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Original Article

Immobilization of *Saccharomyces Cerevisae* cells on water hyacinth stem pieces and application to repeated batch fermentation for ethanol production

Le Nhu Ngoc Ta, Thi Hong Chau Nguyen, and Van Viet Man Le*

Department of Food Technology, Ho Chi Minh City University of Technology, District 10, Ho Chi Minh City, Vietnam.

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Abstract

Water hyacinth (*Eichhorniacrassipe)* stem pieces were used as supports for *Saccharomyces cerevisiae* immobilization. The response surface methodology was implemented to maximize the cell immobilization yield. This methodology optimized the amount of water hyancinth pieces added to yeast suspension and the initial cell concentration in yeast suspension prior to the immobilization. The immobilized yeast on water hyacinth stem pieces was then applied to ethanol fermentation with 12 repeated batches. The ethanol production rate of the immobilized yeast was significantly higher than that of the free yeast. In addition, gradual increase in the metabolic activity of the immobilized cell system was also observed during the 12 fermentation batches. The ethanol production rate of the immobilized yeast in the twelfth batch culture was increased by 29.8% compared to that in the first batch culture.

Keywords: ethanol, fermentation, immobilization, *Saccharomyces cerevisae*, water hyacinth

1. Introduction

Cell immobilization for ethanol production has been intensively studied for the last two decades due to its various economic advantages over the free cell systems (Strehaiano *et al.*, 2006). According to the basic material and the origin, the supports used for the cell immobilization could be classified into two categories: synthetic materials (either inorganic or organic) and natural materials (only in organic form) (Mallouchos *et al.*, 2002). The study of Kourkoutas *et al.* (2004) indicated that inorganic supports exhibited high mechanical stability but low affinity to microbial cells. Synthetic organic supports are manufactured by complex processes; they are stable in chemical compositions but more expensive than the natural organic supports (Baptista *et al.*, 2006).

For industrial application of immobilized cells to ethanol fermentation, the supports must be abundant in nature, easy to use and cost-effective (Kourkoutas *et al.*, 2006). The organic supports from natural sources have been commonly used (Plessas *et al.*, 2006). During the last decade, fragments of vegetables (Kandylisand Koutinas, 2008) and various materials with high cellulosic content such as wild sugarcane stem (Chandel *et al.*, 2009), corn stem (Vucurovic *et al.*, 2008), sorghum baggasse (Yu *et al.*, 2007), loofa sponge (Ogbonna *et al.*, 1997), brewing spent grains (Kopsahelis *et al.*, 2007), and sugarcane stalks (De Vasconcelos *et al.*, 2004) were used as natural supports for yeast immobilization and application to repeated batch fermentation for ethanol production.

Water hyacinth (*Eichhornia crassipes*), a floating plant widely available in tropical and subtropical regions, has been known as a good adsorbent material with a large surface and a high cellulosic content (Tan *et al.*, 2008). As far as we are concerned, no application of water hyacinth as the support for the cell immobilization has been reported. Further study of this plant can introduce an inexpensive and potential material to microbial technology. On the other hand, the application of this plant in the industry provides a good solution to help control various issues caused by a rapid

* Corresponding author.
Email address: lvvman@hcmut.edu.vn

growth of water hyacinth in many countries. The reported issues include choking waterway, reduction in fish populations, proliferation of shelters for mosquitos, and other disease-transmissive organisms (Schneider *et al.*, 1995). In this paper, yeast immobilization on water hyacinth stem pieces was investigated. The application of immobilized yeast was also evaluated in the ethanol production choosing a repeated batch fermentation process.

2. Materials and Methods

2.1 Materials

2.1.1 Media

Medium A proposed by Kopsahelis *et al.* (2007) was used for yeast multiplication. This medium contains *D*-glucose (40 g/L), yeast extract (4 g/L), $(NH_4)_2SO_4$ (1 g/L), KH_2PO_4 (1 g/L) and $MgSO_4$ (5 g/L).Medium B was used for yeast immobilization on water hyacinth stem pieces, and medium C was used for ethanol fermentation. Except that the glucose concentration was adjusted to 120 g/L and 200 g/L, other components in media B and C were kept similar to those of medium A. All media were sterilized at 12°C for 20 min before usage.

