

Evaluating Carriers for Immobilizing *Saccharomyces cerevisiae* for Ethanol Production in a Continuous Column Reactor

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Abstract We evaluated a more practical and cost-effective immobilization carriers for ethanol production using the yeast *Saccharomyces cerevisiae*. Three candidate materials—rice hull, rice straw, and sawdust—were tested for their cell-adsorption capacity and operational durability. Derivatizations of rice hull, rice straw, and sawdust with the optimal concentration of 0.5 M of 2-(diethylamino)ethyl chloride hydrochloride (DEAE · HCl) resulted in > 95% adsorption of the initial yeast cells at 2 hr for DEAE-rice hull and DEAE-sawdust and in only approximately 80% adsorption for DEAE-rice straw. In addition, DEAE-sawdust was found to be a more practical carrier for immobilizing yeast cells in terms of operational durability in shaking flask cultures with two different speeds of 60 and 150 rpm. Furthermore, the biosorption isotherms of DEAE-rice hull, -rice straw, and -sawdust for yeast cells revealed that the Q_{\max} of DEAE-sawdust (82.6 mg/g) was greater than that of DEAE-rice hull and DEAE-rice straw. During the 404-hr of continuous column reactor operation using yeast cells immobilized on DEAE-sawdust, no serious detachment of the yeast cells from the DEAE-sawdust was recorded. Ethanol yield of approximately 3.04 g/L was produced steadily, and glucose was completely converted to ethanol at a yield of 0.375 g-ethanol/g-glucose (73.4% of the theoretical value). Thus, sawdust is a promising practical immobilization carrier for ethanol production, with significance in the production of bioethanol as a biofuel.

Keywords Continuous column reactor, Ethanol production, Immobilization carrier, *Saccharomyces cerevisiae*, Sawdust

Several studies have been conducted on yeast immobilization carriers for ethanol production by using immobilized reactors, particularly for the yeast *Saccharomyces cerevisiae* [1-5]. Operational cost, durability, and availability are crucial factors for selecting an appropriate carrier. Past studies have compared some immobilization methods in terms of ethanol production in a reactor system [3-5].

Among agricultural waste materials, sorghum bagasse [6], sugarcane pieces [7], orange peel [8], apple bagasse [9], corncobs [10, 11], and sawdust [12] have been reported as immobilization carriers for *S. cerevisiae* because of their low price and high availability. In our previous studies, we investigated corncob and cotton as carriers for immobilization of *S. cerevisiae* in ethanol production [13, 14]. However, to further decrease the cost of production of ethanol, it is necessary to explore other carrier options to immobilize yeast cells.

In this study, we analyzed three carrier candidates, namely, rice hull, rice straw, and sawdust, which are abundant and locally available compared to other similar carriers such as corncob and cotton. These three materials have been investigated as feedstock for ethanol production [15] and even as adsorbents for removing metal ions [16-18].

Rice hull, rice straw, and sawdust were delignified and then derivatized with 2-(diethylamino)ethyl chloride hydrochloride (DEAE · HCl) to improve the adsorption of yeast cells onto the carrier. The explanation for performing DEAE · HCl derivatization has been described in detail in a previous study [13]. Next, the maximum cell adsorption by the carrier (Langmuir constant, Q_{\max} ; mg/g) was calculated based on the Langmuir biosorption model proposed by

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Vijayaraghavan and Yun [19]. We then selected the carrier with the highest Q_{\max} for use in a continuous column reactor for ethanol production. We found that the continuous column reactor could be effectively used for continuous ethanol production using the selected carriers for *S. cerevisiae* immobilization.

MATERIALS AND METHODS

Chemicals. DEAE·HCl and dinitrosalicylic acid (DNS) were purchased from Acros (Rockford, IL, USA) and Lancaster (Cambridge, England), respectively. Celite 545 was purchased from Yakuri Pure Chemicals Co. (Kyoto, Japan). The yeast extract and peptone were obtained from Becton Dickinson (Franklin Park, NJ, USA). Corn-steep liquor (CSL) was kindly supplied by Samyang Genex Corp. (Seoul, Korea). All the other chemicals were of reagent grade.

