Immobilization of Yeast on Polymeric Supports

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Review
Received: May 25, 2010
Accepted: October 29, 2010

Biocatalysts (enzymes and whole cells) play a crucial role in industrial processes allowing for efficient production of many important compounds, but their use has been limited because of the considerably unstable nature of enzymes. Immobilization often protects enzymes from environmental stresses such as pH, temperature, salts, solvents, inhibitors and poisons. Immobilization of cells containing specific enzymes has further advantages such as elimination of long and expensive procedures for enzymes separation and purification and it is vital to expand their application by enabling easy separation and purification of products from reaction mixtures and efficient recovery of catalyst. This review focuses on organic polymers (natural and synthetic) used as matrices for immobilization of microorganisms, mainly baker's yeasts and potential application of immobilized cells in the chemical, pharmaceutical, biomedical and food industries.

Key words:
Microorganisms, immobilization, polymer matrices, biocatalyst

Introduction

Industrial application of biotransformations, i.e. reactions with enzymes, has become possible mainly due to the development of techniques that enable their immobilization on solid matrices. Not only can isolated enzymes be immobilized but also the microorganisms that produce them, thus avoiding the high costs of enzyme isolation and purification. Natural or synthetic polymers may serve as a macromolecular base. Such processes were known in the 17th century, when the *Acetobacter* colony immobilized on woodturnings was used for the production of vinegar.[1]

For successful immobilization, the support must be conducive to cell viability as well as have proper permeability to allow sufficient diffusion and transport of oxygen, essential nutrients, metabolic waste and secretory products across the polymer network. Particularly useful forms of carriers are hydrogels which are being investigated for cell immobilization in medicine and biotechnology. Hydrogels are polymers cross-linked via chemical bonds, ionic interactions, hydrogen bonds, hydrophobic interactions or physical bonds. These materials absorb water and swell readily without dissolving.[2] Microorganisms may be immobilized by a variety of methods, which may be broadly classified as physical where weak interactions between support and enzyme exist, and chemical where covalent bonds are formed.[3]

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The physical methods comprise:

– physical or ionic adsorption on a water-insoluble matrix

– inclusion or gel entrapment

– microencapsulation with solid or liquid membranes

– containment of an enzyme or whole cells within a membrane reactor

– formation of enzymatic Langmuir-Blodgett films.

The chemical immobilization methods include:

– covalent attachment to a water-insoluble matrix

– cross-linking with the use of multifunctional, low-molecular mass reagent

– co-cross-linking with other neutral substances, e.g. proteins.

Numerous other methods which are combinations of the ones listed or original and specific of a given support or enzyme have been devised. However, no single method and support is best for all enzymes and their applications. All of the methods present advantages and drawbacks. Adsorption is simple, cheap and effective but frequently reversible; covalent attachment and cross-linking are effective and durable, but expensive and easily worsen the enzyme performance, and in membrane reactor-containment entrapment and microencapsulations diffusional problems are inherent. This re-

view will present polymeric materials used for the immobilization of microorganisms, especially for baker's yeast.

Baker's yeast (*Saccharomyces cerevisiae*) produce many important enzymes, which are used not only in the food industry (mainly in fermentation processes) but also in chemical synthesis.[4] Baker's yeast is an economically attractive biocatalyst due to its availability and low cost, ease of handling and disposal, safety for food and pharmaceutical applications as well as its capability to catalyze a wide range of stereoselective reactions. It is noteworthy that reactions carried out in the presence of baker's yeast are pro-ecological and most of them fit within the concept of 'green chemistry'.

A frequently occurring problem in biocatalytic processes is long reaction time and arduous product recovery from the reaction mixture usually of large volume. The latter problem can be solved by immobilization of microorganisms (in our case baker's yeast) on natural or synthetic polymeric supports.

