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TOXICITY OF ELECTROPLATING METALS ON BENZOATE-DEGRADING GRANULES

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ABSTRACT

Toxicity of five electroplating metals on the activity of biogranules, which were obtained from an upflow anaerobic sludge blanket (UASB) reactor treating benzoate-rich wastewater, was investigated in this study. The methanogenic activities of the biogranules were measured in serum vials using wastewaters containing not only benzoate as substrate, but also individual heavy metals over a wide range of concentration. At 37 °C, the toxicity, which increased with metal concentration, was in the following descending order: nickel > zinc > cadmium > copper > chromium. The biogranules exhibited higher resistance to metal toxicity than the flocculent anaerobic sludge. This is probably due to the densely packed nature of the biogranules, reducing the overall exposure of bacteria to the toxic metals in the bulk solution. This implies that granulation of biomass increases their resistance towards toxicants in the wastewater.

Keywords: Anaerobic, benzoate, electroplating, metal, toxicity.

INTRODUCTION

Microorganisms in nature decompose organic pollutants for energy, carbon and nutrient. Under anaerobic conditions, complex organics pollutants are first hydrolyzed/fermented, producing intermediates which are subsequently converted to simple acids, such as acetate, and hydrogen. Methanogens then convert acetate and H_2/CO_2 into methane. Engineers have applied the same principle for wastewater treatment. In treating high-strength industrial wastewaters, anaerobic process has three intrinsic advantages over the more conventional aerobic process: saving of energy for aeration, producing a readily usable fuel, *i.e.* methane, and generating a lesser amount of sludge.

The anaerobic wastewater treatment technology has become viable in the past decade mainly due to the successful development of high-rate reactors. Among them, the anaerobic filter and the upflow anaerobic sludge blanket (UASB) reactor have probably received most commercial interests [1-3]. The former process is popular in the United States, while the latter is well received in Europe and, more recently, in Asia. The UASB process has been very effective for treating wastewaters from food/beverage and the agricultural industries, of which the pollutants are mainly composed of carbohydrates and fatty acids. Recent research showed that UASB process could also be effective for the treatment of industrial wastewaters containing aromatic chemicals, such as benzoate and phenol [4, 5].

Benzoate is a specialty chemical commonly used in pharmaceutical industry. It has been demonstrated in a recent study that over 97% of benzoate in wastewater could be removed by the UASB process at 37 °C with 9.8 hours of retention time for wastewater containing up to 6300 mg l^{-1} of benzoate, equivalent to 12500 mg·l^{-1} of chemical oxygen demand (COD) [5]. Benzoate can be an intermediate in the anaerobic degradation of complex aromatic chemicals. For examples, benzoate was detected as an intermediate in the degradation of phenol and chlorophenol by anaerobic consortia and in the degradation of 3-hydroxybenzoate and 3-chlorobenzoate in defined syntrophic cocultures or axenic cultures [6-9]. Therefore, the effective removal of benzoate suggests that the application of UASB technology could very likely be broadened to the treatment of wastewaters from chemical industries.

Heavy metals from electroplating industry cause severe water pollution in many places, including Hong Kong. Although many of them are the essential elements in very low concentrations for the microbial growth, heavy metals at elevated concentrations are toxic to microorganisms [10, 11]. In municipal wastewater treatment, heavy metals in sewage could also lead to upset and failure of the anaerobic sludge digestion process [12, 13]. Effects of heavy metals have been studied on the anaerobic digestion of municipal sludge and, more recently, on the UASB treatment of wastewater containing starch from food industry [14, 15]. However, little is known about their toxic effect on the anaerobic treatment

