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Development of Supporting Materials for Microbial Immobilization and Iron Oxidation

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Abstract

We developed the microbial immobilization particle with curdlan and activated carbon, which has great adsorption capacity. The characteristics of porosity and mechanical strength of these supporting particles are dependent on manufacturing method. The supporting particle showed the best performance when the ratio of curdlan and activated carbon was 30 to 6 g/L. Brumauer-Emmett-Teller (specific surface area) and swelling capacity of the carrier were 52.63 m^2/g and 17 (w/w), respectively. The immobilization characteristics of iron-oxidizing bacteria on supporting particles were observed using a scanning electron microscope. The concentration of microorganism on the surface of supporting particle was increased with reaction time. As the number of iron oxidation batch cycles increased, the iron oxidation rate increased.

Index Entries: Curdlan; supporting particle; microbial immobilization; H_2S removal.

Introduction

Many researchers developed bioreactors for biologic treatment of wastewater and offensive odor using microorganisms *(1)*. Although these processes offer the benefits of achieving these goals, many problems arise during this process because the microorganisms are sensitive to environmental conditions such as temperature, pH, and impulse loading, owing to problems related to small amounts of microorganisms and proliferation, and to loss of microorganisms within bioreactors *(2,3)*. To overcome these problems, the concentration of microorganisms should be kept high in

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reactors. To maintain a high cell concentration, the cell immobilization technique using supporting materials is needed, and this method is being studied by many researchers. The biologic treatment method of supporting materials by immobilizing microorganisms increases treatment efficiency because high concentrations of microorganisms are maintained within reactors. Therefore, this method could accommodate the current trend of minimization and high efficiency since the same treatment effect could be achieved by reducing the size of existing reactors. Moreover, microorganisms are immobilized by supporting materials, so they are not released outside the reactor, and microorganisms could be recycled since the microorganism could be separated from the supporting material *(4–6)*.

Because of these benefits, various supporting materials are developed and produced in different products and are used effectively in the bioengineering industry and biologic treatment processes using immobilized cells for the purposes of improving the treatment efficiency of pollutants by immobilizing microorganisms to supporting materials *(7)*. The method of adsorbing microorganisms to supporting materials offers many benefits compared with other methods, such as low cost of immobilization, survival of the microorganism attached to the supporting materials and maintenance of high activity, high rate of recycling, and low resistance of expansion according to the transmission of substrate and byproducts *(8)*. For the supporting materials to be commercialized, they need to satisfy the following characteristics: First, they need to have large specific surface area and porosity for microorganisms to attach sufficiently and allow growth. Second, they need to have little resistance to fluid flow; be able to safely undergo chemical, biologic, and physical changes; and have sufficient mechanical resistance and durability against distortion, destruction, and erosion. Finally, they need to have sufficient volume to entrap suspended microorganisms and be low in cost, stably supplied, and easy to prepare.

There is no supporting material that satisfies all of these requirements. Nonetheless, it is important for supporting materials to have appropriate porosity and sufficient contact surface by increasing the specific surface area of the supporting materials so they will satisfy the conditions for microorganisms to be attached easily and be durable *(5,9)*. Supporting materials applied in recent years for immobilization of microorganisms include ceramics, fibers, synthetic, and plastics *(3,4,10,11)*. Among these supporting materials, ceramic-supporting materials have excellent chemical resistivity, strength, durability, and properties suitable for preparing porous particles having large specific surface area, making these materials preferable in industrial application. The preparation methods of ceramic-supporting materials include incineration, extrusion, sol-gel process, and polymer sponge process, but the most preferred methods are incineration and foam processing because of the ease in manipulating the shape, composition, and density of the supporting materials *(12)*. However, more than two types of additives should be used, and operation should be done at

Table 1
Composition of Supporting Particles for Microbial Immobilization

Method	Curdlan (g/L)	Activated carbon (g/L)
Water bath, 80–100°C, 30 min	30	6
	40	6
	50	6
Autoclave, 1 atm, 121°C, 15 min	30	6
	40	6
	50	6

high temperature, which greatly increases the operating costs in the process of molding and sintering to obtain these high-tech supporting materials.