2.1.2 Preparation of yeast biomass

A strain of *Saccharomyces cerevisiae* supplied by Food Technology Department, Ho Chi Minh City University of Technology was used in this study. Yeast was propagated in medium A in three successive stages: 1) in a 100 mL Erlenmeyer shake-flask containing 10mL of medium, 2) in a 250 mL Erlenmeyer shake-flask containing 100mL of medium, and 3) in a 2L Erlenmeyer shake-flask containing 500 mL of medium. At all stages, yeast was grown at 30°C and 150 rpm for 24 hrs. Yeast cells in the stationary growth phase were harvested by centrifugation at 4°C, 4,000 rpm for 10 min and used for immobilization.

2.1.3 Preparation of support

Fresh water hyacinth plants (*Eichhornia crassipes*) were collected from Saigon River in Ho Chi Minh City. After removing some unnecessary parts (roots and leaves), we cut the stems, whose diameter was roughly 2 cm, into cylindrical pieces with the expected height. Water hyacinth stem pieces were sterilized at 121°C for 20 min. The moisture content and the sugar concentration of the support prior to the immobilization was approximately 90% and 0.7 %, respectively.

2.2 Procedure of yeast immobilization on water hyacinth stem pieces

Yeast biomass was suspended in a 250 mL Erlenmeyer containing 100 mL of medium B to reach a proper cell concentration. Then a predetermined amount of water hyacinth stem pieces was added to the yeast suspension. This mixture was incubated in a thermostat-shaker at 30°C for a determined period. The liquid was then decanted, the support was washed twice with sterile water. The obtained biocatalyst was used for evaluation of yeast cell density on the support (cfu/g support) and ethanol fermentation.

2.3 Effects of technological parameters on yeast immobilization

2.3.1 Effects of support size

The size of water hyacinth stem pieces was determined by the height of cylindrical pieces with 2-cm diameter. In this experiment, the support size was various: 3, 5, 7, 10 and 15 mm. Other parameters were fixed: the weight of support for 100 mL yeast suspension: 19 g; the agitation rate: 100 rpm; the initial yeast cell concentration in the suspension: 2.0×10^7 cfu/mL; the immobilization time: 16 hrs.

2.3.2 Effects of support weight

The weight of support added to 100 mL yeast suspension was changed: 7, 11, 15, 19 and 23 g. The support size was selected from the results of the previous experiment. Other parameters were fixed: the agitation rate of 100 rpm; the initial yeast cell concentration in the suspension: 2.0×10^7 cfu/mL; the immobilization time: 16 hrs.

2.3.3 Effects of agitation rate

The agitation rate was varied: 0, 50, 100, 200 and 300 rpm. The support size and weight were chosen from the results of the previous experiments. The initial yeast cell concentration in the suspension was 2.0×10^7cfu/mL and the immobilization time was 16 hrs.

2.3.4 Effects of initial yeast cell concentration in the suspension

In this experiment, the initial yeast cell concentration in the suspension was ranged from 1.0×10^7 to 7.0×10^7cfu/mL. The support size and weight and the agitation rate were selected from the results of the previous experiments. The immobilization time was fixed at 16 hrs.

2.3.5 Effects of immobilization time

The immobilization time lasted from 12 to 24 hrs. The support size and weight, the agitation rate and the initial yeast cell concentration in the suspension were chosen from the results obtained above. Based on the cell density on the support obtained at the end of the yeast immobilization, the cell immobilization yield Y (%) was calculated according the following formula: $Y = N_1/(N_1 + N_2)$, where N_1 is the yeast

count on the support, and N_2 is the yeast count in the liquid phase at the end of the immobilization.

