Introduction

There is a considerable diversity in bioreactor technology available to wine makers, leading to a wide variety of wines with good productivity. However, the productivity in wine fermentation can also be increased by using high density of yeast cells. This can be achieved by increasing the effective size or density of the cells by aggregation or immobilizing the cells on some support and such systems are termed as high cell density reactors. These systems are more resistant to any accidental change in the feed composition or other operational conditions, because as the biomass is retained, the fermentation activity can be restored once the problem is solved. These are also useful to carry out the fermentation in successive reactors or in concentration gradient reactors. The other advantages of these systems are – (i) Higher fermentation rates due to high cell densities per unit bioreactor volume; (ii) repeated use of the same biocatalyst for extended periods of time; (iii) high flow rates in continuous processes can be used without the risk of cell washout; (iv) high dilution rate in continuous operation decreases the risk of reactor shut-down due to contamination; (v) adaptation to continuous processes can be better optimized and controlled; (vi) easy cell/liquid separations, thus minimizing down-time and separation costs; (vii) a wide variety of microbial strains/genetically modified strains can be used; (viii) improved tolerance or protection of cells from inhibitory products; (ix) sequential reactions can be carried out by connecting various reactors in series; (x) continuous operation and system control is easy; (xi) efficient gas-liquid mass transfer rate; (xii) better product uniformity in continuous systems and (xiii) reduced capital cost for small scale wineries.

The current advances in immobilization techniques have promoted much research in the development of efficient immobilized bioreactors to fully exploit the advantages of immobilized cells and biocatalysts. The exploitation of the immobilization techniques for wine fermentation, therefore, requires the development of a carefully designed and purpose-built bioreactor. There are two major systems, i.e., homogeneous and heterogeneous for confining or immobilizing cell biomass (Fig. 1).
Homogeneous system contains uniform distribution of microbial biomass in the form of free cells in the medium. The repeated use of biomass can be carried out by centrifugation, flocculation of yeast cells with external or internal decanter or retaining the cells in a membrane reactor, whereas the heterogeneous system have two separate phases, i.e., liquid medium which is to be transformed and a solid phase containing the microbial cells. In this system, biomass is confined by means of support, autoflocculation and entrapment in gels. A continuous process for the fermentation of must using serially connected fermentation vats has been developed for continuous wine fermentation. The presence of volatile compounds and sensory tests established the fruity aroma, determinate aroma enhancement. Immobilization of enzymes increases their stability, including their turn-over number and alleviates their removal from the process. Therefore, it is promising to use immobilized enzyme preparations to improve the enzyme consumption and cost of the processes. Another doubtless advantage of applying immobilized enzymes to the food industry is the increase and preservation of clarity of the target product. Immobilized β-glucosidase has been successfully used to improve the quality of Muscat wine in a packed bed reactor at laboratory scale. The stability of β-glucosidase immobilized on chitosan pellets was studied under operational conditions in a continuous stirrer tank membrane reactor at 25°C. Enzyme stability was found not dependent on substrate concentration and was considered satisfactory for an industrial process (half-life 1.2 years).

In the modern wine industry, enzymes are broadly used for grape maceration, extraction improvement, clarification, quality improvement and improvement of wine stability (prevention of colloidal feculence). The most efficient method of wine stabilization and preventing colloidal feculence is the use of specific hydrolysis of biopolymers, particularly enzymatic. The use of pectolytic, hydrolytic, proteolytic, cellulolytic and lignolytic enzyme preparations have been reported for this purpose. The use of polygalacturonase and β-glucosidase has been reported most important for preserving wine from colloidal feculence. β-glucosidase can hydrolyze the monoterpen glycosides which naturally occur in wine and determine aroma enhancement. Immobilization of enzymes increases their stability, including their turn-over number and alleviates their removal from the process. Therefore, it is promising to use immobilized enzyme preparations to reduce the enzyme consumption and cost of the processes. Another doubtless advantage of applying immobilized enzymes to the food industry is the increase and preservation of clarity of the target product. Immobilized β-glucosidase has been successfully used to improve the quality of Muscat wine in a packed bed reactor at laboratory scale.