The microbial polymer curdlan forms gel at high temperature and maintains the gel form at low temperature. When the gel type of curdlan is freeze dried, the volume will be the same but the surface area per unit weight increases, with many pores formed inside owing to moisture evaporation. Curdlan was developed as a food material so that an edible supporting material could also be possible *(13,14)*. Furthermore, curdlan is a biodegradable polymer, making it useful in resolving secondary environmental pollution caused by supporting materials. On the other hand, the functional group of carbon atoms present on the surface of activated carbon exerts magnetism on surrounding liquid or air to adsorb molecules in the surrounding area. Thus, activated carbon is used in overall areas of environmental (air, water) and food industries in filters, decolorant, deodorant, solvent recycling, and gas removal.

To develop a supporting material with a large specific surface area, excellent adsorbing ability, and cost feasibility, we mixed the biopolymer curdlan and activated carbon to increase porosity and adsorption surface and to control the surface area and strength of adsorbent. Using the preparation and immobilization methods for supporting material, we investigated the characteristics of the supporting material and examined the possibility of this material being applied in the oxidation of iron.

Materials and Methods

Preparation of Supporting Material

To prepare a supporting material, the biopolymer curdlan (Takeda Chemical, Tokyo, Japan) and activated carbon (American Norit, Pryor, OK) were used. The supporting material was prepared by varying the compositions of curdlan and activated carbon (Table 1). After 25 mL of 2 *N* NaOH was added to the mixture of 500 mL of distilled water with varying amounts of curdlan, 6 g/L of activated carbon was added. This mixture was neutralized with 25 mL of 2 *N* HCl. The sample was heated in a constant-temperature chamber (100°C, 30 min; LABTECH, Andover, MA) and an

autoclave (121°C, 1 atm, 15 min; DASOL DS-60A, Seoul, Korea). After cooling the gel-type mixture, it was made into 5-mm size and quickly cooled in an ultra-low-temperature freezer (–70°C; Samwon SW-VF-200P, Seoul, Korea). This curdlan and activated carbon supporting material was freeze dried in a freeze dryer (–78.5°C; Samwon SFDSM06, Seoul, Korea) and kept at room temperature.

Immobilized Strain

The strain used for microbial immobilization was the iron oxidation microorganism *Thiobacillus ferrooxidans* (ATCC 19859). This strain, which obtains energy for cell growth by oxidizing iron ions and inorganic compounds, was cultured in Silverman *(15,16)* 9K medium with the initial pH adjusted to 1.8. After 10% (v/v) of cell culture was placed in a 300-mL Erlenmeyer flask containing 150 mL of medium, it was incubated for 3 d at 30°C by stirring at a rate of 200 rpm. The culture medium was then filtered twice using a Whatman No. 2 filter. The filtrate was centrifuged (40,000 *g*, 20 min) to isolate the bacteria, which was suspended in mineral salt solution. This suspension was centrifuged again and used as the inoculums while suspended in 9K mineral salt solution (pH 2.0) containing no $FeSO_4 \cdot 7H_2O$ at 4°C. Because of the problem with jarosite occurring in Silverman 9K medium, we used M16 medium, developed by Kim *(16)*, with the initial pH adjusted to 1.8 for microbial immobilization.