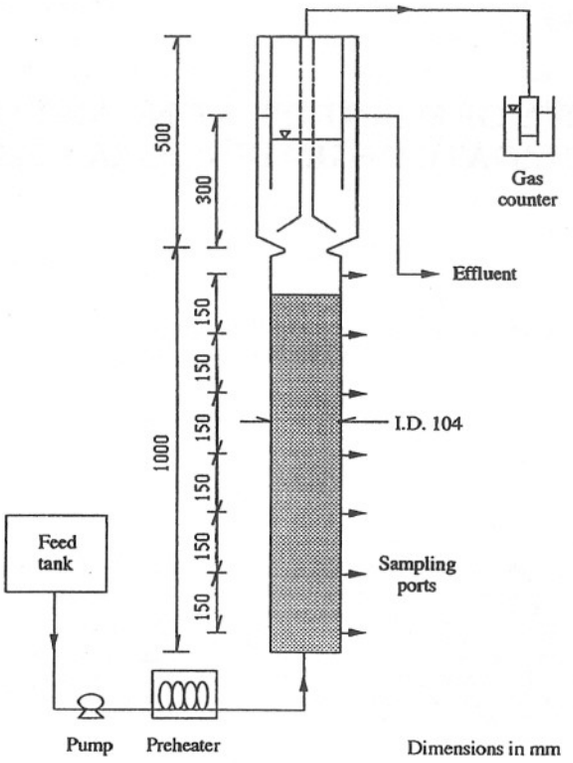

Figure 1. UASB Reactor for Developing Benzoate-Degrading Biogranules.

of wastewater from chemical industry. This study was conducted to investigate the toxic effect of five heavy metals commonly found in the electroplating effluent, including cadmium, chromium, copper, nickel, and zinc, on the bioactivity of benzoate-degrading biogranules.

MATERIALS AND METHODS

An 8.5 liter UASB reactor, as illustrated in Figure 1, was used to treat wastewater which was composed of benzoate as the sole organic substrate, plus proper dosages of alkalinity, nutrients and trace elements as reported in a previous study [3]. Throughout the study, the reactor was kept at 37 °C with 24 hours of hydraulic retention time. Effluent and biogas produced from the reactor were constantly monitored. Steady-state condition was ensured by the constant levels of COD, pH and VFA in the effluent, and the production rate and methane content of the biogas. Concentrations of VFA in effluent and methane in biogas were measured by gas chromatography [3]. The reactor was operated steadily at COD loading rate of 10 $g \cdot l^{-1} \cdot d^{-1}$, consistently removed 98.5-99.5% of COD, comparable to that reported in a previous study [4].

Biogranules from the UASB reactor were then sampled for measurement of methanogenic activity for the degradation of benzoate in the 157 ml serum vials. Each vial was filled with 100 ml of synthetic wastewater containing 1260 $mg \cdot l^{-1}$ of benzoate (equivalent to 2500 $mg \cdot l^{-1}$ of COD) as substrate, along with proper dosages of nutrients, trace metals and vitamins using the formulation reported by Owen, *et al.* [16]. Each vial was then added with about 100 mg of biogranules; the exact amount of biomass in each vial was later measured by the content of volatile suspended solids (VSS) at the end of each test, following the standard methods [17]. Individual heavy metals were also dosed to the serum vials at controlled concentrations (up to 500 $mg \cdot l^{-1}$), except the six vials which served as the controls. The toxicity of individual heavy metals towards the benzoate-degrading granules was reflected by the decrease of bioactivity, as compared to these control granules. After adding the synthetic wastewater with the aforementioned ingredients, each vial was then capped with butyl rubber, and submerged in 37 °C water in a shaking bath. All serum vials were sterilized prior to the test, and all transfers of wastewater and biogranules to the vials were conducted inside an anaerobic chamber.

The biogas production from each vial was monitored every four hours, initially, using a syringe; the monitoring frequency was reduced later on as the biogas production rate gradually decreased. The methane contents in the biogas were measured by a gas chromatograph (Hewlett Packard, model 5890A) equipped with a thermal conductivity detector and a 2m x 2mm (inside diameter) stainless-steel column packed with Porapak N (80-100 mesh). Injector and detector temperatures were kept at 130°C while column temperature was 50°C. Each bioactivity measuring program lasted about

one week after the methane production had levelled off. The initial slope of methane production, which may be expressed as g-methane ·g-VSS^{-1}·day^{-1}, represented the specific methanogenic activity (SMA) of the biogranules using benzoate as substrate [16, 18, 19]. All SMA measurements were conducted in, at least, duplicate.

