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ПРИМЕНЕНИЕ СВЕРХСШИТЫХ ПОЛИМЕРОВ В КАЧЕСТВЕ НОСИТЕЛЕЙ ГЕТЕРОГЕННЫХ БИОКАТАЛИЗАТОРОВ ДЛЯ РЕАКЦИИ ГИДРОЛИЗА ИНУЛИНА

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В данной работе проведено исследование адсорбционной иммобилизации инулиназы на сверхсшитых макропористых сорбентах на основе стирола и дивинилбензола: низкоосновном анионообменнике А100, высокоосновном анионообменнике А500R, сильнокислотном катионообменнике С100Н. Рассмотрено влияние продолжительности процесса сорбции, значений концентраций ионов водорода и белка в растворе на количество иммобилизованного фермента и активность полученных гетерогенных биокатализаторов. Выявлено, что адсорбция достигает своего максимального значения в среднем через 4 ч при pH 4,7-5,0 для рассматриваемых сорбентов. Активность полученных гетерогенных биокатализаторов составляет 64,8-83,5% от активности свободной инулиназы. Хотя активность инулиназы при адсорбции на используемых в работе полимерных носителях снижается, интегральное количество полученного продукта будет выше, чем для нативного катализатора. Изучены изотермы адсорбции инулиназы на сверхсшитых полимерах. Стабильность полученных гетерогенных биокатализаторов объясняется высокой сорбционной способностью сверхсшитых сорбентов. Выявлено, что иммобилизованная инулиназа может применяться в течение 8-11 последовательных циклов реакции гидролиза субстрата. Используя адсорбционную теорию БЭТ, проведен анализ изотерм сорбции фермента и рассчитаны равновесные параметры сорбции: предельное количество сорбированной инулиназы, константы, характеризующие взаимодействие сорбат-сорбент и сорбат-сорбат. Среди рассматриваемых носителей наиболее высокое значение величины константы сорбционного равновесия (KL=4,25±0.04 л/ммоль) соответствует биокатализатору инулиназа-катионообменник, величина сорбционной емкости которого также наибольшая. Полученные данные представляют интерес для оценки эффективности использования сорбентов в качестве носителей инулиназы в последующих технологических процессах переработки инулинсодержащего сырья.

Ключевые слова: сверхсшитые полимеры, инулиназа, иммобилизация, каталитическая активность, гетерогенный биокатализатор

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APPLICATION OF SUPER-CROSSLINKED POLYMERS AS CARRIERS OF HETEROGENEOUS BIOCATALYSTS FOR INULIN HYDROLYSIS REACTION

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In this work the study of adsorption immobilization of inulinase on super-crosslinked macroporous sorbents based on styrene and divinylbenzene: low-base anion exchanger A100, high-base anion exchanger A500R, strong acid cation exchanger C100H was carried out. The influence of the duration of the sorption process, the value of the concentration of hydrogen ions and the concentration of protein in solution on the amount of immobilized enzyme and the activity of the obtained heterogeneous biocatalysts is considered. It was revealed that adsorption reaches its maximum value on average after 4 h at pH of 4.7-5.0 for the sorbents under consideration. The activity of the obtained heterogeneous biocatalysts is 64.8-83.5% of the activity of free inulinase. Although the activity of inulinase during adsorption on the polymer carriers used in the work decreases, the integral amount of the resulting product will be higher than for the native catalyst. The isotherms of inulinase adsorption on super-crosslinked polymers have been studied. The high stability of the obtained heterogeneous biocatalysts is explained by the high sorption capacity of super-crosslinked sorbents. It was revealed that immobilized inulinase can be used during 8-11 consecutive cycles of the substrate hydrolysis reaction. Using the BET adsorption theory, the enzyme sorption isotherms were analyzed and the equilibrium sorption parameters were calculated: the maximum amount of sorbed inulinase, the constants characterizing the sorbate-sorbent and sorbate-sorbate interaction. Among the considered carriers, the highest value of the sorption equilibrium constant (KL=4.25±0.04 l/mmol) corresponds to the inulinase-cation exchanger biocatalyst, the sorption capacity of which is also the largest. The data obtained is of interest for evaluating the effectiveness of the use of sorbents as inulinase carriers in subsequent technological operations of processing of inulin-containing raw materials.

