



# ANOXIC TREATMENT OF LOW-STRENGTH WASTEWATER BY IMMOBILIZED SLUDGE

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## ABSTRACT

Low levels of phenol and m-cresol were effectively removed from wastewater under anoxic condition using immobilized sludge. A 138-day experiment was conducted using wastewater containing  $\text{NO}_3^-$ -N ( $22.3 \text{ mg}\cdot\text{l}^{-1}$ ), phenol ( $10 \text{ mg}\cdot\text{l}^{-1}$ ) and m-cresol ( $5 \text{ mg}\cdot\text{l}^{-1}$ ) at  $30^\circ\text{C}$  using sucrose ( $50 \text{ mg}\cdot\text{l}^{-1}$ ) as co-substrate in an upflow reactor packed with polyvinyl alcohol (PVA) beads entrapped with anoxic sludge and powdered activated carbon (PAC). Throughout the experiment, phenol and m-cresol in the effluent were below the detectable level of  $1.0 \text{ mg}\cdot\text{l}^{-1}$  even when HRT (hydraulic retention time) was as low as 0.55 hour. The effluent quality in general decreased with HRT. At 5.9 hours of HRT, the reactor effluent contained  $5.9 \text{ mg}\cdot\text{l}^{-1}$  of COD (chemical oxygen demand) and  $1.3 \text{ mg}\cdot\text{l}^{-1}$  of  $\text{NO}_3^-$ -N; but at 0.55 hour, they were, 17.3 and  $5.7 \text{ mg}\cdot\text{l}^{-1}$ , respectively. The effluent COD was from the unidentified soluble microbial products. Methane was not produced after the startup, and all PVA beads were not disintegrated. Results of a batch test showed that organic substrate was preferably consumed by the denitrify bacteria in favor of methane-producing bacteria. Scanning electron micrographs showed that most bacteria were populated on the bead surface due to the availability of nitrate and substrate. © 1997 IAWQ. Published by Elsevier Science Ltd

## KEYWORDS

Denitrification; immobilization; low-strength; m-cresol; PAC; phenol; polyvinyl alcohol; wastewater.

## INTRODUCTION

Many surface and ground waters are polluted by chemicals. Aromatic pollutants, such as phenol and cresol, are mostly originated from chemical industry and coal gasification. Although it has been reported in recent studies that these pollutants could be effectively degraded under both anaerobic (Fang *et al.*, 1996) and anoxic conditions, Ramanand and Sulflita (1991) showed that m-cresol was degraded faster under the latter condition. On the other hand, nitrate is a pollutant mainly from untreated domestic wastewater, livestock waste, and run-off from farmland. Nitrate was used to be removed using chemical processes, by which nitrate was first converted to ammonium followed by pH adjustment and air stripping. However, it is more common now to use biological process to remove nitrate (Soares *et al.*, 1988; Gauntlett, 1981). Many heterotrophic bacteria in nature use nitrate as an electron acceptor, but they require organic substrates to provide the carbon source while converting nitrate into nitrogen gas. Organic pollutants provide the carbon needed for this denitrification process in water and wastewater treatment. When carbon is deficient, extra organic chemicals, e.g. methanol, may have to be added to enhance the treatment efficiency.

The efficacy of biological treatment of low-strength wastewater is often determined by two factors: how effective is the separation of sludge from treated effluent, and how short is the HRT (hydraulic retention time) needed to produce a high-quality effluent. Sludge immobilization is a technology developed to enhance the sludge separation (Nilsson *et al.*, 1980; Nilsson and Ohlson, 1982; Kokufuta *et al.*, 1986; Li and Chen, 1993; Yang *et al.*, 1995). Because sludge of high bioactivity is physically entrapped in polymeric films or beads, effluent can be discharged from the reactor without much sludge wash-out. Some researchers recently co-immobilized sludge and powdered activated carbon (PAC) in polymeric beads for wastewater treatment (Lin and Wang, 1991; Siahpush *et al.*, 1992). Toxic pollutants are first adsorbed onto the PAC surface and then degraded by the sludge co-immobilized in the polymeric bead. The PAC is continuously being regenerated by the bacterial action. This process was effective in producing a high-quality effluent from treating low-strength wastewater containing toxic chemicals.

This study was conducted to investigate the effectiveness of an upflow reactor in removing low levels of phenol and cresol from nitrate-containing wastewater using PVA (polyvinyl alcohol) beads co-immobilized with bioactive sludge and PAC.