Homogeneous reactors

The cell recycle batch fermentations (CRBF), whose principal innovation is the multiple successive use of the same yeast starter in different batch fermentations, is the more acceptable non-conventional technique in wine making. Unlike continuous, the CRBF does not require radical changes in the winery procedures nor does it imply capital investment in new equipment. In fact, yeast cells to be recycled can be recovered through natural sedimentation or by filtration or centrifugation with machines already present in most wineries. Five different processes have been reviewed most of these used a single reactor with a centrifugation step for recycling of yeast cells. An increase in cell mass as well as in productivity has been achieved by using a partial vacuum system. The main disadvantages of the centrifugation technique are the decrease...
in viability of the yeast biomass due to the stress to which they are subjected and maintenance of centrifugation machines. The use of membrane bioreactors is an alternative method to centrifugation in homogeneous reactors, where the yeast cells are retained in the reactor having a membrane of pore size of less than 0.45 µm. The material is passed through the membrane bioreactor and the transformed product comes down stream from the membrane. The efficiency of the transformation can be increased by recycling the product through the reactor. However, the main limitation of this is the clogging and unclogging of the membrane.

Heterogeneous reactors

In heterogeneous reactors, microorganisms are confined by immobilization. The basic principle is to increase the density of cells and to maintain their viability for longer periods in repeated use or in a continuous system.

Immobilization techniques

Whole cell immobilization is a process by which “cells are physically confined or localized in a certain defined region of space with retention of their catalytic activities”. The success of an immobilization system in large scale industrial applications depends upon following various factors:

i. The support material must be readily available and affordable.

ii. The system should be efficient, easy to operate and give good yields.

iii. The cells should have prolonged viability in the support, which should not be severely toxic to the cells.

iv. The support material should allow for high cell loading (weight of cells/weight of support).

v. The kinetic behaviour of the loaded support should be understood and not hinder the fermentation. This includes diffusion limitations, local pH and inhibitor accumulation.

vi. Any modification of metabolic processes associated with the carrier should be realized and accounted for.

Cell mobility can be restricted by passive and active immobilization techniques (Fig. 2).

Self-aggregation: Flocculation is a natural phenomenon resulting in a cell aggregation. All the cells don’t flocculate and natural cell aggregates are generally unstable and sensitive to shear. Thus, aggregates are promoted by addition of cross-linking agents. The flocculation of microbial cells to form a dense concentration of biomass has been reported in a number of yeast strains. It is a very attractive method of biomass retention involving decantation, the most basic method of liquid-solid distribution. As no solid support is required, the higher cell density can be achieved. Flocculated cells are particularly compatible for use in fluidized bed reactors. It is possible to use flocculating yeasts by using a laboratory reactor equipped with a simple decanter to treat wine in which fermentation had stopped.

Adsorption: Adsorption is one of the simple and quick immobilization techniques. The affinity of certain microorganisms for growth on solid surfaces is an established phenomenon. Adhesion of different microorganisms on various supports has been reviewed earlier. The choice of an appropriate resin may be expensive and time-consuming task, due to variation to adsorb and retain the cells under fermentation conditions.

