DETERMINATION OF EFFECTIVE DIFFUSION COEFFICIENT OF IMMOBILIZED BAKER'S YEAST INVERTASE IN VARIOUS CONCENTRATION OF PVA-ALGINATE MATRIX

MOHD ZAIN N. A *, SUHAIMI M. S., IDRIS, A.

Department of Bioprocess Engineering
Faculty of Chemical and Natural Resources Engineering
Universiti Teknologi Malaysia
**email: skama_ay@yahoo.com*

ABSTRACT: Baker's yeast invertase is immobilized in PVA-alginate matrix using an improved method. PVA beads were prepared by adding calcium alginate to improve its stability, mechanical and chemical properties. Boric acid was used as the cross-linking agent and additional chemicals consisting of 10% boric acid and sodium sulphate solution was used as a treatment solution to harden the PVA-alginate beads. The determination of the effective diffusion of PVA-alginate matrix is the vital step in optimizing the preparation of immobilized and water-soluble biocatalyst. In this study the two-level full factorial design was used to investigate the effect of PVA and boric acid concentrations on diffusion coefficient. Diffusion coefficient (D_e) is one of the factors that significantly affect the mass transport within the immobilization matrix. D_e value varies for each concentration of PVA and boric acid. The result concluded that both factors significantly affect the D_e. A maximum D_e value of 5.0141 x 10^{-6} cm^2s^{-1} was obtained at boric acid and PVA concentration of 7w/v and 10.5w/v respectively.

Keywords: PVA, Immobilization, Effective Diffusion coefficient, Invertase.

1. INTRODUCTION

Biocatalyst has found numerous applications in industrial domain and it has become reputable ever since. The use of immobilization matrix to entrap biocatalyst has proven to be economical as it offers reusability over a period of time plus simplifying the downstream processing. Numerous types of polysaccharides or polymer has been employed in cell or enzyme immobilization and in this experiment, Poly vinyl alcohol (PVA) was used.

PVA was introduced as an immobilization matrix about 10 years ago. It is a polymer of great interest due to its desirable characteristics specifically for various pharmaceutical and biomedical applications [1]. PVA offers various advantages over the conventional alginate hydrogels including lower cost, higher durability and chemical stability, and non-toxicity to viable cells [2].

Moreover, their non-toxic, non-cryogenic and bioadhesive characteristics as well as their associated ease of processing craft them into an exceptional biomaterial. PVA has a simple chemical structure and modifications are made possible by simple chemical reactions.

ISBN: 978-983-43090-2-2

Furthermore, PVA gels exhibit a high degree of swelling in water (or any biological fluids) and posses rubbery and elastic nature [1]. The elasticity of PVA beads provide them with a better mechanical strength compared to the brittle alginate beads. Besides, PVA beads were very stable over the pH range 1–13, while alginate beads were relatively stable only in the pH range 6–9. A weight loss of up to 20 and 24% were encountered at low and high pH, respectively, for the alginate beads [2].

The transport and consumption of substrates across the beads from the bulk solution control the behavior of many biological systems. It can be conceptualized as the diffusion of small particles in a material containing multiple reactive traps or sinks within an inert continuous phase [3].Molecular coefficient or molecular transport can be defined as the transfer of individual molecules through a fluid by means of the random, individual movements of the molecules. Molecular diffusion is also known as a random-walk process as the molecules travel in a random path.

The measurement of effective diffusivities (D_e) for substrate and metabolic products within gel matrices in the form of beads or large membranes has been reported. The effective diffusion coefficient (D_e) of the substrate molecules within the gel matrices must be known for optimizing the preparation condition of immobilized and in order to characterize them by their kinetical behavior [4].

There were many immobilization methods published by previous researchers using PVA-alginate but the method could not be applied because the chemicals and acids that are not suitable for enzymes and also the beads dissolved in water-based solution [2, 5, 6]. To solve this problem, an innovative method has been developed using chemicals that are suitable for enzymes. In this study, an innovative method was developed to immobilize yeast invertase to compensate the failure of previously published methods when used for this purpose. The PVA-alginate beads preparation were modified by using treatment solution of 10% boric acid and sodium sulphate solution. By using these additional treatment chemicals, the PVA-alginate beads remain insoluble in water and other water-based solution.

In this study, invertase was immobilized in PVA-alginate matrix using a novel method insofar of the treatment method using sodium sulphate. Invertase hydrolyzes sucrose into two different, much more stable sugars which are glucose and fructose. The measurement of glucose concentration is used to calculate the effusion of the solution from the beads. The beads were first equilibrated in 10% sucrose solution (w/v).