Yeast cells. The yeast strain *S. cerevisiae* ATCC 24858 (*MATa/MAT α* , *trp1*, *his3*, *leu2*, *ura3*) was cultivated in a 500-mL Erlenmeyer flask containing 100-mL yeast extract-peptone-dextrose (YPD) medium (10 g/L yeast extract, 20 g/L peptone, and 20 g/L glucose) kept in a shaking incubator at 30°C and 150 rpm.

Rice hull, rice straw, and sawdust. Rice hull, rice straw, and sawdust were obtained from Jeupyung, Chungbuk, Korea. Rice straws were chopped to 0.5-cm lengths before delignification and DEAE·HCl derivatization.

DEAE·HCl derivatization. Rice hull, rice straw, and sawdust were delignified as per the method described previously [13]. The delignified materials were soaked overnight in 18% NaOH at 4°C and then derivatized with 0.125–1.0 M DEAE·HCl at room temperature for 1 hr. The ratio of delignified rice hull, rice straw, and sawdust to DEAE·HCl solution was 1 : 50 (w : v). The DEAE-derivatives of delignified rice hull, rice straw, and sawdust (DEAE-rice hull, DEAE-rice straw, and DEAE-sawdust, respectively) were soaked in distilled water for 1 hr, and the water was decanted to immobilize the cells.

Adsorption kinetic experiment. Three grams each of DEAE-rice hull, -rice straw, and -sawdust were used for the adsorption kinetic experiment. Yeast cell suspension (10 mL) was prepared in YPD medium. For the adsorption kinetic experiment, the yeast suspension was added to a 50-mL conical tube containing the DEAE derivatives and placed on a shaking plate at 60 rpm, with intermittent measurement of the optical density (OD_{600}) of the cell suspension. All the adsorption kinetic experiments were conducted at room temperature.

Shake flask culture using immobilized *S. cerevisiae*. *S. cerevisiae* was immobilized on 3.0 g each of DEAE-rice

hull, -rice straw, and -sawdust separately, and these yeast-immobilized carriers were added to 250-mL Erlenmeyer flask containing 40 mL of YPD medium and kept on a shaking incubator with 60 or 150 rpm speed and 30°C.

Batch experiment for the biosorption isotherms.

Three grams each of DEAE-rice hull, -rice straw, and -sawdust were added to an Econo-Pac polypropylene column (14-cm height \times 1.5-cm ID; Bio-Rad, Hercules, CA, USA). For the experiments, 10 mL of yeast suspensions at different OD_{600} were added to the top of the column. The eluent was collected and its OD_{600} value was measured. All the adsorption experiments were conducted at room temperature.

Vijayaraghavan and Yun [19] elucidated the principle of biosorption in a bacterial system as per the Langmuir model. The following principle was adopted: Yeast cell adsorption by a carrier (e.g., DEAE-rice hull, -rice straw, and -sawdust) can be calculated from the difference between the initial quantity of cells added to the medium and the quantity of cells present in the eluent by using the following equation:

$$Q = \frac{V_o C_o - V_f C_f}{M} \quad (1)$$

, where, Q is cell adsorption by the carrier (mg/g), C_o and C_f are the initial cell concentration and the cell concentration in the eluent (mg/mL), respectively, V_o and V_f are the initial and flow-through volumes (mL), respectively, and M is the mass of the carrier (g).

The Langmuir model can be represented as:

$$Q = \frac{Q_{\max} b_L C_f}{1 + b_L C_f} \quad (2)$$

The Langmuir constant Q_{\max} (the maximum cell adsorption by the carrier) is often used to compare biosorbent performances. Q_{\max} was estimated by plotting $1/Q$ versus $1/C_f$ for the following reciprocal equation of Equation (2).

$$\frac{1}{Q} = \frac{1}{Q_{\max} b_L} \cdot \frac{1}{C_f} + \frac{1}{Q_{\max}} \quad (3)$$

In addition, adsorption kinetic experiments were conducted for DEAE-corn cob and Celite (diatomaceous earth) to compare the biosorbent performances of the DEAE-derivatives of rice hull, rice straw, and sawdust using other carriers.