## MATERIALS AND METHODS

### Immobilization

A sludge of high bioactivity was co-immobilized with PAC using a method modified from a previous study (Lin and Chen, 1993). A mixed solution was prepared by first dissolving 7.5 g of PVA (molecular weight ranging 70,000-100,000; Sigma) in 30 ml of water at 80°C. The solution was then cooled down to 40°C before adding 1 g of PAC (100-400 mesh, Sigma), 2 g of portland cement (strength class 52.5N) and 60 ml of anoxic sludge, which corresponded to 1.2 g of volatile suspended solids (VSS). The cement was added to enhance the mechanical strength and to increase the density of the polymeric beads. The partially granulated sludge was obtained from a UASB reactor treating high-strength industrial wastewater containing 200 mg•l<sup>-1</sup> of phenol, 100 mg•l<sup>-1</sup> of m-cresol and 446 mg•l<sup>-1</sup> of NO<sub>3</sub><sup>-</sup>-N plus 1000 mg•l<sup>-1</sup> of sucrose as a co-substrate. The reactor was operated at an organic loading rate of 1.79 g-COD•l<sup>-1</sup>•d<sup>-1</sup> and a COD/NO<sub>3</sub><sup>-</sup>-N ratio of 4/1 for 195 days. At only one day of HRT, the sludge was able to remove 99% of phenol, 73% of m-cresol and 100% of NO<sub>3</sub><sup>-</sup>-N.

Droplets of the mixed solution was then squeezed out from a syringe into a saturated boric acid solution. After one hour of gentle mixing, the droplets were transformed into spherical beads, which were further hardened in a 1M potassium dihydrogenphosphate solution for one hour. These beads co-immobilized with anoxic sludge and PAC were washed twice by de-ionized water before being packed into the reactor. The average bead diameter was 3 mm.

### Batch test of sludge used for co-immobilization

The bioactivity of the anoxic sludge prior to the immobilization was investigated in a batch test. About 100 mg (as VSS) of disintegrated sludge was added to a serum vial containing 100 ml of a feed solution which was composed of phenol (200 mg•l<sup>-1</sup>), m-cresol (100 mg•l<sup>-1</sup>), sucrose (1000 mg•l<sup>-1</sup>) and NO<sub>3</sub><sup>-</sup>-N (357 mg•l<sup>-1</sup>), plus nutrient, vitamins and trace metals, similar to the test of methanogenic activity developed by Owen *et al.* (1979). The pH was buffered at 7.2~7.6 by bicarbonate. The vial was placed in a 37°C shaking bath. Biogas production and composition, as well as the composition in the mixed liquor, were monitored regularly over 54 days.

### Continuous treatment in upflow reactor

The co-immobilized PVA beads were packed in a 150 ml upflow reactor (Fang *et al.*, 1997) treating wastewater containing phenol (10 mg•l<sup>-1</sup>), m-cresol (5 mg•l<sup>-1</sup>), sucrose (50 mg•l<sup>-1</sup>), and NO<sub>3</sub><sup>-</sup>-N (22.3 mg•l<sup>-1</sup>) at 30°C for 138 days. The COD/NO<sub>3</sub><sup>-</sup>-N ratio of the wastewater was kept at 4/1. The PVA beads was

estimated to contain 0.90 g (as VSS) of anoxic sludge. The HRT was 11 hours initially, and was decreased stepwise eventually reaching 0.55 hour.

### Analytical methods and microscopic examination

Gas composition, including nitrogen, nitrous oxide, and methane, was analyzed by a gas chromatograph (GC, Hewlett-Packard, Model 5890 Series II). Phenol, m-cresol, benzoate and VFAs (from acetic to heptanoic acids) were analyzed by a second GC of same model. Details of operational conditions followed those described in previous study (Fang *et al.*, 1996). Nitrate and nitrite were analyzed by an ion chromatograph (LC-10, Shimadzu) with the Shim-pack IC-A3 column. Sucrose was measured using a spectrophotometer (UV-160A, Shimadzu). Other parameters, such as total solids (TS), VSS and COD, were measured according to the *Standard Methods* (APHA, 1985). After the completion of the continuous test, polymeric beads were sampled from the reactor for microscopic examination using a scanning electron microscope (SEM, Cambridge Stereoscan 360) following the procedures reported previously (Fang and Chui, 1993).