Natural polymers as carriers in the baker's yeast immobilization

A variety of natural substances can be used as support for the immobilization of enzymes. Natural macromolecular polymers have been widely applied in many fields including food fermentation, biological pharmacy, clinical diagnoses, environmental protection and power production. The main natural polymers that have been used are polysaccharides, cross-linked dextrans, starch, agarose, κ-carrageenan, chitin, chitosan and proteins such as collagen, gelatin, albumin, silk fibroin and cotton fibres. The main advantage of natural polymers is low price and absence of impurities coming from chemical reactions.

Polysaccharides

The foremost advantage that makes polysaccharides an excellent base for microorganism immobilization is easiness of forming hydrocolloids. Hydrocolloids in water undergo hydratation and swell coming into colloid solution (hydrogel), in which water molecules do not translate freely. Hydrogel makes up a three-dimensional structure in which covalent, ionic or hydrogen bonds between hydrophilic polymer chains are found. It is characteristic of this structure to absorb a huge amount of water and not interfere with cell functioning (biocompatibility). Low chemical and mechanical stability are two substantial drawbacks of hydrogels as biomaterials.

The following groups of natural and modified polysaccharides are utilized in immobilization processes:

– polyuronides – polymers of uronic acids (the carboxylic group in uronic acids is formed by oxidation of hydroxymethyl group in the sixth position of hexopyranoses), alginic acid, pectins[5]

– galactans – galactose polymers – agar,[6] agarose,[7,8] carrageenan

– glucans – polymers of glucopyranose bound with α or β –1,4-glycosidic bonds, chitin, chitosan, starch,[9] cellulose and its alkyl and carboxylic derivatives[10]

– some polysaccharides containing natural products as for example cashew apple bagasse,[11] corn starch gel[12] or orange peel.[13]

The immobilization process with the use of the mentioned matrices is usually carried out by microencapsulation or entrapment within the fibres for example within the cellulose fibres and its derivatives.[14]

This paper focuses only on the carriers of the greatest application importance.

Alginic acid salts

Alginic acid is a naturally occurring hydrophilic colloidal polysaccharide obtained from the various species of brown seaweed (*Phaeophyceae*). It is a linear copolymer consisting mainly of homopolymeric blocks of 1,4-linked β-D-mannuronate and its C-5 epimer α-L-guluronate residues, respectively, covalently linked together in different sequences or blocks (Fig. 1).

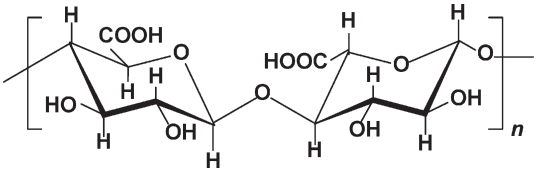


Fig. 1 – *Monomeric unit of alginic acid*

The properties of alginates predispose them to broad applications as matrices in biocatalytic processes.[15] The most important advantages of alginates are: low costs, availability, high affinity to water and capability of gel formation under mild conditions. Calcium alginate is the most frequently used alginate salt.[16,17] Calcium alginate due to its hydrophilic properties is an effective barrier to hydrophobic molecules of organic solvents[18] and in that way enables the reactions under optimal pH and temperature conditions. Typical immobilization with the use of alginate involves mixing with a biocatalyst and then instilling the mixture into the

solution of calcium chloride. By the gel beads entrapping the biocatalyst is formed as the result of the calcium – sodium ion exchange.[19,20] In this kind of immobilization also strontium and barium alginates were used instead of calcium alginate; Sr-alginate or the mixed alginates Ca-Ba or Sr-Ba systems are better entrapping agents for yeast concerning invertase activity.[16] Besides microencapsulation another method of immobilization with alginate is gel entrapment.[20]

Carrageenans

Carrageenans are linear sulphated polysaccharides extracted from red seaweeds. Their sodium salts form sticky water solution but calcium salts form gels. Yeast immobilization with the use of carrageenan carrier proceeds by gel entrapment,[21] which runs more slowly than in alginate because in this case the process is two-stage. The difference in cell colonization in these gels has also been stated. In the case of alginate, colonies of regular, spherical shapes were observed, but in carrageenan the colonies were rather of irregular form. It is suggested that the manner of cell colonization may affect their capability to protect themselves against toxic substances such as phenol[22] and may also influence their catalytic activity.[23]

