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ORIGINAL ARTICLE

Ethanol production potential from fermented rice noodle wastewater treatment using entrapped yeast cell sequencing batch reactor

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Abstract Fermented rice noodle production generates a large volume of starch-based wastewater. This study investigated the treatment of the fermented rice noodle wastewater using entrapped cell sequencing batch reactor (ECSBR) compared to traditional sequencing batch reactor (SBR). The yeast cells were applied because of their potential to convert reducing sugar in the wastewater to ethanol. In present study, preliminary treatment by acid hydrolysis was performed. A yeast culture, *Saccharomyces cerevisiae*, with calcium alginate cell entrapment was used. Optimum yeast cell loading in batch experiment and fermented rice noodle treatment performances using ECSBR and SBR systems were examined. In the first part, it was found that the cell loadings (0.6–2.7 $\times$ 10^8 cells/mL) did not play an important role in this study. Treatment reactions followed the second-order kinetics with the treatment efficiencies of 92–95%. In the second part, the result showed that ECSBR performed better than SBR in both treatment efficiency and system stability perspectives. ECSBR maintained glucose removal of 82.5 $\pm$ 10% for 5-cycle treatment while glucose removal by SBR declined from 96 to 40% within the 5-cycle treatment. Scanning electron microscopic images supported the treatment results. A number of yeast cells entrapped and attached onto the matrix grew in the entrapment matrix.

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Introduction

Fermented rice noodle is one of the most favorite foods in many countries especially in south-eastern Asian countries. To produce the noodle, a large volume of wastewater containing high concentration of complex organic compound (starch) has been generated. The fermented rice noodle production businesses typically are local and in small scale. As a result, the manufacturers normally lack of money, knowledge, and technology to taking care this problem. It was found that the wastewater was discharged to sewer system or receiving water without treatment in many cases (Siripattanakul et al. 2010b). Consequently, it creates the surge of oxygen demand in receiving waters.

In petroleum deficiency situation, bio-ethanol from yeast fermentation has become a promising alternative source for fuel. Agricultural and industrial wastes containing sugar, starch, and cellulose, such as cassava peels, fruit bunches, and the effluents from sugar and pineapple cannery productions were successfully applied for the bio-ethanol production (Khan et al. 1994; Nigam 2000; Patle and Lal 2008; Ibeto et al. 2011; Kassim et al. 2011). In this context, the fermented rice noodle wastewater sounded potential for the fuel production as well. Moreover, the yeast fermentation could preliminarily treat the wastewater before traditional wastewater treatment system. In a previous work, the fermented rice noodle wastewater treatment by the yeast in batch test was preliminarily investigated (Siripattanakul et al. 2010b). It was found that

the yeast fermentation was successfully performed especially by the cells entrapped in calcium alginate (CA).

In practical, wastewater treatment using sequencing batch reactor (SBR) has been generally known about advantages including flexibility, less needed apparatus, and high efficiency. There are numerous SBR applications including traditional organic carbon, nutrient and toxic substance removals (Azimi et al. 2005; Rahman et al. 2007; Ong et al. 2010; Pasukphun et al. 2010; Muhamad et al. 2011). Recently, modified SBR using fixed cells was developed and reported more successful removal efficiencies (Rahman et al. 2007; Muhamad et al. 2011). In the prior works, the attached cells onto plastic or activated carbon media were applied. Thus far, there was no published result on modified SBR using the entrapped cells.

The cell entrapment, which is a technique to immobilize microorganisms in a porous polymeric material, is known as one of effective techniques for environmental applications (Hill and Khan, 2008; Patle and Lal, 2008; Siripattanakul and Khan, 2010) including bio-ethanol production from waste materials (Khan et al. 1994; Sun et al. 1997; Nigam 2000; Najafpour et al. 2004; Siripattanakul et al. 2010b; Yu et al. 2010). The technique can be used to alleviate the limitation associated with the traditional (free) cell wastewater treatment. The entrapped cell system provides high cell loading and stress protection which result in better wastewater treatment efficiency. Moreover, the entrapped cell system does not require the sedimentation process leading to easy and flexible for operation.

Based on the above information, a modified SBR using the entrapped cells called entrapped cell sequencing batch reactor (ECSBR) is a possible alternative for the fermented rice noodle wastewater treatment. The reactor potentially applied as a compact on-site wastewater treatment system which should be suitable for small fermented rice noodle production businesses. It is a combined process between cell entrapment and SBR.

