

## Fermented Rice Noodle Wastewater Treatment and Ethanol Production Potential Using Entrapped Yeast Cells

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### Annotation

*Fermented rice noodle is a major source of food industry generating highly complex organic content (starch) wastewater. This study investigated the treatment of fermented rice noodle wastewater using calcium alginate entrapped yeast cells compared to the free cells. The treatment includes a two-step process: acid hydrolysis for breaking down starch to glucose and fermentation for degrading glucose to ethanol. Yeast culture, *Saccharomyces cerevisiae*, was used in this study. The experiment was conducted to examine optimum acid concentration and cell entrapment condition for fermentation. Sulfuric acid concentrations ranged from 0.25 to 1.00% by volume were tested while the cells entrapped in calcium alginate at cell-to-matrix (alginate) ratios (by volume) of 1:5, 1:10, and 1:20 were varied. The result showed that the optimum acid concentration of 1.00% provided 5-time higher glucose concentration compared to that in raw wastewater. After the batch fermentation, the entrapped cells reduced total chemical oxygen demand (COD) by 33-46% and glucose concentration by 88-90% while the free cells cannot obviously remove COD and reduced glucose concentration by 62%. The entrapped cells at the cell-to-matrix ratio of 1:5 achieved the best glucose biotransformation performance. The entrapped and free yeast cell system potentially produced ethanol of 643 to 801 mg/L.*

**Keywords:** Calcium alginate, cell immobilization, ethanol, fermented rice noodle, wastewater, *Saccharomyces cerevisiae*

### Introduction

Among Asian countries, fermented rice noodle is one of the most favorite foods which produce a large volume of wastewater containing complex biodegradable organic compounds. Although most countries have implemented a stringent regulation to control industrial water pollution, wastewater from fermented rice noodle production still is a problem. This is because the fermented rice noodle production businesses generally are in small scale and spread out in residential area. As a result, the wastewater was discharged directly to sewer system without proper preliminary treatment creating odor issues in the domestic sewer collection and increasing the organic loading to treatment systems. Therefore, compact onsite wastewater treatment system is necessary to fermented rice noodle industry.

Entrapped yeast cell system is a potential alternative for this problematic issue. The microorganism entrapped in porous polymeric material is known as one of effective techniques for environmental applications [1-3]. The technique can be used to alleviate the limitation associated with the traditional (free) cell wastewater treatment. The system provides high cell loading and stress protection which result in better wastewater treatment efficiency. Moreover, the system does not require the sedimentation process leading to easy and flexible for operation. Generally, bacterium is a predominant species used for entrapped microorganism for wastewater treatment application.

There have been only a few studies applied the entrapped yeast cell system for treating wastewater such as pineapple cannery and sugar productions [4-5]. The system successfully treated wastewater and produced ethanol as a product from the fermentation. Currently, with petroleum deficiency situation, ethanol becomes a promising alternative source for fuel; consequently, wastewater treatment by yeast has potential for bio-fuel production.

Thus far, there has been no study on the fermented rice noodle wastewater treatment and ethanol production using the entrapped yeast cells. The aim of this study is to examine the fermented rice noodle wastewater treatment using the entrapped cells. Calcium alginate, a widely used cell entrapment matrix, was selected. Optimum conditions of acid hydrolysis and entrapped cell preparation for fermentation were determined. The free cell system was also studied for a comparative purpose. Potential ethanol production during fermentation was calculated.

## Methods

### *Wastewater and its characteristics*

Wastewater was taken from a fermented rice noodle plant in Ubon Ratchathani, Thailand. The plant producing fermented rice noodle of 300 kg/d, generated wastewater for approximately 3,000 L/d. The wastewater characteristics were shown in Table 1.

**Table 1** Fermented rice noodle wastewater characteristics

Parameter	Range
Chemical oxygen demand (COD) (mg/L)	11,400-12,800
Glucose concentration (mg/L)	4.4-38
pH	3.5-4.0
Appearance/color	Cloudy white

### *Acid hydrolysis*

Wastewater of 100 mL was added with sulfuric acid of 0.25, 0.50, 0.75, and 1.00% (v/v) to investigate the optimum acid concentration. The wastewater without sulfuric acid supplement (0.00%) was also tested as a control along with that with acid addition. The range of sulfuric acid was selected based on a previous study [1]. It was reported that too much acid concentration (more than 1.00%) can not only reduce glucose concentration but also damage a later process (fermentation).

