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Wastewater Treatment with Bacteria Immobilized onto a Ceramic Carrier in an Aerated System

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Biological treatment of the wastewater discharged from a food processing factory was continuously carried out in a packed bed bioreactor under aerobic conditions. The bacterium isolated from the wastewater was immobilized onto a new type of ceramic carrier by a vacuum method and high numbers of bacteria were colonized onto the carrier (2.9×10^9 cfu/g of dry ceramic carrier). The effect of the hydraulic retention time (HRT) and aeration rate on the removal of the chemical oxygen demand (COD) was investigated. The system was able on average to remove more than 82% of the influent COD during 160 d of operation and more than 87% of the influent COD on average was removed when the HRT was 30.17 h and the aeration rate was 2.0 vvm. Aeration rates in the range of 0.4 to 2.0 vvm do not affect the COD removal efficiency.

[**Key words:** biological COD removal, cell immobilization, ceramic carrier, packed bed bioreactor, wastewater treatment]

The treatment of wastewater in packed bed bioreactors using immobilized cells is attracting increasing interest with the application of different immobilization methods and a variety of carriers (1–4). Immobilized systems can provide massive populations of bacteria inside bioreactors and can handle high flow rates. On the other hand, for certain wastewater treatment specifications, smaller bioreactors are required. The retention time in activated sludge processes can be as long as 2 d which demands huge unit sizes for wastewater treatment and there are large quantities of sludge produced for disposal. Because of their characteristics of porosity and reusability, ceramics should be considered as appropriate supports for adsorbed immobilized cells. The simplicity of immobilization and long-term operation capability are the main advantages of such systems (5). Employing ceramics as supports for cell immobilization has been accomplished for the immobilization of yeasts to produce alcohol (6–8) and soy sauce (9). Most of the research in the field of wastewater treatment with immobilized systems using porous carriers is related to anaerobic processes (1, 10, 11). Treatment of synthetic wastewater has been carried out using aerobic processes with microorganisms immobilized on ceramics (12–13). To date, the immobilization of cells onto porous ceramics has been carried out either by the circulation of a cell suspension through a ceramic bed (14, 15) or shaking the cell suspension with the ceramic carrier (16). There is only one report on the application of a vacuum to cell immobilization onto ceramics for soy sauce production (17).

In the present study, a bacterium isolated from the wastewater to be treated was immobilized onto ceramics by a vacuum method and the feasibility of using the actual wastewater from food industries in the packed ceramic bed in an aerated system was investigated.

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MATERIALS AND METHODS

Wastewater Wastewater samples were taken from the Ajinomoto factory located in Morodomi town in Saga prefecture, Japan. The wastewater was stored at 4°C in 20-*l* PVC containers. No processes were applied prior to biological treatment. A typical composition of the wastewater is as follows: 761 mg/*l* total organic carbon (TOC), 1250 mg/*l* BOD, 1100 mg/*l* COD, 270 mg/*l* total nitrogen (TN), 0.01 mg/*l* phosphate, 3050 mg/*l* magnesium, 28 mg/*l* potassium and 350 mg/*l* suspended solids. The pH of the untreated wastewater was 8.18. The effect of the phosphate concentration on COD removal was studied by the addition to test tubes of monobasic sodium phosphate at different concentrations.

Ceramic carrier A cylindrical ceramic support was used. The length, external and internal diameters were 6.2, 4.3 and 1.1 mm, respectively. The ceramic carriers were manufactured using α-quartz which can be found in the northwestern area of Kyushu island, Japan, and is a cheap material. Organic binder (8 wt%) and bentonite powder (3 wt%) were added to the α-quartz and the mixture was fully mixed, molded and fired at 1300°C to obtain the porous ceramics. The synthesized ceramics possessed the following characteristics: 45.7% porosity, 20.0-μm pore size, 0.3676 m^2/g surface area, 0.3264 cm^3/g pore volume and 2.5832 g/cm^3 apparent density. Figure 1A shows the surface of the ceramic carriers.

