Characteristics of Porous Ceramic Carriers made from Fly Ash for Immobilization of Microorganisms

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Abstract: The purpose of this study is to develop the porous ceramic carrier made from fly ash for immobilization of microorganisms which can be applied to the effective removal of nitrogen in wastewater. For the purpose, attempts were carried out to investigate the effects of pore structure in the prepared carrier on the immobilization of microorganisms and to determine the optimum conditions for preparation of the carrier. And the surface of the prepared carriers was treated with NaOH solution at 100°C for 4 hrs to provide the cation exchange capacity by the transformation of a fly ash into a zeolite. As a result, we could control the porosity and pore size of the carrier by varying the amount and particle size of polyethylene added, respectively. The number of immobilized microorganisms increased with the pore volume of the appropriate size for colonization of microorganisms. And it seems that the immobilization capacity depends on the pore size rather than the porosity. The result also showed that immobilization capacities of the prepared carriers were about 3 times as high as the commercial carrier. The carrier which was treated with 4M NaOH solution at 100°C for 4 hrs showed higher cation exchange capacity than the natural zeolite without the change of bulk shape. It is expected that the prepared carrier can be effectively applied to the removal process of nitrogen in wastewater.

Keywords: immobilization, porous carrier, nitrogen removal, fly ash

Introduction

Nitrogenous compounds are major pollutants of water in domestic wastes, animal wastes and industrial wastes from fertilizer production, paper manufacturing and food processing. The excessive input of nutrients such as nitrogen to water bodies causes eutrophication. Furthermore, nitrates in drinking water are harmful to one's health, especially infant's. So many efforts have been devoted to the development of processes for nitrogen removal in wastewater. The biological process is known to be one of the most effective and economical processes for nitrogen removal. But the conventional activated sludge process which has been mostly used as the one of biological processes generally require an extremely long residence time to retain slow-growing microorganisms such as nitrifiers and anaerobic bacteria in the system. And because it has a problem associated with bulking occurrence, Nitrogenous compounds have not been effectively removed from wastewater [1-2]. Consequently, the cell immobilization techniques which can solve the above problems have recently gained much attention.

The nature of the carrier used for immobilization of microorganisms has a significant effect on the reactor performance. For instance, the carrier must supply the enough space as the shelter of microorganisms against the shear stress by flowing water and be environment-friendly in itself. A wide range of materials have been used as carriers for immobilization of microorganisms at laboratory and pilot-scale, including inorganic materials such as glass beads, red drain clay, sand and sintered glass, synthetic fiber and polymers such as needle-punched polyester, polyurethane foam, polyvinyl alcohol bead [3-7].

Meanwhile, because the enormous amount of fly ash
has been generated annually as solid waste in Korea, there is continuing interest in recycling of them. Especially, Attention has been paid to the potential use of zeolite synthesized from fly ash by chemical treatment for adsorbing ammonium ion in waste water [8-11].

The purpose of this study is to develop the porous ceramic carrier made from fly ash for immobilization of microorganisms which can be applied to the effective removal of nitrogen in wastewater. For the purpose, attempts were carried out to investigate the effects of pore structure in the prepared carrier on the immobilization of microorganisms and to determine the optimum conditions for preparation of the carrier. And we treated the prepared carrier with NaOH solution chemically to modify the surface on the carrier and attempted to make a multipurpose carrier which can remove the nitrogen in wastewater simultaneously by adsorption of NH₄⁺ as well as biological process.

