

USING THE IMMOBILIZED YEASTS AS A WAY OF INCREASING THE PERFORMANCE AND THE ECONOMY THE FERMENTATIVE PROCESSES

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Abstract

Immobilization of cells become an important instrument in the biotechnologies of the latest years as an increasing method of the performance and the economy of the fermentative processes.

Using immobilized cells leads to important advantages: the enzymatic activity is maintained at high levels leading to maximum speeds of the substances conversion; the operational stability is generally acceptable; the increased productivity by large concentrations of immobilized cells and also by the possibility of using a high substratum concentration that leads to low reaction times; an easy way to manipulate the products, to control and lead the processes; economizing the processes due to the possibility of using the immobilized cells products in permanent processes on a long time period; the possibility of reusing the biocatalyzers; an easy separation of the immobilized machine from the reaction space; reducing the pollution problems.

The fermentation productivity is limited by the toxicity of the accumulated ethyl alcohol.

The productivity can also be improved by the increasing of the cells concentration obtaining the reactor systems with cells in high density.

Key words: *immobilized yeasts, bioreactor, alginate, alcoholic fermentation, alginate balls, mass transfer, homogeneous reactor, heterogenous reactor.*

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Introduction

Related to the way you use to obtain the immobilized biocatalyzers, there are 4 definite categories:

- the immobilization in the support absence – is realized through the covalent binding by cell to cell attachment [Lee, tsumura, Bungard and others]. The second possibility is the chemical binding directly used with divalent coupled reactors like glutaraldehyde [Lartigue, Prescott, Chibata and others]. The advantages of this method are the high density of cells that can be obtained in gentle circumstances.
- the immobilization of a certain biomass in a performant support – usually there are used supports made up from porous materials and the immobilization is realized through the matrix's immersion in the cells suspension that are to be immobilized [Inoles and others]. The matrix has to have the porous diameter bigger than the cells diameter to allow cells to penetrate into the internal surface of the matrix .
- the immobilization of a certain biomass during the support's preparation- is the most used of the microorganism cells. This technique is in fact formed from 2 methods: entrapment and encapsulation [Mohan, Miyoshi, Fukushima, Chose, Ada, Sakimae and others]. Due to the cells dimension it is quite simple to obtain a porous matrix so that it can guarantee the complete rotation of the cells and the transport processes for the substratum and the reaction products are quickly enough to obtain a high efficiency of the catalytic activity.
- The immobilization through the cells increasing in the support's interior – has a purpose to obtain a biocatalyzer with living cells through increasing the cells concentration in the granules interior. This method is realized through the biocatalyser's incubation that was prepared in certain conditions of increasing or through the biocatalyzer's reactivation after a certain deactivation.

Choosing the materials for the support in case of the biocatalyzer production on a large scale you have to take into consideration some factors:

- a). – the materials have to be accessible and at a convenient price.
- b). – the immobilization process has to be simple and not to affect the medium activity cells.
- c). – the immobilized capacity and efficiency has to be high

d). -the reactor drawing has to be simple and has to respect the manipulation standards of the supports

It's favourable to lead fermentation in successive reactors or in reactors with a concentration gradient.

There are 2 basic system:

- homogeneous system where the biomass is uniformly disposed in the reaction medium. This result can be obtain by using the biomass that already circulated through the centrifugal action or through maintaing the biomass in the membrane reactor.
- Heterogeneous system where the 2 phases are separated, the liquid medium that has to be transformed and the solid phase that contains the biomass.

A). The homogeneous reactors – they are usually used with the membrane. An alcohol level of 10 % could be reached in hours, For the system to be economically viable the specific flux of the membrane must reach 200 to 300 l/h.

B). Heterogeneous reactors – have as main techniques of the enabled biomass the absorption on a support, autofloculation and entrapment in gel.

The use of adsorbed cell reactors has been proposed for beer production as well as for deacidifying wines with *Schizosacharomyces pompe*.

