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RESEARCH ARTICLE

Use of ion-exchange resins for purification of L-lactic acid-containing *Rhizopus oryzae* fermentation broth

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Abstract

Objectives. The work set out to describe conditions for the purification of a model fermentation broth for cultivating the lactic acid-containing micromycete *Rhizopus oryzae* from impurities of inorganic salts using ion-exchange resins under dynamic conditions.

Methods. The solutions collected for analysis were examined using precipitation titration to determine the concentration of chlorides along with a qualitative reaction with Nessler's reagent to ascertain the presence of ammonium ions. The concentration of lactic acid was evaluated spectrophotometrically using iron(III) chloride. The total nitrogen content was determined by high-temperature catalytic combustion on a Formacs HT TOC/TN Analyzer (Netherlands). The content of trace elements and macroelements in the samples was determined using an iCAP 6300 Duo inductively coupled plasma emission spectrometer (United Kingdom).

Results. Purification of the model broth under the described conditions was carried out by successive filtration through the cation exchanger KU-2-8 in the H-form and subsequently through a mixture of weakly basic A847 and strongly basic AV-17-8 anion exchangers in the OH-form taken in a one-to-one ratio. The breakthrough of impurity ions into the solution was shown to occur after passing 30-fold and 10-fold volumes of the model broth relative to the volume of the cation-exchange and anion-exchange resins, respectively. The dynamic exchange capacity prior to breakthrough was determined as follows: 0.35 mmol-eq/cm^3 for the anion-exchange column and 1.61 mmol-eq/cm^3 for the cation-exchange column. The following parameters were defined as column regeneration modes: 3-fold excess of 2 M H_2SO_4, 10-fold excess of distilled H_2O for cation exchange; for anion exchange, 3-fold excess of 2 M NaOH and 20-fold excess of H_2O.

Conclusions. The conducted studies showed that purification of the model fermentation broth of *Rhizopus oryzae* can be successfully implemented using ion-exchange resins. The model fermentation broth passing successively through cation-exchange and anion-exchange columns was shown to be purified from impurities of mineral salts while maintaining the concentration of lactic acid.

Keywords

lactic acid, purification, cation-exchange resin, anion-exchange resin, sorption, model solution

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НАУЧНАЯ СТАТЬЯ

Применение ионообменных смол для очистки ферментационного бульона *Rhizopus oryzae*, содержащего L-молочную кислоту

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Аннотация

Цели. Подбор условий очистки модельного ферментационного бульона для культивирования микромицета *Rhizopus oryzae*, содержащего молочную кислоту, от примесей неорганических солей с использованием ионообменных смол в динамических условиях.

Методы. Отбираемые на анализ растворы исследовали титриметрическим методом (осадительное титрование) для определения концентрации хлоридов и качественной реакцией с реактивом Несслера для определения наличия иона аммония. Концентрацию молочной кислоты определяли спектрофотометрически с применением хлорида железа(III). Содержание общего азота определяли методом высокотемпературного каталитического сжигания на анализаторе Formacs HT TOC/TN Analyzer (Нидерланды). Определение содержания микро- и макроэлементов в исследуемых пробах проводили с помощью эмиссионного спектрометра с индуктивно-связанной плазмой iCAP 6300 Duo (Великобритания).

Результаты. Определили, что очистку модельного бульона в данных условиях необходимо проводить последовательным фильтрованием через катионит КУ-2-8 в Н-форме, а затем через смесь слабоосновного А847 и сильноосновного АВ-17-8 анионитов в ОН-форме, взятых в соотношении один к одному. Установили, что проскок примесных ионов в раствор наступает после прохождения 30-кратного и 10-кратного объемов модельного бульона по отношению к объему катионообменной и анионообменных смол соответственно. Определили динамическую обменную емкость до проскока, которая для анионообменной колонки составила 0.35 ммоль-экв/см^3, а для катионообменной колонки — 1.61 ммоль-экв/см^3. В качестве режимов регенерации колонок определены следующие параметры: 3-кратный избыток 2 M H_2SO_4, 10-кратный избыток дистиллированной H_2O для катионообменной и 3-кратный избыток 2 M NaOH, 20-кратный избыток H_2O для анионообменной колонок.

Выводы. Проведенные исследования показали, что очистка модельного ферментационного бульона *Rhizopus oryzae* может быть успешно реализована с применением ионообменных смол. Установлено, что этот раствор, проходя последовательно через катионообменные и анионообменные колонки, очищается от примесей минеральных солей, при этом концентрация молочной кислоты не снижается.

Ключевые слова

молочная кислота, очистка, катионит, анионит, сорбция

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INTRODUCTION

Lactic acid (LA) is one of the most important chemicals used in various industries and cosmetology. Between 2019 and 2024, the demand for LA on the global market has increased at an average annual growth rate of 18.7% [1]. According to the relevant United States classification, L(+)-LA belongs to Generally Recognized As Safe (GRAS) compounds that are deemed harmless for use in food products [2–3].

