Science Life

ENVIRONMENTALLY HARMLESS AND ECONOMICALLY ATTRACTIVE PROCESSES FOR MICROBIOLOGICAL PRODUCTION OF ORGANIC ACIDS

INTRODUCTION

The costs of a microbiological process can be greatly diminished by the selection of productive microorganisms, application of inexpensive raw materials and other required materials, and use of efficient equipment for production.

At the Experimental Plant of Biochemical Preparations (EPBP), *Aspergillus niger* strain mutants R-6 and *Aspergillus niger* R-5 were selected for production of citric acid from sugar beet molasses and sugar cane molasses, respectively. Applying *Aspergillus niger* R-6, it is possible to achieve a citric acid yield of 100 % from the initial sugar, and a citric acid content of 99 % of the total acid content. The respective indices for *Aspergillus niger* strain R-5 were 80 % and 98 % (Kārkliņš and Skrastiņa, 1995).

At the EPBP, *Aspergillus terreus* strains EUU-417 and G-232 have been selected for biosynthesis of itaconic acid from sugar and molasses utilising surface and submersed fermentation methods. Yields of up to 65 % were obtained for biosynthesis of sugar, raw sugar and molasses, using the surface method, and up to 60 % using the submersed method. The itaconic acid content reached 95–98 % of the synthesised acids.

At EPBP, the yeast *Candida lipolytica* mutants have been applied for biosynthesis of citric, α-ketoglutaric and isocitric acids from n-alkanes, and technological processes have been elaborated for isolation of these acids and their salts from fermentation solutions (Раминя и др., 1984; Пелцмане и др., 1988). Technologies were developed for sodium citrate and citric acid production from fermentation solutions, with technical application mainly for detergents and surface active substances. The technologies for use of these strains in biosynthesis of chemically pure citric acid and sodium citrate have also been elaborated. (Карклинь и др., 1987).

Biosynthesis and end product isolation processes were elaborated for preparation of lysine, gluconic acid, tryptophane and other substances.

The laboratory experiments, followed by subsequent testing at a pilot plant stage, allowed to develop modifications for cheaper sources: molasses or starch in the production of citric acid, molasses for itaconic acid, lysine, gluconic acid production, and oil refinement byproducts (n-alkanes) for technical citric acid and sodium citrate preparation.

Attempts to diminish industrial byproduct yields or to realise their application were also successfully studied, as well as the use of improved methods: ion exchange and electromembrane methods instead of chemical methods in the processes of end product isolation.

STUDIES ON APPLICATION OF INDUSTRIAL BYPRODUCTS IN CITRIC ACID MICROBIOLOGICAL PRODUCTION

The byproducts in citric acid microbiological production from molasses include: *Aspergillus niger* mycelium, fermentation solution after separation of calcium citrate, gypsum, and liquids after crystallisation of citric acid. Using ion exchange for citric acid separation from minerals and dyes, the byproducts include only rinse waters of ionites after sorption and regeneration.

During one cycle of citric acid biosynthesis, 1.8–2.4 kg of mycelium are formed from 1 m^2 of the biosynthesis surface (calculated as dry mycelium). The dry mycelium weight amounts to 100 kg per 1 ton of crystalline citric acid. The dry mass contains 18–25 % of protein, pectolytic and other enzymes (amylase, maltase, etc.) and mineral substances. At the end of the fermentation cycle, mycelium is separated from the fermentation liquid and ground. After this, mycelium with a moisture content of 65–68% is dried to 12–15 % moisture. Prepared in this way, mycelium could be used with good results as a fodder additive mainly in poultry and pig farming, and it has been estimated that live weight growth can be increased by 2.1 times for poultry and by 5.6–18 % for pigs.

Calcium citrate filtrate contains mineral substances, sugars and acids: citric acid (up to 0.6 %), gluconic acid (up to 0.3 %), malonic and adipinic acids (up to 0.15 %), and aminoacids—treonine, glycine, lysine, arginine, leucine, etc. This filtrate, when concentrated up to 80 %, is used as an additive in the production of liquid premixes for application in cattle-breeding. In the production of citric acid, about 7 m^3 of concentrated filtrate is formed per ton of crystalline citric acid. Tests have shown that the chemical composition of the filtrate does not change during its concentration.

The experiments at the plant have shown that the concentrated citrate filtrate can be successfully applied to stabilise the oil soluble vitamins A, E and D. Vitamins A, E and D retain 96–100 % of their initial activities in this medium for 8–13 months.

Liquid premixes based on calcium citrate filtrate can be used for the production of dry premixes.

The citrate filtrate can be used as a base for fodder premix production.