Key words: super-crosslinked polymers, inulinase, immobilization, catalytic activity, heterogeneous biocatalyst

INTRODUCTION

Inulinase (2.1-β-D-fructan-fructanohydrolase, E.C. 3.2.1.7) is widely used in technological processes to obtain fructose from vegetable raw materials and makes it possible to convert inulin into fructose in one stage [1, 2]. Fructose has a twice lower glycemic index, and the assimilation of fructose occurs without the participation of insulin. Due to its properties, syrups with a high fructose content obtained by enzymatic hydrolysis of extracts of inulin-containing plants can be used in the confectionery industry, in therapeutic nutrition as a sweetener for diabetic patients [3, 4].

However, the use of native enzymes has a number of limitations: the impossibility of reuse, low thermal stability, the complexity of separating the biocatalyst and the final product. The effectiveness of enzymatic processes can be improved with the help of immobilized drugs [5-8].

The results obtained during the immobilization of enzymes on super-crosslinked polymer sorbents are of continued interest [9,10]. Previously, we investigated the immobilization of inulinase on sorbents of the “Styrosorb" type [11, 12].

Despite the fact that a large number of enzyme preparations immobilized on different carriers have been already described, it should be noted that the choice of the sorbent and the method of binding the protein to the carrier remains largely empirical. The most important factors on which the adsorption immobilization of enzymes depends are determined, on the one hand, by the surface properties of carriers: their chemical structure, hydrophilicity or hydrophobicity, porosity, swelling, on the other hand – the structure of enzymes, the size of macromolecules, chemical properties.

Super-crosslinked sorbents based on styrene with divinylbenzene have a uniquely high sorption capacity in relation to organic substances in aqueous or airborne media. They have found wide application in large-scale sorption processes in the chemical, food, pharmaceutical industries, to solve a number of environmental problems [13].

The purpose of this work was to study the conditions of adsorption immobilization of inulinase on super-crosslinked ion-exchangers.

EXPERIMENTAL PART

The object of the study was *Aspergillus niger* (Sigma Aldrich) inulinase, inulin (Spofa) was used as a substrate. The sorbents were ion exchangers (Purolite): a weakly basic anion exchanger A100 containing mainly tertiary amino groups; a highly basic anion exchanger A500R; a strong acid cation exchanger C100H with sulfogroups. These carriers belong to the group of super-crosslinked polymer materials based on styrene and divinylbenzene with a highly developed specific surface area and macroporous structure [14]. To prepare for the experiment, the sorbents were previously conditioned [15].

Kinetic experiments were carried out under static conditions with continuous mixing of the solution by the limited volume method. To do this, the air-dry sorbent suspensions weighing 1.0000 ±0.0002 g were placed in conical flasks with a lapped stopper of 1000 ml, and filled with an inulinase solution with a concentration of $2 \cdot 10^{-2}$ mmol/l. Inulinase solutions were prepared on the basis of an acetate buffer. At certain intervals, 1.0 ml samples were taken and the concentration of the substance was determined by the spectrophotometric method. The total amount of protein in native enzyme preparations was determined spectrophotometrically using the Lowry method, in immobilized enzymes – by the modified Lowry method. The process was considered to be completed if the substance content in the solution did not change for some time. The absorption spectra of solutions were recorded on a Shumadzu UV-2401 spectrophotometer, the spectral width of the slit is 0.5 nm, the scanning step is 0.5 nm, the scanning speed corresponded to the Slow mode.

The method of variable concentrations was used to obtain sorption isotherms. Sorbent suspensions (1.0000 ± 0.0002 g) were brought into contact with solutions of various concentrations $((0.25\text{-}2.5) \cdot 10^{-2}$ mmol/l), based on 0.1mol/l acetate buffer pH 4.7. Immobilization was carried out during for 4 h under static conditions at a temperature of 20.0 ± 1.5 °C with periodic stirring. The amount of substance in the sorbent phase was calculated from the difference in the concentrations of the initial and equilibrium solutions. Protein desorption into buffer solutions was no more than 2%.

Experiments to determine the activity of enzyme preparations were conducted in a thermostatically controlled reactor in which the incubated liquid was stirred using a magnetic stirrer. The catalytic activity was measured with the help of the spectrophotometric method by the Selivanov reaction using resorcinol [16]. For a unit of inulinase activity, such an amount of an enzyme was taken that catalyzes the hydrolysis of inulin for 1 min with the formation of 1 μmol of fructose.

Inulinase activity was calculated by the formula:

$$A = \frac{a}{180bt}, \quad (1)$$

where A is the catalytic activity per mg of protein; a – the amount of fructose, mmol; b – the amount of enzyme in the reaction mixture, mg/ml of hydrolysate; t – the hydrolysis time, min; 180 – the molecular mass of fructose.

The standard deviation of the obtained results did not exceed 0.01.

