Asian Journal of Biochemistry

ISSN 1815-9923

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Biochemistry 3 (3): 169-175, 2008
ISSN 1815-9923
© 2008 Academic Journals Inc.

Immobilization of α-Amylase from Acha (*Digiteria exilis*) on Different Cellulose Fibre Materials

E.C. Egwim and O.B. Oloyede
Department of Biochemistry, University of Ilorin, P.M.B. 1515, Ilorin, Kwara State, Nigeria

Abstract: Alpha amylase was obtained from Acha (*D. exilis*) sprouted for 96 h and immobilized on palm wood chips, coconut and cotton wood fibers according to standard procedures. Palm wood chips maintained enzyme activity up to 25 h and still retained about 7×10^{-2} mg glucose mL min^{-1} of residual activity while coconut and cotton wool lost all activity by 12 h. Enzyme half-life improved by 857.0, 21.4 and 28.6 folds using palm wood chips, coconut and cotton wool fibre as carriers, respectively. Palm wood clip improved V_{max} and catalytic efficiency by 177 and 163%, respectively. The results concludes that palm wood chip can be used to immobilize α-amylase from *D. exilis* for several cycles of reuse.

Key words: Immobilization, cellulose fibre, α-amylase, half-life, *D. exilis*

INTRODUCTION

α-Amylase has wide applications in the food industry. For instance, α-amylase is employed in the milling and baking industry (Ruberthaler *et al*., 1965; Anderson and Watson, 1982) to hydrolyse starch to smaller carbohydrates, so as to reduce the dough viscosity and increase sugar levels, prolong freshness, improve softness and crust quality. Similarly, in the brewery and beverage industries, (Shallenberger, 1990; Yeshajahu, 1991), α-amylase is employed in mash thinning, improves runoff of wort and the general quality of the end product. The sweetener and confectionery industries (Johnson, 1976; Wardrip, 1971; Alvin *et al*., 2002) have used α-amylase to control the ratios of different saccharides to achieve specific product qualities. It is not surprising therefore that study into various sources and developments of high quality α-amylase for industrial processes have attracted interests from researchers. Sprouting cereals (Deatherage *et al*., 1955; Finney *el al*., 1972) appear to be one of the popular sources of industrial amylase for some developing economies. Solomon *et al*. (1987) developed standard malt and amylase for the brewery industry from sorghum while Okon and Uwaifo (1985) have demonstrated the malting potentials of different sorghum varieties. It has recently been established that α-amylase from acha (*Digiteria exilis*) is a superior alternative to sorghum α-amylase for industrial processes (Egwim and Oloyede, 2004). Acha is an unpopular cereal that is indigenous to the West African Coast. It grows with reasonable yields in areas of low rainfall and poor sandy or iron stone soils. Farmer believes acha will grow where no other crop will grow (Amonum, 1990). Acha is not yet a popular staple food, but it grows extensively in the northern and middle belt of West Africa, where the soil is not very suitable for the growth of some more popular cereals like maize, sorghum and millet (Okon, 1998).

Currently, industrial enzymes are subjected to immobilization because it offers both technical and economic advantages. Immobilization offers some operational advantages over free enzyme; such as choice of batch or continuous process, rapid termination of reactions, controlled engineering designs (Brahim *et al*., 2002). By careful selection of the matrix, it is also possible to vary the nature of the immobilized derivatives in order to improve enzyme activity and stability and also to enable easier

Corresponding Author: E.C. Egwim, Department of Biochemistry, Federal University of Technology, P.M.B. 65, Minna, Niger State, Nigeria

storage and handling (Mohy *et al*., 2000). The immobilized enzyme can also be used for continuous processes in either fixed or fluidized bed reactors. In this case, it is possible to use higher enzyme dosage per volume of reactor than in the soluble enzyme process. This contributes to high reaction rates and consequently, small reactor size (Bassetti *et al*., 1997). These technical advantages allow a reduction in the operational and capital costs in industrial processes (Zanin and Moraes, 1994).