2.4 Optimization of technological parameters of yeast immobilization on water hyacinth stem pieces

Based on the effects of various parameters on yeast immobilization, two factors which had a great impact on cell immobilization yield were selected for optimization by face centered central composite design. The dependent variable was cell immobilization yield. The Modde software (version 5.0) was used to generate the experimental planning and to process the data. The complete design consisted of 11 experimental points, including 4 factorial points, 4 axial points and 3 center points.

2.5 Ethanol fermentation

The repeated batch fermentation with immobilized yeast on water hyacinth stem pieces was carried out at 30°C in 1 L Erlenmeyer containing 500 mL medium C. The inoculating rate was 10^7cfu/mL. The fermentation was considered completed when the degree of attenuation reached 98% (degree of attenuation is the ratio of the sugar content assimilated by yeast and the initial sugar content in the medium). When the fermentation was completed, the liquid was collected for analysis. The support was washed with 200 mL of sterilized water and then reused for the next fermentation batch. A control sample with free yeast cells was simultaneously realized in the same conditions.

2.6 Analytical methods

Yeast count in the liquid sample was determined by the pour plate method with malt agar medium. Yeast count on the support was determined by homogenizing water hyacinth stem pieces with sterile water in a blender. The obtained mixture was then sampled for yeast count (Kandylis and Koutinas, 2008). Total reducing sugars were quantified by spectrophotometric method, using 3,5-dinitrosalycylic acid reagent (Marsden, 1982).Ethanol concentration was determined by enzymatic method using ethanol kit with a reflectometer model 116970 (MercK KgaA, Germany). Ethanol production rate R_p (g/L.h) was calculated as follow: $R_p = (P_2 - P_1)/T$, where P_1 is the ethanol content in the culture at the beginning of the fermentation (g/L); P_2 is the ethanol content in the culture at the end of the fermentation (g/L); T is the fermentation time (h). The support water hyacinth pieces and immobilized yeast were examined with scanning electron microscope (FESEM, 7410F, Jeol, Japan). The samples were washed with sterile water, dried overnight at 30°C and then sputtered with gold and photographed (Kopsahelis *et al.*, 2007).

2.7 Statistical treatment

All experiments were performed in triplicate. The experimental results obtained were expressed as means ± SD. Mean values are considered significantly different when $p<0.05$ using Multiple Range Test. Analysis of the variances was performed using the software Statgraphic plus, version 3.2.

3. Results and Discussion

3.1 Effects of technological parameters on yeast immobilization

3.1.1 Effects of support size

The effects of support size on yeast cell immobilization on water hyacinth stem pieces are visualized in Figure 1. Decrease in support size from 15 to 10 mm significantly improved both cell immobilization yield and cell density on the support. It can be explained that when the support weight was fixed at 19 g for 100 mL yeast suspension, decrease in support size increased the number of water hyacinth stem pieces as well as the contact surface area between the support and the yeast suspension. The higher the contact surface area, the higher the number of yeast cells adsorbed on the support. Similar result was also noted for yeast immobilization on pineapple fruit pieces (Diep and Le, 2009).

However, the cell immobilization yield and cell density on the support were reduced as the support size decreased from 10 to 3 mm. During the yeast immobilization, it was observed that agitation disintegrated water hyacinth stem pieces and some pieces were unable to be collected at the end of the immobilization. This phenomenon reduced the cell immobilization yield and cell density on the support. The appropriate support size was 10 mm and this value was used for the next experiment.

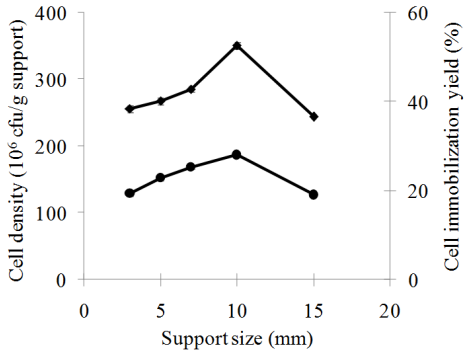

Figure 1. Effects of support size on cell immobilization yield and cell density on the support; (●): Cell immobilization yield; (◆): Cell density on the support.

