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ACETIC ACID BACTERIA – PERSPECTIVES OF APPLICATION IN BIOTECHNOLOGY – A REVIEW

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The most commonly recognized and utilized characteristics of acetic acid bacteria is their capacity for oxidizing ethanol to acetic acid. Those microorganisms are a source of other valuable compounds, including among others cellulose, gluconic acid and dihydroxyacetone. A number of investigations have recently been conducted into the optimization of the process of glycerol biotransformation into dihydroxyacetone (DHA) with the use of acetic acid bacteria of the species *Gluconobacter* and *Acetobacter*. DHA is observed to be increasingly employed in dermatology, medicine and cosmetics. The manuscript addresses pathways of synthesis of that compound and an overview of methods that enable increasing the effectiveness of glycerol transformation into dihydroxyacetone.

INTRODUCTION

Multiple species of acetic acid bacteria are capable of incomplete oxidation of carbohydrates and alcohols to aldehydes, ketones and organic acids [Matsushita *et al*., 2003; Deppenmeier *et al*., 2002]. Oxidation products are secreted outside cells, owing to which they may be isolated directly from a culture medium. A typical trait of acetic acid bacteria is the production of acetic acid from ethanol [Ruiz *et al*., 2000]. Apart from vinegar, the key metabolites produced by those microorganisms include cellulose, gluconic acid, L-ribose and dihydroxyacetone [Kim *et al*., 1996; Charney *et al.*, 1978]. Those compounds are applied in many branches of industry as well as in medicine. Among them, special attention should be paid to dihydroxyacetone (DHA) – a substance sugar in character utilized, among other things, in the treatment of leukoderma and as an active component of self-tanning creams [Erni *et al*., 2006; Green *et al*., 2004; Fesq *et al*., 2001]. Dihydroxyacetone is formed as a result of glycerol oxidation catalyzed by glycerol dehydrogenase – an enzyme linked with a cellular membrane of bacteria [Gätgens *et al*., 2007].

TAXONOMIC, MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF THE FAMILY *ACETOBACTERACEAE*

In the past, affinity of bacterial genera to the family *Acetobacteraceae* was subject to numerous changes. In the year 1898, the genus *Acetobacter* was established and the species *Acetobacter aceti* was affiliated to it. Next, in the year 1935, the genus *Gluconobacter* was postulated and bacteria included to that genus were capable of oxidizing glucose to gluconic acid to a considerably greater extent than of oxidizing glucose to acetic acid [Yamada & Yukphan, 2007]. Another genus, *Acetomonas*, was described in the year 1954. In turn, in the year 1984, *Acetobacter* was divided into two sub-genera: *Acetobacter* and *Gluconoacetobacter*, yet the year 1998 brought another change in the taxonomy and *Gluconacetobacter* was recognized as a separate genus [Yamada & Yukphan, 2007].

Nowadays, the family *Acetobacteraceae* includes 25 genera. Apart from the most recognized ones, *i.e. Acetobacter*, *Acidomonas*, *Asaia*, *Gluconacetobacter*, *Gluconobacter*, *Granulibacter*, *Kozakia*, *Neoasaia*, *Saccharibacter* or *Swaminathania*, some genera have emerged that usually include one species and display characteristics common for the entire family. They are, among others: *Belnapia*, *Leahibacter*, *Oleomonas*, *Rhodopila* or *Rubritepida*. Another 25 species are still awaiting for the confirmation of their affinity to particular taxa [www.ncbi.nlm.nih.gov/Taxonomy].

The most explicitly known and the most widely applied industrial strains of acetic acid bacteria belong to the species *Gluconacetobacter*. Those bacteria occur in vinegar, sugar cane, flowers and fruits [Brenner *et al*., 2005]. Representatives of that genus are Gram-negative aerobic bacteria whose optimal growth temperature accounts for 30°C, and pH ranges from 5.4 to 6.3 [Hommel, 2004]. Cells of bacteria belonging to the genus *Gluconacetobacter* attain shapes from ellipsoidal to more elongated bacilli, usually straight ones, though slightly bended ones are also likely to occur. Their sizes are in the range of 0.6-1.2 × 1.0-3.0 μm. They occur individually, in pairs or in short chains. Only part of them are characterized by peritrichal ciliation which provides their motor capacity. They produce catalase, do not produce oxidase, indole nor hydrogen sulfide, and they do not fluidize gelatin [Brenner *et al*., 2005]. In addition, they transform ethanol into acetic acid, whereas further oxidation to CO_2 and H_2O depends on

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the concentration of that compound in a culture medium. Some strains are also capable of oxidizing lactates to CO_2 and H_2O [Hommel, 2004].