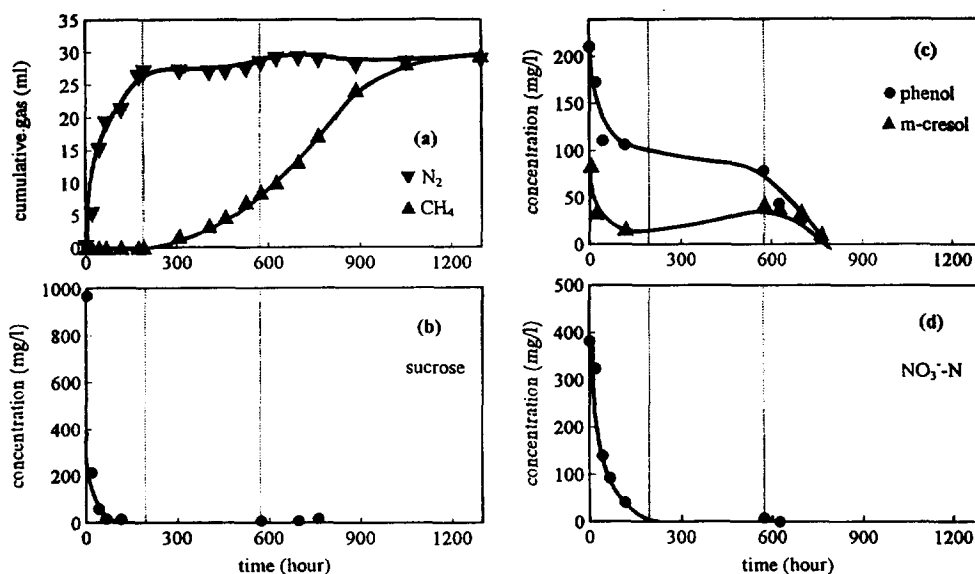


Figure 1. (a) nitrogen and methane production, (b) sucrose removal, (c) phenol and m-cresol depletion, and (d) nitrate reduction in the batch test.

## RESULTS AND DISCUSSIONS

### Bioactivity of anoxic seed sludge

The anoxic sludge appeared to have three phases of bioactivity in the batch test. Figure 1 illustrates (a) the cumulative productions of nitrogen and methane, (b) removal of sucrose, (c) depletion of phenol and m-cresol, and (d) nitrate reduction throughout this 1300-hour test. Over 95% of the nitrate in the feed solution was denitrified in Phase 1, i.e. the first 172 hours of the test, during which over 50% of phenol, 60% of m-cresol and nearly 100% of sucrose were also removed. Nitrogen accumulated in the biogas corresponded closely to the amount of nitrate reduced; however, there was no methane detected in the biogas. This indicated that, in this phase, organic substrates were utilized for denitrification, and not for methane-production. Methane began to accumulate in Phase 2 (from hour 173 to hour 572) after the nitrogen production was ceased. During this phase, phenol and m-cresol concentrations remained unchanged. The

carbon source for methane production was likely from the fraction of sucrose which had been adsorbed by the sludge initially in Phase 1. Results in Phases 1 and 2 indicated that organic substrates were preferentially consumed, in the presence of nitrate, by denitrifiers, in favor of methane-producing bacteria. In Phase 3 (hour 573 to the end), when the adsorbed sucrose became gradually depleted, methane-producing bacteria were then able to switch to utilizing the residual phenol and m-cresol as carbon source.

The wastewater parameter chemical oxygen demand (COD) is a measurement of reducing capacity, namely electrons available in the organic pollutants for chemical oxidation. In an enclosed system, the total quantities of available electrons, as well as COD, remain unchanged. The COD contents originally in the pollutants are transformed into those of the reduced products, such as methane and nitrogen. Each ml of nitrogen and methane produced at 20°C corresponded, respectively, to 2.66 and 3.33 mg of COD. Based on the cumulative production of these two gases, it was found that of the 174 mg of COD in the feed solution, 54.7% was converted by denitrifiers to nitrogen and 45.3% by methane-producing bacteria to methane. There was no nitrous oxide detected in the biogas.

