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Comparative Analysis of Bioethanol Production from Whey by different strains of Immobilized Thermotolerant Yeast

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Abstract- In the present context of increasing demand for energy and biofuel, the microbial synthesis of ethanol using industry waste materials has gained recent importance. The present study deals with the ethanol production from whey-a dairy waste by using potential thermotolerant immobilized yeasts isolates in free and immobilized state. Two species of thermotolerant yeasts strains, *Candida inconspicua* W16, *Candida xylopsoci* W23 and standard culture of *K. marxianus* NCDC 39 were used for bioethanol production. Of the selected thermotolerant yeast species, *Candida inconspicua* W16 exhibited the maximum production of ethanol (3.03±0.02 v/v) on immobilization as well as in free State (1.92±0.08% v/v) within 72 h using whey as a substrate. The experiment revealed that the thermotolerant yeast *Candida inconspicua* W16 is efficient in bioethanol production from whey, when it is immobilized.

Index Terms- bioethanol, whey, thermotolerant, immobilization, lactose

I. Introduction

Whey, the liquid remaining after milk fat and casein have been separated from whole milk, is one of the major disposal problems of the dairy industry, and demands simple and economical solutions. Whey represents about 85–95% of the milk volume and retains 55% of milk nutrients. Among the most abundant of these nutrients are lactose (4.5– 5% w/v), soluble proteins (0.6–0.8% w/v), lipids (0.4–0.5% w/v) and mineral salts (8–10% of dried extract). Whey represents an important environmental problem because of the high volumes produced and its high organic matter content. Whey exhibits a biochemical oxygen demand (BOD) of 35,000 ppm and a chemical oxygen demand (COD) of 68,000 ppm (Guimaraes *et al*., 2010). The search for a satisfactory solution for disposal of the unutilized whey produced in the manufacture of cheese remains an area of intense concern for the dairy industry. Although adequate technology is available to recover selectively and concentrate the nutritious protein portion of cheese whey, no widely accepted method has been developed to utilize the lactose portion. Since conventional waste treatment systems are costly, the ideal solution would entail converting the lactose to a marketable product to defray the operating costs and possibly recover the initial capital outlay. One alternative often proposed is to ferment the lactose into ethanol for use as a fuel or chemical feedstock. High ethanol productivity and reduced energy demand have been two important aspects of most alcoholic fermentation research. To achieve them several techniques have been developed including continuous culture, cell immobilization, cell recycle through sedimentation or membrane retention and two stage reactor systems. Application of immobilized yeast cell is the recent technology used to improve the economics of ethanol production from whey. Immobilization is the technique for the physical or chemical fixation of cells, enzymes or other proteins onto a solid support, into a solid matrix or retained by a matrix in order to increase their stability and make possible their repeated or continued use. The immobilized microbial cell can be reused for a long time and transferred simply by draining; fermentation process can be controlled more easily. The immobilization of microorganisms used for ethanol production by attachment to water-insoluble materials has drawn considerable attention (Kourkoutas *et al*., 2002; Guo *et al*., 2010; Ivanova *et al.*, 2011). Using immobilized cells, different bioreactor configurations were reported with variable success rate. The study on the physiology of immobilized cells and development of non-invasive measuring techniques have remarkably improved our understanding on microbial metabolism under immobilized state. *Saccharomyces cerevisiae* was immobilized in Hollow- Fiber Membrane Bioreactors for ethanol production by following the method of Inloes *et al.* (2008). The ethanol production by free and Ca-alginate immobilized cultures of the thermotolerant yeast was compared. It was found that initial yields produced by the immobilized culture lagged behind those produced by cultures in free suspension. However, in subsequent batch-feed experiments it was demonstrated that the ethanol producing ability of the immobilized preparation increased with successive feeds, while production by the free suspension reduced significantly (Inloes et al., 2008). Since the first report of successful application of immobilized cells in industrial applications, several research groups worldwide have attempted whole-cell immobilization as a viable alternative to conventional microbial fermentations. The advantages associated with the production of ethanol at temperatures higher than those used in conventional systems include reduced operating costs with respect to maintaining growth temperature in large-scale systems, reduced risk of contamination, increased rate of productivity and ease with which the product may be recovered, particularly at the later stages in batch and fed batch reactor systems (Nolan *et al.*, 1994). Other advantages may include biocatalyst recycling and rapid product separation (Banat *et al.*, 1998). The present work was therefore carried out to apply immobilization technology for the production of ethanol from whey using thermotolerant yeast cells.