3.1.2 Effects of support weight

Figure 2 shows that increase in support weight from 7 to 19 g for 100 mL yeast suspension augmented the cell immobilization yield by 76.5%. It was due to an increase in support surface to which the yeast cells were attached. Further increase in support weight from 19 to 23g did not change the cell immobilization yield since some water hyacinth stem pieces were not well immersed in the yeast suspension during the cell immobilization. Otherwise, the cell density on the support gradually decreased when the support weight added to 100 mL yeast suspension increased. The suitable amount of support was therefore 19 g for 100 mL yeast suspension. This result was similar to that of Chandel *et al.* (2009) who immobilized *S. cerevisiae* cells on wide sugarcane pieces. According to these authors, 20g support and 100 mL yeast suspension were mixed for cell immobilization.

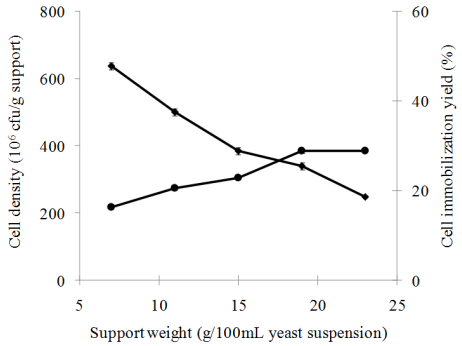

Figure 2. Effects of support weight added to 100 mL yeast suspension on cell immobilization yield and cell density on the support; (●): Cell immobilization yield; (◆): Cell density on the support.

3.1.3 Effects of agitation rate

Figure 3 presents that maximum cell immobilization yield and cell density on the support were 28.8% and 3.5×10^8 cfu/g, respectively at the agitation rate of 100 rpm. Lower agitation rate reduced both cell immobilization yield and cell density on the support since non-uniform distribution of cells in the yeast suspension during the adsorption process. On the contrary, high agitation rate had a negative impact on yeast adsorption on the support due to high centrifugal force. Tang and Le (2011) also reported that 100 rpm was appropriate agitation rate in yeast immobilization on cork root pieces.

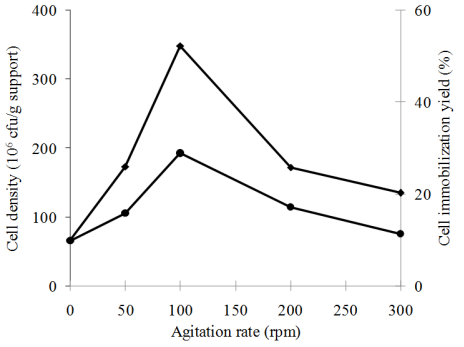

Figure 3. Effects of agitation rate on cell immobilization yield and cell density on the support; (●): Cell immobilization yield; (◆): Cell density on the support.

3.1.4 Effects of initial yeast cell concentration in the suspension

When the initial cell concentration in the yeast suspension augmented from 1.0×10^8 to 3.0×10^8cfu/mL the cell immobilization yield and cell density on the support increased by 44.9% and 40.5%, respectively (Figure 4). Higher cell concentration in the yeast suspension did not change the cell density on the support since the surface area of the support was limited. This phenomenon reduced the cell immobilization yield. Similar observation was mentioned in yeast immobilization on pineapple fruit pieces (Diep and Le, 2009).

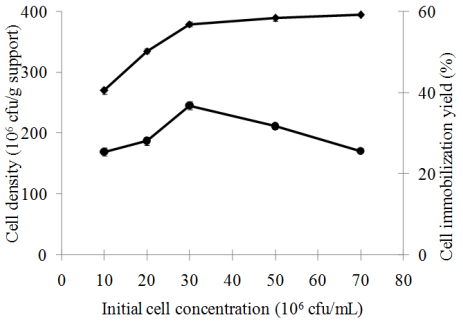

Figure 4. Effects of initial cell concentration in the yeast suspension on cell immobilization yield and cell density on the support; (●): Cell immobilization yield; (◆): Cell density on the support.