There are several carriers or support materials available for enzyme immobilization. These include prefabricated and naturally occurring carrier materials (Johnson, 1979; Wingard *et al*., 1979; Villenouve *et al*., 2000; Kim *et al*., 2005). The use of cellulose derivative as support materials for enzyme immobilization is wide spread. For instance glucoamylase has been bound to halogenacetyl cellulose (Maeda and Suzuki, 1972); glucose isomerase and other industrial enzymes have been immobilized on cellulose acetate fibre (Krenla and Hinko, 1979; Ogunbayo and Bello, 1986) successfully immobilized lactase on palm wood chips for the treatment of whey while Antonio *et al*. (2003) have employed coconut fibre for the immobilization of polyphenol oxidase.

The search for improved immobilized enzymes for technical and economic reasons is currently on the increase. The present study is aimed at immobilization of α-amylase obtained from Acha (*Digitera exilis*), on cellulose carriers like palm wood, coconut and cotton wool fibres.

MATERIALS AND METHODS

All chemicals used for the present study were of analytical grade (Analar) being products of British Drug House (BDH) Chemical Limited Role, England. Acha was obtained from National Cereal Research Institute (NCRI) Badeggi, Niger state, Nigeria.

Palm wood was cut from the bush around while coconut fibre was collected from the waste deposits of coconut sellers in the open market, cotton wool was purchased from the open market.

Preparation and Assay of Crude Amylase Enzyme

Acha was spouted for 96 h and milled in pre chilled 0.05 M citrate buffer, pH 6.0, the resulting homogenate was centrifuged at 10,000×g for 10 min. The supernatant contain the crude α-amylase stored in buffer. α-Amylase activity was assayed by modification of the method described by Gil-Jin *et al*. (1997). Aliquot (0.1 mL) of crude enzyme was pipetted into separate test tube and 0.9 mL of 2% soluble starch was added and incubated in a shaking water bath at 50°C for 30 min. The reaction was stopped by adding DNSA (Dinitro Salicylic Acid) reagent and boiled for at least 3 min for colour development. Absorbance was read at 550 nM against reagent blank. Enzyme activity was thereafter was computed from a standard glucose curve (0.1-1 mM glucose mL^{-1}).

Immobilization of Enzyme

Immobilization carriers used are palm wood (*Raphia hooker*), coconut (*Cocus nucifera*) and cotton wool fibres. Palm wood chip was cut to approximately 1×1×1 cm from the woody portions of the palm fronds of *Raphia hooker*. Coconut fibre was obtained from the fibrous epicarp of coconut fruit, the coconut fibre was cut to smaller shreds for easy pakaging.

The fibres were delignified by boiling in hot water several times until the resulting water was clear. The fibres were air dried and stored. Immobilization of crude α-amylase on the different cellulose fibres followed the method of Ogunbayo and Bello (1986). Dried palm wood chip(250 pieces) were soaked in 400 mL of chilled crude α-amylase for at least 4 h while maintaining cold condition. The excess crude enzyme was drained and the chips were washed and dried for 30 min. Gum arabic (50%) was prepared and used as binder. The binder solution was poured layer by layer into the dried chips to have a thorough mixing with the chips. The chips were then left to dry for at least 5 h. This procedure was

repeated for coconut and cotton wool fibres. The immobilized enzyme was packed into a glass column reactor (60×6 cm), the reactor was maintained at 45°C and 2% starch solution was run through the packed column. The substrate and product valves were adjusted to a steady flow rate. The product of starch hydrolysis was collected and the total reducing sugar was assayed using DNSA reagent and expressed as enzyme activity.

Enzyme Activity

Enzyme activity in the present study is defined as the amount of enzyme required to liberate a unit of glucose per minute. (mM glucose min^{-1}) at reaction condition.

Decay Constant (κ), Half-Life ($T_{1/2}$) and Kinetic Parameters

κ and $t_{1/2}$ of free and immobilized enzymes were computed following the method described by Fabricio *et al.* (2004).

