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Development of heterogeneous preparation with inulinase for tubular reactor systems

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ABSTRACT

We developed highly stable heterogeneous preparations of inulinase immobilized on the ion-exchange matrixes and show that such preparations effectively split the chemically pure inulin and the inulin contained in plant extracts.

The optimal conditions of hydrolysis are created for the migration of inulin solution from top to bottom (the downsteam flow) through the column filled by immobilized compounds extracted from *Kluyveromyces marxianus* and *Helianthus tuberosus* with the flow rate of 3 mL/min. For the bottom-up migration of the inulin solution (i.e. the upstream flow) through the column the flow rate of 5 mL/min was found to be the most optimal for the hydrolysis.

When the extract of *H. tuberosus* was allowed to pass through the column filled by the immobilized plant inulinase from top to bottom with the flow rate of 5–10 mL/min, the conditions for hydrolysis appear to be the most optimal. The conditions for hydrolysis were also found to be favourable for the bottom-up moving of the *H. tuberosus* extract through the column for the flow rate of 3–5 mL/min.

For the yeasts inulinase immobilized on VION KN-1 the most optimal flow rates of the *H. tuberosus* extract in the reactor were found to be similar for the following rates of inulin flow, viz. 3 mL/min for the downstream flow and 5 mL/min for the upstream flow.

A theoretical description of the process of the inulin hydrolysis has been proposed and experimentally verified, which allows predicting the time of the full hydrolysis at different operating conditions with an error not exceeding 20%. It could be applied for the design of continuous reactors.

1. Introduction

Nowadays the development of technologies for preparing foodstuff for diabetic and preventive application gains the increasing relevance. For this reason an interest in fructose, which can be used in dietary food consumed by patients with diabetes, is gradually growing [1,2]. Typically, fructose is obtained from starch. The conventional fructose production by starch hydrolysis includes three steps: the action of α-amylase, amyloglucosidase and glucose isomerase, yielding only 45% of fructose in the final product [3]. Currently, the production of fructose from inulin-containing plant materials, in particular, a *Helianthus tuberosus* [4], is considered to be a very promising approach. In *H. tuberosus* tubers the content of inulin reaches 20–25% and the syrup contains at least 70% of fructose [5–7].

Inulinase [β-D-(2 → 1)-fructan fructanohydrolases, EC 3.2.1.7] breaks down inulin and fructooligosaccharides to fructose. This enzyme is widespread among the plants and microorganisms and may have an application for the industrial preparation of fructose from plant materials. Inulinases are enzymes potentially useful for obtaining fructose syrups by the enzymatic inulin hydrolysis, which have high yields of about 95% [2,4,8–10]. However, the possibilities of utilizing enzymes in practice are very limited at least for two reasons. First, they are unstable during storage due to several factors, such as temperature. Second, multiple repeated use of the enzymes is problematic because of the difficulty in their separation from reagents and products of reaction. Besides, it is not always simple to predict behavior of the enzyme and kinetics of the reaction in industrial fermenters.

In contrast, the advent of the immobilized enzyme technology has led to an opportunity of replacement of the conventional enzymatic process with immobilized enzyme preparations. The

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Table 1
Catalytic activity of the inulinase extracted from *Kluyveromyces marxianus* and immobilized on VION KN-1, using heterogeneous biocatalyser in the model reactor of continuous action.

Substrate flow rate, mL/min	Inulin is used as a substrate		*Helianthus tuberosus* is used as a substrate	
	Downstream flow	Upstream flow	Downstream flow	Upstream flow
2	130	111	155	109
3	169	130	182	120
4	76	173	84	173
5	48	197	39	218
7	–	64	–	62
15	–	32	–	50

Table 2
Catalytic activity of the inulinase extracted from *Helianthus tuberosus* and immobilized on KU-2, using heterogeneous biocatalyser in the model reactor of continuous action.

