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Mechanical properties of Ca-alginate beads for ethanol fermentation with immobilized yeast

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Résumé

Au cours de ces dernières années il y a un intérêt accru accordé à la fermentation de l'éthanol avec des cellules immobilisées. Ces biocatalyseurs immobilisés doivent posséder certaines caractéristiques propres au processus de fermentation en continu - haute stabilité mécanique, fonctionnement à long terme, une forte activité de fermentation.

Dans le présent travail, nous avons étudié les caractéristiques mécaniques des billes d'alginate de Ca-alginate avant et après la procédure d'immobilisation. Les échantillons ont été obtenus à partir des solutions de Na-alginate avec une concentration de 1%, 2%, 3% et 4%. Les propriétés mécaniques des échantillons ont été mesurées avec un rhéomètre DMAQ800. Les échantillons ont un comportement visco-élastique qui dépend fortement de la concentration de la solution en Na-alginate. Il a été constaté que l'ajout de la levure aux suspensions conduit à un changement considérable de l'élasticité de l'échantillon. Les résultats obtenus pourraient être utilisés dans le processus d'analyse et de modélisation de la fermentation de l'éthanol avec des cellules immobilisées.

Abstract

There is an increased interest in the area of ethanol fermentation with immobilized cells in the recent years. These immobilized biocatalysts need to possess certain characteristics that meet the specific characteristics of the continuous fermenting processes - high mechanical stability, long time operation, high fermenting activity.

In the present work the mechanical characteristics of the Ca-alginate beads before and after the immobilization procedure are investigated. For the specific tasks were investigated preparations obtained from Na-alginate solutions with a concentration of 1%, 2%, 3% and 4%. The mechanical properties of the samples were measured with DMAQ800 rheometer. The investigated samples have a viscous-elastic property. These properties strongly depend on the Na-alginate solution concentration. It was found that the added yeast suspensions leads to significant change in the sample elasticity. The received results could be used in the process of investigation and modeling of ethanol fermentation with immobilized cells.

Mot-cles : immobilisation cellulaire, la stabilité mécanique, perles Ca-alginate

Keywords : cell immobilization, mechanical stability, Ca-alginate beads

1. Introduction

The environmental problems caused by the continued and increasing use of fossil feedstock's as energy source and the fact that the supply of many of their more desirable embodiments is approaching exhaustion are two main problems associated with the utilization of fossil fuels. Fermentative production of chemicals and energy production from renewable resources have been seriously considered as an alternative to petrochemical processes in recent years. Probably the best known of the alcohols, which can be an alternative to petroleum, is ethyl alcohol or ethanol. Rapid fermentation and high alcohol levels are desirable to minimize capital costs and distillation energy, while good yields are necessary for process economics [4, 10]. The efficiency of the production processes of ethanol depends on several aspects; operational simplicity, productivity and product concentration in the fermentation, and product recovery. In fermentation, the productivity is proportional to the yeast cell concentration, and techniques to retain the cells in the fermentation section must ensure a high productivity [3, 10].

An upsurge of interest in cell immobilization for alcoholic beverages and potable alcohol production has been taking place recently. This is mainly due to the numerous advantages that cell immobilization offers including enhanced fermentation productivity, feasibility of continuous processing, cell stability and lower costs of recovery and recycling and downstream processing [5]. Cell immobilization may also protect cells against shear force. Industrial use of immobilized cells is still limited however further application will depend on the development of immobilization procedures that can be readily scaled-up [5-11].

The applicability of biocatalysts gel particles in bioreactor systems is highly dependent on the long-term stability of the gel material. It should be inert, insoluble in the actual liquid, non-biodegradable, as well as mechanically stable under reactor operation. The mechanical stability of the support material is a critical factor for the operation of the ethanol fermentation bioreactors, which worked with immobilized cell systems.

The mechanical stability of gel beads in a reactor is largely influenced by their rheological properties of the support material. Many studies have focused on mechanical properties, such as stiffness, fracture resistance and the elasticity of the gels-beads. That is, they depend on not only the size, shape, and density of the particles, but also on their roughness, hardness, elasticity and degree of homogeneity. These properties determined how a biocatalysts particles is able to accommodate the stresses to which is subjected in a reactor [1, 8].

