Characteristics of bacterial populations responsible for uptake of amino acids in activated sludge acclimated to peptone

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Abstract Amino acids (AAs) are produced from the hydrolysis of proteins, which are the major biodegradable organic compounds in municipal sewage. The characteristics of bacterial populations responsible for the assimilation of thirteen AAs into activated sludge (AS) acclimated to peptone are investigated. The results are as follows. (1) The bacterial populations responsible for the uptake of AAs were partly aggregated in AS flocs. (2) The amounts of the bacterial populations responsible for the uptake of leucine, valine, isoleucine, histidine, threonine, lysine and glycine are limited in AS acclimated to peptone. (3) The bacterial populations responsible for the uptake of phenylalanine, leucine and lysine were different. (4) The amounts of bacterial populations responsible for the uptake of aspartate, arginine, alanine, glutamate and phenylalanine are not limited. (5) The functions of the assimilation of these AAs were induced in many bacterial cells as a result of the BOD determination methods applied to these pure AAs.

Keywords Activated sludge; aggregation; amino acids; bacterial population; induction

Introduction
Many researchers have analyzed the bacterial community in activated sludge (AS) and biofilm, using biomarker and molecular methods (quinone profile, PCR-DGGE, nucleic acid probing, etc.). However, the information obtained with these methods does not indicate what type of bacterial populations take up what kinds of organic compounds in influent, even when strains are identified, since most bacteria can take up many organic compounds; for example, Pseudomonas cepacia can take up more than 90 organic compounds as carbon and energy sources (Stanier et al., 1976).

Amino acids (AAs) are produced from the hydrolysis of proteins, which are the major biodegradable organic compounds in municipal sewage. The removal of a mixture of thirteen free AAs by AS acclimated to peptone followed the zero-order reaction kinetics, but the uptake rates of individual AAs during AA mixture removal differed and the uptake rates of some AAs increased during mixture removal (Ubukata, 1998). Therefore, the behavior of bacterial populations responsible for the uptake of AAs had been thought to be complicated. The characteristics of bacterial populations responsible for the uptake of AAs in AS acclimated to peptone were investigated in this study, since AAs are representatives of many other organic compounds and the catabolic pathways of AAs are well known (Lehninger, 1975; Conn et al., 1987).

Experimental methods
AS was acclimated to peptone for more than one month. The acclimating procedures for AS and the methods of measuring the substrate removal rate and the oxygen uptake rate (OUR) of AS during substrate removal have been described previously (Ubukata, 1998). The BOD values of AAs are determined according to the Standard Methods (APHA et al., 1992).
Results and discussion
The uptake rates of thirteen AAs and the OURs of AS during the removal of these AAs from supernatant were measured. The characteristics of AA uptake by AS and OURs of AS during AA removal are shown in Figure 1 (L), although the initial AS concentrations differed.

The uptake rates of glutamate, phenylalanine, aspartate and arginine gradually increased during the removal of these AAs. The OURs of AS during the removal of these AAs also increased, for example, at 8 hours into the experiment, the OURs of AS were about twice the initial rates. However, the uptake rates of leucine, valine, isoleucine, histidine, threonine, lysine, glycine and serine remained constant during the removal of these AAs, and the OURs of AS during the removal of these AAs were constant. In the case of alanine removal, the uptake rate of alanine was constant, but the OUR of AS increased slightly.

The amounts of the bacterial populations responsible for the uptake of leucine, valine, isoleucine, histidine, threonine, lysine, glycine and serine are limited, since both the uptake rates of these AAs and the OURs of AS were constant. On the contrary, the amounts of bacterial populations responsible for the uptake of aspartate, arginine, alanine, glutamate and phenylalanine are not limited, since both the uptake rates of these AAs and the OURs of AS increased during the removal of these AAs. The functions of the uptake of these AAs are thought to be induced during single AA removal in the bacterial cells which are responsible for uptake of other AAs during the removal of peptone (a mixture of AAs) (Gottschalk, 1986).

The characteristics of AA uptake by AS during the removal of single AA, phenylalanine and leucine, and a mixture of phenylalanine and leucine, are shown in Figure 1 (R). The sum total of the removal rates of these substrates, which was calculated from the results obtained during the removal of single AA, phenylalanine and leucine, is also shown in Figure 1 (R) by the filled symbols. The removal rate of the mixture of these AAs was similar to the sum total of the removal rates of single AAs. In this case, two different types of bacteria responsible for the uptake of two different AAs were used during the removal of the mixture of two AAs. The same results are obtained for the combinations for phenylalanine-lysine and leucine-lysine. Therefore, the bacterial populations responsible for the uptake of phenylalanine, leucine and lysine are considered to be different. Similar results were reported during the removal of combinations of other types of organic compounds (Stumm-Zollinger, 1966; Tischler and Eckenfelder, 1969).