II. MATERIALS AND METHODS

A. Microorganism

Kluyveromyces marxianus NCDC 39 was procured from the National Collection for Dairy Cultures (NCDC), Karnal (India). The thermotolerant lactose fermenting yeast isolates (*Candida inconspicua* W16 and *Candida xylopsoci* W23) were isolated by Dahiya *et al.,* 2010 from whey respectively.

B. Immobilization of yeast cells for ethanol production

The calcium alginate gel-entrapping method was used in the present study (Roukas *et al.,* 1991). *Candida inconspicua* W16, *Candida xylopsoci* W23 and standard culture of *K. marxianus* NCDC 39 cells were immobilized with alginate. Pre-cultured cells (of different dry weight) were each mixed with 50 ml 2.5% Na alginate solution. The resulting mixture was added drop-wise to 150ml 2% $CaCl_2$ solution to make cell-embodied beads. $CaCl_2$ solution was gently stirred at room temperature during the process. The mean diameter of the resulting Ca-alginate gel beads was about 2.8 mm.

C. Production of bioethanol

The production of bioethanol was done using immobilized thermotolerant yeast fermentation by following the method outlined by Roukas *et al, (*1991). Batch immobilized cell fermentation and free cell fermentation was performed at 37°C in 500-ml Erlenmeyer flasks. Ten percent (20-mg dry wt) free cells or 20% (w/v) beads carrying immobilized cells were added to 100 ml of whey fermentation medium as the inoculum. Whey (5.0% w/v lactose) was supplemented with specific amount of nutrients (ammonium sulphate (0.25% w/v), yeast extract (0.5% w/v), phosphorus salt (0.5% w/v) and 1% v/v corn steep liquor), pH of whey was adjusted to 5.0. Fermentation was carried out at 37°C. The level of ethanol in all the flasks was estimated at every 24 h time interval of incubation and change in pH during bioethanol production was also analyzed.

D. Lactose estimation

Residual lactose was estimated according to the method of Picric acid method (Perry and Doan, 1950).

E. Ethanol estimation

Ethanol was estimated by the dichromate colorimetric method, which is based on the complete oxidation of ethanol by dichromate in the presence of sulphuric acid to form acetic acid (Caputi *et al.,* 1968).

III. RESULTS AND DISCUSSION

Analysis of ethanol production was carried out in the immobilized and non immobilized thermotolerant yeast strains, respectively. The results obtained from these experiments were shown in Fig. 1 and 2 which showed the comparison between ethanol production by immobilized and non immobilized yeast cells. Alcohol is a source of energy used for heating, cooking, lighting and as a motor fuel. Many researches are at progress in finding an alternative fuel through biological ways. The thermotolerant microorganism would be a potential source of alcohol production. Therefore, this investigation was aimed to study the feasibility of utilizing the thermotolerant yeast for ethanol production. Among the selected thermotolerant yeast isolates *Candida inconspicua* W16, *Candida xylopsoci* W23 and *K. marxianus* NCDC 39, *Candida inconspicua* W16 showed maximum production of alcohol. It was 3.03±0.2% v/v and 1.92±0.08% v/v under immobilized and non immobilized conditions respectively; moreover, the sugar utilization concentration was lower in case of non immobilized yeast strains than immobilized yeast strains (Fig. 3 and 4). There are many studies on bioethanol production from yeast fermentation (Lark *et al.,* 1997; Zhang and Lynd, 2007). Viruthagiri and Sasikumar (2007) produced ethanol using *Trichoderma viride* and thermotolerant yeast *Kluyveromyces marxianus*. *S. cereviseae* is capable of converting only hexose sugars to ethanol. The most promising yeasts that have the ability to use both pentose and hexose sugars are *Pichia stipitis*, *Candida shehatae* and *Pachysolan tannophilus*. Significantly higher fermentation and ethanol yield were obtained from immobilized cells compared to free cells under similar conditions (Alexander *et al*., 2009; Guo *et al*., 2010; Ivanova et al., 2011). This ability of immobilized yeast cells to produce more ethanol than free cells yet remains to be explained but it may be due to the fact that immobilized cells contains significantly higher percentage of saturated fatty acids compared to the free cells which leads to greater ethanol tolerance in the immobilized cells, and hence greater survival and productivity (Alexander *et al*., 2009). High temperature alcoholic fermentation of whey was carried out by Kourkoutas *et al.* (2002) using *Kluyveromyces marxianus* IMB3 yeast. Delignified cellulosic material (DCM) was used for immobilization of yeast cell. They reported that ethanol yield was nearly 7.3 g l^{-1} ethanol at the end of 72 h of fermentation at pH 4.5 and temperature 45°C.