Application of gypsum was also studied. It was found that gypsum, after citric acid separation, treatment and drying, can be used as a building material. Also, gypsum can be used for preparation of tiles. Experiments of gypsum pulp treatment with technical sodium carbonate for sedimentation of calcium carbonate showed that this method could be used in citric acid production.

APPLICATION OF ION-EXCHANGE FOR CITRIC ACID SOLUTION PURIFICATION

The application of ion-exchange and membrane processes presently is growing rapidly in different branches of industry and research. In the production of citric and other food acids, as well as in other organic acid production, ion exchange is used for separation of the main product from mineral and dyes substances, and for separation of different organic acids. At EPBP, a method for separation with ionites of the above mentioned substances from solutions produced during citric acid isolation was developed and applied.

The purification of mother liquids (solutions after citric acid crystallisation) from mineral and dyes substances was applied under production conditions using cation-exchangers KY-1 and KY-2 (ratio 1:1).

By applying cation-exchange, mineral substances are removed from the mother liquids by 95 %, and dye substances by 64 %. The optimum citric acid concentration in the initial solution is 30 %. The crystallisation of the solutions after this treatment yields crystalline citric acid with a purity that meets the demands of standards for food citric acid.

To decrease the amount of chemicals used, sodium hydroxide, sodium carbonate and sulphuric acid were reused in ion exchange. Recycling of sulphuric acid decreases its consumption by twice; sodium hydroxide and sodium carbonate, by 6.6 times. The demineralized water consumption for rinsing of the cationites after sorption and regeneration is 2.5 m^3 per 1 ton of cationites. Half of this water was applied for cationite rinse in the next cycle, and the ramaining part was used for dilution of mother liquids before purification on the cationites.

To increase the time of cationite use under production conditions, regeneration by sulphuric acid and alkali was applied. The sorption capacity after 20 ion-exchange cycles was increased by 3 times.

The investigations have shown that citric acid can be produced cheaper and in a more environmentally friendly fashion.

ELECTRODIALYSIS FOR INDUSTRIAL CITRIC ACID SOLUTION TREATMENT

There are many investigations described in the literature for application of membrane processes in food and other acids industries, for example, for isolation of itaconic acid from fermentation solutions and for concentration, as well as in lactic acid production, in acetic acid preparation, and in the dairy industry (Kobayashi et al., 1972; Khattabi et al., 1996; Boniardi et al., 1997; Strathman, 1985; Biau, 1997).

The membrane processes have the following benefits in comparison with ion exchange: no rinse waters, no need of chemicals in regeneration, more compact equipment, and therefore lower production costs of the end product. At EPBP, the simultaneous use of ion exchange and electrodialysis was tested in the production of several acids to increase the output of the end product.

A technology of citric acid and its salt isolation from n-alkanes fermentation solutions was elaborated. After treatment of sodium citrate solutions with bentonite and activated carbon and application of electrodialysis, a crystalline citric acid was obtained with citric acid content 99.3–99.8 % and yield of 90 % of the initial amount.

Tests were carried out for trisodium citrate concentration by electrodialysis in a four–compartment stack, with the aim to decrease the time of the evaporation process and to decrease costs.

ISOLATION OF ISOCITRATE BY ELECTRODIALYSIS

The isolation of isocitrate from fermentation solution by electrodialysis was studied, and experiments were carried out to separate the mixture of isocitric and citric acids from sulphuric acid in the solutions after potassium isocitrate crystallisation. In these experiments, a crystalline mixture of isocitrate and citrate was obtained. This mixture can be used for technical purposes, for example, as surface active substances or in detergent production. The results of electrodialysis of isocitric acid fermentation liquids and solutions after crystallisation show ways to diminish the wastes of citric and isocitric acid production from n-alkanes.

RECOVERY OF PURE α-KETOGLUTARATE BY ELECTROMEMBRANE PROCESS

The electrodialysis of α-ketoglutaric acid n-alkanes fermentation liquids was carried out with the aim to obtain a pure α-ketoglutaric acid solution with a low content of pyruvic acid, and to produce chemically pure sodium ketoglutarate.

The acids in fermentation liquid usually are in salt form, since during the fermentation, sodium hydroxide is added to maintain the optimum pH, and due to mineral salts of the fermentation medium.

Transformation of the salts to acids is realised by electrodialysis in three- or four-compartment stacks in a flow regime.

The transformation process of salts in the three-compartment unit is faster than in the four-compartment electrodialyser; therefore, it is preferable to use a three-compartment unit on a pilot plant scale and under production conditions.

The comparison of ion exchange and electrodialysis results of α-ketoglutaric acid isolation from n-alkane fermentation liquids showed that chemicals are not needed for regeneration in electrodialysis, but sodium hydroxide is recovered from the fermentation liquid, which is returned to the fermentation process and used to maintain the optimum pH of this process. The consumption of distilled water in electrodialysis is 7–9 times less than in the case of ion exchange.