Microbial Immobilization

The use of a fluidized-bed reactor is expected to increase mass transfer rate because this type of reactor has both mixed flow and plug flow. We used the air-lift type of reactor, which is easy to install, can reduce shear stress, and can increase the mixing effect with little power. The methods of immobilizing microorganisms include the fluidized reactor type, in which microorganisms are immobilized while the supporting material is fluidized. The fluidized reactor type used in the present study was the same as that used in a packed-bed type of reaction. The reactor volume was 2.5 L for the air-lift type (a cylinder-type reactor with 10 cm id, 11 cm od, and 40 cm height). To immobilize the cells, a packed-bed type of cylindrical glass tube (250 mL) filled with supporting material was used. The temperature was kept constant at 30°C in the fluidized-bed reactor and immobilized cell packed-bed reactor. The reactants were circulated in the fluidized-bed reactor and packed-bed reactor using a peristaltic pump (Watson Marlow 101U/R; speed range: 0–32 rpm). The amount of air input was controlled using a mass flow controller (GFC-1109). After adding 200 mL (10% [v/v]) of inoculums diluted with 9K mineral salt solution (pH 1.8) including immobilized cells and supporting materials into the fluidized-bed reactor, the total volume was set to 2 L with the addition of M16 medium *(16)*. The microbial immobilization experiment was done with aeration after placing 100 mL of supporting material in the fluidized-bed reactor. Cell culture was performed for 60 h with the medium and microorganism

circulated with a pump in order to pass through the supporting material in the packed-bed reactor. To increase microbial immobilization with time, repeated-batch operation was applied.

Analytical Methods

A rheometer (COMPAC-100, Tokyo, Japan) was used to measure the compression strength (g/cm^2) with 1 kg of load cell at a table speed of 60 mm/min. To determine the surface characteristics of the supporting material, it was electroplated and observed with a scanning electron microscope (Jeol JSM-840A, Tokyo, Japan). The swelling capacity of the supporting material was measured in dry weight and wet weight. The supporting material was immersed in distilled water for more than 3 h, and the surface moisture was well dried. The material was then weighed.

The amount of immobilized cell was measured with protein assay and scanning electron microscopy. It also can be deduced with the iron oxidation rate. Fe(II) concentration was measured with *O*-phenanthroline solution. The supernatant after centrifugation was diluted with 10-fold distilled water, and 0.4 mL of *O*-phenanthroline was added/0.1 mL of supernatant. Then, 2.5 mL of distilled water was added to make the total volume 3 mL, and color was developed at room temperature for 10 min. Absorbance was measured with a UV/VIS spectrophotometer (Shimazu UV-1201, Columbia, MD) at 560 nm. The total amount of iron was measured by quantifying the amount of Fe(III) reduced into Fe(II) using the strong reductant 10% aqueous hydroxylamine hydrochloride solution. Fe(III) was calculated as the difference between the concentration of total iron and the concentration of Fe(II).

Results and Discussion

Development of Supporting Particles

When the supporting material was prepared with curdlan and activated carbon, the mixture was heated to a high temperature (higher than 80°C) to have elasticity and durability. Once the gel was made, it was maintained as a gel formation even at room temperature. The gelatinized supporting material could easily be processed into desired shapes and sizes. When it was freeze-dried, an excellent supporting material was formed having the same volume as before freeze-drying, many pores, and increased surface area per unit weight owing to moisture evaporation.

Effect of Temperature

When the supporting material was prepared in a constant-temperature chamber (80–100°C, 30 min) or an autoclave (1 atm, 121°C, 5 and 15 min), the results showed that the strength of the supporting material increased but roughness and porosity decreased as the amount of curdlan increased regardless of temperature. The supporting material was heated in a constant-temperature chamber at 80 and 100°C and tested. The supporting material heated at 80°C showed decreased elasticity. The supporting