The aim of this study is to examine the fermented rice noodle wastewater treatment using the yeast cells in the ECSBR system. Optimum cell loading for fermentation was determined in batch experiment. The ECSBR with optimum cell loading were determined for treatability and physiological study of the entrapped cells. Potential ethanol production during fermentation was calculated. The SBR (free yeast cell) system was also studied for a comparative purpose.

Materials and methods

Wastewater characteristics and pre-treatment

Wastewater was taken from a fermented rice noodle plant in Ubon Ratchathani, Thailand. The plant producing fermented rice noodle of 300 kg/day, generated wastewater for approximately 3,000 L/day. The cloudy white wastewater contained chemical oxygen demand (COD) of 11,400–29,000 mg/L, biological oxygen demand (BOD) of 9,462–23,200 mg/L, glucose concentration of 650 mg/L, and pH of 3.5–4.0. Before treatment, the wastewater mainly containing starch was preliminarily treated using acid hydrolysis process. The process was performed for breaking down starch into reducing sugar (glucose). After adding sulfuric acid of 1.00% (v/v), the wastewater was mixed and autoclaved at 121°C for 15 min. Note that the sulfuric acid of 1.00% was previously optimized for the fermented rice noodle wastewater in earlier study (Siripattanakul et al. 2010b). The autoclaved wastewater was then shaken at 150 rpm and 37°C for 3 h. The glucose concentration of the pre-treated wastewater was measured and the pH was adjusted to approximately 4.5–5.0.

Microorganism and medium

A yeast strain, *Saccharomyces cerevisiae*, was used for the wastewater treatment. The strain was chosen because it was an effective culture, which was previously isolated for beverage production at Faculty of Science, Ubon Ratchathani University, Ubon Ratchathani, Thailand. The culture was grown in a sterile yeast peptone dextose (YPD) medium with the condition previously described (Siripattanakul et al. 2010b). The culture was grown at 150 rpm and 37°C for 10–12 h to reach a stationary phase before using in the wastewater treatment process. The YPD medium contained yeast extract of 5 g/L, peptone of 20 g/L, and dextrose of 20 g/L.

Calcium alginate cell entrapment

For entrapped cell preparation, the yeast cells were entrapped in calcium alginate (CA) according to a technique adjusted for the rice noodle wastewater treatment by yeast cells (Siripattanakul et al. 2010b). The yeast cells (approximately 6×10^9 cells/mL) were homogenously mixed with sodium alginate solution of 2% (w/v). The cell-to-matrix ratio of 1:5 (volume of wet cells:volume of sodium alginate) was applied followed the optimum entrapped yeast cell preparation described earlier (Siripattanakul et al. 2010b). The mixtures were manually dropped into a calcium chloride solution of 3.5% (w/v) using a sterile syringe (bead size of 3–5 mm). The droplets remained in the solution for 2 h to form and harden spherical beads.

Wastewater treatment experiments

This study was divided into two parts: (1) batch experiment for optimization of the yeast cell loading and (2)

Table 1 Batch experiment description

No.	Reactor	Description	Reactor volume (mL)	Initial innoculum size (mL of entrapped cells)[a]	Final cell concentration in reactor (cells/mL)
1	LL	Low cell loading	200	10	0.63×10^8
2	ML	Medium cell loading	200	20	1.38×10^8
3	HL	High cell loading	200	40	2.70×10^8
4	CTRL	No cell (control)	200	0	0

[a] Entrapped cell density was approximately 10^9 cells/mL of calcium alginate

sequencing batch experiment for demonstration of the entrapped yeast cell system for the application. In the first part, duplicate laboratory scale batch experiment was conducted. Four 200-mL vial bottles containing different cell loadings (designated LL, ML, HL, and CTRL) were prepared as described in Table 1. The acid pre-treated wastewater of 150 mL (initial pH of 4.5–5.0) was filled in the yeast inoculated reactors. All reactors were shaken at 150 rpm and 37°C for 10 h. During the test, samples of 5 mL were taken consecutively to measure glucose (reducing sugar) concentration.

In the second part, the cell loading that yielded highest wastewater treatment efficiency was selected. Duplicate sequencing batch tests were performed. Three 200-mL vial bottles for ECSBR, SBR, and CTRL (no cell) tests were applied. The reactors were consecutively run for 5 cycles. Each cycle included five periods followed traditional SBR cycle: (1) fill of 0.25 h, (2) react of 8 h, (3) settle of 1 h, (4) draw of 0.25 h, and (5) idle of 0.50 h. Influent and effluent from each cycle were taken for glucose and pH measurement. The beads from ECSBR reactor (before and after use) were taken for the entrapped cell physiological observation.