3.1.5 Effects of immobilization time

Figure 5 shows that more cells were adsorbed on the support during the time. Both cell immobilization yield and cell density on the support achieved maximum as the immobilization time was 20 hrs. Longer time slightly reduced the cell immobilization yield as well as the cell density on the support due to cell desorption. The appropriate time of yeast immobilization was 20 hrs.

In summary, the conditions for yeast immobilization on water hyacinth stem pieces were as follows: the height of water hyacinth pieces (cylindrical form with 2-cm diameter): 1 cm, the weight of water hyacinth pieces added to 100 mL of yeast suspension: 19 g, the initial cell concentration in the yeast suspension: 3.0×10^7cfu/mL, the agitation rate: 100 rpm,

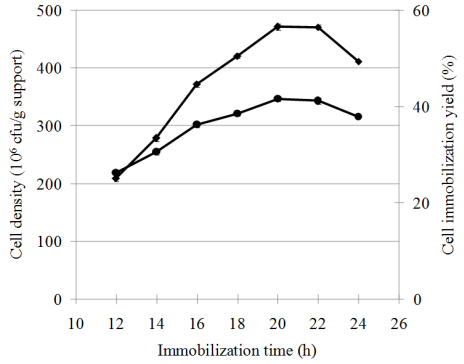

Figure 5. Effects of immobilization time on cell immobilization yield and cell density on the support; (●): Cell immobilization yield; (◆): Cell density on the support.

and the immobilization time: 20 hrs. Under these conditions, the cell immobilization yield and cell density on the support were 41.6% and 4.7×10^8cfu/g, respectively.

3.2 Optimization of technological parameters of yeast immobilization on water hyacinth pieces

It can be noted that change in the weight of support added to 100 mL yeast suspension and the initial cell concentration in yeast suspension in the examined ranges led to a significant variation of the cell immobilization yield. These two factors were therefore chosen for optimization by face centered central composite design with X_1 (g) the amount of water hyacinth stem pieces added to 100 mL of yeast suspension, X_2 ($\times10^6$ cfu/mL) the initial cell concentration in yeast suspension, and Y (%) the cell immobilization yield.

Based on the results obtained, the support weight of 19g (for 100 mL yeast suspension) and the initial cell concentration of 3.0×10^7cfu/mL were chosen as central levels of the face centered central composite design. The experimental ranges and different levels of the independent variables for response surface of the immobilization yield are shown in Table 1. Table 2 reports the cell immobilization yield of each run according to the experimental planning.

Table 1. Experimental ranges and levels of the independent variables for response surface of the immobilization yield.

Independent variables	Range and level			
	Symbol	-1	0	1
Weight of the support added to 100 mL of yeast suspension (g)	X_1	15	19	23
Cell concentration in yeast suspension ($\times10^7$cfu/mL)	X_2	1.0	3.0	5.0

Table 2. Face centered central composite design and experimental results. Y (%): cell immobilization yield; X_1 (g): weight of water hyacinth stem pieces added to 100 mL of yeast suspension; X_2 ($\times10^7$cfu/mL): initial cell concentration in the yeast suspension.

Experiment number	X_1	X_2	Y
1	-1	-1	16.6
2	+1	-1	29.8
3	-1	+1	26.1
4	+1	+1	41.1
5	-1	0	32.5
6	+1	0	45.4
7	0	-1	28.4
8	0	+1	38.5
9	0	0	43.6
10	0	0	43.4
11	0	0	43.6

With experiment number 1 to 4: factorial runs, 5 to 8: axial points with á =1,9 to 11: center points in cube.