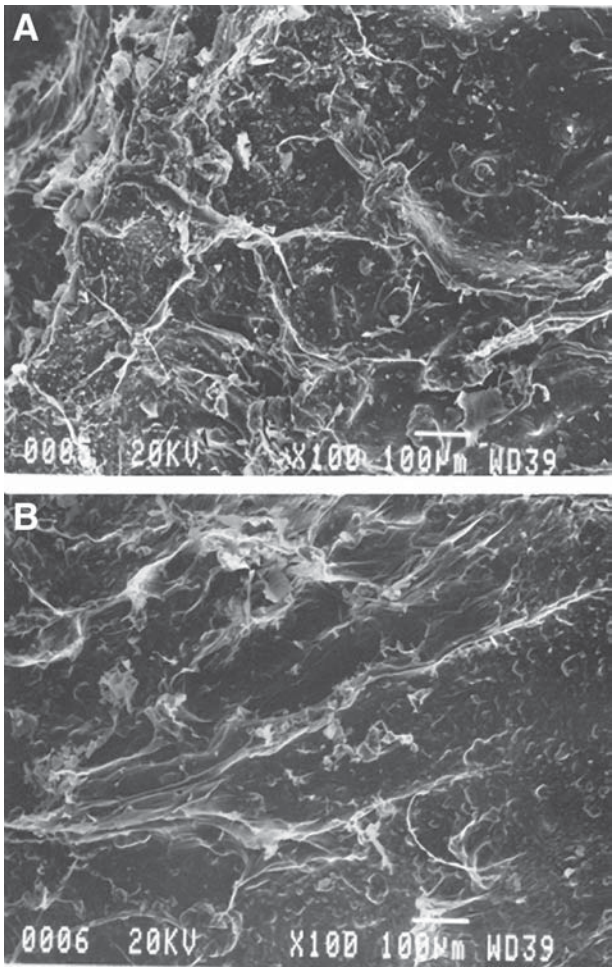

Fig. 1. Surface of particles with different ratios of curdlan to activated carbon manufactured in boiled water (100°C) for 30 min: **(A)** 30 g/L of curdlan and 6 g/L of activated carbon; **(B)** 50 g/L of curdlan and 6 g/L of activated carbon.

material heated at 100°C showed excellent elasticity and solidification capacity, but not many pores were present when the heating time was long. The supporting material prepared in an autoclave changed its characteristics according to the period of heating. Thus, we compared the heating periods of 5 and 15 min. The supporting material heated for 15 min was excellent but was crackling compared with that heated for 5 min. Overall, the supporting material heated for 15 min showed better elasticity and strength compared with that heated for 5 min. Regarding pore characteristics, the supporting material heated in an autoclave showed more pores compared with that dried in the constant-temperature chamber. The size of the pores was compared in the supporting material dried in a constant-temperature chamber at 100°C and that in an autoclave at 121°C for 15 min through scanning electron microscope images (*see* Figs. 1 and 2).

