EFFECT OF INITIAL SULFUR DIOXIDE CONTENT IN MUST ON THE KINETICS OF WINE PRIMARY FERMENTATION, USING YEAST IMMOBILIZED IN CALCIUM ALGINATE GEL

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ASTRACT: *Effect of initial sulfur dioxide content in must on the kinetics of wine primary fermentation, using yeast immobilized in calcium alginate gel was examined. Initial sulfur dioxide content in must was adjusted from 12ppm to 312ppm. Our experimental results indicated that the maximum specific growth rate of the immobilized yeast was 1-2 times less than that of the free yeast. On the contrary, the sugar uptake rate and ethanol production rate of the immobilized yeast were 1.23-1.90 times and 1.22-1.81 times, respectively, more than those of the free yeast. The volatile acid content and pH value of the final wine fermented by immobilized yeast were lower. Thus, the organoleptic properties and biological stability of the wine were ameliorated. In general, the yeast immobilized in calcium alginate gel was more resistant to sulfur dioxide than the free yeast.*

Key words: *alginate, immobilization, fermentation, sulfur dioxide, yeast*

1. INTRODUCTION

Cell immobilization in alcoholic fermentation is a rapidly expanding research area because of its attractive technical and economic advantages compared to the free cell system such as enhanced fermentation productivity, feasibility of continuous processing, cell stability and lower costs of recovery and recycling processing as well as tolerance to disadvantage factors [5,6,8].

Although many immobilization supports have been proposed for use in wine-making, industrial application of the technology is still uncertain and should be investigated. This is mainly due to difficulty in finding a low-cost material that is abundant, durable, of food-grade purity and has the ability to be preserved for a long period [5].

Alginate has been considered as a potential support for cell immobilization because of simple immobilization procedure, high cell density in the gel and non-toxic carrier [1,7].

The aim of this study is to investigate the effect of initial sulfur dioxide content in must on the kinetics of wine primary fermentation, using yeast immobilized in calcium alginate gel.

2. MATERIALS AND METHODS

Yeast: A Saccharomyces cerevisiae strain of Food Microbiological Laboratory Collection (Ho Chi Minh City University of Technology) was used in the present study. Grape juice was used for yeast multiplication. Preculture was prepared by two successive inoculations: 1) in 250ml erlenmeyer shake flask containing 100ml of grape juice for 24 hours, and 2) in a 2000 ml erlenmeyer shake flask containing 500ml of grape juice. For both periods, the inoculum was grown at 28°C and 250rpm.

Alginate: Sodium alginate was supplied by Biotechnology Center, Nha Trang University of Fisheries. The viscosity (2% alginate solution, 25°C) was 423.6cp.

Must: Fermentation medium was prepared from Red Cardinal grape (Ninh Thuan province, Vietnam). It was adjusted to 240g/L of glucose, 195ppm of ammonium nitrogen, pH 4.0. Initial sulfur dioxide content was alternatively adjusted to 12ppm, 112ppm, 212ppm, 216ppm and 312ppm.

Yeast immobilization: The immobilization procedure was carried out by the traditional external gelation method [1]. Yeast concentration was 25x106 cells/ml of gel bead.

Fermentation: was conducted at 22-25°C in an erlenmeyer containing 500ml of grape must. The inoculating rate was $5x10^6$ cells/ml. Free yeast cells were used in the control sample.

Analytical methods:

Yeast cell number was quantified by haemocytometry, using Thoma counting chamber. For counting yeast cells inside the gel beads, the beads were dissolved in a 2%w/v Na-EDTA solution [1].

Reducing sugar content was determined by spectrophotometric method, using 3,5-disalycylic acid reagent [4].

Ethanol concentration was determined by a method based on distillation and density quantification [4].

Volatile acid content was measured by the following method: Firstly, wine sample was steam-distilled. Then the obtained distillate was titrated by the 0.1M NaOH solution [9].

Statistical treatment

The presented results were the average of three independent experiments. The obtained results were subjected to analysis of variance (ANOVA), $p<0.05$ using Statgraphics plus, version 3.2.

3. RESULTS AND DICUSSION

3.1.Effect of initial SO_2 content in must on yeast growth

Figure 1 showed that when the sulfur dioxide content in must increased from 12 to 112ppm, the maximum specific growth rate of the free and immobilized yeasts increased slightly, the maximum cell density of the free and immobilized yeasts augmented aproximately 19% and 5%, respectively. In wine fermentation, sulfur dioxide has been used for controlling oxidation reactions and restricting the growth of indigenous micro flora in must [2]. According to Fleet (1993), increase in sulfur dioxide content in must reduced the multiplication of wild yeasts and bacteria [2]. The growth of the wine yeast was therefore improved.