## Experimental

### Preparation of Porous Ceramic Carrier Using Fly Ash

Fly ash used in this work was supplied by Seo-Chon thermoelectric power plant in Korea, and it was composed of SiO₂ (46.03%), Al₂O₃ (30.73%), Fe₂O₃ (4.51%), K₂O (4.27%), Na₂O (0.83%) and CaO (0.70%). The fly ash was sieved to 180 mesh and was used without any treatment. Porous ceramic carrier was prepared by combustion method of polymer particle mixed with fly ash. The polymer used in this work was polyethylene (LDPE), and was sieved under 80 mesh (≤ 180 μm). And water glass was used as inorganic binder. In order to reinforce the strength of the carrier in the water, glass fiber powder was added. The preparation of porous ceramic carrier using fly ash is summarized as follows; Fly ash of 60 g, glass fiber powder of 10 g and polymer powder of 5-15 g were mixed with water glass of 40 g. Then the mixture was put into extruder to make the cylindrical form. It was then dried at 110°C, and calcined at 800°C for 5 hrs to make the pore in the carrier by combustion of the polymer mixed with fly ash. The nomenclature with the mixing ratio in this work is listed in Table 1.

### Immobilization of Microorganisms on Porous Ceramic Carrier

To ascertain the immobilization capabilities of microorganisms on the prepared carriers, the immobilization tests of microorganisms were carried out as batch experiments. The microorganisms used in this work were two kinds of bacillus species which have the average size of 1 μm (designated as “BS-U1”) and 5 μm (designated as “BS-J1”), respectively. Nutrient broth of 300 mL was put into baffled flask, and it was maintained at 121°C for 20 min under 1.5 atm. The microbes were then inoculated into the baffled flask, and it was cultivated in the shaking incubator at 28°C for 24 hrs. The prepared cylindrical carriers of 3 g was then put into the test tube with 10mL of cultivated nutrient broth, and it was maintained in the shaking incubator at 28°C for 12 hrs. To ascertain the immobilized microbes inside the carrier, the carrier was dried at room temperature and crushed in the mortar. The crushed powder of 1 g was added to the test tube with 9 mL of the sterilized water and shaken vigorously by a shaker for 20 min. The samples of 1 mL were then taken and diluted with 9mL of the sterilized water until 10⁻⁴ - 10⁻⁶ order. The respective samples of 100 μL were smeared on nutrient agar (NA) plate and cultivated in a B.O.D incubator at 28°C for 3 days. Finally, the colonies formed on the NA plate were counted. The measurements were carried out for 5 times, and the mean numbers of attached colonies were calculated arithmetically.

### NaOH Treatment of Prepared Carriers

To modify the surface of porous carrier made from fly ash, the carriers were chemically treated with NaOH solution at the concentration of 2 M to 6 M. The prepared carrier of 10 g and NaOH solution of 100 mL were put into the flask which was equipped with reflux condenser, heated to 100°C and maintained for 4 hrs without stirring. The carrier was then cooled and washed with the deionized water for several times to adjust the pH = 8. And the carrier was dried in an oven at 100°C for 12 hrs.

### Analyses

The pore size distribution and porosity of the prepared carriers were measured by Hg porosimeter (Micro-
meritics, Autopore II 9220). Scanning electron microscope (SEM, Philips XL-30) was used to observe the pore structure of surface on porous carrier. And the microorganisms immobilized in the carrier were observed using SEM after treatment as follows; Specimen were fixed with 5% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.2) for 3 hrs at room temperature. They were then post fixed overnight in similarly buffered 2% osmium tetroxide at 4°C. Fixed material was dehydrated in a graded ethanol series, 15 mins in each of 10% steps. This was followed by a gradual substitution of the alcohol with acetone before critical point drying of the specimens (Valtech. CPD030). Dried sections were mounted on aluminum stubs with double sided adhesive tape (Scotch), gold coated (600 Å thickness) in a sputtering system (Valtech. SCD005 coater) and observed by a scanning electron microscope (Philips XL302).

To determine the cation exchange capacities (CEC) of the carriers which were chemically treated with NaOH solution, the general method using 1N-NH4OAc (pH = 7.0) solution was used. And the capacity of the commercial carrier (S company) and natural zeolite (clinoptilolite type, Po-hang) were measured for comparison. The sample was saturated with NH4+ using 1N-NH4OAc (pH = 7.0) solution. It was then mixed with solutions of methyl red, methylene blue and bromo cresol green as indicator after leaching and distillation using 4% boric acid solution and MgO powder. Finally, It was titrated with 1N-H2SO4 standard solution. The CECs of samples were calculated by the following equation.

\[
\text{CEC(meq/100 g)} = \frac{V_i}{W_s} \times C_i \times 100
\]

Where, \(V_i\) is the volume of H2SO4 solution (mL), \(W_s\) is the weight of samples (g), and \(C_i\) is the concentration of H2SO4 solution (N).