Sucrose, commonly known as table sugar, is a disaccharide composed of an alpha-D-glucose molecule and a beta-D-fructose molecule linked by an alpha-1,4-glycosidic bond. When this bond is cleaved in a hydrolysis reaction, an equimolar mixture of glucose and fructose is generated. Sucrose can be hydrolyzed in the presence of invertase or sucrose. Invertase officially known as beta-fructofuranosidase (EC3.2.1.26) which implies that the reaction catalyzed by this enzyme is the hydrolysis of the terminal non-reducing β-fructofuranoside residues in β-fructofuranosides.

2. THEORETICAL BACKGROUND

External diffusion is caused by an unstirred Nernst layer which thickness depends on the relative velocity of the beads to the bulk solution. Diffusion of molecules in a ball shaped beads with radius R can be appropriately modeled by the expression:

ISBN: 978-983-43090-3-2

$$\frac{c_t - c_\infty}{c_0 - c_\infty} = \frac{6}{\pi^2}\sum_{n=1}^{\infty} n^{-2} \exp\left(-\frac{n^2 \pi^2 D_e t}{R^2}\right) \quad (1)$$

For an amply extended period of time, the expression can be simplified as

$$\frac{c_t - c_\infty}{c_0 - c_\infty} \cong \frac{6}{\pi^2}\exp\left(-\frac{\pi^2 D_e t}{R^2}\right) \quad (2)$$

Thus by plotting the graph $\ln[(C_t - C_\infty)/(C_0 - C_\infty)]$ versus time, the diffusion coefficient D_e could be obtained.

The effusion process for the spherical beads can be divided into 4 different stages. Based on Fig 1, at the beginning of the experiment (Fig 1a, $t = 0$), the whole substrate is within the beads, thus the concentration 'c' in the bulk solution is zero ($c = 0$). At time 't', (Fig 1 b, $c = c_t$) the substrate concentration within the bulk solution increases as the result of substrate diffusion from the beads. When 't' reaches ∞, ($1/t = 0, c = c_\infty$) the substrate concentration in both phases is at equilibrium (Fig 1c).

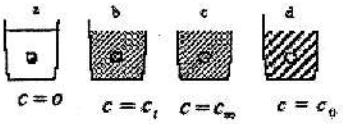

Fig. 1: Scheme for substrate effusion from ball-shaped immobilized cells into the surrounding solution.

If the substrate concentration in the bulk solution is measured by the electric conductivity 'G' then a hypothetically complete diffusion of the substrate out of the beads (Fig. 1d) can be expressed by 'Go' (=c_0) whereas for $t \to \infty$, G_∞ is obtained. Taking this into account, the c-values in Eq. (1) can be replaced with conductivity values (or any other measured quantity, being equivalent to 'c'): $c_0 = G_0$, $c_t = G_0 - G_\infty$ and $c_\infty = G_0 - G_\infty$ Substitution into Eq. (1) yields:

$$\ln\frac{G_\infty - G_t}{G_\infty} = \frac{\pi^2 D_e t}{R^2} + const \quad (3)$$

3. MATERIAL AND METHOD

3.1 Materials

Bakers yeast Invertase (b-fructofuranosidase, EC 3.2.1.26) and sucrose grade V, practical grade were purchased from Sigma-Aldrich Co, St Louis, Mo, USA. Polyvinylalkohol 72000 for synthesis and boric acid for analysis were purchased from Merck Schuchardt OHG, Darmstadt, Germany, sodium alginate from Fluka Chemie GmbH, Buchs.Sodium sulphate was purchased from GCE Laboratory Chemicals and calcium chloride from R&M Marketing, Essex, U.K.

3.2 Experimental design

By using factorial design, the concentration of PVA and boric acid were varied simultaneously while the sodium alginate and calcium chloride concentration were maintained. Boric acid concentration was also varied to find the optimum concentration in solidifying PVA. Table 1 depicts how the concentration of PVA and boric acid was varied for each run.

3.2 Immobilization of invertase

Invertase (10ml, 5% w/v) was mixed with 90 ml of PVA (10.5% and 12% w/v) sodium alginate (1% w/v) solution. The mixed solution was then dropped into a mixed solution of 100ml boric acid (5% and 7% w/v) and calcium chloride (2% w/v) using a syringe to form beads and stirred for 30 to 50 minutes. The beads were stored at 4°C for 24 hours. After 24 hours, the beads were stirred in 10% boric acid solution for 30 minutes and were treated with sodium sulphate solution for another half an hour. Then the beads were kept at 4°C for further use. This technique was performed in sterile conditions. The concentration of PVA and boric acid used can be viewed in table 1.

