CHEMIJA. 2006. Vol. 17. No. 4. P. 74–89
© Lietuvos mokslų akademija, 2006
© Lietuvos mokslų akademijos leidykla, 2006

Application of polyurethane-based materials for immobilization of enzymes and cells: a review

Tatjana Romaškevič[1],

Saulutė Budrienė[*1],

Krzysztof Pielichowski[2] and

Jan Pielichowski[2]

[1]*Department of Polymer Chemistry, Vilnius University, Naugarduko 24, LT-03225 Vilnius, Lithuania*

[2]*Department of Chemistry and Technology of Polymers, Cracow University of Technology, Warszawska 24, 31-155 Cracow, Poland*

Polyurethane as a functional material has a wide application in many areas. This paper is designed to the application of polyurethanes in biochemical and biotechnological fields, concerning its biocompatibility and stability. The synthesis of different kinds of polyurethanes, their application for the immobilization of enzymes and whole cells, and their employment in the constructions of biosensors are reviewed. The basic concepts of such application are described.

Keywords: polyurethanes, microspheres, immobilization, enzymes, cells, biosensors

INTRODUCTION

Polyurethanes are one of the most versatile materials in the world today. They are known for being a perfect material for footwear, machinery industry, coatings and paints, rigid insulation, elastic fiber, soft flexible foam, medical devices [1]. The unique properties of PU deserve popularity as PU offers the elasticity of rubber combined with the toughness and durability of metal, it is resistant to oils, solvents, fats. Products, coated with PU last longer, PU adhesives provide strong bonding advantages, PU elastomers can be molded into any shape, they are lighter than metal and offer good resistance to environmental factors [2]. Rigid PU foam is used as water heaters, insulation for buildings, commercial and residential refrigeration, for flotation and for energy management. Flexible PU foam is irreplaceable in the industries of furniture, bedding, carpet, packaging and machinery. Thermoplastic PU is highly elastic, flexible and resistant to many environmental factors, that is why it has found its application in footwear production, wire and cable coatings, architectural glass lamination, auto-body side molding, medical tubing and biomedical apparatus, etc. [3].

Some time ago PU was found to be applicable in the biochemical and biotechnological fields as a perfect support for enzyme immobilization, a membrane in analytical biosensors. Natural, artificial and synthetic high-molecular substances with different structures can be used for enzyme immobilization, and one of the best supports for this purpose is PU. It can be used in the following states: foam, microspheres and microcapsules, nanocomposites and membranes. PU applications for those purposes will be described in this paper in detail.

POLYURETHANES

Foam structure and properties

Foam is a microcellular structure, produced by the internal generation or liberation of a gas in a fluid medium that simultaneously polymerizes while expanding in volume [4].

The final product is either an open-, closed- or mixed-cell foam [3]. The mixed-cell structure is characterized by the percentage of the closed-cell. The closed-cell foam has better thermal isolation and higher resistance to external factors. The open-cell structure foam has a better acoustic insulation, permeability of water vapour and gases [5].

PU foams can be polyether- and polyesterurethanes. The first one is resistant to hydrolysis and chemical treatment, however it is not resistant to the oxidation process. Polyesterurethanes have a high mechanical stability, but have no resistance to chemical factors [5].

PU foams by their types are classified into flexible, semi-flexible and rigid foams. The flexible foam show

* Corresponding author. E-mail saulute.budriene@chf.vu.lt

relatively low-bearing properties with high recovery properties, similar to a coiled metal spring, while the rigid foam displays high load-bearing (but a definite yield point) properties and a subsequent cellular collapse and lack of recovery. The semiflexible foam possesses a mixture of these characteristics. Any cellular product that falls between the values for the rigid and flexible foam curves can be classified as semiflexible. Microcellular urethanes are high-density elastomers of cellular composition, which has a linear load deformation. They are designed for heavy-duty mechanical applications [3].

The flexible PU foam yields open-cell materials that allow a free movement of air throughout the materials when flexed. They remain flexible down to –18 °C. In general, the flexible foam is based on polyether (the most important are the polyoxypropylene derivatives [5]) and polyester polyols. The latter is less resilient and less stable to hydrolysis but exhibit higher strength and elongation. The working temperature of these foams varies from 50 to 100 °C, depending on the application. The flexible foam is unstable to UV radiation. [3].

Semiflexible PU foam is produced by using suitable combinations of polyesters and isocyanates. This foam is somewhat thermoplastic and do not melt, but it becomes notably softer with a moderate increase in the temperature, however this foam does not distort under its own weight below 90 °C, and hence can be used at higher temperatures if not under stress. The semiflexible foam is composed of open cells, and water can be mechanically absorbed. Acoustic insulation is one of the advantages of the open-cellular structure [2].

