A COMPARATIVE STUDY OF KINETIC IMMOBILIZED YEAST PARAMETERS IN BATCH FERMENTATION PROCESSES

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ABSTRACT

The rate of biochemical reactions in immobilized cell systems usually is lower compared to free cell fermentation. Kinetic parameters modified resulting in different mathematical models, productivity and rate. This paper is focused in kinetic parameters study (μ_{max}, K_s, K_i) of immobilized yeast cells in alginate matrix compared to free yeast cells parameters in batch fermentation processes. Using different beads diameters, cell density, including substrate and product inhibition conditions. We have evaluated kinetic parameters with three different linearization methods and mathematical models that fits with experimental results. At non – inhibitory conditions immobilized yeast ferment similiary to free yeast for small bead diameters. Due to bead diffusion resistance the differences compared to free yeast system are notable with beads size increase. In inhibitory condition, productvity and reaction rate are higher compared to free yeast fermentation. This is linear with beads size increasing.

Keywords: Immobilized yeast, kinetic parameters, linearization, inhibition constant.

INTRODUCTION

The "white biotechnology", the new concept proclaims that the use of renewable raw materials for biofuels production by low expensive and ecofriendly biotechnologies constitutes one of the priority of industrial activities. However, the economic feasibility of the ethanol industry is still questioned and much effort should be put into improving the process, especially resistance to the main inhibition factors.

To eliminate inhibition caused by high concentrations of substrate and product as well as to enhance yield, cell immobilization approaches have been applied in ethanol production. The advantages of immobilized cell over free cell systems have been extensively reported. Immobilized cell fermentation has been shown to be more effective than the free yeast process, mainly due to the enhanced fermentation productivity, feasibility for continuous processing, cell stability and lower costs of recovery and recycling and downstream processing. However, immobilized cells still have limited industrial application. The process of immobilization changes not only the environment, but also the physiological and morphological characteristic of cells, and the catalitic activity of enzymes. Therefore the fermentation conditions (kinetics) of the free yeast fermentation and of the immobilized cell process are different.

Simulation investigations are proven to be powerful tools for evaluating the fermentation processes alternatives that decrease spending of expenses on pilot experiments. The quality of the simulation itself depends on the quality of the underlying mathematical model used fot prediction of the responses of a given system to changes in environmental and operating conditional. Hence the mathematical models should describe with sufficient accuracy the mechanisms of the processes under consideration. For the purpose of bioprocesses simulation, kinetic models based on mass balance of the main compounds in the bioreactor. Modeling batch fermentation process by the yeast *Saccharomyces Carlsbergensis in* immobilized in Na-alginate gel beds were considered. They

describe the main factors affecting the glucose concentration – substrate and product inhibition, but none of them can account simultaneously for all of these factors. However there is no model universal structure that could perfectly suit glucose fermentation by all possible kinds of strains since each particular strain has its specifics that require an individual approach to kinetics modeling. The yeast posses the ability to converse glucose under anaerobic conditions and the main final products being the ethanol and carbon dioxide. The efficiency of the ethanol production by yeasts can be affected by glucose or ethanol concentration, due to the specific phenomenon of substrate and product inhibition. An interesting result has been obtained that the addition of ethanol in a culture of *Saccharomyces Carlsbergensis* induces less toxic effect than that generated by ethanol biosynthesized during the fermentation, the cells death occurring with lower rate in the former case. This result could be explained by the presence of other metabolic products beside ethanol, these secondary products contributing to the amplification of the inhibitory phenomenon.