### Continuous treatment in upflow reactor

Figure 2 illustrates (a) HRT in the continuous treatment of wastewater in the upflow reactor, and several parameters related to the reactor performance, including (b) effluent  $\text{NO}_3^-$ -N concentration, (c) nitrogen production rate, and (d) effluent COD level. Concentrations of residual phenol and m-cresol in the effluent were below the detectable level of  $1.0 \text{ mg}\cdot\text{l}^{-1}$  after day 10. Sucrose concentration in the effluent was below the detectable level of  $1 \text{ mg}\cdot\text{l}^{-1}$  after day 21. These results showed that the co-immobilized PVC beads were very effective for the removal of phenol and m-cresol even at only 0.55 hour of HRT. The residual COD in the effluent was contributed by the unidentified soluble microbial products.

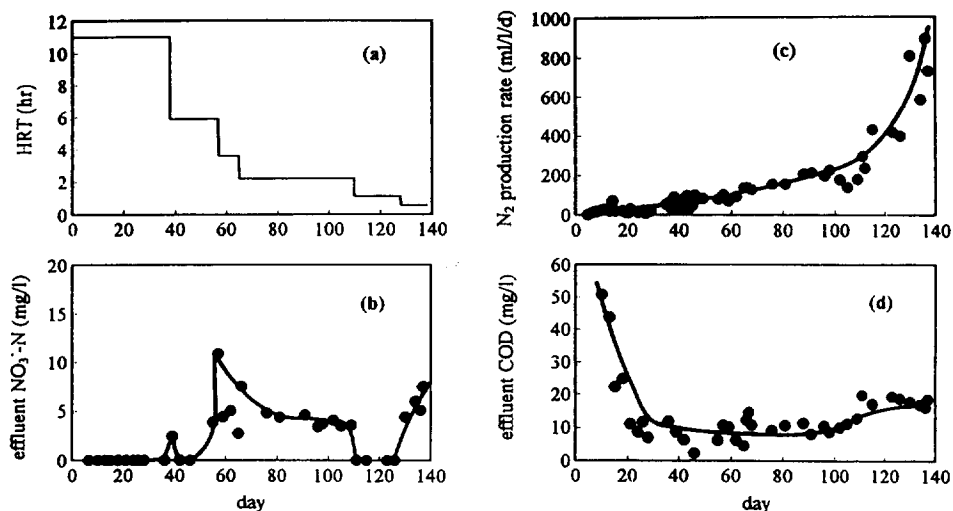


Figure 2. (a) HRT, (b) effluent  $\text{NO}_3^-$ -N, (c) nitrogen production rate, and (d) effluent COD level during the continuous treatment in the upflow reactor.

The PVA beads were mechanically stable against attrition, and there was no bead disintegrated during the test, resulting in very little sludge wash-out. Even at only 0.55 hour of HRT, the effluent TS and VSS averaged only  $17.4$  and  $13.1 \text{ mg}\cdot\text{l}^{-1}$ , respectively. Throughout the test, the pH of the influent was controlled at 8.0, and the effluent pH was stable in the range of 6.9-7.5. The volumetric loadings to the reactor were proportional to the HRT because of treating the same wastewater. As the HRT was step-decreased from 11.0 to 0.55 hours, the COD loading was increased from  $0.20$  to  $4.23 \text{ g}\cdot(\text{l}\cdot\text{d})^{-1}$ , and the  $\text{NO}_3^-$ -N loading was increased from  $0.043$  to  $0.96 \text{ g}\cdot(\text{l}\cdot\text{d})^{-1}$ . At 11.0 hours of HRT, nearly all the influent nitrate was denitrified

producing an effluent containing less than  $0.1 \text{ mg}\cdot\text{l}^{-1}$  of  $\text{NO}_3^- \text{-N}$ ; during this period, there was a small amount of methane produced at a rate of less than  $0.1 \text{ ml}\cdot(\text{l}\cdot\text{d})^{-1}$ . The  $\text{NO}_3^- \text{-N}$  concentration was gradually increased in the effluent as the HRT was shortened. As illustrated in Figure 2(b), the residual averaged  $5.7 \text{ mg}\cdot\text{l}^{-1}$  for HRT levels ranging from 5.9 hours to 0.55 hour. Methane production was completely inhibited starting day 38 after residual  $\text{NO}_3^- \text{-N}$  began to show up in the effluent. This clearly indicated that methane-producing bacteria were unable to compete with denitrifiers with substrate. Figure 3(a) illustrates that nitrate removal efficiency in general increased with the increase of HRT. The nitrate reduction rate was  $0.04 \text{ g}\cdot\text{NO}_3^- \text{-N}\cdot(\text{l}\cdot\text{d})^{-1}$  at 11 hours of HRT, and  $0.71 \text{ g}\cdot\text{NO}_3^- \text{-N}\cdot(\text{l}\cdot\text{d})^{-1}$  at 0.55 hour. Nitrous oxide production was nil at higher HRT; at 0.55 hour of HRT, it could be detected at an average production rate of  $5.4 \text{ ml}\cdot\text{N}_2\text{O}\cdot(\text{l}\cdot\text{d})^{-1}$ .