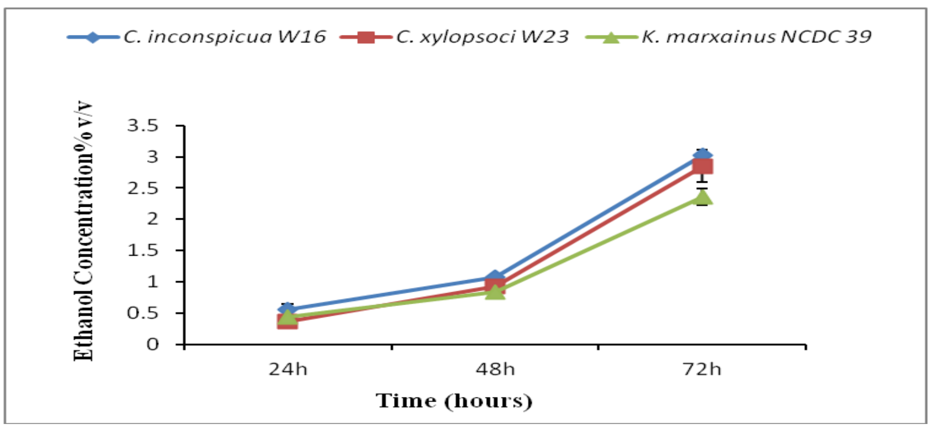

Fig. 1 Ethanol production by immobilized thermotolerant yeast strains from whey

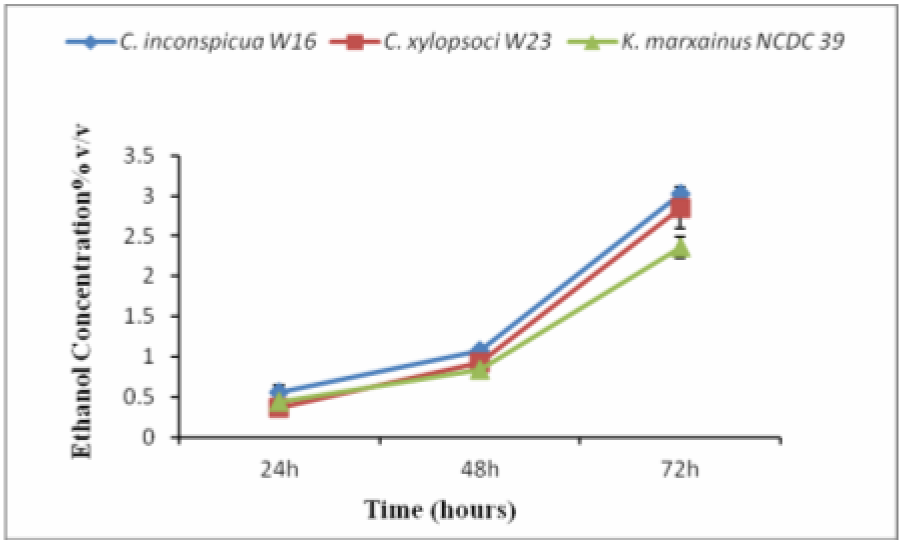

Fig. 2 Ethanol production by non-immobilized thermotolerant yeast strains from whey