In terms of industrial usability a valuable representative of the genus *Gluconacetobacter* is *G. xylinus* species. Literature references provide a number of synonyms that describe the same organism, *e.g. Acetobacter xylinum*, *Bacterium xylinoides*, *Bacillus xylinum*. Cells of *G. xylinus* occur individually, in pairs, chains or in small clusters. Those bacteria are observed to grow well in the presence of D-glucose and to produce acids from ethanol, D-glucose and D-xylose [Brenner *et al*., 2005].

G. xylinus is a model organism in biochemical and genetic investigations into mechanisms of cellulose synthesis. The quantity of cellulose synthesized by that species is proportional to an increase of biomass, whereas the yield of that process is determined by the source of carbon [Embuscado *et al*., 1994a, b]. Once ethanol is the major source of carbon, then multi-stage oxidation of that substrate occurs that results in the formation of acetic acid. Once the culture medium contains glycerol, *G. xylinus* oxidizes it to dihydroxyacetone. A research conducted by Nabe *et al*. [1979] demonstrated that *G. xylinus* (*Acetobacter xylinum*) was characterized by a high, as compared to the other acetic acid bacteria, activity of the enzyme catalyzing aerobic transformation of glycerol into dihydroxyacetone. Those observations induced a study into the adaptation of that species to the production of dihydroxyacetone on an industrial scale.

APPLICABILITY OF THE METABOLIC POTENTIAL OF ACETIC ACID BACTERIA

The use of acetic acid bacteria (AAB) dates back to distant years of our civilization. Probably, as early as in the Babylonian times (VI century BC) use was made, though unconsciously, of their capability for the preservation of food [Hommel, 2004]. Some notes on the consumption of vinegar have been encountered in the Old Testament. The production of vinegar consisted in filling jars half-full with wine, grape pomace and comminuted date fruits. Those components were next subjected to spontaneous fermentation. Vinegar was contemporarily used as a seasoning, a refreshing drink and a medicine [Allgeier & Hildebrandt, 1960]. In countries in which the production of vinegar was not disseminated it was recognized as an expensive and luxurious beverage. In the Middle Ages, France became its greatest producer.

Contemporary knowledge on biochemical, physiological and genetic characteristics of AAB enables their well-thought-out and targeted application in a number of industry branches.

The capability of bacteria from the genus *Acetobacter* to ferment ethyl alcohol to acetic acid is employed in the food industry for the production of vinegar [Hommel, 2004]. Vinegar accelerates the process of meat tenderization and loosening, softens fish bones, is a component of marinades and pickles applied in the fruit and vegetable industry as well as in the preparation of fish and sea fruits. Vinegar is additionally used for the preparation of sauces, mayonnaises and mustards. It improves sensory attributes of a ready product and, by acidifying medium, it enables preservation of food.

Apart from the production of vinegar, an increasing interest has been observed in the application of those bacteria for the production of vitamin C (ascorbic acid). Nowadays, vitamin C is synthesized in a seven-step Reichstein's cycle, with D-glucose used as a substrate. That processes covers six chemical syntheses and one reaction of microbiological oxidation of D-sorbitol to L-sorbose. The proper course of that transformation requires high pressure and temperature [Hancock & Viola, 2002]. In recent years, research have focused on the elaboration of alternative methods for the production of ascorbic acid, especially the biotechnological ones. In order to minimize production costs a study was undertaken to modify methods applied so far with the use of industrial strains of acetic acid bacteria. It was proved that the application of enzymes originating from *G. oxydans* enabled facilitating that process and additionally minimized production costs [Hancock & Viola, 2002].

