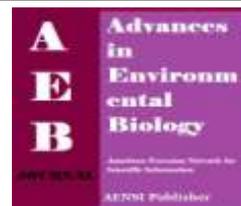




AENSI Journals

Advances in Environmental Biology

Journal home page: <http://www.aensiweb.com/aeb.html>



## Performance of Encapsulated Enzymes within Calcium Alginate-clay Beads in a Stirred Bioreactor for Biosugar Production

<sup>1</sup>Siti Noraida Abd Rahim, <sup>1</sup>Alawi Sulaiman, <sup>2</sup>Ku Halim Ku Hamid

<sup>1</sup>Tropical Agro-Biomass Research Group, Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia

<sup>2</sup>Faculty of Chemical Engineering, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia

### ARTICLE INFO

#### Article history:

Received 11 September 2013

Received in revised form 21

November 2013

Accepted 25 November 2013

Available online 3 December 2013

#### Key words:

Encapsulation; Enzymes; Tapioca slurry; Stirred bioreactor; Hydrolysis.

### ABSTRACT

Enzymatic hydrolysis of starch and cellulose from natural sources has a very big potential for commercial glucose production. In this study, the encapsulated of multi-enzymes (alpha-amylase, glucoamylase and cellulase) onto calcium alginate-clay beads were applied using a stirred bioreactor to hydrolyze tapioca slurry into glucose. The performance of encapsulated enzymes in terms of reusability, ideal pH, temperature and reaction time was investigated. The optimum pH and temperature for encapsulated enzymes were found at pH 6 and 50 °C, respectively. Under optimal conditions, the maximum conversion of glucose was obtained after 6 hrs. The encapsulated enzymes were able to retained 12% of its activity after five hydrolysis cycles. The result from the enzymes leaching test showed that the reduction of enzymes activity after each cycle was consistent with the residue of enzymes loading in the beads.

© 2013 AENSI Publisher All rights reserved.

**To Cite This Article:** Siti Noraida Abd Rahim, Alawi Sulaiman, Ku Halim Ku Hamid., Performance of Encapsulated Enzymes within Calcium Alginate-clay Beads in a Stirred Bioreactor for Biosugar Production. *Adv. Environ. Biol.*, 7(12), 3783-3788, 2013

## INTRODUCTION

Bioethanol is a renewable fuel which is becoming increasingly important due to depletion of oil reserves, rising crude oil prices and greenhouse effect. The bioethanol can be produced from the fermentation of sugars, starches or cellulose [10]. Cassava root is considered as an attractive raw material for bioethanol production because it contains high amount of starch and fibre [9]. Cassava root is also inexpensive and abundantly available in Malaysia and other Asian countries [4,12]. In the enzymatic hydrolysis process, alpha-amylase, glucoamylase and cellulase are commonly used for the glucose production. Alpha-amylase is usually used to hydrolyze starch by cleaving  $\alpha$ -(1 $\rightarrow$ 4) glycosidic bonds to produce maltodextrin while glucoamylase is able to hydrolyze  $\alpha$ -(1 $\rightarrow$ 4) and  $\alpha$ -(1 $\rightarrow$ 6) glycosidic bonds in starch to produce glucose [18]. For the cellulose or fibre hydrolysis, the cellulase can be used due to it has ability to attack  $\beta$ -(1 $\rightarrow$ 4) linkages in fibre to produce glucose [17].

Recently, the enzyme immobilization technology is widely applied in food and biotechnology, biomedicine and also analytical chemistry. The immobilized enzyme technology offers many advantages over free enzyme technology such as easy separation from the reaction media, easy recovery and reuse of the enzymes by repeatedly or continuously [19]. The enzymes can be immobilized onto various supporting materials by adsorption, covalent binding, encapsulation, entrapment and cross-linking [5]. Each technique has advantages and disadvantages, however, the encapsulation of enzyme within alginate beads is commonly used due to easy for formulation, mild gelation conditions, non-toxic, biocompatibility, low cost and resistance to microbial attack [20,16]. Unfortunately, the alginate beads have a low mechanical strength, large pore size and leakage of enzymes. To improve these properties, the surface of alginate beads is coated with cross-linked biopolymers [6,8]. Besides that, the alginate also can be blended with clay to solve this problem [1]. Gülay and Sanlı-Mohamed reported that the modification of alginate beads gives higher reusability and prevent the leakage of enzymes. Therefore, in this study, the alginate is blended with local kaolinite clay to obtain the higher performance of encapsulated enzymes.