In the most studies dealing with the support materials for cell immobilization, beads are compared with each other on the basis of their resistance to compression or tension, but this was not related to the stability in the bioreactor. In the present study, some mechanical properties of gel-beads were investigated. Because of very little is known in this area, we used standard rheological methods for that purpose. For the rheological investigation, we used "Dynamic Mechanical Analyzer", DMAQ800 [2, 9], which was supplied with special software. That analyzer could investigate different rheological characteristics and mechanical stability parameters. The results will be integrated to the full model of the ethanol fermentation process with immobilized cells.

The purpose of this work is to investigate the mechanical characteristics of Ca-alginate beads for yeast immobilization for ethanol fermentation. For this purpose were obtained four types beads from Na-alginate solution with 1 %, 2 %, 3 % and 4 % concentration. To study the impact of the yeast suspension on the mechanical characteristics of the beads the pearls are subject to the studies before and after the immobilization procedure.

2. Materials and methods

2.1. Yeast strain

Dried Saccharomyces cerevisiae 46 EDV, which is widely used in industrial ethanol fermentation, was used in the present study. Before the immobilization procedure the yeast was rehydrated in 2 % glucose solution.

2.2 Gel-beads preparing

Na-alginate „Algogel 6021" -„Degussa"-France with molecule weight 30-50 CP was used to prepare alginate gel solutions with concentration 1 %, 2 %, 3 % and 4 %. For the ionic gelation 2 % $CaCl_2$ solution was prepared. The rehydrated yeast was gently mixed with the alginate solutions. The yeast concentration was 2% (w/v alginate solution). 20 ml from the obtained solutions was put in the dropping device (Fig.1). The dropping device is consists from piston to which is joint a syringe. The device is supplied with velocity controller for regulation of the flow rate of the solution. Below the dropping device is put the gelation solution. The dropping velocity was 100 ml/h [12].

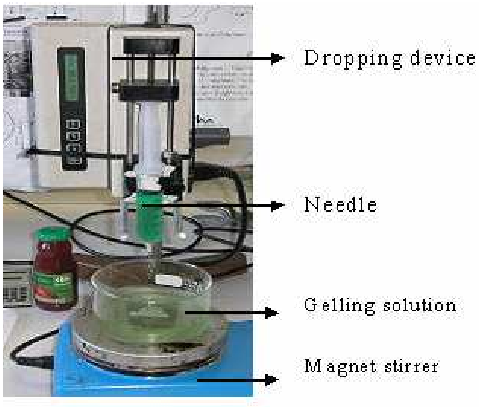


Figure 1. Device for immobilized cell preparation.

The gel-beads were ready 2-3 hours, after all quantity was dropped in the gelling bath. The mean diameter of the beads was around 2 mm [12].

The average diameters of the pearls are measured by DMAQ800. Based on this calculated diameter DMA Q800 calculated the area on which is applied the necessary force to achieve the determine value of stress or deformation of the samples [2, 9].

2.3 Dynamic Mechanical Analyzer DMAQ800 and rheological experimental procedures

The TA Instruments Q800 Dynamic Mechanical Analyzer (DMAQ800) (fig.2) was used for the measuring of the mechanical stability of the gel-beads. DMAQ800 is a thermal analytical instrument used to test the mechanical properties of many different materials. To make measurements, the test specimen is mounted on one of several clamps, all of which have been designed using Finite Element Analysis to minimize mass and compliance. The DMA instrument works in conjunction with a controller and associated software to make up an analysis system [2, 9].

A functional DMA system has several major parts - the DMA cabinet, which contains the system electronics, the DMA assembly, a computer controller for analysis and control of the instrument, and an optional Gas Cooling Accessory (GCA).

The following components make up the DMA assembly:

- • The mechanical section enclosure contains the air bearings, optical encoder, drive motor, and the associated electronics.
- • The clamp assembly (called the "clamp") is interchangeable for making mechanical measurements in a variety of deformation modes to accommodate a wide array of

sample shapes and materials. Several different types of clamps are available for the DMA, see page 27 for a list.

- • The furnace assembly envelops the clamp assembly and provides temperature control. The furnace temperature is monitored by the control thermocouple.
- • The CHROMEL®*/ALUMEL®* sample and reference thermocouples sense the temperature of the sample and heater and relays the readings to the instrument. The position of the sample and reference thermocouples can be changed to accommodate the various clamp assemblies.

The mechanical stability of the gel-beads was investigated by the follow working modules of DMAQ800 [2, 9].