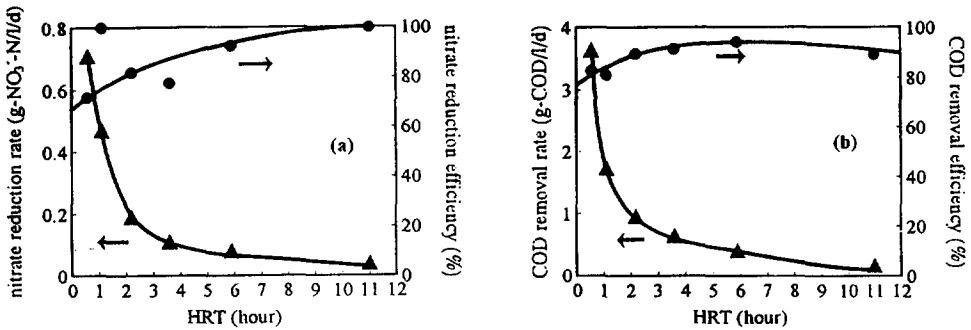


Figure 3. Reduction rates and removal efficiencies of (a) nitrate and (b) COD at various HRTs.

Figure 2(d) illustrates that the effluent COD remained low after first 20 days of acclimation period. The average COD in the effluent slightly increased with HRT, from  $5.9 \text{ mg}\cdot\text{l}^{-1}$  at 5.9 hours of HRT to  $17.3 \text{ mg}\cdot\text{l}^{-1}$  at 0.55 hour. Figure 3(b) illustrates that, although the COD removal rate decreased with HRT (from  $0.018 \text{ g}\cdot\text{COD}\cdot(\text{l}\cdot\text{d})^{-1}$  at 11 hours to  $3.64 \text{ g}\cdot\text{COD}\cdot(\text{l}\cdot\text{d})^{-1}$  at 0.55 hour), the COD removal efficiency remained in the range of 80-94%.

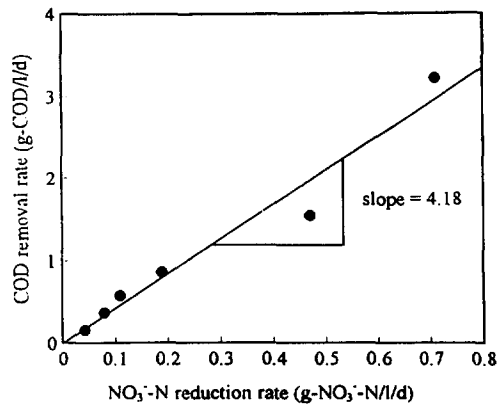
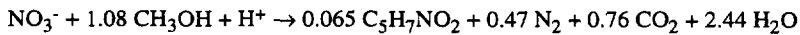


Figure 4. Correlation between removed COD and denitrified  $\text{NO}_3^- \text{-N}$ .

Figure 4 illustrates that, in this study, each gram of  $\text{NO}_3^- \text{-N}$  denitrified corresponded to an average of 4.18 grams of COD removal. McCarty *et al.* (1969) described denitrification, assuming methanol was the carbon source, using the following equation:



According to this equation, each gram of  $\text{NO}_3^-$ -N denitrified would require 2.47 g of methanol that is equivalent to 3.70 g of COD. Yang *et al.* (1995) showed that the ratio of COD/ $\text{NO}_3^-$ -N ratio ranged from 3.62 to 3.68 in a reactor containing immobilized sludge treating wastewater also containing methanol as substrate. Using ethanol as substrate for denitrification, Delanghe *et al.* (1994) reported a similar ratio of 3.73. The COD/ $\text{NO}_3^-$ -N ratio of 4.18 found in this study was about 13% higher than these data in literature. The discrepancy could be due to the difference in substrate.