RESULTS AND DISCUSSION

Kinetic sorption curves were obtained to determine the time to achieve equilibrium in the sorbent enzyme solution system. The equilibrium value of inulinase adsorption for the studied sorbents is reached on average within 250 min (Fig. 1).

To use polymer carriers in real technological processes, an important requirement is not only a high sorption capacity of the sorbent relative to the sorbate, but also a short time period to achieve equilibrium in the system. Considering the size of the inulinase (radius – 12.6 nm, height of the molecule – 6.4 nm) [17], it is possible to note good kinetic properties of the sorbents used.

The conducted studies have shown that the highest value of inulinase sorption is observed in the pH range 4.5-5.2 (Fig. 2), at this value heterogeneous

biocatalysts showed the highest value of catalytic activity. The activity of inulinase preparations immobilized on the exchanger C100H at pH 4.7 is 83.5%, on A500R –78.4%, on A100 – 64.8% of the activity of the native enzyme (2500 U/g). It can be assumed that the active centers of inulinase after immobilization become sterically less accessible to the high-molecular substrate – inulin. On the other hand, adsorption immobilization stabilizes the inulinase molecule, primarily the mobility of the tertiary structure which is responsible for the formation of the enzyme-substrate complex, thus protecting the protein from the effects of denaturing factors, pH in particular. Despite the decrease in the maximum reaction rate of inulin hydrolysis and catalytic activity compared to free inulinase, a significant advantage is the possibility of reuse of a heterogeneous biocatalyst.

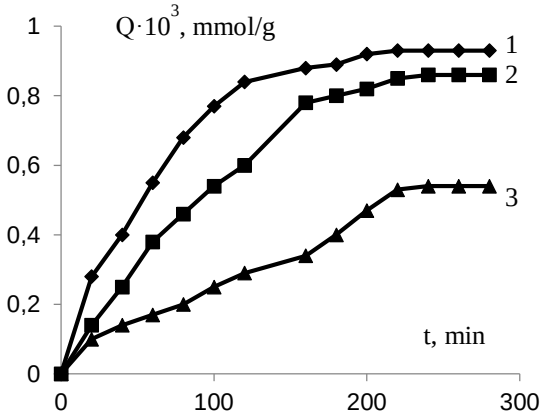

Fig. 1. Kinetic curves of inulinase sorption on C100H (1), A500R (2), A100 (3). Q – the amount of sorbed inulinase, mmol/g; t – is the duration of the process, min

Рис. 1. Кинетические кривые сорбции инулиназы на C100H (1), A500R (2), A100 (3). Q – количество сорбированной инулиназы, ммоль/г; t – продолжительность процесса, мин

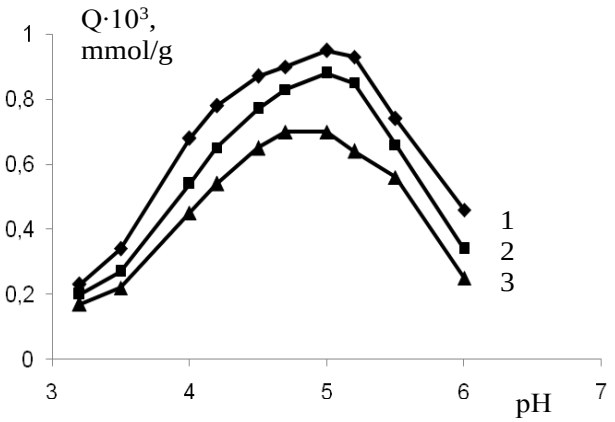

Fig. 2. Dependence of the amount of sorbed inulinase (Q, mmol/g) on C 100H (1), A 500R (2), A 100 (3) on the pH of the equilibrium solution

Рис. 2. Зависимость количества сорбированной инулиназы (Q, ммоль/г) на C100H (1), A500R (2), A100 (3) от pH равновесного раствора

The isotherms of inulinase sorption on the carriers under consideration, obtained at pH 4.7, are shown in Fig. 3. In the region of low concentrations at sorption isotherms, we observe an almost linear dependence and the subsequent appearance of a plateau, which corresponds to the formation of a monomolecular sorbate layer. Further, immobilization occurs due to the interaction of inulinase molecules with each other, which leads to the formation of polymolecular layers on the surface of the sorbent.