2.3.1 „Strain Rate" module

That module can measure the deformation of the samples at constant clamp velocity of 100 µm/min. By that we can measured the critical stress, where the sample is started to destroy.

2.3.2 „Creep" module

The samples (gel-beads) were placed between the plates of the clamps. At the initial time t1 the static force (initial pressure 0.1 MPa; 0.2 MPa; 0.3 MPa) was applied. The deformation of the sample and the stress were measured as a function of the time. The results were representing by the DMAQ800 software. After 2 min. the static force was remove and the sample start two recover. During this period the deformation recovery was measured. The creep module is represented at Figure 4.

The samples (gel-beads) were placed between the plates of the clamps. At the initial time t1 the initial deformation (5%, 10%, 20%, 30% from the maximal) was applied on the sample. The deformation of the sample and the stress were measured as a function of the time. The results were represented by the DMAQ800 software. The time of the experiment was 5 min. This module is represented at Fig.5.

2.3.3 Measured parameters

- Stress

$$\sigma = \frac{Force}{Area}, MPa$$

where: σ – normal stress;

- Deformation

$$\varepsilon = \frac{(h_0 + \Delta h)}{h_0}.100,\%$$

where: h_0 – initial sample height; Δh – sample height change. All parameters are calculated and recorded in the DMAQ800 software [2, 9].

3. Result and discussion

3.1 Mechanical characteristics of pure alginate beads

3.1.1. Destruction characteristics of the Ca-alginate beads

Recognizing that the destruction of the beads in the operating conditions due to continuous strikes between them and hit with the working parts of the bioreactor is necessary first to establish border tensions and deformations in which the destruction occurs. Destruction of the samples is mainly result from large deformations, which lead to irreversible separation of the material parts. In viscose-elastic bodies, such destruction is related to leakage of the material in the application of stresses on it. This means that the constantly increasing pressure on the material, he first began to expire and be destroyed later. By DMAQ800 this is done by loading modules "Strain rate".

Figure 2. Q800 Dynamic Mechanical Analyzer

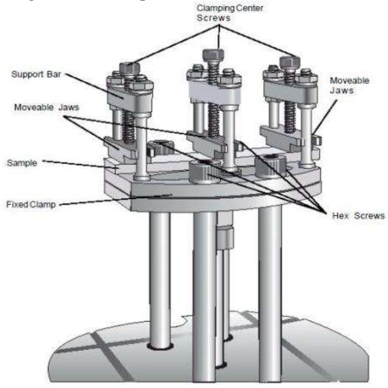
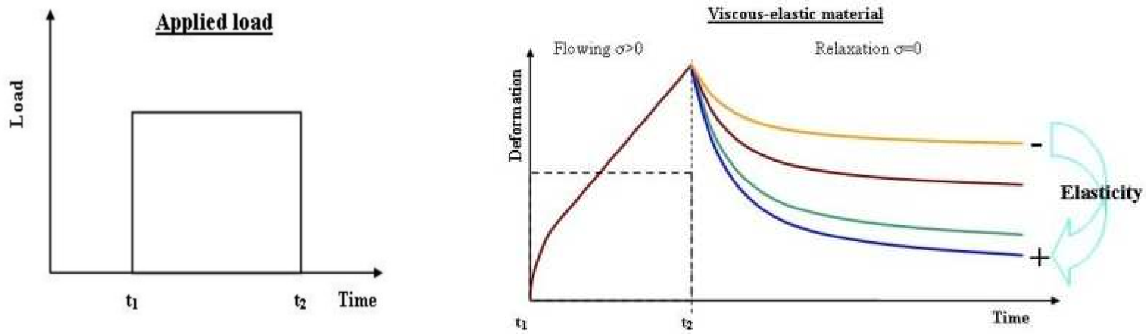