Analytical procedures

Chemical oxygen demand (COD) and pH were measured according to standard method (APHA, AWWA and WEF 1998). Total COD was measured by potassium dichromate digestion method whereas pH was measured using a pH meter (inoLab pH level 1, WTW GmBH, Weilheim, Germany). Yeast cell number was counted using a hemacytometer. Glucose concentration was measured using a 3,5-dinitrosalicylic acid (DNS) method (Najafpour et al. 2004). For the entrapped cell physiological observation, SEM was conducted in which the method for sample preparation was described elsewhere (Siripattanakul et al. 2010a).

Potential ethanol production was calculated from the fermentative conversion of glucose to ethanol shown in Eq. 1 (Glazer and Nikaido, 2007). Based on the Eq. 1, ethanol was theoretically produced for 51.1% by weight of glucose. Potential ethanol production of 0.511 g/g glucose utilization was used.

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \quad (1)$$

Results and discussion

Batch experiment: investigation of optimum cell loading

Sulfuric acid hydrolysis was applied as preliminary treatment of the wastewater since it is appropriate to treat several types of feedstock for bioethanol production including wastewater (Siripattanakul et al. 2010b; Kassim et al. 2011). Starch in the fermented rice noodle wastewater was broken down to reducing sugars which mainly is glucose. The concentration of glucose obtained from the acid hydrolysis process was approximately 67.4 g/L. The glucose concentration in hydrolysate was much higher than the initial concentration of 0.65 g/L. Then, the entrapped yeast cells were applied for treating the glucose-containing wastewater. Figure 1 presents normalized glucose concentrations in the systems at different entrapped yeast cell loadings.

Glucose reduction was observed for all the entrapped cell loadings. At low cell loading (LL reactor), glucose concentration decreased steadily during the first 6 h and then slightly dropped in the last 4 h period. On the other hand, it was noticed that glucose concentrations decreased sharply during the first 2 h for the ML reactor and slowly decreased in later period. Likewise, the HL reactor showed the similar trend as the ML reactor. In case of the HL

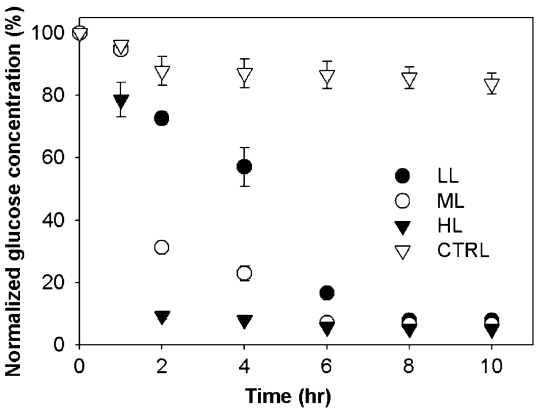

Fig. 1 Normalized glucose concentrations in the systems with different yeast cell loadings

reactor, the glucose concentration substantially dropped in the first 2 h and remained relatively unchanged during the rest of experiment. It appeared that the increase of cell loading caused higher glucose removal rate. This phenomenon is reasonable since high numbers of cell could create more sorption of substrate (glucose) to the cells and eventually degrades the substrate. Although different cell loadings provided the different glucose removal rates, about the same reductions (>90%) were observed at the end of the experiment (10 h). The glucose reduction by the entrapped cells at different cell loadings (LL, ML, and HL) was 92, 93, and 95%, respectively. The result suggested that there were sufficient yeast cells in all reactors resulting in similar glucose removal efficiencies.

For the control reactor (CTRL), approximately 20% reduction of glucose was observed. As expected, the glucose reduction should be from adsorption process of the CA matrix. A previous publication reviewed adsorption ability of entrapment matrices which was commonly found at the early stage of matrix utilization (Siripattanakul and Khan, 2010). In case of the entrapped cell reutilization, no or less adsorption would be observed (Siripattanakul et al. 2008). It was clear that the entrapped yeast cell systems successfully reduced glucose by matrix adsorption (glucose immobilization) and biodegradation (glucose bioconversion) but the main process was biodegradation. In other words, the entrapped yeast cells potentially produced ethanol.

Based on kinetic stand point, the glucose removal trends followed the second-order kinetics (Table 2). From the batch experimental results, it was clear that the entrapped cell systems performed high treatment efficiency. Higher cell loading resulted in higher glucose removal rate. Therefore, the optimum cell loading for this application is 2.7×10^8 cells/mL since it provided the fastest rate of glucose reduction (0.020 L/mg/h). The cell loading of approximately 2×10^8 cells/mL of wastewater was chosen for later experiment.

