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Preparation of guava wine using immobilized yeast

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Abstract

Saccharomyces cerevisiae (NCIM 3287) was immobilized in sodiumalginate beads to produce a biocatalyst for use in guava wine making. The immobilized biocatalyst was found to be suitable for guava juice fermentation at ambient temperatures. The study included effects of alginate concentrations, initial bead loadings and bead diameters on fermentation. The optimized parameters were 2% (w/v) alginate concentration, 60g/l initial bead loading and3 mm bead diameter for effectivefermentation ofguava must.

Key words: Guava wine, Immobilization, *Saccharomyces cerevisiae* NCIM 3287, Sodium alginate

Introduction

An upsurge of interest in cell immobilization for alcoholic beverages and potable alcohol production has taken place recently. This is mainly due to the numerous advantages that cell immobilization offers including enhanced productivity, feasibility of continuous processing, cell stability and lower costs of recovery and recycling and downstream processing (Sevda et al. 2011a, Stewart et al. 1986, Kourkoutas et al. 2004, Fumi et al. 1988). Cell immobilization may also protect cells against shear force (Colagrande et al. 1994). Industrial use of immobilized cells is still limited andfurther applications will depend on the development of immobilization procedures that can be readily scaled-up. Cell immobilization in food and alcoholic fermentation is an attractive and rapidly expanding research area because of its technical and economical advantages compared to the free cell system (Margaritis et al 1984 and Riboulet 1986, Ezquerro and Tena 2005). Guava juice extraction from guava pulp is done by treating it with enzyme pectinase (Vijayanand et al. 2009, Sevda et al.2012) and is then used for fermentation.

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Commercial preparations of yeasts immobilized on alginates or confined by microfiltration membranes are available for sparkling wine production for secondary fermentation in the bottle according to the traditional Champagne method (Iconomou et al. 1996 and Bardi et al. 1996, Kourkoutas et al. 2001). To satisfy the demand for clean technologies and consumer acceptance, various food grade, natural materials like cellulose (Kourkoutas et al. 2001, Fumi et al. 1987 and Silva et al. 2002), gluten (Silva et al. 2003) and fruit pieces (Lemonnierand Duteurtre 1986 and Kosseva et al. 1998) have been proposed as supports for yeast immobilization for use in wine making and brewing processes. However, for application of immobilized system in the food industry, the support should be FDA complaint, cheap, abundant in nature, suitable for low temperature fermentation, and should not change the taste and aroma of the final product.The influence of heat shock treatment on yeast cell and its effect on glycerol production in guava wine (Sevda et al. 2011b) and pomegranate wine (Sevda et al. 2011c) improved the glycerol content and quality of wine.After heat shock treatment, cells can be immobilized for better quality of fruit wine. Many researchers have proposed various supports for cell immobilization in the wine-making process (Hamdy 1990; Ageeva 1985 and Bakoyianis 1992). Immobilized cells have been successfully used for production of useful compounds by various types of bioreactions, such as transformation, synthesis or degradation.

Guava *(PsidiumguajavaL.)* wine is the product of anaerobic fermentation by yeast in which the sugars are converted into alcohol and carbon dioxide (Anderson et al.2005 and Sevda et al. 2011d).The aim of the present study was to investigate the suitability of immobilized cells entrapped in sodium alginate beads for alcoholic fermentation and guava wine making and to study the effect of alginate concentration, initial bead loading and cell bead diameter for guava wine production.

Material and methods

Yeast and Fermentation Substrate

Saccharomyces cerevisiae wine yeast (NCIM 3287) was used in all experiments. Yeast cultures stored in slants were

reactivated in YEPD Medium (Yeast extract 10 g/L, Peptone 20 g/L, Dextrose 20g/L) for 48 hr at 25 °C. Fermentation was carried out on guava juice. Potassium metabisulphate, diammonium phosphate and sugar were added in guava juice before fermentation. All experiments were performed in 250 ml shake flasks.

Preparation of Na-alginate solution

Different concentrations of Na-alginate solution were prepared by dissolving Na-alginate in hot water. The solution was cooled to the room temperature. Appropriate amount of the cell suspension was mixed with Na-alginate solution. Mixing was done with cyclone mixer for 5ml or less solution. In case of higher suspension volume, orbital shaker was used.

Cell Immobilization

Immobilized beads were prepared by drop wise extruding cell-alginate suspension in 0.1M $NaCl_2$solution with help of needle and syringe. To form even sized and perfectly spherical beads, drop wise extrusion was done from approx. 28 cm height. Needles with different size (#18, #22, #24) were used to vary diameter of the beads.

