionization degree of PCP increased with pH increasing, making enhancement of hydrophily, low possibility to move through the cytomembrane and low toxicity. But when pH was too high such as pH=9.0, most microorganisms activity was inhibited resulting in a comparatively low PCP removal efficiency and accumulation of the dechlorinated intermediate products. So the microorganisms' activity and the system needed long time to recover at the normal pH value.

Effect of pH shock on physical-chemical characters of the microaerobic granules

Microbial activities were affected by the changes of pH values, which led to the changes in the amounts and composition of microbial secretions, and had impact on some physicochemical properties of granular sludge such as flocculation and settling performances. As shown in

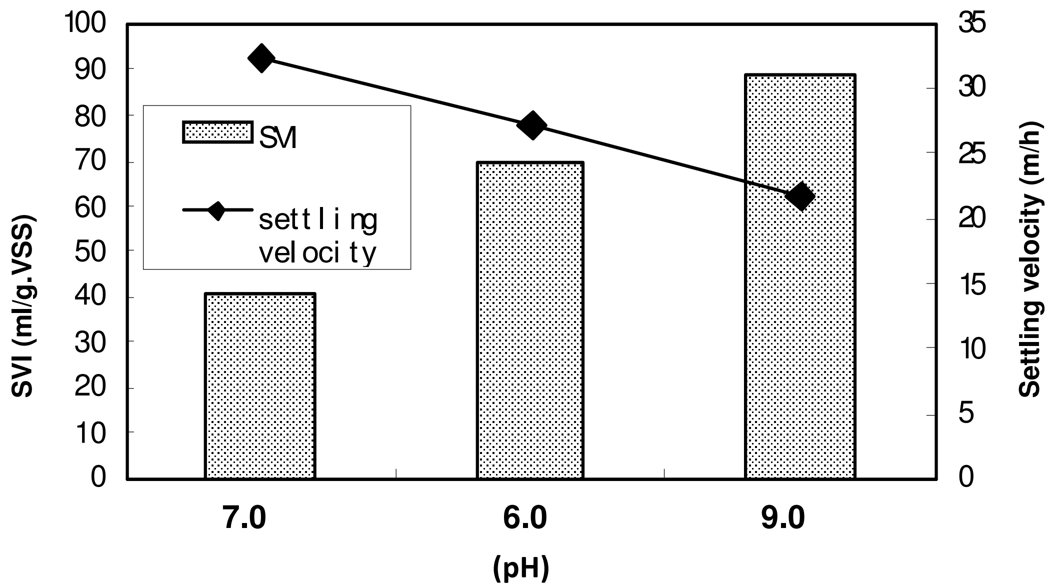

Figure 4. Effect of pH shock on SVI and settling velocity of the granular sludge

Figure 3 (the results of the 8th day under pH shock), both the amounts and proportions of the main components of extracellular polymers secreted by microorganisms have changed for pH shock. The total amount of extracellular polymers (ECPs) and their components including proteins (PN) and polysaccharides (PS) reduced by pH shock (PN, PS and PN/PS was 4.08 mg/g VSS, 3.04 mg/g VSS and 1.34 under the normal condition of pH 7.0 respectively). The decrement of PN, PS and PN/PS was more serious under the shock of pH=9.0 that decreased to 1.86mg/g VSS, 1.85 mg/g VSS and 1.00 respectively. Compared with PS, PN was reduced significantly, in other words, the ratio of PN and PS decreased obviously.

Pavoni (1972) speculated that ECPs was the direct cause leading to bacterial flocculation since 1972. Based on further research of Liao et al. (2001), it has been seen that the flocculation performance was affected by not only the total amount of ECPs , but also by the ratios of PN and PS which also had an important impact on hydrophobicity and charge characteristics of sludge. The hydrophobicity of PN was better than that of PS, so the increase of the PN/PS ratio reduced the retention water in the sludge and resulted in the separation of sludge and body water to make close cell-bindings. In addition, for its stronger bonding capabilities with positive ionic, PN could constitute a three-dimensional structure by the means of cationic bridge effects to keep the stable performance and integrated structure of granular sludge (Delia et al., 2003; Frank et al., 2003). Therefore, the deterioration of flocculation performance and tightness of granular sludge at pH of 6.0 and 9.0 shock made PCP come into somatic cells easily, and caused higher concentration of PCP in the cells, which were harmful for microorganisms. Especially for the sharp decrease of PN in ECPs, flocculation performance, hydrophobic properties and tightness of granular sludge were significantly worse which reduced the ability of granular sludge to resist to toxic substances, so the removal effects of PCP and COD_{Cr} were not good.

