

some condensed by-products, inhibitory to fermenting microorganisms along with the sugars. Inhibitors of fermentation mainly include furan derivatives, such as furfural and 5-hydroxymethyl-furfural (5-HMF); low-molecular-mass aliphatic acids, such as acetic acid, formic acid, and levulinic acid; and phenolic compounds^[7]. Detoxification is often necessary to enhance fermentability, which would increase the final cost of the xylitol, while it is difficult to detoxify the hydrolysates completely. Besides, furfural along with other inhibitors at very low concentration may greatly inhibit yeast cell growth^[8]. Secondly, fermentable sugar concentrations in hydrolysates are too low to provide enough nutrition for yeast cell growth, so condensation of hydrolysates is needed. However, through this way the concentration of inhibitors is unavoidably increased^[9].

The aim of the present work is to investigate how to enhance resistance to fermentation inhibitors in corn cob fiber condensation hydrolysates of *Candida tropicalis*, isolated from a soil sample, without any detoxifications.

1 Materials and Methods

1.1 Preparation of corn cob hydrolysate

The corn cob hydrolysate used was provided by the HNYR (Hunan Yu-run Biotechnology Co., LTD). The hydrolysates were prepared by mixing with 1% (W/V) sulfuric acid at a ratio of 1 g biomass to 10.0 mL acid solution to be loaded into a 1000 liter reactor. Steam was then added to a pressure of 12 $\times 10^5$ Pa (190 \times). This pressure was kept constant for 10 min. The liquid fraction was separated by filtration from solid fraction then condensed^[7]. It contains 11.955 \pm 0.1 g/L glucose, 98.525 \pm 0.5 g/L xylose, 8.537 \pm 0.08 g/L arabinose, 1.100 \pm 0.3 g/L galactose, 1.326 \pm 0.07 g/L mannose, 1.274 \pm 0.07 g/L acetic acid, 0.621 \pm 0.05 g/L furfural, 0.778 \pm 0.08 g/L 5-HMF, 0.066 \pm 0.01 g/L ethanol (measured by HPLC), and 1.223 \pm 0.05 g phenolic compounds (determined spectrophotometrically by using the Prussian blue method with vanillin as the standard). The hydrolysates used throughout these experiments were stored at 4 \times before use.

1.2 Strain and medium

Candida tropicalis LF01 was isolated from a soil sample and identified and confirmed by Microorganism Graduate School of CAS, China, and was used in all experiments^[10]. The strain was maintained on agar plates made from yeast-peptone-dextrose (YPD) medium containing 10 g/L yeast extract, 20 g/L peptone, 20 g/L dextrose, 20 g/L agar while subcultured every 4 weeks. This medium without agar was used for

growth of *Candida tropicalis*. Adaptation and selection were used to define synthetic media with condensed corn cob fiber hydrolysate by adding 5g/L yeast extract, 10 g/L peptone, 0.5g/L KH_2PO_4 , 0.5g/L MgSO_4 and 2g/L $(\text{NH}_4)_2\text{SO}_4$.

1.3 Adaptation and encapsulation

The cells of *Candida tropicalis* LF01 were cultured at 30 \times overnight in YPD medium, and recovered by centrifugation and suspended in defined media for 13 cycles (Fig. 1). Then the adapted strain LF03 was obtained (Fig. 1). LF03 was used for inoculation and incubated for 48 h at 30 \times on a rotary shaker at 250 r/min before being encapsulated. The capsules were prepared according to Farid Talebnia and Mohammad J. Taherzadeh^[11]. In this method, the inoculum's cells were centrifuged and re-suspended in 1.3% CaCl_2 solution containing 1.3% carboxymethyl-cellulose (CMC). This solution was added drop-wise into 0.6% sterile sodium alginate solution containing 0.1% Tween 20 to create the capsules with mean diameter of 3.9 \pm 4.2 mm and 0.17 \pm 0.02 mm in membrane thickness. The capsules were then washed with distilled water and hardened in 1.3% CaCl_2 solution for 30 min. Then encapsulated strains LF04 were obtained^[11].