Microorganism A sample taken from the aeration tank of the conventional activated sludge wastewater treatment process of the factory was diluted and spread over a solidified medium prepared by the addition of agar (1.5%) into the wastewater and incubated at 30°C. Eight different types of bacteria were isolated from the plate and cultured in the wastewater at different temperatures and pHs. The bacterium with a very high COD removal efficiency (80%) was used for immobilization. It was observed that this bacterium had the highest COD removal efficiency at 37°C with no

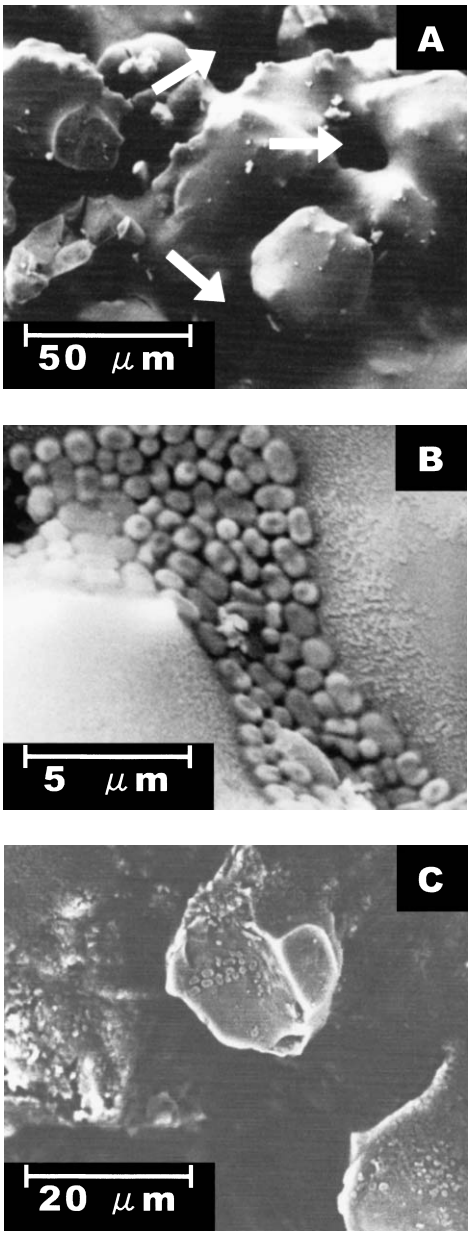

FIG. 1. Scanning electron micrographs taken of ceramics used for the treatment of wastewater in a packed bed bioreactor; (A) before immobilization (arrows indicate the position of pores), (B) after immobilization, (C) after use in the wastewater treatment process for 160 d.

initial pH adjustment. Phylogenic identification of the bacterium was carried out based on 16S rDNA sequence analysis (using the GenomeNet data base) and with more than 98% compatibility the genus of the bacterium was determined as *Acinetobacter*.

Immobilization method Inside a beaker-shaped vessel, 380 g of ceramic carrier was placed over a net so that a free space was available for a magnet stirrer in the bottom section. Then, 1000 ml of a cell culture suspension (cultivated in 5 g/*l* meat extract, 5 g/*l* Polypepton, 1.5 g/*l* yeast extract and 2.5 g/*l* NaCl, pH adjusted to 7, at 30°C, 200 rpm for 12 h) were added to the vessel. The entire system was placed inside a vacuum chamber for 30 min while the cell suspension was stirred after which the ceramics were separated and transferred into the bioreactor. Cell loading of the ceramics was calculated by measuring the cell concentration in the cell suspension medium before and after immobilization. It was observed that the cell loading rate was 2.9×10^9 cfu/g of dry ceramics, which is superior to that, 9×10^8 cfu/g of carrier, for type Z biocarriers consisting of silica, alumina and zeolite molecular sieves for *Pseudomonas* cells (18). Figure 1B shows the surface of the carrier just after immobilization of bacteria.