**Results and Discussion**

**Pore Structure of the Prepared carrier**

As the biological treatment of wastewater takes place at the carrier surfaces by the immobilized microorganisms, the knowledge for physical and chemical properties of the carrier is of crucial importance. It has been recognized that the carrier performance is influenced by the various factors such as the composition of carrier, particle size, carrier size, roughness, surface area, pore size, porosity, and so on [12]. In the previous works by Huysman and Murray, it has been reported that the immobilization capacity of porous carrier is especially influenced by the pore size and porosity of it. It has been known that the pore size of carrier must be appropriate for colonization of microorganisms and be several (about five) times as large as microorganisms which have the size of around 5 μm. Also, the large porosity of the carrier is required because the nutrient of microorganisms must not be intercepted from the outside [13,14]. Therefore, we focused the control of the pore size and porosity appropriate for immobilization of microorganisms in this work. Figure 1 shows the scanning electron micrographs of FPE-1, 2, 3 and FO. The left shows an internal section and the right shows an external surface respectively. As shown in Figure 1, with polyethylene added increasing, the macro-pores averaging about 100 μm on the internal section and external surface of the carrier increased. On the other hand, FO carrier that was prepared without addition of polyethylene didn’t show such the macro-pores. In addition, Figure 1 showed that the porous ceramic carriers were successfully prepared without destruction of the cylindrical shape and closure of the pore on external surface during the forming process. Figure 2 shows the plots of cumulative pore volume as a function of mean pore diameter in the prepared carriers. As shown in Figure 2, FO carrier had about 2-3 μm of mean pore diameter that took 60% of total pore volume. It may be due to evaporation of water inside carrier during the calcination process and the aperture between fly ash particles bound with water glass. But as mentioned above, it was expected that such small pore size of FO carrier would not be suitable for the immobilization of microorganisms. Whereas as the amount of polyethylene added increased, it was observed that the pore size distribution was gradually shifted to larger than FO carrier and that pore volume of the size averaging about 20-30 μm which may be effective for immobilization also increased. These results may be due to the formation of macro-pores by the combustion of polyethylene particle added. It also means that the porous ceramic carrier with the ordered pore size distribution can be obtained by varying the amount of polyethylene added to fly ash.

And we carried out the experiments to ascertain the change of pore structure with the varying the particle size of polyethylene (FPE-4, 5 and 6). Figure 3 shows the scanning electron micrographs of the prepared carriers. As shown in Figure 3, it was observed that as the particle size of polyethylene decrease, the pore size also decreased but the number of pores increased. Pore size distributions were shown in Figure 4. These plots showed that volume of the pore size over about 10 μm between all the carriers was alike. With the particle size of polyethylene decreasing, pore size distribution over about 10 μm was shifted to the smaller. But volume of the pore size below about 10 μm which would not be effectively used for immobilization decreased. The physical properties of the carriers are listed in Table 2. All the samples had the
Figure 1. Scanning electron micrographs of FO, FPE-1,2 and 3.
same diameter without any shrinkage after calcination process. The results had the tendency that as the amount of polyethylene increased and the particle size of polyethylene decreased, the porosity and total pore area also increased.

Figure 5 is the enlarged micrographs showing the connection between pores in the carrier. It seems that the immobilized microorganisms can be supplied with the shelter against the shear stress by flowing water and nutrients from the outside through the connection between pores.