Adsorption depends on the chemical nature of the surface of the cell wall where constituents such as peptides, hexosamines, and diaminopimelic acid provide the necessary ionic sites for attachment to a charged support. Carriers and biocatalysts are kept together through Van der-waals bonding forces, ionogenic bonds or H-bridges. Several steps like adaptation of the polymer (polysaccharide, protein, etc.) to serve as the support conditions to promote adsorption of the microbial cells, reversible adsorption and irreversible adsorption are considered for this technique. The use of adsorbed cell reactors has been proposed for deacidifying wines with Schizosaccharomyces pombe.
**Entrapment:** The most widely spread cell immobilization technique is entrapment in a matrix. Entrapment involves the confinement of the living cells in a rigid network which permits the diffusion of the substrates and the products. The system also ensures the growth and maintenance of viability of yeast cells. The typical examples of polymers used for entrapment of cells are polyacrylamide, collagen, cellulose acetate, alginate, κ-carrageenan, chitosan, gelatin, agar, agarose, epoxy resin and silica solution. Amongst these, alginate is the most common polymer used for entrapment of yeast cells in wine making. Synthetic polymers like polyacrylamide may not be a useful support for use in food industry in view of its toxicity. The higher productivity with alginate beads than polyacrylamide cubes may be due to the ratio of specific surface area based on gel particle size. The use of entrapped cells for conducting the alcoholic fermentation of wine is now confirmed by substantial amount of research, but no industrial installation is yet operational. This technique offers interesting advantages for wine production because of mechanical properties and chemical stability of the matrix. Entrapment has been extensively used for the immobilization of cells, but not for enzymes. The major limitations of this technique for the immobilization of enzymes are the possible slow leakage during continuous use in view of the small molecule size as compared to cells, diffusion limitation as well as steric hinderance. The diffusional limitations create substrate and product concentration gradients within the matrix that affects the kinetics and efficiency of transformations by immobilized cells. The development of so-called hydrogels and thermo reactive water-soluble polymers, like albumin-poly(ethylene glycol) hydrogel that have attracted attention of researchers.

**Containment behind a barrier:** Membrane supports can be used to maintain ten times more cell densities as compared to alginate and it allows greater volumetric productivity also. But, diffusional limitations involving the build-up of inhibitory products are one of the major limitations of these membrane supports. The membrane should be hydrophilic for easy exchange of nutrients and products and, mechanically strong to withstand the pressure differentials. The membranes are typically made up of polymers like polyvinyl chloride, polypropylene or polysulphone. Sheets and hollow fibre cartridges are two basic configurations used for this technique. The sheet-type usually have low strength and require additional support. Hollow fibre cartridges give the membrane increased strength and a higher surface to volume ratio. Typical problems associated with the hollow fibre systems are supply and the removal of gases is difficult, as CO₂ gas bubbles may block the flow in the tubes. The unrestricted cell growth can restrict the nutrient flow and unchecked adhesions can cause fouling the membranes. An interesting variation of standard containment methodology is called encapsulation. Usually, cells are first entrapped in spherical gel and then, the coating of the sphere is carried out with a polymer such as polyethyleneimine.

**Immobilized bioreactor design**

The main function of the reactor is to retain the immobilized complex and to ensure efficient and controlled contact between the catalysts in order to maximize product formation. Immobilization may in fact present the engineer with more processing options since the biocatalyst is in a concentrated, easily handled form. Typical reactors used for immobilized cells are continuous stirred tank reactor (CSTR), packed bed reactor (PBR), fluidized bed reactor (FBR), rotating disc reactor (RDR) and air lift reactor (ALR) shown in Fig. 1. The FBR provides a compromise between good mixing conditions (CSTR) and low shear stress (PBR) on the immobilized biocatalyst. Membrane reactors using biological catalysts can also be used in production, processing and treatment operations.

The choice of the reactor depends upon the type of immobilization, metabolism of the cells, resistance of the matrix to shear stress and the mass transfer requirement. Most of the industrial fermentations use batch system. However, continuous fermentation with immobilized cells may enhance productivity because cell separation, recycle or growth is not required to maintain high cell density in the reactor and minimum risk of contamination. The performance of a bioreactor using immobilized cells cannot be expressed by the intrinsic volumetric productivity, as the total biomass is difficult to determine. Thus, the volumetric productivity is used either by volume of liquid or volume of reactor.

