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# Acetic Acid Separation from Anaerobically Treated Palm Oil Mill Effluent by Ion Exchange Resins for the Production of Polyhydroxyalkanoate by *Alcaligenes eutrophus*

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**Separation of acetic acid from palm oil mill effluent (POME) to increase its concentration by an anion exchange resin was examined as a preliminary study for its recovery from POME that had been anaerobically treated by sludge from a palm oil mill. This paper concerns the acetic acid thus separated for producing bacterial polyhydroxyalkanoate (PHA) by *Alcaligenes eutrophus*. It was found that sludge particles in POME strongly inhibited the adsorption of acetic acid on the anion exchange resin. Removing the sludge particles from the POME facilitated the separation of acetic acid from the POME efficiently. The concentrated acetic acid thus obtained from anaerobically treated POME could be used as a substrate in the fed-batch production of polyhydroxyalkanoate by *Alcaligenes eutrophus*.**

**Key words:** acetic acid; ion-exchange separation; palm oil mill effluent; polyhydroxyalkanoates; fed-batch culture

Currently palm oil mill effluent (POME) is mainly treated anaerobically in lagoons, producing biogas that is released into the atmosphere uselessly.[1,2] Apart from contributing to the greenhouse effect, this treatment method wastes large amounts of carbonaceous matter[3,4] that could be used as profitable raw materials. We have previously examined the effects of organic acids from POME on biodegradable plastics or polyhydroxyalkanoate (PHA) production.[5] By maintaining the pH at 7, mainly acetic and propionic acids are produced from POME by the partial anaerobic treatment with sludge. In that research, it was confirmed that more than 7000 ppm of organic acids could be obtained from POME. Instead of wasting large amounts of biological materials from the palm oil industry, a large amount of organic acids could be produced which can be used as raw materials for other industries; thus providing an opportunity to produce something useful from POME. In an earlier report,[6] we have described the use of organic acids produced from POME as raw materials for the continuous production of PHA in a coupled system by the photosynthetic bacterium, *Rhodobacter sphaeroides*.

However, the organic acid concentrations obtained were too low[5] for use as raw materials for the bacterial PHA production on an industrial scale because a much larger size of the PHA production reactor would be required than a reactor for the normal bioplastic production. The concentration of organic acids should be increased. Acetic acid forms a monovalent anion, especially at high pHs, and can be separated by an anion exchange resin. Presently, ion exchange resins are widely used in wastewater treatment and in the food industry.[7]

Once the organic acids are separated in a concentrated form, they could be used for the production of PHA industrially. The bacterial strain used for producing PHA industrially is the *Alcaligenes eutrophus* strain H16, which could accumulate higher than 90% of its dry weight of PHA.[8]

The objective of this study is to separate acetic acid from POME and increase the acid concentration by using a anion exchange resin for use in the fed-batch culture of PHA by the industrial strain of *Alcaligenes eutrophus* to confirm the validity of our strategy to convert the otherwise wasted POME to raw materials for bioplastics production.

## Materials and Methods

*Materials.* Unless otherwise stated, all chemicals were obtained from Wako Pure Chemicals Industry, Osaka.

*Samples.* A 0.25 M acetic acid solution and 0.25 M sodium acetate solution were used. POME was obtained from Bukit Raja Palm Oil Estate Co., Ltd., Klang, Malaysia. Anaerobic sludge was taken from the final pond for wastewater treatment from the same factory. POME was first treated anaerobically by the sludge.[5] POME, sludge, and tap water were mixed in the ratio of 5 : 3 : 2 in a 1 liter bioreactor with the pH controlled at 6.5.[6] Acetic acid was added to the treated POME so that the acid concentration became 0.25 M and 0.5 M because at most 0.5 M acetic acid would be potentially produced judging from the carbonaceous concentration in POME (30,000 ppm BOD sources).[1,2] One M HCl was used for the desorption of the acid.

The treated POME with sludge as well as a clarified supernatant of the POME treated were used for the separation of acetic acid in the POMEs. The clarified supernatant was easily obtained by sodium hydroxide precipitation at pH 12.[9] The sludge easily sedimented and the supernatant was separated by decantation. The pH of the supernatant was not returned to 7.0.