Materials and methods

For yeast cells immobilization was used the calcium Alginate Gel. Alginic acid a heteropolysaccharide of L- guluronic acid and D- mannuronic acid extracted from various species of algae. Depending on the source, the composition and the sequence in L- guluronic acid and D- mannuronic acid varies widely.

The monomers are arranged in a pattern of blocks along the chains. After processing the alginate is available as water – soluble sodium alginate, when the water – soluble sodium polyelectrolyte is mixed with multivalent counterions (Ca^{2+} , Al^{3+} , Zn^{2+} , Ba^{2+} , Fe^{2+}) gelation occurs.

Sodium alginate soluble in water, in a mixture of CaCl_2 forms a calcium alginate gel. Alginate beads are formed by droplet or emulsification methods. The stability of alginate beads is quite low in the presence of chelating agents such as phosphat, lactate and citrate due to shared affinity for calcium destabilizing the gel.

Obtaining the immobilized yeast in balls of alginate can be obtained in different ways:

- Extrusion under gravity force:

The cell – polymer mixture is passed through a needle speed sufficiently low to prevent the formation of a jet so it is obtained a droplet which falls into a hardening solution under gravity force. The droplet diameter is mainly influenced by the needle diameter and the alginate composition. Scale – up of this procedure is performed by using a large number of needles.

- Extrusion under coaxial liquid or air jet:

Polysaccharide solution containing cells may be extruded through a needle and the flow a liquid or a concentric air around the needle outlet facilitates the droplet's detach.

With the coaxial air jet the bead diameter depends mainly on the air jet speed. A high gas flow rate induces a high shear rate at the droplet surface promoting early release of the droplet, resulting in a smaller diameter.

- Extrusion under electrostatic potential

The cell – polymer suspension is extruded through a capillary subjected to high static potential between the capillary and the hardening solution. Increasing potential causes a decrease in the droplet size and an air increase in drop frequency, obtaining droplets of several hundred micrometers.

- Droplet formation by jet break-up:

In this technique the polymer-cell suspension exists from a vibrating nozzle as a jet, causing break up of the jet. The frequency was stabilized using a coupled stroboscopic light source to visualize the droplet.

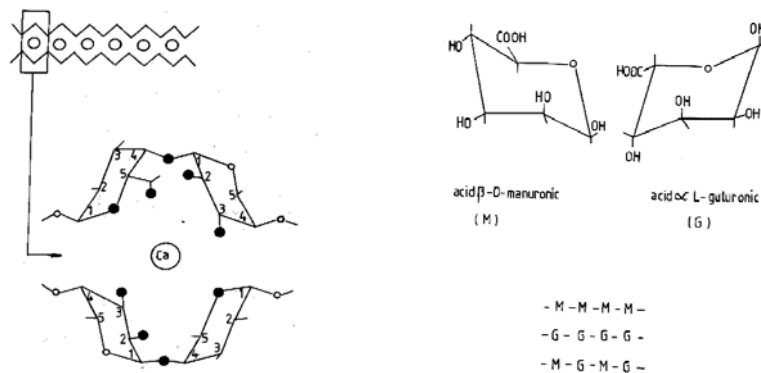


Figure no. 1 – The alginates gelification scheme

- Emulsion Technique:

Beads and microcapsules may be formed using emulsification to disperse the cell – polymer suspension. Parameters influencing particle diameter depends on the rotational speed of the impeller, the polymer concentration, the aqueous phase viscosity and also the alginate composition.

- Rotating Nazzle – Ring:

The technique is based an extrusion of the polymer-cell suspension through a rotating nazzle ring. The mixture is forced under presure to pass through a vertical hallow shaft equipped with a nazzle head. Shaft rotation ejects the droplet from the different nazzles into a hardening solution. The bead diameter

depends on the nozzle internal diameter, distance between 2 nozzles, rotational speed and the polymer solution viscosity.