1.4 Fermentation experiments

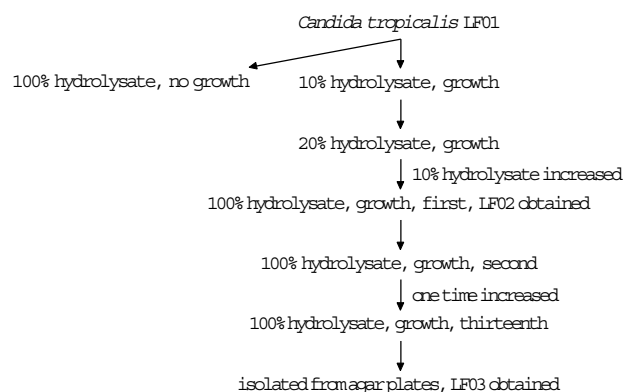


Fig.1 Adaptation of *Candida tropicalis* in hydrolysates

In order to prepare biomass inside the capsules, LF04 was treated by continuous anaerobic cultivation for 20 h and the temperature and pH were controlled at 30 \times and 5.5 respectively in all the experiments unless additionally state. Then inoculated 3000 mL defined medium to obtain into 5 L *B. Braun* jar, to obtain the initial $\text{OD}_{600}=1.0$, cultivated with the stirring rate of 450 r/min for 96 h under oxygen aeration 0.10 vvm.

1.5 Analysis of substrates and products

The fermentation samples containing glucose, xylose, xylitol, glycerol, acetic acid and ethanol filtered through 0.45 μm filters were separated with a Bio-RAD Aminex HPX-

87H hydrogen form cation exchange resin-based column (300 ; 4.8 mm) packed with sulfonated divinyl benzene-styrene copolymer of a particle size of 9 μm. The column temperature was maintained 55 ; æ. The mobile phase consisted of 0.005 mol/L sulfuric acid aqueous solution. The flow rate was set at 0.4 ml/min. The samples were injected with a 10 μl loop ^[12]. The HPLC was produced by Shimadzu, Japan.

Cell concentration was determined by spectrophotometer at a wavelength of 600nm on the process of fermentation, with distilled water used as control.

2 Results and Analysis

2.1 Analysis of yeast growth

Yeast growth phases were determined by colony formation in YPD agar plate with corn cob hydrolysates and OD₆₀₀ of fermentation solutions. YPD agar plates were incubated with 4 μl different strains suspended solution at 30 ; æ for 2 days and fermentations were performed in duplicate and in defined medium for 40 h. The results presented in Fig. 2 and Fig. 3.

The growth phases demonstrated the potential of

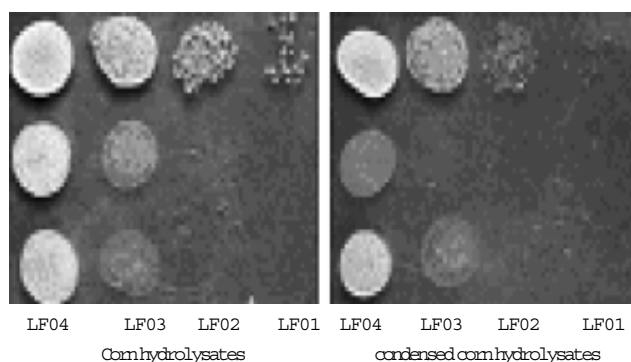


Fig. 2 Growth phases of yeast strains on agar plates with hydrolysates

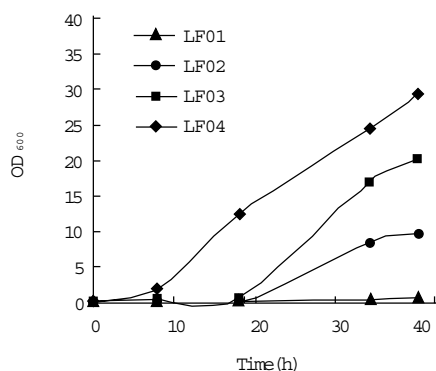


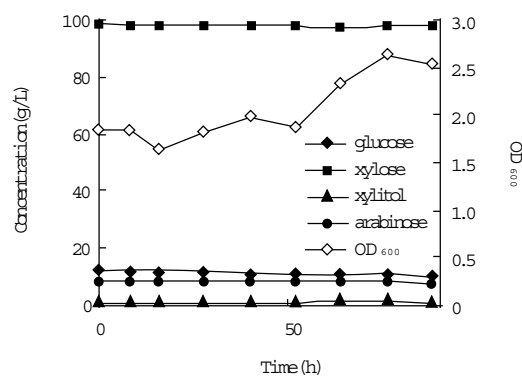
Fig. 3 Growth phases of yeast strains in fermentor with hydrolysates

enhancing resistance to corn cob hydrolysates of yeasts by adaptation and encapsulation. Parent strains LF01 hardly lived in hydrolysates; and adapted strains LF02 and LF03 showed similar fermentation patterns, but there was a great difference in growth between LF02 and LF03. Adapted and encapsulated strains LF04 had the fastest growth and reached a higher cell mass in 20 hours. Since both better growth and higher cell mass were obtained, LF04 were expected to have perfect performance of fermenting corn cob hydrolysates.