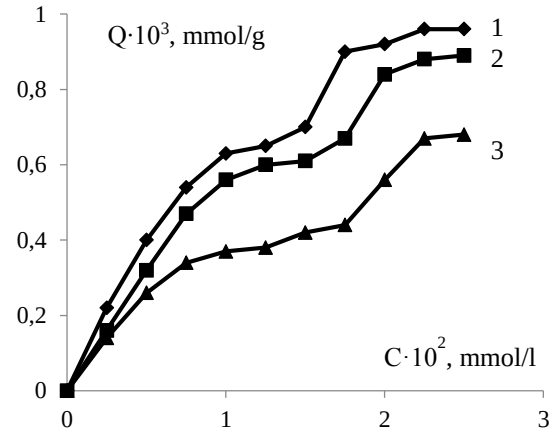

Fig. 3. Isotherms of inulinase sorption at C100H (1), A500R (2), and 100 (3) at pH 4.7. C – equilibrium protein concentration in solution, mmol/l

Рис. 3. Изотермы сорбции инулиназы на C100H (1), A500R (2), A100 (3) при pH 4,7. C – равновесная концентрация белка в растворе, ммоль/л

Hydrophobic interactions and emerging hydrogen bonds between molecules, leading to the formation of supramolecular complexes, probably play a decisive role in the binding of inulinase to the carrier. The three-dimensional structure of super-stitched polystyrene used as carriers is a polymer mesh with a developed inner surface (up to 1500 m^2/g) and a certain mobility capable of forming structured phases in such a polymer matrix. This assumption has been noted during the sorption of a number of organic substances [18-20]. The presence of reactive functional groups also makes it possible to implement electrostatic interactions to ensure stronger carrier – enzyme bonds.

During immobilization, the catalytic activity of a heterogeneous biocatalyst was controlled for each value of the enzyme concentration. It was proved that despite the decreasing of the inulinase activity during immobilization, the total amount of the resulting product will be higher than that of the native enzyme.

The study of the mechanism of formation of the enzyme-carrier complex is necessary for the targeted creation of immobilized enzyme preparations. It is proved that enzymes are able to form supramolecular

complexes when their microenvironment changes. This phenomenon must be taken into account when selecting conditions and methods of immobilization, since the association-dissociation processes are one of the key mechanisms for regulating the catalytic activity of the biocatalyst [21]. Theoretical processing of sorption isotherms allows us to draw a conclusion that the nature of the interaction in the sorbate-sorbent, sorbate-sorbate, sorbate-solvent system can be used to optimize immobilization processes.

To describe quantitatively the isotherms of inulinase sorption by super-crosslinked sorbents, the BET model was used [22] (Table). The amount of immobilized enzyme was calculated by the formula:

$$Q = \frac{Q_\infty K_L c_e}{(1 - K_S c_e)(1 - K_S C_e + K_L c_e)}, \qquad (2)$$

where $Q \cdot 10^{-3}$ is the amount of sorbed protein, mmol/g; $Q_\infty \cdot 10^{-3}$ – the maximum amount of sorbed protein (the amount of the monolayer protein to be sorbed), mmol/g; $c_e \cdot 10^{-2}$ – the equilibrium concentration of the solution, mmol/l; K_L – the sorption equilibrium constant characterizing the intensity of the sorption process, l/mmol; K_S – the sorption equilibrium constant for the polymolecular layer, l/mmol.

The values of the sorption equilibrium constants included in the BET isotherm equation were calculated using the linearized equation:

$$\frac{c_e}{Q(1 - K_S c_e)} = \left(\frac{K_L - K_S}{Q_\infty K_L} \right) c_e + \frac{1}{Q_\infty K_L}. \qquad (3)$$

The maximum quantity of the immobilized enzyme that composes the monomolecular layer was determined from equations (4,5):

$$Q_\infty = \frac{1}{b \cdot K_L}, \qquad (4)$$

$$K_L = \frac{k}{b} + K_S. \qquad (5)$$

The sorption equilibrium constant for the polymolecular layer (K_s) was determined from the maximum authentic value of the line approximation

$$c_e - c_e / \ Q(1 - K_S c_e). \qquad (6)$$

Table

Values of sorption parameters calculated using BET equations

***Таблица.* Значения сорбционных параметров, рассчитанных с использованием уравнений БЭТ**

Sorbent	K_L, l/mmol	$Q_\infty \cdot 10^{-3}$, mmol/g	K_S, l/mmol	R^2
A 100	3.58±0.05	0.42±0.01	0.72±0.06	0.95
A500R	4.05±0.02	0.60±0.03	0.85±0.01	0.97
C100H	4.25±0.04	0.65±0.02	0.92±0,02	0.96

The calculated values of sorption parameters are presented in Table. It follows from the data obtained that the maximum value of the sorption equilibrium constant (K_L) has the cation exchanger C100H, which indicates a greater affinity of the carrier to the enzyme, compared to the anion exchangers studied. Since the values of K_L for all carriers are higher than K_s, it is possible to assume a greater role of the sorbent-sorbate interaction, compared to the formation of complexes of protein molecules. The obtained values of the constants are consistent with the values of the constants K_L and K_S for organic substances during sorption by carriers [23, 24].