Sequencing batch experiment: investigation of ECSBR and SBR performance

In this section, the experiments were conducted to compare long term performance between ECSBR and SBR. Normalized glucose concentrations remaining in the EC-SBR, SBR, and CTRL systems are presented in Fig. 2. The treatment efficiencies by ECSBR and SBR were 66–92% and 40–96%, respectively, while glucose concentrations remaining relatively stable at approximately 100% (no removal at all) in the control system. It was observed that the treatment efficiencies in both ECSBR and SBR declined as number of cycles increased; however, ECSBR performance was more stable than SBR (Fig. 2).

In the first and second cycles, SBR provided slightly better glucose removal efficiencies than ECSBR (96 vs. 94%). The glucose degradation rates by ECSBR and SBR of 7.92 and 8.09 g/L/h, respectively, were not obviously different. This could be from limitation of substrate diffusion in the entrapped cell systems leading to lower treatment efficiencies. Similar situation also reported in previous studies (Kim et al. 2001; Song et al. 2005; Siripattanakul et al. 2008). However in later cycles (3–5), ECSBR (removal of 75 ± 10%) significantly performed superior over SBR (removal of 51.5 ± 11.5%). The glucose degradation rate by ECSBR was 6.32 g/L/h while the rate by SBR was 4.34 g/L/h. In comparison, ECSBR could degrade glucose 45% over SBR. This should be because the ECSBR system could reduce cell washout leading to higher number of the cells retained in the system. The limitation in terms of lower substrate diffusion did not play an important role for the rice noodle wastewater treatment compared to influence of the cell washout. This should be because the entrapped cell structure did not obviously obstruct the substrate transport (more detail of entrapped cell morphology presented in later section) (Siripattanakul and Khan 2010).

Otherwise, the results suggested that though the entrapped cells needed more preparation materials and time reflecting higher cost, the entrapped cells obviously achieved higher removal efficiency. For long term, ECSBR would be more profitable. In practical, concept of ECSBR could be applied for both small and large factories in either modified SBR or an alternative of moving bed bioreactor.

Table 2 Glucose removal kinetic equations and rates

No.	Reactor	Glucose removal kinetic equation[a]	R^2	Glucose removal rate (L/mg/h)
1	LL	$y = 0.013x + 0.007$	0.881	0.013
2	ML	$y = 0.017x + 0.000$	0.902	0.017
3	HL	$y = 0.020x + 0.029$	0.865	0.020

[a] y = 1/glucose concentration (L/mg) and x = time (h)