The microstructure of benzoate-degrading biogranules and their microbial composition were examined using a scanning electron microscope (SEM) and transmission electron microscope (TEM). Biogranule samples were prepared for SEM and TEM examinations following the reported procedures [20].

RESULTS AND DISCUSSION

Figure 2 illustrates the methane production of control biogranules at 37 °C. Initially, the supply of benzoate substrate was not a limiting factor for the methanogenesis. However, as benzoate became depleted, the methane production rate gradually levelled off. The maximum slope of specific methane production represented the SMA of the biogranules on the degradation of benzoate.

For the control biogranules,the SMA using benzoate as substrate was 0.264 g-methane·g-VSS^{-1}·day^{-1}.

The slope of specific methane production rate

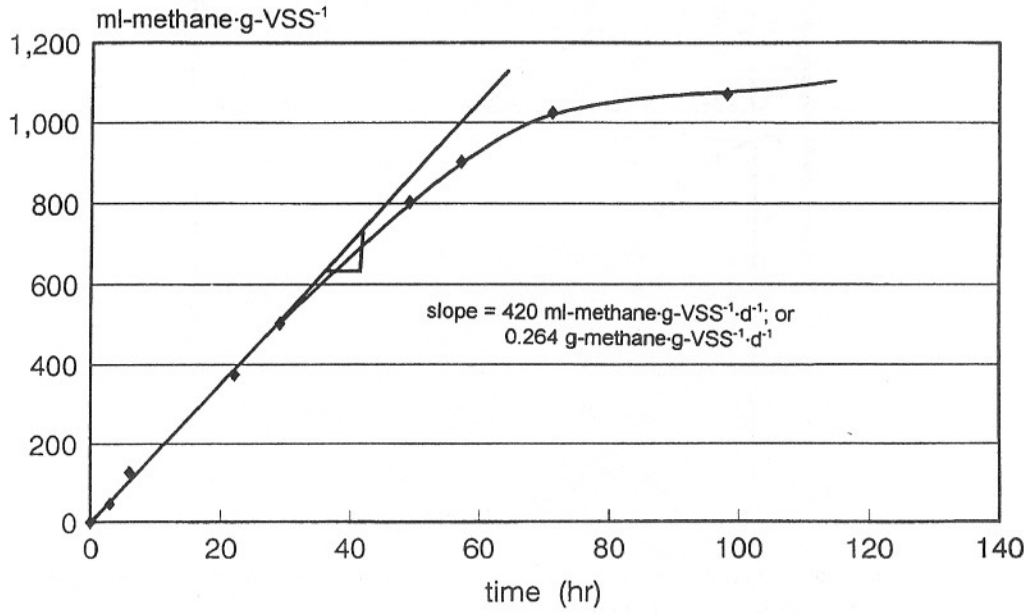

Figure 2. Specific Methane Production of Control Biogranules.

Table 1. $C_{I,50}$ for UASB Biogranules and Digester Sludge.

	$C_{I,50}$ (mg·l^{-1})		
metal	B-granule[a]	S-granule[b]	Fl-sludge[c]
cadmium	150	> 550	7.7
chromium	210	630	14.7
copper	175	158	12.5
nickel	100	118	400
zinc	110	97	16

[a]Benzoate-degrading biogranules (present study)
[b]Starch-degrading biogranules [15]
[c]Flocculent digester sludge [14]

decreased with the increase of heavy-metal concentration. The toxicities of individual metals over a wide range of concentration were indicated by the decrease of SMA in percentage as compared to the controls, as illustrated in Figure 3a-3e, respectively, for cadmium, chromium, copper, nickel and zinc. The methanogenic activity of benzoate-degrading biogranules decreased with the increase of the heavy metal concentration. The $C_{I,50}$, which represents the heavy-metal concentration at which the methanogenic activity of biogranules was reduced to 50% of the controls, was 150 mg·l^{-1} for cadmium, as illustrated in Figure 3a. Figure 3b-3e illustrate the similar plots of relative methanogenic activity as compared to the controls for the other four heavy metals. The respective $C_{I,50}$ were 210, 175, 100 and 110 mg·l^{-1}, for chromium, copper, nickel and zinc. In general, nickel and zinc are more toxic than cadmium, chromium and copper. Table 1 summarizes the $C_{I,50}$ of each heavy metal for the benzoate-degrading biogranules. Also listed for comparison are the corresponding data for starch-degrading UASB biogranules and the flocculent anaerobic digester sludge on the degradation of mixed volatile fatty acids [14, 15].