Selected strains of acetic acid bacteria have been shown to be capable of cellulose biosynthesis [Zhou *et al*., 2007; Chao *et al*., 2000], which affords new possibilities of that biopolymer's production. Investigations have shown that *Acetobacter xylinum* is the most efficient producer of bacterial cellulose (BC). That property has also been demonstrated for *Gluconobacter oxydans*, yet in its case the biosynthesis of cellulose is not that efficient as in the case of *A. xylinum* [Jia *et al*., 2004]. Bacterial cellulose has turned out to be a versatile biomaterial. Paper produced from such a cellulose is highly elastic, resistant and, more importantly, completely biodegradable [Shah & Brown, 2005]. Apart from the paper industry, BC may also be applied for the electrophoretic separation of DNA. Due to an internal, three-dimensional network of microfibres, cellulose may be used for the separation of DNA fragments with sizes of even 10-100 pz [Tabuchi & Baba, 2005]. Yet the greatest emotions and hopes are fostered by the possibility of applying that easily-available bacterial biopolymer in medicine. Attempts are underway to apply BC for reconstruction of destroyed or damaged organs, fragments of skin, for healing wounds and for the synthesis of artificial tissues [Czaja *et al*., 2007]. Low production costs and the possibility of synthesizing unlimited quantities of bacterial cellulose prompt scientists to search for new possibilities of its application in medicine.

Out of multiple compounds produced by acetic acid bacteria, worthy of special notice is dihydroxyacetone (DHA). It is produced as a result of glycerol oxidation catalyzed by glycerol dehydrogenase (EC 1.1.1.6) – an enzyme bound with a cellular membrane. Dihydroxyacetone is an important component of self-tanning creams and a secondary metabolite on the pathway of obtaining other chemical compounds [Draelos & Zoe, 2002; Claret & Bories, 1994].

With development of agricultural economy a need has appeared for the elimination or partial reduction of artificial fertilizers used in farming. It is common knowledge that nitrogen is an element indispensable for the growth of plants. Intensification of plant production requires doses of artificial fertilizers to be increased, which poses ecological risk. In order to provide a high crop yield and, simultaneously, to reduce environmental hazard use may be made of the so-called "plant growth-promoting bacteria" (PGPB) that are

capable of binding nitrogen in cells and of reducing atmospheric nitrogen to ammonia [Pedraza, 2007]. In the family *Acetobacteraceae* such properties have been demonstrated for three genera of bacteria: *Gluconacetobacter*, *Acetobacter,* and *Swaminathania*.

Gluconobacter oxydans possesses capability to reduce toxicity of patulin [Ricelli *et al*., 2007]. Patulin is a mycotoxin produced by moulds that belong to the following species: *Penicillium*, *Aspergillus* and *Byssochlamys* [Draughon & Ayres, 1980; Northolt *et al*., 1979]. In humans, intoxication with patulin usually occurs after the consumption of mould-infested apples and apple juices [Moake *et al*., 2005]. High concentrations of that mycotoxin were also reported on other fruits (pears, peaches, strawberries, berries, cherries, apricots and grapes) as well as in cheese [Piemontese *et al*., 2005; Majerus & Kapp, 2002; Pittet, 1998]. Patulin poses a severe risk to the health of potential consumers, infants and children in particular. Depending on its concentration, intoxication with patulin may induce, among other things: ulceration, convulsions, damage to the neural and immune systems, activation of tumor cells for neoplasia, and – at a cellular level – inhibition of DNA synthesis [Moake *et al*., 2005]. Strains of *Gluconobacter oxydans* have been shown capable of transforming patulin (with 96% effectiveness) into ascladiol, *i.e.* its less toxic precursor [Ricelli *et al*., 2007; Sekiguchi *et al*., 1983].