The immobilized enzyme bioreactor can be designed as stirred, packed, fluidized and membrane reactor

**Corresponding Author:** Alawi Sulaiman, Tropical Agro-Biomass Research Group, Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia  
E-mail: [dr\\_alawi@uitm.edu.my](mailto:dr_alawi@uitm.edu.my)

that can be operated either in batch or continuous system [21]. The stirred bioreactor is a commonly employed in bench scale and industrial scale application. This bioreactor is a versatile and easy to operate. Besides, the stirred bioreactor has some advantages compared to packed bed bioreactor where it provides lower construction costs and the efficiency of stirring will eliminate the presence of concentration and temperature gradient [15]. Thus, the stirred bioreactor can be expected to be effective for the hydrolysis of tapioca slurry into glucose by using encapsulated enzymes.

The present study demonstrates the performance of enzymes encapsulation onto calcium alginate-clay beads in a stirred bioreactor. The factors that affected the encapsulation such as temperature, pH and reaction time were investigated in order to obtain the optimum condition for hydrolysis process. Besides that, the reusability and leaching of encapsulated enzymes were also studied.

## MATERIALS AND METHODS

### 1. Materials:

Alpha-amylase from *Bacillus subtilis*, glucoamylase from *Rhizopus niveus* Lyophilized and cellulase from *Aspergillus niger* were purchased from MP Biomedicals, United States. All other chemicals reagent used were analytical grade.

### 2. Preparation of tapioca slurry:

Cassava root was purchased from local market as a substrate. It was washed free of dirt, hand peeled and sliced to small pieces. The chips were dried in oven at 65 °C for 24 hr to remove moisture and then ground into powder. 1 % (w/v) of tapioca slurry was prepared using citrate phosphate buffer solution and then boiled in the water bath with continuously agitated for 1 hr to gelatinize the starch before further use.

### 3. Preparation of clay powder:

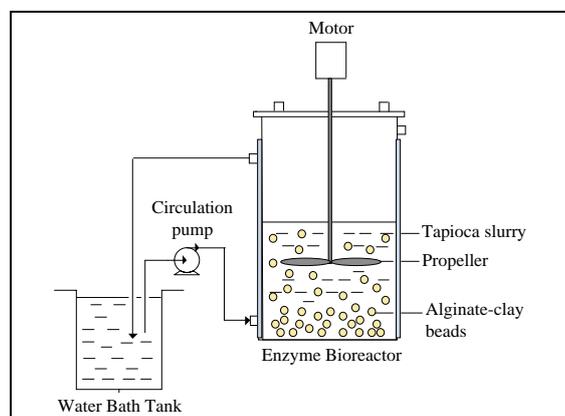
The local kaolinite clay was obtained from Sg. Sayong, Perak, Malaysia was dried at 90°C for 24 hr, ground into fine powder and lastly sieved through 150 µm mesh before further use.

### 4. Encapsulation of enzymes within calcium alginate-clay beads:

The encapsulation of enzymes onto alginate-clay beads was carried out by following method reported by Adzmi *et al.* (2012). 2.5 g (2.5 % w/v) of previously prepared kaolinite clay was dissolved into 100 mL of citrate phosphate buffer solution and stirred for 1 hr at room temperature. Then, 2.5 g of alginate powder was added to the clay solution and stirred for 4 hr. Next, 2.5 mL of glycerol was added to the alginate-clay solution. 1 mL of each enzyme solution (1 mg solid/mL of alpha-amylase, glucoamylase and cellulase) was mixed with 12 mL of alginate-clay solution which produced the final clay concentration 2 % w/v. The mixture solution was stirred thoroughly to ensure complete mixing and dropped into 0.2 M CaCl<sub>2</sub> solution by using syringe. After 3 hr of hardening, the beads were collected and then washed with buffer solution several times to remove any unbound enzymes. Finally, the beads were stored at 4 °C until use.

### 5. Bioreactor set-up:

The schematic diagram of enzyme bioreactor to hydrolyzed tapioca slurry into glucose is illustrated in Fig 1. The bioreactor is made from glass where the size of bioreactor is 27 cm in height and 10 cm in inner diameter (with a total volume of 2.1 L). The water jacket is supplied in bioreactor to control the reaction temperature by circulating the water from the water bath tank. It is also equipped with propeller where it was mounted on the shaft that is connected to the agitator motor (IKA, RW 20 Digital, Germany).