However, when the initial sulfur dioxide content in must increased from 112 to 312ppm, the maximum specific growth rate of the free and immobilized cells decreased aproximately 14 times and 7 times, respectively; the maximum cell density of the free and immobilized yeasts reduced aproximately 1.35 times and 1.24 times, respectively. It can be explained that when the initial sulfur dioxide content in must was high enough, the wine yeast was also inhibited.

When the initial sulfur dioxide content in must varied from 12 to 112ppm, the maximum specific growth rate and maximum cell density of the free yeast were higher than those of the immobilized yeast. On the contrary, when the initial sulfur dioxide content varied from 212 to 312ppm, the difference in maximum specific growth rate and maximum cell density in both free and immobilized yeast cultures were nearly similar. Therefore, the immobilized cells were more resistant to sulfur dioxide than the free cells. It was due to protection role of alginate gel for the immobilized yeast.

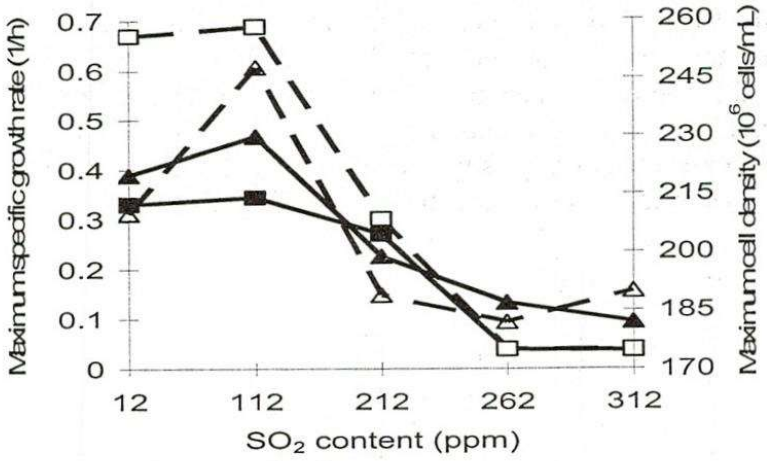

Figure 1. Effect of initial SO_2 content of must on maximum specific growth rate and maximum cell density. Symbol: (□■), maximum specific growth rate, (△▲) maximum cell density. Opened symbol: free yeast, filled symbol: immobilized yeast

3.2.Effect of initial SO_2 content in must on the fermentation time

In this experiment, the fermentation was considered as completed when the fermentation productivity reached approximately 97%. Fermentation productivity was the ratio between the reducing sugar content consumed by yeast during the fermentation and the initial reducing sugar content in the medium. Our results showed that the higher the sulfur dioxide content in must, the longer the fermentation time (Table 1). When the sulfur dioxide content in must increased from 12 to 112ppm, the change in fermentation time was insignificant. However, when the content of sulfur dioxide augmented from 112 to 312ppm, the fermentation time of the free and immobilized yeasts increased. It should be noted that the fermentation time of the immobilized yeast was always shorter than that of the free yeast in all cases. Therefore, yeast immobilization in calcium alginate gel improved its fermentation activity.

Table 1.Effect of initial SO_2 content of must on the fermentation time

SO2 (ppm)	Fermentation time (h)	
	Immobilized yeast	Free yeast
12	84 ± 3,61a	112,8 ± 2,31c
112	84,5 ± 3,04a	117,6 ± 3,33c
212	100,8 ± 2,97b	132 ± 3,61d
262	100,3 ± 3,54b	196,8 ± 3,54f
312	184,8 ± 3,70e	228 ± 4,36g
Various superscripts indicate significant differences ($p < 0.05$)		

3.3.Effect of initial SO_2 content in must on the fermentation rate

The fermentation rate was evaluated by the average sugar uptake rate and average ethanol production rate of yeasts. Figure 2 and 3 presented the obtained results. Analysis of variances

showed that when the sulfur dioxide content in must increased from 12 to 112ppm, both sugar uptake rate and ethanol production rate of the immobilized and free yeasts changed insignificantly. On the contrary, when the sulfur dioxide content augmented from 112 to 312ppm, the fermentation rate of the immobilized and free cells decreased approximately 50% of initial value. In all cases, the average sugar uptake rate and average ethanol production rate of the immobilized yeast were always higher than those of the free yeast.