Modification of the Surface on the Prepared Carrier

The surface of the prepared carrier (FPE-3) was treated with NaOH solution at 100°C for 4 hrs to provide the cation exchange capacity. Although treated with the
strong base, the carriers maintained the cylindrical shape. Figure 6 shows scanning electron micrographs of the surface on the carriers before and after NaOH treatment at the concentration of 4 M. As shown in Figure 6, the surface of the prepared carrier was smooth before NaOH treatment but rough after the treatment. Furthermore, it was observed that the spherical fly ash particles have been transformed into a material with high crystallinity. It may be due to the transformation into zeolite through hydrothermal reaction of fly ash with NaOH solution [15]. To confirm the transformation to zeolite, an X-ray diffraction measurement was performed in the range of diffraction angles of 5° to 60°. Figure 7 shows the X-ray diffraction patterns of each sample. As shown in Figure 7, the sample was transformed to phillipsite at the concentration of 4 M and then to sodalite at the concentration of 6 M. This result shows that the surface on the carrier was successfully transformed to zeolite by

Figure 4. Cumulative intrusion volumes of FPE-4, 5 and 6.

Figure 5. Scanning electron micrographs showing the connection between pores in the carrier.

Figure 6. Scanning electron micrographs of the surface on the carriers before and after NaOH treatment for 4 hrs at 100°C. a) before (FPE-3) and b) after (ZPE-3).

Figure 7. X-ray diffraction patterns of the carriers treated with NaOH solution: (a) without NaOH treatment (b) 2 M-NaOH (c) 4 M-NaOH and (d) 6 M-NaOH
Table 2. The Physical Properties of the Prepared Porous Carriers

<table>
<thead>
<tr>
<th>Nomenclature</th>
<th>Diameter(mm)</th>
<th>Bulk density(g/cm³)</th>
<th>Porosity(%)</th>
<th>Total pore area(m²/g)</th>
<th>Total pore volume(mL/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPE-1</td>
<td>1.96-1.98</td>
<td>1.2929</td>
<td>35.695</td>
<td>23.844</td>
<td>0.2761</td>
</tr>
<tr>
<td>FPE-2</td>
<td>1.96-1.98</td>
<td>1.2011</td>
<td>37.097</td>
<td>24.948</td>
<td>0.3089</td>
</tr>
<tr>
<td>FPE-3</td>
<td>1.96-1.98</td>
<td>1.1513</td>
<td>47.599</td>
<td>29.361</td>
<td>0.4135</td>
</tr>
<tr>
<td>FPE-4</td>
<td>1.96-1.98</td>
<td>1.2071</td>
<td>45.090</td>
<td>31.743</td>
<td>0.3735</td>
</tr>
<tr>
<td>FPE-5</td>
<td>1.96-1.98</td>
<td>1.2126</td>
<td>40.888</td>
<td>23.889</td>
<td>0.3372</td>
</tr>
<tr>
<td>FPE-6</td>
<td>1.96-1.98</td>
<td>1.1432</td>
<td>39.228</td>
<td>18.480</td>
<td>0.3431</td>
</tr>
<tr>
<td>ZPE-3*</td>
<td>1.96-1.98</td>
<td>0.8738</td>
<td>58.213</td>
<td>40.430</td>
<td>0.6662</td>
</tr>
<tr>
<td>FO</td>
<td>1.96-1.98</td>
<td>1.4134</td>
<td>36.306</td>
<td>24.186</td>
<td>0.2569</td>
</tr>
</tbody>
</table>

*the sample after the treatment with 4M NaOH solution for FPE-3 sample.

Figure 8. Cumulative intrusion volumes of FPE-3 and ZPE-3.

The simple treatment with NaOH solution without the change of cylindrical bulk shape. Figure 8 shows the change of pore size distribution before and after NaOH treatment. The result shows that volume of the pore size over about 10 μm decreased, whereas increased below about 10 μm of pore size. And the pore volume and porosity of ZPE-3 increased in comparison with FPE-3 as shown in Table 2. It seems that the macro-pores over about 10 μm was destroyed by dissolution and transformed into zeolite which has the pore size of several angstroms through the NaOH treatment. Cation exchange capacities of the carriers treated with 2 M, 4 M and 6 M of NaOH solution were 70, 112 and 85 meq/100 g, respectively. These values were higher in comparison with that of the commercial carrier (about 18 meq/100 g) and about the same as natural zeolite (about 90 meq/100 g). In contrast, cation exchange capacity of the untreated carrier was not detected.