The acid-added wastewater was vigorously mixed and autoclaved at 121°C for 15 min. The heated wastewater was then shaken at 150 rpm and 37°C for 3 hr. The hydrolysate was measured glucose concentration and adjusted pH to 4.5-5.0 before the fermentation. Note that after obtaining optimum acid concentration for the wastewater hydrolysis, the optimum acid concentration was applied for the rest of the experiment.

### *Microorganism and medium*

A yeast strain, *Saccharomyces cerevisiae*, was used for fermentation. The strain 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 Dextrose (YPD) medium at 150 rpm and 37°C for 10-12 hr to reach a stationary phase before using in the fermentation 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**

The culture was entrapped in calcium alginate according to a technique adapted from Smidsrod and Skjak-Braek [6]. The technique was chosen because of several successful prior applications [2, 7]. Sodium alginate (Fluka, Singapore) was dissolved into sterile de-ionized water (DI) at 2% (w/v). The yeast cells were centrifuged at the highest speed for 10 min to obtain concentrated yeast cells. To prepare the contents for reactor (described in Table 2), the concentrated cells were resuspended in sterile DI of 5 mL and homogenously mixed with sodium alginate solutions. 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 hr to form and harden spherical beads.

### **Batch fermentation**

Optimization of the cell entrapment preparation condition was focused. Among the conditions tested, the effect of the cell-to-matrix (calcium alginate) ratios in substrate diffusivity and contaminant removal ability was one of major concerns previously reported in several studies [3, 8]. In the present study, eight reactors containing different content of the cells and the matrix were prepared to test the effect of cell-to-matrix ratio (Table 2).

**Table 2** Inoculated content of fermentation reactors

No.	Reactor	Description	Cell-to-matrix ratio (mL of cells: mL of calcium alginate)	Volume of cells (or DI) (mL)	Volume of calcium alginate (mL)	Total inoculated volume (mL)
1	EC5	Entrapped cells	1:5	5*	25	30
2	EC10	Entrapped cells	1:10	5*	50	55
3	EC20	Entrapped cells	1:20	5*	100	105
4	FC	Free cells	1:0	5*	0	5
5	CTRL	Control	0:0	0	0	0
6	CA5	Calcium alginate	0:5	5**	25	30
7	CA10	Calcium alginate	0:10	5**	50	55
8	CA20	Calcium alginate	0:20	5**	100	105

\* The yeast cells of 5 mL had approximately  $3 \times 10^{10}$  cells.

\*\* Sterile DI of 5mL did not have any cells.

Duplicate 24-hour batch fermentation tests were performed. The hydrolysate of 150 mL (initial pH of 4.5-5.0) was filled in a reactor inoculated with the content shown in Table 2. All reactors were shaken at 150 rpm and 37°C. During the 24-hr test, samples of 5 mL were taken at 0, 1, 2, 3, 4, 6, 8, 10, and 24 hr to measure COD and glucose concentration. Potential ethanol production was calculated based on reduction of glucose concentration.

### **Analytical procedures**

COD and pH were measured according to standard methods [9]. Total COD was measured by potassium dichromate digestion method whereas pH was measured by 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 [10]. The DNS solution was prepared by dissolving DNS of 10 g and sodium hydroxide of 16 g into DI of 450 mL. While heating the solution in boiled water bath, sodium potassium tartrate of 300 g and sodium sulfite of 0.5 g were added. The solution was then made up to 1,000 mL by DI. Sample (or calibration solution) of 1 mL was boiled with DNS of 2 mL for 5 min and immediately submerged in cooled water bath. The sample was then added with DI of 20 mL and measured absorbance at

wavelength of 520 nm. The calibration curve was prepared with glucose solution between 0 to 1,000 mg/L.

#### **Potential ethanol production calculation**

Potential ethanol production was calculated from the fermentative conversion of glucose to ethanol shown in equation 1 [11].