Rigid foam has a high strength and low weight, good resistance properties, an excellent adhesion to metal, wood, and ceramics. The percentage of the closed cells in the rigid foam depends on the degree of cross-linking and the surfactant used during the foaming as well as on the polyol equivalent weight. The strength of the rigid PU foam increases with the increasing density and decreases with the increasing temperature, particularly at higher densities [3].

Chemical and physical properties of PU foams depend on many factors: functional groups, bond strength, crosslink density and flexibility, the effect of isocyanates, polyol, and their ratio.

PU foams are obtained by a reaction of isocyanates (di-, tri-, and other) with glycols (polyglycols, polyester polyols, or polyether polyols). However, urethane foams contain a large number of functional groups other than urethane linkages. For example, in addition to ester and ether groups, urea, biuret, allophanate, and imide groups may be found in these polymers [4].

The stability of the foams derived from aliphatic diisocyanates is greater than of their aromatic counterparts.

The regulation of the crosslink density allows the urethane polymer molecule to vary from the uncrosslinked linear polymers (fibers and thermoplastics) to a moderate crosslinking (elastomers, flexible coatings and foams) and finally to the very highly crosslinked structures (rigid thermosetting resins and foams). Crosslinking can be controlled by [4]:

1. Molecular weight and functionality of the polyol component;
2. Functionality of the isocyanate component;
3. Structure and functionality of a chain extender.

For the flexible foam, the most commonly used isocyanate is toluene diisocyanate (TDI) in various 2,4- and 2,6-isomer molar ratios. Diphenylmethane diisocyanate (MDI) in various forms has been found to be increasingly used, particularly in high-resiliency flexible and semiflexible foam [3]. The diisocyanates used in the rigid foam production are modified TDI, MDI or polymethylene polyphenyl diisocyanate.

Diisocyanates are mostly used for the synthesis of PU foams (about 75% of TDI). A lot of PU foams are available as commercial products. Foams can be produced by mixing commercial prepolymers, containing different amounts of active NCO groups with diol-containing commercial composition [6]; or it is possible to use a commercial hydrophilic foamable PU prepolymer which is a water-activated derivative [7–11].

The source of hydroxyl groups for almost all commercial uses of urethane polymers are polyethers, polyesters and naturally-occuring hydroxyl-bearing oils such as castor oil [3]. Polyols can vary in equivalent weight, functionality, and the degree of rigidity or flexibility contributed by the different chain units in the polyols. Polyol determines whether foam is rigid or flexible, brittle or non-brittle, and the extent of its permeability of gas and moisture. The flexible foam is produced from polyols of a moderately high molecular weight and a low degree of branching, while the rigid foam is prepared from a lower-molecular-weight highly branched resin.

The functionality of polyols has a substantial effect on the properties of the rigid foam. The higher polyol functionality favors the greater heat resistance and dimensional stability [4].

At a higher ratio of NCO/OH, the compressive strength increases, but the foams tend to become more brittle. In general, the isocyanate index (ratio of NCO/OH·100) of about 105 produces the best cost and performance ratio [4].

Microspheres. Synthesis and properties

Usually PU microspheres can be prepared by a one- or two-step suspension (dispersion) method [12–18].

The synthesis of the spherical particles of PU is patented in [12]. PU microspheres with a controlled particle size which comprises the reaction of diol (such as ethylene glycol [EG], 1,2-propylene glycol, 1,3-propylene glycol, diethylene glycol, 1,4-butane diol, 1,3-butane diol, 1,6-hexane diol, 2-ethyl-1,3-hexane diol and others) and an organic diisocyanate (for example 2,2,4-trimethyl hexamethylene diisocyanate, 1,4-tetramethylene diisocyanate, 1,6-hexamethylene diisocyanate,

isophorone diisocyanate, 4,4'-methylene-bis-(cyclohexane diisocyanate), meta or para-tetramethyl xylene diisocyanate, α'-xylylene diisocyanate, TDI, 1,4 phenylene diisocyanate, MDI, etc.), and optionally a multifunctional hydroxyl compound in the presence of a polycondensable macromonomer, having a long chain hydrophobic moiety and reactive hydroxyl groups at the chain terminal, and a catalyst (which is selected from the group essentially consisting of triethylene diamine, morpholine, N-ethylmorpholine, piperazine, triethanolamine, triethylamine, dibutyltindilaurate, stannous octoate, dioctyltin diacetate, lead octoate, stannous tallate and dibutyltin dioxide) in an organic solvent at temperatures ranging from 40 to 100 °C for a period ranging between 2 and 12 hours, separating the spherical polyurethane particles from the reaction mixture by a resin reactor. The particle size was ranging from 150 nm to 500 μm, depending on the concentration of the macrodiol stabilizer.