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*Separation and concentration of organic acids.* Breakthrough experiments were done at 25°C to find the amount of acetic acid adsorbed on the anion exchange resin. Chromatographic columns with 1.4 cm inside diameter and 14.7 cm, 32.5 cm, and 67.5 cm in length were used for packing the ion exchange resins. We adopted 1.3 cm $min^{-1}$ of superficial linear velocity of sample solutions. Dowex SBR anion exchange resin (Muromachi Kagaku Kogyo Kaisha, Ltd., Tokyo) with particle sizes between 0.5 and 0.8 mm diameter was used for the separation and concentration of acetic acid. Dowex 88 cation exchange resin (Muromachi Kagaku Kogyo Kaisha, Ltd., Tokyo) with particle sizes between 0.5 and 0.8 mm diameter was also used for the treatment of the sodium acetate and the treated POME to remove sodium ions and reduce the pH.

*Fed-batch culture for PHA production. Alcaligenes eutrophus* strain H16 (ATCC 17699) was used for the fed-batch culture using the concentrated organic acids obtained from POME. The cells were first grown in 30 ml of growth medium consisting of 10 g $l^{-1}$ each of polypeptone and yeast extract, and 5 g $l^{-1}$ each of meat extract and $(NH_4)_2SO_4$, for 20 hours in a shake flask at 30°C before being inoculated in a 150 ml of the same growth medium in a shake flask for another 20 hours. The cells were then centrifuged to obtain the inoculum for the fed-batch bioreactor operation. The aerobic fermentation was done in a stirred-tank Mini Jar Fermentor, Model MAO, supplied by Sanki Seiki Co., Ltd., Tokyo. The medium used was 0.3 g $l^{-1}$ yeast extract, 1.0 g $l^{-1}$ $NaHCO_3$, 0.01 g $l^{-1}$ $CaCl_2$, 0.123 g $l^{-1}$ $MgSO_47H_2O$, 0.024 g $l^{-1}$ $Fe(NH_4)_2(SO_4)_26H_2O$, and 0.1 ml of trace elements solution containing 0.713 g $l^{-1}$ $NiCl_26H_2O$, 0.682 g $l^{-1}$ $CuCl_22H_2O$, 1.33 g $l^{-1}$ $CrCl_36H_2O$, 0.396 g $l^{-1}$ $MnCl_24H_2O$, 0.2 g $l^{-1}$ $CuSO_45H_2O$, 0.863 g $l^{-1}$ $ZnSO_47H_2O$, 0.484 g $l^{-1}$ $Na_2MoO_42H_2O$, and 0.019 g $l^{-1}$ $CoCl_26H_2O$. The initial culture volume was 1.5 liters. The fed-batch PHA production phase was done by the pH-stat method with feeding of acetic acid obtained from POME. Consumption of acetic acid resulted in an increase of pH; thus in controlling the pH at 7.0, acetic acid was automatically fed *via* a peristaltic pump. The rotation speed was set at 400 rpm, with an aeration rate of 0.75 l $min^{-1}$ and the temperature was controlled at 30°C by an internal coil connected to a water bath. Two-ml samples were taken every 2 to 3 hours to measure the dry cell weight, acetic acid and ammonium concentrations in the medium, and PHA content in the cells.

*Assay.* Acetic acid was measured by an HPLC system with an $H^+$ form strong acid ion exchanger (TSK GEL SCX($H^+$), Tosoh, Tokyo), 0.8 cm i.d. and 30 cm long column with 7 mM sulfuric acid as the mobile phase. The acid concentration was also confirmed by UV spectrophotometry at 280 nm.[10,11]

The optical density of the fermentation broth at 660 nm and the values were converted to dry cell weights by using a calibration curve obtained from previous experiments. For the cell PHA content, 1 ml of broth containing the cells were first centrifuged and the supernatant discarded. One ml of concentrated $H_2SO_4$ was then added to break the PHA into its monomers. The mixture was placed at 100°C for 12 hours. The PHA content was then measured by the same HPLC system as mentioned above, and the concentration was obtained by comparison with a PHB standard obtained from Sigma Chemical Company, St. Louis, U.S.A.

Ammonia was measured by an ammonium test kit obtained from Wako Pure Chemical Industry, Osaka, Japan.

