Obtaining stabilized enzymes and their application in the food industry

Baxtiyor Baxshilloyevich Toxirov
Mukhayo Bafoevna Tagaeva
Shakhina Shukurova
Bukhara State University

Abstract: The article describes the traditional technological method for the production of cognacs and alcoholic beverages with the participation of immobilized enzymes, based on the fact that the resulting immobilized enzymes are stable in an organic environment. In addition, the article provides recommendations for reducing the amount of alcohol in alcoholic beverages using immobilized invertase and esterase enzymes to convert them into alkyl fructosides and esters.

Keywords: activated carbon, esterase, invertase, stabilized enzyme, alkyl fructosides, sorbent

Introduction

It is known that stabilized enzyme preparations are widely used in various areas of the food industry. Stabilized enzymes provide the required indicators and properties for the product and reduce the amount of losses at various technological stages of production.

It is known that the convenience and economic efficiency of using stabilized enzymes on an industrial scale compared to native enzymes. However, obtaining stabilized enzymes and finding convenient, ecologically clean and economical ways to implement the process is one of the urgent tasks of modern biotechnology [1]. These processes are important for their stable and long-term operation.

Application of stabilized enzyme preparations for the food industry has its own challenges. It is necessary to determine the optimal parameters of a bioreactor operating on an industrial scale and to maintain this regime until the end of the process. Taking into account the above-mentioned problems and the wide range of applications of stabilized enzymes, this thesis describes the problem of purification of alcohol from toxic alcohols by yeast invertase and esterase enzymes.

Main body

In the production of alcoholic beverages, activated charcoal of the BAU brand obtained from a birch tree is mainly used. This coal is activated by secondary thermal treatment. Coal-sirs holds in its fragments a significant amount of tar and other pyrolysis products. In the process of activation, these substances are burned [2], and the inner surface of the coal expands several times. The structure of wood activated carbon consists of very small graphite lattice crystals. According to V.S. Veselovsky, there are 200 carbon atoms in activated carbon crystals burned at a temperature of 1000°C, and it is impossible to measure their size. In coal burned at 1000°C, the size of the approximate crystallites is equal to 10, and the boundary atoms of the crystallites have saturable free valence and bind oxygen well.

A number of scientists have conducted a lot of scientific work on activated carbon and note that activated carbon consists of three different types of fragments-macro, permeable and micro-fragments. Macro fragments have an outer radius of 2000 nm and an inner radius of 100 nm, permeable fragments have an outer radius of 100 nm and an inner radius of 1.5 nm, and micro fragments have a radius of less than 1.5 nm.

In litre of BAU brand activated carbon has fragments with a specific capacity of 260g and the total number of fragments is 1.50 of which macro fragments are 1.19, permeable fragments are 0.08, and micro fragments are 0.23 [3-5]. The specific surface area of permeable fragments is 52 m/g. BAU brand activated carbon is chemically bonded with constant oxygen. Therefore, the surface of activated carbon is covered with a thin layer of oxygen atoms. At the maximum amount of oxygen, i.e. at 12% of the total mass, 19% of the coal surface is covered with a thin layer of oxygen atoms.

In addition, BAU brand activated carbon contains organic substances, of which carbon is up to 96%, hydrogen is up to 1-2.5%, nitrogen is up to 1.5%, and sulfur is up to 0-1% and the minerals as iron, aluminum, magnesium, potassium, calcium and silicon is included.

Despite the extent to which BAU brand activated carbon has been studied, its adsorptive properties have not been fully studied. Only a few of research have been done. Among them, M.S. Shulman studied the adsorption properties of BAU brand activated carbon more than the other. It completely adsorbs acetic aldehyde, acetic ethyl ether, and isoamyl alcohol in aqueous medium compared to water-alcohol medium when examining the adsorption properties of acetic aldehyde, acetic ethyl ether [6-9], and isoamyl alcohol from water and 50% water-alcohol media in the presence of BAU brand activated carbon and the water-alcohol mixture adsorbs up to 12-17% of the total mass of the three additives. However, there is no enough data and studies conducted on this manner, it will be applicable to conduct many more studies on this BAU brand activated carbon.

The development of nanotechnology at the end of the 20th century and the beginning of the 21st century brought about a big change in the field of sorbents. As a result, new age sorbents were developed. Among them, STRG (thermally decomposed graphite) and USVR (highly reactive carbon mixture) are currently considered sorbents in great demand all over the world [10].