In addition, ECPs have an important impact on the physiochemical properties of sludge such as SVI, and settling rates. The amount of ECPs and the PN/PS ratio decreased, furthermore, the abnormal pH values made the proliferation of sludge so slow that the death rate increased, which all made the granules loose. Therefore, as shown in Figure 4, SVI values increased but settling rates decreased which had the same results of the research of Chen et al. (2010) about the correlation relation between the content of pN in ECPs and settling rate. At the same time, it could be observed that flocculent sludge increased in the reactor, and a few granular sludge disintegrated while the granulation of new sludge was restrained. The above phenomenon were even more serious under pH of 9.0 shock.

Under pH of 6.0 shock, the departure degree of ECPs from normal condition (pH = 7.0) and its composition, as well as SVI and settling rate were smaller compared with that under pH of 9.0 shock.

Although, it has gone beyond the optimal scope of some bacteria such as methanogens, pH of 6.0 did not restrain some microorganism in aerobic zone, acid-producing bacteria in anaerobic zone and sulfate reducing bacteria, so the physical and chemical characteristics of micro-aerobic granular sludge had no serious deterioration, the removal of PCP was not as low as that under pH of 9.0 as shown in Figure 2. The advantages of micro- aerobic

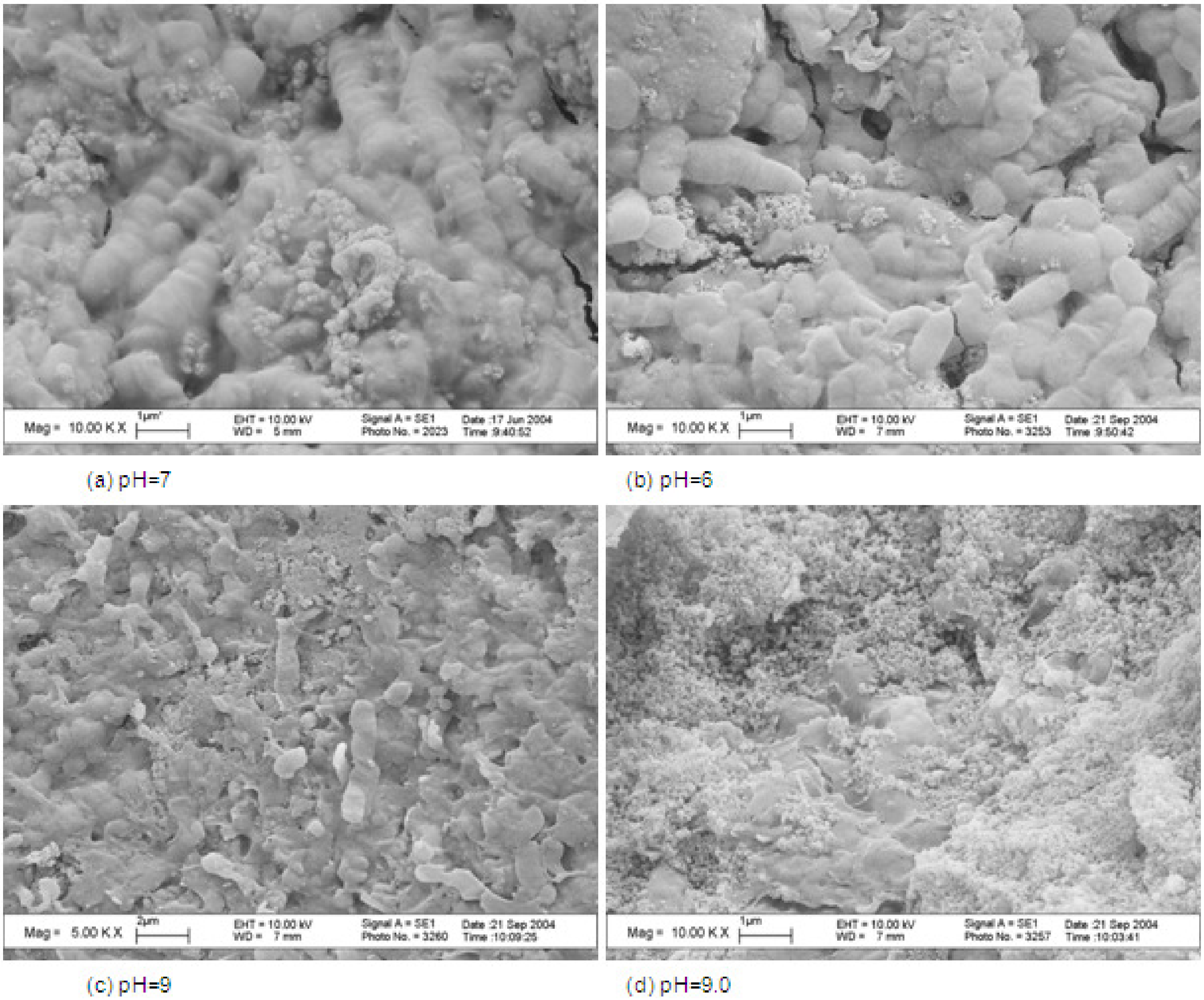

Figure 5. The microbial morphology of bacteria at different pH value.

granular sludge system were exhibited fully in which aerobic and anaerobic microenvironments existed simultaneously. Combined with the restoring experiment shown in Figure 2, although it experienced 8 day pH shock, the system would recover soon under suitable pH.

The shock of pH on microbial morphology of granular sludge

Figure 5 shows the results of internal sections of sludge detected by SEM under different pH shocks. Photo (a) shows the situation of pH was 7.0, photo (b) showed the situation of 8 days' shock when the pH was 6.0, photo (c) and photo (d) showed the situation of 8 days' shock when the pH was 9.0. We could know from the photos that when the pH was controlled at 7.0, the bacteria were plump and had regular shape, most of them were bacillus and only a few were coccus. ECPs were obviously packed within the outer layer. When the pH was 6.0, a part of bacteria decayed in morphology with irregular shape formed for a long time pH shock, though the most microbes were still bacillus. There were no obvious polymers observed, but the bacteria flocked tightly together. When the bacteria were shocked for a long time under pH of 9.0, coccus was preponderant and bacillus was rare, as