Multiple regression analyses were performed on the experimental data and the coefficients of the model were evaluated for their significance using Student t-test. Table 3 shows that the linear coefficients (X_1, X_2) and pure quadratic coefficients (X_1^2, X_2^2) were significant but the interaction coefficient was not. Consequently, the number of water hyacinth pieces and the initial cell concentration in the yeast suspension had a positive effect on cell immobilization yield, while their obvious quadratic effects were also observed, but were all negative.

The analysis of variance of the fitted model is presented in Table 4. Given a satisfactory correlation coefficient, the regression model was deemed significant at the considered confidence level. The predictive equation was finally obtained as below:

$$Y = 43.63 + 6.85X_1 + 5.15X_2 - 4.83X_1^2 - 10.33X_2^2.$$

The effect of the weight of water hyacinth stem pieces added to 100mL of yeast suspension and the initial cell concentration in yeast suspension to the cell immobilization yield is illustrated in Figure 6. From the model, the cell immobilization yield reached its maximum (46.8%) when the weight of water hyacinth stem pieces added to 100 mL of yeast suspension (X_1) was 22g and the initial cell concentration in the yeast suspension (X_2) was 3.5×10^7cfu/mL.

According to our literature review, there have been very few studies showing immobilization yield of yeast cells on organic supports from natural sources. The cell immobilization yield of46.8% in this study was lower in comparison with the findings of Nguyen *et al.* (2009), who immobilized yeast on bacterial cellulose (62.6%). It could be explained by the difference in support structure and adsorption ability of various yeast strains. The structure of bacterial cellulose that is a pellicle composed of a small amount of nanofibrils (Backdahl *et al.*, 2006) is very different from the porous capillary structure of water hyacinth (Schneider *et al.*, 1995).

Under optimal conditions of immobilization, the yeast concentration reached 5.0×10^8 cfu/ g of wet water hyacinth stem pieces. This value is relatively close to the number of yeast cells immobilized on grape skins (4.5×10^8 cfu/g of wet support) (Mallouchos *et al.*, 2002). A higher level of immobilized yeast cells was observed with some organic supports from natural sources such as bacterial cellulose (6.0×10^8cfu/g, Nguyen *et al.*, 2009) or potato pieces (7.1×10^8cfu/g, Kandylis and Koutinas, 2008). Nevertheless, both bacterial cellulose and potato pieces required a special treatment procedure before being used as supports for yeast cell immobilization (Krystynowics and Czaja, 2002; Kandylis and Koutinas, 2008). That would increase the preparation cost of immobilized biocatalyst in the ethanol industry.

Figures7a and b show that the structure of water hyacinth stems was highly porous, with a lot of wrinkles on both the inside and outside surfaces of the stems. This porous structure facilitates yeast adsorption on water

Table 3. Estimated effect of independent variables on cell immobilization yield.

Factor	Effect	SE	P
Intercept	43.63	0.19	2.85E-11
X_1	6.85	0.15	9.48E-08
X_2	5.15	0.15	3.93E-07
X_1^2	-4.83	0.23	4.61E-06
X_2^2	-10.32	0.23	1.05E-07
$X_1 x X_2$	0.45	0.18	0.05899

SE: Standard error; P: Indicates significance of linear regressions. Significant factors at 95% of confidence level.

Table 4. Analysis of variance for the model representing the cell immobilization yield (Y)

Source	DF	SS	MS	F	P	SD
Total	11	14625.70	1329.61			
Constant	1	13756.50	13756.50			
Total Corrected	10	869.27	86.93			9.32
Regression	5	868.59	173.72	1288.32	0	13.18
Residual	5	0.67	0.13			0.37
Lack of Fit (Model Error)	3	0.65	0.22	16.19	0.059	0.46
Pure Error (Replicate Error)	2	0.03	0.01			0.12
N = 11	Q2 =	0.993	Cond. no. =	3.0822		
DF = 5	R2 =	0.999	Y-miss =	0		
	R2 Adj. =	0.998	RSD =	0.3672		