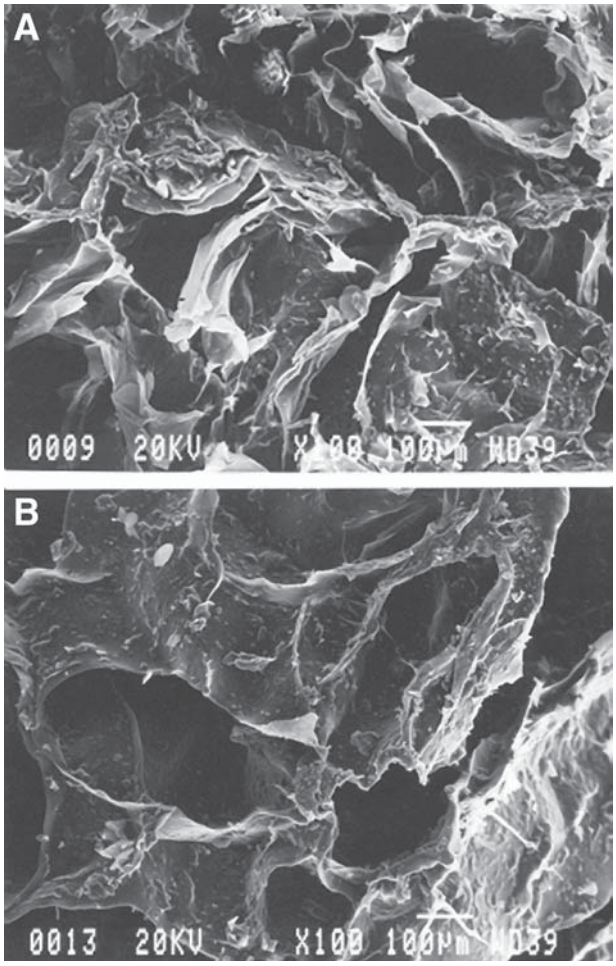

Fig. 2. Surface of particles with different ratios of curdlan to activated carbon manufactured in autoclave (1 atm, 121°C) for 15 min: **(A)** 30 g/L of curdlan and 6 g/L of activated carbon; **(B)** 50 g/L of curdlan and 6 g/L of activated carbon.

Effect of Particle Composition

The effect of the ratio of curdlan to activated carbon on particle character was studied. Curdlan is a biopolymer and harmless to humans and becomes a strong gel at high temperatures. Activated carbon has excellent adsorption ability. Among several types of supporting materials prepared, we measured malleability, dried strength, and porosity to select excellent supporting materials and tested the microbial immobilization ability. Although solidification became easier with more curdlan, the adsorption capacity decreased slightly. On the other hand, when the amount of activated carbon was increased, the adsorption capacity became excellent but the supporting material crumbled within and outside the reactor. Therefore, when the ratio of curdlan to activated carbon was adjusted at 30 g/L to 6 g/L, the resulting supporting material showed the best properties,

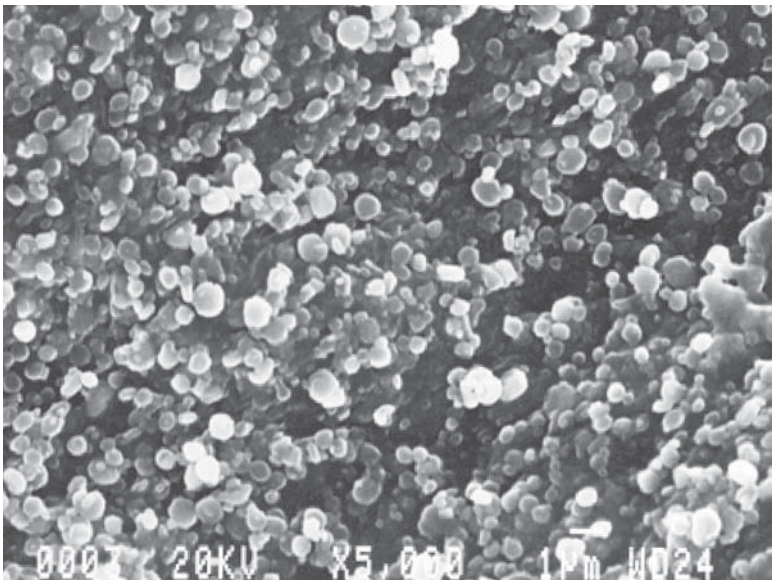

Fig. 3. Surface of supporting particles in bubble column reactor.

being cost-effective and having excellent adsorption capacity (Figs. 1 and 2). The specific surface area (Brumauer-Emmett-Teller) of the supporting material was 52.63 m^2/g, and the swelling capacity increased with time when it was immersed in distilled water. The swelling capacity measured after the supporting material completely adsorbed water showed an average of 17 (w/w) (the average was obtained after measuring the swelling capacity of several supporting materials). As shown in Fig. 2A, pore sizes of 5–200 μm were observed at low magnification. This supporting material showed wider specific surface area adsorbing more microorganisms and had excellent adsorption capacity owing to activated carbon.

Cell Immobilization

Fluidized-Bed Reactor

After placing 250 mL of cells into a 2.5-L fluidized-bed reactor, the total volume was brought up to 2 L, and 100 mL of supporting material was placed. While flowing with bubbles, microorganisms were immobilized. Since the specific gravity was smaller than those of regular supporting materials, the flow state of the supporting material was relatively stable but broke off with time. Furthermore, Fe(II) ions were not oxidized, and the medium also turned slightly light green with minute increases in pH. These changes made the medium inhabitable for microbial growth, and thus the microorganism did not grow within the fluidized-bed reactor. Although no activity of microorganisms was seen, salts were adsorbed on the surface of the supporting material owing to the strong adsorption capacity of the supporting material (Fig. 3), showing the possibility of immobilizing other microorganisms.