**Fig. 1:** Schematic diagram of stirred tank bioreactor

#### 6. Determination of enzymes activity:

The hydrolysis of tapioca slurry catalyzed by encapsulated enzymes within calcium alginate-clay beads was carried out in stirred tank bioreactor. 27 g of alginate-clay beads was added to 1000 mL of 1 % (w/v) of tapioca slurry. The reaction mixture was agitated at 120 rpm. After complete the reaction, the activity of enzymes was measured by using spectrophotometer at 540 nm with 3,5-dinitrosalicylic acid (DNS) as an indicator (Bernfeld, 1955). Theoretically one unit of alpha-amylase activity is defined as the quantity of enzyme that releases 1 mg of reducing sugar as maltose per minute at pH 6.6 and 30 °C. One unit of glucoamylase is expressed as the amount of enzyme releasing 10 mg of reducing sugar (glucose) per minute at pH 4.5 and 40 °C. One unit of cellulase activity is liberated as the amount of enzyme that produces 1  $\mu$ mole of reducing sugar (glucose) at pH 5 and 37 °C in 1 min.

#### 7. Effect of temperature and pH:

The effect of temperature on the activity of encapsulated enzymes within alginate-clay beads was determined at various temperatures (30 – 70 °C) by fixing other conditions. Meanwhile, the effect of pH on the activity of encapsulated was measured at different pH (3 – 7) by using citrate phosphate buffer solution. The relative activity was calculated in percentage with reference to the activity of the optimum temperature or pH (100 %).

#### 8. Effect of reaction time:

The effect of reaction time for the hydrolysis process was investigated by varying reaction periods from 0.5 – 7 hr. The product produced was measured based on the glucose content by using DNS method as described above.

#### 9. Reusability:

The reusability of encapsulated enzymes was evaluated by repeated enzymatic hydrolysis several times. The hydrolysis reaction was conducted at optimum condition. After each cycle of hydrolysis, the beads were separated and washed with citrate phosphate buffer. Next, the fresh tapioca slurry solution was added to start the next round. The activity of each round was compared with the first run which was defined as 100 %.

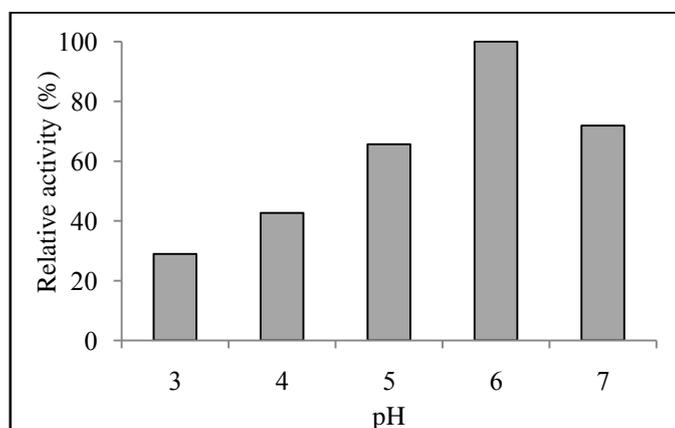
#### 10. Enzymes leaching study:

After each batch of recycling process, the amount of enzymes leached into bulk solution was determined by Lowry's procedure modified by Hartree[7] to measure the change of enzymes loading efficiency.

## RESULTS AND DISCUSSION

#### 1. Effect of pH:

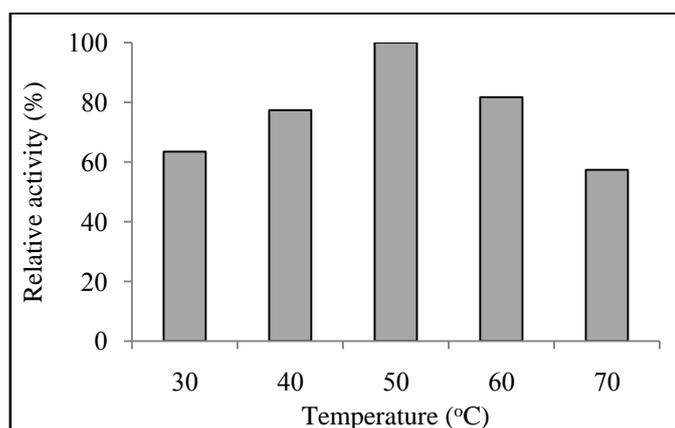
pH is one of the major parameters that can influence the enzyme activity in the reaction mixture. In this study, the encapsulated of enzymes within calcium alginate-clay beads were assayed at different pH ranging from 3 to 7. The relative activity of the encapsulated enzymes was depicted in Fig. 2. From the figure, the activity encapsulated enzymes increased from pH 3 to 6 and then decreased at pH 7. Therefore, it can be deduced that the optimum pH for the encapsulated enzymes was at pH 6. According to Anwar *et al.* [2], the surfaces of the beads which localize the enzymes have a cationic and anionic nature that will produce microenvironment charges and then it will affect the nature of active enzyme. Thus, the increasing or decreasing of pH from pH 6 will reduce the activity of enzymes due to charge acquired by the support.