Analytical method

Determination of ethanol: Ethanol in a dilute sample can be separated from other wine components by Gas Chromatography. In this experiment, a hydrogen flame ionization detector (FID) was used. When the carrier gas and the sample emerged from the column, hydrogen and air (synthetic) were added to the carrier gas to produce a flame of about 2100 °C. The pike of the flame served as cathode and a collector electrode as anode. The carrier gas and the sample substances were ionized so that the conductivity of the flame increased. The electric current among flame and electrode were proportional to the absolute mass of the substance.

Settings of the Gas Chromatograph Equipment:

- Temperature of Column Oven: 60 °C
- Temperature of Detector: 150 °C
- Temperature of Splitter: 150 °C
- Carrier gas : Helium;
- Detector: FID; and
- Method: isocratic

To improve quantification, 2-propanol (used as internal standard) solution was used to dilute quantitatively the sample. The peak area ratio for the two chromatographic peaks was compared with the area ratio obtained from injection of standard ethanol-internal standard mixture (Zoecklein et al 1995).

Results and discussion

Effect of alginate concentration

To study the effect of alginate concentration on alcohol production, alginate solutions of 1% to 5% were prepared. To each 40 ml alginate solution with different alginate concentration, 10 ml of cell suspension containing9 g/L of yeast cells was added. Anaerobic fermentation was carried with 8 gm of immobilized beads and 100 ml of guava must for the production of alcohol.

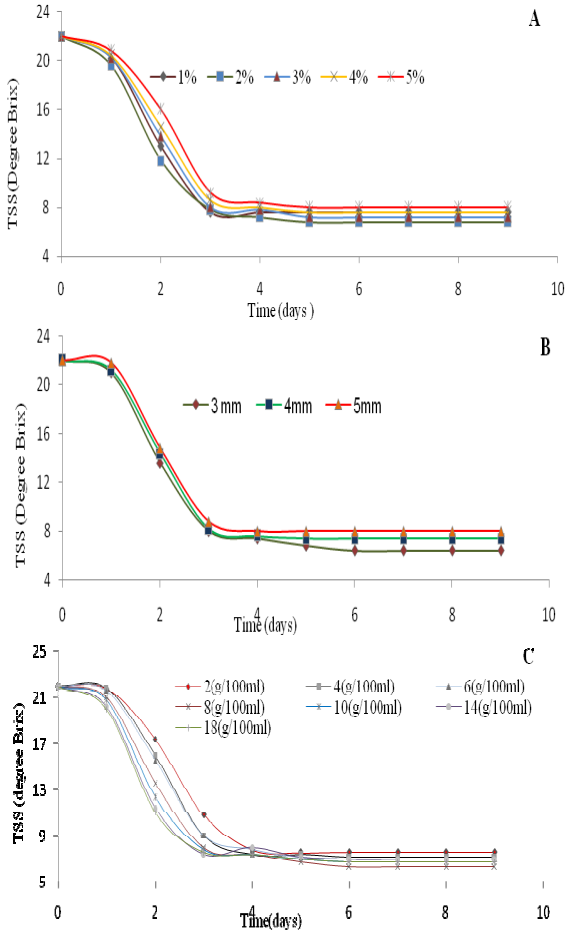

Figure 1: Study on reduction of Total soluble solids (TSS) during guava wine fermentation by immobilized yeast cell with alginate beads with (A) Effect of alginate concentration. (B) Effect of bead diameter of alginate beads and (C) Effect of initial cell loading (n=2, all experiments done in duplicate and average value is used).

Figure 1A shows the reduction in Total soluble solids (TSS) during guava wine fermentation by yeast cells immobilized on alginate beads with varying concentration of sodium alginate. The maximum reduction in TSS during fermentation was observed at 2 % alginate concentration.

Figure2Adepicts the effect of alginate concentration on production of alcohol by immobilized yeast cells*Saccharomyces cerevisiae* NCIM3287. As alginate concentration was varied from 1 to 5%, it caused significant effect on alcohol production: alginate at 2% and 3 % showed maximum alcohol production 8.3 (%v/v). Lower alginate concentration had the problem of cell leakage whiles the beads with higher alginate concentrations had the good mechanical properties as compared to the low concentrations.

Mechanical properties like hardness, mechanical strength etc is essential to operate them in a reactor. This would be expected since there should be a defined pore structure that is dependent on the type of gel material and on the gel concentration. Since the gel forms a quasi-solid structure, it is expected to hinder transport of the solute and, thus, reduce the diffusion coefficient. Na-alginate and $CaCl_2$ reacts and forms the Ca-

alginate gels. This gel involves formation of a porous structure in the beads. The increase in the Na-alginate concentration results in denser structure. Thus, higher alginate concentration decreases the pore size in the bead. It results in more retardation of substrate molecule i.e., increase in internal mass transfer resistance and decrease in effective diffusivity of the substrate molecule and thus resulting in lower rates of fermentation.