Chitin and chitosan

Chitin and chitosan are natural polyaminosaccharides, chitin being one of the world's most plentiful, renewable organic resources. Chitin is a major constituent of the shells of crustaceans, the exoskeleton of insects and the cell walls of fungi where it provides strength and stability. Chemically, chitin is composed of β-1,4 linked 2-acetamido-2-deoxy-β-D-glucose units (Fig. 2), forming a long chain linear polymer. Chitosan, the principal derivative of chitin, is obtained by partial or complete *N*-deacetylation and is consequently a polymer of *N*-acetyl-D-glucosamine and D-glucosamine.

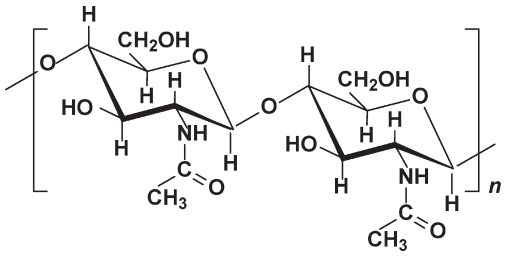


Fig. 2 – *Monomeric unit of chitin*

Chitin and chitosan can be chemically considered as analogues of cellulose, in which the hydroxyl at carbon-2 is replaced by acetamido and amino groups, respectively. Chitin and chitosan – based materials are used in the form of powders, flakes and gels[24] as enzyme immobilization supports. Chitosan gels in the form of membranes, coatings, capsules and fibres are the most frequently used in laboratory work. The methods of chitosan gel preparation can be divided into four groups: solvent evaporation, neutralization method, cross-linking method and ionotropic gelation method.[24,25]

The solvent evaporation method is mainly used for the preparation of membranes and films, the latter being especially useful in preparing minute enzymatically active surfaces (in biosensors) deposited on the tips of the electrodes. In the neutralization method an acidic chitosan solution is mixed with alkali, an increase in pH results in precipitation of solid chitosan.[26]

In the cross-linking method an acidic chitosan solution is subjected to straightforward cross-linking by mixing with a reticulating agent, which results in gelling. Overwhelmingly, as a cross-linking and surface activating agent glutaraldehyde[27] or glyoxal[28] is used. In such gel, the yeast cells may be entrapped or immobilized among formed membranes.

The application of polyelectrolytes in the form of microcapsules or membranes has also gained a lot of attention. By virtue of the attraction of oppositely charged molecules, chitosan, owing to its cationic polyelectrolyte nature, spontaneously forms water-insoluble complexes with anionic polyelectrolytes.[29]

Protein carriers

Similar to polysaccharides, proteins form hydrocolloides and are very effective and frequently used matrix for the immobilization of enzymes and whole cells.[30] The most often employed proteins are: albumin, gelatine, gluten, cotton and silk fibroin.[31–34] In this case immobilization of yeast cells can be carried out by encapsulation or by entrapment inside the fibres (e.g. cotton).

The use of silk fibroin as a support for enzyme immobilization has numerous advantages other than natural proteins. Fibroin protein is non-toxic and has certain nutritive value to humans. The preparation procedure or process using fibroin as a carrier for the immobilization of enzyme is simple and easy. The silk fibroin consists of a variety of aminoacid residues, so that there are many reaction sites such as amino, carboxyl, phenol and imidazole groups. Thus, several kinds of chemical modification methods are available to immobilize enzymes. Fibroin supports are usually prepared for immobilization in the form of fibres, powder or membranes. An attempt has also been made to combine fibroins with a synthetic polymer, i.e. polyethylene glycol.[35]

