XXI SINAFERM
XII SHEB
Aracaju - Sergipe

XXI SIMPÓSIO NACIONAL DE BIOPROCESSOS
XII SIMPÓSIO DE HIDRÓLISE ENZIMÁTICA DE BIOMASSA

3 a 6 de setembro de 2017
Aracaju, Sergipe, Brasil

MULTIPOINT COVALENT IMMOBILIZATION OF DEXTRANSUCRASE ON AN EPOXY ACTIVATED AGAROSE – OLIGOSACCHARIDES SYNTHESES

Priscila M.P. Souza[1], Rhonyele M. Silva [1], Sueli Rodrigues[1] and Luciana R. Gonçalves [2]

[1] Universidade Federal do Ceará, Departamento de Engenharia de Alimentos

[2] Universidade Federal do Ceará, Departamento de Engenharia Química

E-mail: sueli@ufc.br

ABSTRACT

Dextransucrase is an industrial enzyme used to the production of dextran and oligosaccharides. This enzyme presents many difficulties to immobilize, due to dextran masking its reactive groups. To facilitate immobilization dextransucrase was co-immobilized with dextranase in the epoxy-agarose carrier. The synthesis of oligosaccharides was evaluated at different temperatures (4 °C and 25 °C). The results showed that the better conditions of synthesis to the immobilized enzymes was at 25 °C, resulting in low levels of dextran and oligosaccharides with a degree of polymerization up to 10.

1. INTRODUCTION

Dextransucrase (EC 2.4.1.5) is a bacterial extracellular glucosyltransferase produced by several *Leuconostoc mesenteroides* strains, and it is involved in the synthesis of prebiotic oligosaccharides through the acceptor reaction. Also, the enzyme catalyzes the formation of dextran. For the industrial application of dextransucrases, an effective immobilization method is required to ensure continuous processing and the reuse of the biocatalyst. The main difficult to achieve dextransucrase immobilization is due to the presence of a covalently bound dextran layer covering the enzyme surface. Several groups have studied the advantages of co-immobilization of dextransucrase (DS) and dextranase (DN) because it facilitates immobilization and allows the production of low molecular weight dextran and isomalto-oligosaccharides (Tanriseven and Dogan, 2002). The use of cheap, nontoxic, available carriers for covalent immobilization of enzymes for food processes is of great interest. The best results reported to dextransucrase immobilization were in Eupergit C, but this carrier production was discontinued. As an alternative to Eupergit C, epoxy-agarose carriers are proposed because of their high reactive groups density and the easy chemistry for attachment of the

enzyme to the support (De Segura et al., 2004). This work proposes co-immobilized dextransucrase (DS) and dextranase (DN) onto the epoxy-agarose carrier to synthesize oligosaccharides.

2. MATERIALS AND METHODS

2.1. Materials

L. mesenteroides NRRL B-512F dextransucrase (DS) was obtained as previously described (Rodrigues et al., 2003).

2.2. Co-Immobilization of Dextransucrase and Dextranase in Epoxide-Agarose Support

The epoxy-agarose carrier was obtained as reported by Souza (2015). The co-immobilization was carried out under optimum conditions according to previously published work (de Segura et al., 2004). DS (1000 µL) and 0,5µL of DN (Sigma-Aldrich) were mixed in 20 mM sodium acetate buffer with 0,05g/L of $CaCl_2$ (4 mL, pH 5.2). Afterward, the enzymatic solution was added to 300mg of epoxide-agarose support. The mixture was incubated for 24h at 4 °C in a tube rotating mixer.

2.3. Determination of Dextransucrase Activity

DS and DN (both enzymes soluble or immobilized) were incubated with 450 µL of the activity solution containing sucrose as substrate (Rodrigues et al. 2003). The reaction mixture was kept in agitation at 30 °C for 10 min. The amount of fructose released from sucrose was quantified by DNS method (Miller, 1959). The activity unit (IU) is defined as the amount of enzyme needed to release 1µmol fructose.

2.4. Oligosaccharide Synthesis

Oligosaccharide synthesis was carried out using DS and DN free and immobilized in epoxide-agarose (1 IU/ mL, 30 IU/g). The enzyme was diluted in 20 mM sodium acetate buffer with 0.05g/L of $CaCl_2$. The amount of reducing sugar (glucose and fructose) was 75g/L and sucrose (75g/L) was selected as optimum conditions to produce oligosaccharides according to previously published work (Fontes et al., 2015). The synthesis was carried out at 25 °C and 4 °C and pH 5.2. During the synthesis, samples were collected at 6 h, 12 h and 24 h.

2.5. Determination of Carbohydrates

Dextran was precipitated by adding three volumes of ethanol 96% (v/v). The pellet was re-suspended in distilled water, and the dextran formed during the fermentation was assayed as total carbohydrate

(Dubois et al., 1956). The supernatant containing the oligosaccharides was assayed by Thin Layer Chromatography (TLC). The oligosaccharides that were produced were characterized by its Degree of Polymerization (DP) by TLC on Silica Gel 60 TLC aluminum plate (20 x 20cm, Merck, Germany) as described by Fontes et al. (2015).