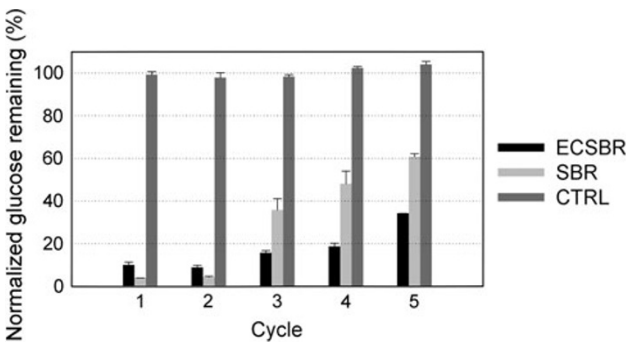

Fig. 2 Normalized glucose concentrations in sequencing batch systems

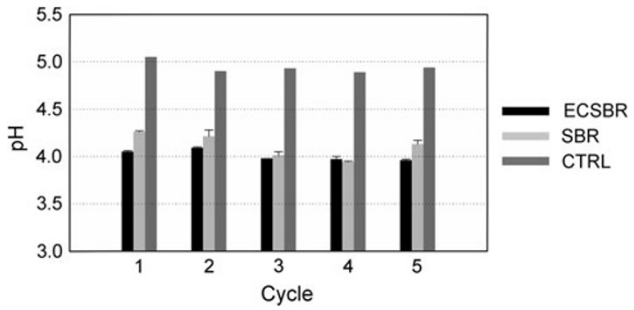

Fig. 3 pH in sequencing batch systems

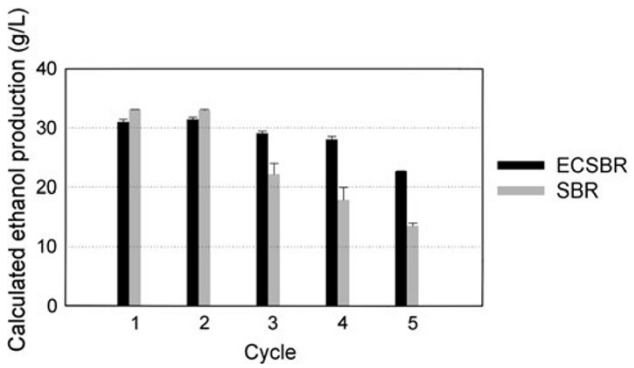

Fig. 4 Calculated ethanol production from sequencing batch systems

Figure 3 shows the pH of systems for all 5 cycles. It is noticed that pH of ECSBR and SBR systems ranged from 4.0 to 4.3 while pH of 4.8 to 5.1 was observed in the control system. The decreasing of pH values in ECSBR and SBR could be from CO_2 production during glucose degradation as shown in Eq. 1 (Glazer and Nikaido 2007; Mellati et al. 2010). It was noticed that the ECSBR system had more stable pH than SBR. Once again, this result showed the advantage of entrapped cell system that provided better environmental condition than the free cell system (SBR). The similar result was reported in a previous work (Mellati et al. 2010). The entrapped *S. cerevisiae* performed better than the free cells. The entrapped cells had 1.5 times higher bioactivity than the free cells.

Potential ethanol production from the ECSBR and SBR systems was estimated (Fig. 4). The ECBSR and SBR systems were able to produce ethanol up to 23–32 and 13–34 g/L, respectively. From the 5-cycle test, the average potential ethanol production rates by ECSBR and SBR were 3.4 and 2.9 g/L/h, respectively. In practice, ethanol of 2–5 g/L/h was generally produced from batch reactors (Sun et al. 1997). This indicated that the novel reactor, ECSBR, was in acceptable level.

Based on the information of the model fermented rice noodle production plant, the wastewater was produced for approximately 3,000 L/day. Ethanol could be produced by ECSBR and SBR for 69–96 and 39–102 kg/day, respectively. This result suggested that the fermented rice noodle wastewater treatment by the yeast cells is promising as one of the efficient wastewater treatment techniques as well as an alternative for bio-fuel production. Moreover, operation in ECSBR mode enhanced treatment efficiency and system stability. The system has the potential for real wastewater treatment practice.

The SEM images of calcium alginate beads with yeast cells before and after experiment are presented in Fig. 5. These images are used to examine the entrapped cell structure and durability of calcium alginate. Theoretically, calcium alginate entrapment is a cross-linking reaction (Siripattanakul and Khan, 2010). Calcium and alginate linkage is net structure. The images confirmed that a large number of net linkages were reacted resulting in sheet structure presented in Fig. 5. Figure 5 indicated that there were numerous voids inside the entrapped matrices. This supported that the cell entrapped in

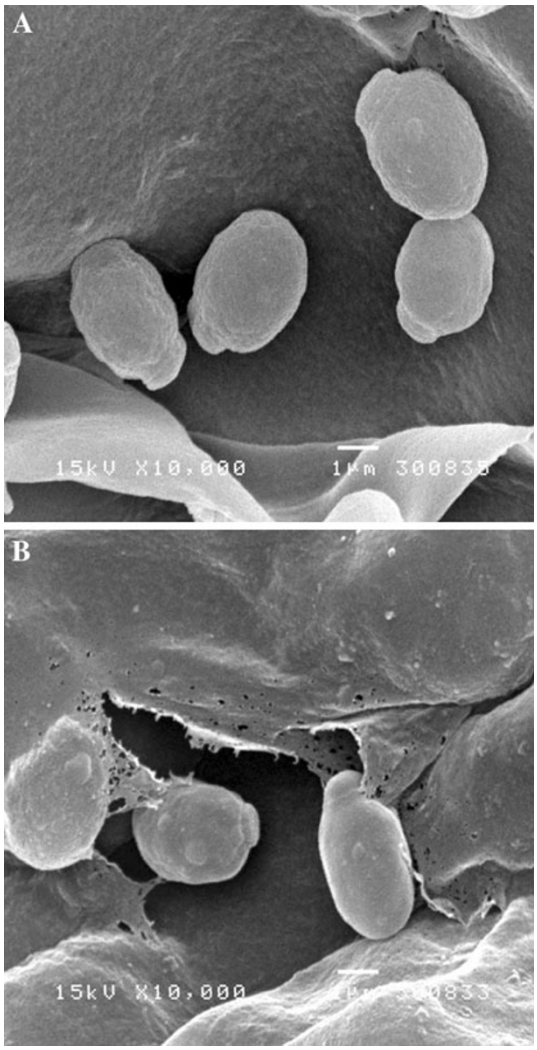

Fig. 5 SEM images of calcium alginate beads with yeast cells **a** before and **b** after experiment at ×10,000

CA matrices did not substantially get the limitation of the substrate diffusion resulting in high glucose removal as described in earlier section.

