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*Full Length Research Paper*

# Comparative analysis of bioethanol production by different strains of immobilized marine yeast

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**Yeasts are well known for bioethanol production. However, marine yeasts are less known for the activity. In the present context of increasing demand for energy and biofuel, the microbial synthesis of ethanol using cellulosic waste materials has gained recent importance. The present study deals with the identification of potential marine yeasts for ethanol production. Ten species of marine yeasts were cultured for 24, 48, 72, and 96 h for bioethanol production. Of the ten species, *CANDIDA ALBICANS* exhibited the maximum production of ethanol (47.3±3.1 g/L) within 96 h, when glucose was used as carbon source. The ethanol production by this species was found higher when the yeast cells were immobilized in sodium alginate compared to suspension culture. This experiment was also conducted with both immobilized yeast cells and non-immobilized cells. The experiment revealed that the marine yeast *C. ALBICANS* is efficient in bioethanol production, when it is immobilized.**

**Key words:** Bioethanol, marine yeast species, monoclonal antibodies, thermotolerant.

## INTRODUCTION

Immobilization in biotechnology is the technique used for the physical or chemical fixation of cells, organelles, enzymes or other proteins (e.g. monoclonal antibodies) onto a solid support, into a solid matrix or retained by a membrane, in order to increase their stability and make possible their repeated or continued use. Therefore, it is expected that the microenvironment surrounding the immobilized cells is not necessarily the same

experienced by their free-cell counterparts. Immobilization of microbial cells in biological processes can occur either as a natural phenomenon or through artificial process. While the attached cells in the natural habitat exhibit significant growth, the artificially immobilized cells are allowed restricted growth. 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.

Using immobilized cells, different bioreactor configurations were reported with variable success rate. The study on the physiology of immobilized cells and development of noninvasive measuring techniques have remarkably improved our understanding on microbial metabolism under immobilized state. We have presented an overview of this field.

*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).

## MATERIALS AND METHODS

### Marine yeast species

Ten species, *Candida albicans, Candida tropicalis, Debaryomyces hansenii, Geotrichum* sp., *Pichia capsulata, Pichia fermentans, Pichia salicaria, Rhodotorula minuta, Cryptococcus dimennae* and *Yarrowia lipolylica* isolated from mangrove sediments were used in the present study. After identification, screening was done to

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**Table 1.** Ethanol production in culture filtrates of immobilized marine yeast species.

| S/N | Name of marine yeast | Ethanol production by non-immobilised marine yeasts (g\L) | Ethanol production by immobilised marine yeasts (g\L) |
|---|---|---|---|
| 1. | *Candida albicans* | 28.12±2.14 | 47.3±3.1 |
| 2. | *Candida tropicalis* | 14.13±1.89 | 25.00±1.7 |
| 3. | *Debaryomyces hansenii* | 18.76±2.65 | 24.00±0.7 |
| 4. | *Geotrichum.sp* | 26.79±3.65 | 33.00±0.2 |
| 5. | *Pichia capsulata* | 9.98±1.23 | 14.00±1.6 |
| 6. | *Pichia fermentans* | 13.98±2.54 | 22.00±2.0 |
| 7. | *Pichia salicaria* | 28.50±4.32 | 38.00±1.2 |
| 8. | *Rhodotorula minuta* | 12.34±3.54 | 16.00±1.9 |
| 9. | *Cryptococcus dimennaea* | 14.32±2.98 | 24.00±1.6 |
| 10. | *Yarrowia lipolytica* | 9.80±1.32 | 13.00±2.2 |

identify the potential strain for ethanol production; *C. albicans*, and it sequenced their 18s rDNA and it conformed with *C. albicans* (AC No: Jf292449) with the sequence analysis, submitted to the NCBI.

#### Immobilization of yeast cells for ethanol production

The calcium alginate gel-entrapping method was used in the present study (Inloes et al., 2008). The spherical gel method was employed for the preparation of calcium alginate gels. This spherical gels can be readily obtained by adding sodium alginate solution to calcium chloride solution using a nozzle. No special granulation apparatus was used at the time of equipment assembling, but a gel-dropping nozzle was provided at the top of the fermenter. The fermenter was filled with a calcium chloride solution and substrate was pretreated as innoculum prior to fermentation, and sodium alginate solution was added drop wise to form granules. The culture medium was then supplied to the fermenter to initiate the fermentation. This procedure was carried out to simplify the gel preparation process.