Results of this study showed that a reactor containing PVA beads immobilized with sludge and PAC was able to produce high-quality effluent at a HRT as short as 0.55 hour. The effectiveness of this process for the removal of phenol and m-cresol could be attributed, to a large degree, to the adsorbing characteristics of PAC (Ehrhardt and Rehm, 1985; Hutchinson and Robinson, 1990; Schultz and Keinath, 1984). The PVA beads packed in the reactor contained only 0.75 g of PAC, but the reactor removed in total 11.64 g of sucrose, 2.33 g of phenol, and 1.16 g of m-cresol throughout the test. Ehrhardt and Rehm (1985) reported that one gram of activated carbon was able to adsorb about 0.5 g of phenol. Thus, the removal of phenol and m-cresol in this study could not be solely due to PAC adsorption. Those adsorbed on the PAC surface must have been further degraded by the bacteria, and the PAC was constantly being regenerated as a result.

Although the seed sludge was uniformly entrapped inside the PVA beads at the beginning, SEM micrographs of bead samples taken on day 138 illustrate that bacteria were mostly populated on the PVA bead surface, instead of in the interior of the bead. This implies that most of the bioactivities were taken place on the bead surface, because of the availability of substrate and nitrate in the mixed liquor. Figure 5 is a SEM micrograph illustrating that a rod-shaped bacterium, presumably a denitrifier, was predominant on the PVA bead surface.

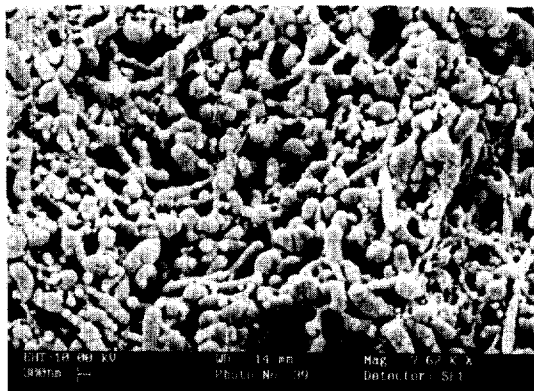


Figure 5. SEM micrograph of the surface of a PVA bead.

## CONCLUSIONS

Low levels of phenol and m-cresol, two common toxic industrial pollutants, in wastewater could be effectively removed under anoxic condition at 30°C in an upflow reactor packed with PVA beads co-immobilized with anoxic sludge and PAC. At 5.9 hours of HRT, treating a wastewater containing  $\text{NO}_3^-$ -N ( $22.3 \text{ mg}\cdot\text{l}^{-1}$ ), phenol ( $10 \text{ mg}\cdot\text{l}^{-1}$ ) and m-cresol ( $5 \text{ mg}\cdot\text{l}^{-1}$ ) using sucrose ( $50 \text{ mg}\cdot\text{l}^{-1}$ ) as co-substrate, the reactor effluent contained  $5.9 \text{ mg}\cdot\text{l}^{-1}$  of COD and  $1.3 \text{ mg}\cdot\text{l}^{-1}$  of  $\text{NO}_3^-$ -N. The effluent quality decreased with HRT. However, at HRT as low as 0.55 hour, the effluent COD was still only  $17.3 \text{ mg}\cdot\text{l}^{-1}$ , and  $\text{NO}_3^-$ -N  $5.7 \text{ mg}\cdot\text{l}^{-1}$ . The residual phenol and m-cresol were below the detectable level of  $1.0 \text{ mg}\cdot\text{l}^{-1}$  in all HRTs. On average, each gram of COD removal corresponded to 0.24 g of  $\text{NO}_3^-$ -N reduction. The PVA beads exhibited satisfactory mechanical strength without any disintegration throughout the 138-day experiment. Batch test on the seed sludge showed that it contained both denitrifiers and methane-producing bacteria. However, organic substrate was preferably consumed by the former, as evidenced by the observation that methane

production began only after nitrate had been depleted. Scanning electron micrographs showed that bacteria were mostly populated on the bead surface, probably due to the availability of nitrate and substrate.

#### ACKNOWLEDGEMENT

The writers would like to thank the Hong Kong Research Grants Council for the partial financial support of this study, and the Electron Microscope Unit of the University of Hong Kong for their technical assistance.

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