It was observed that the yeast cells were entrapped in the calcium alginate sheet before utilization (Fig. 5a). After application, it looked like that the yeast cells got adhered in the structure (Fig. 5b). This observation implied that yeast cells could attach on calcium alginate cross-linkage causing better cell retention in the system. This may also be a reason why the smooth sheet structure bead (Fig. 5a) turned slightly rough and torn (Fig. 5b) after utilization. The rupture of sheet structure could result in detachment of cell from calcium alginate matrix and leading to the cell lost. This should explain why the treatment performances dropped as numbers of operating cycle increased (Fig. 2).

Budding of the yeast cells is presented in Fig. 5. This indicated that the yeast cells well grew in the entrapment matrix. After application, the yeast cells entirely proliferated in the alginate matrices (Fig. 6a). In Fig. 6b, the matrix showed rupture and void between layers which could be from interference by gas production in the fermentation process. Based on this image, it was reminded about durability of the calcium alginate entrapped cells. For future study or practice, the method to improve durability of the entrapped cells needed to be investigated. These SEM images supported the wastewater treatment results presented above. In ECSBR, the yeast cells were immobilized (entrapped and attached) and proliferated in the alginate matrices. Consequently, ECSBR performed higher glucose removal efficiencies compared to SBR.

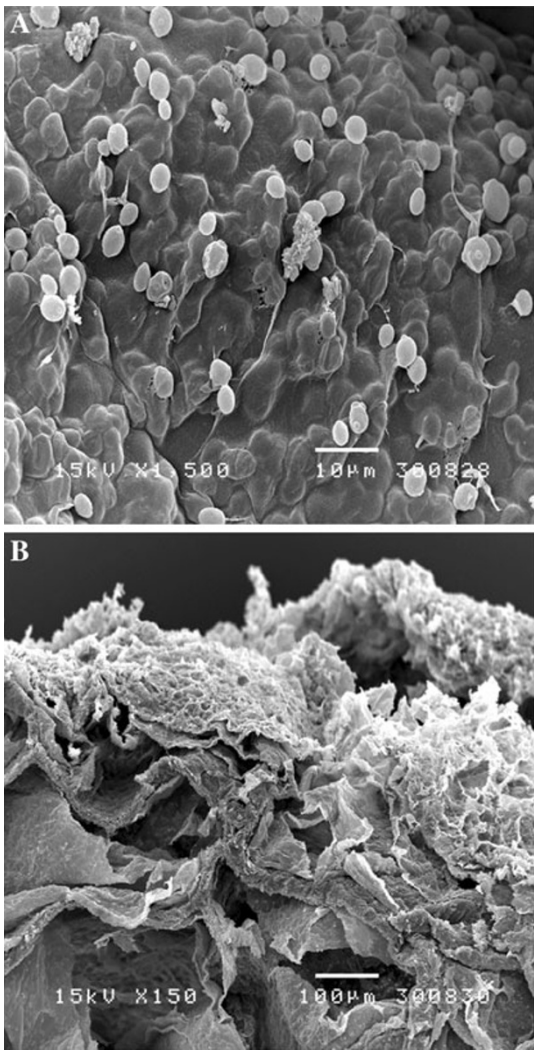

Fig. 6 SEM images after 5-cycle experiment in **a** facial view (×1,500) and **b** cross-sectional view (×150)

Conclusions

ECSBR is a novel system for treating fermented rice noodle wastewater and producing ethanol. In fermentation process by yeast, the entrapped cells reduced glucose concentration of 92–95%. The system could produce ethanol up to 3.4 g/L/h. The cell loadings applied in this study did not affect treatment efficiency; however, boarder range of the cell loading should be performed to ensure the optimum cell loading before practice. The treatment kinetics followed second-order reaction mechanism. In sequencing batch mode, ECSBR performed better than SBR in both treatment efficiency and stability perspectives. For future work, investigation of operating conditions for the novel system and entrapped cell durability is recommended. Insight investigation on the entrapped cells during wastewater treatment should be performed as well.

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