

Starchy Supports: Immobilization and Wine Making

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Whole cell immobilization was defined as “the physical confinement or localization of intact cells to a certain region of space with preservation of some desired catalytic activity” [1]. Immobilization often mimics what occurs naturally when cells grow on surfaces or within natural structures. A lot of different techniques for the immobilization of cells have been proposed, which can be divided into four major categories based on the physical mechanism employed: (a) attachment or adsorption on solid carrier surfaces, (b) entrapment within a porous matrix, (c) self aggregation by flocculation (natural) or with cross-linking agents (artificially induced), and (d) cell containment behind barriers [2].

In last decades cell immobilization for alcoholic fermentation is a rapidly expanding research area and several immobilized cell systems have been proposed and studied. However, applications of this technology at industrial scale are limited. It is important the supports that will be used for immobilization in food industry to be of food grade purity in order the final product to be suitable for consumption. Therefore many research works have been published concerning the use of food grade purity supports in wine making. Some examples are the use of gluten pellets [3], dried raisin berries and grape skins [4,5], and fruits such as quince, apple, pear [6], guava [7], watermelon [8] and dried figs [9]. The use of these immobilized supports led to the production of high quality wines with improved aroma. In addition the aroma of these products, produced using fruits as immobilization supports, was characterized fruity.

Nowadays another important aspect for successful industrial application of this technology that should be taken into consideration during selection of supports suitable for immobilization is that they must ideally be abundant in nature and cost effective. The use of the above mentioned supports may lead to an increase in the price of the final product something that is not preferred. Therefore in such products, like wine and beer, it is important to use as immobilization supports products abundant in nature, ease to handle and especially of low cost. A promising proposal for such supports is starchy supports. Starchy supports are mainly referred to products such as potato, corn, wheat, barley and products that derive from them.

Potato

The use of potato pieces as immobilization support of yeast for wine making has been investigated and the results were very promising [10]. More specifically this biocatalyst retained its operational stability for a long period and in a wide range of fermentation temperatures ranging from 25 to 2°C producing wines of fine clarity. Regarding the effect of the biocatalyst in the aromatic profile of the wines the SPME-GC-MS (Solid Phase Micro-Extraction – Gas Chromatography – Mass Spectroscopy) analysis showed that the immobilized cells produced wines with improved aroma compared to the wines produced by free cells. In addition the percentages of the total esters on total volatiles were increased by the drop in temperature, while percentages of alcohols were reduced. Finally a possible catalytic effect of the potato pieces in alcoholic fermentation was reported and was proved by the calculation of activation energies. The results showed that the activation energy of

the immobilized cells was 44% smaller than that of free cells while the corresponding fermentation rate constant k was higher in immobilized cells.

Barley and Wheat

Whole barley and wheat grains have been used as immobilization support of yeast for wine making [11,12]. The barley and wheat supported biocatalysts proved capable for wine making in a wide range of temperatures producing quality wines with great and improved aroma as was indicated by SPME-GC-MS analysis. More specifically the formation of esters was accelerated, giving higher numbers and concentrations compared to free cells. In addition, a decrease of temperature led to further increase in the percentages of esters compared to alcohols something that is considered to have a positive impact to wine aroma. These two supports have almost the same characteristics for immobilization. More specifically offer a large area for immobilization since the yeast cells can be attached in the surface of grains and can also be mixed and entrapped inside them in the starch granules. This characteristic is important in immobilization technologies since larger numbers of immobilized cells means higher productivities.

Corn

Corn grains have been also evaluated as support for yeast immobilization [13]. The immobilization was confirmed by electron microscopy, showing yeast cells attached on the surface of corn grains and also mixed and entrapped inside them, showing also physical flocculation of yeast cells. Thus the available area of immobilization is increased making possible the immobilization of a large number of cells per grain. The biocatalyst retained its operational stability for a long period and it proved able to produce dry wines of fine clarity, even at extremely low temperatures (5°C). The produced wines were analyzed for volatile byproducts by GC and SPME-GC-MS analysis. The results showed an increase in the number and amount of esters produced when immobilized cells were used. In addition, an increase in the percentages of esters and a decrease in those of alcohols with the decrease of fermentation temperature were observed. Finally a model, appropriate for describing sugar consumption and the simultaneous ethanol formation by immobilized cells, was proposed. The results of this model fit almost perfectly with the experimental data. This model is necessary for possible designing of a process suitable in larger industrial scale.