Bioreactor A 500-ml bioreactor (MBR-053 F; EYELA, Tokyo) which was jacketed and equipped with an aeration pump was used for the experiments. The bioreactor was randomly packed with 380 g of ceramics to which bacteria had been immobilized. The ceramics, including the total internal pore volume, occupied 147 cm^3 of the bioreactor. Thus, 353 cm^3 of the bioreactor volume was available for the liquid phase. HRT calculation was conducted based on this volume for the liquid phase. The bioreactor was operated as an aerated up-flow packed bed bioreactor during the experiments.

Wastewater treatment process Wastewater was introduced into the bottom of the bioreactor by a peristaltic pump. The aeration rate was kept at less than 0.4 vvm at the beginning, while the influent flow rate was 67.1 cm^3/h. The temperature was adjusted to 37°C and the pH was not controlled. The influent flow rate to the bioreactor was 67.1 cm^3/h up to the 52nd day, 190 cm^3/h up to the 66th day, 90 cm^3/h up to the 79th day, 11.7 cm^3/h up to the 123rd day and 175.8 cm^3/h up to the 160th day, which corresponded to HRT values of 5.26, 1.86, 3.92, 30.17 and 2.01 h, respectively. The aeration rate was less than 0.4 vvm up to the 14th day, 0.4 vvm up to the 20th day, 2.0 vvm up to the 35th day, 1.0 vvm up to the 58th day and 2.0 vvm up to the 160th day. A schematic diagram of the wastewater treatment system is shown in the Fig. 2. The system was operated continuously for 160 d.

Analytical methods Samples were taken from the influent wastewater and the output of the bioreactor every day and the COD, viable cells and turbidity were measured. Each sample was centrifuged for 20 min at 3000 rpm before measuring the COD concentration. The COD was measured using the closed reflux colorimetric method according to 5220 D of the standard methods (19). The viable cell count was determined by dilution and spreading of the samples on solidified medium (described in the Immobilization method), culturing over night at 30°C and counting the number of colonies. The optical density was directly measured at 660 nm using a spectrophotometer (Spectronic 20^+; Spectronic Instruments, Rochester, NY, USA). Scanning electron micrographs were taken using a Hitachi S-4500. The ceramics used in the bioreactor were freeze-dried for 12 h before scanning electron microscopy (SEM) was carried out.

RESULTS AND DISCUSSION

The performance of the bioreactor was investigated under different values of HRT and aeration rates (Fig. 3D). Since the samples were taken from the factory discharge, there were fluctuations in the influent COD concentration. Continuous operation of the bioreactor was carried out for 160 d. Figure 3 shows the results of this experiment.

Effect of aeration rate on COD removal To better understand the effect of aeration rate on the COD removal efficiency, a similar system was operated under anaerobic conditions for 35 d. The influent flow rate was 67.1 cm^3/h (HRT of 5.26). As indicated in Fig. 4, only 42.6% of the COD could be removed on average. Although the aeration rate was less than 0.4 vvm in the aerated system, more than 86% COD was removed between days 10 to 14, following transient behavior of the bioreactor in the start-up period