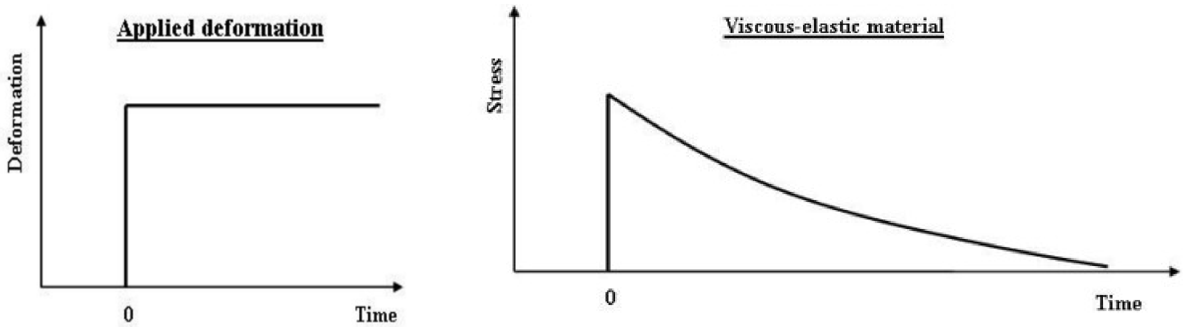
Figure 3. Clamp assembly of DMAQ800



Creep

Material reaction in creep module

Figure 4. Graphic representation of creep module



Stress relaxation

Material reaction in stress relaxation module

Figure 5. Graphic representation of stress relaxation module

The results are summarized in Table 1.

Gel type	Destruction characteristics	
	Maximal deformation, %	Maximal tension, MPa
Alginate 1%	95	0.365
Alginate 2%	90	0.653
Alginate 3%	90	0.673
Alginate 4%	93	0.753

Table 1. Destruction characteristics of pure alginate beads.

*There is a strong plastic deformation of the material before the destruction of the samples.

In all tested variants was observed strong plastic deformation of the samples before their destruction. A strong leaking of the samples was observed in the rheograms (fig.6), which is led to high plastic deformations. In the first moments of the experiment the material has elastic properties, but reaching the relative deformation around 50% began to observe a sharp increase of both studied parameters. Destruction of the material is due rather to the high plastic deformation than the shear load applied as a result.

Based on these studies have defined the experimental parameters for the modules "Stress relaxation" and "Creep", as follows:

- module for "Creep" - static load of 0.1 MPa, 0.2 MPa, 0.3 MPa;
- module for "Stress relaxation" - 5%, 10%, 20% and 30% of the maximum deformation.

3.1.2. Module „Stress relaxation”

The results from the experiments in module “Stress relaxation” are represented at Fig.7 and Fig.8. From the obtained results could be made different conclusions for the stability of the pure gel-beads.

With increasing of the concentration of the alginate solution increased and the necessary tension for reaching of the set deformation. In addition, with this increased and the residual stress in the samples. This is probably due to the gel-beads formation method – ionic gelation in $CaCl_2$ bath.

At the dissolving in water the Na-alginate molecule starts to rehydrate and to obtain viscous solution. The viscosity of the solution increases with the increasing length of the alginate molecules i.e increases with the number of monomers, which formatted the alginate molecule. The alginate chain has a good flexibility, which depends on the alginate block structure. The presence of bivalent cations ($Ca^{2+)}$ leads to cross-linking of the alginate molecules [6, 11, 12].

From the above it can be concluded that when the pressure was applied on the samples, their chain is contracted due to its flexibility. When the load was taken down from the samples, we observed residual tension in the samples. The Ca-alginate beads, which were produced from solution with higher concentration has a longer chains, so the residuals stress was higher.

The different alginate beads show relatively high flexibility despite increasing residual stress. This is confirmed by the fact that after the experiment the tensions decreased significantly, which means that the material resist to the deformation.

3.1.3. Module „Creep”

According to studies made in 3.1.1 the stress (corresponding static loads) of 0.1 MPa, 0.2 MPa, 0.3 MPa were selected as parameters to measure in this module. Time to conduct the experiment was 5 minutes, as in the first 2 minutes the samples were subjected to the load. After taking down the load from samples the stain relaxation were measured by DMAQ800. The results are summarized in Fig.9.

The four types of Ca-alginate beads showed similar levels of relaxation at the selected strains. For applied low static load of 0.1 MPa, the relaxation degree was between 50 and 60 percent for alginate concentration of 2%, 3% and 4%. When the pearls formed by 1% alginate solution alginaten the relaxation degree is about 10%. This is most likely due to short chain of the polymer.

The obtained results show that Ca-alginate beads have a relatively good elasticity. They show plasticity, so we may refer them to the viscuose-elastic bodies. It can be expected that, they will have resistance to the applied stress at the working conditions in the bioreactor.

