INNOVATIVE MATERIALS FOR THE IMMOBILIZATION OF MALOLACTIC STARTERS. TECHNOLOGIES, BIOLOGICAL EFFECTS AND EXPERIMENTAL FERMENTATIONS WITH OENOCOCCUS OENI STRAINS IMMOBILIZED IN HYBRID MATRICES (SILICA/ALGINATE).

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Introduction

The use of immobilized microbial cultures can improve the efficiency of industrial fermentations [1, 2]. In recent years, the use of immobilized winemaking starters has become a reasonable alternative to free cell cultures [3, 4]. Wines and musts have a chemical composition which can limit microbial activity given various factors: high initial sugar content, high acidity, low pH and inhibiting effects of ethanol and SO2. These obstacles can lead to sluggish fermentations and to the production of secondary metabolites that can reduce the quality and safety of the wine [5]

Microbial culture immobilization can resolve these problems: given that it is possible to adapt the chemical composition of the immobilization matrix to meet the particular needs of the microbial species used, hence reducing the environmental effects on the cellular activity. Furthermore, if the cell immobilization within the solid substrate is efficient, cells will not be released into the fermentation medium. This guarantees a strict control over the microbial development in the wine and an easier biomass recovery at lower process costs.

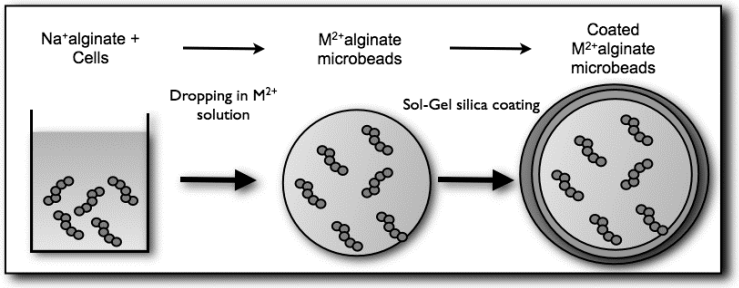

Figure 1. Proposed immobilization scheme (by Callone et al.,1998 [6])

Results and Discussion

The study presented aimed to improve and perfect immobilization techniques for microorganisms used in winemaking.

The proposed process was recently published [6] and foresees 3 distinct phases (Figure 1):

1. The cells are suspended in a 2% Na^+-alginate solution;
2. The cell/alginate suspension is extruded into a 0.1M M^{2+} (M = Ca, Ba) solution, the alginate immediate solidifies trapping the cells in M^{2+}- alginate microspheres.
3. The microspheres are coated with a silica layer, sol-gel derived from two different precursors, tetraethoxysilane and methyltriethoxysilane.

Due to this particular production process and the type of precursors used, the silica layer is chemically inert, allowing for free exchange of mass between the cells and the environment. It also has physical properties (elasticity and rupture resistance) that prevent the release of cells into the medium [7-9].

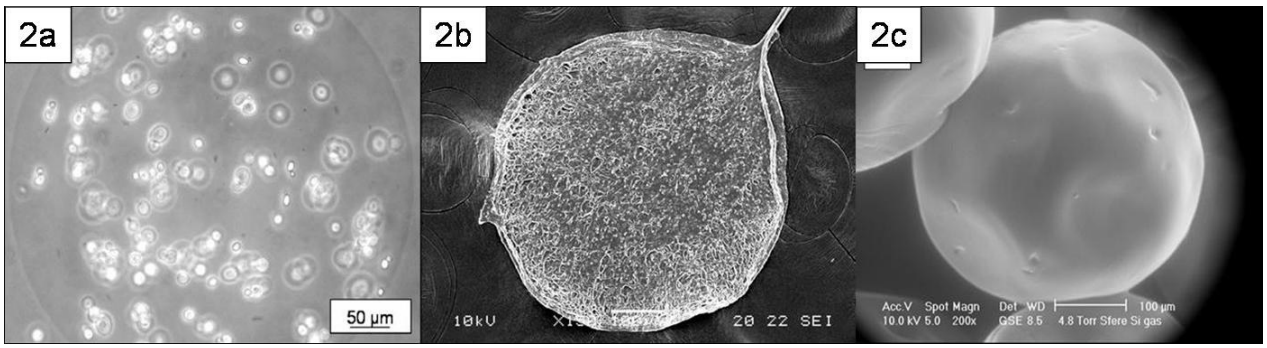

Figure 2°: S. cerevisiae cells immobilized in silica alginate microspheres (optical microscope 25x). 2b. SEM image of alginate microspheres. 2c. ESEM image of silica/alginate microspheres. The morphological change in the surface due to the sol-gel deposited silica layer is evident (from Callone et al., 1998 [6])