The study of the cyclical action of the obtained heterogeneous biocatalysts was carried out. To do this, the immobilized enzyme (100 mg) was placed in test tubes with an inulin solution (10 ml; 1 mmol/l) and hydrolysis was performed, the substrate was removed every hour. It was revealed that a heterogeneous biocatalyst based on the sorbent C100H can be used for an average of 11 cycles (Fig. 4), A500R – 9 cycles, A100 – 8 cycles, which indicates a sufficiently high stability of the obtained preparations.

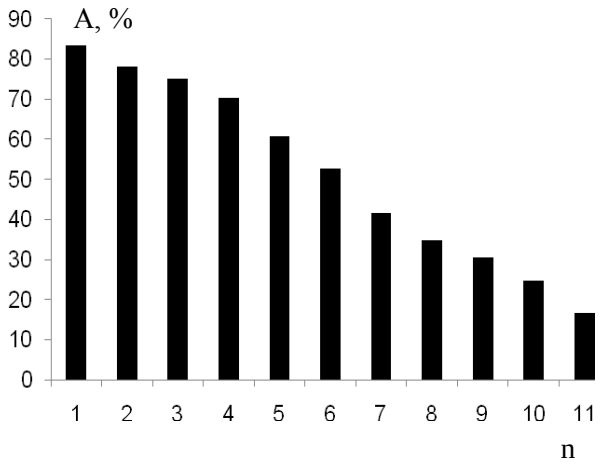

Fig. 4. The multiplicity of the use of immobilized inulinase in the reaction of hydrolysainulin. A– the activity (% of the maximum); n – the number of reaction cycles

Рис. 4. Кратность использования иммобилизованной инулиназы в реакции гидролиза инулина. А – активность (% от максимальной); n – количество реакционных циклов

Despite the decrease in the maximum reaction rate of inulin hydrolysis and catalytic activity compared to free inulinase, a significant advantage is the possibility of reuse of a heterogeneous biocatalyst.

The results obtained during the experiment confirm the relevance and prospects of further investigation of biocatalysts based on super-crosslinked sorbents.

CONCLUSION

The paper considers the regularities of adsorption immobilization of inulinase on macroporous super-crosslinked ion exchangers. Optimal conditions for the immobilization process were established: time – 4 h, pH – 4.7, sorbate concentration – $(2\text{-}2.5)\cdot10^{-2}$ mmol/l. The catalytic activity of heterogeneous biocatalysts is 64.8-83.5% of the activity of the native enzyme. One of the advantages of immobilized enzymes is the possibility of their reuse. It has been established that immobilized inulinase can be used during 8-11 cycles in the inulin hydrolysis reaction.

It is noted that inulinase sorption isotherms on the carriers under consideration have a polymolecular character. The values of sorption parameters for prediction and description of polymolecular sorption on carriers are calculated using the BET equations. The data obtained in this work confirm the expediency of using super-crosslinked ion exchangers C100H and A500R for the immobilization of inulinase.

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Авторы заявляют об отсутствии конфликта интересов, требующего раскрытия в данной статье.