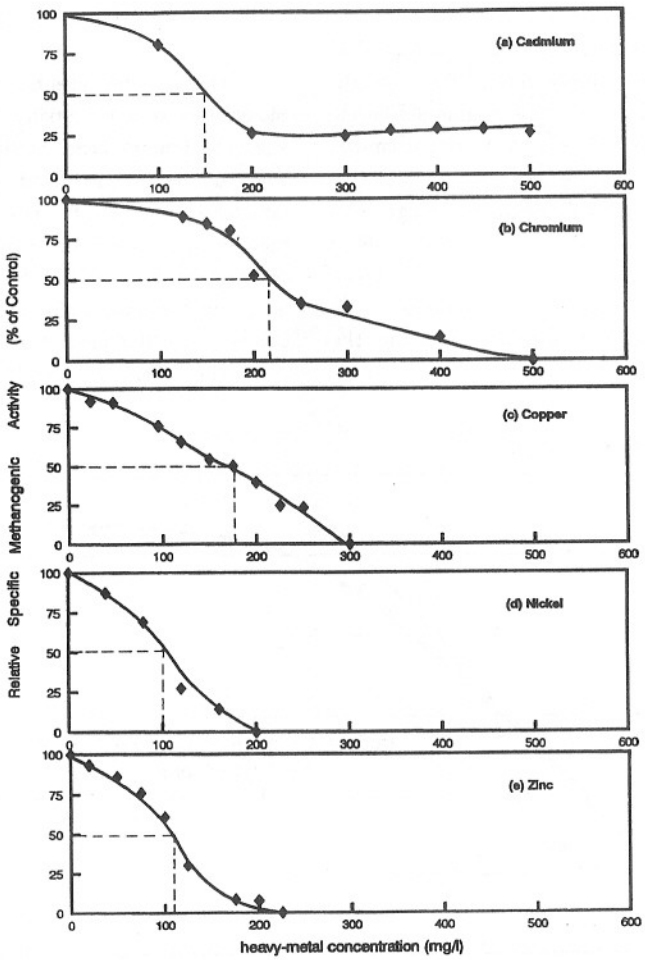

Figure 3. Methanogenic Activity of Biogranules as Compared to the Controls for Wastewaters Containing (a) Cadmium, (b) Chromium, (c) Copper, (d) Nickel, and (e) Zinc.

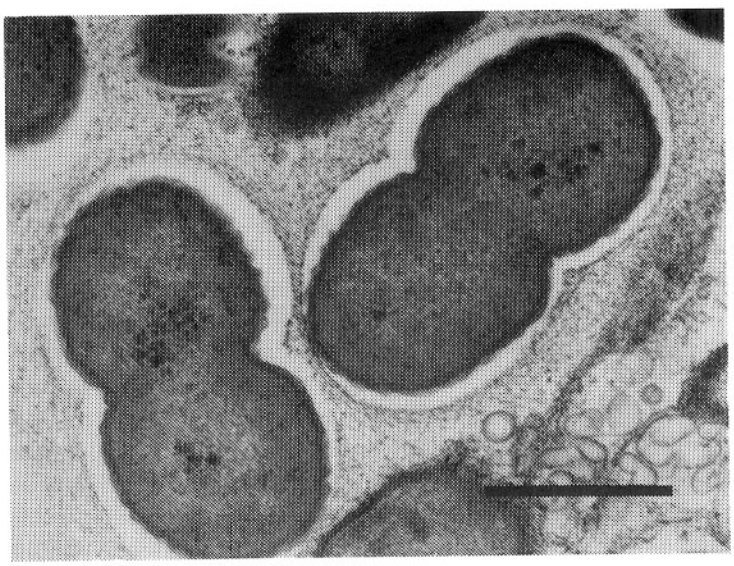

Figure 4. Bacteria Resembling Benzoate-Degrading *Syntrophus buswellii* (bar = 0.65μm).