Based on the above equation, ethanol was theoretically produced for 51.1% by weight of glucose. Potential ethanol production of 0.511 g/g glucose utilization was used.

## **Results and Discussion**

#### **Optimum acid hydrolysis**

Table 3 presents glucose concentrations before and after hydrolysis. Glucose concentrations in hydrolysates increased with increasing of the amount of acid added. The highest acid concentration (1.00%) provided the highest glucose concentration. This trend is similar to a previous study [1]. In the previous study, sulfuric acid of 0.25 to 4.00% was added for hydrolyzing Thippi (a starchy feedstock for ethanol production). It was found that glucose concentrations went up with the increasing of sulfuric acid concentration in the tests between 0.25 to 1.00%. The hydrolysates with acid concentrations of higher than 1.00% provided less glucose concentration since a portion of glucose was destroyed by acid.

**Table 3** Glucose concentration before and after sulfuric acid hydrolysis

No.	Description	Glucose concentration (mg/L)	Glucose increment (time)
1	Before hydrolysis	73.0±8.0	-
2	After hydrolysis with 0.00%	0.0±0.0	-1.00
3	After hydrolysis with 0.25%	306.5±15.5	3.20
4	After hydrolysis with 0.50%	373.5±22.5	4.12
5	After hydrolysis with 0.75%	403.5±44.5	4.53
6	After hydrolysis with 1.00%	430.5±28.5	4.90

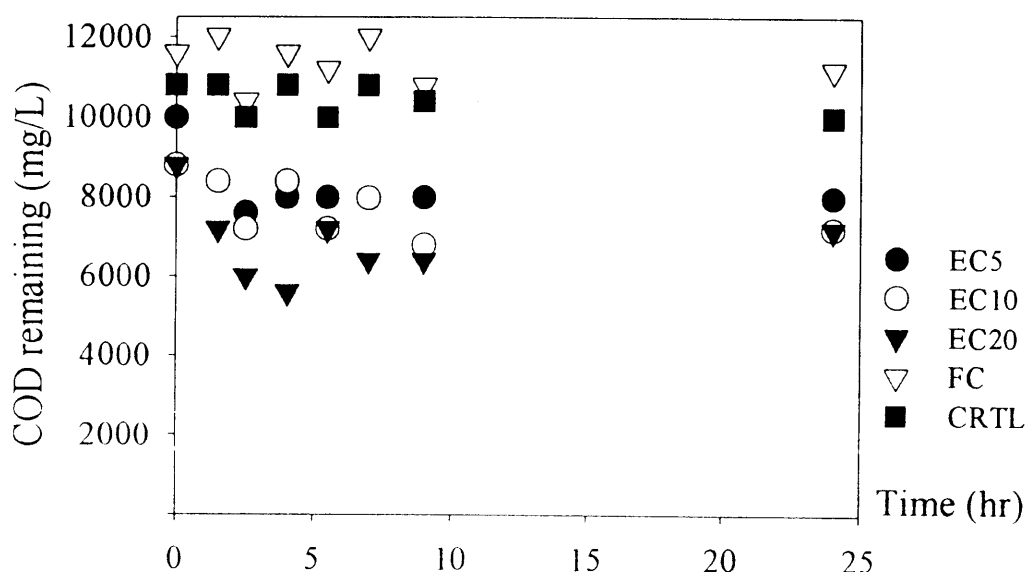
The optimum acid concentration for hydrolyzing the fermented rice noodle wastewater was 1.00% (v/v). Otherwise, it is noticed that after hydrolysis without sulfuric acid addition (0.00%), glucose concentration decreased. This could be due to glucose destruction from heat and pressure during hydrolysis process.

#### **Optimum cell entrapment for batch fermentation**

##### **COD reduction**

Figure 1 presents average COD reduction (from duplicate experiments) by the yeast cells entrapped at different cell-to-matrix ratios compared to the free cells and control (reactor no. 4 and 5, respectively). The trends of COD reduction by all entrapped cell conditions were similar. Chemical oxygen demand dramatically decreased during the first 5 hr and slightly dropped off soon after. At the end of the experiment (24 hr), COD reduced 33, 41, and 46% for the entrapped cells at the cell-to-matrix ratios of 1:5, 1:10, and 1:20, respectively. On the other hand, the free cells removed COD of only less than 5% after testing for 24 hr (Figure 1) which closed to COD values observed in the control reactor.