MATERIALS and METHODS

The aim of our study was to carry out a comparative analysis of different mathematical structures known by far for modeling of batch alcoholic fermentation with free and immobilized cells of *Saccharomyces Carlsbergensis* using real experimental data. The two type of processes (with free and immobilized cells) were compared with respect to the main model parameters that determine the main interactions in the culture – inhibition and transformation of sugar to ethanol and biomass. Conclusions were drawn about the influence of cell immobilization on the batch process. The main purpose of this study was to choose the best model that will be further refined and used for control synthesis of the process in order to increase its productivity. The equipment consists in eight conical bioreactors (vessels) 250ml, where was placed the immobilized yeast with the entrapment method and free yeast, in respective amount 1 and 2 g/l. For yeast cell immobilization solution of Na – alginate was used. It was prepared by dissolving alginate in distilled water at constaint stirring until a homogeneous solution was obtained.The immobilization has been carried out by cells inclusion into the alginate matrix, respecting the entrapment method. The following diameters of the biocatalyst spherical particles have been obtained: 4, 5.3 and 7 mm.The fermentations have been carried out until a constant amount of glucose was taken, at the ambient temperature.

RESULTS and DISCUSSION

Glucose consumption and ethanol production

The experimental studies presented in this paper have been carried out at various glucose concentrations, including here inhibitory and non-inhibitory substrate conditions. Immobilized batch fermentation was analysed for three different bead size (4, 5.3 and 7 mm) and fermentation performance was compared with free yeast fermentation.

Figure 1. Extract plot versus Time in 12 ^{0} Plato batch fermentation

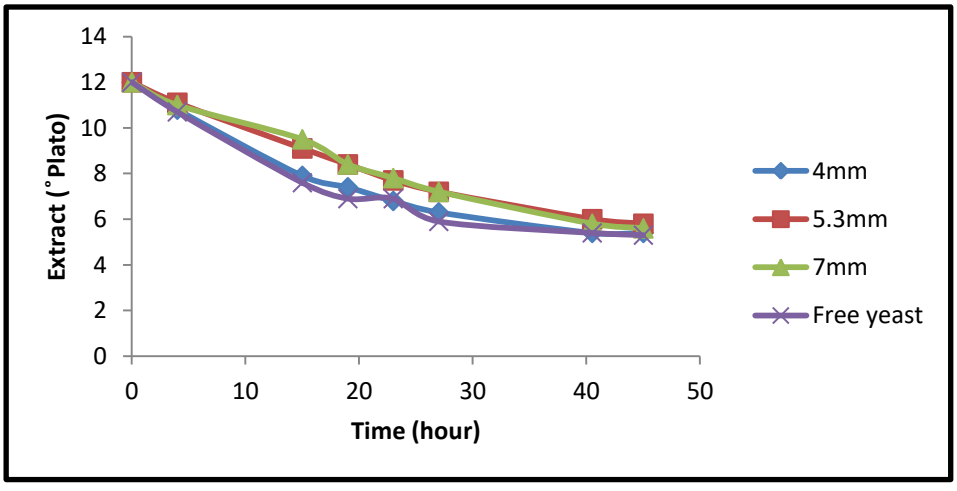
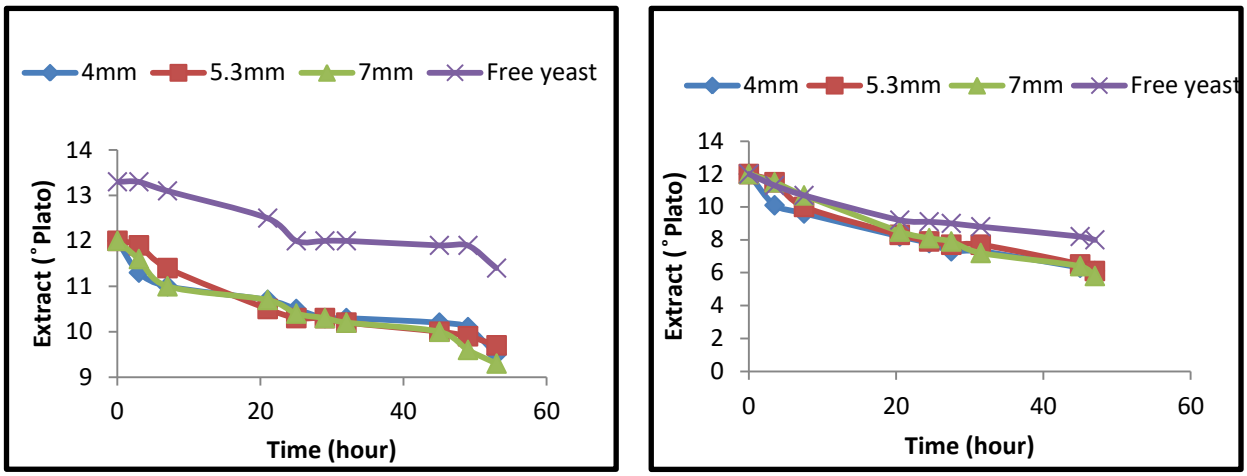