Kinetic parameters were determined by varying the substrate concentration (0.1-1%) and V_{max} and K_m were extrapolated from Hane's Plot ([s]/vo vs [s])

RESULT AND DISCUSSION

The result of the activity-time profile of α-amylase immobilized an difference cellulose fibre supports is shown in Fig. 1. Enzymes activity in coconut fibre showed an initial increase in activity within the first 4 h, activity dropped sharply by the 8 h and then reduced to zero. The cotton wool maintained enzyme activity for up to 10 h and dropped sharply to zero by the 12 h, while the palm wood chip maintained fairly steady enzyme activity up to 15 h and there after activity reduced gradually to a residual activity of about 53×10^{-4} mM glucose min^{-1} at 25 h. The present observation indicates that palm wood chip may be the cellulose fibre of choice amongst the three for immobilizing α-amylase from *D. exilis*.

Enzyme molecules may have leached from coconut and cotton wool fibres since the fibres are in tread and shred forms. While palm wood chips may have created a microenvironment within the matrix of the palm wood chip cut into cubes. Ogunbayo and Bello (1986) have successfully immobilized lactase on palm wood chips and achieved up to 50% stability while Antonio *et al.* (2003) have studied the effectiveness of polyphenol oxidase naturally immobilized on coconut fibre (*Cocus nucifera*). The present observation indicates that palm wood chips are the most suitable carrier for α-amylase immobilization. Indeed, the immobilization confers stability on the enzyme as portrayed by the extended half-life (Table 1).

- The extended half-life as shown by immobilization on palm wood chips show its superiority over coconut and cotton wood fibre.
- The half-life of crud α-amylase immobilized on palm wood chip is 40 and 30 times greater than that of coconut and cotton wood fibre, respectively. This observation is in line with the finding of Bassetthi *et al.* (1997) who had earlier reported an increase in invertase half-life when immobilized.

Table 1: Decay constant and half-life of α-amylase immobilized on different cellulose fibre supports

Support	Decay constant (h)	Half-life (h)
Free enzyme	16	0.14
Palm wood chip	5.8×10^{-3}	120.00
Coconut fibre	23×10^{-3}	3.00
Cotton wool	17.8×10^{-3}	4.00

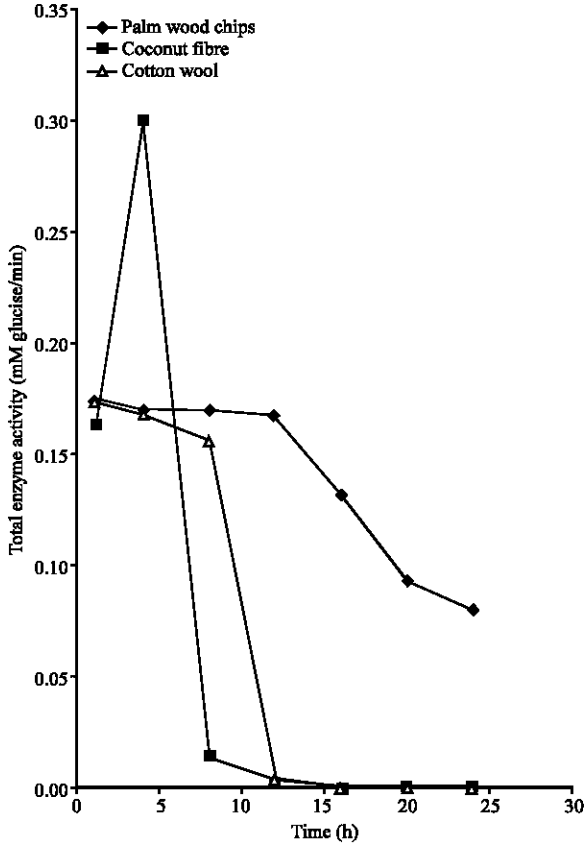

Fig. 1: Activity-time profile of alpha amylase from *D. exilis* immobilized on different supports

Fabricio *et al.* (2004) have shown that the operational stability of lipase immobilization on chitin is quite favourable. While Adriano *et al.* (2005) have shown that immobilization of G acylase improves operational stability. It seems obvious therefore that one of the main targets in immobilization is to improve enzyme stability and half-life for possible reuse. The present study has achieved enzyme stability by immobilization on palm wood chip, coconut and cotton wool fibres.