Although the COD reduction by the entrapped cells was apparently better than that by the free cells, the reduction was not as high as expected. The low COD reduction efficiencies by either the entrapped or free cells could be from the interference on the COD analysis by ethanol and the yeast cells. In the entrapped cell system, most of the yeast cells were in the matrices; COD was likely from glucose and ethanol. In the free cell system, COD comprised glucose, ethanol, and the cells resulting in low COD reduction in the system.



**Figure 1** COD reduction by the entrapped and free yeast cells

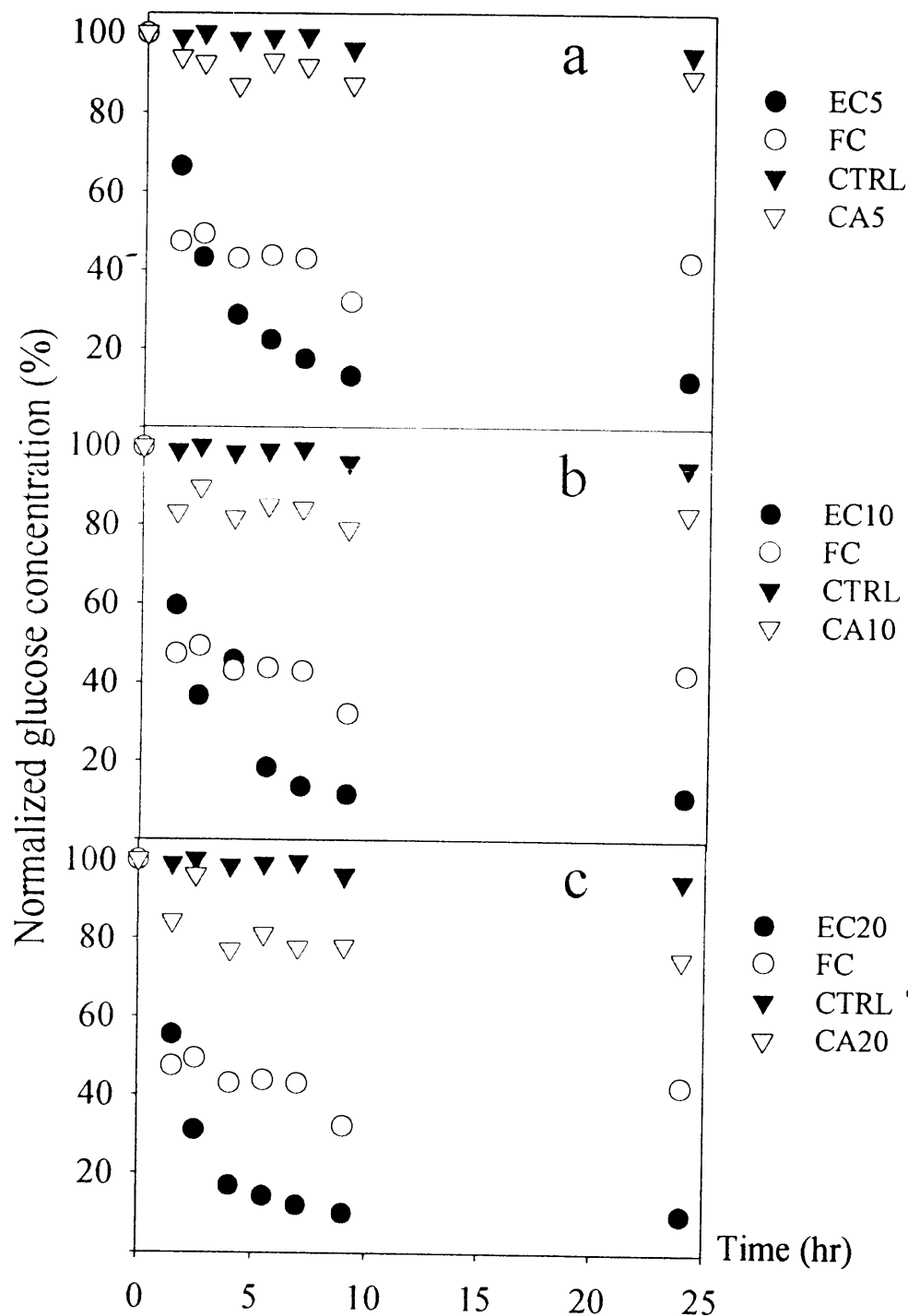
#### *Glucose reduction*

Figure 2 presents normalized glucose concentration in the systems. Average initial glucose concentration from duplicate experiment was 2,045 mg/L. The trends of glucose reduction by the entrapped cells, the free cells, and the matrices were similar. The glucose concentration rapidly decreased within the first 5 hr and slightly decreased in a later period.

In Figure 2a, at the end of the experiment (24 hr), the glucose concentration reduced for 88, 62, 10, and 4% in the entrapped cells at the cell-to-matrix ratio of 1:5, the free cells, the matrices (calcium alginate), and control (reactor no. 1, 4, 6, and 5), respectively. The result by the matrices indicated that calcium alginate could adsorb glucose for some certain portion associating to a better glucose reduction by the entrapped cell system. The similar result reported in a previous study [3]. The adsorption by matrices could take place at the first time exposed to contaminant. This suggests that the glucose reduction by the entrapped cells was from biotransformation and adsorption processes. However, after subtracting the glucose reduction by the entrapped cells and the matrices, the glucose reduction was still higher than the free cells by 10%. Therefore, it is obvious that the entrapped cells successfully decreased glucose compared to the free cells. This may be from the matrices give a better environment for the yeast cells which worked well in anaerobic condition. Also, the matrices may protect some environmental stresses, such as acid residue in the wastewater (hydrolysate).