Figure 2. Extract plot versus Time in 16 ^{0}Plato and 20 ^{0}Plato batch fermentation



Free fermentation performance in 12 Plato (Figure 1) compared to immobilized yeast fermentation is better in terms of rate of substrate consumption and remain extraxt. A good similiarity is noticed with 4 mm bead size fermentation. Higher the bead size larger are the differences between imobilized and free yeast fermentation. In the case of substrate and product inhibition (Figure 2 and 3) this dependence is the opposite. Residual extract remains higher in the case of free yeast fermentation and the plot is distlinctly isolated from the others. Higher the bead size lower is the residual extract.

Figure 3. Extract plot versus Time in 12 ^{0} Plato + 5 % Alcohol and in 12 ^{0} Plato batch fermentation

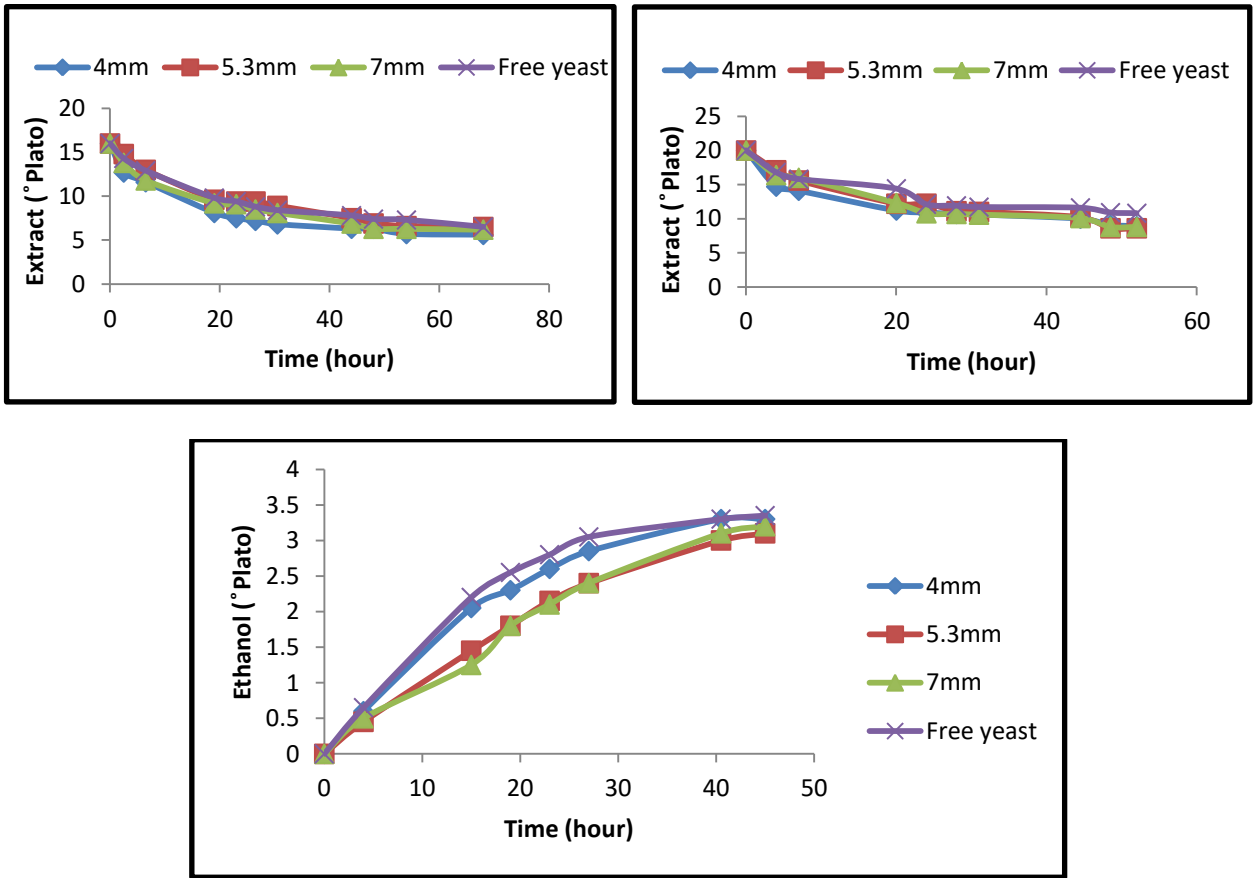


Figure 4. Ethanol plot versur Time in 12 ^{0} Plato batch fermentation

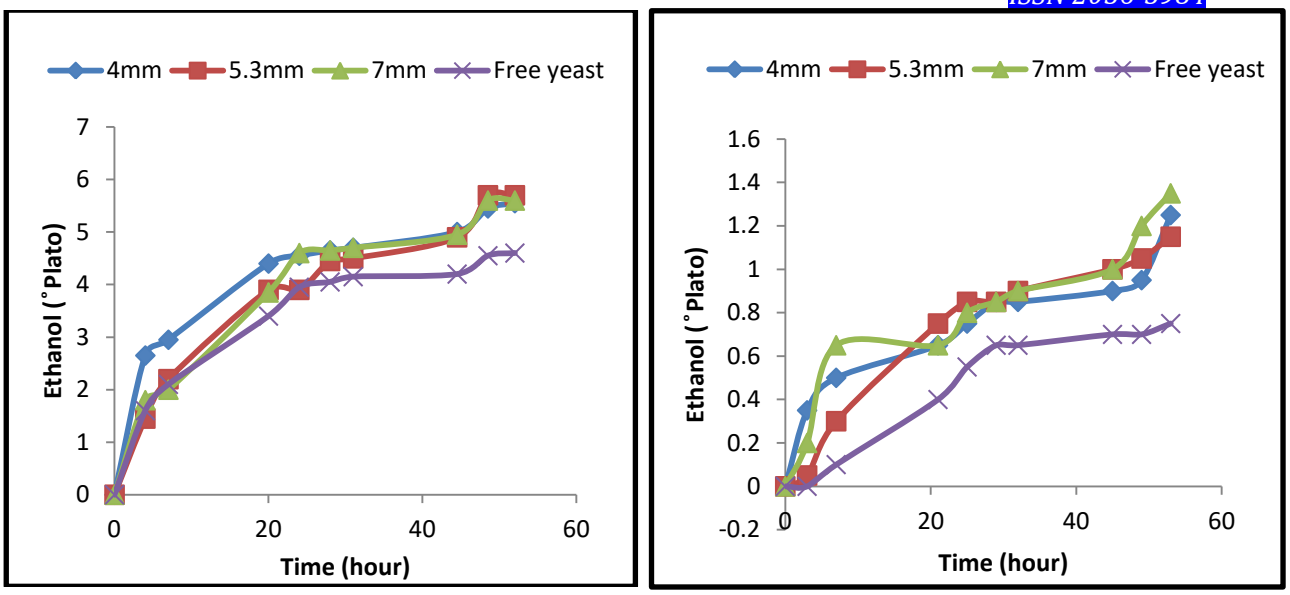


Figure 5. Ethanol plot versus Time in 16 ^{0} Plato and 20 ^{0} Plato batch fermentation

