Acetate uptake by PHA-accumulating and non-PHA-accumulating organisms in activated sludge from an aerobic sequencing batch reactor fed with acetate

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ABSTRACT

The present study was conducted to evaluate the specific acetate uptake rates of microorganisms with and without polyhydroxyalkanoates (PHA) accumulation. Activated sludge was aerobically incubated with 75 mg C L\(^{-1}\) radiolabeled or non-labeled acetate, and acetate consumption and PHA accumulation were monitored. Microorganisms were quantified as follows: all microbial cells by DAPI staining, whole acetate utilizing organisms by microautoradiography, and PHA-accumulating organisms by staining with Nile blue A. The abundance of acetate-utilizing organisms without PHA accumulation was also calculated from the outcomes. The estimate of acetate utilized by PHAAOs included both the acetate converted to PHA and that used to supply reducing power and ATP. Acetate utilized by PHAAOs and non-PHAAOs were divided by their respective abundances to obtain their respective specific acetate uptake rates: PHAAOs ranged between 5.3 and 8.0 \(\times\) 10\(^{-10}\) mg C cell\(^{-1}\) h\(^{-1}\), and non-PHAAOs ranged between 2.8 and 4.2 \(\times\) 10\(^{-10}\) mg C cell\(^{-1}\) h\(^{-1}\).

Key words | activated sludge, non-PHA-accumulating organisms, PHA-accumulating organisms, specific acetate uptake rate

INTRODUCTION

The removal of easily biodegradable organic matter is one of the most important objectives of biological wastewater treatment. Some parts of biodegradable organic matter are thought to be stored in bacterial cells as temporal carbon storage material. For example, organic matter removed from the anaerobic zone of enhanced biological phosphorus removal (EBPR) processes is typically stored as polyhydroxyalkanoates (PHA) (Mino et al. 1998). The accumulation of temporal carbon storage materials can be found not only in EBPR processes but also in aerobic activated sludge processes (Van Loosdrecht et al. 1997). And, the formation of temporal carbon storage materials has been suspected as a key factor in microbial selection of floc forming and filamentous microorganisms in the selector tanks, a small aeration tank located upstream of the main aeration tank (Chudoba et al. 1973). Also, biological carbon adsorption, in such forms as PHA, should be an important process in the contact stabilization activated sludge process. In addition, PHA accumulation by microorganisms in activated sludge has attracted research interest because PHA is a biodegradable plastic (Serafim et al. 2004; Johnson et al. 2009). In order to understand the details of carbon removal and PHA accumulation by microorganisms in activated sludge, and better apply the temporal carbon storage phenomena in such forms as PHA for wastewater treatment, the specific substrate uptake rates of microorganisms with and without PHA accumulation should be evaluated, as they are the key in microbial competition over carbon sources.

The present study was conducted to evaluate the specific acetate uptake rates of the microorganisms with
PHA accumulation (PHAAOs) and without PHA accumulation (non-PHAAOs). For this purpose, aerobic batch experiments composed of cold incubation, using non-radiolabeled acetate, and hot incubation, using tritium labeled acetate, were performed. Cold incubation was performed to estimate the amount of acetate taken by PHAAOs and non-PHAAOs, and the acetate consumption and PHA accumulation were determined during the incubation. In estimating the acetate utilized by PHAAOs, both the acetate converted to PHA and that used to supply reducing power and ATP were considered. Hot incubation was performed to enumerate the abundance of whole acetate utilizing organisms and PHAAOs by microautoradiography (MAR) and chemical staining with Nile Blue A. Based on the outcomes of the hot and cold incubations, the specific acetate uptake rates of PHAAOs and non-PHAAOs were calculated.