Continuous column reactor for immobilized *S. cerevisiae*.

DEAE-sawdust (16 g) was added to the column reactor (glass column; 10-cm height \times 5.0-cm ID; Bio-Rad) and 200 mL of *S. cerevisiae* suspension prepared in YPD medium ($OD_{600} = 5.0$) was passed through the column reactor twice. After *S. cerevisiae* was immobilized on the DEAE-sawdust, 200 mL of YPD medium was circulated at a flow rate of 1.0 mL/min in a recycling mode [13]. After recycling, YCD medium (20 g/L CSL, 5 g/L yeast extract, 1.2 g/L $(NH_4)_2SO_4$, 2.4 g/L KH_2PO_4 , 1.2 g/L $MgSO_4 \cdot 7H_2O$, and

glucose) was supplied continuously at 0.69 mL/min to the reactor for ethanol production. During the operation, eight batches of YCD medium were prepared, with an average glucose concentration of 8.1 ± 1.1 g/L ($n = 8$). The entire reactor system was operated in a 30°C incubator. Industrial-grade glucose and yeast extract were used.

Electron microscopy. The yeast cell-immobilized DEAE-sawdust was washed with deionized water and dried for 24 hr at 60°C [13, 14], followed by observations under scanning electron microscope (SEM; FEI Quanta 400; FEI, Hillsboro, OR, USA).

Analytical methods. Yeast cell concentration was estimated by measuring the suspension at OD_{600} by using a spectrophotometer (Spectronic; Thermo Scientific, Rockford, IL, USA). If necessary, the dry cell weight was estimated by using a calibration curve generated on the basis of dry cell weight versus OD_{600} value. The residual glucose concentration was measured by the DNS method [20] or by quantitative thin layer chromatography [21]. Ethanol concentration was measured by gas chromatography, as described previously [22].

RESULTS AND DISCUSSION

Adsorption of *S. cerevisiae* on DEAE-rice hull, -rice straw, and -sawdust. We conducted the adsorption experiment using *S. cerevisiae* (initial $OD_{600} = 8.0$) on a shaking plate reaction of 0.125–1.0 M DEAE·HCl, with 3.0 g of delignified rice hull, rice straw, and sawdust to investigate the optimal concentration for DEAE·HCl derivatization (Fig. 1). When the delignified materials were derivatized with 0.5 M DEAE·HCl, >80% of the initial cells were adsorbed on the DEAE-derivatives after 1 hr. Although complete adsorption was not observed at any concentration, 0.5 M DEAE·HCl was determined to be the optimal concentration of DEAE·HCl for derivatization, which is the same as that of corncob used for derivatization in our earlier study [13].

To accomplish complete adsorption of yeast cells, the initial OD_{600} value of the yeast cell suspension was decreased from 8.0 to 4.0 and 6.0, the same amounts of DEAE-derivatives were added, and 0.5 M DEAE·HCl was used for derivatization (Fig. 2). Due to the decrease in the initial OD_{600} value of yeast suspension, DEAE-rice hull and DEAE-sawdust adsorbed >95% of the initial cells at 2 hr, whereas DEAE-rice straw adsorbed only approximately 80% of the initial cells. In addition, we observed almost complete adsorption of the yeast cells after >3 hr on DEAE-rice hull and DEAE-sawdust. In other words, DEAE-rice hull and DEAE-sawdust adsorbed more yeast cells than DEAE-rice straw. When the OD_{600} value of the initial yeast suspension was decreased to <4.0 with DEAE-rice straw, the yeast cells may be adsorbed completely on the DEAE-rice straw. Thus, we tentatively concluded that