Synthetic polymeric matrices in the process of yeast immobilization

Matrices used in the immobilization of enzymes and microbes should exhibit high chemical and biological stability, mechanical resistance to abrasion, appropriate permeability to reagents and large surface, capacity and porosity. Synthetic polymeric carriers meet all of the mentioned criteria and moreover, by comparison with natural polymers their chemical stability is higher and they exhibit lower susceptibility to abrasion. The main groups of polymers used for immobilization are: acrylic polymers, vinyl polymers, amide polymers,[36] polyuretans,[37,38] poly(ethylene-oxide),[39] different co-polymers[40,41] and conductive polymers.[42,43]

Poly(vinyl alcohol) supports

Poly(vinyl alcohol) (PVA) is non-toxic to organisms and can be cheaply produced at industrial scale using poly(vinyl acetate) as a substrate. Apart from the mentioned features, such properties as porosity, chemical, physical, biological and mechanical stability have contributed to the employing of poly(vinyl alcohol) in immobilization processes.

Since PVA became a potential carrier for microorganisms, three basic methods of immobilization have been used. The first method applied was cell entrapment in gel prepared under the influence of UV irradiation.[44] Another was the so-called 'freezing-thawing' technique, which involved cell lyophilisation and several cycles of cooling and heating of gel-biocatalyst mixture, which subsequently entailed high costs and work consumption.[45] A modification of the freezing-thawing technique was introduced by Lozinsky,[46] who conducted cell immobilization avoiding their lyophilisation and employing only one cycle of freezing-thawing.

The third method of microorganism immobilization in PVA matrix is the application of highly acidic solution (for example a concentrated solution of boric acid) in the gel-forming process.[47] This method involves low costs and easy handling but on the other hand boric acid is toxic and some problems with PVA gel agglomeration occur. To prevent agglomeration a small addition of calcium alginate[48] was used, whereas the harmful influence of boric acid was limited by reducing the immersion period of the beads from 24 h to 2 h as well as applying additionally orthophosphoric acid solution as a binding agent.[49]

The lengthening of the process by another gelation stage is uneconomical therefore it was decided to replace both acids with sodium nitrate (III) solution,[50] which led to simplification of the process and to higher mechanical stability of the matrix.

Another yeast immobilization technique on PVA matrices is the Lentikat® process,[51] commercialised by geniaLab (Braunschweig, Germany).[52] The patented Lentikat® liquid (a solution of 10 %, w/v PVA) offers the possibility to entrap cells in stable hydrogels obtained by dehydration in the absence of chemical reaction starters. The lenticular form of the gel particle (named Lentikat®) obtained following gelation of the PVA solution has an optimised geometry (3–4 mm diameter and 200–400 μm thickness) which is claimed to reduce mass transfer resistance in the matrix. Moreover, using a Lentikat®Printer a reproducible large-scale production of gel particles of the same size can be obtained. This immobilization technique was reported to preserve cell viability in the case of bacterial cells. Lentikats® of different yeast strains showed to be suitable for the production of beer without noticeable changes in the activity over 6 months as well as for the production of D-galactose[53] and continuous production of glucoamylase and interleukin 1β.[54]

Polyacrylamide matrices

Acrylic polymers are polymers obtained from acrylic acid (acrylic series) or methacrylic acid (methacrylic series) or their derivatives such as amides, esters and others.

In yeast immobilization, apart from acrylic polymers, acrylic copolymers obtained during free-radical copolymerization can also be used. These kind of carriers to which belong copolymers such as 2-hydroxyethylmethacrylate/acrylamide,[55] acrylamide/maleic acid[56] or acrylamide/sodium acrylate[57] are the most frequently used in the production of ethyl alcohol. Often used is Eupergit®C, a copolymer of methacrylamide and glicydyl methacrylate cross-linked with *N,N*'-methylenebisacrylamide, which is produced on an industrial scale. Eupergit®C contains epoxy groups which function as active components for the covalent binding of ligands containing amino, mercapto or hydroxyl groups.[58] Covalent binding of a ligand introduces no alteration of electric charge into the matrix or the ligand, i.e. no electric charge is lost or generated upon binding, which is suitable for protein molecules and allows the immobilization of enzymes with high activity yields.