The increased interest in LA can be largely attributed to the demand for polylactide, of which the former is a monomer. Polylactide or polylactic acid is widely used as a biodegradable plastic in the food industry, cosmetics, and medicine [1–7].

Fermentation is generally considered to be a preferred method for LA production as compared to chemical synthesis due to the possibility of obtaining specific LA isomers. However, extraction and separation of LA from microbial fermentation media represent relatively complex and expensive processes. Since the L-isomer is the only biologically active form of LA, i.e., capable of being quickly and completely assimilated by human or animal organisms, the main efforts in LA biosynthesis are aimed at using strains that form the L-isomer.

Various representatives of bacteria and fungi have the ability to synthesize LA. Among microscopic fungi, special attention is paid to mucoral fungi of the genus *Rhizopus*. The *R. oryzae* and *R. arrhizus* species, which can be grown on cheap and simple media with hexoses and pentoses or glycerol as a carbon source, are most resistant to low pH [8, 9].

The costs of isolating LA from fermentation broth (FB) and purification can reach 50% of the total costs of the process. Until now, the most common production method involves the neutralization of the acid formed in the biotechnological process with calcium compounds and the subsequent precipitation of calcium in the form of sulfate, which forms a large amount of gypsum, resulting in LA of low purity. Various approaches to the isolation and purification of LA aimed at overcoming these drawbacks include solvent extraction, membrane separation, ion-exchange chromatography, and reactive distillation [8, 10]. These separation technologies have both advantages and disadvantages. In any case, it is difficult to effectively separate and purify LA using a single method; in many cases, better results can be obtained by integrating several technologies.

Under actual conditions, FB purification is generally carried out over several stages, in particular, when using membrane technologies [9, 10]. Membrane filtration, which represents an important stage of LA isolation, is often used in combination with other methods to obtain high-purity LA. The first stage involves the removal of suspended particles (first of all, biomass) using ultrafiltration. The second stage can be nanofiltration, which is used to separate LA from carbohydrates (whose molecules have a higher molecular weight and are additionally binded with an additional hydration shell) and a significant portion of the salts contained in the FB. Since membrane filtration does not remove all impurity ions, further purification of the LA solution using ion exchange technology is usually required.

In recent decades, ion exchange technology has become a key technological process in various industries, resulting in a proliferation of available synthetic ion exchange materials marketed under different brands and having various properties. In Russia, as elsewhere, ion exchange filter methods for softening and purifying water are used in around 75% of cases [11, 12]. Ion exchange processes occupy an important place in the technologies for extracting almost all rare, widely dispersed, and radioactive elements, especially in the nuclear industry. Ion exchange resins are used to separate multicomponent mixtures of acids and their salts, extract and remove various metals in the metallurgical and chemical industries, as well as to purify target components in the food, pharmaceutical, and medical industries [1, 3, 11–18]. The efficiency of the separation and sorption process during ion exchange depends on the type of ion exchanger, its charge and size, structural characteristics, the size of ions in hydrated and dehydrated states, and the temperature of the environment.

There are two main approaches to the recovery and purification of LA by adsorption using ion-exchange resins. Thus, the works of various research groups demonstrate the possibility of adsorbing LA from the FB by ion-exchange resin [19–21]. This strategy involves neutralization (pH control) of the FB by exchanging lactate ions for the counterions obtained from an anion exchanger. The subsequent elution of resin-bound lactate, e.g. using 1 *M* hydrochloric acid solution, leads to complete LA extraction [22].

In the second case, a type of ion-exchange system is selected that does not sorb lactate ion or absorbs it in an amount of no more than 10%, but removes impurity ions after the FB passes through a cation exchanger and then through an anion exchanger. For example, in [23] a two-stage LA purification process was proposed: first, a strongly acidic cation exchange resin was used to convert sodium lactate obtained after fermentation into LA and remove cations present in the nutrient medium, then LA was separated from other anions from the broth using a weakly basic anion exchanger. However, one volume of cation exchanger was only sufficient for three-fold volume of the broth; subsequently, regeneration was required.

Since the extraction and separation of LA from the microbial fermentation medium are labor-intensive processes, the problem of simplifying the technology and improving the quality of purification of the target product is acute. Our research group is currently developing a highly effective strain-producer for enantiomerically pure L-LA involving optimal cultivation modes for the LA purification membrane technology (LA isolation and purification is significantly simplified due to the absence of an alkaline neutralization agent). At the second stage, the additional purification of LA from impurities of inorganic ions is carried out using ion exchange approaches.

In the present work, the ion exchange purification of LA on a model system using organic resins was studied. This approach can be used to remove impurity anions and cations from FB, while LA with a concentration of 8–10% remains in solution. The proposed process, which uses no additional chemical reagents, can be used to obtain LA as a high-purity aqueous solution, which can be subsequently concentrated to obtain LA of commercial quality (50–90%).