MATERIALS AND METHODS

Activated sludge

A 10-L sequencing batch reactor (SBR) was operated in the laboratory. Conventional activated sludge, obtained from a domestic wastewater treatment plant, was used for seeding. The SBR was operated in 6 h cycles: 12 min feeding, 288 min aerobic reaction, 30 min settling, and 30 min effluent discharge. In the feeding phase, the SBR was fed with a 5 L synthetic nutrient medium containing: acetate 150 mgC L\(^{-1}\), CaCl\(_2\)-2H\(_2\)O 8.8 mg L\(^{-1}\), MgCl\(_2\)-6H\(_2\)O 90.8 mg L\(^{-1}\), KCl 42.0 mg L\(^{-1}\), NH\(_4\)Cl 17.6 mg L\(^{-1}\), (NH\(_4\))\(_2\)SO\(_4\) 21.6 mg L\(^{-1}\), K\(_2\)HPO\(_4\) 18.0 mg L\(^{-1}\), and KH\(_2\)PO\(_4\) 14.0 mg L\(^{-1}\). The composition of this synthetic nutrient medium is the same as the one described by Fukushima et al. (2007) except for acetate concentration. Acetate concentration in the synthetic nutrient medium was at 150 mgC L\(^{-1}\) to set the value of volumetric COD loading rate in SBR at 0.4 gCOD L\(^{-1}\) day\(^{-1}\). The hydraulic retention time in SBR was at 12 h, and the value of volumetric COD loading rate was around 0.4 gCOD L\(^{-1}\) day\(^{-1}\). The solid retention time was 12.5 days and the reactor was operated at room temperature at 22 ± 2°C. pH control was not conducted during the reactor operation but bulk pH was around at 8.5.

The reactor was operated for four months, and complete acetate removal was observed during the entire operating period. The concentration of mixed liquor volatile suspended solid (MLVSS) was between 1,000 and 1,500 mg L\(^{-1}\).

Batch experiments

On days 45, 50, and 89, activated sludge was collected at the end of the aerobic phase, and batch experiments were conducted with the sludge. Each batch experiment consisted of a pair of cold and hot incubations. In the hot or cod incubation, radiolabeled or non-radiolabeled substrate was fed, respectively. Cold incubation with natural acetate as the feed was performed to monitor acetate consumption and PHA accumulation by activated sludge. The total number of cells in the activated sludge was also enumerated in cold incubation. Hot incubation with tritium-labeled acetate was performed to enumerate the abundance of whole acetate utilizing organisms. The abundance of PHAAOs was also enumerated in hot incubation.

Cold incubation and chemical analysis

Activated sludge collected at the end of an aerobic phase of the SBR was diluted with effluent water to a final MLVSS concentration of around 500 mg L\(^{-1}\) with 2 L volume, and put in a plastic beaker. Natural sodium acetate (at a final concentration of 75 mgC L\(^{-1}\)) was added, and aerobically incubated. During incubation, air was supplied by an air pump through an air stone, and mixing was done using a magnetic stirrer. Samples for determining acetate and PHA concentrations were collected every 15 min. During incubation, the oxygen uptake rate (OUR) was monitored, and the steep drop of OUR was used as an indicator of the depletion of acetate in the supernatant. Incubation was stopped when a steep drop of OUR was detected. The depletion of acetate was also confirmed by ion chromatographic analysis of acetate. Incubation was performed at room temperature (22 ± 2°C), and the pH was not controlled. Samples for MLVSS measurement and the total cell count were collected at the end of incubation.

Acetate concentrations were determined by ion chromatography, and a DX-AQ1110 equipped with an AS-9HC column (Dionex, Japan) was used for the analysis.
PHA concentrations were determined by gas chromatography after methanolytic degradation as per the procedures stated by Takabatake et al. (2002). A Shimadzu GC2010/FID (Shimadzu, Japan) equipped with an Inert Cap1 capillary column (GL science, Japan) was used for the gas chromatographic analysis, and sodium 3-hydroxybutyrate (Tokyo Chemical Industry, Japan) was used as the standard for 3-Hydroxybutyrate (3HB) unit in PHA. MLVSS determination was carried out according to the Standard Methods (2005).

Total cell numbers in activated sludge was determined as follows. Activated sludge was sonicated at 4 W for 3 min (Branson sonifier, USA), and filtered through a black 0.2 µm polycarbonate filter (Millipore, Japan). Microorganisms on the filter were stained with 2 µg mL⁻¹ of DAPI solution for 5 min, washed with Milli-Q water, and quantified by epifluorescent microscopy.