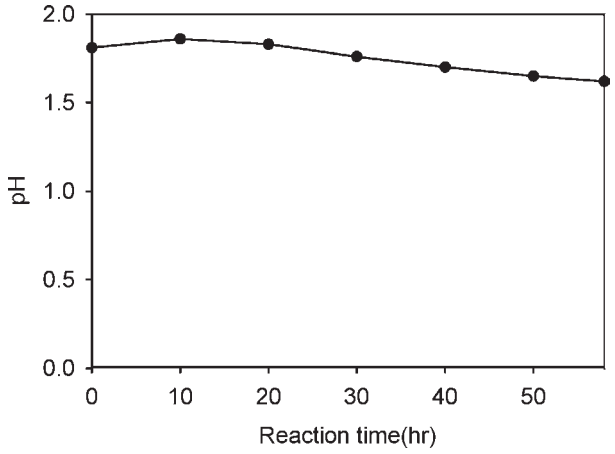

Fig. 4. pH profiles during iron oxidation reaction with *T. ferrooxidans*.

Packed-Bed Reactor

Because of the difficulty in microbial immobilization with a fluidized-bed reactor, we used a packed-bed reactor by immobilizing while the supporting material was filled into the column and the medium containing microorganism was circulated through the reactor. As a result, microbial immobilization was well achieved although the response was somewhat slow, and specific surface area was wide so a repeat test was done, confirming the immobilization of high concentrations of microorganism to supporting material. During the growth stage of the microorganism, pH decreased while Fe(II) was oxidized to Fe(III). The initial pH of 1.8 was increased slightly to 1.87, but decreased to 1.6 with the maximum growth of the strain (with minimum Fe[II]) (Fig. 4). Although the culture pH of the microorganism decreased slightly under pH 1.5, the iron oxidation rate and cell growth rate were slightly changed with culture pH change.

Iron Oxidation With Repeated-Batch Culture

To examine the adsorption capacity of supporting material, the supporting material was passed through a bubble column reactor initially to test microbial immobilization. In the flowing state, no change in adsorption was present, but the collapse effect was seen with time as the activated carbon and curdlan broke off. Thus, we used a packed-bed reactor for microbial immobilization employing the repeated-batch method. Microorganisms attaching to the surface of the supporting material during repeated-batch immobilization were observed using a scanning electron microscope. Figure 5 shows the surface of the particles before and after immobilization of the microorganism. The surface before attachment only showed some salt, but the microorganism attached was increased with operation time.

About 9 g/L of Fe(II) was completely oxidized in the first batch within 60 h, and the time required to oxidize Fe(II) decreased significantly as the