- Immobilization on palm wood chips extends α-amylase half-life from 0.14 h (free enzyme) to 120 h, which is about 857 folds increase, while coconut and cotton wool fibres achieved 21.4 and 28.7 folds extension of enzyme half-life.
- Varavinit *et al.* (2002) achieved an extension of α-amylase half-life (10 cycles reuse) by immobilization on cellulose fibre from bagasse. Torchilin *et al.* (1978, 1979) have shown that stability of immobilized enzymes is due to intramolecular linkage conferred by the carriers and depends on the length of such linkage. This may explain why palm wool chip is a better carrier in the present study.

The result of varying substrate concentration on the activity of α-amylase immobilized on palm wood chips is shown in Fig. 2. The result shows a normal Mechealis-Menten's pattern. This observation suggests that immobilization has not altered the reactive site of the enzyme.

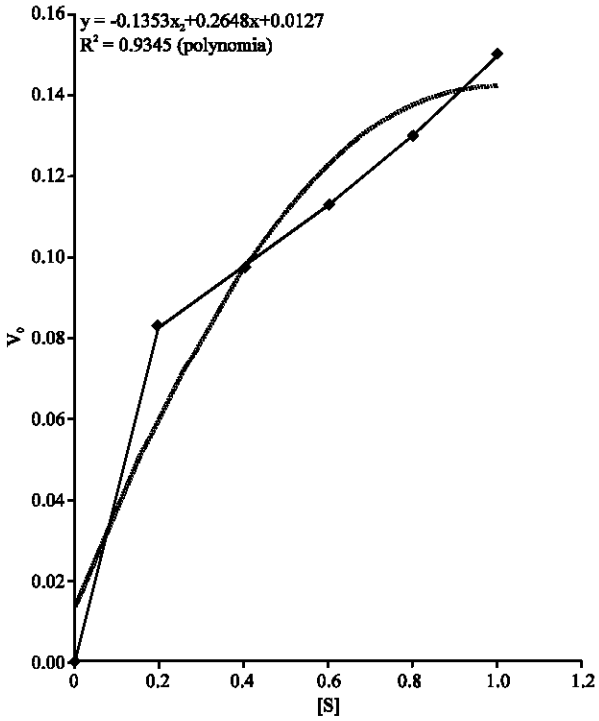

Fig. 2: Effect of substrate concentration on alpha-amylase activity immobilized on palm wood chips

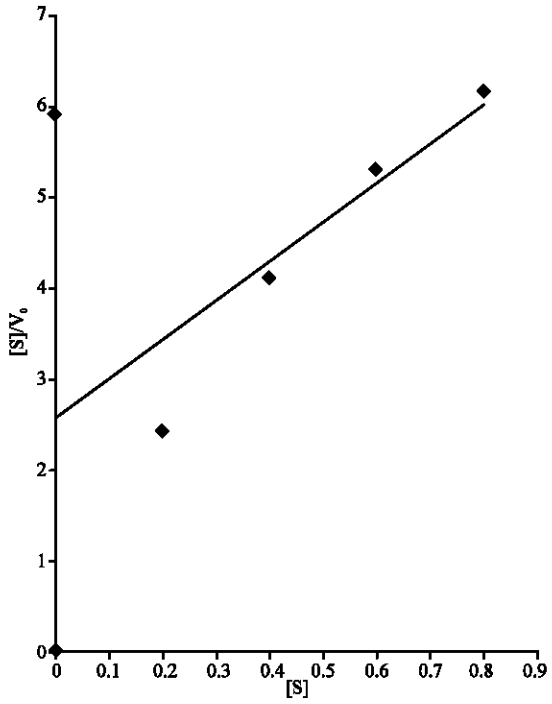

Fig. 3: Hans plot to determine Km and Vmax of alpha-amylase from *D. exilise*

Table 2: Kinetic parameters of free and immobilized α-amylase from *D. exlis* immobilized on palm wood chips.