Apart from benefits that result from the application of AAB, they have also been demonstrated to act as pests. They are the cause of a number of faults in a ready wine or beer as they evoke turbidity, souring, vinegar aroma and a film on their surface [Silva *et al*., 2006]. They may also evoke spoilage of fruit and vegetable marinates as well as disturb the apt course of the production process of baker's yeast. Recently, those bacteria have been isolated from, among other things, apple juices in the Italian Alps [Dellaglid *et al*., 2005], from fruits in Senegal [Ndoye *et al*., 2007] and Thailand [Seearunruangchai *et al*., 2004], from grapes grown in Chile [Prieto *et al*., 2007], as well as from vegetable silages of corn [Mehnaz *et al*., 2006], wheat and sugar beets [Elferinck *et al*., 2001]. The occurrence of AAB in various regions of the world may point to a great adaptive potential of those microorganisms.

Pathogenic characteristics are ascribed to bacteria of *Granulibacter bethesdensis*. That species has been isolated from human lymph nodes. It is likely to be one of the factors inducing chronic granulomatous disease (CGD) which is the cause of diminished immune resistance to staphylococci and mycotic infections [Greenberg *et al*., 2007]. Fortunately, so far it is the sole example of a pathogenic species of the family *Acetobacteraceae*.

PROPERTIES, APPLICATIONS AND METHODS FOR SYNTHESIZING DIHYDROXYACETONE

Dihydroxyacetone (DHA) is a ketotriose occurring in sugar beets and sugar cane as a product of glycerol oxidation. The full name of that compound stipulated by the International Union of Future and Applied Chemistry (IUPAC) is 1,3-dihydroxy-2-propanone ($C_3H_6O_3$), its molecular weight accounts for 90.08 g/mol and melting point – for 75-80°C [www.iupac.org]. It is a white, hygroscopic powder with sweet, refreshing taste and characteristic aroma [Rabinowitch, 1925]. It is the simplest representative of known ketoses, it does not posses a chiral centre nor shows optical activity [Erni *et al*., 2006]. Dihydroxyacetone is a water-soluble substance susceptible to microbiological factors and capable of further oxidation. The most widely recognized and utilized characteristics of dihydroxyacetone is its ability to form colour compounds in contact with human skin [Draelos & Zoe, 2002]. That phenomenon was first observed by Eva Wittgenstein from the University of Cincinnati in the year 1950 [Wittgenstein & Guest, 1961]. Being a compound from a group of saccharides, dihydroxyacetone was a constituent of syrup administered to children treated for congenital glycogen storage disease. While taking the medicine, some children were accidentally spilling it onto their skin. After a few hours, brown spots were observed at the site of syrup contact with skin [Wittgenstein & Guest, 1961]. That interesting observation prompted Eva Wittgenstein to continue experiments on DHA reactions with human skin.

Nowadays, DHA is known to react with several compounds, including ammonia and amino acids, thus yielding colour compounds. Brown coloration of skin results from a reaction of dihydroxyacetone with free amino acid groups of arginine occurring in cuticle [Wittgenstein & Berry, 1961]. The effect of skin browning is analogous to the mechanism of Maillard reaction; it usually proceeds upon the influence of heat between amino acids and reducing sugars. Increasing the concentration of dihydroxyacetone in a reaction with amino acids enables obtaining colours from yellow to brown [Draelos & Zoe, 2002]. In the year 1970, dihydroxyacetone was included by the Food and Drug Administration (FDA) onto the list of cosmetic components [www.cfsan.fda.gov]. Currently, it is an indispensable component of self-tanning creams, the so-called bronzers [Green *et al*., 2004]. A too long exposure of skin to the action of UV radiation may be the reason of painful burns as well as accelerates processes of ageing and neoplasia [McCook *et al.*, 2004]. Cosmetic concerns that promote safe tan all year round encourage to use the self-tanning creams as safe alternatives to the excess of sunrays.

It turns out that dihydroxyacetone may be used successively not only in the cosmetics but also in dermatology as a therapeutic agent in the treatment of leukoderma [Fesq *et al*., 2001]. Most of known methods of leukoderma treatment are long-term processes that do not assure complete regression of symptoms of that disease. Hence, new, certified and acceptable means of fighting that disease are still needed. Investigations conducted on a group of patients affected by leukoderma demonstrated that a preparation containing DHA affected the alleviation of effects of that disease in 80% of the patients examined [Fesq *et al*., 2001].