- Rotating Flat Disc Atomizer:

The automatization system consists of a variable speed motors on which a flat disc is mounted. The cell- polymer suspension is dropped on top of the rotating disc and disintegrates into droplets that fall into the hardening solution.

In the effected experiments to obtain the alginate balls the extrusion under gravity force was used: 4% sodium alginate gel was sterilized and then added a special yeast cells suspension (*Saccharomyces cerevisiae*), then mixture being homogenized.

The gel beads were obtained by drop suspension of alginate cells through a needle in a calcium brine. So the gel is polymerized as a sphere having the diameter of several millimeters.

Letting the Ca^{2+} ion free beads to the acetic addition until a 6,5 pH, the obtained balls were washed for the calcium brine to be eliminated. So, the yeasts are included in the gel.

For the security of the yeasts immobilization and to avoid the increasing medium turbidity of the fermentation, the immobilized yeast balls were again introduced in a 4% sodium alginate gel and then passed in a calcium brine. So, there were obtained double closed balls.

For studying the immobilized yeast comportation, it was used a seal machine with a thermometer, pressure indicator, a cooling cloak (for maintaining at constant temperatures the fermentation process), samples collecting tops, in the machine's interior sodium alginate balls were introduced in a nutritional medium made up of a 150 g/l solution

The alginate balls with immobilized yeasts have a 4 mm diameter.

The temperature at which was the fermentative process was at 16°C.

In the same medium conditions was watched the alcoholic fermentation of the same type of substratum, but using the free immobilized yeasts like *Saccharomyces cerevisiae*.

Results and discussions

The main factor which influences the cells conduct is the mass transfer limitation, according to oxygen substratum and product these changes can increase or decrease the cells metabolism, so the immobilization can influence the cell physiology and its activity.

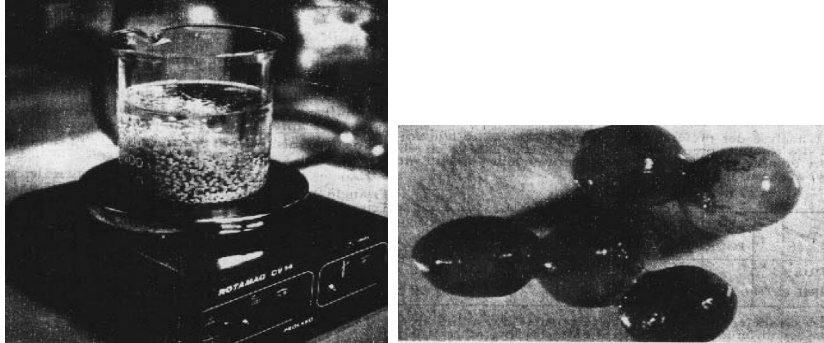


Figure no. 2– Balls in the preparation solution. Figure no. 3 –Alginate balls.

The main transfer limitation affects the oxygen preluation but also the removeness formed products resulting in the products acumulation around the immobilized cells.

The polymer's properties, used as a gel affects the cinetic fermentation through the modification of the mass transfer of the final matrix.