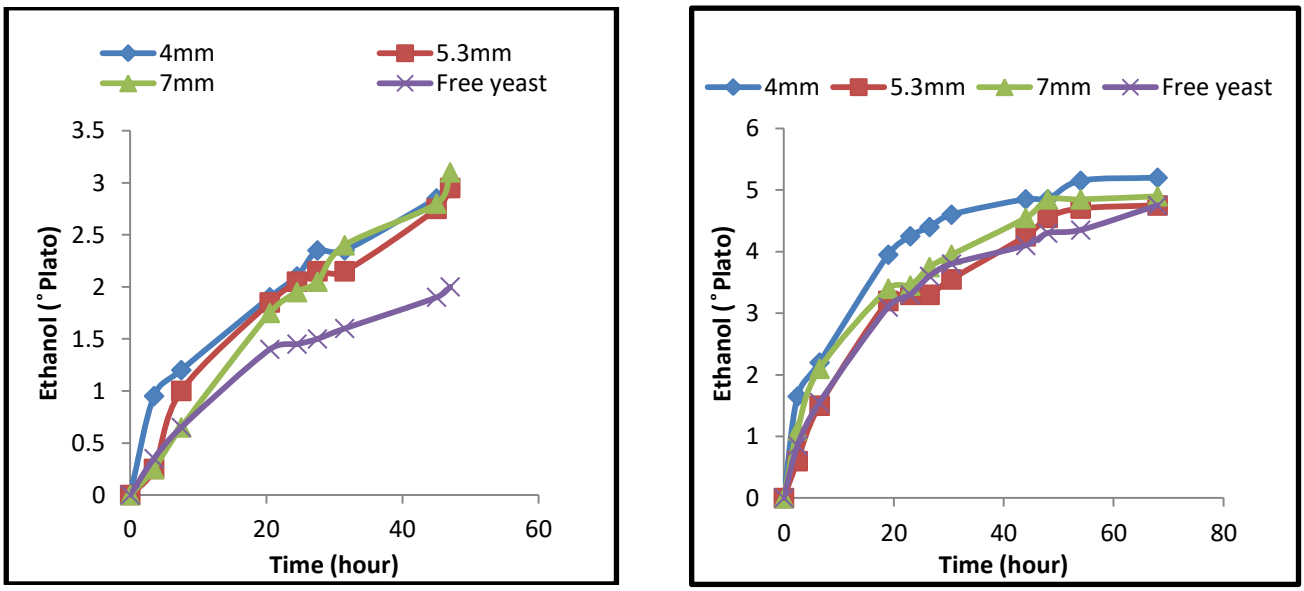


Figure 6. Ethanol plot versus Time in 12 ^{0} Plato + 5 % Alcohol and 12 ^{0} Plato + 20 % Alcohol batch fermentation

Ethanol production in 12^{0} Plato free yeast fermentation is very close to 4mm fermentation (Figure 4).

In inhibitory conditions, substrate and product inhibition (Figure 5 and 6), productivity is higher in immobilized yeast fermentation due to diffusion resistance of alginate matrice. The internal diffusion reduces significantly the inhibitoty effect of glucose. But, in this case, the product inhibition could become important, due to the low diffusion rate of ethanol towards the outer medium and to its accumulation inside the particle.

Kinetic Parameters

The rate of biochemical reactions in heterogeneous systems is lower than that recorded for homogeneous media, owing to the radial decreasing of the substrate concentration inside of the

biocatalyst particles. For the heterogeneous systems, not only the value of the biochemical reaction rate is affected, but also the kinetic model is modified compared to the ideal models describing the substrate cunsumpition or product formation.

For these reasons, the kinetic parameters of the biochemical reactions which involve immobilized cells differ from those of homogeneous environments. For the analyzed fermentation systems, the ethanol formation can be mathematical described by Monod equation:

$$\mu = \mu_{max} \frac{1}{s + K_s} \quad (1)$$

The equation (1) can be used for fermentation systems without inhibitory phenomena. But the inhibitory effects occur also in the case of alcoholic fermentation with immobilized yeast cells, the most important being that induced by glucose. Therefore, taking into account the substrate inhibition, the Monod equation can be written for the immobilized cells as follow as:

$$\mu = \mu_{max} \frac{s}{s + K_s} exp\left(- \frac{s}{K_i}\right) \quad (2)$$

When we have product inhibition the equation can be:

$$\mu = \mu_{max} \frac{s}{s + K_s} exp(- K_i * p) \quad (3)$$

After the determination of the kinetic parameters, various mathematical models were ploted for each fermentation. These plots were compared with the experimental plot. An example of mathematical modeling is shown in Figure 7.