Smart polymers

Stimulus-responsive or smart polymers undergo strong conformational changes when only small changes in the environment (e.g. pH, temperature, electric or magnetic field, ionic strength,

some chemical compounds, light) occur.[59,60] Such polymers occur naturally (e.g. alginate, chitosan) but can also be synthesized by chemical methods (e.g. methyl methacrylate polymers available commercially as EudragitTM).[61] Linking the enzyme to these polymers obtains a biocatalyst which can be recovered and reused by applying appropriate stimulus. The most frequently used smart polymers are thermosensitive materials due to the easiness of monitoring the stimulus, and the most frequently used materials are cross-linked or reversible hydrogels, micelles or modified surfaces.[62] To this group belong mainly *N*-substituted acrylamides: the thermosensitive hydrogel of poly(*N*-isopropylacrylamide) was applied to on-chip cells immobilization and monitoring system.[63]

Apart from thermoresponsive polymers, also a wide range of pH-responsive materials are used in the immobilization processes.[64]

Conductive polymers matrices in the immobilization processes

Conductive polymers have backbones of spatially extended π-bonding system. The electrons in these delocalized orbitals have high mobility when the material is doped by oxidation, which removes some of these delocalized electrons. The same materials can be doped by reduction, which adds electrons to an otherwise unfilled band. In practice, most organic conductors are doped oxidatively to give π-type materials, although some are doped by reduction to create *n*-type materials. Conductive polymers can combine high electrical conductivity with the mechanical properties (flexibility, toughness, malleability, elasticity, etc.) and processability of plastics. Additionally, their properties can be fine-tuned using the methods of organic synthesis.

Well-studied classes of organic conductive polymers include poly(acetylene)s, poly(pyrrole)s, poly(thiophene)s, polyanilines, poly(*p*-phenylene sulphide)s and poly(*p*-phenylene vinylene)s.

The conducting organic molecular electronic materials have attracted much attention largely because of their many projected applications in solar cells, lightweight batteries, electrochromic devices, sensors and molecular electronic devices. In biosensors, organic conductive polymers are a convenient component, forming an appropriate environment for the immobilization of yeast cells at the electrode surface. The most frequently used electrochemically prepared conducting polymers are polypyrrole, poltyhiophene, polyindole, polyaniline.[65,66]

Yeast immobilization on conductive carriers takes place by physical methods (van der Waals forces, hydrogen bonds)[67,68] as well as by covalent binding and electro-polymerization.[69–71]

Exemplary application of immobilized yeast

Thanks to its many advantages, immobilized yeast finds application in many life areas,[72] mainly in the food industry (alcohol-distilling industry,[25,73] winemaking and brewing,[12,74] baking[75] but also in biotechnological fuel production,[76,77] pharmaceutical[78] and chemical industries[79–81] as well as in agriculture,[82,83] electronics (biocells) and medicine (biosensors)).[43] Because of the interactions between yeast cells and carriers some differences in the survivability and catalytic activity of the released enzymes may occur – both advantageous and disadvantageous when taking chemical reactions into account. These changes may be caused by both the type of a carrier or by the method of cell binding and may be the effect of:

– disturbances in the growth pattern of cells and their morphology due to immobilization

– changes in osmotic pressure and water activity

– altered membrane permeability and media components availability.

Moreover, the changes are difficult to predict *a priori*. For example immobilized yeast cells in calcium, strontium or barium alginate showed lower activity of invertase than in mixed system Ca-Ba and Sr-Ba.[16] Melzoch *et al*.[84] observed differences in the shape and morphology of immobilized cells and attributed them to insufficient space for growth in the support. In the case of the most frequently used polysaccharide gels, the type of microcolonies formed during cells growth depends, among other, on the used concentration and gelation method.[19]