**Hot incubation and enumeration of microorganisms**

Hot incubation was performed with tritium labeled acetate. A 10 mL aliquot of activated sludge was sonicated to 4 W for 3 min (Branson sonifier, USA), and filtered through a black 0.2 µm polycarbonate filter (Millipore, Japan). Microorganisms on the filter were stained with 2 µg mL⁻¹ of DAPI solution for 5 min, washed with Milli-Q water, and quantified by epifluorescent microscopy.

The microorganisms in the activated sludge were classified into three groups, as shown in Figure 1, and the abundance of whole acetate utilizing organisms, PHAAOs and non-PHAAOs in the total cells were enumerated. Whole acetate utilizing organisms were detected by MAR. Before the MAR procedures, the sample was stained with 2 µg mL⁻¹ of DAPI for 5 min and washed with Milli-Q. The MAR procedures were performed as per the procedures stated by Chua et al. (2006), with an exposure time of 4 weeks.

Dual staining with Nile blue A and DAPI was performed to determine the PHAAOs. Because PHA granules emit strong fluorescence when they are bound with Nile blue A, PHAAOs are easily identified by staining with Nile blue A (Ostle & Holt 1982). Dual staining with Nile blue A (certified dye content 81%, Kodak, Japan) and DAPI was performed as per the procedures stated by Oshiki et al. (2008). In the present study, it was assumed that the amount of PHAAOs without acetate utilization was negligible. The abundance of non-PHAAOs in the total cells was calculated as the difference between all the acetate utilizing organisms and the PHAAOs.

An Olympus BX51, equipped with a CCD camera DP70 (Olympus, Japan), was used for microscopy. The fluorescence signal of DAPI was observed through a WU filter. The fluorescence signal of Nile blue A was observed through a WIG filter. Microautoradiographs were observed under bright fields, and when a cell was covered with silver grains with an area over 2 µm², the cell was regarded to have utilized acetate. To determine the total number of cells, whole acetate utilizing organisms, and PHAAOs, at least ten randomly selected fields, containing more than one thousand total cells, were used for the quantification. The number of cells was counted manually from the captured images.

**Specific acetate uptake rates for PHAAOs and non-PHAAOs**

Specific acetate uptake rates for PHAAOs ($q_{\text{PHAAOs}}$) and non-PHAAOs ($q_{\text{non-PHAAOs}}$) were calculated by Equations (1) and (2), respectively.

$$q_{\text{PHAAOs}} = \frac{\Delta C_{\text{PHAAOs}} \cdot MLVSS^{-1} \cdot TCN^{-1} \cdot X_{\text{PHAAOs}}^{-1} \cdot T^{-1}}{1}$$

$$q_{\text{non-PHAAOs}} = \frac{\Delta C_{\text{AS}} - \Delta C_{\text{PHAAOs}} \cdot MLVSS^{-1} \cdot TCN^{-1}}{X_{\text{non-PHAAOs}}^{-1} \cdot T^{-1}}$$

where $\Delta C_{\text{PHAAOs}}$: acetate utilized by PHAAOs during cold incubation (mg C L⁻¹), $\Delta C_{\text{AS}}$: acetate utilized by the whole activated sludge during cold incubation (mg C L⁻¹),
MLVSS:MLVSS concentration (mgVSS L\(^{-1}\)), TCN: total cell number in activated sludge (cells mgVSS\(^{-1}\)), \(X_{PHAAOs}\): abundance of PHAAOs in total cells (%), \(X_{non-PHAAOs}\): abundance of non-PHAAOs in total cells (%), and \(T\): incubation time of cold incubation (hour).

The value of \(\Delta Ac_{PHAAOs}\) was calculated from Equation (3):
\[
\Delta Ac_{PHAAOs} = \Delta Ac_{accumulated} + \Delta Ac_{(H)} + \Delta Ac_{ATP}
\]
where \(\Delta Ac_{accumulated}\): acetate accumulated as PHA during cold incubation (mgC L\(^{-1}\)), \(\Delta Ac_{(H)}\): acetate consumed for the production of reducing power to convert acetate to PHA, and \(\Delta Ac_{ATP}\): acetate consumed for the ATP production to transport acetate into the cell and synthesize acetyl-CoA from acetate. In the present study, the simultaneous growth and PHA accumulation by PHAAOs was ignored because above 90% of acetate was used for PHA accumulation or oxidized for the ATP production and the amount of acetate utilized for bacterial growth was thought to be small during the batch experiment.