An important benefit of electrodialysis is: the process produces no waste waters, in contrast to ion exchange. With application of electrodialysis, 87–95 % of α-ketoglutarates are transformed into acid, and this acid is separated from sulphate ions without the use of barium hydroxide, but there are insignificant losses of α-keto--glutaric acid. The combination of electrodialysis with the ion-exchange could be useful. Salts transformation into acid produced a yield of 85–95 % by electrodialysis, which was increased by ion exchange for complete transformation.

The conducted studies showed that the application of highly active microbial producers, using inexpensive raw materials (sugar cane molasses and n-alkane), coupled with contemporary methods of isolation of end products (ion-exchange and electrodialysis) and lower energy and materials consumption, guarantee ecologically harmless and economically attractive processes for production of some organic acids (citric, isocitric, α-ketoglutaric). The technologies for further use of product and waste treatment were successfully applied.

All of the investigations described in this article were put into practice in organic acid production.

REFERENCES

Biau, A. S. (1997) Conception, realization and use of an industrial unit for tartrate stabilization. *Rev. Un. Oenol.*, **162**, 18–20.

Boniardi, N., Rota, R., Nano, G., Mazza, B. (1997) Lactic acid production by electrodialysis. *J. Appl. Electrochem.*, **27** (2), 125–133.

Kārkliņš, R., Skrastiņa, I. (1995) *Aspergillus niger* sējmateriāla ieguves metode citronskābes ražošanai [A method of *Aspergillus niger* seeding material obtainment for citric acid production]. *Latvijas Republikas patents 11342 LR. 11.01.1995* (in Latvian).

Kārkliņš, R., Skrastiņa, I. (1995) Mikroskopisko pelējuma sēņu *Aspergillus niger* celms R-5 — citronskābes producents [Microscopic fungus *Aspergillus niger* strain R-5 as a producer of citric acid]. *Latvijas Republikas patents 11340 LR. 11.01.1995* (in Latvian).

Khattabi, M. O., Alaoui H. M., El Midaoui, A. (1996) Reduction of mellassigenic ions in cane sugar juice by electrodialysis. *Desalination*, **7** (2), 140–157.

Kobayashi, T., Nakamura, I., Nakagawa, M. (1973) Itaconic acid. *Japan Patent 7356,890 Jap. 09.08.1973.*

Strathmann, H. (1985) Electrodialysis and its application. *Separ. Purif. Method.*, **14** (1), 41–66.

Карклинь Р., Лемба Я., Раминя Л. (1987) Метод получения цитратов из ферментативных растворов [A method of citrate recovery from fermentation solutions]. В кн.: *Материалы Всесоюзной конференции: Методы получения и анализа биохимических реактивов, Юрмала, 26–28 октября 1987.* Кузнецова Т., Демидова Л. (ред.). Рига, Зинатне, с. 171 (in Russian).

Пелцмане И., Раминя Л., Фипогенова Т., Карклинь Р. (1988) Биосинтез и выделение изолимонной кислоты из растворов н-алканов [Biosynthesis and isolation of isocitric acid from n-alkane solutions]. *Изв. АН ЛатвССР*, Т. 2 с. 93–95 (in Russian).

Раминя Л. Я., Озолиня М. Я. (1984) Получение цитратов из ферментационного раствора н-алканов [Citrate production from n-alkane fermentation liquid]. В кн.: *Биосинтез оксикислот и кетокислот микроорганизмами.* Арс Р., Бекер М., Лиепиньш Г., Якобсон Ю. (ред.). Рига, Зинатне, с. 35–42 (in Russian).

Jānis Lemba
Rīga Technical University

Romāns Kārkliņš, Iveta Lapele, Lidija Ramiņa
Experimental Plant of Biochemical Preparations

EKOLOĢISKI NEKAITĪGI UN EKONOMISKI IZDEVĪGI PROCESI ORGANISKO SKĀBJU MIKROBIOLOĢISKĀ RAŽOŠANĀ

Bioķīmisko preparātu eksperimentālajā rūpnīcā ir izpētīti ekoloģiski nekaitīgi un ekonomiski izdevīgi procesi un to ieviešana dažādu skābju (citronskābes, izocitronskābes, α-ketoglutārskābes, itakonskābes) ieguvē, pielietojot augstražīgus producentus, lētākas izejvielas (cukurniedru melase, n-alkāni), energo- un materiālmazietilpīgus izdalīšanas procesus (jonapmaiņu un elektrodialīzi). Izpētīta arī šo procesu atkritumprodukti izmantošana lopkopībā (kā barības piedeva) un celtniecībā.