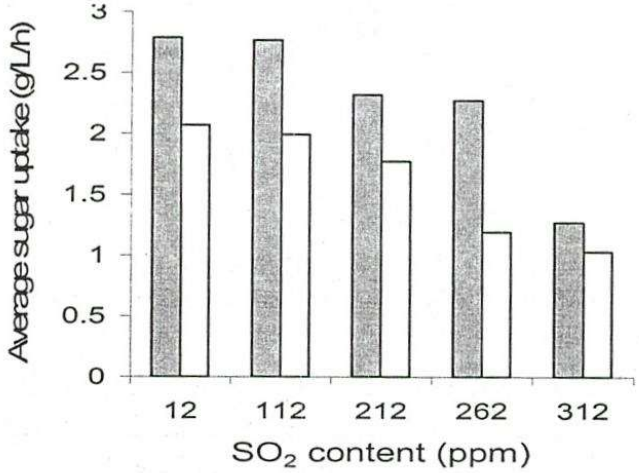

Figure 2. Effect of initial SO_2 content of must on average sugar uptake rate. Symbol: (■) immobilized yeast, (□) free yeast.

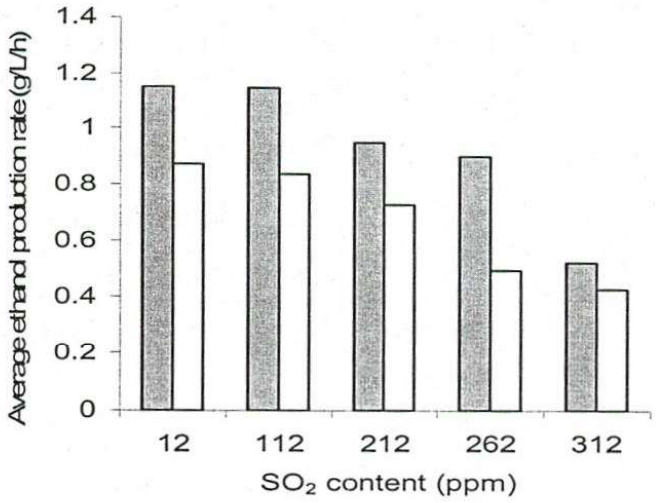

Figure 3. Effect of initial SO_2 content of must on average sugar uptake rate. Symbol: (■) immobilized yeast, (□) free yeast.

3.4.Effect of initial SO_2 content in must on pH and volatile acid content in the product

Figure 4 showed that increase in sulfur dioxide content in must augmented the pH value at the end of the primary fermentation. In addition, the pH value of wine fermented by the immobilized yeast was always lower than that in the control sample.

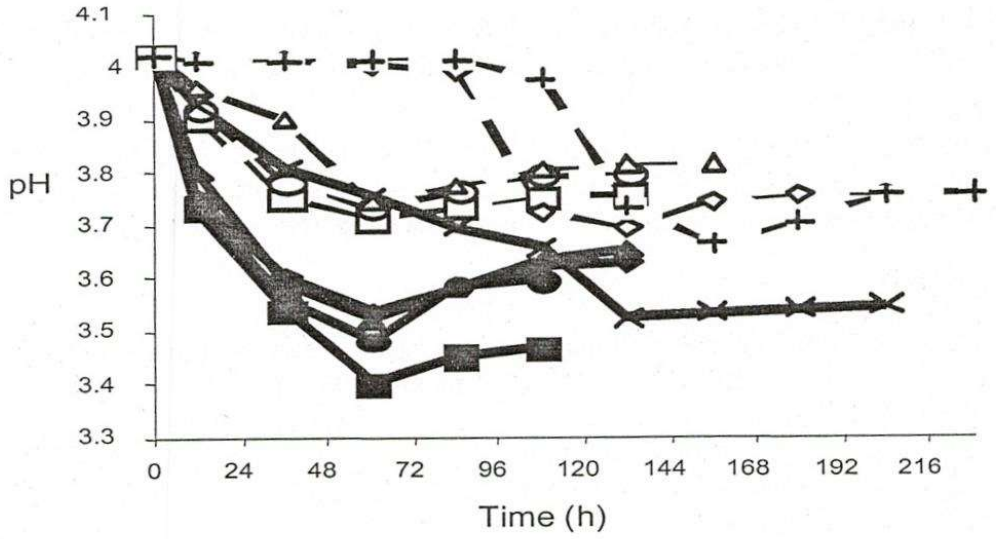

Figure 4: Change in pH value during must fermentation. The initial sulfur dioxide content in must was varied: 12ppm (□■), 112ppm (○●), 212ppm (△▲), 262ppm (◇◆), 312ppm (+×). Opened symbol and (+): free yeast, filled symbol and (x): immobilized yeast.

