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Assessment of gum Arabic and agar gum as binders for the immobilization of α -amylase

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Abstact

The use of gum arabic and agar gum as binders for the immobilization of α -amylase on carrier support was studied in a pilot glass reactor. The result showed that gum arabic binder sustained enzyme activity up to 24hrs while agar gum could not exceed 20 hrs. The $t_{1/2}$ and residual activity after 4 cycles of refuse were 94hrs, 98% (R^2=93%) and 5.3hrs, 13% (R^2=89%) for gum arabic and agar gum systems. The work conclude that gum arabic is superior to agar gum both for immobilizing α -amylase on a support carrier and for predicting system operations.

Key words: Gum arabic, agar gum, immobilization, α-amylase, reactor system.

Introduction

Alpha-amylase has a long history of industrial applications. While new versions of the traditional enzymes used for starch processing are being engineered (Grabb and Shetty 1999), already existing enzymatic processing techniques are still being improved for industrial and economic purposes. One of such existing enzymatic processing techniques is immobilization. Among the techniques is the adsorption of alpha amylase on fibrous cellulosic materials (Egwim and Oloyede 2007). Saiyavit (2002) has reported several advantages of using cellulosic fiber for immobilizing alpha amylase which includes cheapness, easy separation of the reactants, products and reaction media; easy recovery of the enzymes and repeated or continuous reuse. Different binders have been employed to covalently bind enzymes to the surface of support carriers, particularly gel forming polysaccharides (Wang 2004), while Ogunbanyo and Bello (1986) have shown the use of different gums as binders for the immobilization of lactase on solid supports. In the present study, the possibility of using gum arabic or agar gum as binders for immobilization α -amylase on solid support is assessed.

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Materials and Methods

All chemicals used for the present work were of analytical grade (Analar) being products of British Drug House (BDH) chemical limited, Pole, England.

Alpha-amylase

α -amylase was obtained from supporting acha (*Digiteria ibura*). *D. Ibura* was supplied by National Cereals Research Institute (NCRI) Badeggi, Niger State, Nigeria; sprouting acha has been shown to contain a high quality α -amylase which can be employed for industrial starch processing (Egwim and Oloyede 2006).

Binders

Gum arabic and agar gums were purchased from Foremost Daires limited, Lagos, Nigeria. 50% (W/V) gum arabic or agar gum were prepared by stirring in hot water till gum dissolves with homogeneity.

Enzyme immobilization

α -amylase was immobilized by gel entrapment into porous matrix raphia palm (*Raphia hookeri*) wood chips (1cm x 1cm x 1cm) as described by Egwim and Oloyede (2007);in summary, the α -amylase and binding agent were mixed thoroughly before the wood chips were soaked in the mixture for at least three hours. The chips were the removed and left to dry at room temperature, the immobilized enzyme was then packed in a glass column reactor (60 cm x 6cm), the reactor was maintained at 45^{o}C while 2% (W/V) 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 at 4hrs interval for 25hrs. Total reducing sugar was assayed by the DNSA reagent method (Miller 1959) and expressed as enzyme activity.

Enzyme activity

Enzyme activity in the present work is defined as the amount of enzyme required to liberate a unit of glucose per min (mg glucose/ml/min) at reaction condition of 45^0C and pH 6.0.

Decay constant (κ) and half -life ($T_{1/2}$)

The κ and $T_{½}$ values for immobilized enzyme using gum arabic or agar gum binders were computed following the method of Fabricio *et al*. 2004.

$$de/dt = -KE \quad (1)$$

At t =0; E =E_o

Integrating

$$E = E_o e^{(-Kt)} \quad (2)$$

Where Eo is the initial active enzyme concentration and t is time elapsed during reaction. The residual enzyme activity Ar is directly proportional to the concentration of the active enzyme (E):

$$A_r \; A_o = E/E_o \quad (3)$$

From equation 2&3, the residual enzyme activity follows as:

$$A_r = A_o e^{(-Kt)} \quad (4)$$

Equation 4 is known as the exponential decay model.

From equation 4; $K = 2.303/t \log A_r/A_o$ (5)

E enzyme half life ($t_{1/2}$) is the time lapse for the enzyme activity to decrease 50% of the initial value. From equation (5) it means that half life ($t_{1/2}$) can be calculated.

$$T_{1/2} = \text{In } (0.5)/K = 0.693/K \quad (6)$$

Models Mathematical models for predicting system operations were generated using computer excel package.

Results and Discussion

Fig 1 shows the influence of gum arabic and agar gum binders on the activities of α-amylase. The result shows that gum arabic maintained higher enzyme activity over that shown for a gar gum up to 16 hrs of running the reactor.