#### Production of bioethanol

The production of bioethanol was done using immobilized marine yeast fermentation by following the method outlined by Inloes et al. (2008). 1 ml of the yeast was enriched in yeast malt broth (dextrose-5.0 g, peptone-5.0 g, yeast extract-3.0 g and malt extract-3.0 g in 1000 distilled water added with 50% seawater). The fermentation was carried out in 500 ml Erlenmeyer flasks using 100 ml of medium. It was kept for fermentation at 28°C for 120 h on a shaker at 120 rpm. The level of ethanol in all the flasks was estimated at every 24 h time interval of incubation and effect of pH on bioethanol production was also analyzed.

#### Estimation of bioethanol by using gas chromatography

Ethanol concentration in the samples was estimated using a Hewlett Packard 5890 Series II gas chromatography with nitrogen as a carrier gas. The temperature of the injection port, oven and detection port were 250,120 and 250°C, respectively. For the analysis, 2 ml of liquid samples was withdrawn from the fermentation broth by using gas tight syringes and then the sample was injected into gas chromatography. The ethanol concentration was determined by using ethanol standard plot and was expressed in percentage of ethanol.

## RESULTS

### Determination of ethanol production by immobilized and non-immobilized marine yeasts

Ethanol production in culture filtrates of immobilized and non-immobilized marine yeast species was determined. Results are tabulated in Table 1 which shows the comparison between immobilized and non immobilized yeasts. Maximum ethanol production was obtained from *C. albicans (*47.3±3.1) which was subjected to calcium alginate entrapment immobilization.

### Changes in pH during the screening of marine yeasts for alcohol production

The changes in pH of ten species of marine yeasts, which were screened for ethanol production during various time intervals, are depicted in Figure 1. The results revealed that *C. albicans* was showing the lowest pH and the highest ethanol production.

### Effect of ethanol production in immobilized yeast and incubation period

Effect of incubation period on immobilization was determined. Maximum ethanol production was obtained in immobilized marine yeast *C. albicans*, maximum bioethanol production was recorded at 96 h of incubation, but there was no significant changes after 96 h incubation (Figure 2).