ЛИТЕРАТУРА

1. **Кайшев В.Г., Лукин Н.Д., Серегин С.Н., Корниенко А.В.** Рынок инулина в России: возможности развития сырьевой базы и необходимые ресурсы для создания современного отечественного производства. Пищ. промышл. 2018. № 5. С. 8-17.
2. **Singh R.S., Singh R.P.** Production of Fructooligosaccharides from Inulin by Enddoinulinases and Their Prebiotic Potential. *Food Technol. Biotechnol.* 2010. V. 48. N 4. P. 435-450.
3. **Singh R.S., Chauhan K.** Production, purification, characterization and applications of fungal inulinases. *Curr. Biotechnol.* 2018. V. 7. N 3. P. 242-260. DOI: 10.2174/2211550105666160512142330.
4. **Zhen-Ming Chi, Tong Zhang, Tian-Shu Cao, Xiao-Yan Liu, Wei Cui, Chun-Hai Zhao.** Biotechnological potential of inulin for bioprocesses. *Bioresour. Technol.* 2011. V. 102. N 6. P. 4295-4303. DOI: 10.1016/j.biortech.2010.12.086.
5. **Neeraj G., Ravi S., Somdutt R., Ravi S. K., Kumar V.** Immobilized inulinase: a new horizon of primary importance, stimulating the production of sweeteners and prebiotics. *Critical Rev. Biotechnol.* 2018. V. 38. N 3. P. 409-422. DOI: 10.1080/07388551.2017.1359146.
6. **Mateo C., Palomo J.M., Fernandez-Lorente G., Guisan J.M., Fernandez-Lafuente R.** Improvement of enzyme activity, stability and selectivity via immobilization techniques. *Enzyme Microb. Technol.* 2007. V. 40. N 6. P. 1451-1463. DOI: 10.1016/j.enzmictec.2007.01.018.
7. **Сидоров А.И., Ожимкова Е.В., Тихонов Б.Б.** Ферменты: свойства, иммобилизация, применение. Тверь: ТГТУ. 2010. 139 с.
8. **Сазыкин Ю.О., Орехов С.Н., Чакалева И.И.** Биотехнология. М.: Академия. 2008. 256 с.
9. **Holyavka M.G., Kayumov A.R., Baydamshina D.R., Koroleva V.A., Trizna E.Yu., Trushin M.V., Artyukhov V.G.** Efficient fructose production from plant extracts by immobilized inulinases from Kluyveromyces marxianus and Helianthus tuberosus. *Int. J. Biol. Macromol.* 2018. V. 115. P. 829–834. DOI: 10.1016/j.ijbiomac.2018.04.107.

REFERENCES

1. **Kaishev V.G., Lukin N.D., Seregin S.N., Kornienko A.V.** Inulin market in Russia: opportunities for the development of the raw material base and the necessary resources for the creation of modern domestic production. *Pishch. Promyshl.* 2018. N 5. P. 8-17 (in Russian).
2. **Singh R.S., Singh R.P.** Production of Fructooligosaccharides from Inulin by Enddoinulinases and Their Prebiotic Potential. *Food Technol. Biotechnol.* 2010. V. 48. N 4. P. 435-450.
3. **Singh R.S., Chauhan K.** Production, purification, characterization and applications of fungal inulinases. *Curr. Biotechnol.* 2018. V. 7. N 3. P. 242-260. DOI: 10.2174/2211550105666160512142330.
4. **Zhen-Ming Chi, Tong Zhang, Tian-Shu Cao, Xiao-Yan Liu, Wei Cui, Chun-Hai Zhao.** Biotechnological potential of inulin for bioprocesses. *Bioresour. Technol.* 2011. V. 102. N 6. P. 4295-4303. DOI: 10.1016/j.biortech.2010.12.086.
5. **Neeraj G., Ravi S., Somdutt R., Ravi S. K., Kumar V.** Immobilized inulinase: a new horizon of primary importance, stimulating the production of sweeteners and prebiotics. *Critical Rev. Biotechnol.* 2018. V. 38. N 3. P. 409-422. DOI: 10.1080/07388551.2017.1359146.
6. **Mateo C., Palomo J.M., Fernandez-Lorente G., Guisan J.M., Fernandez-Lafuente R.** Improvement of enzyme activity, stability and selectivity via immobilization techniques. *Enzyme Microb. Technol.* 2007. V. 40. N 6. P. 1451-1463. DOI: 10.1016/j.enzmictec.2007.01.018.
7. **Sidorov A.I., Ozhimkova E.V., Tikhonov B.B.** Ferments: properties, immobilization, application. Tver': TGTU. 2010. 139 p. (in Russian).
8. **Sazykin Yu.O., Orekhov S.N., Chakaleva I.I.** Biotechnology. M.: Academiya. 2008. 256 p. (in Russian).
9. **Holyavka M.G., Kayumov A.R., Baydamshina D.R., Koroleva V.A., Trizna E.Yu., Trushin M.V., Artyukhov V.G.** Efficient fructose production from plant extracts by immobilized inulinases from Kluyveromyces marxianus and Helianthus tuberosus. *Int. J. Biol. Macromol.* 2018. V. 115. P. 829–834. DOI: 10.1016/j.ijbiomac.2018.04.107.
10. **Grebennikova O.V., Mikhailova A.N., Molchanov V.P., Sulman A.M., Doluda V.Yu., Matveeva V.G.** Polymeric supports