EXPERIMENTAL

The following reagents were used in the work: metal salts (chemically pure and analytical grade, *Khimreaktivsnab*, Russia) $K_2HPO_4 \cdot 3H_2O$, NaCl, NH_4NO_3, $MgSO_4 \cdot 7H_2O$, $ZnSO_4 \cdot 7H_2O$, NaOH, H_2SO_4, $BaCl_2$, $AgNO_3$, phenolphthalein, Nessler reagent. The following types of resins were used in the experiments: Purolite A847, AV-17-8, KU-2-8. Weakly basic gel anion exchanger Purolite A847 (*Purolite*, Romania) is manufactured in hydroxide form with a polyacrylic matrix containing a tertiary ammonium functional group (type II). This resin is an analog of AN-31, Granion AWA-G1, etc. The strongly basic gel anion exchanger AV-17-8 (GOST 20301-74[1], *Tokem*, Russia) having a matrix based on a styrene-divinylbenzene copolymer is produced in chloride form and contains a functional group of quaternary ammonium (type I). This resin is an analog of Purolite A400/A300, Lewatit M-500, Amberlite IRA 402/420, Dowex SBR-P/Maraton A, etc. Strongly acid cation gel exchanger KU-2-8 (TU 6-07-493-95, *Tokem*) produced in sodium form is a sulfonated copolymer of styrene and divinylbenzene with an SO_3H-active group. This resin is an analog of Purolite C100, Lewatit S-100, Amberlite IR-120, Amberjet 1200, Dowex HCR-S/S, Dowex Marathon C. The granule size of all the resins is 0.4–0.6 mm. These commercially available resins are widely used in various separation and purification processes in Russia.

The choice of gel-type ion exchangers is due to the fact that, when using porous anion-exchange resins, a significant proportion of LA will be retained in their pores, having an average size of 600–1500 µm.

The preparation of ion exchangers for work was carried out in accordance with GOST 10896-78[2]. The static exchange capacity of anion exchangers in the OH form was 0.9 mmol-eq/cm^3 for AV-17-8 and 1.1 mmol-eq/cm^3 for A847. The static exchange capacity of KU-2-8 in the H form was 2.1 mmol-eq/cm^3.

The model medium composition (in g/dm^3) is as follows: $K_2HPO_4 \cdot 3H_2O$ — 1.78; NaCl — 0.41; NH_4NO_3 — 2.56; $MgSO_4 \cdot 7H_2O$ — 0.074; $ZnSO_4 \cdot 7H_2O$ — 0.0086; LA — 81.25.

To prepare 1000 cm^3 of the medium, 100 cm^3 of 80% LA (sold by *Paritrade* Russia; manufactured in China) were diluted with distilled water in a 1-dm^3 measuring flask without bringing the volume to the mark. Following the addition and dissolution of pre-weighed salts, the volume was brought to the mark with distilled water. The pH value of the model FB was 1.9.

The process was studied under dynamic conditions as follows. A cotton plug was placed on the bottom of a burette (ion exchange column) with a working volume of 25 cm^3. Next, the first burette was filled with 25 cm^3 of the prepared cation exchange resin, and the second one—with anion exchange resin. The resins were placed in a swollen state. The model FB was successively passed through columns having a bed height of 350 mm and a diameter of 10 mm using the descending flow method at a rate of 5 cm^3/min. The ion and LA content, as well as the solution pH, were monitored at the outlet of the anion-exchange column. The process diagram is shown in Fig. 1a.

The column regeneration modes were investigated after completing the experiments on purifying the model FB (Fig. 1b). Thus, 100 cm^3 of 2 *M* NaOH solution were passed through an anion-exchange column containing a resin with a volume of 25 cm^3. 100 cm^3 of 2 *M* H_2SO_4 solution were passed through a cation-exchange column with a resin volume of 25 cm^3. The flow rate was 5 cm^3/min. Three portions of 33 cm^3 were collected at the outlet of each column and analyzed. Then, distilled water was passed through the columns to determine the amount of water required to

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[1] GOST 20301-74. State Standard of the USSR. Ion-exchange resins. Anionites. Specifications. Moscow: Izdatelstvo standartov; 1992. https://meganorm.ru/Data2/1/4294833/4294833041.pdf. Accessed June 06, 2025.

[2] GOST 10896-78. Interstate Standard. Ion-exchange resins. Preparation of specimens for test. Moscow: IPK Izdatelstvo standartov; 1999. https://meganorm.ru/Data2/1/4294840/4294840000.pdf. Accessed June 06, 2025.

wash the resins from the regeneration solution. Samples of 25 cm^3 were collected at the outlet of each column. A total of 20 column volumes of water were passed through each column. The samples were analyzed for alkalinity (using phenolphthalein) and for sulfate ions (using barium chloride); pH was also monitored.