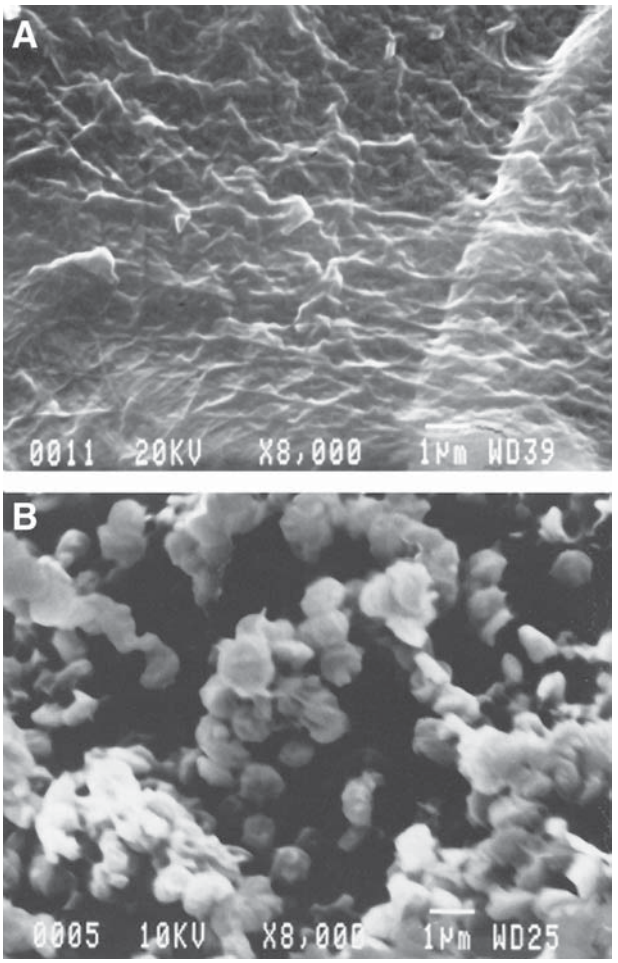

Fig. 5. Surface of supporting particles **(A)** before and **(B)** after cell immobilization.

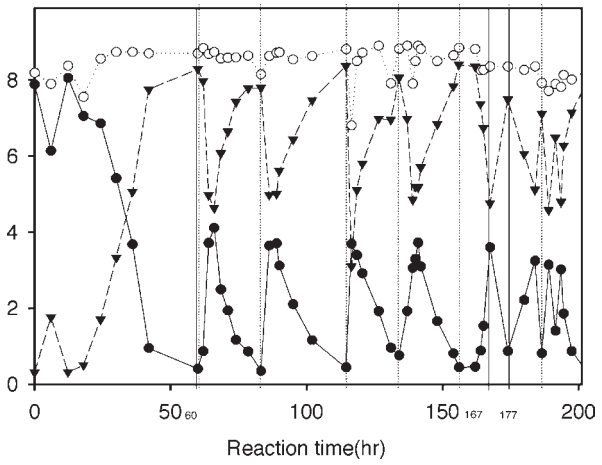

Fig. 6. F(II) oxidation by immobilized *T. ferrooxidans* in iron oxidation reactor with repeated fed-batch operation (packed-bed type of immobilized cell reactor was used). ●, Fe(II); ▼, Fe(III); ○, Fe(total).

number of batches increased. This amount of Fe(II) was completely oxidized within 10 h at the sixth batch cycle (Fig. 6). When expressed in the average oxidation rate, the oxidation rate in the first batch based on the amount of reactant was 0.15 g/(L·h), and the oxidation rate was maintained constantly from the sixth batch, showing a sixfold increase in the sixth batch compared with the first batch. Therefore, the increase in iron oxidation rate in the repeated batch was the result of increased concentration of immobilized cells.

Conclusion

Among the particles of curdlan and activated carbon, by varying the composition of each component, the supporting material prepared by 30 g/L of curdlan and 6 g/L of activated carbon in an autoclave (121°C, 1 atm, 15 min) showed excellent properties for immobilizing cells. However, the fluid layer of this supporting material showed a tendency to be destroyed and changed the microbial medium, but it showed an excellent adsorption rate. When the microbial adsorption property was tested after immobilizing the supporting material in a packed-bed reactor, the results showed that the iron oxidation rate increased with the increased immobilized bacteria with time. When the supporting particles were observed under a scanning electron microscope, we confirmed that the concentration of bacteria attached to the supporting material increased with time. The supporting material used was mainly composed of biodegradable polymer so that secondary environmental pollution could be resolved and the cost of maintenance, management, and exchange could be minimized. Compared with existing supporting materials, the supporting material studied had a relatively large specific surface area, and because biopolymers are not harmful to humans, it could be applied in food products. However, compared with other supporting materials, the tested supporting material lags in durability, and therefore, efforts need to focus on its improvement.

Acknowledgment

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