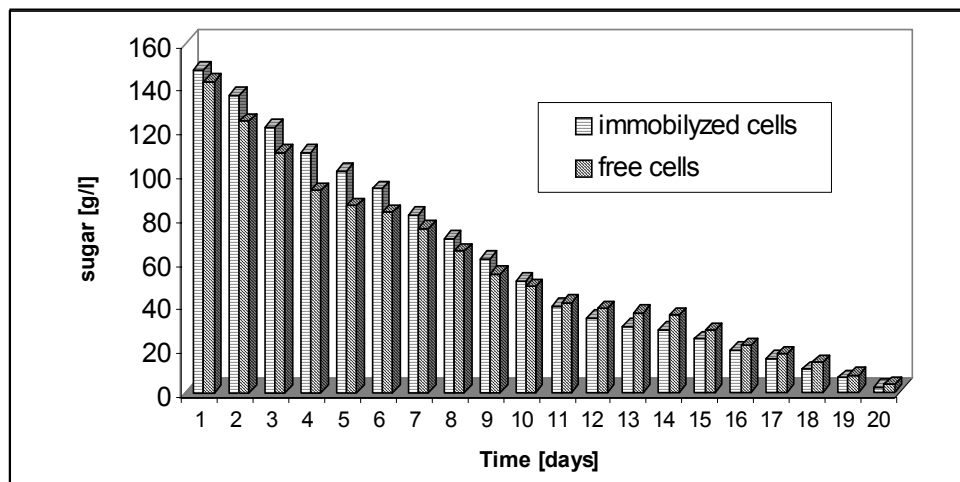
In case of some thiner gels, it is facilitated a higher level of the substratum and product transportation, through the gel's network increasing the fermentation achivement. The cells density in the interiour of the matrix can affect the mass transfer properties of the beads, so at a low charging of the alginate beads, the cinetic fermentation was similarly not only for the free yeasts utilization but also for the immobilized yeast, and a higher charging with cells derminates a transfer limitation that affects the cinetic of the immobilized cells reactions.

The increasing of the cells concentration isn't uniformly in all the gel particles through scanning, electromical transmittion, the cells density can be determinated as a localization purpose, the increasing of the yeast cells take place mainly at a 4 mm diameter beads exteriour with an easy increase in the middle of the bead.

To obtain immobilized microorganisms with high performances is recommended to allow these to increase directly in the gel than to prepare gels with a high cells concentration. The immobilization protects the yeasts against the ethyl alcohol, also against hardmetals, phenol, acidity and extreme temperatures. The yeasts used in experimntes can assure the alcoholicol fermentation beading in condition when the pressure inside the fermentative recipients is increasing.

Table no. 1 - The sugar metabolism evolution and the mathematical programme.

days	immobilized cells		free cells	
	experimental data	determined data	experimental data	determined data
1	148.1	148.1007	142.2	142.1997
2	136.2	136.2028	124.3	124.2996
3	121.5	121.5103	109.9	109.8999
4	109.9	109.9344	92.5	92.5008
5	101.2	101.2975	85.5	85.5
6	93.2	93.4372	82.2	82.2
7	81.1	81.0349	75.1	75.1
8	70.3	70.1832	64.5	64.5
9	61.2	61.0071	53.9	54.6146
10	51.1	50.8	48.1	47.11
11	39.1	39.0648	40.9	41.636
12	33.8	33.7048	38.1	37.9928
13	30.1	29.9128	36.2	36.2
14	28.2	27.8808	34.9	34.9
15	24.3	24.2375	28.1	28.1
16	19.1	19.0168	21.2	21.2
17	15.2	15.0929	17.2	17.2
18	10.1	10.2344	13.3	13.2344
19	6.3	6.4653	7.5	7.4653
20	2.1	2.3	3.2	3.2