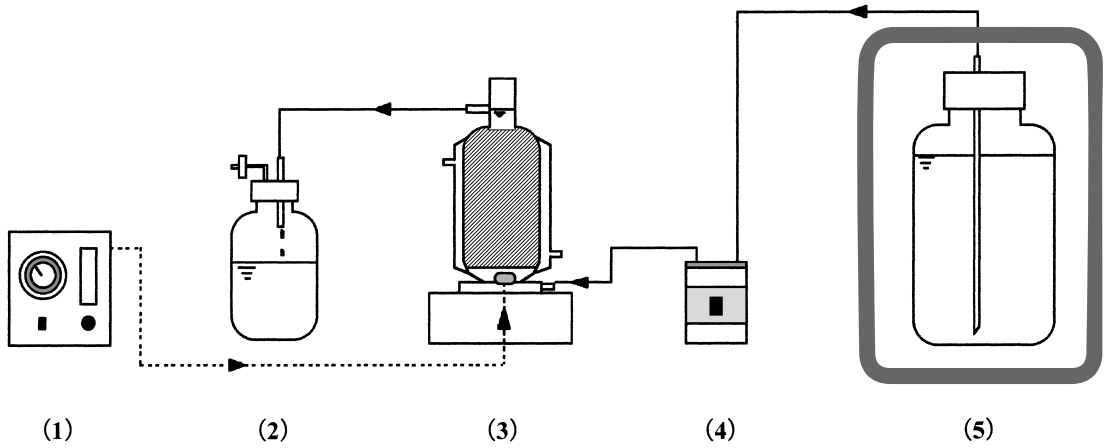

FIG. 2. Schematic diagram of the set up used for wastewater treatment. 1, Aeration pump; 2, storage tank; 3, packed bed bioreactor; 4, peristaltic pump; 5, wastewater tank placed in a cold box.

which lasted for 9 d. Higher aeration rates did not markedly influence COD removal. However, the aeration rate was maintained at the high values of 1.0 or 2.0 vvm.

Effect of HRT on COD removal When the HRT was decreased from 5.26 to 1.86 h on the 52nd day, the COD removal decreased to 53.7% (Fig. 3A). However, the system was only operated at the lower HRT for 12 d although not more than 76.5% COD removal could be achieved. Interestingly, when the HRT was decreased again from 30.17 to 2.01 h on the 123rd day, no sharp decrease in COD removal was observed but the COD removal efficiency began to decrease. This could be due to the washout of bacteria from the bioreactor (Fig. 3C). It is also consistent with the suggestion that a real approach to wastewater treatment systems employing adsorbed cells must be based on the amount of cells inside the reactor (20). At an HRT of 30.17 h which started from the 79th day, the COD removal efficiency had increased up to 90% or higher, 32 d later. The highest COD removal efficiency (92.8%) was achieved on day 130. The COD loading rate decreased and COD removal efficiency increased by increasing the HRT.

Effect of viable cell count and turbidity on COD removal Both the viable cell count and turbidity were influenced by the influent COD concentration and flow rate. A trend was observed whereby the higher the influent COD concentration and the shorter the HRT, the higher the viable cell count and turbidity. The viable cell count was maximum at an HRT of 5.26 h when the influent COD concentration was around 1800 mg/*l*, but decreased one order of magnitude when the HRT was reduced to 1.86 h. COD removal is partially related to the conversion of influent COD to bacterial cells. At the HRT value of 30.17 h, the average OD value was 0.13 which is equivalent to a COD of 78.2 mg/*l*. At this HRT, the system could effectively operate when the influent COD concentration was as high as 1800 mg/*l*. At a shorter HRT of 2.01 h, the influent COD concentration was also relatively high, and therefore the effluent turbidity was 0.82 on average which is equivalent to a COD of 632.8 mg/*l*. According to this observation, considering the bacteria inside the output stream of the bioreactor, on average 47.3% and 81.8% of the influent COD was removed inside the bioreactor at HRTs of 2.01 and 30.17 h, respectively. Based on the morphological shape of bacterial colonies, *Acinetobacter* appeared to be the dominant bacterium.

Scanning electron micrograph of the carrier surface A scanning electron micrograph was taken of the surface of the ceramic support on the 160th day of the process. As shown in the micrograph, the ceramic surface was covered by the adsorbed bacteria (Fig. 1C).

Effect of phosphate concentration It was observed in batch experiments that the bacterium could grow in the wastewater and remove the same amount of COD (80%) at higher phosphate concentrations up to 50 mg/*l*. Therefore, it could be guaranteed that the phosphate concentration would not affect the bioreactor operation in this range.

