Low temperature fermentation of wine and beer by cold-adapted and immobilized yeast cells

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1 Introduction

Over the last 20 years considerable research and development have been made in wine making and brewing technology with the aim of improving the productivity and quality, taste and aroma of wine and beer. Use of selected cultures and genetically modified yeasts with desired traits, use of immobilized cells on several supports, enzymatic treatments, addition of adjuncts to malt, modern development in fermentor design and low-temperature fermentation are some technological innovations in alcoholic fermentation. Among these technological innovations, low-temperature fermentation by cold-adapted and immobilized yeast cells is reviewed here.

The genus *Saccharomyces* is the most important yeast to mankind. It is used as wine yeast, brewer’s yeast, distiller’s yeast and baker’s yeast. Yeast is an important contributor to flavor development in fermented beverages. The major flavor compounds produced by yeast during fermentation are ethanol, higher alcohols, aldehydes, esters, sulphur compounds, vicinal diketones and others. Some of these compounds are highly desirable even in very small quantities while others are not. So, strains possessing valuable properties, such as low-temperature and ethanol tolerance, may give high fermentation rate, produce desirable aroma and taste, and may give hygienic composition of beverages, high cell viability and easy separation from alcoholic beverage at the end of fermentation. In addition, cell immobilization has advantages over a free cell system, such as (i) higher cell density inside a bioreactor, thereby offering a higher fermentation rate, (ii) immobilization of cells by entrapment within polymers or adsorption to solid carriers which protects the cells and so maintains their viability and activity more, with or without a limited increase in cell population, (iii) after the end of batch fermentation the alcoholic beverage is removed and the fermentor is refilled with substrate for a new batch. In the case of continuous fermentation the process can be monitored and optimized.

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The capital cost is minimized. A basic problem in the cell immobilization is the nature of support, which must be hygienic for food production. Also the support must be stable, cheap, easily prepared or abundant in nature for probable industrial application.

1.1 Influence of temperature on alcoholic fermentation

The process of the alcoholic fermentation depends directly on the temperature. Temperature is a factor over which the winemaker and brewer have great control.\textsuperscript{1,2} The upper, the lower and the optimum fermentation temperature differ depending on the different yeast strains as regards to the fermentation rate, the yeast growth, the yeast metabolism, the alcohol-tolerance of the yeast, fermentation by-products, etc.

When the fermentation is going to be carried out at the lower temperature limit, there is risk of yeast death. Also at low temperature, the fermentation start, process, cell growth and metabolism of yeast may be delayed, but the evaporation of ethanol and volatile by-products is minimized. It has been reported by Nagodawithana et al.\textsuperscript{3} that when the fermentation temperature was increased between 15 and 30°C, the fermentation rate is also increased, but the cell viability decreased. The optimum fermentation temperature is higher than optimum temperature growth of yeast and the optimum process temperature must lie between the two above temperatures. The optimum fermentation temperature and the optimum temperature growth of yeast are decreased at high ethanol concentrations because the alcohol tolerance of the yeast is decreased at high temperatures.\textsuperscript{3,4} Thermotolerant strains of \textit{Saccharomyces} were isolated from samples collected at the end of the sugarcane harvest season in distilleries.\textsuperscript{5} Their main characteristic was the maintenance of high cell viability, so they fermented sugar juice at 38–40°C in less than 10 h and without continuous aeration of the culture. Thermotolerant yeasts have been defined as those capable of growth at temperature $\geq 45^\circ$C and/or fermentation at 40°C. In general, \textit{Kluyveromyces} strains were more thermotolerant than \textit{Saccharomyces} and \textit{Candida}, but \textit{Saccharomyces} strains produced higher ethanol yields.\textsuperscript{6} Research on the influence of temperature, dilution rate and sugar concentration on the establishment of steady-state in continuous ethanol fermentation of molasses was carried out in laboratory-scale vessels at 27, 32 and 37°C by Perego et al.\textsuperscript{7} At 27°C the system attained a steady state. Steady states were never reached at 37°C, but at 32°C the system response depended on the values of the dilution rate and sugar concentration.

The effect of temperature on must fermentation in the laboratory and at an industrial scale ($2.5 \times 10^4$ l) was studied by Caro et al.\textsuperscript{8} Factors such as specific microbial (yeast) growth rate, conversion of substrate to other products except ethanol, ethanol evaporation and lower alcohol yield were investigated. Table 1 shows the ethanol amount evaporated during fermentation, the final ethanol concentration, the specific microbial growth rate, and the percentage fraction of substrate converted to other products at different fermentation temperatures for batch laboratory fermentors. The data in Table 1 show that there is important loss of ethanol...