There are known a few compounds that act similarly to dihydroxyacetone. They cannot, however, replace DHA due to their irritating and sometimes toxic effect on skin. In turn, some compounds exist that may be applied in a combination with dihydroxyacetone, *e.g.* erytrulose, owing to which it is possible to reduce DHA concentration in cosmetic preparations. Due to DHA capability for further oxidation and its instability, self-tanning preparations should be utilized before the expiry date declared by the producer. A method for protecting DHA

molecules against a detrimental effect of environmental factors was patented in the year 1995 [Durand, 1995]. It postulated encapsulation of dihydroxyacetone molecules in the structure of a water-proof material that releases dihydroxyacetone stepwise during the application of a self-tanning cream onto skin. The water-proof coat protects DHA against the contact with water and, simultaneously, eliminates its oxidation.

Only a few laboratory methods of DHA synthesis have been applied under industrial conditions [Fakley *et al.,* 1988]. The method of microbiological transformation of glycerol to dihydroxyacetone, patented in 1978, involves the application of properties of the strain *Acetobacter suboxydans* ATCC 621 [Charney *et al.*, 1978]. Its inventor assured a high reaction yield once appropriate culture conditions and composition of a culture medium are maintained. The suggested medium should contain 9-12% of glycerol and 0.5% of a yeast or fish hydrolysate. Biotransformation spanned for *ca.* 30 h and the resultant dihydroxyacetone was isolated from the culture medium with known methods, through filtration of the culture medium, removal of inorganic ions present in the medium, and next concentration of the solution and crystallization. Another concept of the industrial production of DHA was linked with re-utilization of previously proliferated biomass of acetic acid bacteria [Ohrem *et al.*, 1998]. Various strains of *Gluconobacter* and *Acetobacter* genera are characterised by a high activity of membraneous glycerol dehydrogenase, catalyzing the transformation of glycerol to dihydroxyacetone, in an appropriately-composed culture medium. After several dozens of hours of culturing, cells lose their capability for further divisions, which however does not mean the loss of the activity of glycerol dehydrogenase. Based on those observations, Ohremi and Westmeier postulated a new method for the synthesis of dihydroxyacetone [Ohrem *et al.*, 1998]. According to that method, cells of acetic acid bacteria should be proliferated and, simultaneously, should be provided appropriate conditions for the transformation of glycerol into DHA. Biomass, incapable of further proliferation is not removed from a tank but utilized, as a whole or in part, for the subsequent production cycle. It enables a substantial reduction of production costs. Apart from determining the optimal parameters of dihydroxyacetone biosynthesis, additional difficulties are posed by the process of its isolation from the post-fermentation culture medium. The processes of DHA extraction and purification usually involve many stages. The specific crystallization of DHA should be preceded by multiple filtration, evaporation and incubation of the sample. However, those methods cannot be applied for the isolation of DHA from organic mixtures, they are additionally time-consuming and difficult to proceed, and the quality of the end product does not always reach the required level of purity [Fakley *et al.*, 1988].

Fakley and Lindsay have contented with difficulties accompanying the purification of dihydroxyacetone on a large scale. They elaborated a method based on thin-layer evaporation in which DHA is distilled off the residue of a reaction mixture [Fakley *et al.*, 1988]. They claimed that standard distillation of DHA resulted in partial dehydration of that compound and that precipitation was not an appropriate treatment since, apart from dihydroxyacetone, also other sugars were likely to precipitate from the medium and contaminate the end product. Although a few methods of dihydroxyacetone synthesis are in use, studies are underway to elaborate easier and more cost-effective methods for obtaining that compound, whose role in, among others, cosmetic and dermatological industry cannot be overestimated.

SYNTHESIS OF DIHYDROXYACETONE WITH THE USE OF FREE AND IMMOBILIZED CELLS OF ACETIC ACID BACTERIA

Dihydroxyacetone (DHA) may be synthesized by means of two methods, namely: chemical and microbiological synthesis [Gehrer *et al.*, 1995; Charney *et al.*, 1978].