**Fig. 2:** Effect of pH on the activity of encapsulated enzymes

### 2. Effect of temperature:

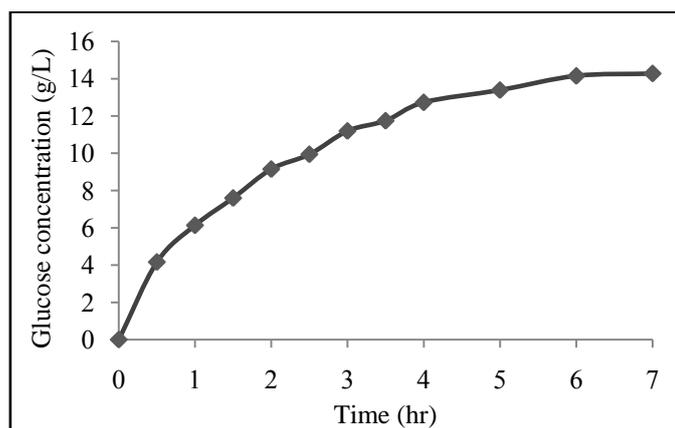
The changes in the reaction temperature can affect the activity and stability of the encapsulated enzymes. In this study, the effect of temperature on the activity of encapsulated enzymes was investigated at various temperatures in the range of 30 to 70 °C. As shown in Fig. 3, the maximum activity of encapsulated enzymes that gives 100 % of relative activity was observed at 50 °C. From the figure, there was a slight increased in the enzymes activity from temperature 30 to 50 °C and then a gradual decreased from 50 to 70 °C. The enzymes activity decreased above 50 °C which can be explained due to the vibration and movement of the enzyme molecule which can affect the hydrogen and other bonding in the enzymes structure. Therefore, the enzymes molecule will unfold and alter its tertiary and quaternary structure where it will reduce the catalytic power of enzymes and lastly it will tend to the denaturation of enzymes [13,11].



**Fig. 3:** Effect of temperature on the activity of encapsulated enzymes

### 3. Effect of reaction time:

The time course is another important parameter to determine the necessary time in the reaction process in order to obtain a good yield and minimize the process cost [13,14]. In this study, the effect of retention time on the activity of encapsulated enzymes was determined by varying the time course from 0.5 to 7 hr at optimum conditions. The profile of glucose production at various time intervals is presented in Fig. 4. From the figure, the glucose concentration increased with increasing reaction time. This is because the reaction time is required for the substrate to penetrate into the beads and reach the active site of enzymes so that the substrate can be converted into glucose [2]. After 6 hr, the glucose concentration was relatively constant due to the reaction has achieved the equilibrium state.

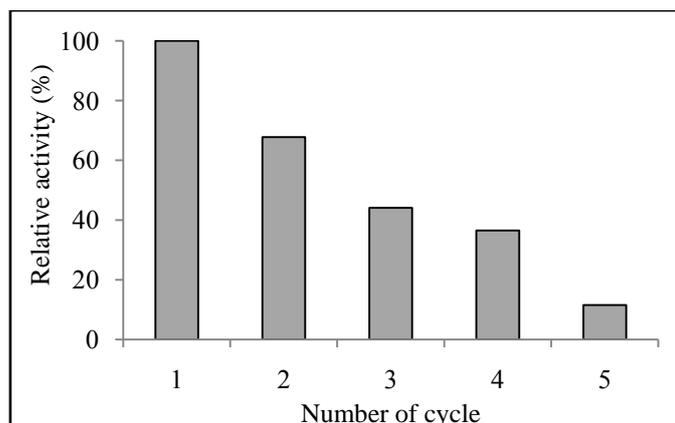


**Fig. 4:** Effect of reaction time on the activity of encapsulated enzymes

### 4. Reusability:

The reusability of encapsulated enzymes was determined by using recovered enzymes from different cycles. The reaction was conducted under optimum condition as obtained above and the reusability pattern of encapsulated enzymes is depicted in Fig. 5. It was observed that the encapsulated enzymes retained 68, 44 and 37 % of its activity for second, third and fourth cycles, respectively. After the fifth cycles, the remaining activity