## DISCUSSION

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

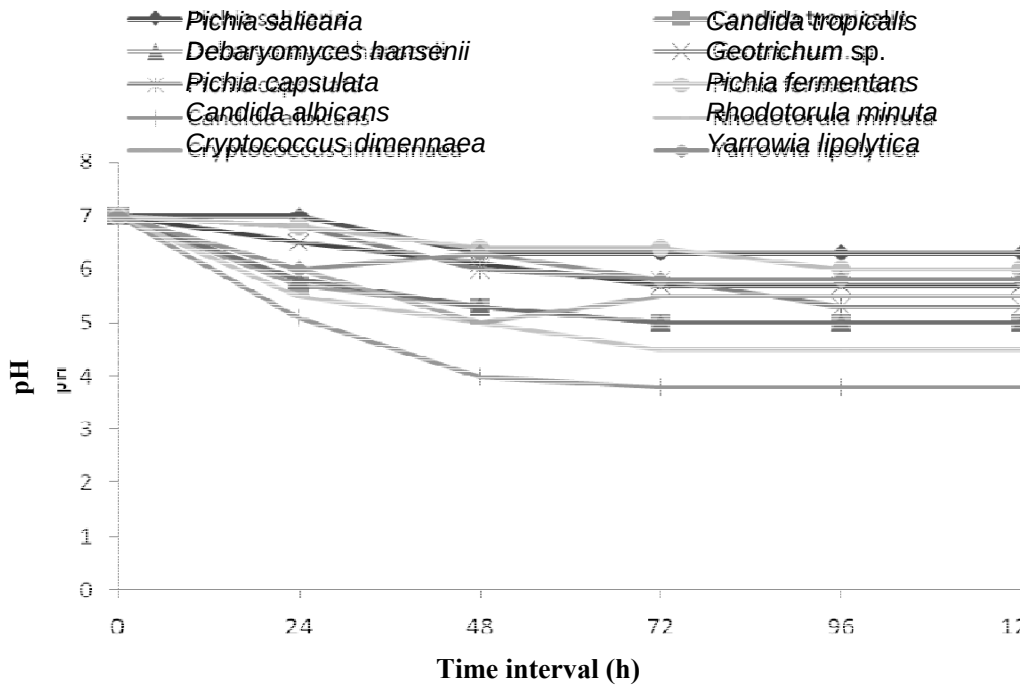


**Figure 1.** Change in pH of yeast strain cell filtrate.

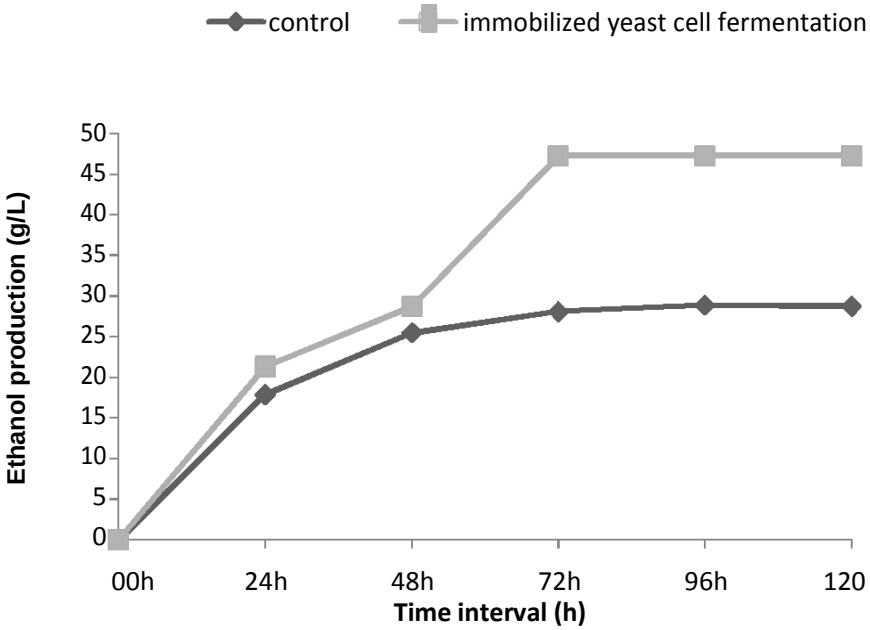


**Figure 2.** Effect of incubation period on ethanol production by immobilized yeast.

ways. The marine microorganism would be a potential source of alcohol, but they are largely ignored for biofuel studies. Therefore, this investigation was aimed to study the feasibility of utilizing the marine yeast for ethanol production. Among ten species of the marine yeast screened for alcohol production, *C. albicans* showed maximum production of alcohol. It was 28.12±2.14 and 47.3±3.1 g/L under non immobilized and immobilized conditions respectively. 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 C5 and C6 sugars are *Pichia stipitis*, *Candida shehatae* and *Pachysolan tannophilus*.

However, ethanol production from sugars derived from starch and sucrose has been commercially dominated by the yeast *S. cereviseae* (Lin and Tanaka, 2006). Thermotolerant yeast could be more suitable for ethanol production at industrial level. In high temperature processes energy savings can be achieved through a reduction in cooling costs. The results of the present study revealed that the bioethanol production varied significantly between the species ($P<0.05$). The changes in pH of ten species of marine yeasts that were screened for ethanol production during various time intervals revealed that *C. albicans* was showing the lowest pH, hence highest ethanol production. Optimization is an important aspect to be considered in the development of fermentation technology. Sree et al. (2000) reported ethanol production of 3.24% (v/v) at 30°C, whereas at 40°C, it produced 1.92% (v/v) by *Zymomonas mobilis*. Jyothi et al. (2005) reported the ethanol production from *Candida intermedia.* Benschoter and Ingram (1986) reported that *Z. mobilis* showed maximum ethanol production and sugar utilization at 30°C.

In the present study, incubation period of 96 to 120 h did not show alcohol production in any of the yeast strains. The calcium-alginate gel is an efficient matrix for the entrapment of yeast cells. These studies and others demonstrate that *C. albicans* cells maintained about 10.0% viability. Less than 1.0% of the entrapped cells were viable after 1 month. The enzyme systems were responsible for alcohol production from glucose function in the yeast cells for as long as 90 days in the fermentation. Our data strongly suggest that the efficient use of immobilized microorganisms for ethanol production has outstanding potential for making future alternative industrial and domestic fuels.


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