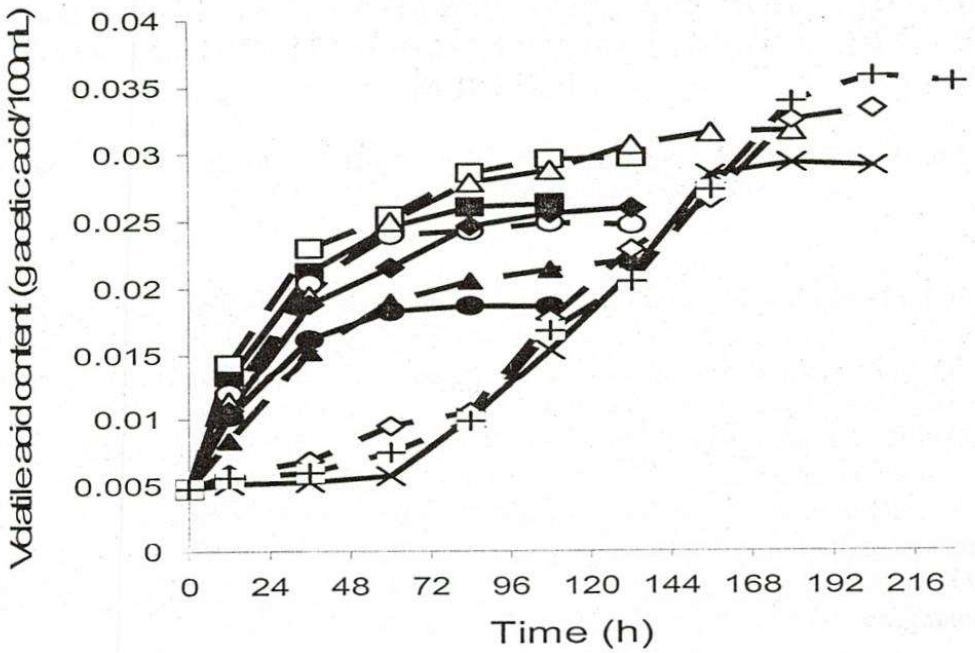

Figure 5. Change in volatile acid content during must fermentation. The initial sulfur dioxide content in must was varied: 12ppm (□■), 112ppm (○●), 212ppm (△▲), 262ppm (◇◆), 312ppm (+×). Opened symbol and (+): free yeast, filled symbol and (x): immobilized yeast.

Figure 5 indicated that volatile acid content augmented during the fermentation. When the initial sulfur dioxide in must was 112ppm, the volatile acid content in the produced wine was the lowest. It can be explained that in must with low sulfur dioxide content, the growth of non-*Saccharomyces* yeasts and bacteria that produced volatile acid was ameliorated. Thus the volatile acid content in the final product increased. In addition, some genera if wild yeasts such as *Kloeckera, Candida* were resistant to sulfur dioxide. According to Heard G.M. et al., (1993), the growth of these wild yeasts was not inhibited by total SO_2 concentrations of 100-

150ppm or higher [3]. So in some cases, high sulfur dioxide content in must did not completely inhibit the wild yeasts and this phenomenon increased the volatile acid content in the culture.

It can be noted that the volatile acid content of the produced wine fermented by the immobilized yeast was always lower than that in the control sample. Thus the application of immobilized yeast in wine making improved the sensory properties of the product.

4. CONCLUSION

Yeast immobilization in calcium alginate gel increased the fermentation activity of cells as well as their resistance to sulfur dioxide. In wine making, the immobilized yeast had higher fermentation activity than the free yeast, especially when must contains high initial sulfur dioxide content. So the application of immobilized yeast in wine fermentation was very potential when initial must needs to be processed with high sulfur dioxide content for inhibiting wild yeast and bacteria.

ẢNH HƯỞNG HÀM LƯỢNG SULFUR DIOXIDE BAN ĐẦU TRONG DỊCH NHO ĐẾN ĐỘNG HỌC QUÁ TRÌNH LÊN MEN CHÍNH TRONG SẢN XUẤT RƯỢU VANG, SỬ DỤNG NẤM MEN CỐ ĐỊNH TRONG GEL ALGINATE CALCIUM

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TÓM TẮT: *Bài báo này khảo sát sự ảnh hưởng của hàm lượng sulfur dioxide ban đầu trong dịch nho đến động học quá trình lên men chính trong sản xuất rượu vang, sử dụng nấm men cố định trong gel calcium alginate. Hàm lượng sulfur dioxide ban đầu trong dịch nho được hiệu chỉnh trong khoảng từ 12 đến 312ppm. Kết quả thực nghiệm cho thấy tốc độ sinh trưởng riêng cực đại của nấm men cố định thấp hơn từ 1-2 lần so với nấm men tự do. Ngược lại, tốc độ sử dụng đường và tốc độ sinh tổng hợp ethanol của nấm men cố định lần lượt cao hơn từ 1.23-1.90 lần và 1.22-1.81 lần so với nấm men tự do. Hàm lượng acid bay hơi và giá trị pH của rượu vang được lên men bằng nấm men cố định thì thấp hơn. Do đó, các tính chất cảm quan và độ bền sinh học của rượu vang được cải thiện. Nhìn chung, nấm men cố định trong gel calcium alginate bền với tác nhân sulfur dioxide hơn so với nấm men tự do.*

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