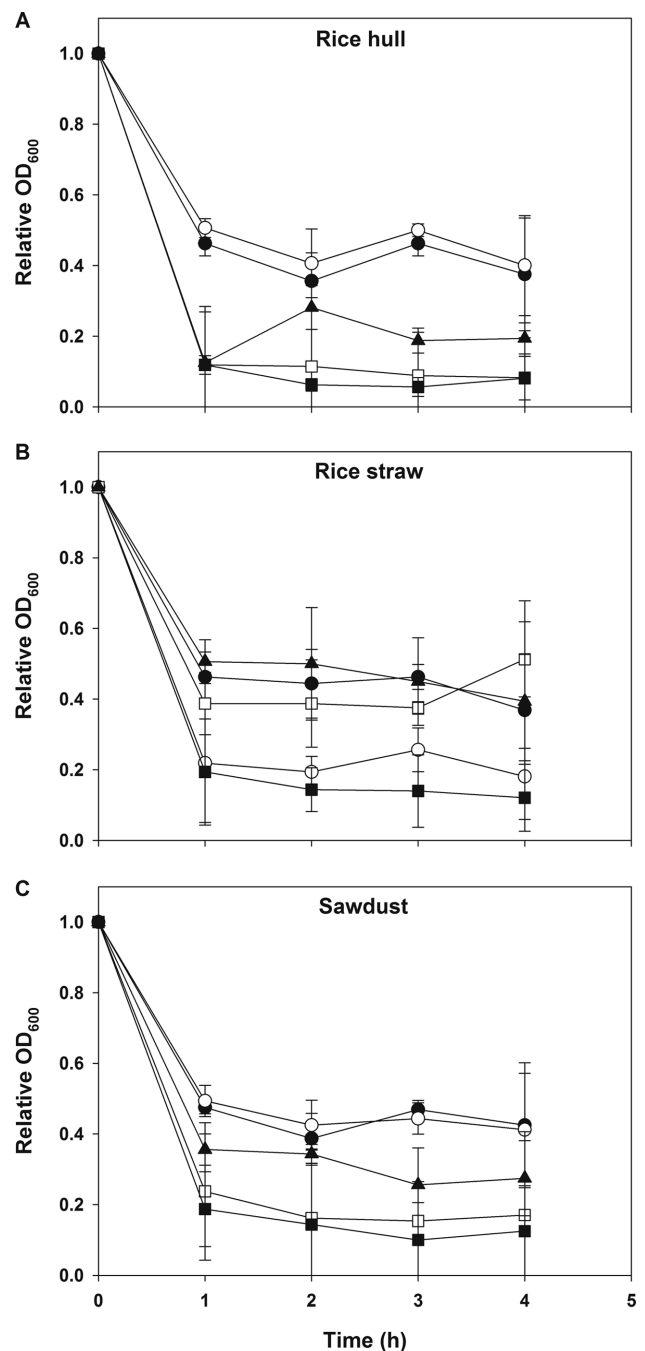


Fig. 1. Optimal concentration of DEAE·HCl for derivatization of rice hull (A), rice straw (B), and sawdust (C) for adsorption of yeast cells. DEAE·HCl was used at concentrations of 0.125 M (▲), 0.25 M (□), 0.5 M (■), 0.75 M (○), and 1.0 M (●), respectively. Three grams each of delignified rice hull, rice straw, and sawdust were derivatized. For adsorption of yeast cells, a 10-mL yeast cell suspension ($OD_{600} = 8.0$) was added to a 50-mL conical tube containing DEAE-rice hull, -rice straw, and -sawdust. The relative OD_{600} values were normalized to the values measured at time zero, in which the relative OD_{600} values were calculated based on an initial OD_{600} value of 1.0. Triplicate samples were used for OD_{600} measurement, and the average and standard deviation of relative OD_{600} values were calculated.