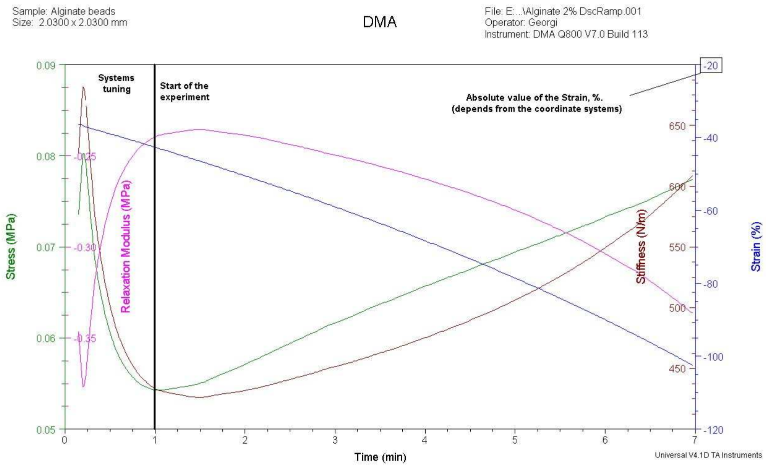


Figure 6. A typical rheogram from DMAQ800л

These results, alone in themselves are unusable because they do not take into account the impact of the yeast suspension on the beads' stability. It should be made immobilization of the yeast cell in the alginate matrix and to trace the same parameters after the immobilization procedures. We used dry yeast with a high reproduction capability, specifically selected for ethanol fermentation. For the immobilization was used 2% (w/v) yeast suspension. The concentration was chosen on the basis of carried out fermentation processes.

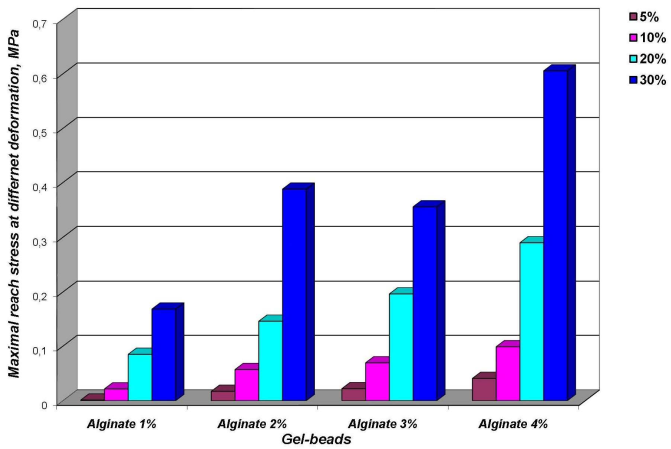


Figure 7. Necessary stress for reaching the set deformationл

3.2 Characteristics of immobilized preparation

3.2.1. Destruction characteristics of immobilized preparation

The obtained results are summarized in Table 2. The adding of yeast suspensions to the Na-alginate solution leads to stabilization of the samples and to increases of the maximum destruction stress of the samples. The samples behave as viscose-elastic bodies. When was reached 50 % of the deformation, it was observed a sharp increase of the stress in the samples. Hence it can be concluded that the adding of yeast suspension a little changed the nature of the immobilized beads. We can be separated two zones - the first by about 50% of the deformation, where the samples are with elastic properties, and after 50%, where the samples have a plastic nature with high plastic deformation.

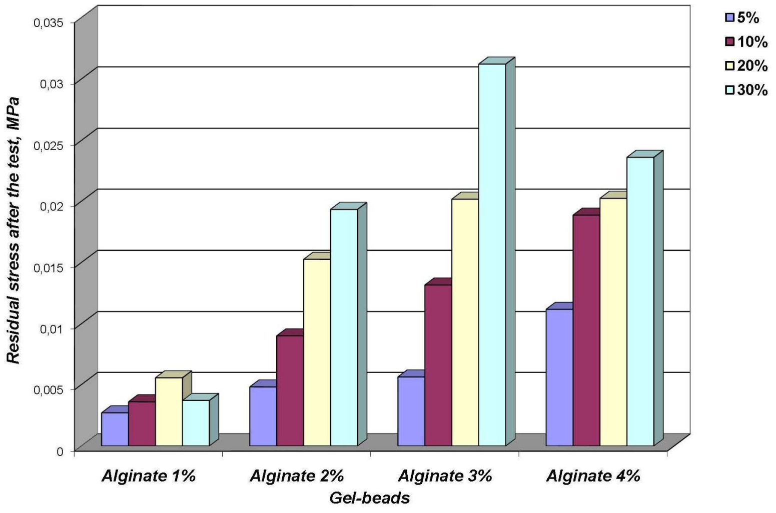


Figure 8. Residual stress in the samples after the experiments.