Parameters	$V_{Max}(sec^{-1})$	$K_M(mM)$	Efficiency ($V_{Max}/K_{M.}$)
Free enzyme	6.66×10^{-4}	2.725×10^{-3}	24.4
Immobilized	11.81×10^{-4}	3.028×10^{-3}	39.0

Hane's plot is shown in Fig. 3 to obtain the enzyme kinetic parameters. Table 2 compares the kinetic parameter of free and immobilized α-amylase on palm wood chips. The result shows that immobilization improves both the V_{max} and catalytic efficiency by 177 and 163%, respectively. This observation suggests that the interaction between the carrier and the enzyme exposed the enzymes active site to a better orientation for maximum turn over.

Similarly, Cellulose frbres can be used to immobilize α-amylase from *D. exilis* for better application. The study there concludes that immobilization improves stability, enzyme half-life and maximizes turn over. Palm wool chips (1×1×1 cm) are better carrier for immobilizing α-amylase from *D. exilis* than coconut and cotton wool fibres.

REFERENCES

Adriano, W.S., E.H.C. Filho, J.A. Silva, R.L.C. Giordano and L.R.B.C. Goncalves, 2005. Stabilization of penicillin g acyclase by immobilization on glutaraldehyde-activated chitosan. Braz. J. Chem. Engr., 22: 1-14.

Alvin, S.A., K. Karthik and L. Xiao-xing, 2002. Textural and sensory properties of α-amylase Treated Poi. Stored at 4°C. J. Food Process. Preserv., 26: 1-10.

Amonum, J.I., 1990. The Performance Of Acha (*Digiteria exilis*). B.Sc. Thesis, University of Jos.

Anderson, R.A. and S.A. Watson, 1982. He Corn Milling Industry. In: Hand Book of Processing and Utilization in Agriculture, Wolf, I.A. (Ed.). CRC press, Boca Raton, Florida, 2: 31-61.

Antonio, W.O.L., S.B. Pushkar and M.R. Eduardo and A. Lucio, 2003. Application of a new continuous flow spectrophotometric method for the characterization polyphenol oxidaze. J. Food Process. Preserv., 1: 10-18.

Bassetti, F.J., F.F. Moraes and G.M. Zanin, 1997. Hydrolise enzimative da sacarose em reactor de leito fixo e fluidizado. Anais xxiv congresso Brasil; Eiro d sistemas paraticulados (ENEMP), pp: 705-710.

Brahim, S., D. Narinesingh and A. Guiseppi-Elie, 2002. Kinetics of glucose oxidase immobilized in P(HEMA)-hydrogel microspheres in a packed-bed reactor. J. Mol. Catalysis Enzymatic, 18: 69-80.

Deatherage, W.L., M.M. MacMasters and E.C. Rist, 1955. A partial survey of any lose content in starch from domestic and foreign varieties of corn, wheat and sorghum and from some other starch-bread-plants. Trans. Am. Assoc. Cereal Chem., 13: 31-42.

Egwim, E.C. and O.B. Oloyede, 2004. Comparison of alpha-amylase activity in some sprouting Nigeria cereals. Brokemistry, 18: 15-20.

Fabricio, M.G., B.P. Ernandes and F.D. Heizor, 2004. Immobilization of lipase on chitin and its use in nonconventional brocatahysis. Biomacromolecules, 5: 17-23.

Finny, K.F., M.O. Shogren, Y. Pomeranz and L.C. Bolte, 1972. Cereal malts in bread making. Bakers Deg., 46: 36-38, 55.