**Continuous stirred tank reactor (CSTR):** Stirred tank reactors consist of an agitated tank in which fresh substrate is continuously fed and corresponding volume of the liquid contents are removed. They are well mixed by the use of impellers. With immobilized cells, high...
fluid velocities are needed to achieve a constant supply of substrate and product removal. CSTRs or back-mix reactors as they are sometimes called are cheap versatile and especially suitable when liquid phase reactions are being carried out. Supply of gas, control of pH and temperature are easy, fresh catalysts can be easily added to the reactor and substrates containing particulate materials can be tolerated without causing fouling. However, the relatively high power input required to give efficient agitation in CSTR is clearly a disadvantage and it may result in abrasion damage to the immobilized catalyst because of high shear forces at the impeller surface. Nevertheless, the CSTR offers the best mixing characteristics and oxygen transfer. The substrate concentrations in a CSTR are typically lower than the packed bed and fluidized bed reactor, resulting in lower average reaction rates. However, lower substrate concentration may be advantageous for inhibited cell culture. The stability of β-glucosidase immobilized on chitosan pellets was studied in a continuous stirred tank reactor and the enzyme stability was not found dependent upon substrate concentration and was also considered satisfactory for an industrial process development. Fast stirring rates in CSTR result in high shear stresses, thus increasing cell leakage from alginolate or carrageenan beads cell detachment from ion exchange resins and floc disruption.

**Packed (fixed) bed reactor:** The packed bed reactor (PBR) is the most frequently used type of immobilized cell bioreactor. The immobilized particles are packed in a column through which the substrate solution passes. If the fluid velocity profile is perfectly flat, the PBR operates as a plug flow reactor that is an ideal behaviour. High cell loadings are often achieved by entrapment resulting in improved productivity. The particle size for the cell attachment also had a strong influence on productivity. In principle, it is possible to achieve total conversion into product so that such reactors are ideal where total removal of a substrate is essential, e.g. detoxification. Psychrophilic yeast immobilized on apple cuts was used in a packed bed reactor (2l) to accomplish continuous fermentation of grape must. The immobilized β-glucosidase has been used to improve the aromatic quality of Muscat wine in a packed bed reactor.

The packed bed reactors have the advantage of simplicity of operation and low cost. However, the main difficulty in operating a packed column is ensuring good flow through out the bed. But, it also has serious limitations, as it can be unstable during long-term operations because of continuous biomass accumulation, mass transfer limitations and CO₂ holdup that become responsible for channeling and the creation of dead spaces and even matrix disruption. Gas build-up may also result in back mixing, resulting deviation from ideal plug flow behaviour. Horizontal packed bed reactors have been used to aid gas removal, thus reducing channeling and gas hold up problems in the continuous system.

**Fluidized bed reactor (FBR):** Fluidized bed reactors provide conditions that are intermediate to those of the CSTR and PBR. Mixing is better than in the PBR and lower levels of shear are encountered compared with the CSTR. Fluidized bed reactor consists of a column in which the biocatalyst particles are maintained in suspension relative to each other by a continuous flow of the substrate or gas at the high flow rates. The pressure drop of the fluid flow supports the weight of the bed. The fluidized bed reactor offers higher productivity than CSTR because liquid approximates plug flow similar to the PBR. However, the FBR is more advantageous for fermentation with substrate inhibition than the PBR because of the mixing caused by fluid flow. Such bioreactors promote good mass transfer, the dead cells are removed from the system and large volumes of CO₂ can be released without channeling and minimizes pressure drop. Fluidization avoids such problems as contamination, shear damage and limits to scale up, associated with impeller shafts and blades in stirred tanks. FBR can expand to accommodate growing biomass so they are less sensitive to plugging. This type of reactor is more useful for cultures for which oxygenation is needed.