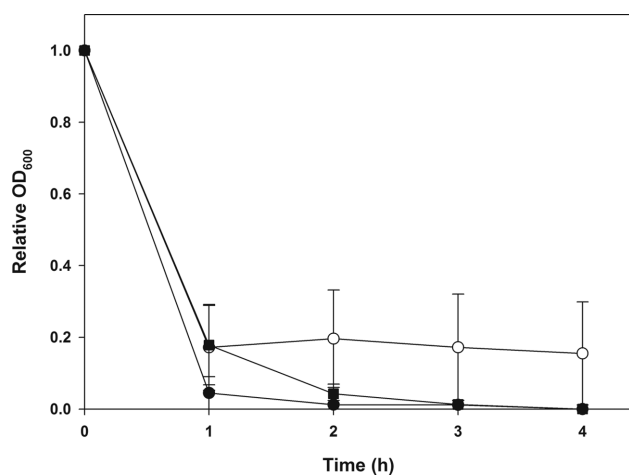


Fig. 2. Adsorption kinetics of yeast cells on DEAE-rice hull (●), -rice straw (○), and -sawdust (■). Three grams each of delignified rice hull, rice straw, and sawdust and 0.5 M DEAE · HCl were used for derivatization. Two different yeast cell suspensions were prepared, with the initial OD_{600} values of 4.0 and 6.0. After the adsorption kinetic experiments were conducted using each cell suspension, the relative OD_{600} values were calculated as described in Fig. 1. The average and standard deviation were calculated for each relative OD_{600} values. Triplicate samples were used for the OD_{600} measurements.

DEAE-rice hull and DEAE-sawdust were more preferable carrier candidates for yeast cell immobilization.

In our previous study [13], we had observed that, when 3.0 g delignified corncob grit was derivatized with 0.5 M DEAE · HCl, the yeast cell suspension of $OD_{600} = 3.0$ was adsorbed at > 90% of the initial concentration. Comparing the results of our previous and the present studies, we conclude that DEAE-rice hull and DEAE-sawdust are comparatively more efficient carriers than corncob grit in terms of yeast cell adsorption.

Shake flask culture using immobilized yeast cells.

To investigate the durability of immobilizing yeast cells onto the carriers, we conducted shake flask cultures at two different shaking speeds of 60 and 150 rpm (Fig. 3). The yeast cell growth in the culture medium was estimated to determine the growth of detached cells from the carriers with respect to the time elapsed after culturing. In the shake flask culture at 60 rpm, the detached cell growth using the three DEAE-derivatives reached an OD_{600} of approximately 2.0 during culturing (Fig. 3A). However, higher growth of detached yeast cells was observed in the shake flask culture at 150 rpm using the DEAE-derivatives, except DEAE-sawdust (Fig. 3D). For yeast cells immobilized on DEAE-sawdust, the OD_{600} of the culture broth was maintained steadily at approximately 2.0. Thus, from among the three derivatives, DEAE-sawdust was found to be the most practical carrier to immobilize yeast cells, in terms of its adsorption capacity and operational durability.

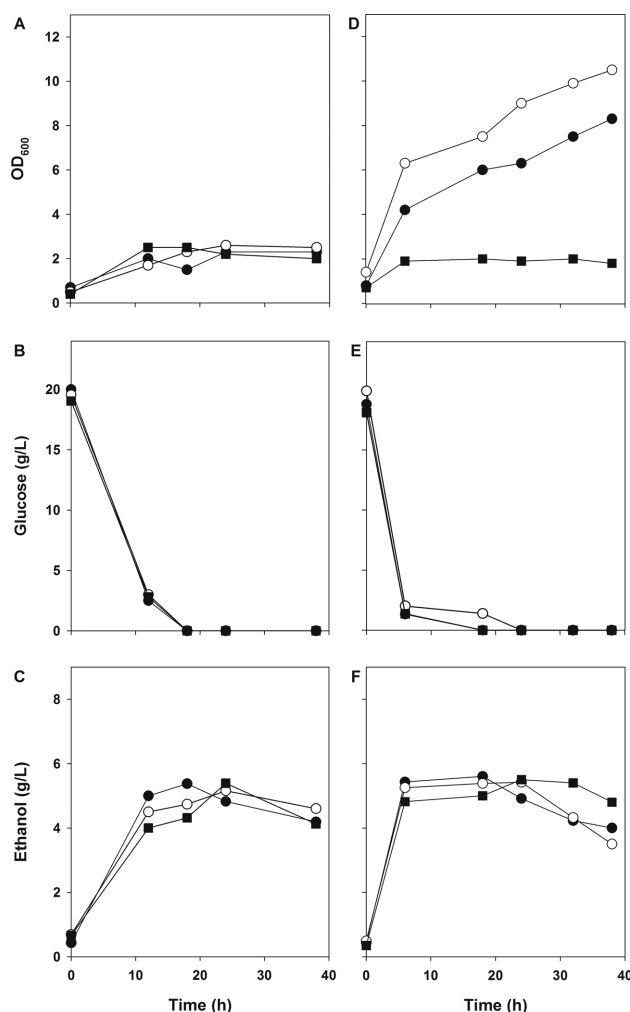


Fig. 3. Shake-flask culturing was performed for yeast cells immobilized on DEAE-rice hull (●), -rice straw (○), and -sawdust (■). A, D, Cell growth in culture medium; B, E, Residual glucose concentration (DNS method); C, F, Ethanol production. Shaking speed was (A~C) 60 rpm and (D~F) 150 rpm, respectively.