**Immobilization of Microorganisms on Porous Ceramic Carrier**

The results of immobilization tests are listed in Table 3. The microorganisms used in this work were two kinds of *bacillus species* which have the average size of 1 μm (BS-U1) and 5 μm (BS-J1). In comparison, the commercial carrier (designated as “s”) was tested at the same condition. As shown in Table 3, with the amount of polyethylene increasing, the number of immobilized colonies increased. In contrast, FO carrier had little immobilization capacity. As mentioned above, these results may be caused by increase of volume of pore size (>10 μm) appropriate for colonization of microorganisms and porosity. As the particle size of polyethylene increased, the number of immobilized colonies decreased considerably in spite of the small differences in the porosity. And the immobilization capacity of ZPE-3 simply treated with NaOH solution decreased markedly after the increase of porosity. Also the prepared carrier showed higher capacity for BS-J1 (5 μm) than that for BS-U1 (1 μm). It seems that the pore size had larger effect on the immobilization capacities than the porosity. These results were consistent with that of the previous works [10-11]. The result also showed that immobilization capacities of the prepared carriers (FPE-3 and 4) were about 3 times as high as that of the commercial carrier. And the capacity of ZPE-3 was about the same in comparison with the commercial carrier. Therefore it is expected that ZPE-3 carrier can be economically applied as a multipurpose carrier used in the removal of nitrogen in wastewater simultaneously by adsorption of NH₃ as well as biological process. Microorganisms attached on the surface of the prepared carrier (FPE-3) were shown in Figure 9. As shown in

Table 3. The Number of Microorganisms Immobilized in Porous Ceramic Carrier

<table>
<thead>
<tr>
<th>Nomenclature</th>
<th>Immobilized microorganisms (CFU/g. × 10⁵)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BS-U1</td>
</tr>
<tr>
<td>FPE-1</td>
<td>3.9</td>
</tr>
<tr>
<td>FPE-2</td>
<td>9.5</td>
</tr>
<tr>
<td>FPE-3</td>
<td>31.5</td>
</tr>
<tr>
<td>FPE-4</td>
<td>15.8</td>
</tr>
<tr>
<td>FPE-5</td>
<td>10.1</td>
</tr>
<tr>
<td>FPE-6</td>
<td>6.6</td>
</tr>
<tr>
<td>FO</td>
<td>1.2</td>
</tr>
<tr>
<td>ZPE-3</td>
<td>11.5</td>
</tr>
<tr>
<td>S</td>
<td>13.2</td>
</tr>
</tbody>
</table>
Figure 9, it was confirmed that the colonies of microorganisms were desirably formed on the surface of the carrier.

Conclusions

This study investigated the characteristics of porous ceramic carrier made from fly ash for immobilization of microorganisms. The porous ceramic carriers were successfully prepared, and we could control the pore size and porosity by varying the amount and particle size of polyethylene added, respectively. Immobilization capacities of the prepared carriers (FPE-3 and 4) were about 3 times as high as that of the commercial carrier. It was observed that pore size had a great effect on the immobilization capacity rather than the porosity. The surface of the prepared carrier was treated with NaOH solution at 100°C for 4 hrs to provide the cation exchange capacity. As a result, the carrier which was treated with 4M NaOH solution had 112 meq/100 g of cation exchange capacity owing to the transformation of a fly ash into a zeolite and showed higher capacity than the natural zeolite without the change of bulk shape. It is expected that the prepared carrier can be applied economically as a multipurpose carrier used in the removal of nitrogen in wastewater simultaneously by adsorption of NH₄⁺ as well as biological process.

Acknowledgement

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References