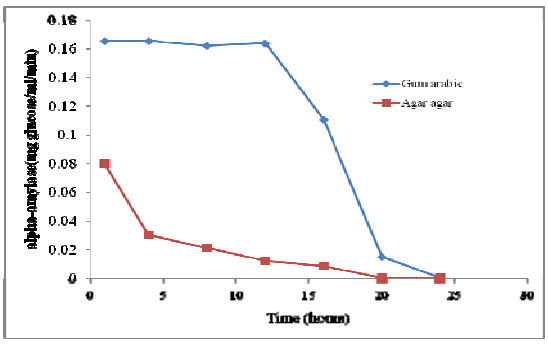

Figure 1. Influence **of** gum arabic and agar-agar binders on the activity of immobilized alpha amylase

Enzyme activity in the reactor using gum arabic tailored down to 24hrs, while agar gum did not sustain enzyme active above 20hrs. The present observation shows that gum abrabic may be the binder of choice for the immobilization α -amylase on palm wood chip. Ogunbayo and Bello (1986) have shown that agar gum is a better binder for the immobilization of lactase. The reason for the present discrepancy is not understood yet, but the reason may be due to any or all of the following. Difference in α -amylase and lactase molecular constitution, difference in the three dimensional structures of the enzymes thereby exposing different amino acid residues which confers different interactive side groups to the enzyme; and the degree of crossing linking in the gum polymers in relation to the enzyme size. The later suggestion seems very possible because Wang (2004) have shown that these is a statistical relationship between the mesh size and diffusion of enzyme molecules, such that some enzyme molecules gradually diffuse toward the outer shell of the gel and eventually leak into the surrounding medium. The present result (fig 1) shows that gum arabic maintain relativity constantan activity from 1-12 hrs. Thereafter, enzyme activity dropped sharply, which may be attributed to diffusion and the leaking of enzymes molecules from the gel. Another possible reason for the sharp drop in enzyme activity may be due to mass transfer resistance. This may be so because the average path length of the enzyme carrier (1cm x 1cm x 1cm) is much larger compared to the average diffusion length. Substrate may not diffuse deep into the carrier matrix. At the same time, diffusion resistance encountered by the product molecule may have caused product to accumulate in the matrix of the carrier. There by hindering the substrate to have access to the enzyme. In either case, the system can be improved by studying the relationship between binder: enzyme ratios and carrier size.

Table 1. Influence of different binders on decay constant (κ). Half- life ($T_{1/2}$) and Residual activity of immobilized α -amylase.

Binders	κ	$T_{1/2}$(hrs)	Residual activity after 4 cycles of reuse.
Gum Arabic	0.15×10^{-2}	94	98%
Agar gum	12×10^{-2}	5.3	13%

Table 1 shows the influence of the different binders on the κ, $T_{1/2}$ and Residual activity of immobilized α -amylase. The result shows that gum arabic induced a higher $t_{1/2}$ and Residual activity (94 hrs and 98% respectively). This result reveals that immobilization

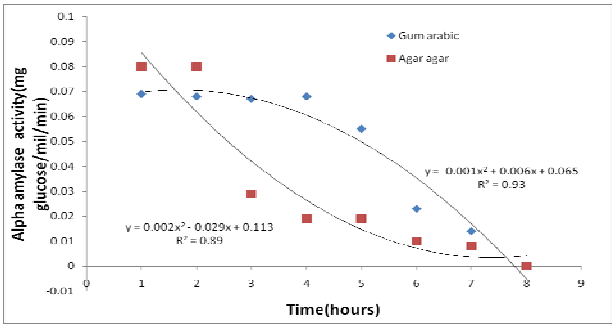

Figure 2. Model equation for predicting scale-up operation of binders for the immobilization of alpha amylase

system using gum arabic as binder lost only 2% of its initial activity after 4 cycles of reuse which lasted for almost 16hrs, while the immobilization system using agar gum has lost as much as 87% of its initial activity. This observation further suggests that that gum

arabic may be better binder for α -amylase immobilization on palm wood chips.

We have earlier shown that immobilization of α -amylase on palm wood chips may be a cheap sources of industrial immobilization (Egwim and Oloyede 2007). Using gum arabic as binder can further improve this process.

Fig. 2 shows a second order polynomial equation for predicting the influence of different binders on the activity of immobilized α -amylase. The equations show an R^2 of 93% and 89% for the systems using gum arabic and agar gum respectively. The present observations shows that the immobilization system using gum arabic yielded a better model fit for predicting system operations compared to that using agar gum.

Conclusion

The present work concludes that gum arabic is a cheap and better than agar gum both for immobilization of α -amylase and for predicting system operations. Hence, may be employed in an industrial bioreactor design.

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