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Received April 19, 2012; **Accepted** April 20, 2012; **Published** April 23, 2012

Citation: Kandylis P (2012) Starchy Supports: Immobilization and Wine Making. *Ferment Technol* 1:e107. doi:10.4172/2167-7972.1000e107

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Corn Starch Gel

In addition to potatoes, barley, wheat and corn grains pure starch has been also investigated as a possible support for yeast immobilization [14]. The corn starch was used in the form of gel produced after gelatinization of starch granules at high temperatures. The yeasts were attached on the surface of gel and were also mixed and entrapped in the interior of gel matrix. The immobilized biocatalyst was used in the form of small cakes having different shapes with lengths ranging from 0.2 to 1 cm. The biocatalyst retained its operational stability for a long period and in a wide range of fermentation temperatures producing wines of fine clarity. The biocatalyst retained its form during the whole fermentation process without dissolving. The SPME-GC-MS analysis showed that the produced wines had improved aromatic profile compared to free cells and an increase in the percentages of total esters and subsequently decrease of alcohols was observed with the decrease of temperature. Finally the catalytic effect of corn starch was also revealed by calculating the activation energies and fermentation rate constants. More specifically the activation energy of immobilized cells was 36% smaller than that of free cells while the reaction rate constant of immobilized cells was 3.6-fold higher than that of free cells at 15°C.

Freeze-Drying

In order to be of practical use, immobilized biocatalysts must be formulated as products capable of storage, distribution and application in agricultural and food marketplace. Formulation is necessary in order to present the product in a marketable form and in order to optimize the efficacy, stability, safety and ease of application of the product. Freeze-drying is the most convenient and successful method of preserving yeasts, sporulating fungi and bacteria due to its numerous advantages such as protection from contamination or infestation during storage, long viability and ease of biocatalyst distribution. Taking all this into account the freeze-drying of starchy supports is necessary in order to make them attractive to market. The freeze-drying of wheat supported biocatalyst has reported and the effect of several protective agents and storage at 5°C on viability and fermentative activity of yeast cells were studied [15]. Glycerol was the best protective agent that preserved the viability of immobilized yeast cells at high levels even after 9 months of storage. The freeze-dried biocatalyst proved capable to produce dry wine in a wide range of temperatures. The SPME-GC-MS analysis showed that the freeze-dried biocatalyst produced wines with higher formation of esters than free cells, having at least similar aromatic profile to those produced by wet biocatalyst. This biocatalyst can be stored by wine making industries until the next wine making period without significant loss of cell viability and most importantly capable to produce wines with similar organoleptic characteristics to those of wet cultures.

Scale-up

An important step for the transfer of immobilization technologies and especially new biocatalysts in industrial level is to prove that they can retain their fermentation characteristics (that they had in laboratory level) also in a semi-industrial level without, if it is possible, any loss. Therefore biocatalysts prepared after immobilization of yeast cells on wheat grains has been used for wine making in a scale-up system of 80 L [12]. The scale-up process is necessary in order to determine the effect of such scale on fermentation characteristics of biocatalyst. The wheat supported biocatalyst was used for repeated batch fermentations and was capable to produce dry wines for a long period. The scale-up process did not affect the fermentation ability of

biocatalyst, even at low temperatures, while the produced wines had improved aromatic profile compare to free cells as revealed by GC and SPME-GC-MS analysis. In addition an increase in the percentages of total esters and a decrease in those of higher alcohols were observed in lower fermentation temperatures.

Nowadays the advantages of cell immobilization in alcoholic fermentation and especially in wine making such as increased productivities, reduced risk of contamination, biocatalyst recycling and rapid product separation are well established and known. Therefore the research should be focused on cheap, abundant and food grade purity immobilization supports and especially on their effects on the quality of the final products. Studies should be carried out covering all the aspects, as in the case of starchy supports, from laboratory to industrial scale and even in studies evaluating the possible storage of biocatalysts for long periods.

In rapidly expanding technologies like immobilization the use of open access journals is becoming a necessity. Open access journals make new knowledge available instantly and more importantly to all without the prerequisite of expensive subscription. The open access journals in combination with the unique features offered by OMICS Group such as 50 world leading language translation of manuscripts, audio version of published paper, digital article etc making the submission and reading of papers more attractive, easy and enjoyable. In addition the submission and review process is rapid (21 days), qualitative (15,000 editorial team) while possible publication offers indexing in high quality organizations such as PubMed, Scopus, DOAJ, EBSCO etc. Finally the plethora of scientific conferences organized annually by OMICS Group, covering all the aspects of modern research, is also helpful for knowledge distribution and exchange.

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