Biosorption isotherms of DEAE-rice hull, -rice straw, and -sawdust. We conducted a batch experiment to assess the biosorption isotherms and to estimate the Q_{max} values of DEAE-rice hull, -rice straw, and -sawdust. This experiment was also conducted using DEAE-corn cob and Celite for comparison purpose. DEAE-corn cob was previously

Table 1. Q_{max} values and linear regression coefficients from reciprocal plots of the biosorption isotherm of *Saccharomyces cerevisiae*, represented by the Langmuir model

	Q_{max} (mg/g)	Regression coefficients (r^2)
DEAE-rice hull	65.8	0.917
DEAE-rice straw	32.2	0.980
DEAE-sawdust	82.6	0.901
DEAE-corn cob	25.6	0.849
Celite	174.4	0.960

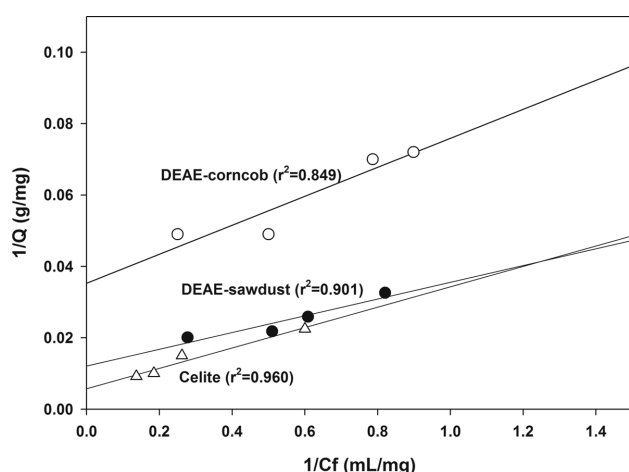


Fig. 4. Reciprocal plots of the biosorption isotherms of *Saccharomyces cerevisiae* on DEAE-sawdust (\triangle), DEAE-corn cob (\circ), and Celite (\bullet), represented by the Langmuir model. Linear regression coefficients are shown in parenthesis.

investigated as a yeast carrier [10, 11, 13] and Celite as an immobilization carrier [23, 24]. As shown in Table 1, the Q_{\max} of DEAE-sawdust (82.6 mg/g) was greater than that of DEAE-rice hull (65.8 mg/g) and DEAE-rice straw (32.2 mg/g). The Q_{\max} of DEAE-corn cob was found to be 25.6 (mg/g), which is almost the same as that estimated in our previous study [13]. In particular, the Q_{\max} value of Celite was the greatest at 174.4 mg/g. However, Celite was excluded as a candidate carrier because it can clog the continuous column reactor due to its fine particle size (20–100 μm).

We could not compare the Q_{\max} values for *S. cerevisiae* with those reported in earlier studies because of the lack of studies reporting the Q_{\max} of biosorption isotherms for immobilized yeast cells. We compared the Q_{\max} values of DEAE-rice hull, -rice straw, and -sawdust to assess their adsorption capacity, and concluded that DEAE-sawdust was the best choice for the immobilization of the yeast cells. Fig. 4 shows the reciprocal plots of *S. cerevisiae* biosorption isotherms on DEAE-sawdust, DEAE-corn cob, and Celite to estimate the Q_{\max} values.

Operation of the continuous column reactor packed with immobilized yeast cells.