Conclusion The results obtained for the cells immobilized onto the ceramic carrier in the packed bed bioreactor show that the system can effectively remove COD under certain conditions. Over the operation period, the system not only retained its performance level but also became more tolerant to external shocks. This might be due to the fact that cell immobilization occurs gradually on the carriers and the total cell loading inside the reactor increases with time. Considering the long-term usability of ceramics and the use of untreated wastewater with a high COD concentration in this study, the major advantage of the process proposed here is evident. Among the HRT values used, the bioreactor showed the best performance at 30.17 h. However, there might be an optimum HRT between the values of 5.26 and 30.17 h for practical applications. The bioreactor would exhibit good performance at shorter HRTs if the influent COD concentration is below 1000 mg/*l*. The COD removal was also high at HRT of 5.26 h but due to the rather high turbidity of 0.56, a bacterial separation system such as filtration would be required after the bioreactor operation. To achieve the ideal performance of the bioreactor, the operation should be started with longer HRTs to provide suitable conditions for bacterial adsorption to the carrier. The HRT then could be reduced step-wisely. In the adsorbed cell systems, dead cells can be replaced by viable bacteria to prolong the time period of operation of the bioreactor. In this study, the amount of bacteria in the effluent was at least 100

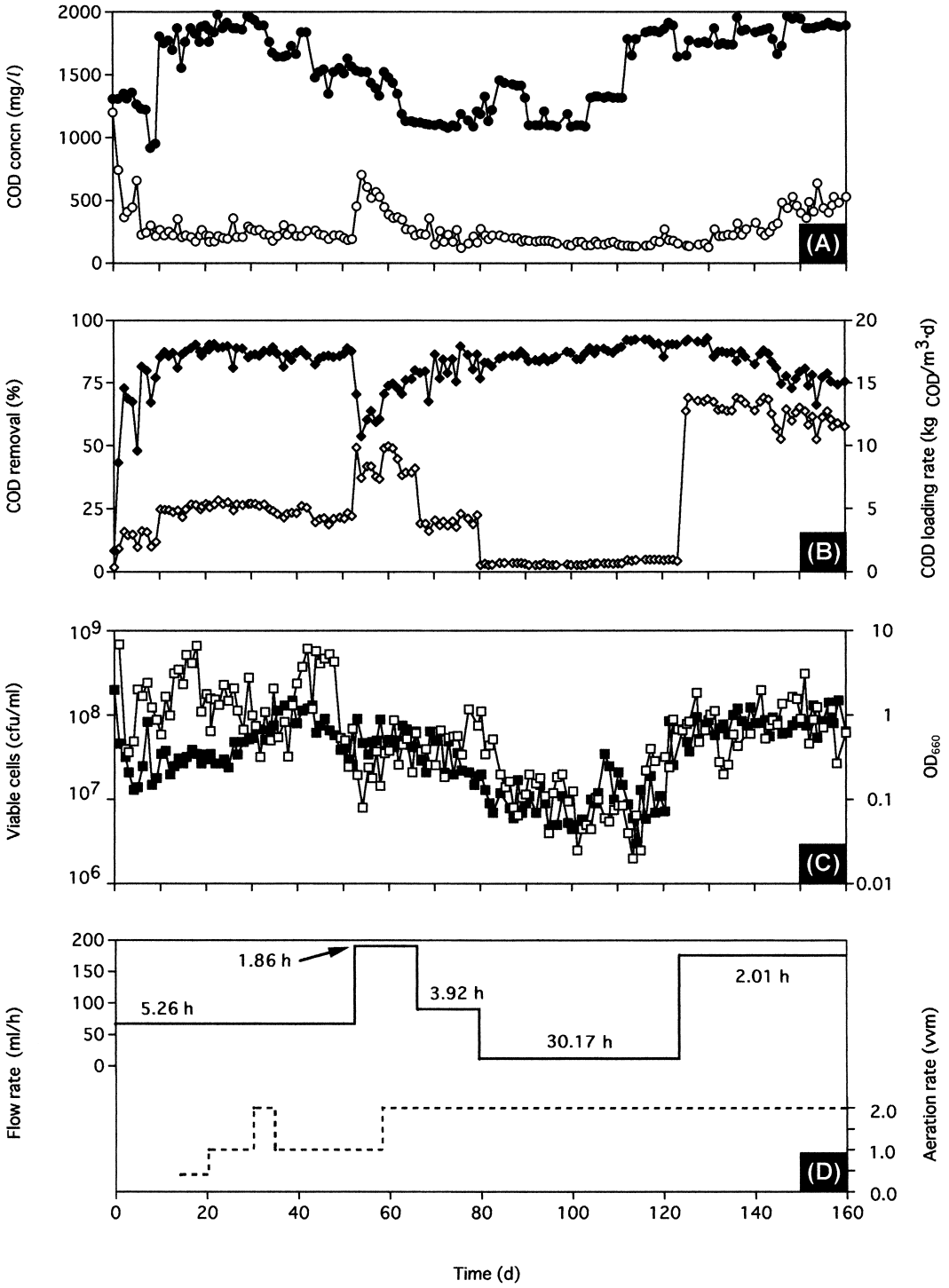

FIG. 3. Performance of the bioreactor at different volumetric flow rates of wastewater and aeration rates. Symbols: solid circles, COD concentration input to the bioreactor; open circles, COD concentration output from the bioreactor; solid diamonds, COD removal efficiency; open diamonds, COD loading rate; open squares, viable cell output from the bioreactor; solid squares, optical density output from the bioreactor; solid line, influent flow rate of wastewater into the bioreactor; dashed line, aeration rate.

times less than the total initial amount of the immobilized cells. This is because the number of adsorbed bacteria increased during the process and the free sites on the carrier surface were occupied by the bacteria.

Although output COD concentration did not comply with the environmental regulations, this process has the potential to be improved toward that goal in further studies.

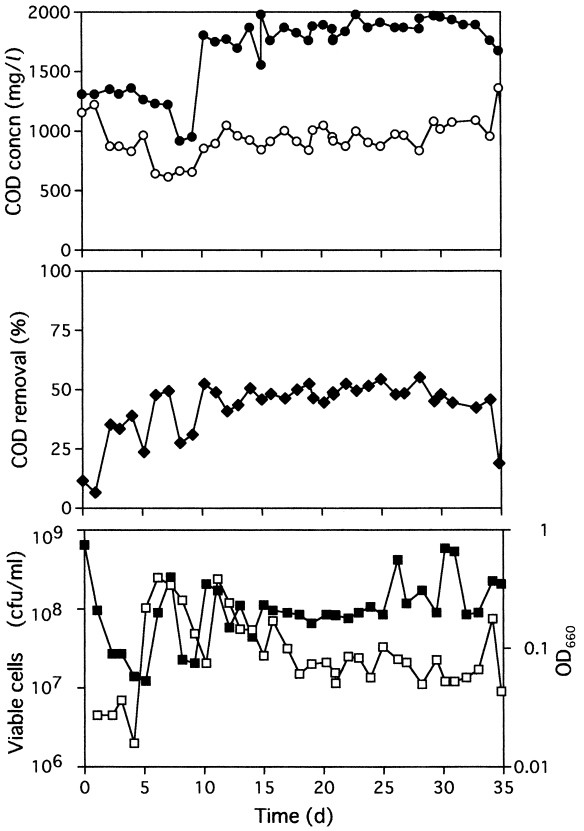

FIG. 4. Performance of the non-aerated bioreactor at an influent flow rate of 67.1 cm^3/h (HRT of 5.26 h). Symbols: solid circles, COD concentration input to the bioreactor; open circles, COD concentration output from the bioreactor; solid diamonds, COD removal efficiency; open squares, viable cell output from the bioreactor; solid squares, optical density output from the bioreactor.

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