## Results

### *Separation of organic acids*

Figure 1 shows a breakthrough curve of 0.25 M acetic acid onto the anion exchange resin. The pH of the acetic acid solution was 2.6. The horizontal and vertical lines are the dimensionless time ($u_0t/Z$, where $u_0$ is the superficial linear velocity, $t$ is the time variable, and $Z$ is the column length) and dimensionless concentration ($C/C_0$, where $C$ is the concentration at time $t$ and $C_0$ is the concentration at the inlet), respectively. Unity at the dimensionless time indicates the time required for the column volume of the effluent to flow out. The arrow in Fig. 1 indicates the time at which 1.0 M HCl was introduced to desorb the adsorbed acetic acid. It is seen that 0.25 M acetic acid is strongly adsorbed on the anion exchange resin and desorbed from

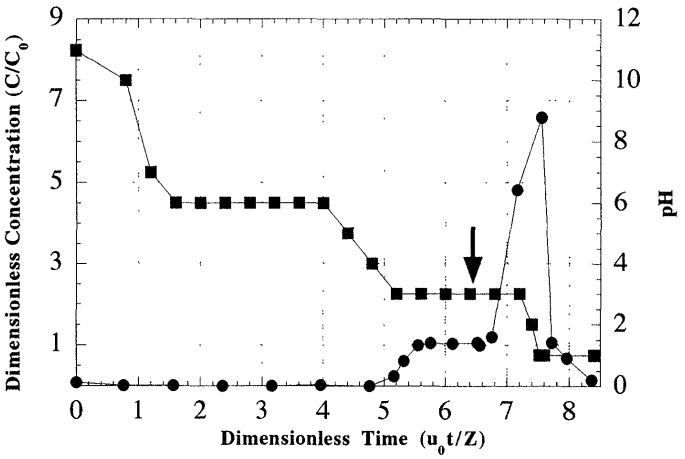


**Fig. 1.** Breakthrough Curve for 0.25 M Acetic Acid (pH 2.6) in Dowex SBR Anionic-exchange Column.

$u_0 = 1.3$ cm $min^{-1}$; $Z = 32.5$ cm.
●, dimensionless concentration; ■, pH.

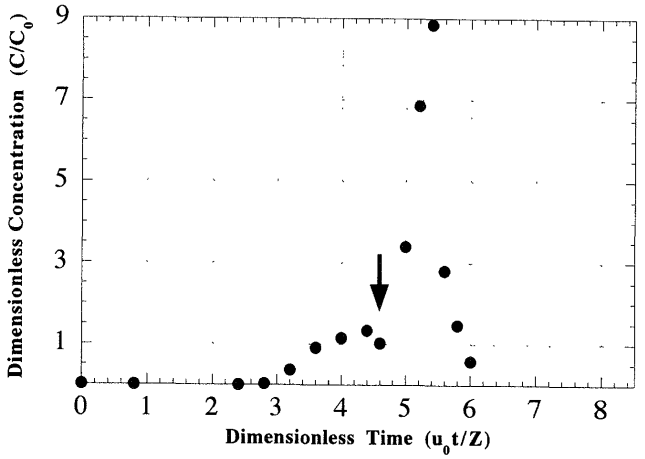


**Fig. 2.** Breakthrough Curve for 0.25 M Sodium Acetate (pH 11.6) in Dowex SBR Anionic-exchange Column.

$u_0 = 1.3$ cm $min^{-1}$, $Z = 32.5$ cm.

the resin to obtain more than 1.0 M concentrated acetic acid. Figure 2 shows the breakthrough curve of 0.25 M of sodium acetate solution at pH 11.6. Sodium acetate is formed in a clarified supernatant POME because sludge is removed from anaerobically treated POME by supplying NaOH. The arrow in the figure also indicates a position where 1.0 M HCl was added to desorb the acetic acid. Arrows in the following figures have the same meanings. Comparing Figs. 1 and 2, it is found that less acetic acid was adsorbed on the resin when it was applied in the form of sodium acetate. Sodium acetate was then treated by a cation exchange resin to remove the sodium ions, which also resulted in reduction of pH from 11.6 to 2.6. When this solution was introduced into the anionic exchange column, the amount of acetic acid adsorbed on the resin was increased, as shown in Fig. 3, to a similar level to that in Fig. 1.

On the other hand, when 0.25 M acetic acid in the treated POME containing sludge was separated by the anion exchange resin, very little acetic acid was adsorbed on the resin as shown in Fig. 4 which indicates that a much shorter time for the breakthrough of acetic acid is required compared with those from Figs. 1 to 3. The pH of the treated POME without sludge was very high at around 12 because the sludge was removed by NaOH sedimentation.