3. RESULTS AND DISCUSSION

Table 1 shows the dextran formed from free and immobilized enzymes. Figure 1 shows the oligosaccharides formed.

Table 1. Dextran synthetized by free and immobilized dextransucrase and dextranase

T (°C)	Run	Time (h)	Dextran (g/L)	
4	**1**	**6**	35.59 ±0.30	**Immobilized**
	2	**12**	27.25 ±0.86	
	3	**24**	42.96 ±1.14	
	4	**6**	25.31 ±0.48	**Free**
	5	**12**	39.34±1.61	
	6	**24**	70.10 ±2.23	
25	**7**	**6**	37.53 ±0.41	**Immobilized**
	8	**12**	32.52 ±1.85	
	9	**24**	48.27 ±1.45	
	10	**6**	41.49 ±0.48	**Free**
	11	**12**	39.21 ±1.11	
	12	**24**	47.47 ±1.49	

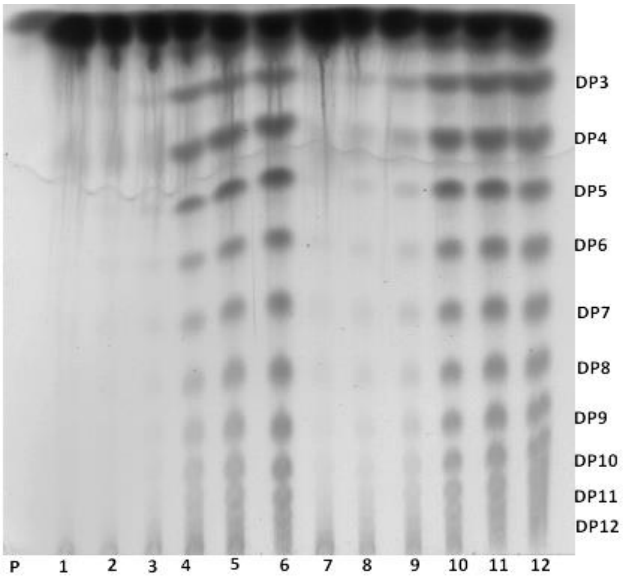

Figure 1. Degree of Polymerization (DP) of oligosaccharides synthetized by free and immobilized enzymes (see Table 1).

In the production of dextran, co-immobilized enzyme presented similar behavior of free enzymes at 25 °C. While at 4 °C, free enzyme (70.10 g/L ± 2.23, 24h) synthesized more dextran than co-immobilized (42.96 g/L ± 1.14, 24h). This values can be explained due to diffusion limitation and low temperature that may decrease the stability of dextranase retarding dextran hydrolysis (Erhardt and Jördening, 2007). Besides, the free enzyme does not contain dextranase, which also affects the dextran hydrolysis.

According to Figure 1, the free enzyme presented the same results for (DP up to 12) at both temperatures. Long chain oligosaccharide is important to human health because it reaches the colon. The higher DP for co-immobilized enzymes was at 25 °C and 24h with DP=10. The immobilized enzyme present operational stability at 25 °C. At 4 °C, the number of oligosaccharides was lower, and it was not detected in TLC plate. The co-immobilization of DS/DN on alginate beads synthesized oligosaccharides with a DP > 5, depending on the synthesis conditions (Kubik et al., 2004). The use of the prepared agarose-based epoxide support is an alternative to replace the commercial discontinued Eupergit C.

4. CONCLUSION

In conclusion, co-immobilized DS/DN in epoxy-agarose carrier had better stability at 25 ºC synthesizing oligosaccharides with a degree of polymerization up to 10.

5. REFERENCES

de Segura, A.G., Alcalde, M., Yates, M., Rojas-Cervantes, M.L., Lopez-Cortes, N., Ballesteros, A., Plou, F.J., 2004. Immobilization of Dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F on Eupergit C Supports. Biotechnology Progress 20, 1414–1420.

Erhardt, F.A., Jördening, H.-J., 2007. Immobilization of dextranase from *Chaetomium erraticum*. Journal of Biotechnology 131, 440–447.

Fontes, C.P.M.L., da Silva, J.L.A., Rabelo, M.C., Rodrigues, S., 2015. Development of low caloric prebiotic fruit juices by dexransucrase acceptor reaction. Journal of Food Science and Technology 52, 7272–7280.

Kubik, C., Sikora, B., Bielecki, S., 2004. Immobilization of dextransucrase and its use with soluble dextranase for glucooligosaccharides synthesis. Enzyme and Microbial Technology 34, 555–560.

Miller, G.L., 1959. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Analytical Chemistry 31, 426 – 428.

Rodrigues, S., Lona, L.M.F., Franco, T.T., 2003. Effect of phosphate concentration on the production of dextransucrase by *Leuconostoc mesenteroides* NRRL B512F. Bioprocess and Biosystems Engineering 26, 57–62.

Sousa, M. de, 2015. Obtenção de um catalisador insolúvel para a produção de D-tagatose por L-arabinose isomerase. Universidade Federal do Ceará.

Tanriseven, A., Dogan, S., 2002. Production of isomalto-oligosaccharides using dextransucrase immobilized in alginate fibres. ResearchGate 37, 1111–1115.