10. **Гребенникова О.В., Михайлова А.Н., Молчанов В.П., Сульман А.М., Долуда В.Ю., Матвеева В.Г.** Полимерные носители для иммобилизации ферментов в синтезе биологически активных соединений. *Изв. вузов. Химия и хим. технология*. 2021. Т. 64. Вып. 1. С. 67-72. DOI: 10.6060/ivkkt.20216401.6223.
11. **Шкутина И.В., Стоянова О.Ф., Селеменев В.Ф.** Гидролиз инулина с помощью гетерогенных биокатализаторов. *Химия растит. сырья.* 2012. № 2. С. 2 –31.
12. **Shkutina I.V., Stoyanova O.F., Selemenev V.F.** Biocatalytic Properties of Immobilized Inulinase. *Russ. J. Bioorganic. Chem.* 2016. V. 42. N 7. P. 64–67. DOI: 10.1134/s1068162016070153.
13. **Davankov V.A., Tsyurupa M.P.** Hypercrosslinked polymeric networks and adsorbing materials, synthesis, structure, properties and application. New York: Elsevier. 2011. 648 p.
14. **Цюрупа М.П., Блинникова З.К., Павлова Л.А., Пастухов А.В., Даванков В.А.** Гиперкросшитый полистирол: полвека от нетривиальной идеи до промышленной реализации. *Лаборатория и пр-во*. 2020. Т. 11. № 1. С. 86-96. DOI: 10.32757/2619-0923.2020.1.11.86.96.
15. **Селеменев В.Ф., Славинская Г.В., Хохлов В.Ю., Иванов В.А., Горшков В.И., Тимофеевская В.Д.** Практикум по ионному обмену. Воронеж: Изд-во Воронеж. гос. ун-та. 2004. 160 с.
16. **Nakamura T., Nakatsu S.** General properties of extracellar inulase from Penicillum. *J. Agr. Chem. Loc. Jap.* 1997. V. 1. N 12. P. 681–689. DOI: 10.1271/nogeikagaku1924.51.12_681.
17. **Холявка М.Г., Артюхов В.Г., Макин С.М.** Исследование надмолекулярной организации инулиназ из продуцентов рода Aspegillus с помощью некоторых численных и экспериментальных методов. *Биофизика*. 2015. Т. 60. № 4. С. 639-645. DOI: 10.1134/S0006350915040144.
18. **Филиппов О.А., Тихомирова Т.И., Цизин Г.И., Золотов Ю.А.** Динамическое концентрирование органических веществ на неполярных сорбентах. *Журн. аналит. химии.* 2003. Т. 58. № 5. С. 354–479. DOI: 10.1023/A:1024796928538.
19. **Ланин С.Н., Рычкова С.А., Виноградов А.Е., Вирясов М.Б., Востров И.А., Шаталов И.А.** Сорбция водорастворимых витаминов на сорбентах различной природы. *Сорбц. и хроматограф. проц.* 2015. Т. 15. № 2. С. 179–195.
20. **Шафигулин Р.В., Константинов А.В., Буланова А.В., Ильин М.М., Даванков В.А.** Сорбция фениламидных производных адамантана на сверхсшитом полистироле из водно-ацетонитрильных элюентов. *Журн. физ. химии.* 2016. Т. 90. № 11. С. 1729–1733. DOI: 10.7868/s0044453716110236.
21. **Варфоломеев С.Д.** Физическая и структурная химия ферментативного катализа. Системный подход: от первичной стуктуры до элементарного акта. *Кинетика и катализ.* 2007. Т. 48. № 4. С. 499-509. DOI: 10.1134/S0023158407040039.
22. **Amanollah E., Jafar S., Soltan M.** What is the correct form of BET isotherm for modeling liquid phase adsorption? *Adsorption*. 2009. V. 15. N 1. P. 65–73. DOI: 10.1007/s10450-009-9151-3.
23. **Котова Д.Л., Фам Тхи Гам, Крысанова Т.А., Васильева С.Ю., Тимченко Ю.А., Бекетов Б.Н.** Описание изотермы сорбции пиридоксина на клиноптилолитовом туфе. *Сорбц. и хроматограф. проц.* 2014. Т. 14. № 4. С. 572-577.
24. **Смусева С.О., Мироненко Н.В., Селеменев В.Ф., Бородкина Т.А.** Изотерма сорбции сапонина Quillaja Saponaria Molina на полимерном сверхсшитом сорбенте MN-200. *Сорбц. и хроматограф. проц.* 2017. Т. 17. № 4. С. 557-563. DOI: 10.17308/sorpchrom.2017.17/413.