SS: Sum of squares; DF: Degrees of freedom; MS: Mean square; F: F-value at 95% of confidence level; SD: Standard deviation; RSD: Relative Standard deviation

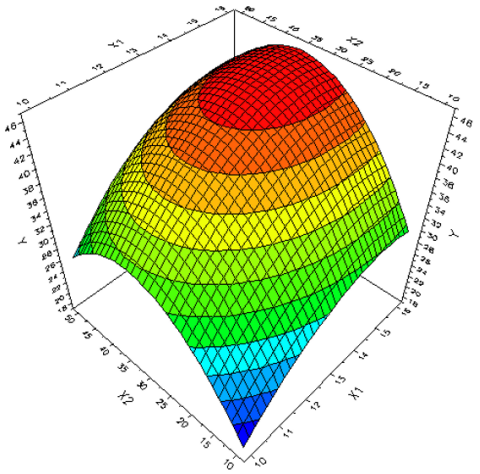

Figure 6. Response surface plot for maximizing cell immobilization yield Y (%), X_1: weight of water hyacinth stem pieces added to 100 mL of yeast suspension (g), X_2: initial cell concentration in yeast suspension ($\times 10^7$cfu/mL).

hyacinth pieces and mass transfer between the support and the medium. After immobilization, yeast cells were fixed not only in various pores (Figure 7c) but also on the stem surface of water hyacinth (Figure 7d).

3.3 Application of immobilized yeast to repeated batch fermentation for ethanol production

In this experiment, the immobilized yeast on water hyacinth pieces was reused for 12 fermentation batches while the free yeast was only used for one batch (Table 5). In the second batch with the free cells, the fermentation was stuck at the residual sugar concentration of 83.7 g/L.

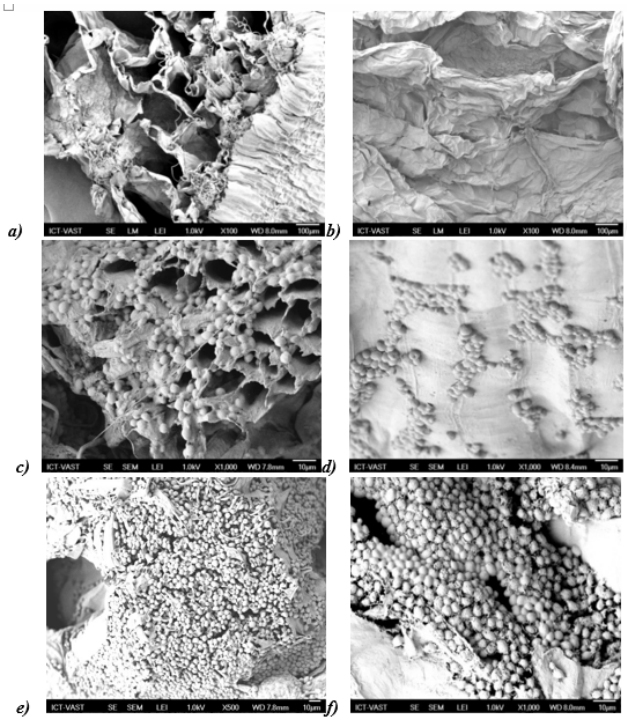

Figure 7. Electron micrographs of water hyacinth stem pieces; (a,b): at the beginning of the yeast immobilization; (c,d): at the end of the yeast immobilization; (e,f): at the end of the 12th fermentation batch; (a,c,e): outer surface of water hyacinth stem; (b,d,f): cross-section of water hyacinth stem.

In the first batch, the ethanol production rate of the immobilized yeast was significantly higher than that of the free yeast. As a result, the fermentation time of the immobilized cells was significantly shorter than that of the free cells.