Effect of Cell Loading

Cell loading was varied between of 2 g/100ml to 20 g/mL with 3 mm bead diameter and 2 % alginate concentration for guava wine. Immobilized alginate beads were washed three times with 100 ml of saline to remove the cells, which can leak. Then, 8gm of beads were mixed with 100 ml of guava juice and subjected to anaerobic fermentation and analyzed for alcohol content.

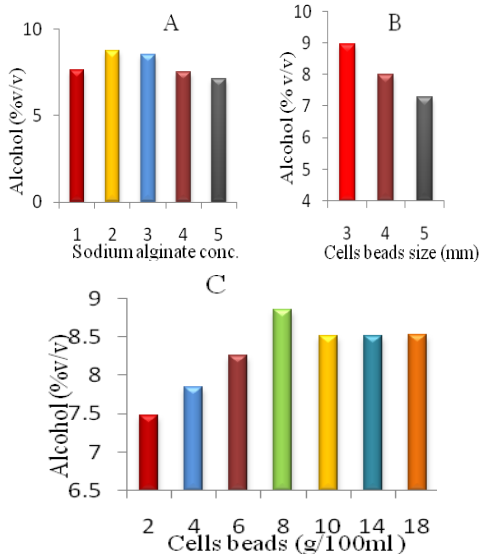

Figure 2: Immobilized yeast cells *Saccharomyces cerevisiae* NCIM 3287 showing(A) Effect of alginate concentration (B) Effect of bead diameter of alginate beads and (C) Effect of initial cell loading on production of alcohol in guava wine production(n=2, all experiments done in duplicate and average value is used).

Figure 1C shows effect of initial cell loading on reduction of TSS. Figure2C indicates the effect of initial cell loading on alcohol production by the immobilized system. As the cell loading was decreased from 18 g/100ml to 8 g/ml, it had no effect on alcohol production whereas further reduction in cell loading from 8 to 2 g/ml decreased the alcohol production. The reason forthe low production rate can be attributed to lower cell density in the bead at low cell loadings. At an optimum concentration of 8 g/ml of cells, it showed maximum reduction in TSS with a constant rate of fermentation. The cells were present to such an extent that further increase in cell density resulted in only marginal increase in productivity.

Effect of bead diameter

Beads with various sizes (2, 3 and 4 mm diameter) were prepared in 3% alginate solution. 10ml of 9 g/l cell suspension was mixed in 40 ml of alginate solution. 8 gm of these beads were added to100 ml of fruit juice and subjected to anaerobic fermentation. After completion of fermentation, the samples were analyzed for alcohol content Figure 1B shows the effect of cell bead diameter on alcohol production with immobilized cells. There was a gradual decrease in alcohol production with increasing bead diameter. As the diameter of the bead is increased, the substrate molecule has to travel more to reach the center of the bead. At the same time, cells present inside the matrix reacts with the molecule and form product. Due to product formation, outward flow of product towards outside bulk liquid stream starts. It additionally lowers the diffusion coefficient and internal mass transfer limitation occurs. This results in overall reduction in the production. Smaller beads (3 mm) have more surface area per unit volume and hence more productivity.

Figure 2B shows the effect of cell bead diameter on alcohol production by immobilized cells. It shows that lower cell bed diameter exhibits higher alcohol production. The immobilization of yeast cells in sodium alginate spherical beads was made by entrapment of cells. The biocatalyst was found effective for guava wine making, and the process was feasible using a modified bioreactor, designed to keep separately the grape skins from the immobilized biocatalyst. Good operational stability, as observed during repeated fermentation batches, shall give the possibility to remove the biocatalyst after a longer time.

Conclusions

The above study demonstrates that sodium alginate can be used for the development of a cost effective wine-making process involving immobilization of yeast. They are cheap, safe, and easily available raw material. Their use demands no pre-treatment and the immobilization technique is a natural process. The immobilized biocatalyst is operationally stable, which makes possible its use at commercial scale. Guava wine making using yeast cells immobilized entrapped in sodium alginate spherical beads.

Various physical factors like alginate concentration, bead diameter and cell loading play important role in the success of immobilized systems as all the factor has impact on the mass transfer rate of the substrate and product formed. The optimized parameters were alginate concentration 3 %(w/v), initial cell loading 8g/100ml and cell bead diameter of 3mm. Also, because of their shape and size, they could be applied in continuous processes and fluidized bed bioreactor systems.

Acknowledgements

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