\(\Delta Ac_{(H)}\) and \(\Delta Ac_{ATP}\) were estimated by Equation (4), which is derived from Equations (5)–(8).
\[
2.0 \text{Ac} + (0.25 \text{Ac} - 0.5 \text{CO}_2) + (0.45 \text{Ac} + 0.9
\]
\[
\text{O}_2 - 0.9 \text{CO}_2 + 4.5 \text{ATP} \rightarrow 1.0 \text{3HB} + 2.4 \text{H}_2\text{O}
\]
\[+ 4.5 (\text{ADP} + \text{P}_i) \]
\[
2.0 \text{acetyl-CoA} + 1.0 (\text{H}) \rightarrow 1.0 \text{3HB} \]
\[
0.25 \text{acetyl-CoA} \rightarrow 1.0 (\text{H}) + 0.5 \text{CO}_2 - 0.75 \text{H}_2\text{O} \]
\[
1.0 \text{Ac} + 2.0 \text{ATP} \rightarrow 1.0 \text{acetyl-CoA} + 1.0 \text{H}_2\text{O}
\]
\[+ 2.0 (\text{ADP} + \text{P}_i) \]
\[
1.0 \text{acetyl-CoA} + 2.0 \text{O}_2 + 12 (\text{ADP} + \text{P}_i) \rightarrow 12 \text{ATP}
\]
\[+ 2.0 \text{CO}_2 + 1.0 \text{H}_2\text{O} \]

where Ac: acetate, 3HB: 3-hydroxybutyrate, acetyl-CoA: acetyl coenzyme A, (H): reducing power as hydrogen equivalent, ATP: adenosine triphosphate, ADP: adenosine diphosphate and P\(_i\): inorganic phosphate. Equation (5) indicates the synthesis of the 3HB monomeric unit from acetyl-CoA and Equation (6) the production of reducing power from acetyl-CoA by the citric acid cycle. Equation (7) is composed of the following two reactions: (a) transport of acetate across cell membrane and (b) activation of acetate to acetyl-CoA in the cell. 1ATP is required at each reaction and thus a total of 2ATP is consumed in Equation (7). Equation (8) indicates ATP production by oxidative phosphorylation from acetyl-CoA. In Equation (8), it is assumed that 3ATP, 3ATP, and 2ATP are formed during oxidation of NADH, NAD(P)H, and FADH, respectively, when they are processed in the electron transport chain with oxygen as the electron acceptor; 1 ATP equivalent is formed in the conversion of succinyl-CoA to fumarate when acetyl-CoA is oxidized by the citric acid cycle.

\[\Delta Ac\_{(H)}\] is 0.25 mol\(_{\text{ac}}\) mol\(_{-1}\)\(3\text{HB}\) and \(\Delta Ac_{ATP}\) and 0.45 mol\(_{\text{ac}}\) mol\(_{-1}\)\(3\text{HB}\), as in Equation (4).

**RESULTS AND DISCUSSION**

Cold incubation

Activated sludge was aerobically incubated after the addition of natural acetate. Activated sludge linearly consumed acetate and accumulated PHA during cold incubation, as shown in Figure 2. As summarized in Table 1, \(\Delta Ac_{\text{AS}}\) and \(\Delta Ac_{\text{accumulated}}\) were 77.0–83.6 mgC L\(^{-1}\) and 39.0–43.2 mgC L\(^{-1}\), respectively. The outcome of gas chromatographic analysis indicated that accumulated PHA consisted mainly of 3HB unit and other monomeric unit of PHA other than 3HB was not accumulated. The initial pH was 8.5 and increased to 9.0 towards the end of incubation.