Numerous techniques were used to characterize the chemical and physical properties of the proposed immobilization system. The measurement of the silica/alginate spheres with an optic micrometer demonstrated that the process guarantees dimensionally consistent microspheres (average diameter 450±80 µm) [6]. This is fundamental in order for the immobilized cells to maintain efficient activity (Figure 2a).

The immobilization process does not alter the microbial load inside the microspheres: cellular density measurements inside the silica/alginate microspheres show that this treatment does not decrease yeast or bacteria viability. The final microbial load is greater than 10^9 cells/gram of microsphere [6]. The examination of the microspheres with an electron scan microscope (ESEM and SEM) (Figure 2b, 2c), the elemental analysis of silica and the NMR analysis (Nuclear Magnetic Resonance) of the solid isotope ^{29}Si [6, 10] allowed for an accurate characterization of the silica coating layer. The silica coating layer is an continuous and uniform film, 10µm thick, formed of completely polymerized silicate units (R-Si$(OSi)_3$ and Si$(OSi)_4$) that are further anchored to the alginate matrix.

Experimental fermentations were completed with different substrate concentrations (glucose for yeast, L-malic acid for malolactic bacteria) in order to evaluate the fermentation activity of the cells enclosed in the silica/alginate matrix. The experimental results were analyzed following a method proposed by Lineweaver and Burk, which is used to measure the effect of the immobilization on substance transport. The K_m (affinity constant) and V_{max} (maximum fermentation speed) calculated for fermentations with either free or immobilized cells (Table 1) showed that the immobilization treatment does not interfere with the cellular activity, and that substances are transported across the microsphere [6].

Table 1. Kinetic parameters of alcoholic fermentations (S.cerevisiae) and malolactic fermentations (O. oeni), with either free or immobilized cells, in a synthetic medium (modified from Callone et al., 1998 [6]).

Sample	10^6 V_{max} (M sec^{-1} × g of cells)	K_m (M^{-1})
Free *S. cerevisiae* cells	48 ± 5	0.3 ± 0.1
Immobilized *S. cerevisiae* cells	33 ± 1	0.1 ± 0.1
Free *O. oeni* cells	4.2 ± 0.4	4 ± 0.1
Immobilized *O. oeni* cells	4.0 ± 0.2	2 ± 0.6

The efficiency of immobilized *Oenococcus Oeni* cells during malolactic fermentation (MLF) was studied on volumes between 5 and 50 liters with different operative conditions.

For all the trials completed, the immobilized and free bacteria showed analogous fermentation activity. The immobilized biomass was used in three serial MLF for a total of 48 consecutive fermentation days (Figure 3), hence fermenting 3 times the amount of wine as the homologous free

culture. There is a low release of cells into the wine, less than 10^5 cell/ml. Furthermore there is a significant cost saving in selected bacterial starter usage.

	Etanolo (%)	pH	Acidità totale /g(l)	Acidità volatile (g/l)	Ac. Malico (g/l)	Ac. Lattico (g/l)
VINO BASE	11,39	3,34	5,99	0,32	2,35	0,20
FINE 1ª FML	11,24	3,48	4,60	0,32	0,18	2,07
FINE 2ª FML	11,28	3,48	4,40	0,42	0,24	2,24
FINE 3ª FML	11,08	3,45	4,50	0,45	0,20	2,27