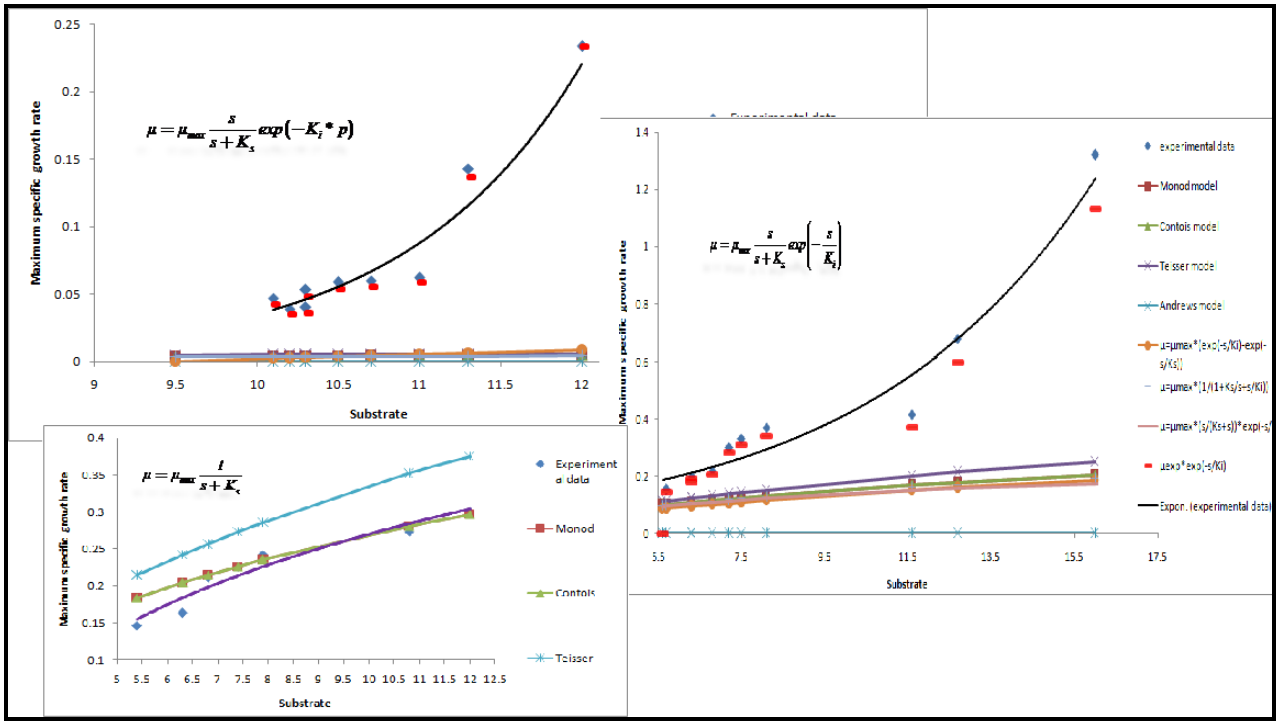


Figure 7. Example of mathematical modeling

From the obtained results it is difficult to choose a single best fitting model. Good approximation potencial was shown by the model of Monod, Teisser, Aiba. For substrate and product inhibition the most approximate models were exponential. Non-inhibitory fermentations fit with the Monod model, while product and substrate inhibition fermentation fits with exponential models.

Three linearization methods were used to determine μ_{max} and Ks, which are:

- Lineweaver – Burk

$$\frac{1}{\mu} = \frac{K_s}{\mu_{max}} \frac{1}{s} + \frac{1}{\mu_{max}} \quad (4a)$$

- Hans Woolf

$$\frac{s}{\mu} = \frac{1}{\mu_{max}} s + \frac{K_s}{\mu_{max}} \quad (4b)$$

- Eadie Hofslee

$$\mu = - K_s \frac{\mu}{s} + \mu_{max} \quad (4c)$$

And the one with the highest correlation coefficient was chosen, and K_i was determined by the mathematical method trial and error.