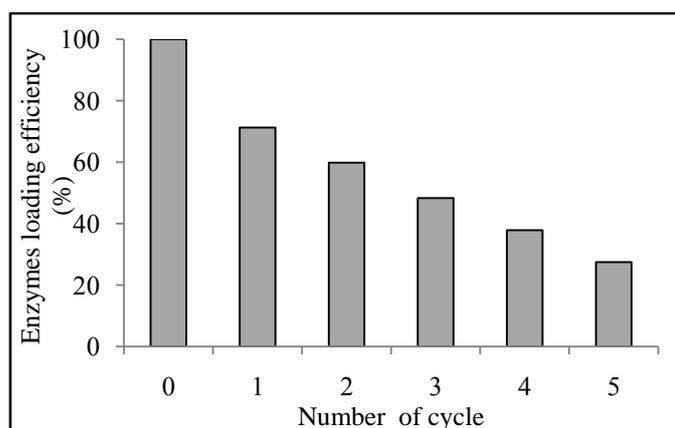
was 12 %. The decrease in activity may due to the leakage of enzymes from the beads along with the process and washing steps at the end of each cycle [2].



**Fig. 5:** Reusability of encapsulated enzymes

#### 5. Enzymes leaching behavior:

The leaching of enzymes from calcium alginate-clay beads was examined by measuring enzymes content in the solution after each reaction batch (Fig. 6). The enzymes were encapsulated onto alginate-clay beads lost more than 21 % of its initial amount after the first cycle. After fifth cycles, the amount of enzymes that still available in the beads was only 27 %. The decreasing of enzymes loading trend was in agreement with the result of reusability where the activity of enzymes was also decreased after each cycle. Therefore, it was proved that the leaching of enzymes from the beads occurred during the hydrolysis process.



**Fig. 6:** Enzymes loading efficiency in the beads after recycling process

#### Conclusion:

In the current study, alpha-amylase, glucoamylase and cellulase was immobilized onto calcium alginate-clay beads by using encapsulation technique. The encapsulated enzymes were successfully carried out into a stirred bioreactor to hydrolyzed tapioca slurry into glucose. The optimal conditions for enzymes encapsulation including pH, temperature and reaction time were examined. The encapsulated enzymes showed optimum activity at pH 6 and 50 °C, respectively. As the purpose of encapsulation, the reusability of enzymes was also observed. The work has demonstrated that encapsulated enzymes can be used at least five hydrolysis cycles. Thus, the application of encapsulated enzymes in the stirred bioreactor still has a potential for industrial application.

#### ACKNOWLEDGEMENT

The authors are grateful for the financial support provided by Universiti Teknologi MARA (UiTM) Excellent Funds (600-RMI/ST/DANA 5/3/Dst 495/2011 & 475/2011) and Ministry of Higher Education, Fundamental Research Grant Scheme (600-RMI/ST/FRGS 5/3 Fst 2/4/2010).