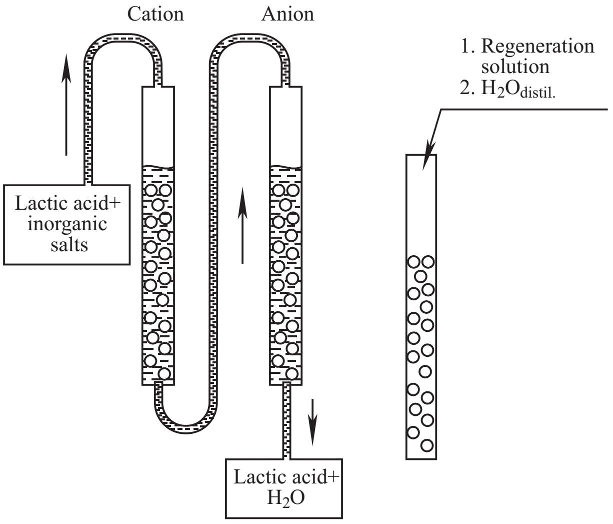

Fig. 1. (a) Scheme of ion-exchange purification of the model fermentation broth (FB) of *R. oryzae*; (b) scheme of regeneration of ion-exchange columns

The dynamic exchange capacity before breakthrough (DEC_{br}, mmol-eq/cm^3) was obtained according to the relationship presented in general form as:

$$DEC_{br} = \frac{\left(C_{init} - C_{res}\right)\cdot V_{FB}}{M_e \cdot V_{resin}}, \qquad (1)$$

where C_{init} is the initial concentration of the impurity ion in the model FB, mg/dm^3; C_{res} is the residual concentration of the impurity ion at the outlet of the column, mg/dm^3; V_{FB} is the volume of the model solution that passed through the column before breakthrough, dm^3; V_{resin} is the volume of resin in the column, cm^3; M_e is the molar mass of the ion equivalent, g/mol-eq; ½Mg^{2+}, ½Zn^{2+}, NH_4^+, K^+, Na^+, Cl^-, NO_3^-, HPO_4^-, ½SO_4^{2-}.

The solutions collected for analysis were examined by the titrimetric method (precipitation titration) to determine the concentration of chlorides [RD 52.24.407-2017] (detection limit is 2 mg/dm^3) and by the qualitative reaction with Nessler reagent to determine the presence of ammonium ion. The concentration of LA was determined spectrophotometrically using iron(III) chloride [24]. The content of total nitrogen was determined by high-temperature catalytic combustion on a Formacs HT TOC/TN Analyzer (*Skalar*, Netherlands) (detection limit is 0.008 mg/dm^3).

The content of trace- and macroelements in the studied samples was determined using an iCAP 6300 Duo inductively coupled plasma (ICP) emission spectrometer (*Thermo Scientific*, United Kingdom). Samples for analysis were diluted 100-fold and acidified with hydrochloric acid (special purity grade, *Sigma Tek*, Khimki, Russia) in a ratio of 1/100. The device was calibrated using multi-element standards (ICP multi-element standard solution IV and IX, *Merck*, Germany; ICP multi-element standard solution VI, *Fluka*, Switzerland) and single-element standards P (CGP10) and Mg (CGMG10) (*Inorganic Ventures*, USA), as well as CaO and Na_2SO_4 (special purity grade). Scandium (5 mg/dm^3) (Scandium ICP standard, *Fluka*, Switzerland) was used as an internal standard. Deionized water (18 MOhm) was used to prepare calibration standards and dilute samples. Concentrated hydrochloric acid (1/100) was added to the standards. The detection limit (3×SD, mg/dm^3) was 0.003 for Ca (λ = 317.933), 0.03 for K (λ = 769.896), 0.03 for Mg (λ = 279.079), 0.03 for Na (λ = 589.592), 0.004 for P (λ = 213.618), 0.03 for S (λ = 182.034), and 0.4 for Zn (λ = 213.856), where SD is the standard deviation, and λ is the element detection wavelength, nm. A detailed description of the spectrometer characteristics is presented in [25].

The solutions pH measurements were carried out on a tabletop pH meter AQUASEARCHER^{TM} AB41PH (*OHAUS*, China) with a resolution of 0.1 to 0.001.

RESULTS AND DISCUSSION

In this work, we studied the processes of purification of model FB using ion-exchange resins under dynamic conditions. Provided that neutralizing agents were not used, the composition of the model FB was close to the composition of the mineral medium for cultivating *R. oryzae*. Table 1 shows data on the composition of impurities in 8% LA from different manufacturers. LA purchased from *Paritrade*, Russia, was used to prepare the FB.