Kinetic parameters μ_{max} and K_s were determined by three different linearization methods. An example of linearization calculation is shown in Figure 8.

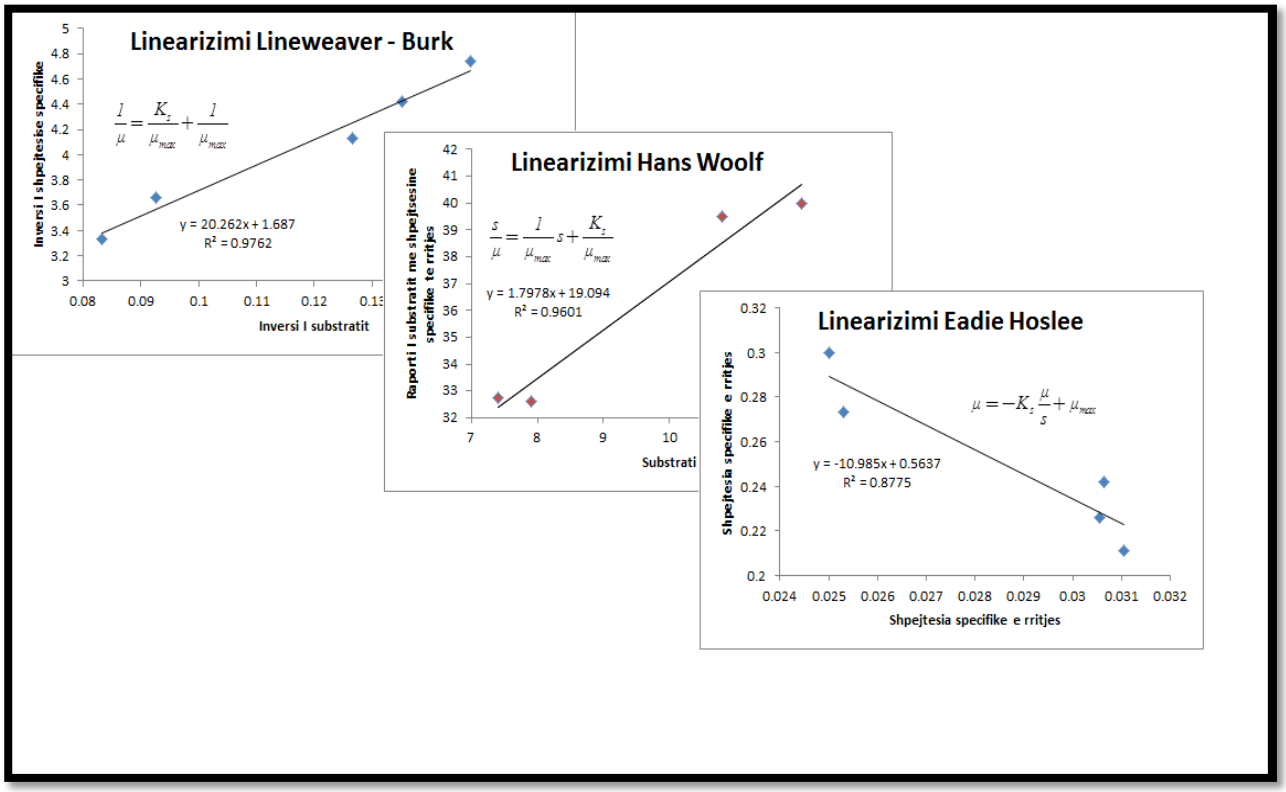


Figure 8. Example linearization determination of constants

The values obtained for the kinetic parameters are presented in Table 1.