Based on the results given in Figs. 2 and 3 and Table 1, we concluded that DEAE-sawdust was the best carrier for immobilizing *S. cerevisiae*. Using this immobilization system, the continuous column reactor was operated for continuous ethanol production. The optimal operation conditions with respect to the amount of carrier material and the medium flow rate for the continuous column reactor using DEAE-corn cob for ethanol production were investigated previously [14]. In addition, a 24-hr-long operation yielded an average ethanol concentration of approximately 4.6 g/L in the effluent. The 10.5 g/L of glucose added to the feeding medium was completely consumed by the end of the

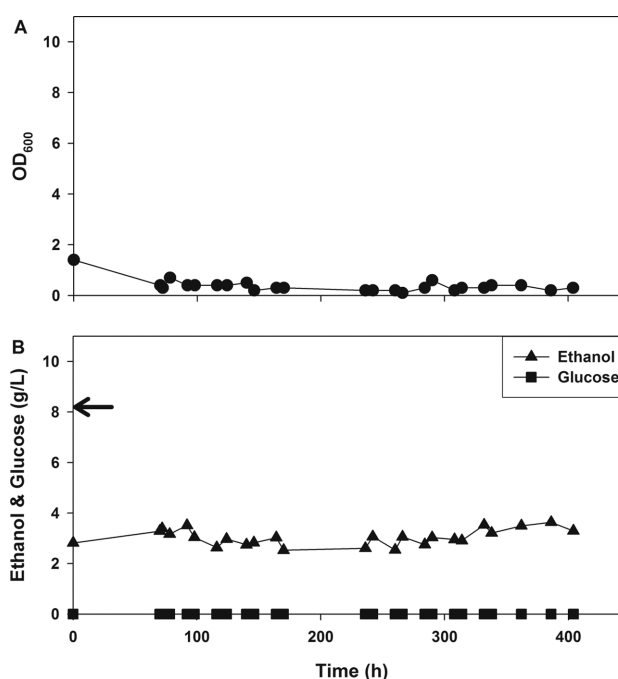


Fig. 5. Operation of continuous column reactor packed with yeast cells immobilized on DEAE-sawdust. A, Cell concentration; B, Residual glucose (\blacksquare) (quantitative thin layer chromatography), and (\blacktriangle) ethanol concentration of the effluent. DEAE-sawdust (16 g) was used as the immobilization carrier. The glucose concentration in the medium reservoir (YCD medium) was 8.1 g/L (average; indicated by the arrow in panel B and the medium flow rate was 0.69 mL/min).

reaction. Particularly, in this study, we used DEAE-sawdust as the carrier for yeast cell immobilization. The continuous column reactor was packed with 16 g DEAE-sawdust and continuously fed with YCD medium (glucose = 8.1 ± 1.1 , g/L) at the rate of 0.69 mL/min. The relationship between the amount of carrier packed in a column and the medium feeding rate was determined previously [14].

As shown in Fig. 5A, no detachment of yeast cells was observed from the DEAE-sawdust during a continuous operation of 404 hr. The residual glucose concentration in the effluent was not measured (Fig. 5B), and the ethanol concentration in the column reactor effluent was maintained steadily at an average of 3.04 ± 0.32 g/L ($n = 25$) (Fig. 5B). The glucose fed to the reaction medium was completely converted to ethanol with a yield of 0.375 g-ethanol/g-glucose (73.4% of the theoretical value). Use of DEAE-corn cobs as the immobilization carrier in our previous study yielded 0.438 g-ethanol/g-glucose [14]. In other words, the ethanol yield was approximately 14% less with DEAE-sawdust than with DEAE-corn cob. However, the ethanol yield using DEAE-sawdust showed higher Q_{\max} value, suggesting that higher ethanol yield is not related to higher Q_{\max} value. Alternatively, it was deduced that the higher Q_{\max} value of the DEAE-sawdust may result in improved ethanol productivity during the operation of a