The adsorption capacity of ion-exchange resins with respect to impurity ions from a model FB was studied as follows. A model FB with a volume of 500 cm^3 was passed successively through a burette filled with a strongly acidic gel cationite KU-2-8 in the H-form, and then through a weakly basic gel anionite Purolite A847 in OH-form. The solution feed rate was 5 cm^3/min is concomitant with state-of-the art technological processes. Preliminary experiments showed that an increase in the flow rate decreases the breakthrough (appearance) time of ions, which increases the amount of impurities remaining in the solution after purification. At the outlet, the solution was collected for analysis in portions of 50 cm^3, which is double the volume from each ionite. Thus, the samples with the phase volume ratios V_{FB}/V_{resin} = 2, 4, 6, 8, 9, 10, 12, 14, 16, 18, and 20 were obtained.

Table 1. Elemental composition of 8% LA from various manufacturers

Type of solution	Concentration, mg/dm^3							
	Ca	K	Mg	Na	P	S	Zn	N
LA (*Paritrade*, Russia)	0.4	BDL[3]	0.1	7.9	1.1	4.7	0.1	13.7
LA (chemically pure, *NevaReaktiv*, Russia)	0.3	1.5	BDL	17.2	6.2	14.8	BDL	18.0
LA (*Panreac*, Spain)	0.1	BDL	BDL	0.5	BDL	7.2	0.0	0.7

To assess the sorption characteristics of the cation exchanger and anion exchanger, the degree of ion sorption was calculated from the V_{FB}/V_{resin} ratio (Figs. 2 and 3). It can be seen that most of the analyzed impurities are almost completely removed from the model FB with a volume of 500 cm^3 ($V_{FB}/V_{resin} = 20$; Figs. 2 and 3). Even in the last portion, their content did not exceed 11 mg/dm^3 (0.001%). The exception was sulfur (represented in the studied medium by sulfate ions). Even in the first fractions (V_{FB}/V_{resin} = 2–4), the sulfur content did not fall below 50 mg/dm^3 (sorption degree 80%, Fig. 3); in the subsequent ones, it even increased. This means that the efficiency of this type of anion exchanger in relation to sulfates is insufficient.

Since lactate ion is also capable of being sorbed on the anion exchanger, its concentration was monitored in each sample. According to the obtained results, following the passage of 50 cm^3 of FB, the LA concentration dropped sharply; subsequently in the sample $V_{FB}/V_{resin} = 6$, it was restored and its concentration further increased. This can be explained by the fact that the initial content of all anions in FB is much lower in relation to the anion exchanger capacity. As the concentration of strong acid anions increases, the weaker lactate ion passes into solution as a result of exchange for chlorides, nitrates, phosphates and sulfates.

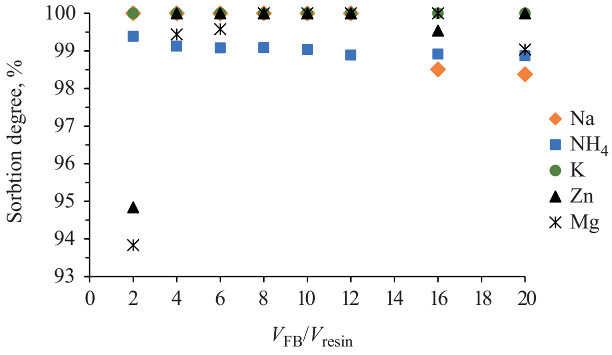

Fig. 2. Dynamics of sorption of impurity cations from a model FB by cation exchanger KU-2-8 (H)

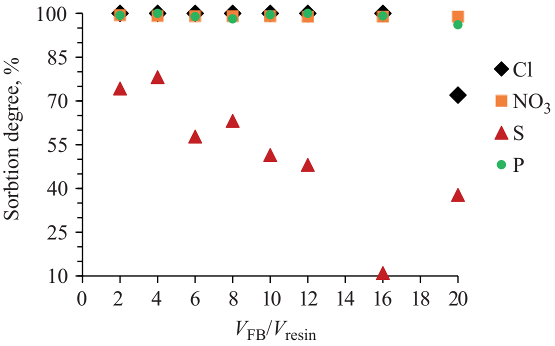

Fig. 3. Dynamics of sorption of impurity anions from a model FB by the anionite Purolite A847 (OH)

Additional qualitative analysis was performed using the Nessler method to determine ammonium ions. Since this method is sufficiently sensitive to determine microscopic amounts (up to 0.001% by volume) of ammonia, trace amounts of NH^{4+} ions were found in all samples. However, with a fairly high nitrogen concentration in the initial FB (i.e., NH^{4+} and NO^{3-} ions), its concentration in the obtained samples did not exceed 11 mg/L according to the ICP analysis data. This indicates the effectiveness of ion-exchange purification. Chloride ions begin to be detected only in the sample with a phase volume ratio of $V_{FB}/V_{resin} = 20$.