**Rotating disc reactor (RDR):** It consists of immobilized cell units such as polyurethane foam sheets or fibre discs attached to a rotating shaft. The reactor is slowly stirred, thus allowing good mixing and removal of dead cells, debris and evolved CO₂. The energy required for RDR is less than for STR because of its slow mixing speed. This bioreactor has the potential to accept industrial substrates containing suspended solids to achieve high productivity. No difficulties with high solid media in this type of bioreactor have been observed. **Air (gas) lift reactor:** In air lift reactor (ALR), the fluid volume of the vessel is divided into two interconnected zones by
means of a baffle or draft tube. One zone is sparked with air or gas called riser and other zone that receives no gas is the down comer. Gas bubbles carry the liquid, resulting in a reduction in liquid bulk density. Gas escapes from the top and the liquid cascades down in a down comer. An external loop system may replace the inner draft tube for the recirculation of liquid in some bioreactors. Agitation in the ALR due to gas flow results in low shear with efficient mixing and mass transfer. The size of the draft tube influences the hydrodynamics of the fermenter, such as the gas hold up. Air lift bioreactors are highly energy efficient relative to stirred fermenters, but the productivities of both the types are comparable.

Cider production

Cider is an alcoholic beverage produced by fermentation of apple juice. The cider making process is similar to the wine production and results in products that range from sweet to very dry, usually containing 2-8% (v/v) alcohol. The production of cider is a complex process that combines two successive fermentations; the first one is the alcoholic fermentation by yeast and second is the malolactic fermentation which occurs during maturation process by lactic acid bacteria. In earlier times, natural fermentation of apple juice was the common practice. Spontaneous fermentation begins within a few hours, if the temperature of the juice rises above 10°C. This process is usually very slow. It requires 2-3 weeks to accomplish the main fermentation and several months for the maturation. That is why, it is difficult to control the flavour formation. Due to the risk of spontaneous fermentation by indigenous microbial flora, the cider making industry is now-a-days adopting the use of selected pure starter cultures. The optimum temperature for cider fermentation ranges from 15-18°C.

Traditional wooden fermentation casks are used without temperature control, whereas modern commercial vessels are usually large sized (2000-9000 l) capacity, constructed of lined concrete, lined mild steel or stainless steel. Fermenter depths in excess of 14.5m, which produce hydrostatic pressure of 1.5atm, have been shown to impair cider yeast performance. The use of immobilized cells in fermentation processes offers many well known advantages over the use of free cells. In recent years, the use of immobilized/co-immobilized cell systems in cider production has been an area of research.

Cider making by co-immobilized yeast and bacteria

The co-immobilization of yeast and bacteria has been carried out in/on a number of supports. Ca-alginate matrix was used to co-immobilize Saccharomyces bayanus and Leuconostoc oenos in one integrated biocatalytic system in order to perform simultaneously alcoholic and malolactic fermentation of apple juice to produce cider in a continuous packed bed bioreactor (2.5 l). The schematic presentation of the experimental set-up is shown in Fig. 3 and the chemical composition of the reconstituted apple juice is given in Table 1. Bioreactor loaded with co-immobilized alginate beads (0.985l volume of liquid and 1.225l volume of beads) was operated at 30°C and upstream feed flow of apple juice through the bioreactor was adjusted by peristaltic pump with normal flow rates between 0.05 and 0.55 l/h. Bioreactor was connected to a stainless steel cylindro-conical vessel (150 l), in which

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Concentration</th>
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<tbody>
<tr>
<td>Total sugar (equivalent glucose), g/l</td>
<td>7.1</td>
</tr>
<tr>
<td>Malic acid, g/l</td>
<td>5.2</td>
</tr>
<tr>
<td>Ethanol, % v/v</td>
<td>0.03</td>
</tr>
<tr>
<td>Diacetyl, ppb</td>
<td>71</td>
</tr>
<tr>
<td>2,3-Pentanedione, ppb</td>
<td>4.0</td>
</tr>
<tr>
<td>Acetaldehyde, ppm</td>
<td>0.4</td>
</tr>
<tr>
<td>Propanol, ppm</td>
<td>7.7</td>
</tr>
<tr>
<td>Isobutanol, ppm</td>
<td>0.3</td>
</tr>
<tr>
<td>Isoamylalcohol, ppm</td>
<td>0.9</td>
</tr>
<tr>
<td>Isoamylacetate, ppm</td>
<td>0.1</td>
</tr>
</tbody>
</table>