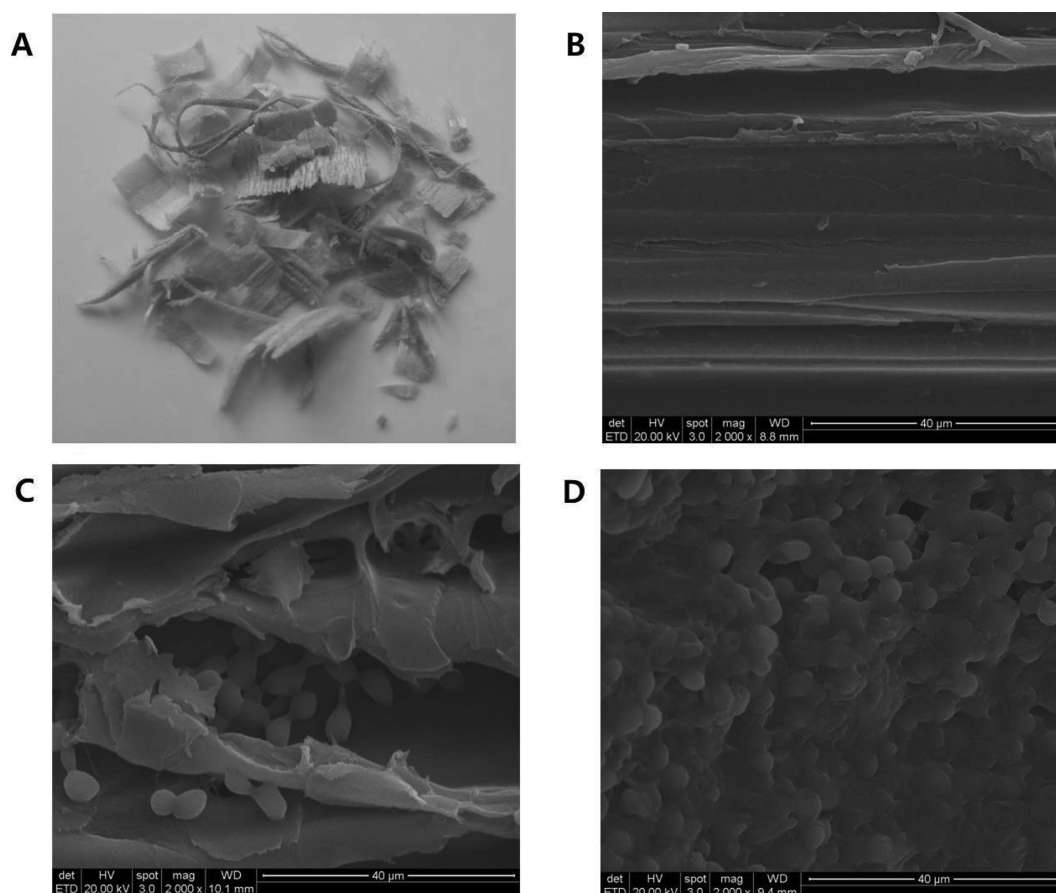


Fig. 6. Photograph of sawdust (A) and electron micrographs of sawdust (B) and yeast cells (C, D) immobilized on DEAE-sawdust. Samples for observation were collected at 48 hr (the early hours of operation) (C) and 404 hr (the last hours of the operation) (D) ($\times 2,000$).

continuous column reactor. It is probable that more immobilization of yeast cells on DEAE-sawdust increased the medium feeding rate, which may be attributed to the increased ethanol productivity. However, when a higher number of yeast cells were immobilized on the carrier, a greater portion of the immobilized cells faced hindered nutrient access and/or ethanol inhibition. After completion of the continuous operation, the yeast cells adsorbed onto DEAE-sawdust were observed under SEM (Fig. 6). Consequently, the final density of the yeast cells attached to the DEAE-sawdust was found to be greater than the initial density.

In conclusion, we successfully conducted a 404-hr continuous operation using immobilized *S. cerevisiae* and obtained a 3.04 g/L ethanol in the effluent (16.72 L). We conclude that sawdust can be practically used as an immobilization carrier for ethanol production. Because sawdust is a waste of the wood industry, it is a cheap and abundant carrier material, making its use more practical in terms of reducing the operation cost and ease in ethanol production. Moreover, if the sawdust derivatization process with DEAE leads to an increase in the total process cost, sawdust without DEAE-derivatization may also be used to

immobilize the yeast cells. However, in the latter case, more cell recycling for continuous medium feeding and more time will be needed to obtain a dense cell growth on the sawdust surface.

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