Next, we studied the effect of multiple passes of the model FB through ion-exchange resins on the degree of its purification. For this, we used the KU-2-8 and A847 ion exchange resins. The model FB with a volume of 250 cm^3 (a tenfold excess to the volume of each resin) was passed through the columns at a rate of 5 cm^3/min, and then returned to the original container. Fractions of 5 cm^3 were collected for analysis every hour. According to the analysis of the obtained samples (given in Table 2), most elements were almost completely removed with residual concentrations not exceeding 12 mg/dm^3. This is consistent with the data of the previous experiment for the sample $V_{FB}/V_{resin} = 10$.

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[3] Here and below BDL means "below the detection limit."

As in the previous experiment, the sorption of sulfates was not complete, but only 50–60%. This may be due to both their very high initial concentration compared to most other elements and the insufficient specific affinity of the anion exchanger to sulfates. In addition, when carrying out purification by this method, LA losses of 6–20% are observed. Thus, multiple passing of the model FB through ion-exchange resins is ineffective and labor-intensive; since the sorption rate is quite fast, the purification was carried out in subsequent experiments by descending flow to pass the model FB once through the resins.

To assess the possibility of improving the sorption of sulfate ions, experiments were carried out using a combination of strongly basic and weakly basic gel-type anion exchangers with different ratios. AV-17-8 (OH) was chosen as the strongly basic anion exchanger. A previous study of the selectivity of the AV-17-8 anion exchanger with respect to the anions SO_4^{2-}, NO_3^-, and Cl^- [13] showed that the main factor influencing the selectivity of ion exchange is electroselectivity, i.e., the preferential sorption of ions with a higher charge—in our case, the sulfate ion. In the case of anions having the same charge, selectivity is determined by the radius of the ion and its hydration energy. It was shown in [13, 26] that increased ion size results in a decreased tendency to hydration. Therefore, a strongly basic anionite exhibits greater selectivity with respect to nitrate ions as compared to Cl ions.

In the next experiment, the first burette was filled with the KU-2-8 cation exchanger (25 cm^3), while the second was filled in turn with two types of anion exchangers such that the total volume was 25 cm^3. The ratio of the A847 and AV-17-8 anion exchangers was 50/50 or 75/25. The obtained results were compared with the data of the experiment in which only A847 was used. Samples obtained after passing 250 cm^3 (V_{FB}/V_{resin} = 10), 400 cm^3 (V_{FB}/V_{resin} = 16), 500 cm^3 (V_{FB}/V_{resin} = 20), and 600 cm^3 (V_{FB}/V_{resin} = 24) of the model FB were collected and analyzed.

It is evident from the obtained data presented in Table 3 that most elements were almost completely extracted from the solution (~100% sorption) after passing even a 24-fold excess of FB relative to the volume of each resin and that the residual concentrations ranged from 0 to 13 mg/cm^3. However, the sorption of phosphates deteriorated sharply after replacing part of the A847 anion exchanger with AV-17-8 and increasing the volume of the passed solution (experiments 2 and 3, Table 3). At the same time, the efficiency of sulfate removal increased to 94%. Thus, a 10-fold excess of the passed solution is sufficient for effective purification of the model FB. In this case, the concentration of lactate ions does not decrease. Since the lowest residual content of impurities was noted for the KU-2-8+A847/AV-17-8(50/50) system with the ratio V_{FB}/V_{resin} = 10, these parameters were used for further purification of the real solution.

Table 2. Ionic composition of samples after cyclic purification of model FB depending on time

Time, h	Concentration, mg/dm^3								
	LA, %	Ca	K	Mg	Na	P	S	Zn	N
0	8.4	1.4	586.2	15.4	190.7	261.7	224.7	3.3	942.2
1	6.6	0.4	BDL	0.1	2.6	1.9	125.6	BDL	11.7
2	7.5	0.3	BDL	BDL	2.3	4.1	117.1	BDL	8.1
4	7.9	2.8	0.1	0.1	0.8	12.5	78.6	BDL	7.7

Table 3. Ionic composition of samples depending on the ratio of phase volumes V_{FB}/V_{resin} and the type of anion exchange column

V_{FB}/V_{resin}	Concentration, mg/dm^3								
	LA, %	Ca	K	Mg	Na	P	S	N	Zn
FB	8.4	1.4	586.2	15.4	190.7	261.7	224.7	942.2	3.3
1. KU-2-8+A847(100)									
10	9.6	BDL	BDL	BDL	BDL	0.8	115.2	9.2	BDL