The amounts of \(\Delta Ac\_{(H)}\) and \(\Delta Ac_{ATP}\) were estimated based on Equation (4). As seen in Equation (4), \(\Delta Ac\_{(H)}\) and \(\Delta Ac_{ATP}\) account for about 9 and 17% of \(\Delta Ac_{PHAAOs}\), respectively. \(\Delta Ac_{PHAAOs}\) the sum of \(\Delta Ac_{\text{accumulated}}\), \(\Delta Ac\_{(H)}\), and \(\Delta Ac_{ATP}\)...

\[
\text{Figure 2} \quad \text{An example of the monitoring results for acetate consumption and PHA accumulation during cold incubation.}
\]
Table 1 | Acetate consumption during cold incubation and the total cell count in activated sludge

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>1st batch experiment</th>
<th>2nd batch experiment</th>
<th>3rd batch experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>D\text{Ac}_{\text{AS}} (mgC L^{-1})</td>
<td>83.6</td>
<td>77.0</td>
<td>77.7</td>
</tr>
<tr>
<td>D\text{Ac}_{\text{accumulated}} (mgC L^{-1})</td>
<td>43.2</td>
<td>39.0</td>
<td>42.0</td>
</tr>
<tr>
<td>D\text{Ac}_{\text{ATP}} (mgC L^{-1})</td>
<td>5.4</td>
<td>4.8</td>
<td>5.2</td>
</tr>
<tr>
<td>D\text{Ac}_{\text{PHAAOs}} (mgC L^{-1})</td>
<td>58.3</td>
<td>52.6</td>
<td>56.6</td>
</tr>
<tr>
<td>Total cell number (cells mgVSS^{-1})</td>
<td>3.6 \times 10^8</td>
<td>2.6 \times 10^8</td>
<td>4.4 \times 10^8</td>
</tr>
</tbody>
</table>

and D\text{Ac}_{\text{ATP}}, ranged between 52.6 and 58.3 mgC L^{-1}, and accounted for seven tenths of D\text{Ac}_{\text{AS}} as shown in Table 1.

Total cell numbers in activated sludge were counted after the DAPI staining, and ranged between 2.6 and 4.4 \times 10^8 cells mgVSS^{-1}, as shown in Table 1.

**Hot incubation**

Whole acetate utilizing organisms in activated sludge collected after hot incubation were enumerated by the MAR technique. The occurrence of dense silver grains was confirmed under microscopic observation, as shown in Figure 3. Whole acetate utilizing organisms accounted for about three fifths of the total cells in batch experiments, as shown in Table 2. On the other hand, silver grains could not be seen when the MAR experiment was performed after Nile blue A staining; therefore, the detection of whole acetate utilizing organisms by MAR and PHAAOs by Nile blue A staining were performed separately.

The abundance of PHAAOs in total cells was examined by dual staining with Nile blue A and DAPI. As shown in Figure 4, PHAAOs giving signals of both Nile blue A and DAPI were found in activated sludge. As shown in Table 2, about half of the whole acetate utilizing organisms was PHAAOs. On the other hand, the abundance of non-PHAAOs in total cells was calculated as the difference between whole acetate utilizing organisms and PHAAOs. Non-PHAAOs accounted for about half of the whole acetate utilizing organisms as shown in Table 2.

**Specific acetate uptake rates**

The specific acetate uptake rates of PHAAOs (q_{PHAAOs}) and non-PHAAOs (q_{non-PHAAOs}) were calculated from Equations (1) and (2), respectively. The values of D\text{Ac}_{PHAAOs}, D\text{Ac}_{AS} and the total cell number are shown in Table 1 and the abundances of PHAAOs and non-PHAAOs are shown in Table 2. The values of q_{PHAAOs} and q_{non-PHAAOs} ranged from 5.3 to 8.0 \times 10^{-10} mgC cell^{-1} h^{-1} and from 2.8 to 4.2 \times 10^{-10} mgC cell^{-1} h^{-1}, respectively, as shown in Table 3. PHAAOs showed a specific acetate uptake rate about twice that of non-PHAAOs.

