

THESIS INFORMATION

INTRODUCTION

PhD student: **TÔN NỮ MINH NGUYỆT**
Thesis topic: **Research on the establishment of appropriate conditions for *Saccharomyces cerevisiae* immobilization in calcium alginate gel and on bacterial cellulose in wine fermentation.**
Major: **Food and beverage processing**
Major code: **62540201**
Training institute: **Ho Chi Minh City University of Technology, VNU-HCMC**
Scientific supervisor: **Associate Prof. Dr. Lê Văn Việt Mẫn**

CONTENT

Research objective: Improvement in economic efficiency for wine fermentation by using the immobilized yeast on a new carrier.

Research subject: Bacterial cellulose carrier (*Acetobacter xylinum* BC12), calcium alginate carrier, *Saccharomyces cerevisiae* TSV09, Shiraz and Red Cardinal grape juice were used.

Research content: Determination of technological parameters for yeast immobilization, using a popular carrier (alginate) and a new carrier (bacterial cellulose); comparison of wine fermentation ability of the fixed and free yeast; investigation of the reuse of immobilized yeast and establishment of stoichiometric equations of the primary fermentation in wine – making.

Analytical methods: Haemocytometry (for determination of yeast cell number), spectrophotometric technique (for reducing sugars, nitrogen, tannins), chemical titration (for SO₂, acidity, Ca²⁺), gas chromatography (for flavor compounds) and dynamic flash combustion (for C, H, O, N) were used.

Data analysis: Analysis of variance (Statgraphics plus 3.2) and response surface methodology (Modde 5.0) were used.

Scientific contributions:

- Technological parameters for yeast immobilization in alginate gel and for yeast immobilization on bacterial cellulose by Face Centered Central Composite Design were determined; the dependent variable was yield of cell immobilization.
- Fermentation ability of the immobilized yeast in alginate gel, the immobilized yeast on bacterial cellulose and the free yeast were evaluated: Yeast growth, substrate uptake and metabolite production were determined and compared.
- The repeated batch fermentation was investigated with the reuse of the immobilized yeast.
- Stoichiometric equations of wine fermentation were established for the immobilized yeast in alginate gel, the immobilized yeast on bacterial cellulose and the free yeast.

RESULTS

From the fundamental science point of view:

Establishment of stoichiometric equations of wine fermentation: The biomass yield of the immobilized yeast on bacterial cellulose was 8,0% higher than that of the free yeast. Meanwhile, the biomass yield of immobilized cells in alginate gel was 15,4% lower than that of the free cells. The ethanol yield of the fixed yeast in alginate gel and on bacterial cellulose was 2,4% and 1,5%, respectively, lower than that of the free yeast.

From the applied science point of view:

Technological parameters for yeast immobilization in alginate gel: Cell concentration in yeast suspension: $108 \cdot 10^6$ cfu/mL; alginate concentration: 4,3%; CaCl_2 concentration: 2%; retention time of the gel beads in CaCl_2 solution: 2h. The cell immobilization yield was 95,0% and the cell concentration in the gel beads was $7,2 \cdot 10^7$ cfu/g.

Technological parameters for yeast immobilization on bacterial cellulose by adsorption-incubation method: Cell concentration in yeast suspension: $185 \cdot 10^6$ cfu/mL; immobilization time: 4h 45min; pH of yeast suspension: 4; carrier size: 1cm x 1cm x 0,7cm; weight of carrier: 40g/200mL of yeast suspension; agitation speed: 200rpm; incubation time: 2 days at room temperature. The cell immobilization yield at the end of the adsorption was 62,6%. After 2 day incubation, the cell concentration on bacterial cellulose was $1,4 \cdot 10^9$ cfu/g.

Wine fermentation ability of the immobilized yeast in alginate gel, the immobilized yeast on bacterial cellulose and the free yeast: As the sugar concentration in grape juice varied from 200 to 280g/L, the cell concentration and growth rate of the immobilized yeast on bacterial cellulose were higher than those of the free yeast. Meanwhile, the fixed yeast in alginate gel demonstrated the lowest cell concentration and growth rate. The immobilized yeast fermented sugar faster, assimilated more free amino and ammonium nitrogen than the free yeast. Moreover, the fixed yeast produced ethanol faster and produced less volatile acids than the free yeast.

Application of immobilized yeast to the repeated batch fermentation in wine-making: The treatment of alginate gel beads in CaCl_2 solution at the end of each batch run reduced the ratio of released cells of 30-64% and increased the ethanol production rate of 9-27 % in comparison with the control; The immobilized yeast in alginate gel and on bacterial cellulose could be reused at least for 10 batch runs. During the reuse process, the fermentation time of the immobilized yeast was always shorter while the sugar uptake rate and ethanol production rate were higher than those of the free yeast.

Scientific supervisor

PhD student