(Source: Ref. 49)
The maturation process of the partially fermented juice was accomplished at 10°C. Cider produced by continuous process was compared with two-step batch fermentation with suspended *S. bayanus* and *L. oenos* at 30°C in a continuously stirred tank reactor (working volume 15 l) with a stirring speed of 150 rpm. The continuous process permitted much faster fermentation compared with traditional batch process. The flavour formation was also better controlled. By adjusting the flow rate of feeding substrate through the bioreactor i.e. its residence time, it was possible to obtain either “soft” or “dry” cider. However, the profile of volatile compounds in the final product was modified compared to the batch process, especially for higher alcohols, isoamyl acetate and diacetyl linked to different physiological states of yeast in two processes. Nevertheless, the taste of cider was quite acceptable.

The feasibility of immobilized/co-immobilized *S. bayanus* and *L. oenos* was studied in different bioreactor configurations (Table 2) for cider making50. Four of these reactor configurations (A, B, C & D) were studied with respect to the spatial distribution of the two microorganisms. Two bioreactors (A & B) were very similar with respect to their capacity to use glucose or malic acid. In reactor B, *S. bayanus* in the first stage showed more ethanol productivity, while in the second stage low lactic acid was produced by *L. oenos*. These four bioreactor configurations performed efficient alcoholic fermentation with concentrated apple juice. In the fluidized bed reactor (E), the inhibition of glycolytic flow in the yeast has been made. In the mono-reactor configurations (F, G, H & I), fresh matrix or rehydrated beads of *L. oenos* and *S. bayanus* were used. The volumetric productivities of alcoholic and malolactic fermentation were twice, when rehydrated matrix was used in reactors F and H which may be due to the narrower geometry of the beads after dehydration allowing a better surface exchange per unit volume of matrix. A thermostable tubular APV bioreactor (total volume 250ml) with upstream feed flow has been successfully used for cider making at 30°C using co-immobilized *S. bayanus* and *L. oenos*51. The production of ethanol, consumption of malic acid and residual sugars were taken as indicator for the biological activity of

Table 2 : Bioreactor configurations used for cider making

<table>
<thead>
<tr>
<th>Reactor</th>
<th>Configurations</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Two tubular fixed bed reactors (270 ml)(^a) associated in series, the first was charged with <em>L. oenos</em> (1.9 \times 10^{12} cfu/l) and second with <em>S. bayanus</em> (10^6 cfu/l).</td>
</tr>
<tr>
<td>B</td>
<td>Two tubular fixed bed reactors (270 ml)(^a) associated in series, the first was charged with <em>S. bayanus</em> (10^6 cfu/l) and second with <em>L. oenos</em> (1.9 \times 10^{12} cfu/l).</td>
</tr>
<tr>
<td>C</td>
<td>One tubular fixed bed reactor (135 ml)(^a) charged with two different types of beads containing <em>L. oenos</em> (1.9\times10^{12} cfu/l) or <em>S. bayanus</em> (10^6 cfu/l).</td>
</tr>
<tr>
<td>D</td>
<td>One tubular fixed bed reactor (135 ml)(^a) charged with beads containing co-immobilized <em>L. oenos</em> (1.9 \times 10^{12} cfu/l) and <em>S. bayanus</em> (10^6 cfu/l).</td>
</tr>
<tr>
<td>E</td>
<td>One reverse conical fluidized bed reactor (201 ml)(^a) charged with beads containing co-immobilized <em>L. oenos</em> (1 \times 10^{12} cfu/l) and <em>S. bayanus</em> (10^6 cfu/l).</td>
</tr>
<tr>
<td>F</td>
<td>Two tubular fixed bed reactors (270 ml)(^a) associated in series, the first was charged with <em>S. bayanus</em> (10^6 cfu/l) and second with <em>L. oenos</em> (1.9 \times 10^{12} cfu/l).</td>
</tr>
<tr>
<td>G</td>
<td>Two tubular fixed bed reactors (270 ml)(^a) associated in series, the first was charged with <em>L. oenos</em> (1.9 \times 10^{12} cfu/l) and second with <em>S. bayanus</em> (10^6 cfu/l).</td>
</tr>
<tr>
<td>H</td>
<td>One tubular fixed bed reactor (250 ml)(^a) charged with rehydrated beads containing <em>L. oenos</em> (1.9 \times 10^{12} cfu/l).</td>
</tr>
<tr>
<td>I</td>
<td>One tubular fixed bed reactor (250 ml)(^a) charged with fresh beads containing <em>L. oenos</em> (1.9 \times 10^{12} cfu/l).</td>
</tr>
<tr>
<td>J</td>
<td>One tubular fixed bed reactor (250 ml)(^a) charged with rehydrated beads containing <em>S. bayanus</em> (10^6 cfu/l).</td>
</tr>
<tr>
<td>K</td>
<td>One tubular fixed bed reactor (132 ml)(^a) charged with fresh beads containing <em>S. bayanus</em> (10^6 cfu/l).</td>
</tr>
</tbody>
</table>