Attention was drawn to the influence of the matrix on the functioning in alcoholic fermentation. Systematic research concerning hydrogels such as acrylamide-sodium acrylate[57] and acrylamide-maleic acid[56] was undertaken. The changes in the composition and in the method of polymer cross-linking affected the hydrophilicity, the size of the pores and the conditions of reagents diffusion, and finally, the yield of ethanol production. Many scientific reports substantiate that immobilized yeast cells show higher tolerance to the growth of alcohol concentration,[85] which in the case of poly(hydroxyalkylmethacrylic) gel is attributed to the alteration of the composition of cell membrane (the growth of saturated acids content, the decrease of unsaturated acids content and the higher amounts of phospholipids and ergosterol) and for poly(acrylamide-hydrazide) (PAAH) crosslinked by glyoxal[86] to the formation of a polymer coating onto yeast cells.

Immobilization can affect enzymes activity by pH alteration – immobilized yeast shows slightly

higher pH values inside cells due to the increased permeability of cytoplasm membrane in relation to protons, which intensifies the glycolytic activity of yeast.[87] Every change in metabolism is crucial to the food industry, not only because of the overall process yield but also because of the changes in the synthesis of flavour and fragrance compounds which determine the organoleptic quality of the product.[30]

Food industry

The course and the effectiveness of the fermentation taking place in the presence of immobilized microorganisms depends on the method of their immobilization, the type of the bioreactor and the applied technique.[88] Calcium alginate, carrageenan, gelatine, polyacrylamide and epoxy resin are considered the most suitable supports in alcoholic fermentation. The cells immobilized on a solid carrier form a thin film usually of the range from one layer of cells to 1 mm or more. The entrapment within porous matrix is based on inclusion of cells within a network, and in this case, the cell growth depends on diffusion limitations. Cell flocculation and mechanical containment behind a barrier are also applied in alcoholic beverages and potable alcohol production.[30]

Brewing and winemaking are the branches of the food industry that are directly based on alcoholic fermentation. In brewing immobilized yeasts were used for the first time at the end of the 60s. Several organic materials were used as immobilization supports for the production of beer, such as polysaccharides (calcium alginate, carrageenan, pectins), poly(vinyl alcohol) as well as modified polystyrene and modified polyethylene. The last two mentioned are usually employed in the production of non-alcoholic beer.[74] In winemaking, cell immobilization on natural supports such as alginate, cellulose, carrageenan, agar, pectine, chitosan and gelatine contributes to inhibiting of toxic influence of the produced ethanol on microbes. In both brewing and winemaking the cell immobilization on polymeric support has a positive impact on the condition of the process as well as on the properties of the obtained products, among other, on the quality of their flavour.[30] Immobilization of yeast cells is a promising method for efficient continuous industrial-scale production of fermented beverages[89] and continuous beer fermentation.[90]

Another branch of the food industry that exploits immobilized baker's yeasts is baking. In this case, immobilization also has a positive effect on the fermentation process. The advantage stems mainly from the possibility of running the process at low temperature (< 5 °C), which promotes amylase activity – an enzyme responsible for the reduction of sugars present in flour. The amylolitic activity of yeasts was a crucial factor in selecting a proper support for their immobilization. Alginate inhibits both enzyme activity and yeast metabolism. Gelatine showed no inhibitory effects even at high concentrations, while carrageenan was not tested since it gels at the measurement temperature. Alginate and gelatine have thus antagonistic effects on the fermentation process. However, gelatine did not ensure a proper aggregation of micro-beads therefore another strategy was used that involved micro-beads formed of alginate and gelatine in the ratio 1:12.5. Such a solution induced a proper aggregation and at the same time increased enzyme activity.[33]

Biotransformations

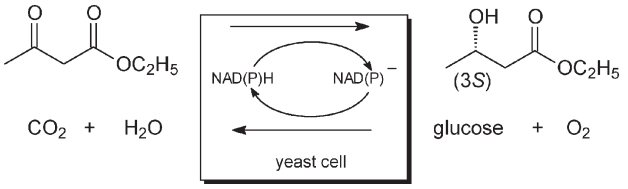
The use of whole microorganisms to carry out stereospecific and stereoselective reactions has taken on greater significance. These reactions have proven useful in the asymmetric synthesis of molecules with important biological activities. Additionally, biotransformation reaction technology is deemed economically and ecologically competitive in the search for new useful compounds for the pharmaceutical and chemical industries.