PU powder consisting of spherical microspheres with a relatively narrow particle size distribution in the range of 1–100 μm and preferably between 10 and 50 μm can be synthesized in a non-aqueous medium at low temperatures. Suspension (by some authors entitled as a dispersion polymerization) step type polymerization of PU microspheres in paraffin oil was presented by Ramanathan and associates [13] and patented in [14]. Dispersion polymerization of 2-ethyl 1,3-hexanediol and TDI was carried out in paraffin oil in the presence of different amounts and composition of macrodiol stabilizers. Nearly monodisperse particles in nanometer size are obtained using 10% of the weight of a long-chain stabilizer. Micron size PU particles are formed with a broad particle size distribution, using 5% of the weight of macrodiol [13].

PU microparticles were also obtained by a suspension process in paraffin oil from ethylene glycol and TDI using an amphiphilic block copolymer (poly(butadiene-*b*-ethylene oxide)) as a steric stabilizer [13]. This stabilizer was used in respect that it contained a hydrophilic anchor block (polyethylene oxide segment) and a freely soluble stabilizing moiety (polybutadiene segment) in the dispersion medium. An increase in the stabilizer concentration has no significant effect on the particle size. However, the increasing stabilizer concentration change from a bimodal to an unimodal size distribution was observed.

PU microspheres prepared according to [13] were covered by gold nanoparticles. The obtained nanogold-PU composites were employed for the immobilization of several enzymes.

PU microspheres were also produced by a polyaddition of EG and TDI in cyclohexane at 60 °C, in the presence of dibutiltin dilaurate as a catalyst. PU synthesis in organically dispersed media was carried out using functional homopolymers such as ω-hydroxypolystyrene as a steric stabilizer. The authors [15] noticed, that the ability of hydroxy-therminated polystyrene to play the role of a stabilizer depended on its concentration and size as well as the method of addition. The stabilization of the particles was obtained by *in situ* formation of polystyrene-*b*-polyurethane block copolymers insoluble in the reaction media. When ω-hydroxypolystyrene of a low molar mass was used as a reactive stabilizer of the dispersion, a narrow size distribution of PU microspheres (range 0.2–5 μm) was obtained. Polystyrene-*b*-poly(ethylene oxide) block copolymers were also used as steric stabilizers in the production of PU microspheres, however such block copolymers were not well suited for this purpose.

A one-step suspension process for the preparation of essentially linear PU-urea granules consists of adding to water a solution from about 2 to about 20% of weight of a water-soluble inertia-suspending agent and an organic diisocyanate (bis(4-isocianatocyclohexyl)methane), a water-immiscible polyol (polycaprolactone diol) and a catalyst (dibutiltin dilaurate) [16].

The spherical PU particles were prepared by a suspension (dispersion) process using water and/or alcohol as a dispersing medium. Polyisocyanate, polyol and other additives/reagents were first dissolved in an organic solvent which was dispersed in water in the presence of a stabilizer such as poly(ethyleneoxide-propyleneoxide) block copolymer, gelatin, poly(vinyl alcohol), methyl cellulose or sodium alkyl sulphate [17].

Jabbari and Khakpour [18] presented a two-step suspension process method with MDI as isocyanate, polyethylene glycol (PEG 400) as diol, and 1,4-butanediol (BD) as a chain extending agent. In the first step, the PU prepolymer from MDI, PEG 400 and BD was formed. Next, the prepolymer was added dropwise to the aqueous phase. 1,4-butenediol was used to increase the ratio of hard to soft segments of the PU network, and its effect on the microsphere morphology was studied with SEM. As the amount of the chain extending agent increased from zero to 50% by mol, the number of pores decreased and the typical pore diameter decreased from 950 to 600 nm (Figs. 1 and 2). With the further increase of the chain-extending agent to 60–67%, the microspheres became non-porous. PU microspheres were as a connection point of using nanomaterials in the immobilization fields.

PU particles could be obtained by a criogenic grinding of the thermoplastic PU [19].

Immobilization of enzymes

Enzymes are biologic polymers that catalyze the chemical reactions that make biological life possible. They have a wide variety of biochemical, biomedical, pharmaceutical and industrial applications. Enzymes exhibit a number of features such as [20]:

• High level of catalytic efficiency;

• High specificity (e. g. substrate specificity, regiospecificity, stereospecificity etc.);

• Non-toxicity;

• Water solubility.