The chemical synthesis may proceed through catalytic oxidation of glycerol or condensation of formaldehyde with calcium carbonate. Such a process of glycerol transformation into DHA proceeds in a inhomogeneous mode, additionally results in the formation of complex and hard to remove compounds, and the yield of reaction is not satisfactory [Hekmat *et al*., 2003].

In turn, the microbiological methods consist in partial oxidation of glycerol to DHA by selected strains of acetic acid bacteria characterized by a high activity of an enzyme catalyzing that reaction, *i.e.* glycerol dehydrogenase [Claret, 1992; Nabe, 1979].

Chemical syntheses of organic compounds, run on an industrial scale, require considerable financial input. Often, such types of reactions result in the formation of by-products being detrimental to natural environment. Processes conducted with the use of microorganisms are usually less expensive than the chemical ones; what is more their control is easier and they do not lead to the production of substances posing risk to environment [Hekmat *et al*., 2003].

Some strains of acetic acid bacteria, including *Gluconobacter melanogenus*, *Gluconobacter oxydans*, and *Acetobacter xylinum* [Wei *et al*., 2007a, b; Nabe *et al*., 1979; Flickinger & Perlman, 1977; Asai, 1968], are capable of partial oxidation of glycerol to DHA on condition that strictly specified parameters of that transformation are provided.

Oxidation of glycerol by acetic acid bacteria may follow according to two pathways. The first pathway, independent of adenosinotriphosphate (ATP) and nicotinamide adenine dinucleotide (NAD), proceeds at pH 6 and results in the production of dihydroxyacetone [Flickinger & Perlman, 1977; Hauge *et al*., 1954]. The only enzyme that catalyzes this transformation is membraneous glycerol dehydrogenase [Claret & Bories, 1994]. Under the effect of ATP and in the presence of Mg^{2+} ions the resultant DHA may be transformed into the phosphorylated form in a reaction catalyzed by dihydroxyacetone kinase [Hauge *et al*., 1954]. The second pathway of glycerol transformation proceeds at pH 8.5 and requires the presence of ATP and Mg^{2+} ions and additionally of an enzyme – glycerol kinase. A reaction product is triphosphoglycerol which is next oxidized in the presence of NAD-dependent dehydrogenase to the phosphorylated form of DHA [Flickinger & Perlman, 1977; Hauge *et al*., 1954]. In a number of subsequent biochemical transformations, the phosphorylated DHA is being transformed into fructoso-1,6-diphosphate, which is followed by the formation

of glucoso-6-phosphate which, in turn, is incorporated into the pentosophosphate cycle [Hauge *et al*., 1954].

Investigations conducted on the mechanisms of DHA synthesis from glycerol by acetic acid bacteria have demonstrated that, apart from appropriate pH and sufficient aeration of the culture, the apt course of the reactions requires determining the concentration of glycerol in the production medium [Claret *et al*., 1992]. Its high content may inhibit the growth of some strains of acetic acid bacteria and disturb mechanisms of cells division [De Muynck *et al*., 2007]. The excessive concentration of glycerol may impair its biotransformation to dihydroxyacetone or even make it impossible [Claret *et al*., 1992]. The proper course of biotransformation is additionally determined by the quantity of the formed DHA. A too high concentration of that product may exert an inhibiting effect on the growth of, among others, *Gluconobacter oxydans.* Some research have shown that the strain *G. oxydans* was capable to grow until DHA concentration in the medium did not exceed 8% [Bauer *et al*., 2005].

Independent observations have demonstrated that, despite a high effectiveness of glycerol biotransformation to DHA, of crucial significance is the selection of an appropriate growth phase of acetic acid bacteria. Only cells being in the stationary growth phase (to be more specific – being in the last stage of that phase) are capable of rapid and effective biosynthesis of DHA once appropriate pH, aeration and medium composition are provided [De Muynck *et al*., 2007; Hauge *et al*., 1954].