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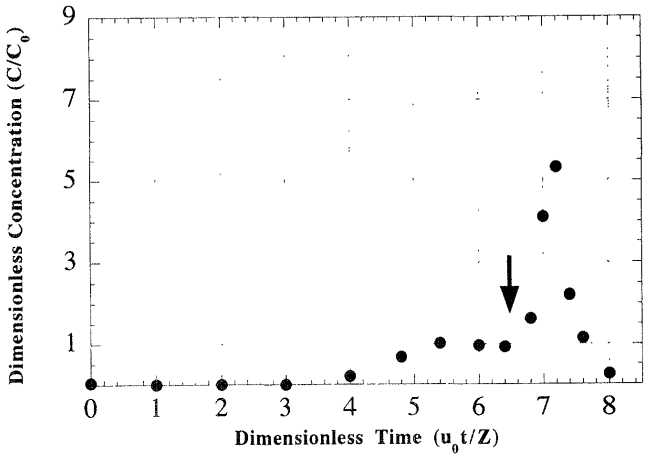


**Fig. 3.** Breakthrough Curve for 0.25 M Sodium Acetate (pH 11.6) Subjected to Dowex SBR Anionic-exchange Column after Separation by Dowex 88 Cationic-exchange Column.

$u_0 = 1.3\,\mathrm{cm\,min^{-1}}$; $Z = 32.5\,\mathrm{cm}$.

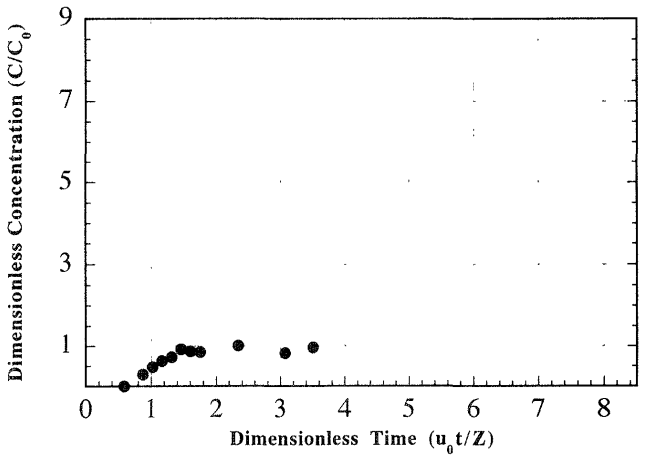


**Fig. 4.** Separation of 0.25 M Acetic Acid in Treated POME Containing Sludge in Dowex SBR Anionic-exchange Column.

$u_0 = 2.2\,\mathrm{cm\,min^{-1}}$, $Z = 14.7\,\mathrm{cm}$.

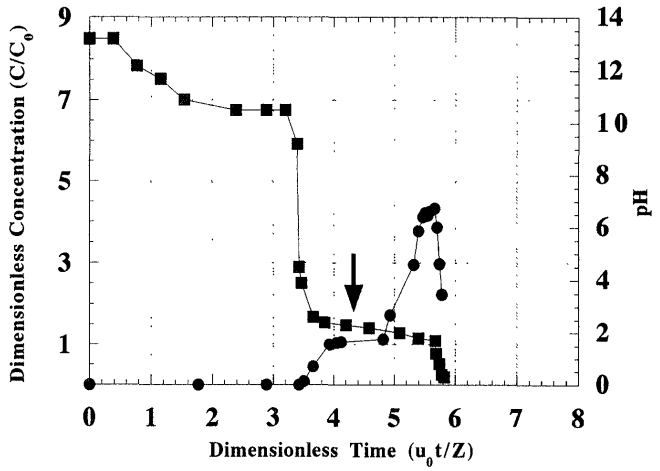


**Fig. 5.** Separation of 0.5 M Acetic Acid in Treated POME without Sludge in Dowex SBR Anionic-exchange Column after Removal of Sodium Ions in Dowex 88 Cationic-exchange Column.

$u_0 = 2.6\,\mathrm{cm\,min^{-1}}$; $Z = 67.5\,\mathrm{cm}$.
●, dimensionless concentration; ■, pH.