shown in photo (c). Bacteria, in many areas were packed with dissolved substances and the polymers packed outside layer disappeared, and totally appeared in incompact structure, as shown in photo (d).In the experiments of anaerobic sludge domesticated by chlorophenols, Ye et al. (2004) found that bacillus possessed the higher capacity of degrading chlorophenols. The study about the microorganism community of pentachlorophenol (PCP) degrading in the coupled granules by Chen et al. (2009) indicated that the major of cultured microbes in the anaerobic area of granular sludge were bacillus. When the pH was 6.0, though the major of bacteria inside the granular sludge were bacillus, part of them changed in morphology and their regular function were restrained which was adverse to the degradation of PCP. When the pH was 9.0, the shock led bacillus (which was the chief contributor for the degradation of PCP) to decrease in large amount which resulted in the obvious reduction of the PCP degradation efficiency.

Conclusion

PCP and COD_{Cr} removal efficiency with microaerobic granular sludge could reach the normal level after short-term pH shock for 3 days recovery no matter the pH was controlled at 6.0 nor 9.0. While long-term shock led the treatment efficiency of the system to decrease obviously. For 8 days shock of pH 6.0, PCP and COD_{Cr} removal efficiency descended obviously. The content of PN and PS in the ECPs outside the cells, PN/PS and the settling velocity had an obvious decrease compared with that at the situation of normal pH, value, and SVI had a large increase. The bacillus, the major of the bacteria inside the granular sludge, changed obviously in morphology. But after recovering for 6 days, PCP and COD_{Cr} removal efficiency reached above 80% and the system ran normally. For 8 days shock of pH 9.0, PCP and COD_{Cr} removal efficiency decreased to under 20%. Physical and chemical characters of the sludge got seriously worse. The coccus replaced the bacillus to be the preponderant bacteria and a large amount of inside dissolved substance occurred. After recovering for 6 days, PCP and COD_{Cr} removal efficiency did not reach normal level. The experiments showed that microaerobic granular sludge system had certain pH shock resistance but long-term shock caused the system to get worse which needed a long period to recover. So in the application of wastewater treatment using microaerobic granular sludge, short-term shock of pH had little effect on the system, which was the superiority compared to active sludge system. But it must be noted the system should be refrained from long-term shock of pH, or serious effect on the system would occur.

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