At the beginning of the 21st century, these sorbents obtained on the basis of nanotechnology created by Russian scientists were called the miracle of the 21st century. They have a high adsorption capacity and have the ability to adsorb organic and inorganic substances mixed with water, as well as radioactive elements. STRG sorbent is mainly considered a sorbent discovered at the end of the 20th century, and its application dates back to the beginning of the 21st century. This sorbent is obtained by thermal decomposition of natural graphite. Graphite is first oxidized with concentrated sulfuric and nitric acids, and then thermally decomposed at high temperature [11]. Oxidation of graphite is already known, but much work has been done on thermal decomposition. During thermal decomposition of oxidized graphite, free radicals of sulfur and nitrogen were preserved in graphite. Therefore, special equipment was created by the Russian company Nordragment, and graphite is thermally decomposed at a temperature of 1250°C, as a result, free radicals of nitrogen and sulfur are significantly reduced.

STRG sorbent is a sorbent mainly used for cleaning oil and organic synthesis waste from water. It has not yet been used in the alcohol industry, but it is considered a new sorbent in this field. If you talk about the properties of STRG sorbent, the specific density of this sorbent is very small, it occupies a volume of 1 kg and 22 m. Depending on the structural grid of graphite, it holds organic compounds such as oxygen, sulfur, nitrogen, tar residues, and holds heavy metals such as iron, copper, lead, arsenic from minerals, and is a sorbent consisting mainly of macro fragments and micro fragments. The adsorption capacity of STRG sorbent is 6-7 times greater than that of activated carbon, and 1 kg of sorbent can absorb 30 kg of acetone in water, 35 kg of benzene, 20 kg of butyl alcohol, 20 kg of methanol, and up to 100 kg of 96% sulfuric acid. have In the conducted studies, it was shown that 1 g of sorbent has the ability to absorb 88 grams of oil in 10 seconds [12-17]. Many aspects of the sorbent solution have not been opened and it is considered a new sorbent for the field of alcohol, and we think it is appropriate to conduct many scientific studies on its adsorption capacity and processing capacity, activity and technological indicators. In addition, there are other parts of the sorbent where the fragments are not deep and open. USVR is a high-reactivity carbon mixture, which is considered a high innovation of the 21st century. The author of this sorbent is the Russian Academy of Sciences Victor Ivanovich Petrik, and the sorbent is considered a product of the perfect technology of modern nanotechnology.

Graphite is an inorganic substance found in nature. Graphite and diamond are allotropic products of carbon. The graphite grid is connected by covalent bonds with

10-9 meter carbon atoms and has a strong structure. It is very difficult to break it up, and it requires a fusion explosion to break it apart.

V.I.Petrik was the first to separate the carbon atoms in graphite using nanotechnology and separate the carbon powder in its individual state. For this innovation, V.I.Petrik won 6 international patents. Academician Petrik was the first to prove that when graphite decomposes, its volume increases 5000 times, and developed a USVR sorbent with different characteristics.

According to USVR, it is hydrophobic, electrically conductive, chemically inert, stable in aggressive environments, an environmentally friendly sorbent and contains 99.4% carbon, the specific density is 0.01-0.001 g/cm3, the specific surface area of 1 gram of sorbent is 2000 m^2, internal temperature from 60°C to Q 3000°C. It has the ability to re-desorb 98% of adsorbed substances. Today, this sorbent is used to clean the world's oceans from oil and other organic compounds, to clean wastewater from radioactive isotopes and radicals, and to clean waste water from nuclear reactors to the level of drinking water. The most demanding countries are the USA, European countries, Japan and Russia. As a result of the demonstration of the sorbent, many ecologically threatening situations were eliminated. In addition, the possibilities of the sorbent began to be widely used in medicine, pharmacology, food industry, production of drinking water and other fields [18-25].