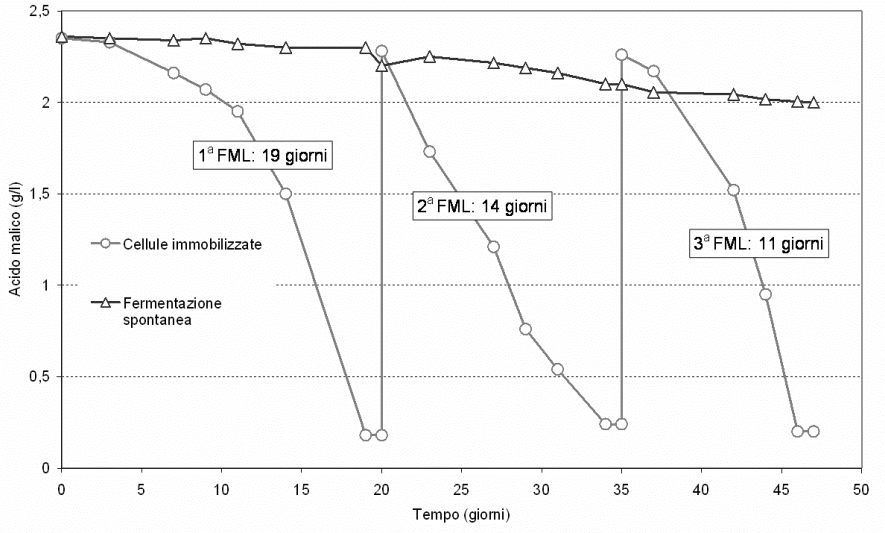

Figure 3. Serial malolactic fermentation with *O.oeni* cells immobilized in silica/alginate microspheres. The immobilized biomass showed a high fermentative activity for 48 consecutive days of MLF.

The immobilized bacteria were also co-inoculated with free yeasts, in the must, hence resulting in a MLF that ended at the same time as the alcoholic fermentation, without altering the enological parameters of the wine (acetic acid, tartaric acid, residual sugar) (Figure 4).

	Etanolo (%)	pH	Acidità totale (g/l)	Acidità volatile (g/l)	Ac. Tartarico (Ac. Malico (g/l)		Ac. Lattico (g/l)
Dati iniziali	0,2	3,43	5,5	0,19	7,22	3,58	0
Test 17 Brix fine fermentazione	10,29	3,54	4,1	0,47	1,68	0,23	2,66
Test 20 Brix fine fermentazione	12,11	3,56	4,3	0,52	1,81	0,27	2,34
Test 22 Brix fine fermentazione	13,2	3,54	4,6	0,56	1,78	0,64	2,04

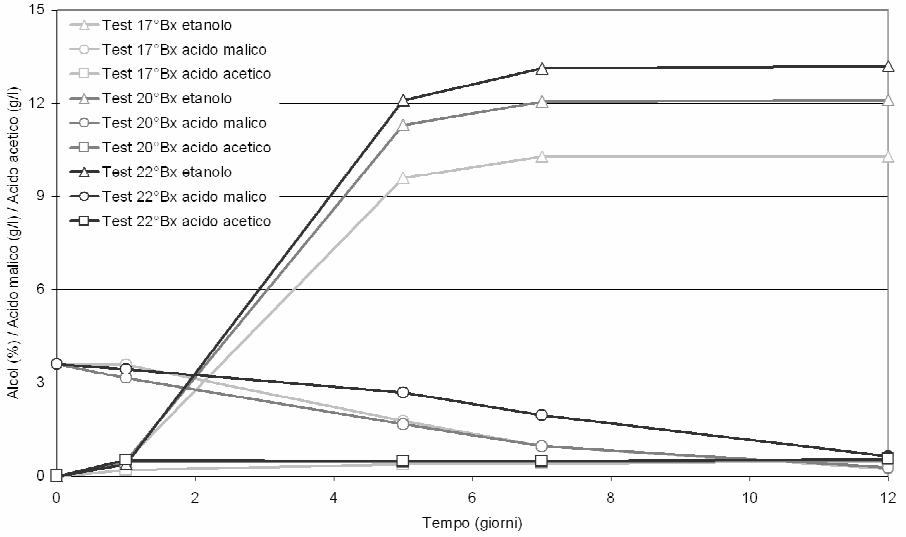

Figure 4. Alcoholic fermentation (Free S. cerevisiae cells) and malolactic fermentation (Immobilized O. oeni cells) completed simultaneously in the must.

Conclusions

The use of bacterial cultures immobilized in hybrid silica/alginate matrices is an efficient alternative to the use of free bacterial cultures for MLF management. Thanks to these developed materials, applied for the first time in winemaking, immobilized cells within silica/alginate microspheres give successful MLF and allow for a strict control over bacterial development in the wine. The cells can be removed immediately at the end of the process, hence reducing the risk of fermentation deviations and improving the overall wine quality.

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