The glucose reduction by the entrapped cells at the cell-to-matrix ratios of 1:10 and 1:20 and the matrices (reactor no. 2, 3, 7, and 8) were 89, 90, 15, and 25% respectively. The trends of the result were similar to the entrapped cells at the cell-matrix ratio of 1:5. The entrapped cells gave better glucose reduction efficiencies than the free cells. It is noticed that the matrices analogous to the cell-to-matrix ratio of 1:20 gave the highest glucose adsorption. This is understandable since the matrices at the ratio had the highest volume attributing to the highest adsorption (Table 2). After

subtracting the adsorption ability by the matrices, it turned out that the entrapped cells at the cell-to-matrix ratio of 1:5 give the best glucose biotransformation (Table 4). This is similar to the previous studies that although the cell number in reactors was the same, the cell entrapment condition would affect the wastewater treatment ability [3, 8].



**Figure 2** Normalized glucose concentration in the systems

#### *Potential ethanol production*

Potential ethanol production was calculated by glucose utilization (Table 4). Ethanol possibly produced for 643-801 mg/L. Wastewater generated from the model plant was 3,000 L/d. Ethanol

could be produced for 58 to 72 kg ethanol/month. Based on this calculation, it could state that the fermented rice noodle wastewater is promising as an alternative for bio-fuel production.

**Table 4** Potential ethanol production

Cell-to-matrix ratio	Initial glucose concentration (mg/L)	Glucose reduction (mg/L) at 24 hr by			Potential ethanol production (mg/L)
		Entrapped cells	Calcium alginate	Glucose biotransformation	
1:5	2,045±15	1,790±10	220±10	1,570	801
1:10	2,045±15	1,825±15	385±25	1,440	734
1:20	2,045±15	1,865±15	605±45	1,260	643
Free cells	2,045±15	1,405±15	0	1,405	717

### Conclusions

The calcium alginate entrapped yeast cell system is a novel technique for treating fermented rice noodle wastewater and producing ethanol. Sulfuric acid concentration of 1.00% provided the best condition for wastewater hydrolysis. In fermentation process by yeast, the entrapped cells reduced COD of 33-46% and glucose concentration of 88-90%. The entrapped cells at the cell-to-matrix ratio of 1:5 gave the best glucose biotransformation and possible ethanol production. For future work, operating conditions for the novel system, such as wastewater or solid retention time are recommended. Insight investigation on the entrapped cells during wastewater treatment should be performed as well.

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