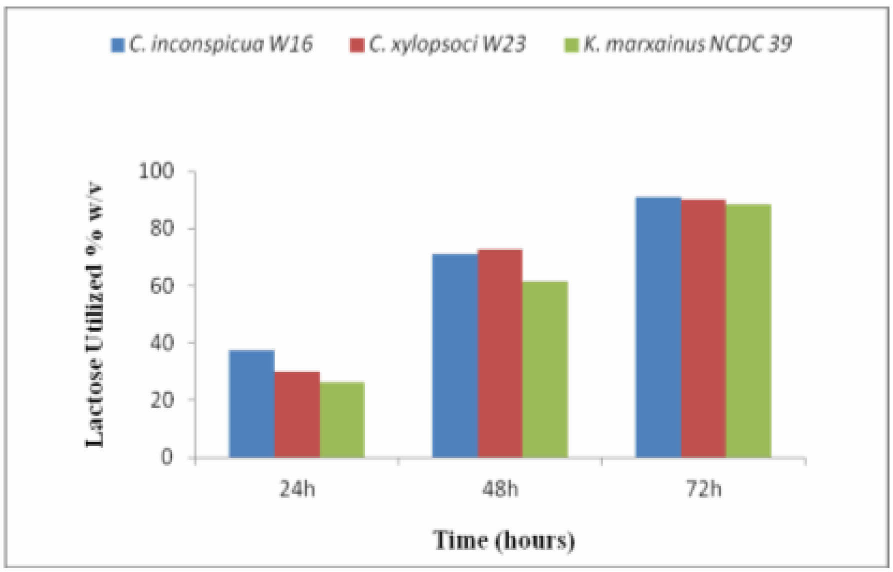

Fig. 3 Lactose utilization by immobilized thermotolerant yeast strains from whey

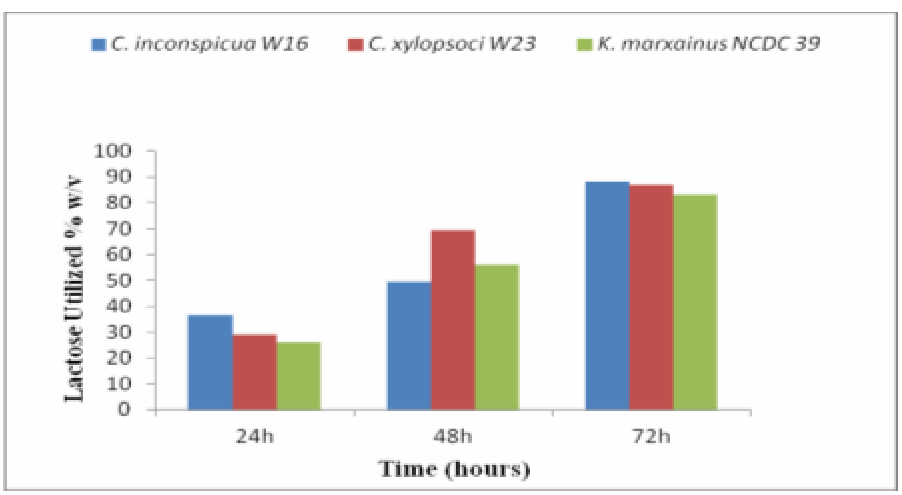

Fig. 4 Lactose utilization by non-immobilized thermotolerant yeast strains from whey

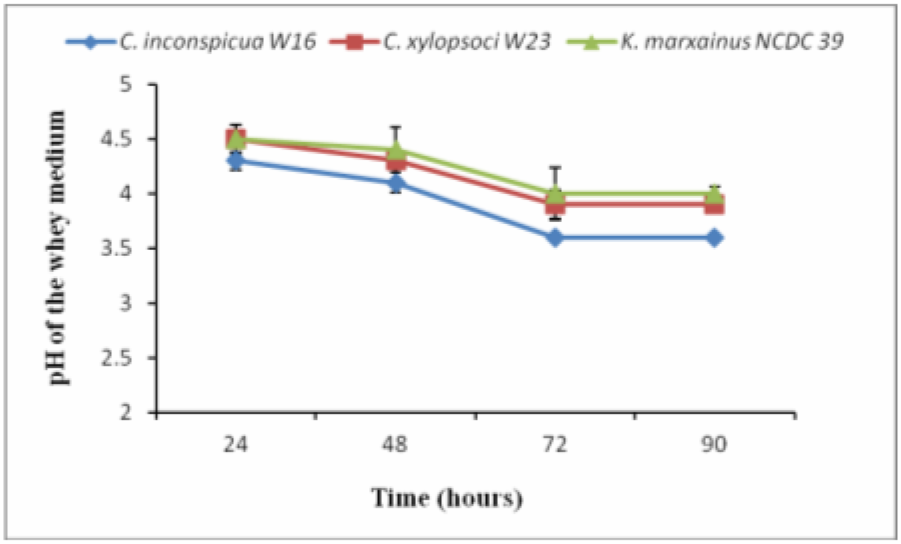

Fig. 5 Change in pH during fermentation

Change in pH of whey medium during ethanol production by immobilized thermotolerant yeast strains

The changes in pH of selected thermotolerant strains, were screened for ethanol production during various time intervals, are depicted in Figure 5. The results showed the relationship between pH and ethanol production by the immobilized yeast cells. *Candida inconspicua* W16 showed the lowest pH and the highest ethanol production.

IV. CONCLUSION

The use of immobilized thermotolerant yeast cells can help to overcome the problems/cost associated ethanol production from whey. Among the selected thermotolerant yeast strains and standard culture of NCDC 39, *Candida inconspicua* W16 thermotolerant yeast strain showed the maximum ethanol production (3.03±0.02 v/v) within 72 h in immobilized state as well as in free cell state (1.92±0.08% v/v). *Candida inconspicua* W16 is a potential yeast strain for producing ethanol production from whey.

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