\(^a\)Total volume of reactor (Source: Ref. 50)
both the microorganisms (Table 3).

A sponge like material has been used to immobilize both S. cerevisiae and L. plantarum for carrying out fermentation and partial maturation of alcoholic cider. Cider medium (2 l) was taken in a fermenter (2.5 l) and operated at 20°C. The fermenter was connected via a side-loop to a temperature controlled glass column (1.5 cm i.d.) packed with sponge (6g wet weight) preloaded with S. cerevisiae (2.2 ± 0.1 × 10⁹ cells/g sponge). The medium was circulated continuously upwards through the non-fluidized sponge at 60 ml/min for 28 days. L. plantarum (10⁹ cells/ml) were added at set periods (0, 3 & 10 days) to some of the fermenters, while others were left only with yeast. This pliable sponge material has been proved an excellent immobilizer for both yeast and bacteria. The fermentations carried out with immobilized yeast and sequential addition of lactic acid bacteria indicated a route to both enhancing rate of fermentation and controlling positive flavour development. The influence of long term continuous apple wine fermentation on two strains of S. bayanus immobilized on foam glass was investigated in a 4-column reactor for 3.5 months. Various shapes of cells were isolated from the carrier at the end of the fermentation. It can be concluded from the forgoing account that the use of co-immobilized bioreactors will offer the opportunity to perform the cider fermentation in a fast way in one reactor containing two microorganisms.

### Cider deacidification by immobilized lactic acid bacteria

Traditional ciders are frequently subjected to malolactic fermentation. In cider making it is a complex process and difficult to control, because many nutritional and physico-chemical factors affect the growth and metabolism of lactic acid bacteria. Essential growth factors in the apple juice promote, whereas products of yeast metabolism such as fatty acids and ethanol inhibit the growth of lactic acid bacteria. The use of starter cultures in cider fermentation might allow cider makers to produce a uniformly high quality product, to be maintained during successive processes and seasons. Although, the use of starter cultures to control industrial fermentations is well established in brewing and wine production, it has not been widely adopted in cider making. In French cider making, where the primary fermentation is very slow, the malolactic change may occur concurrently with the yeast fermentation, whereas in UK cider making, it is most likely to occur once the yeast fermentation has finished and the cider is in bulk store. Work in France in recent years has shown that the malolactic fermentation can be encouraged by an appropriate inoculum of L. oenos into maturing ciders. In modern UK factory of cider making, the malolactic fermentation is generally regarded as a nuisance and is not encouraged.