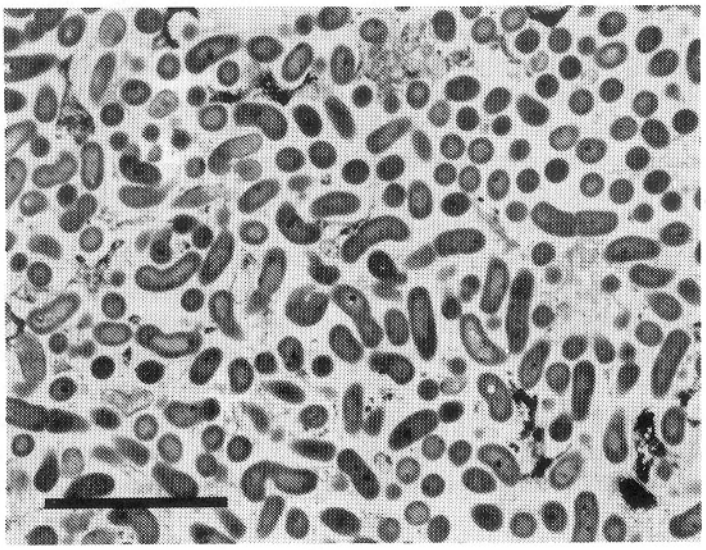

Figure 5. Bacteria Resembling Hydrogenotrophic *Methanobrevibacter* (bar = 2.5μm).

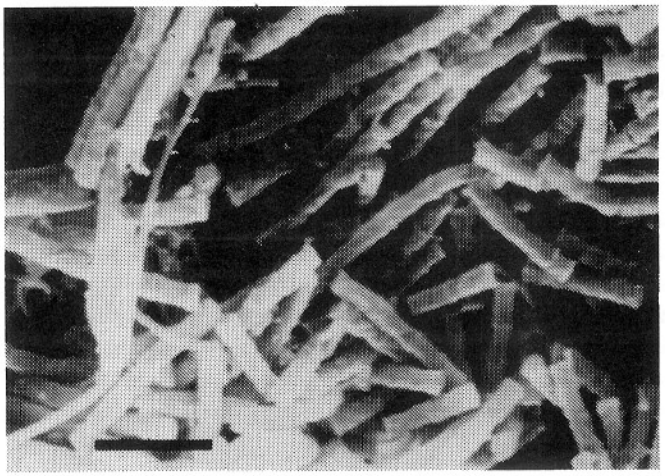

Figure 6. Bacteria Resembling Acetotrophic *Methanothrix* (bar = 3.5μm).

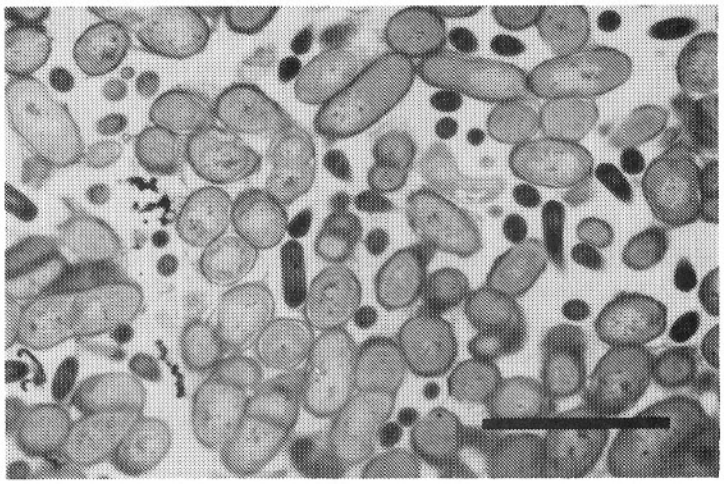

Figure 7. Juxtapositioned Syntrophic Association Between Bacteria Resembling *Syntrophus buswellii* and *Methanobrevibacter* (bar = 2.5μm).