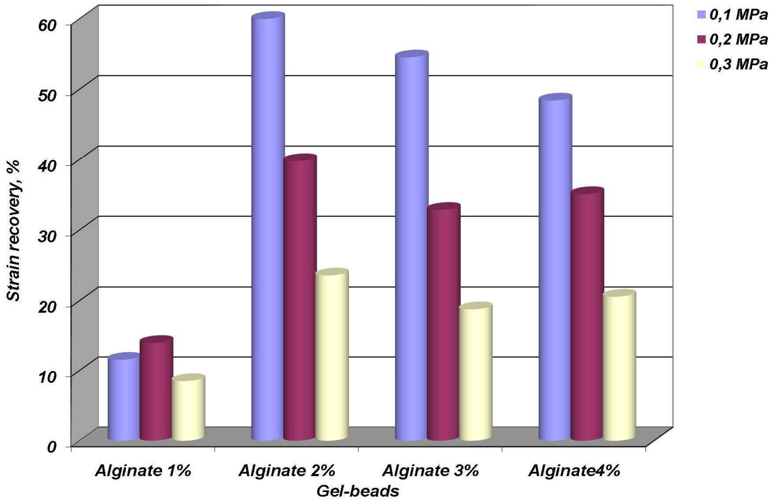


Figure 9. Relaxation degree for different Ca-alginate beads.

3.2.2. Module „Stress relaxation"

The results from that part of the experiments shows, that the added yeast suspension in the solution did not change significantly the stress in the samples in compare with pure preparations. The samples have viscose-elastic nature. It was observed non significant change in the investigated parameters with that module. The obtained stresses in some points are bigger, and in others are smaller, but the difference compared with pure samples is negligible (Fig.10 and Fig.11).

Gel type	Destruction characteristics	
	Maximal deformation, %	Maximal deformation, %
Alginate 1%	95	0.385
Alginate 2%	90	0.683
Alginate 3%	90	0.713
Alginate 4%	93	0.793

Table 2. Destruction characteristics of Ca-alginate beads with immobilized yeasts

From the results it can be concluded that the immobilized cells has no influence on the value of maximum and residual stresses. The beads structure was stabilized by the suspension, but not drastically change the sample properties. The difference in the examined samples is only due to the larger diameter. The immobilized beads have a greater diameter, but the difference in compared with the pure beads was within 0.1-0.2 mm.

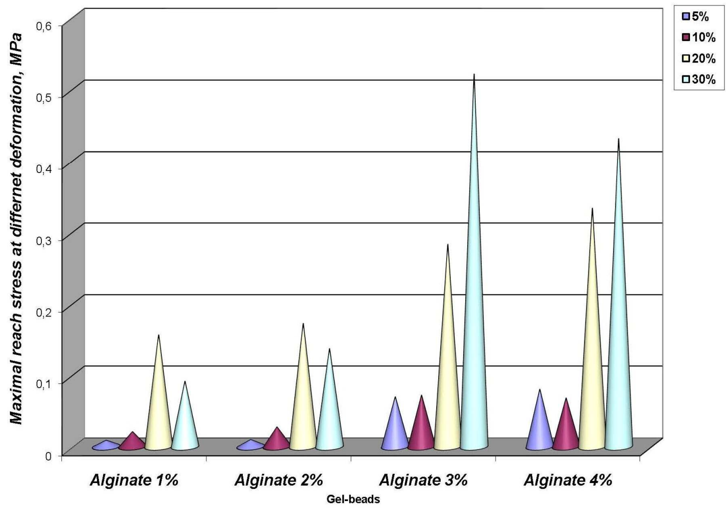


Figure 10. Necessary stress for reaching the set deformation for immobilized preparation.