Table 1: The Boric Acid And PVA Concentration For Each Run Of Experiment

Experiment no	Boric acid concentration, w/v	PVA concentration, w/v
1	5.00	12.0
2	7.00	12.0
3	7.00	10.5
4	5.00	10.5

3.3 Analytical methods

5 g of the beads are equilibrated for 4 h in 50 ml of 10% sucrose solution (w/v) and then transferred into 50 ml of sterile distilled water. After 50 min, sampling was done (1 ml) every 2 h interval to determine the amount of substrate effused from the beads into the bulk solution. Biochemistry analyzer YSI 2700 was used to determine the glucose concentration. The amount of sucrose is equal to the difference ΔE of the extinction values E_2, and E_1 for the blank. The ΔE values are substituted into equation (3).

4. RESULT AND DISCUSSION

Previously, the immobilization method was applied to entrap *Lactobacillus delbrueckii* ATCC 9649 and the diffusion coefficient of the PVA-alginate bead was determined compared to the very common immobilization matrix which is calcium alginate [7]. While in this study, the method to immobilize the invertase was carried out using the very same

technique but in various concentrations of PVA and boric acid. PVA was varied because it is the main material in the immobilization matrix. It is non-toxic, non-cryogenic and bioadhesive characteristics as well as their associated ease of processing. It also exhibits a high degree of swelling in water (or biological fluids) and a rubbery and elastic nature [8].

PVA is highly hydrophilic. It must be crosslinked either chemically or physically to make it become hydrophobic. PVA is usually chemically crosslinked with difuntional gluteraldehyde in the presence of sulfuric acid, acetic acid or methanol. But, these chemical are toxic and can not be used in medical or pharmaceutical applications. In this study, boric acid is used to crosslink the PVA. Due to this scenario, in this experiment the concentration of boric acid has been varied to investigate the effect of changing the concentration of the cross-linking agent to D_e.

By using the method proposed by Grunwald *et al.* (1997) [4], the effective diffusion coefficient for various concentration of PVA-alginate beads immobilizing Baker's yeast invertase have been determined. In figure 2 and figure 3 the results presented were determined by measuring the glucose concentration effused from the various concentration of PVA-alginate beads immobilized invertase. ΔE_∞ could be determined straight from the ordinate intercept by the extinction difference ΔE_t versus the reciprocal effusion time plot. The effective diffusion coefficient, D_e can be calculated from the slope of the linear relationship between $\ln[(c_t - c_\infty)/(c_0 - c_\infty)]$ and the effusion time 't'.

Sucrose, known as small molecules diffused into the spherical beads. Then, after the beads were transferred into the distilled water, the glucose and fructose start to diffuse into the bulk solution. The determination of effective diffusion coefficient of the beads is based on glucose concentration which in turn was determined using Biochemistry analyzer YSI 2700. The D_e for each run from run 1 to run 4 respectively are 3.0923 x 10^{-5} cm^2s^{-1}, 3.9201 x 10^{-5} cm^2s^{-1}, 5.0141 x 10^{-5} cm^2s^{-1} and 4.1024 x 10^{-5} cm^2s^{-1}.

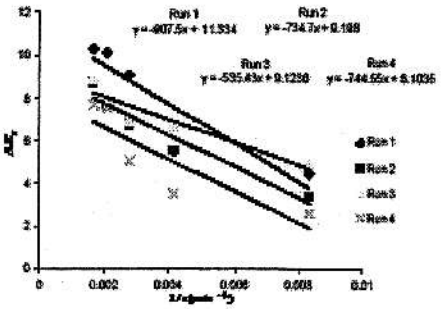

Fig 2: Plot of ΔE versus reciprocal effusion time from the experiments with invertase immobilized in PVA-alginate matrix

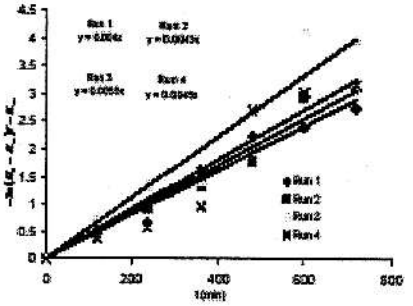

Fig 3: An evaluation of experimental data from fig 3, D_e could be calculated from the slope. The mean diameter of the beads was 0.3 cm

Table 2 presents the output from Minitab for the least squares regression models for enzyme activity data. The regression equation that models the relationship between variables was also presented. The table also presents the value of p of the t-test for each coefficient in the regression model. Based on the regression analysis, both PVA and boric acid concentration acts as significant factors that affect D_e as the p value for both factors are less than 5%. The regression equation that relates the variables is De = 9.31 + 0.435 Boric acid Concentration - 0.701 PVA concentration.