## REFERENCES

- [1] Adzmi, F., S. Meon, M.H. Musa and N.A. Yusuf, 2012. Preparation, characterisation and viability of encapsulated *Trichoderma harzianum* UPM40 in alginate-montmorillonite clay. *Journal of Microencapsulation*, 29(3): 205-210.
- [2] Anwar, A., S. Ali, U. Qader, A. Raiz, S. Iqbal and A. Azhar, 2009. Calcium Alginate: A Support Material for Immobilization of Proteases from Newly Isolated Strain of *Bacillus subtilis*. *World Applied Sciences Journal*, 7(10): 1281-1286.
- [3] Bernfeld, P., 1955. Amylases alpha and beta. *Methods in Enzymology*, pp: 149-158.
- [4] García, N.L., L. Famá, A. Dufresne, M. Aranguren and S. Goyanes, 2009. A comparison between the physico-chemical properties of tuber and cereal starches. *Food Research International*, 42(8): 976-982.
- [5] Gopinath, S. and S. Sugunan, 2007. Enzymes immobilized on montmorillonite K 10: Effect of adsorption and grafting on the surface properties and the enzyme activity. *Applied Clay Science*, 35(1-2): 67-75.
- [6] Gülay, S. and G. Şanlı-Mohamed, 2012. Immobilization of thermoalkalophilic recombinant esterase enzyme by entrapment in silicate coated Ca-alginate beads and its hydrolytic properties. *International Journal of Biological Macromolecules*, 50(3): 545-551.
- [7] Hartree, E.F., 1972. Determination of protein: A modification of the lowry method that gives a linear photometric response. *Analytical Biochemistry*, 48(2): 422-427.
- [8] Haufová, P., Z. Knejzlík, J. Hanuš, A. Zdražil and F. Štěpánek, 2011. Reversible buckling and diffusion properties of silica-coated hydrogel particles. *Journal of Colloid and Interface Science*, 357(1): 109-115.
- [9] Kearsley, M.W. and S.Z. Dziedzic, 1995. *Starch Hydrolysis Products and Their Derivatives*, Blackie Academic & Professional, Springer.
- [10] Kumar, A., L.K. Singh and S. Ghosh, 2009. Bioconversion of lignocellulosic fraction of water-hyacinth (*Eichhornia crassipes*) hemicellulose acid hydrolysate to ethanol by *Pichia stipitis*. *Bioresource Technology*, 100(13): 3293-3297.
- [11] Kumar, V., B. Joseph, P.W. Ramteke, A. Mani and F. Jahan, 2011. Cold active lipase catalyzed production of biodiesel from *Jatropha* oil in a solvent free system. *Journal of Chemical and Pharmaceutical Research*, 3(2): 226-233.
- [12] Lee, S.Y., H. Chen and M.A. Hanna, 2008. Preparation and characterization of tapioca starch-poly(lactic acid) nanocomposite foams by melt intercalation based on clay type. *Industrial Crops and Products*, 28(1): 95-106.
- [13] Mat Radzi, S., M. Basri, A.B. Salleh, A. Ariff, R. Mohammad, M.B. Abdul Rahman and Raja Abdul R.N.Z. Rahman, 2005. High performance enzymatic synthesis of oleyl oleate using immobilised lipase from *Candida antarctica*. *Electronic Journal of Biotechnology*, 8(3): 291-298.
- [14] Muhamad, S.K., S. Mat Radzi, S.S. Othman, M.B. Abdul Rahman and H. Mohd Noor, 2010. Optimization of lipase-catalyzed synthesis of flavour esters in solvent free system. *Journal of Fundamental Sciences*, 6(1): 31-36.
- [15] Murty, V., J. Bhat and P. Muniswaran, 2002. Hydrolysis of oils by using immobilized lipase enzyme: A review. *Biotechnology and Bioprocess Engineering*, 7(2): 57-66.
- [16] Quiroga, E., C.O. Illanes, N.A. Ochoa and S. Barberis, 2011. Performance improvement of araujain, a cystein phytoprotease, by immobilization within calcium alginate beads. *Process Biochemistry*, 46(4): 1029-1034.
- [17] Sukumaran, R.K., R.R. Singhanian and A. Pandey, 2005. Microbial cellulases - Production, applications and challenges. *Journal of Scientific & Industrial Research*, 64: 832-844.
- [18] van der Maarel, M.J.E.C., van der B. Veen, J.C.M. Uitdehaag, H. Leemhuis and L. Dijkhuizen, 2002. Properties and applications of starch-converting enzymes of the  $\alpha$ -amylase family. *Journal of Biotechnology*, 94(2): 137-155.
- [19] Varavinit, S., N. Chaokasem and S. Shobsngob, 2001. Covalent immobilization of a glucoamylase to bagasse dialdehyde cellulose. *World Journal of Microbiology and Biotechnology*, 17(7): 721-725.
- [20] Won, K., S. Kim, Kim, P. Kwang-Je, W. Hong and S.J. Moon, 2005. Optimization of lipase entrapment in Ca-alginate gel beads. *Process Biochemistry*, 40(6): 2149-2154.
- [21] Zhang, C. and X.H. Xing, 2011. 2.23 - Enzyme Bioreactors. *Comprehensive Biotechnology (Second Edition)*. Burlington: Academic Press.