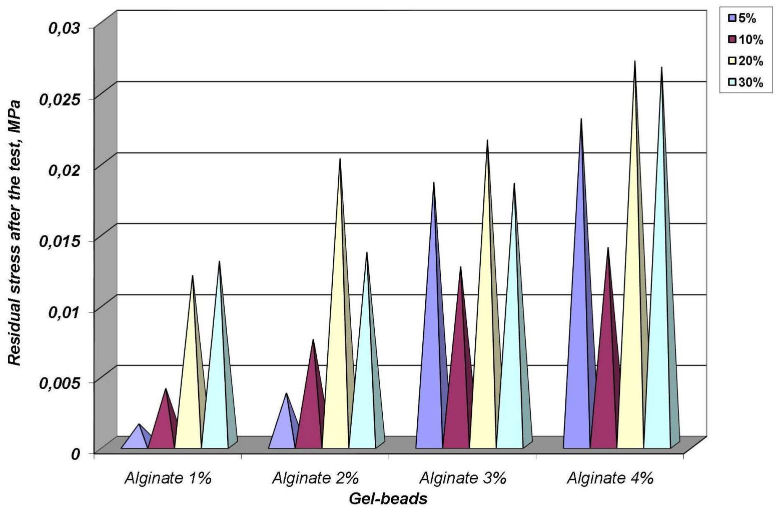


Figure 11. Residual stress in the samples after the experiments for immobilized preparation.

3.2.3. Module „Creep"

The results of the studies are shown in fig.12. In the immobilized samples had a reduced elasticity in compared with pure preparations subjected to the same studies. This is probably due to the inclusion of yeast cells in the alginate matrix. Thus reduces the elastic component of viscose-elastic bodies. The cells are located in the pores of the matrix, as in this way reduces the ability of the body to resist at the applied stress.

Although research in the module "Stress relaxation" is not observed significant changes in the sample properties, from the module of "Creep" can be concluded that the bodies become with more plastic properties. From the obtained results in this module can be concluded that immobilized samples will be more susceptible to the impact of strokes in the working parts of the apparatus and will keep tensions within them. Probably placed in the working conditions the immobilized preparations will destroy more easily.

Although the elasticity of the samples decreases, it can be concluded that the immobilized beads behave like viscose-elastic bodies with predominantly plastic properties. Plastic properties are reflected in reducing of the stain relaxation for the immobilized beads.

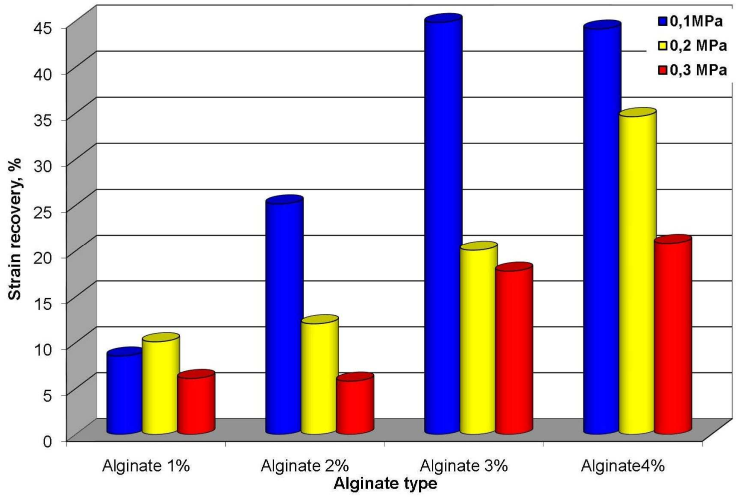


Figure 12. Strain recovery for immobilized samples.

4. Conclusion

The mechanical properties of Ca-alginate pearls for alcoholic fermentation with immobilized cells were studied in this work. Certain parameters of the deformation of the samples before and after the immobilization of yeast cells were investigated. From the results can make some important conclusions:

- The alginate beads could be regarded as viscose-elastic bodies. They occur as elastic properties (strain relaxation), and plastic properties (observed residual stress in samples) ;

- The pearl elastic properties decrease with the addition of yeast suspension. This is probably due to the inclusion of yeast cells in the free space of the matrix and the reduction the possibility of relaxation of the deformation ;

- The immobilized and the pure samples had closer values for the maximum and residual stresses. From here it can be concluded that the samples retain their plastic properties, as opposed to the change in elastic properties.

The results may be useful in the development and implementation of systems for ethanol fermentation with immobilized cells. To obtain a complete picture of the operation of such systems, it is necessary to make such studies in working conditions. Similar studies of the mechanical characteristics of the pearls in the operating conditions, integrated with the results of the impact of alginate concentration on the accumulation of ethanol in the medium will lead to a better selection of optimal alginate concentration. The latter is a good direction for future work in this field.

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