Gil-Jin, K., M.K. Jeon, W. Kim and K. Park, 1997. Immobilization of thermostable maltogenic anylase from *Bacillus strearothermophilus* for continous production of branched oligosacharide. J. Agric. Food Chem., 45: 4168-4172.

Johnson, J.C., 1976. Specialized sugars for the food industries. Noyes Date Corporation, Partk Ridge, New Jersey.

Kim, B.C., S. Nair, J. Kim, J.H. Kwak, T.W. Grate, S.H. Kim and M.B. Gu, 2005. Preparation of biocatalytic nanofirbre with high activity and stability via enzyme aggregate coating on polymer nanofirbres. Nanotechnology, 16: S382-S388.

Krenla, H.H. and Hinko, 1979. Second international congress on engineering and food. August Helsinki University of Technology.

Maeda, H. and H. Suzuki, 1972. Preparation and properties of gluloamylase bound to halogenacetyl cellulose. Arch. Biol. Cliem., 36: 1581-1593.

Mohy, E.M.S., C.G.P.H. Schroen, A.E.M. Jassen, D.G. Mita and J. Tramper, 2000. Immobilization of penicillin G. Acylase on chemically grafted nylon particles. J. Mol. Catalisis: Enzymatics, 10: 445-451.

Ogunbayo, A.O. and R.A. Bello, 1986. Immobilization of lactase on palm wood chips for lactose Hydrolysis. J. Nig. Soc. Chem. Eng., 5: 1-6.

Okon, E.U. and A.O. Uwaifo, 1985. Evaluation of malting sorghum. The malthing potentials of Nigerian varieties of sorghum. Brewers Dig., 60: 24-27.

Okon, P., 1998. Cereal Grains. In: Nutritional Qualities of Plants Foods, Osagie, A. and O. Eka (Eds.). Post Harvest Research unit Publication, Benin City Nigeria, pp: 38.

Ruberthaler, G.L., K.F. Fininey and Y. Pomeran, 1965. Effect on loaf volume and bread characteristics of alpha-amylase from cereal, fungal and bacterial sources. Food Technol., 19: 239-241.

Shallenberger, R.S., 1990. Introduction to sweeteners chemistry. Cereal Foods World, 35: 377-381.

Solomon, B.O., S.K. Layokem and S. Oladimeji, 1987. Development of malt for the Nigeria brewing industries. Malting of Sorghum Grain J. Nig. Soc. Chem. Eng., 6: 60-64.

Torchilin, V.P., A.V. Maksimenko, H.N. Smirnov, I.V. Berezin, A.M. Klibanov and K. Martinek, 1978. The principles of enzyma stabilization the effect of intramolecular cross-linkages on thermostability of enzymes. Brochimica et Biophysica Acta, 522: 277-283.

Torchilin, V.P., A.V. Malcsimenko, V.N. Smirnow, I.V. Berezin and K. Marchiek, 1979. Principle of enzyme stabilization v. The possibility of enzyme self stabilization under the action of potentially reversible intermolecular cross-linkages of different length. Biochmica et Biophysica Acta, 568: 1-10.

Varavinit, S., N. Chaokasem and S. Shobsrigob, 2002. Immobilization of a thermostable alpha-amylase. Science Asia, 28: 247-251.

Villenouve, P., J.M. Muderhwa, J.M. Graille and M.J. Hass, 2000. J. Mol. Catal. B. Enzymology, 4-6: 113-148.

Wardrip, E.K., 1971. High fructose corn syrup. Food Technol., 25: 47.

Wingard, L.B., J.E. Katchalshi-katzir and S.C. Glod, 1979. Enzymatic Technology. In: Applied Biochem. Bioeng. Academic press, New York, 2: 122.

Yeshajahu, P., 1991. Functional Properties of Food Components. Academic Press Inc. London, pp: 116-121.

Zanin, G.M. and F.F. de Moraes, 1994. Performance of fixed and fluidized reactors with immobilized enzyme. Applied Biochem. Biotechnol., 45/46: 627-640.