A controlled malolactic fermentation in cider using Oenococcus oeni free and immobilized cells in alginate beads in flask culture showed the similar rates of malic acid consumption. But lower ethanolic acid content and higher concentration of alcohols was detected with immobilized cells which have beneficial effects on the sensory quality of cider. Based on the comparison of the kinetics obtained by means of a diffusion model for malic acid consumption in immobilized and free cells, both systems can be modeled by the same kinetic equation:

\[
\frac{dS}{dt} = \frac{kS}{K' + S} X
\]

Where, \( S \) = malic acid concentration; \( X \) = biomass concentration; \( k \) and \( K \) are constants. It was observed that the value for \( k \) is approximately 5.6

### Table 3 : Outlet profile of the cider produced in a thermostable tubular bioreactor loaded with beads containing Saccharomyces bayanus and Leuconostoc oenos

<table>
<thead>
<tr>
<th>Flow rate L/h</th>
<th>Ethanol % (v/v)</th>
<th>Residual sugars (g/l)</th>
<th>Malic acid (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.110</td>
<td>5.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.031</td>
<td>5.5</td>
<td>3.2</td>
<td>0.11</td>
</tr>
<tr>
<td>0.045</td>
<td>4.7</td>
<td>15.3</td>
<td>0.23</td>
</tr>
<tr>
<td>0.059</td>
<td>4.8</td>
<td>17.2</td>
<td>0.71</td>
</tr>
<tr>
<td>0.091</td>
<td>3.9</td>
<td>18.3</td>
<td>1.32</td>
</tr>
<tr>
<td>0.118</td>
<td>3.4</td>
<td>45.9</td>
<td>1.58</td>
</tr>
<tr>
<td>0.154</td>
<td>1.9</td>
<td>73.6</td>
<td>2.90</td>
</tr>
</tbody>
</table>

(Source: Ref. 51)
times higher in free than in immobilized cells.

A lab scale continuous process to deacidify apple juices and cider was developed by entrapped _Oenococcus oeni_ in a new type of polyvinyl alcohol hydrogel (Lentikats) in a tubular glass reactor (170 ml)\(^6\). With a residence time of 0.55h and a volume of 50ml of gel, the specific malic acid consumption in apple juice at pH 3.95 was 11.4g/h for the lentikats bioreactor and 2.5g/h for the alginate beads bioreactor. The better performance of lentikats bioreactor is due to the increase of the ratio of external surface to volume by allowing better mass transfer.

### Conclusions and future perspectives

Some of the biotechnological innovations like selected yeasts, improvement of microbial starters, enzymatic treatment, bioreactor designing and microorganism immobilization are of fundamental importance in wine making. In the past, major emphasis of research has been on immobilized techniques and characterization of immobilized systems, and successful application of this technology in various areas of wine making led it to become a center of attraction. The feasibility with respect to overall productivity of immobilized biocatalyst however warrants consideration of appropriate bioreactor design. Moreover, the carrier cost is a determining factor in the feasibility of the immobilized system. Therefore, the design of immobilized reactor must be taken into consideration for its successful operation in wine making and to make it cost effective. The use of several pilot scale bioreactors have also established the feasibility of immobilized bioreactors for malolactic fermentation and proved to be cost effective\(^5\). The developments of bioreactor technology with respect to fruit wines other than grape are scarce and limited data is available in the literature. The technology of entrapped yeast for must fermentation requires further optimization of immobilized systems in the areas of mass transfer, reactor design and yeast physiology along with most logical strategy for providing the means of increasing productivity without changing the sensory quality of wine. It is expected that future would see the innovations in wine-making which would allow one to maintain not only the quality of the product but would take into account the hygienic and legal aspects also. The use of co-immobilized biocatalysts in cider making will be a real improvement of the industrial process for reducing processing time as well as cost. Intensified research aimed at developing improved reactors, a better understanding of the physiology of immobilized cell systems and the immobilization mechanisms, together with the search for novel, innovative and cheap carrier materials should enable a more general implementation of this promising technology.

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