From the regression equation, it is clear that the D_e is proportional to boric acid concentration and inversely proportional to PVA concentration.This circumstance implies that the beads with lower PVA concentration would give higher reading of D_e. With a lower PVA concentration, more substrate can be stored by the beads. Moreover, as more substrate is stored inside the beads, the fugacity of the substrate increases and this would drive the substrate to diffuse at a much higher rate to the bulk solution.

This observation implies that beads with lower density of PVA would allow substrate to diffuse at a much faster rate to the bulk solution hence a higher D_e. It is also clear that D_e is proportional to the concentration of boric acid used. This circumstance implies that the concentration of boric acid would improve the beads quality in terms of substrate diffusivity through the pore of the beads. Table 3 contains the numerical estimates of the regression coefficients. The coefficient of determination (R-sq) is 99.9% which ensured a satisfactory model. In addition, the value of σ^2 was also presented. The table shows that the estimate of σ^2 for the D_e values which is equal to 0.00176.

Table 2: T-Value And P-Value For The Multiple Regression Models

Predictor	Coef	SE Coef	t	P
Constant	9.3134	0.3395	27.43	0.023
Boric acid Concentration	0.43487	0.02097	20.73	0.031
PVA concentration	-0.70137	0.02797	-25.08	0.025

Table 3: ANOVA For Multiple Regression Model

Source	DF	SS	Ms	F	P
Regression	2	1.86327	0.93164	529.40	0.031
Residual error	1	0.00176	0.00176		
Total	3	1.86503			

S = 0.04195 R-Sq = 99.9% R-Sq(adj) = 99.7%, SS, sum of squares; DF, degree of freedom; MS, Mean square.

5. CONCLUSION

Immobilization of invertase Bakers'a yeast was done by using an innovative method using PVA-alginate matrix. By the determination of glucose concentration, it is known that the PVA-alginate beads allow molecules such as sucrose, glucose and fructose diffused from the beads and beads with different PVA and boric acid concentration would allow these molecules to diffuse at different rate. The constant of proportionality, i.e the D_e could be directly determined from the plot after the substrate effusion from the beads was measured. Beads with lower PVA and higher boric acid concentration would give a higher D_e thus a better rate of diffusion of substrate to the bulk solution.

6. REFERENCES

[1] Hassan, C. M. and Peppas, N. A. (2000). Cellular PVA Hydrogels Produced by Freeze/Thawing. *Journal of Applied Polymer Science*. 76: 2075-2079.

[2] Khoo, K.M. and Ting, Y. P. (2001). Biosorption of Gold by Immobilized Fungal Biomass. *Biochemical Engineering Journal*. 8: 51–59.

[3] Riley, M. R., Muzzio, F. J. and Buettner, H. M. (1995). The Effect of Structure on Diffusion and Reaction in Immobilized Cell Systems. *Chem. Eng. Science*. 50: 3357-3367.

[4] Grunwald P., Hansen K. and Gunβer W. (1997). The Determination of Effective Diffusion Coefficient in a Polysaccharide Matrix Used for the Immobilization of Biocatalyst. *Solid State Ionics*. 101-103: 863-867.

[5] Chen, K. C., Wu, J. Y., Huang, C. C., Liang, Y. M.and Hwang, S. C. J.(2003). Decolorization of Azo Dye Using PVA-Immobilized Microorganisms. *Journal of Biotechnology* 101: 241-252.

[6] Long, Z., Huang, Y., Cai, Y., Cong, W. and Ouyang, F. (2004). Immobilization of Acidithiobacillus ferrooxidans by a PVA-boric Acid Method for Ferrous Sulphate Oxidation. *Process Biochemistry*. 39: 2129-2133.

[7] Mohd Zain N.A and Idris A. Determination of effective diffusion coefficients in a PVA-alginate matrix used for the immobilization of biocatalyst. KUSTEM 5th Annual Seminar. Proc. Pp. 218.-.220, 2006

[8] Hassan,C.M and Peppas, N.A (2000). Structure and Application of Poly (Vinyl Alcohol) Hydrogel Produced by Conventional Crosslinking or by Freezing/Thawing Methods. *Advanced in Polymer Science*. Springer-Verlag Berlin Heidelberg 153.