In general, heavy metals with the exception of nickel were less toxic to the two UASB biogranules than to the flocculent digester sludge. Between the two UASB biogranules, the benzoate-degrading biogranules were more sensitive to cadmium and chromium than the starch-degrading ones, while the toxic effects of copper, nickel and zinc were similar.

Micrographs of SEM and TEM illustrate that the biogranules were densely populated with bacteria resembling benzoate-degrading *Syntrophus buswellii* (Figure 4), syntrophically associated with those resembling hydrogen-consuming *Methanobrevibacter* (Figure 5) and those resembling acetotrophic *Methanothrix* (Figure 6) [23-25].

Benzoate degradation is a multi-step process. A recent study suggested that benzoate is first converted to acetate inside the bacterial cell of *Syntrophus buswellii*, as expressed in the following equation [4]:

$$\text{benzoate}^- + 7H_2O \rightarrow 3Ac^- + 3H_2 + 3H^+ + HCO_3^-$$
$$G^{o'} = +89.4 \text{ kJ}$$

where $G^{o'}$ represents the change of standard Gibbs free energy at pH 7. Both acetate and hydrogen are then transferred to the outside of the cell for further conversion to methane. Acetate is converted by *Methanothrix* and hydrogen by *Methanobrevibacter*. However, thermodynamically the above acetogenic equation is unfavorable, because of positive $G^{o'}$ (+ 89.4 kJ). Based on chemical thermodynamics, the reaction is feasible only if the change of Gibbs free energy is negative. In order to lower the change of Gibbs free energy, the product hydrogen has to be kept at a very low concentration. This can be accomplished when the produced hydrogen is readily converted to methane by *Methanobrevibacter*, as expressed by the following equation:

$$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O \qquad G^{o'} = -135.6 \text{ kJ}$$

The overall reaction can thus be expressed as:

$$4 \text{ benzoate}^- + 19H_2O \rightarrow 12Ac^- + 3CH_4 + HCO_3^- + 9H^+$$
$$\Delta G^{o'} = -49.2 \text{ kJ}$$

Thus, in the biogranules, bacteria resembling benzoate-degrading *Syntrophus buswellii* are in juxtaposition with the bacteria resembling hydrogen-consuming *Methanobrevibacter* so that hydrogen can be readily transferred between these two species of bacteria. Figure 7 illustrates the microscopic evidences of such a syntrophic relationship between these two groups of bacteria.

The resistance to toxicity exhibited by the benzoate-degrading biogranules in this study and the starch-degrading biogranules in a previous study can be attributed to the physical nature of the biogranules [15]. Up to millions of bacteria are densely packed forming a biogranule. Only a small number of bacteria are exposed to the toxic metals in the bulk solution, and thus considerably limits the overall metal toxicity to the microorganisms. Such a finding implies that granulation of biomass in a wastewater treatment system increases the overall resistance towards toxicants in the wastewater.

CONCLUSION

Heavy metals in electroplating effluent inhibited the methanogenic activity of benzoate-degrading biogranules in the following order: nickel (most toxic) > zinc > cadmium > copper > chromium (least toxic). At 37 °C, the bioactivity of granules was reduced by 50% when the wastewater contained 100 $mg \cdot l^{-1}$ of nickel, 110 $mg \cdot l^{-1}$ zinc, 150 $mg \cdot l^{-1}$ cadmium, 175 $mg \cdot l^{-1}$ copper, or 210 $mg \cdot l^{-1}$ chromium, individually. The biogranules exhibited high resistance to metal toxicity because of bacteria were densely packed and thus their exposure to the toxic metals in the bulk solution was limited. This implies that granulation of biomass increases their overall resistance towards toxicants in the wastewater.

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