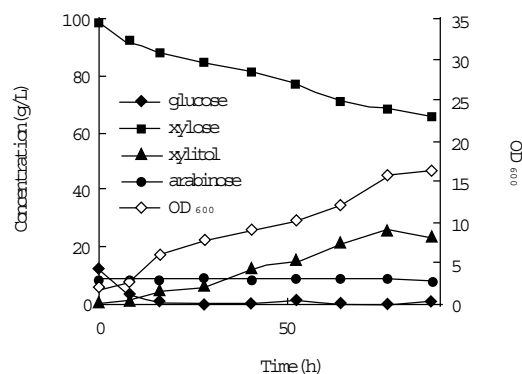
2.2 Batch fermentation in hydrolysate

Batch fermentations in defined synthetic media were carried out by LF01, LF02, LF03 and LF04 respectively. LF04 was able to ferment xylose to xylitol in condensed hydrolysates, which were considered a success in cultivation without any prior detoxification. The average xylitol yield is 0.83 ; 0.03 g/g, much higher than the LF02, LF03, especially parent strain LF01 (Fig. 4).

Combination of adaptation and encapsulation seems to be a promising method to keep the cells viable in toxic environments such as corn cob hydrolysates. Cultivation with encapsulated cells showed clear advantages over cultivation with free cells even though the cells were adapted for many



Concentrations of hydrolysates by LF01 fermenting and its OD₆₀₀



Concentrations of hydrolysates by LF02 fermenting and its OD₆₀₀

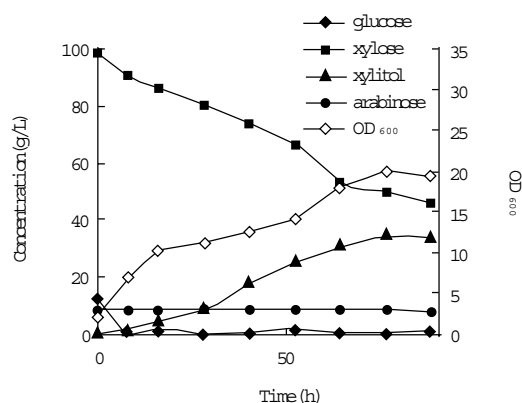
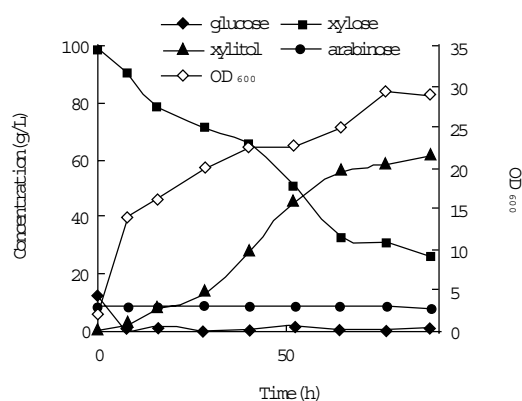
Concentrations of hydrolysates by LF03 fermenting and its OD₆₀₀Concentrations of hydrolysates by LF04 fermenting and its OD₆₀₀

Fig.4 Batch fermentation by strains in hydrolysates

times, and also over the detoxification fermentation. After all detoxification always brought about sugar loss^[13-14].

3 Discussion

In this study, it successfully demonstrated the efficient xylitol fermentation from corn cob hydrolysates by isolated and encapsulated strains. Although the experiments were carried out in the laboratory, yet the commercialization of lignocellulose hydrolysate fermentation would provide possibly strains exhibiting a high growth rate, a rapid fermentation rate, and a high resistance to inhibitors in hydrolysates^[15]. In the fermentation of condensed hydrolysates, LF04 had a slight lower growth than parent strain in YPD medium. It was considered that the rate of C/N of cell wall was changed and the proportion of C and N decreased with increasing adapted times, so the thickness of cell wall was increased. It resulted in reduction of growth rate of the adapted strains.

It could also be concluded that the method of effective combination of adaptation and encapsulation might be used

for bio-ethanol production from the acid hydrolysates of lignocellulosic biomass, such as agricultural and forestry residues, waste papers, industrial wastes and so on.

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