Substrate flow rate, mL/min	Inulin is used as a substrate		*Helianthus tuberosus* is used as a substrate	
	downstream flow	upstream flow	downstream flow	upstream flow
1	103	96	129	122
2	104	102	132	125
3	120	102	134	132
4	115	105	136	134
5	90	110	146	134
7	81	105	148	126
10	72	89	145	108

advantages of immobilization technology include: (a) enzymes ability to process large amounts of substrate, since they can be separated easily from the mixture of substrate and product(s), thus enabling the enzymes to be reused [11]; (b) imparting greater stability to the enzyme [12–14], so that it can be used for the development of the continuous process; (c) affording greater control of the catalytic process; (d) possibility of cost reduction [15]. Currently, there is a number of papers dealing with optimisation of the packed-bed bioreactors configuration for inulinase production [16], although the available data on immobilized inulinases in the tubular reactor systems are very limited. An increasing interest has also been expressed for the search of the best operating conditions in fructose production by inulin enzyme hydrolysis [17] in terms of the immobilization technique [18–20] and the reactor type [21–23]. To date a wide range of practical aspects associated with the use of immobilized enzyme in continuous reactors need further elaboration. Thus, only few studies have appeared on continuous processes with immobilized inulinase. In most of them packed bed reactors were used with few exceptions, such as the work by Diaz et al. [21] who employed a shell-tube membrane reactor. Among the most recent works on packed bed reactors remarkable results were obtained by Gupta et al. [24] who used inulinase immobilized on DEAE-cellulose, Nakamura et al. [19] who used Amino-cellolufine as the support, Wenling et al. [20] who used macroporous ionic polystyrene beads, and Gill et al. [23] who tested different supports: chitin, QAE-Sephadex and ConA linked-amino activated silica beads.

Alginate-chitosan beads [25], carbon nanotubes [26], poly-D-lysine coated $CaCO_3$ micro-particles [27], different composite membranes composed of chitosan/nonwoven fabrics [28], concanavalin A-attached super macroporous cryogel [29], montmorillonite [30] were also used as the carriers for inuinase immobilization.

A new type of support for enzyme immobilization has drawn a great attention in recent years, viz. Sepabeads (a class of methacrylic polymers particularly suitable for enzymes immobilization in industrial purposes) [31,32]. Basso et al. [33] reported a rational immobilization of inulinase on Sepabeads based on homology modeling, docking and molecular dynamics. The new preparation of immobilized enzyme was tested under conditions close to industrial and a novel reactor configuration for fructose production from inulin was proposed and tested. Experiments were run in batch and continuous reactors.

The copper and cobalt aluminates exhibit high inulinase immobilization efficiencies, which makes them promising supports for enzyme immobilization [34]. New glycidyl methacrylate copolymers containing different numbers of epoxy groups were synthesized and used to develop effective procedures for inulinase immobilization. The yield of immobilization of this enzyme on the investigated type of microspheres was higher than that on the commercial carrier, Eupergit C [35].

Adsorptive and covalent ways of immobilization for inulinase on synthetic cation-exchanger and anion-exchanger were developed, allowing to preserve 80–85 % of activity of the native enzyme [36].

The majority of the works mentioned above report experimental results confirming a good productivity and versatility of the process under study. However, the stability and performance in vast ranges of operating conditions have not been thoroughly investigated. The knowledge of the reaction kinetics, deactivation rates and their overall effect on the reaction progress could be a very useful tool to further improve the fructose production process by inulin enzymatic hydrolysis and enable proper optimization of the operating conditions.

In the present work a complete model accounting for the reaction kinetics of the Michaelis–Menten type (for immobilized inulinase) and the enzyme deactivation is proposed as a tool to understand and predict enzyme performance within the reaction medium and reaction progress in the tubular reactor systems.

2. Experimental

Inulinases were isolated from yeast *Kluyveromyces marxianus* and plant *H. tuberosus*. Their homogeneous fractions were prepared using the method published in Ref. [37]. Stages of purification are provided below.

2.1. K. marxianus *yeast cultivation*

Submerged cultivation of yeast *K. marxianus* Y-303 was carried out in 500 mL flasks containing 50 mL of culture medium on a laboratory shaker for 72 h. For the cultivation of the *K. marxianus* yeast, a medium containing peptone (1.0%), yeast extract (0.5%), and inulin