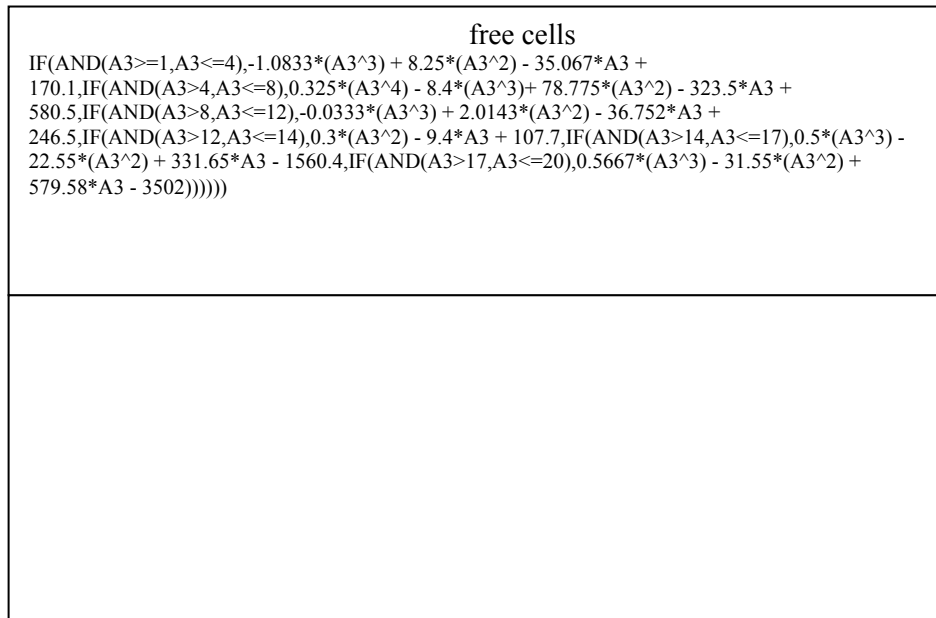
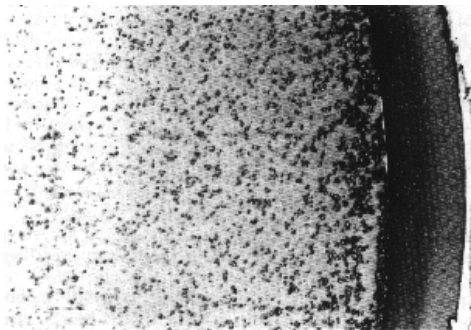


Figure no. 4 - The experimental results and obtained by a certain programme.

After analysing the sugar metabolism while the alcoholic fermentation, it can be noticed that the fermentation with free yeasts is quicker than in case of using immobilized yeasts, but at the end of the alcoholic fermentation, the fermentative process is slower, its reactivation being necessary for the alcoholic fermentation process by using a stirring palette machine.

Figure no. 5 – Microscopic view of a section through a ball with double cover.



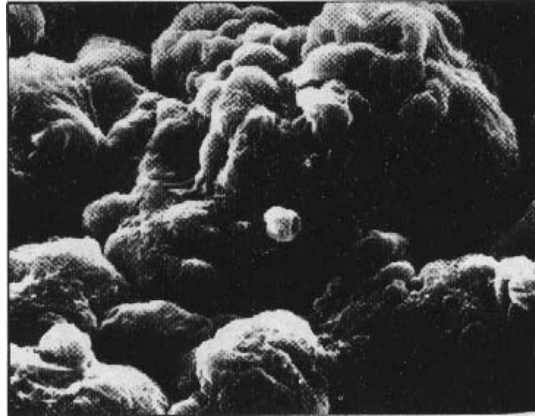


Figure no. 6 – A ball surface. Detail with electronical microscope.

By using the immobilized yeasts, even if at the beginning the substratum metabolism is slower, the alcoholic fermentation is more constantly and quicker, not being necessary for the fermentative medium to be agitated.

Conclusion

1. The cells immobilization became an important instrument in the biotechnologies of the latest years as an increasing performance method and the economy of the fermentative process, the operational stability is generally high assuring a great productivity by high immobilized cells concentration but also by the possibility of using high substratum concentration, which leads to lower time reactions manipulating the products easily, in the control and leading processes, the processes economy, the possibility of using the immobilized cells products in permanent long time processes, the possibility of reusing the biocatalyzers, the easy separation of the immobilized medium from the reaction reducing the pollution problems.
2. The immobilization protects the yeasts against the ethyl alcohol toxicity, also against hard metals, phenols, acidity.
3. In case of using the immobilized yeast even if at the beginning the substratum metabolism is slower, the alcoholic fermentation is more constantly and quicker not being necessary for the fermentative medium to be agitated.

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