The specific acetate uptake rates of PHAAOs and non-PHAAOs were calculated from D\text{Ac}_{PHAAOs}, which is the sum of D\text{Ac}_{\text{accumulated}}, D\text{Ac}_{(H)}, and D\text{Ac}_{ATP} as shown in Equation (3). That is, acetate utilized by PHAAOs was calculated by adding acetate consumption for the supply of reducing power and ATP to the amount of acetate converted to PHA. As seen in Equation (4), the sum of D\text{Ac}_{(H)} and D\text{Ac}_{ATP} accounts for about 26% in D\text{Ac}_{PHAAOs}. That is, in the estimation of D\text{Ac}_{PHAAOs}, the omission of D\text{Ac}_{(H)} and D\text{Ac}_{ATP} causes a significant underestimation.

Here, the rationality for the estimation of D\text{Ac}_{(H)} and D\text{Ac}_{ATP} should be discussed. The D\text{Ac}_{(H)} value is determined stoichiometrically from the redox balance of the

Figure 3 | An example of sample observation by MAR after DAPI staining: (left) MAR, (right) DAPI. Scale bar: 10 \mu m.
reaction. As for $\Delta A_{\text{ATP}}$, the amounts of ATP required for the activation of acetate to acetyl-CoA is known $1.0 \text{ mol}_{\text{ATP}} \text{ mol}_{\text{Ac}}^{-1}$ (Gottschalk 1979). On the other hand, it is difficult to evaluate the true value of $\Delta A_{\text{ATP}}$ due to uncertainties in (i) the efficiency of ATP production in microbial cells and (ii) the amount of ATP consumed for acetate transportation across cell membranes. In Equation (8), the authors assumed that the efficiency of ATP production is 3 and 2 mol when 1 mol of NADH or NAD(P)H and 1 mol of FADH are oxidized with oxygen. This efficiency is the theoretical maximum (Madigan et al. 2002), and thus, its uncertainty will result in an underestimation of $\Delta A_{\text{ATP}}$. On the other hand, as for (ii), this uncertainty causes the overestimation of $\Delta A_{\text{ATP}}$. The amount of ATP consumed for acetate transportation across cell membranes ranges between 0.0 and 1.0 mol$_{\text{ATP}}$ mol$_{\text{Ac}}^{-1}$ and increases with an increase of bulk pH (Gottschalk 1979). In the present study, the value of 1.0 mol$_{\text{ATP}}$ mol$_{\text{Ac}}^{-1}$ was adopted because bulk pH during the batch experiment was 8.5–9.0. On the other hand, Filipe et al. (2001) reported the value of 0.29 mol$_{\text{ATP}}$ mol$_{\text{Ac}}^{-1}$ at bulk pH 8.5 for glycogen accumulating organisms. Therefore, our assumption, 1.0 mol$_{\text{ATP}}$ mol$_{\text{Ac}}^{-1}$, might be an overestimation, and thus, this uncertainty will cause an overestimation of $\Delta A_{\text{ATP}}$.

Based on the above discussions, the value of $\Delta A_{\text{ATP}}$ include underestimation and overestimation. On the other hand, it is possible to estimate the possible minimum amounts of $\Delta A_{\text{ATP}}$ 0.0 mol$_{\text{ATP}}$ mol$_{\text{Ac}}^{-1}$ by assuming passive transport of acetate across cell membrane. In this case, the value of $\Delta A_{\text{ATP}}$ shown in Table 1 will decrease by half. Yet, even in this case, $q_{\text{PHAAs}}$ will still remain 1.5 times higher than $q_{\text{non-PHAAs}}$. Therefore, PHAAOs are thought to take up acetate more rapidly than non-PHAAs.

In the next step, it would be needed to establish a mathematical model to explain the competition between PHAAOs and non-PHAAs. For this purpose, the specific acetate uptake rates obtained in the present study needs to be converted from unit cell basis to unit biomass basis. “Unit biomass” here is meant for unit biomass excluding PHA. Actually, the authors attempted to convert cell-specific acetate uptake rate into biomass-specific one by using a conversion rate (mgVSS cell$^{-1}$). This conversion rate can be calculated by dividing MLVSS concentration excluding PHA by the number of total cells in biomass. There was, however, significant difference of cell volume between PHAAOs and non-PHAAs due to the presence of PHA granules inside the cells. It would be necessary to measure the cell volumes of PHAAOs without PHA accumulation and the one of non-PHAAs to obtain a conversion rate and to calculate mass specific acetate uptake rates.