for enzymes immobilization in synthesis of biologically active compounds. *ChemChemTech [Izv. Vyssh. Uchebn. Zaved. Khim. Khim. Tekhnol.]*. 2021. V. 64. N 1. P. 67-72. DOI: 10.6060/ivkkt.20216401.6223.
11. **Shkutina I.V., Stoyanova O.F., Selemenev V.F.** Hydrolysis of inulin using heterogeneous biocatalysts. *Khimiya Rastit. Syr'ya.* 2012. N 2. P. 2 -31 (in Russian).
12. **Shkutina I.V., Stoyanova O.F., Selemenev V.F.** Biocatalytic Properties of Immobilized Inulinase. *Russ. J. Bioorganic. Chem.* 2016. V. 42. N 7. P. 64–67. DOI: 10.1134/s1068162016070153.
13. **Davankov V.A., Tsyurupa M.P.** Hypercrosslinked polymeric networks and adsorbing materials, synthesis, structure, properties and application. New York: Elsevier. 2011. 648 p.
14. **Tsyurupa M.P., Blinnikova Z.K., Pavlova L.A., Pastukhov A.V., Davankov V.A.** Hypercrossed polystyrene: half a century from a non-trivial idea to industrial implementation. *Laboratoriya Pr-vo*. 2020. V. 11. N 1. P. 86-96 (in Russian). DOI: 10.32757/2619-0923.2020.1.11.86.96.
15. **Selemenev V.F., Slavinskaya G.V., Khokhlov V.Yu., Ivanov V.A., Gorshkov V.I., Timofeevskaya V.D.** Workshop on ion exchange. Voronezh: Izd. Voronezh. Gos. Univ. 2004. 160 p. (in Russian).
16. **Nakamura T., Nakatsu S.** General properties of extracellar inulase from Penicillum. *J. Agr. Chem. Loc. Jap.* 1997. V. 1. N 12. P. 681–689. DOI: 10.1271/nogeikagaku1924.51.12_681.
17. **Kholyavka M.G., Artyukhov V.G., Makin S.M.** Investigation of the supramolecular organization of inulinases from producers of the genus Aspegillus using some numerical and experimental methods. *Biofizika*. 2015. V. 60. N 4. P. 639-645 (in Russian). DOI: 10.1134/S0006350915040144.
18. **Filippov O.A., Tikhomirova T.I., Tsizin G.I., Zolotov Yu.A.** Dynamic concentration of organic substances on nonpolar adsorbents. *Russ. J. Analyt. Chem*. 2003. V. 58. N 5. P. 454–479 (in Russian). DOI: 10.1023/A:1024796928538.
19. **Lanin S.N., Rychkova S.A., Vinogradov A.E., Viryasov M.B., Vostrov I.A., Shatalov I.A.** Sorption of water-soluble vitamins on sorbents of various nature. *Sorbts. Khromatograf. Prots*. 2015. V. 15. N 2. P. 179–195 (in Russian).
20. **Shafigulin R.V., Konstantinov A.V., Bulanova A.V., Ilyin M.M., Davankov V.A.** Sorption of phenylamide derivatives of adamantane on super-stitched polystyrene from water-acetonitrile eluents. *Zhurn. Fizich. Khim.* 2016. V. 90. N 11. P. 1729–1733 (in Russian). DOI: 10.7868/s0044453716110236.
21. **Varfolomeev S.D.** Physical and structural chemistry of enzymatic catalysis. A systematic approach: from the primary structure to the elementary act. *Kinetika Kataliz*. 2007. V. 48. N 4. P. 499-509 (in Russian). DOI: 10.1134/S0023158407040039.
22. **Amanollah E., Jafar S., Soltan M.** What is the correct form of BET isotherm for modeling liquid phase adsorption? *Adsorption*. 2009. V. 15. N 1. P. 65–73. DOI: 10.1007/s10450-009-9151-3.
23. **Kotova D.L., FamThi Gam, Krysanova T.A., Vasilyeva S.Yu., Timchenko Yu.A., Beketov B.N.** Description of the pyridoxine sorption isotherm on clinoptilolite tuff. *Sorbts. Khromatograf. Prots.* 2014. V. 14. N 4. P. 572-577 (in Russian).
24. **Smuseva S.O., Mironenko N.V., Selemenev V.F., Borodkina T.A.** Isotherm of saponin sorption Quillaja Saponaria Molina on polymer super-crosslinked sorbent MN-200. *Sorbts. Khromatograf. Prots.* 2017. V. 17. N 4. P. 557-563 (in Russian). DOI: 10.17308/sorpchrom.2017.17/413.

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