Table 3. Continued

V_{FB}/V_{resin}	Concentration, mg/dm^3								
	LA, %	Ca	K	Mg	Na	P	S	N	Zn
16	10.1	0.4	BDL	BDL	2.72	1.5	211.1	10.4	BDL
20	9.7	0.4	BDL	0.14	2.95	7.1	147.6	10.8	BDL
2. KU-2-8+A847/AB-17-8(50/50)									
10	9.4	BDL	BDL	0.01	1.84	3.2	13.2	9.8	BDL
16	9.2	1.6	BDL	0.04	0.20	67.6	47.9	11.0	BDL
20	8.9	0.2	BDL	0.07	1.10	126.4	11.1	11.3	BDL
24	8.9	BDL	BDL	0.07	BDL	231.3	121.8	13.0	BDL
30	8.9	BDL	BDL	BDL	6.1	544.7	11.5	29.1	BDL
3. KU-2-8+A847/AB-17-8(75/25)									
10	9.3	0.6	BDL	0.1	3.4	1.1	14.6	9.3	BDL
16	9.2	2.1	BDL	0.3	0.2	25.3	28.8	12.5	BDL
20	9.2	0.1	BDL	BDL	2.3	63.3	12.6	10.1	BDL
24	9.5	0.1	BDL	BDL	1.9	186.4	111.2	12.8	BDL

Since the cationite did not reach full adsorption capacity, the model FB was continued to be passed through the KU-2-8+A847/AV-17-8(50/50) system. According to the ICP analysis, in the sample $V_{FB}/V_{resin} = 30$, the concentration of only Na cations increased to 6 mg/L (Table 3, experiment 2). This will be considered as the threshold concentration (before breakthrough). This assumption does not contradict the data on the sodium ion content in industrial LA samples, whose amount varies from 0.52 to 17.2 (Table 1). Since the qualitative reaction to the ammonium ion was negative, the increase in the nitrogen amount to 29.14 (Table 3, experiment 2) is probably due to the saturation of anionite with nitrate ions and their release into the solution.

Thus, under these conditions, in the case of using KU-2-8 in the H-form, effective purification from impurity cations is possible for a model FB that is 30 times larger than the volume of the cation exchanger.

Ion-exchange purification processes are impossible without effective regeneration of the resins used. As a result of contact with a model FB containing impurities, anion-exchange resins saturated with counterions extracted from the solution are deactivated to lose their original adsorption characteristics. However, it is possible to remove sorbed ions and restore the activity of the resin layer with the help of the regeneration process. Regeneration can be carried out repeatedly with little or no loss of capacity.

Next, we regenerated the column with KU-2-8 (25 cm^3) and the anion exchange column filled with a mixture of anion exchangers AV-17-8 (~12.5 cm^3) and A847 (~12.5 cm^3) (the anion exchangers were taken in a 50/50 ratio) after an experiment, in which 750 cm^3 of model FB passed through these resins. The columns were regenerated in two stages using the technique described in the experimental section. First, a three- to four-fold excess of the regenerate solution (eluent) was passed through the cation exchanger (anion exchanger). Next, repeatedly distilled water was passed in order to remove the regeneration products and excess regeneration solution from the ion exchanger layer.

As can be seen from Tables 4 and 5, the concentration of elements in the first portion of eluates is the highest, i.e., extraction (desorption) is effective. In subsequent samples, the concentration drops sharply; therefore, the amount of eluent can be reduced to a two- to three-fold excess relative to the resin volume. From Table 4 it is clear that the solution pH is quite high with a 5- to 15-fold excess of distilled water, decreasing to 7 only after passing

Table 4. Content of elements after regeneration of anion exchange column

Stages of filtration	Concentration, mg/dm^3								pH
	Ca	K	Mg	Na	P	S	N	Zn	
Eluate, portion 1	0.5	44.4	BDL	30880.0	463.3	2383.6	6835.5	BDL	14.8
Eluate, portion 2	5.0	51.6	BDL	38844.6	41.4	143.1	3296	0.1	14.7
Eluate, portion 3	4.0	69.1	1.1	39783.9	42.6	138.4	1266	0.1	14.4
5× H_2O	–	–	–	–	–	–	–	–	14.2
10× H_2O	–	–	–	–	–	–	–	–	12.3
15× H_2O	0.3	9.8	BDL	15.9	1.33	9.6	1.1	0.1	10.0
20× H_2O	0.2	2.7	BDL	1.6	BDL	BDL	0.4	BDL	7.2

Table 5. Content of elements after regeneration of cation exchange column

Stages of filtration	Concentration, mg/dm^3							pH
	K	Mg	Na	P	S	N	Zn	
Eluate, portion 1	11666.0	175.4	3881.4	29.5	41480.0	10760.0	41.4	2.1
Eluate, portion 2	3283.5	111.9	538.9	3.9	57953.8	1607.0	27.7	2.1
Eluate, portion 3	769.5	60.5	101.5	0.8	49813.2	151.1	15.7	2.1
5× H_2O	17.6	0.1	2.7	BDL	53.4	2.7	BDL	3.7
10× H_2O	3.4	0.1	1.2	BDL	BDL	0.7	0.1	5.8
15× H_2O	4.5	BDL	1.1	BDL	BDL	0.7	BDL	6.8

a 20-fold excess of water through the column. The solution contained virtually no impurities, i.e., regeneration was effective. In the case of a cation-exchange column, a sharp decrease in sulfate ions is observed already after a 5-fold excess of distilled water (Table 5). After a 10-fold excess of water, the test solution basically does not contain ions, and its pH increases to 5.8. Thus, under these conditions, a 10-fold excess of distilled water is sufficient.