S.Kh.Abdurazaqova was the first to put forward the information that sivukh alcohols contained in wine, cognac and liqueurs are transformed into alkylfructosides under the influence of β-fructofuranosidase enzyme of yeasts. According to this idea, it was confirmed in practice that yeast conducts various reactions with -fructofuranosidase and produces ethers, antioxidants, and enzyme-saccharide compounds in the environment. As a result of the research, the scientists explained that it is possible to activate sivukha alcohols in the enzyme system through biochemical processes, reduce the toxic activity of sivukha alcohols, and obtain various pharmacological and perfume products from them [26-30]. By the 21st century, the work in this field has intensified, and great steps have been taken in the purification of sivukha spirits. One of them is the work done by D.T.Mizarakhmetova. D.T.Mirzarakhmetova isolated and immobilized the yeast b-fructofuranosidase enzyme in her scientific research and was the first to show that the transferase functions of yeast invertase can be used to convert toxic and bitter alcohols into alkyl fructosides. Currently, he proved that invertase can be effectively used in its immobilized state and studied its conversion of alcohols into alkylfructosides. D.T.Mirzarakhmetova studied the hydrolytic and transferase functions of immobilized and native invertase from a kinetic point of view, developed optimal parameters of biochemical purification, and they allowed to remove 87% of isoamyl alcohol contained in brandy alcohol [31-33].

These scientific innovations will pave the way for the creation of the most advanced theoretical and practical technology for the purification of sivukh alcohols contained in alcohol, and will create wide opportunities for the use of sivukh alcohols in pharmacology, the food industry, and the perfumery industry.

Conclusion

1. Invertase was covalently immobilized on chemically modified capron and activated carbon. The immobilization process was carried out in the presence of sodium borohydride under conditions of pH 7.6 for 24 hours (40C) in a medium with an initial enzyme amount of 1.2 mg per 1 g of carrier. In this case, 50% of the total amount of the enzyme was bound to the carrier, and the activity of 1 g of immobilized invertase drug was 225.5 units [34-35]. Esterase is covalently immobilized on chemically modified oak granules. Enzyme immobilization was carried out at the ratio of 30 mg of enzyme per 1 g of carrier for 24 hours (40C) in the environment with pH value of 7.5. In this process, 56% of the total amount of the enzyme was bound to the carrier, and the activity of 1 g of the immobilized esterase drug was 16 units.

2. Studying the properties of native and immobilized invertase enzymes in water and water-organic medium, the water-organic medium changed the transferase properties of enzymes. Although immobilization did not affect the optimal substrate concentration (62.5 mM) of the enzyme in the aqueous medium, the optimal alcoholic substrate (isoamylol) of the enzyme in the transferase reactions was 30 mM for the native enzyme and 70 mM for the immobilized enzyme. It was determined that the KM index for transferase reactions of the immobilized enzyme was three times higher, 30Mm [36-39], and the optimal time duration for transferase reactions of the drug increased by 2 times to 12 hours. For the native enzyme, the maximum conversion of isoamylol (26%) was observed in 40% ethanol medium, while for the immobilized enzyme it was 40% in 60% ethanol medium.

3. The optimal conditions for immobilized esterase to convert higher alcohols into esters in the ethanol-water model system are as follows: pH-optimum-6.0, optimal concentration of alcohol (isoamylol) substrate -30 mM, optimal concentration of acidic (caproic acid) substrate -30 mM, optimal incubation time for enzyme - 48 hours. Maximum (48%) conversion of isoamylol alcohol was observed in 40% volume ethanol medium. In the ethanol-water model system, the optimal concentration of ethyl ether (ethylbutyrate) for the enzymatic transesterification reaction using yeast esterase was 30 mM.

4. The substrate specificity of the enzymes was determined, according to which invertase and esterase were active against alcohols with the ON-group in α-state. Spatial location of the ON-group on the first carbon atom, branching of the carbon chain and separation of the secondary carbon atoms from the alcohol hydroxyl group

were considered criteria for the enzyme to "recognize" the substrate. Esterase showed high activity and specificity for isoform alcohols such as isoamyl (42.4 units/mg) and isobutyl (24 units/mg) alcohols. It is observed that the high activity of the enzyme in the medium of volatile acidic substrates such as oil (36 units/mg), isovalerian (30 units/mg), capron (16 units/mg) and caprylic (8 units/mg) is important in the formation of the enzyme brand bouquet.

5. A method of synthesizing boiling esters at a high temperature was developed as a result of treatment of higher alcohols contained in the sorting with the presence of immobilized invertase into alkylfructosides and brandy with the help of immobilized esterase. Optimum kinetic indicators for the operation of the bioreactor based on enzymes immobilized in the model system were determined to match the technological scheme of vodka and brandy production. The laws of transformation of higher alcohols into alkylfructosides and esters were consistent with the results of the practical samples and the model system.

6. The expected economic efficiency of production of 100,000 dal brands based on immobilized esterase will be 168 million soums per year by reducing the shelf life of cognac spirits.

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