It will also be necessary to anyhow make the mathematical model in a way so that it can explain the coexisting situation of PHAAOs and non-PHAAs. In fact, in the present study, even after four months (equivalent to about 10 SRTs) operation of the SBR, there were significant amount of both PHAAOs and non-PHAAs in the sludge. It will be necessary to measure specific acetate uptake rates

| Abundance of whole acetate utilizing organisms, PHAAOs, and non-PHAAs in total cells |
|---------------------------------|---------------------------------|---------------------------------|
|                                 | 1st batch experiment (%)        | 2nd batch experiment (%)        | 3rd batch experiment (%)        |
| Whole acetate utilizing organisms | 55 ± 5                          | 65 ± 6                          | 61 ± 6                          |
| PHAAOs                          | 25 ± 2                          | 36 ± 4                          | 36 ± 5                          |
| non-PHAAs                       | 30 ± 4                          | 29 ± 8                          | 25 ± 1                          |

Figure 4 | An example of sample observation by dual staining of DAPI and Nile blue A: (left) Nile blue A, (right) DAPI. Scale bar: 10 µm.
of these two groups of microorganisms under different conditions to obtain relevant kinetic parameter values.

CONCLUSIONS

The specific acetate uptake rates of PHAAOs \( \dot{q}_{\text{PHAAOs}} \) and non-PHAAOs \( \dot{q}_{\text{non-PHAAOs}} \) were evaluated by the combination of cold and hot incubation. In calculating the amount of acetate taken by PHAAOs, the amount of acetate used to produce reducing power and ATP for the conversion of acetate into PHA was included, in addition to the amount of acetate that was converted to PHA. Acetate taken up by PHAAOs to produce reducing power and ATP accounted for at least 26% of the acetate utilized by PHAAOs. The abundance of whole acetate utilizing organisms and PHAAOs was enumerated by hot incubation followed by MAR and staining with Nile blue A. Whole acetate utilizing organisms constituted three fifths of the total cells, and about half of the whole acetate utilizing organisms was PHAAOs. The values of \( \dot{q}_{\text{PHAAOs}} \) and \( \dot{q}_{\text{non-PHAAOs}} \) were calculated by dividing the acetate taken up by PHAAOs and non-PHAAOs with their respective abundances. The values of \( \dot{q}_{\text{PHAAOs}} \) and \( \dot{q}_{\text{non-PHAAOs}} \) were \( 5.3-8.0 \times 10^{-10} \text{mgC cell}^{-1} \text{h}^{-1} \) and \( 2.8-4.2 \times 10^{-10} \text{mgC cell}^{-1} \text{h}^{-1} \), respectively. These data set will be helpful for the development of a mathematical model to explain the competition between PHAAOs and non-PHAAOs. Prior to the development of such mathematical model, the specific acetate uptake rates obtained in the present study needs to be converted from unit cell basis to unit biomass basis.

ACKNOWLEDGEMENTS

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REFERENCES


Table 3 | Specific acetate uptake rates of PHAAOs \( \dot{q}_{\text{PHAAOs}} \) and non-PHAAOs \( \dot{q}_{\text{non-PHAAOs}} \)

<table>
<thead>
<tr>
<th>mgC cell (^{-1})h (^{-1})</th>
<th>1st batch experiment</th>
<th>2nd batch experiment</th>
<th>3rd batch experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \dot{q}_{\text{PHAAOs}} )</td>
<td>( 8.10 \times 10^{-10} )</td>
<td>( 7.3 \times 10^{-10} )</td>
<td>( 5.3 \times 10^{-10} )</td>
</tr>
<tr>
<td>( \dot{q}_{\text{non-PHAAOs}} )</td>
<td>( 2.9 \times 10^{-10} )</td>
<td>( 4.2 \times 10^{-10} )</td>
<td>( 2.8 \times 10^{-10} )</td>
</tr>
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