The factors affecting the useful exchange capacity of resins include:

- the type of ions removed from the aqueous solution;
- the ratio of salt components in the aqueous solution;
- the pH value;
- the height of the layer;
- the flow rate of the filtered water;
- the intensity of operation.

In practice, the ion-exchange capacity of resins is determined by the degree of their saturation. This is due to the fact that, at a certain stage, the number of breakthroughs reaches the maximum permissible level, at which the quality of purification is significantly reduced. The formula for calculating the sorption capacity is given in the experimental section.

Table 6 shows the calculated values of the dynamic exchange capacity of ion-exchange resins for each impurity element contained in the model FB before

Table 6. Dynamic exchange capacity before the breakthrough (DEC_{br}) of cation exchange and anion exchange columns

FB components	$C_{initial}$, mg/dm^3	C_{res}, mg/dm^3	DEC_{br}, mmol-eq/cm^3
K	586.21	0	0.45
Mg	15.43	0	0.04
Na	190.84	6	0.24
Zn	3.39	0	0.01
S	224.71	13.2	0.13
P	261.82	3.2	0.08
Cl	250.03	0	0.07
N (NH_4^+)	522.41	0	0.87
N (NO_3^-)	420.75	9.2	0.07
∑anions	–		0.35
∑cations			1.61

breakthrough. They were obtained based on the results of the best experiment for the anion-exchange column (Table 3, experiment 2). The ratio for the cation-exchange column V_{FB}/V_{resin} = 30, for the anion-exchange V_{FB}/V_{resin} = 10. In this work, the breakthrough value was taken to be a residual concentration of the impurity element of no more than 14 mg/L. Thus, DEC_{br} for the anion exchange column was 0.35 mmol-eq/cm^3, and for the cation exchange column, DEC_{br} was 1.61 mmol-eq/cm^3. The DEC_{br} value depends on the concentration and nature of the sorbed ions and cations, as well as on the pH of the medium, the solution filtration rate, the size of the ion exchanger grains, the ratio of the ion exchanger layer height to its width, and other factors. As a rule, its value is significantly lower than the static exchange capacity.

CONCLUSIONS

The conducted studies demonstrate that purification of the model FB of *R. oryzae* can be successfully implemented using ion-exchange resins. The model FB passed successively through cation-exchange and anion-exchange columns was shown to be purified from mineral salt impurities while maintaining the concentration of LA. In the work, a strongly acidic gel cation exchanger KU-2-8 in the H-form was used as a cation absorber. For the sorption of anions, it is necessary to use a mixture of weakly basic gel A847 anion exchanger and strongly basic gel AV-17-8 anion exchanger in the OH-form taken in a ratio of 1/1. It is shown that the breakthrough of impurity ions into the solution occurs after passing a 30-fold and 10-fold volume of the model FB relative to the volume of the cation-exchange and anion-exchange resins, respectively. For the resins used in the work, the dynamic exchange capacity before breakthrough was determined to be 0.35 mmol-eq/cm^3 for the anion-exchange column and 1.61 mmol-eq/cm^3 for the cation-exchange column.

According to the ion-exchange column regeneration parameters established in this work, the regenerate solution (eluent) must be used in an amount of two to three times excess relative to the resin volume. Then, in order to remove the regeneration products and excess regeneration solution, a 20-fold excess of distilled water must be passed through the anion-exchange column, while a 10-fold excess of distilled water is sufficient to pass through the cation-exchange column.

It is worth noting that the model medium used in the work was not subjected to preliminary nanofiltration purification, i.e., the salt content corresponded to the original FB. Under conditions of additional nanofiltration purification, a significant increase in the productivity of ion-exchange resins can be expected, depending on the efficiency of the implemented nanofiltration process. The relevance of the work consists in the possibility of using the obtained experimental data in the development of technology for the production and purification of LA.

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Authors' contributions

E.V. Pikurova—methodology development, conducting experiments, analysis of literary sources, writing and editing the text of the article.

A.N. Boyandin—methodology development, conducting experiments, analysis of literary sources, writing and editing the text of the article.

D.R. Serebryakov—conducting experiments, analysis of literary sources.

N.L. Ertiletskaya—conducting experiments, analysis of literary sources.

O.V. Anishchenko—conducting experiments, determination of research objects, provision of equipment, processing experimental data, and consultation on the instrument base.

A.A. Sukhanova—scientific consulting, discussion of the results, editing the article.

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