The current interest in applying baker's yeast in organic synthesis is mainly related to their chemo- and stereoselectivity[91–93] under environmentally friendly conditions. Significant attention has been paid to the stereo- and enantioselective synthesis of enantiomerically pure compounds of chiral synthons needed under the increasing demand for the development of modern drugs and agrochemicals. From among the chiral compounds pure alcohols are particularly useful as building block for the synthesis of pharmaceuticals and agrochemicals.

The carbonyl group reduction[94,95] is probably the most extensively studied baker's yeast mediated biotransformation. The use of whole microbial cells is particularly advantageous for carrying out reductions of ketones since they do not require the addition of cofactors for their regeneration. This is important in alcohols oxidations as well.[96]

The change in the preparation of the biocatalyst by immobilization, for example in calcium alginate, makes the purification of products much easier, moreover the enantioselectivity of the reduction is usually higher (from 85 % to 98 % for ethyl 3-oxobutanoate[97]) (scheme 1) and the activity of immobilized baker's yeast could be retained for a long period of time.[98]

A higher enantiomeric yield is sometimes accompanied by a slower reaction rate – the reaction is hindered by the diffusion resistance, which in the



Scheme 1 – *Reduction of carbonyl group in the presence of baker's yeast*

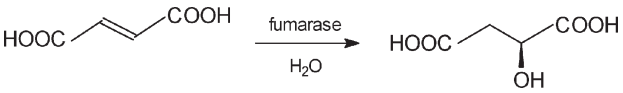
case of the synthesis of (*R*) – mandelic acid from phenylglyoxal acid, could have been compensated by more vigorous stirring.[99] The yield of the reaction largely depends on the polymeric support in which the cells were immobilized.[100] In the reduction of α-diketones, better results were obtained in the presence of microencapsulated yeast in polyamide matrix then using yeast immobilized in alginate.[36]

The yeast immobilization in calcium alginate[101] or microencapsulation in polyamide[102] was also effective in protecting the cells against the lethal effects of the organic solvent and maintaining their viability. The tolerance of sol-gel immobilized *Saccharomyces cerevisiae* increases with the logP value of the solvent.[18] A similar correlation was stated in the case of the viability of yeast immobilized in the polyhydroxylated silane network in organic solvents such as ethanol, propanol, butanol, pentanol, hexanol, heptanol and octanol.[103] The authors ascribe it to the increased diffusion easiness of polar solvent compounds by a hydrophilic barrier that forms on the phase boundary. Matrices that bind water very tightly will help protect the biocatalyst against the water distorting activity of the surrounding organic solvent, and hence increase viability and biocatalytic properties. Immobilization of microorganisms for application in organic media not only has the advantage of enhanced tolerance but also allows for their easy recovery, reduction of microbial contamination problems, as well as increases solubility of non-polar substrates.

The positive influence of immobilization in alginate on yeast viability permits the reduction reaction to be carried out in the presence of solvents accepted by green chemistry such as glycerol,[104] perfluorooctane,[105] ionic liquids[106] and enables continuous production (ethyl benzoyl formate reduction).[107] Baker's yeast immobilized in nanoporous silicates has been employed in the reduction of aromatic nitro compounds,[108] in alginate to reduce carbon – nitrogen double bonds[109] and also as a catalyst in esters hydrolysis.[110]

Moreover, other strains of immobilized filamentous fungi were applied in the reduction of ethyl benzoylacetate[111] or substituted acetophenones[112] and the alginate immobilized cells of *Candida lipolytica* accelerated the degradation of petroleum derived hydrocarbons,[113] which can be applied in the biodegradation processes.