In order to adjust conditions of dihydroxyacetone synthesis by means of microbiological methods, a comparative analysis was conducted for processes with the use of free and immobilized cells of acetic acid bacteria [Raška *et al*., 2007; Nabe *et al*., 1979]. Such investigations were carried out for the strain *Acetobacter xylinum* immobilized on polyacrylamide gel [Nabe *et al*., 1979]. It was demonstrated that pH 4-4.5 appeared to be optimal for the immobilized cells whereas pH 5.5 for the free cells. The immobilized cells were less susceptible to temperature fluctuations, as compared to the free ones [Nabe *et al.*, 1979]. Cells collected from the stationary phase and then immobilized were observed to oxidize glycerol considerably faster than those collected from the logarithmic phase of growth [Gullo *et al*., 2006; Allgeier & Hildebrandt, 1960]. The yield of the biotransformation process of glycerol to DHA with the use of immobilized cells of *A. xylinum* was higher even by 18% in respect of the yield of reaction run with the presence of free cells [Nabe *et al*., 1979].

The utilization of immobilized cells in the industrial production of DHA, though promising, has been arising some doubts. Problems encountered in their use on a large scale included insufficient aeration of cells entrapped in structures of the gel as well as diminished activity and bacterial stability of glycerol dehydrogenase in cells re-used for the reaction [De Muynck *et al*., 2007]. A similar research was conducted with the use of *Gluconobacter oxydans* bacteria immobilized in polyvinyl alcohol gel [Raška *et al*., 2007]. An attempt was made to determine the initial concentration of glycerol and yeast extract as well as temperature optimal for the biotransformation process. That experiment enabled determining the most favorable conditions for the apt and effective course of the process. The most effective biotransformation proceeded at a temperature of 25-30°C, at yeast extract content of 2-4 g/L and at glycerol concentration of 20-50 g/L [Nabe *et al*., 1979]. According to other investigations carried out with cells of *Gluconobacter oxydans* immobilized in polyvinyl alcohol gel, effective synthesis of DHA from glycerol occurred at pH 6 and temperature of 30°C [Wei *et al*., 2007a]. Such cells displayed an oxidative activity against glycerol even after 14 days of storage, yet the activity was lower by 10% as compared to that of cells subjected to immediate immobilization. The re-used immobilized cells were observed to run the reaction with effectiveness of *ca*. 86%, and gel capsules they were entrapped in remained non-deformed even after five production cycles [Wei *et al*., 2007a].

Research addressing glycerol oxidation to DHA with the use of enzymes produced by acetic acid bacteria still focus mainly on reducing production costs, facilitating the process and yielding the greatest effectiveness of biotransformation. Hence a growing interest is observed in the use of immobilized cells that may be applied repeatedly in the reaction while omitting the stage of re-proliferation of biomass. Recently, agricultural wastes in the form of maize pulp have been postulated as a source of vitamins and nutrients for acetic acid bacteria [Wei *et al*., 2007b]. Such natural components would enabled minimizing costs of DHA production even by 75%. In addition, it has turned out that the activity of glycerol dehydrogenase in a culture medium containing apart from glycerol also the maize pulp is almost as high as that observed in the medium with sorbitol and yeast extract.

SUMMARY

Advance in biotechnology and emergence of innovative research methods are accompanied by increasing knowledge on physiological, biochemical and genetic properties of acetic acid bacteria. They are extensively applied for the synthesis of specified substances, including dihydroxyacetone. Demand for DHA and new possibilities of its practical application encourage scientists to elaborate more effective, faster and less expensive methods of its production.

The process of glycerol biotransformation to dihydroxyacetone may be enhanced by constructing a strain incapable of further transformations of DHA. Deletion or disruption of gene continuity of dihydroxyacetone kinase, responsible for phosphorylation of dihydroxyacetone, would enable high yield production of that compound. Nevertheless, processes of microbiological synthesis of DHA are linked with some constrictions. Bacterial metabolites present in post-fermentation fluids impair the isolation of a product with a required level of purity. An alternative means would be the application of an immobilized preparation of bacterial glycerol dehydrogenase that catalyzes oxidation of glycerol to dihydroxyacetone. Knowing the characteristics of that enzyme, an attempt might be undertaken to isolate it from a mixture of bacterial proteins, purify and apply it in an immediate reaction with glycerol. Owing to that method, crystallization of DHA would become much more simplified for the reaction mixture would contain only the product and residues of a substrate of that enzymatic biotransformation.

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