Table 5. Ethanol formation of the immobilized *Saccharomyces cerevisiae* cells on water hyacinth stem pieces in repeated batch fermentation

Batch number	Ethanol concentration (g/L)	Ethanol production rate (g/L.h)	Ethanol yield (g ethanol produced/ g glucose consumed)
1	$90.50^{a} \pm 0.71$	$1.04^{a} \pm 0.01$	$0.464^{a} \pm 0.004$
2	$90.66^{ab} \pm 0.58$	$1.05^{b} \pm 0.03$	$0.465^{ab} \pm 0.003$
3	$91.05^{abc} \pm 0.48$	$1.08^{c} \pm 0.01$	$0.467^{abc} \pm 0.002$
4	$91.37^{bc} \pm 0.28$	$1.09^{d} \pm 0.07$	$0.469^{bc} \pm 0.001$
5	$90.66^{ab} \pm 0.87$	$1.12^{de} \pm 0.01$	$0.465^{ab} \pm 0.004$
6	$90.73^{ab} \pm 0.39$	$1.15^{e} \pm 0.01$	$0.465^{ab} \pm 0.002$
7	$90.74^{ab} \pm 0.24$	$1.17^{f} \pm 0.03$	$0.465^{ab} \pm 0.001$
8	$90.42^{a} \pm 0.40$	$1.21^{fg} \pm 0.02$	$0.464^{a} \pm 0.002$
9	$90.10^{a} \pm 0.36$	$1.25^{g} \pm 0.04$	$0.462^{a} \pm 0.002$
10	$90.02^{a} \pm 0.49$	$1.29^{h} \pm 0.04$	$0.462^{a} \pm 0.003$
11	$90.74^{a} \pm 0.46$	$1.34^{i} \pm 0.01$	$0.465^{a} \pm 0.002$
12	$90.58^{a} \pm 0.53$	$1.35^{i} \pm 0.02$	$0.464^{a} \pm 0.003$
Free cells	$89.55^{a} \pm 0.48$	$0.87^{j} \pm 0.01$	$0.459^{a} \pm 0.003$

Various small letters in each column represent statistically significant difference ($p<0.05$)

Strehaiano *et al.* (2006) explained that the support could protect the microbial cells from inconvenient factors during fermentation and this would improve metabolic activity of the immobilized biocatalyst. In addition, the ethanol production rate of the immobilized yeast on water hyacinth pieces in all batches was 19.5-55.2% higher than that of the free yeast in the control sample. Similar results were also observed by different researchers with the fixed yeast on corn stem (Vucurovic et al., 2008) and on potato pieces (Kandylis and Koutinas, 2008).

Table 5 also demonstrates that the ethanol production rate of the immobilized yeast increased from 1.0 to 29.8% during the reuse for 12 repeated batches. Figures 7e and f show the fixation of many yeast cells in the pores and on the surface of the support at the end of the 12th batch; the number of yeast cells on water hyacinth pieces reached 5.5×10^{9}cfu/g. Increase in cell concentration on the support during the repeated batch fermentation would lead to a gradual improvement in ethanol production rate. In this study, the ethanol production rate of the immobilized yeast in the 12 repeated batches varied from 1.04 to 1.35 g/L/h; these values were 1.73-2.25 times higher than that in the study of Chandel *et al.* (2009) with immobilized yeast on the support *Saccharum spontaneum* (wild sugarcane), 0.601 g/L/h.

For 12 repeated batches, the ethanol concentration at the end of fermentation with immobilized yeast was nearly similar to that of the control sample with the free cells. Moreover, the ethanol yield of the immobilized and free cells was also similar. Therefore, yeast immobilization on water hyacinth stem pieces did not change ethanol content in the fermentation broth as well as ethanol yield in comparison with the free yeast.

4. Conclusions

The immobilization procedure employing water hyacinth stem pieces was simple and inexpensive. Immobilized yeast on the stems of this plant exhibited much higher ethanol formation rate than that of the free yeast and could be reused for many batches. It can be concluded that water hyacinth pieces can be considered as a promising carrier for the application of immobilized yeast to ethanol fermentation.

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