Apart from the reduction of carbonyl compounds, the synthesis of L-malic acid is a useful biotransformation catalyzed by baker's yeast. L-Malic acid is the second most popular general-purpose food acid and holds about 10 % of the market. The enzymatic conversion of fumaric acid to L-malic acid is catalyzed by fumarase from different *Saccharomyces* species[114] and thus immobilized cells of *Saccharomyces cerevisiae* and *Saccharomyces bayanus* were applied in this reaction.[115,116]



Scheme 2 – *Biotransformation of fumaric acid to L-malic acid*

The yeast were immobilized in beads of composite silicate-alginate matrix[117] or agarose beads and microspheres.[118] Baker's yeast immobilized on various polymeric materials (eg polystyrene, polytetrafluoroethylene, perfluoroalkoxy and fluorinated ethylene-propylene) were applied to the construction of microreactors, which can be used for the development of the biotransformations in microscale.[119]

Environment protection and biosensors

Toxic heavy metal pollution has become a central environmental problem of today. The biological methods for their remediation, including biosorption with the use of microorganisms (fungi, algae, bacteria)[120,121] are considered promising for the treatment of high volume and low concentration complex wastewaters. Immobilized baker's yeast is an ideal biomaterial widely used in this field.[122] *Saccharomyces cerevisiae* were applied in the biosorption of Cd(II) and Zn(II)[123] (immobilized on calcium alginate), as a new magnetic adsorbent for the adsorption of Cu(II) from aqueous solution[124] (immobilized on the surface of chitosan-coated magnetic nanoparticles (SICCM)), and as environmentally friendly biosorbents to evaluate the uptake process of anionic and cationic mercury(II) species as well as other metal ions[125] (immobilized on Dowex anion exchanger).

Immobilized viable cells have gained considerable importance recently in the fabrication of biosensors,[126] which are finding applications in a variety of analytical fields.[127,128] They provide a rapid and convenient alternative to conventional methods for monitoring chemical substances in

fields such as medicine, environment, fermentation and food processing. The basic requirement of a biosensor is that the biologic material brings the physicochemical changes in close proximity to a transducer. In this direction, immobilized cell technology has played a major role. Immobilization not only helps in forming the required close proximity of the biomaterial with the transducer but also in stabilizing them for reuse. The major limitation of immobilized cell-based biosensors has been the slow response compared with the enzyme sensors.[129] This has been attributed mainly to the mass transfer resistance offered by the cell membrane, especially for the intracellular enzymes. Nevertheless, the immobilized yeast-based biosensors can be successfully employed in studying some metabolic characteristics,[130] estimation of BOD (Biological Oxygen Demand),[131] determination of lysine[132] or vitamin B,[133] heavy ions, penicillin, urea, creatinine and different alcohols.

Immobilized yeast, that produce oxidoreductase, is used in microbial fuel cells that convert chemical energy into electrical energy by the reaction of microorganisms.[134] The most obvious target for biofuel cells research is still for *in vivo* applications where the used fuel could be withdrawn virtually without limit from the blood to provide a long-term or even permanent power supply for such devices as pacemakers, glucose sensors for diabetics or small valves for bladder control.[135]

Summary

This review indicates the broad possibilities of applying immobilized baker's yeast. The proper immobilization facilitates for conducting of the process, enables recycling of the catalyst and makes the cells more resistant to external factors which may have a detrimental effect on their viability. Moreover, when taking the chemical reaction in an organic solvent into account, immobilization protects the viable cells against the lethal effect of the solvents. Therefore, technologies that employ immobilized cells allow for higher effectiveness of bioreactors, while at the same time, a reduction in the operational volume is observed. Such technologies accelerate the course of processes and diminish the biomass volume necessary for conducting them, and what is more, they fully meet the Green Chemistry requirements.

The application of natural supports is especially attractive in the food processing industry, whereas synthetic matrices are particularly required in analytical and synthetic chemistry because of the possible structure optimization depending on the requirements.

No correlation between the support structure and the activity of immobilized baker's yeast has been stated so far, but some processes connected with mass transfer can be described by mathematical modelling.[136]

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