The time courses of the removal of four types of AAs are shown in Figure 2 (L). The concentrations of AAs during their removal were determined from the concentrations of TOC and BOD; in this case, the size of AS flocs was smaller than that obtained in other experiments. The correlations between the TOC and BOD values during the removal of these AAs are shown in Figure 2 (R). During the removal of glutamate and glycine, the BOD values were strongly correlated with the TOC values, and during the removal of phenylalanine, the

Figure 1  Characteristics of AA uptake by AS (left) and OURs of AS (center) during removal of glutamate (circles), phenylalanine (triangles) and leucine (squares). (R), Characteristics of AA uptake by AS during removal of phenylalanine (circle) and leucine (triangle), and phenylalanine-leucine mixture (square), (Xo = 1.1 g/l). Calculated sum total (filled symbols)
BOD values were correlated to the TOC values, although the coefficient of the correlation between the BOD values and TOC values was not high. However, during the removal of leucine, no correlation between the BOD values and the TOC values was observed.

Why was no correlation observed between the BOD values and the TOC values only during the removal of leucine, although the measurement methods were the same as for other AAs? The key point in solving this irregularity in the results between the TOC values and the BOD values is assumed to be the aggregation of bacterial populations responsible for the uptake of AAs, since the size of the AS used as the seeding AS for BOD determination was greater (0.3 mm) than that of the AS used to treat municipal sewage.

The effects of both the seeding rates of AS and the conditions of the seeding AS for the BOD determinations on the BOD values are shown in Table 1. One AS was completely homogenized using an ultrasonic homogenizer, and the other was roughly homogenized using a blender homogenizer. Five BOD bottles were used for the determination of the BOD values per AA and peptone sample.

When the seeding AS was completely homogenized, the BOD values for each AA were not scattered, even though the amounts of seeding AS were one-hundredth that for the roughly homogenized AS. In contrast, when the seeding AS was roughly homogenized, the BOD values of AAs for each AA were scattered. In the case of leucine, phenylalanine and glycine, extremely low BOD values of AAs were determined. These results are considered to indicate that the bacterial populations responsible for the assimilation of these AAs are partly aggregated in the AS flocs and that the numbers of aggregated bacterial populations responsible for the assimilation of these AAs are limited; in this case, some parts of the bacterial populations in the seeding are not capable of the assimilation of these AAs.

In the case of leucine, the BOD values obtained with the completely homogenized AS were scattered slightly compared with the cases of other AAs. These results are thought to indicate that the amounts of the bacterial populations responsible for the assimilation of leucine in the seeding AS are smaller than those for other AAs, and that the five-day incubation period is too short for the growth of the bacterial populations responsible for the assimilation of leucine. In the case of glutamate, the difference in the BOD values due to the AS conditions was small compared with that for other AAs. This means that most of the bacteria in the seeding AS are capable of glutamate assimilation.

**Table 1** Effects of seeding rates of AS (4 g/l) and AS conditions on BOD values (%, mg BOD/mg AA)

<table>
<thead>
<tr>
<th>AS seeding rate 0.01 ml/10 L</th>
<th>AS seeding rate 1 ml/10 L</th>
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<tbody>
<tr>
<td>AS conditions</td>
<td>completely homogenized</td>
</tr>
<tr>
<td>Leucine</td>
<td>16 22 25 29 43</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>103 104 106 106 107</td>
</tr>
<tr>
<td>Glycine</td>
<td>32 33 33 33 33</td>
</tr>
<tr>
<td>Glutamate</td>
<td>45 45 45 48 51</td>
</tr>
<tr>
<td>Peptone</td>
<td>80 81 81 83 83</td>
</tr>
</tbody>
</table>
The effects of the seeding rates of AS on the BOD values of AAs are shown in Table 2; in this case, the seeding rates are widely varied. The conclusions based on the results obtained from these experiments are as follows. (1) The bacterial populations responsible for the assimilation of lysine are extremely limited. (2) The bacterial populations responsible for the assimilation of leucine are limited, although the function of leucine assimilation was induced in some bacterial cells. (3) The function of phenylalanine assimilation was induced in many types of the bacterial cells. (4) The functions of glutamate and alanine assimilations were induced in many bacterial cells, even for the natural bacterial contamination of BOD bottles (no seeding).

Conclusions

Amino acids (AAs) are produced from the hydrolysis of proteins, which are the major biodegradable organic compounds in municipal sewage. The characteristics of bacterial populations responsible for the assimilation of thirteen AAs in activated sludge (AS) acclimated to peptone are investigated. The results are as follows. (1) During the removal of glutamate, phenylalanine and glycine, the BOD values were correlated with the TOC values, but during the removal of leucine, no correlation between the BOD values and the TOC values was observed. (2) The bacterial populations responsible for the uptake of AAs were shown to be partly aggregated in AS flocs, since the BOD values of pure AAs varied with the seeding AS conditions (completely or roughly homogenized). (3) The amounts of the bacterial populations responsible for the uptake of leucine, valine, isoleucine, histidine, threonine, lysine and glycine are limited in AS acclimated to peptone. (4) The bacterial populations responsible for the uptake of phenylalanine, leucine and lysine were shown to be different. (5) On the contrary, the amounts of bacterial populations responsible for the uptake of aspartate, arginine, alanine, glutamate and phenylalanine are not limited, since both the uptake rates of these AAs and the oxygen uptake rates of AS increased during the removal of these AAs. (6) The function of the assimilation of these AAs was shown to be induced in many bacterial cells in AS as a result of the BOD determination methods applied to these pure AAs.

References


*Standard Methods for the Examination of Water and Wastewater* (1992), 18th edn, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.