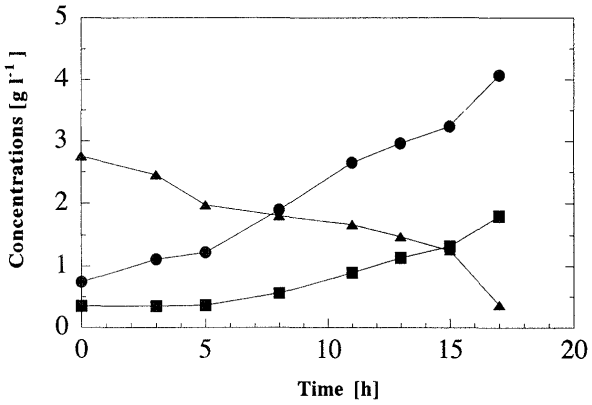


**Fig. 6.** Production of PHA by *A. eutrophus* Using Concentrated Acetic Acid Separated from POME.

●, cell density; ■, PHA conc.; ▲, acetic acid conc.

The POME was, therefore, pre-treated by the cation exchange resin to remove sodium cations and reducing the pH at a low level. Then, 0.5 M acetic acid from anaerobically treated POME was separated to obtain acetic acid for the subsequent PHA production by *Alcaligenes eutrophus*. A long column was used to obtain enough acetic acid for the fed-batch culture. Figure 5 shows the breakthrough curve of 0.5 M acetic acid in the POME. It was found that acetic acid was successfully recovered from the POME and concentrated to yield 89 ml of 1.5 M acetic acid.

*Fed-batch production of PHA*

The time course of the PHA production stage is shown in Fig. 6, using acetic acid separated from anaerobically treated POME shown in Fig. 5. Acetic acid levels were maintained quite low between 1.5 to 2.5 g l$^{-1}$ for most part of the culture by the pH-stat control to avoid substrate inhibition. Cell dry weight increased from 0.8 to 4.0 g l$^{-1}$ within the 17 hours of the culture course, with a maximum of 1.8 g PHA l$^{-1}$ (initial PHA concentration; 0.3 g l$^{-1}$). This corresponds to 45% of bacterial dry cell weight. Finally the volume of the medium attained 1.7 liters. Since 8 g of acetic acid was added in total, the yield of PHA from acetic acid is 0.32. The overall volumetric productivity of PHA is etimated as 0.09 g-PHA l$^{-1}$ h$^{-1}$. Ammonium concentrations remained low below 0.3 g l$^{-1}$ throughout the culture as is required for the PHA production phase.[12,13]

## Discussion

Figures 1 and 2 indicate that more acetic acid could be adsorbed on an anion exchange resin when the pH of the acetic acid solution was low. In the anion exchange process of acetic acid the following anion exchanges are mainly completing:

$$R^+ + CH_3COOH = R^+\text{–}CH_3COO^- + H^+$$
$$R^+ + CH_3COONa = R^+\text{–}CH_3COO^- + Na^+$$
$$R^+ + NaOH = R^+\text{–}OH^- + Na^+$$

where $R^+$ is the ion exchange resin. At a high pH obtained by adding NaOH, more hydroxide ions occupy the adsorptive sites on the anion exchange resin because the equilibrium of $OH^-$ shifted to the resin side at the pH, resulting in less acetic adsorbed on the resin at high pHs.

When acetic acid in the treated POME with sludge was separated by an anion exchange resin, very little acetic was

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adsorbed on the resin. Since sludge particles are negatively charged and would be adsorbed on the anion exchange resin, the pores in the resin particles would be blocked by the sludge particles and fewer acetate ions could penetrate into the resin pores. Therefore, it is necessary to remove the sludge particles from the treated POME to separate and concentrate acetic acid from it.

As shown in Fig. 6, the concentrated acetic acid obtained from treated POME could be used in the fed-batch culture of *Alcaligenes eutrophus* for producing PHA. About 45% PHA content in the dry cells could be obtained, corresponding to a yield of 0.32 from acetic acid. The overall volumetric productivity of PHA is estimated as 0.09 g PHA $l^{-1} h^{-1}$. The PHA content is comparable with the batch, and fed-batch PHA production by *Alcaligeneses*,[14-18] where the PHA contents were from 18% to 76%. However, the overall PHA productivity obtained here was far less than those obtained by others[14-18] (around 0.5–3 g PHA $l^{-1} h^{-1}$), due to the low cell concentration. This indicates that our strategy is confirmed to be effective if a high density culture is realized using organic acids from POME; acetic acid produced anaerobically in POME is separated by an anion exchange resin to be a raw material for the PHA production.

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