Table 1. Kinetic parameters for each fermentation

		μ_{max}	K_s	K_i	$Y_{p/s}$	$Y_{x/s}$	R
	Unit	1/hour	°Plato	°Plato	-	-	%
Fermentation 1	4 mm	0.593	12.01	-	0.5	0.00114	97.6
	5.3 mm	0.34	7.145	-	0.5	0.0021	82.8
	7 mm	0.385	10	-	0.5	0.0023	66.3
	Free yeast	0.711	14.413	-	0.5	0.09881	89.3
Fermentation 2	4 mm	5.78	118.844	-	0.5	0.0039	82.7
	5.3 mm	0.087	12.38	-	0.5	0.0118	63.6
	7 mm	2.924	69.941	-	0.5	0.0118	79.3
	Free yeast	0.077	13.06	-	0.5	0.1079	71.3
Fermentation 3	4 mm	0.471	20.94	104.7	0.5	0.0117	93.8
	5.3 mm	0.839	42.718	213.6	0.5	0.0126	93.1
	7 mm	0.406	22.94	114.7	0.5	0.0127	97.3
	Free yeast	0.384	24.894	124.47	0.5	0.0669	91.6
Fermentation 4	4 mm	0.321	22.793	113.965	0.5	0.0101	95.17
	5.3 mm	0.425	29.701	148.505	0.5	0.0132	91.01
	7 mm	0.412	27.629	138.145	0.5	0.0134	87.58
	Free yeast	0.208	23.638	118.19	0.5	0.0624	95
Fermentation 5	4 mm	0.135	14.79	0.1	0.5	0.0143	98.3
	5.3 mm	0.018	9.316	0.09	0.5	0.0162	68.1
	7 mm	0.167	0.613	0.006	0.5	0.0217	80.7
	Free yeast	0.11	18.54	0.18	0.5	0.1148	97.1
Fermentation 6	4 mm	0.009	12.37	0.12	0.5	0.0059	99.9
	5.3 mm	0.005	11.84	0.11	0.5	0.0095	94.4
	7 mm	0.011	12.51	0.12	0.5	0.0064	99.6
	Free yeast	0.00008	12.3	0.12	0.5	0.1975	99

The results indicate that the inhibition constant has an unique value indifferent of the biocatalyst particles size, but depending on their concentration. The Monod constant is influenced by the size and concetration of biocatalyst particles. The maximum rate of ethanol production is favorably influenced by the increase of the biocatalysts concentration, and is affected by their size.

The Monod constant is greater compared with homogeneous systems fermentation by the internal diffusion. Besides the positive exhibited by the attenuation of the inhibitory phenomena, the immobilization of yeast cells affects the fermentation rate compared to the systems without inhibition contationing free yeast cells.

In the case of the free cell fermentation, we have high values of the parameters K_s and $Y_{X/S}$. In case of substrate inhibition, we have the impact of the inhibition constant K_i, that reduces the maximum specific growth rate and increases the semi saturation constant K_s. In case of product inhibition, the values of K_i parameter is very low. This is because the obtained ethanol concentration is much lower than the concentration that causes complete cell growth inhibitionfor our experimental data.

CONCLUSIONS

The studies on the substrate consumption and product formation rates during the alcoholic fermentation with immobilized yeast cells compared to free yeast performance show that immobilized yeast ferment similiary to free yeast for small bead diameters. Higher the bead size larger are the differences between imobilized and free yeast fermentation.

In inhibitory conditions, substrate and product inhibition productivity is higher in immobilized yeast fermentation. Due to other diffusion inside the biocatalyst particles, the inhibitory phenomena are avoided, the microbial activity being preserved.

Using a specific mathematical model for ethanol formation in the investigated systems, the kinetic parameters μ_{max}, K_s and Ki have been estimated and compared with their values reported for alcoholic fermentation in homogeneous media in presence or not of inhibitory effects.

It was considered also modeling of batch fermentations with free and immobilized yeasts. Non-inhibitory fermentations fits with the Monod model, while product and substrate inhibition fermentation fits with exponential models.

Based on fermentation performance, kinetic parameters and mathematical models we conclude that immobilised yeast cells in normal conditions ferment similarly to the free yeast. Immobilization decrease the substrate and product inhibition phenomena compared to free yeast fermentation.

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