The major advantage is that the catalyzed reaction is not perturbed by a side-reaction, resulting in the

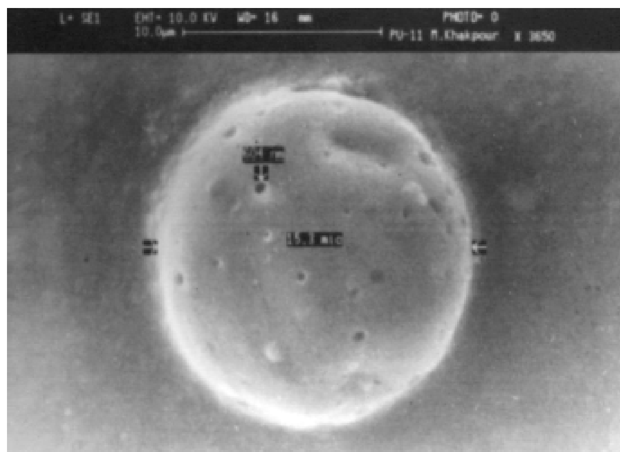


Fig. 1. Scanning electron micrograph of a microsphere prepared by suspension polycondensation without a chain-extending agent at magnification of 2660. The homogenization speed was 13500 rpm. The emulsifer was polyvinyl pyrrolidone with a concentration of 1% w/w of the aqueous phase. A typical pore size was 950 nm, as marked in the micrograph [18]

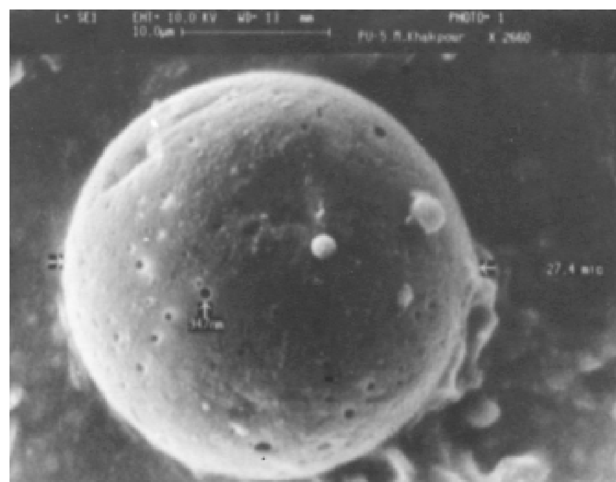


Fig. 2. Scanning electron micrograph of a microsphere prepared by suspension polycondensation with 50 mol% of the PEG 400 diol substituted with a chain-extending agent, 1,4-butanediol, at magnification of 3650. The homogenization speed was 13500 rpm. The stabilizer was polyvinyl pyrrolidone with a concentration of 1% w/w of the aqueous phase. Typical pore size was 600 nm, as marked in the micrograph [18]

production of one required end-product. In addition, the enzymatic reaction took place at mild conditions of temperature, pressure and pH with the reaction rates of the order of those achieved by chemical catalysts at more extreme conditions [20]. However, there are some practical disadvantages of the use of enzymes, and one of the major is that most enzymes operate dissolved in water in homogeneous catalysis systems, and the main problem is the separation of enzymes, especially the separation of their active form, from the reaction media for reuse.

A possible solution of this problem can be enzyme immobilization. This is a method of keeping the enzyme molecules confined or localized in a certain defined region of space with a retention of their catalytic activity. In comparison with their native form, the immobilized enzymes offer several advantages such as [9]:

- Enhanced stability;
- Easier product and enzyme recovery and purification;
- Possibility of a repeated usage of enzyme;
- Continuous operation of the enzymatic processes;
- Rapid termination of reaction.

Several techniques may be applied to immobilize them on a solid support. They are based on chemical and physical methods [21].

The chemical immobilization where covalent bonds with enzyme are formed include:

- Enzyme attachment to the matrix by covalent bonds;
- Cross-linking between enzyme and matrix;
- Enzyme cross-linking by multifunctional substances.

The physical immobilization where weak interactions between the support and enzyme exist include [22–28]:

- Entrapment of the enzyme molecules;
- Microencapsulation with a solid or liquid membrane;
- Adsorption on a water-insoluble matrix.

Both physical and chemical immobilization methods offer some advantages and disadvantages. During the chemical methods, a loss of the activity of enzyme is observed; covalent bonds formed as a result of the immobilization can perturb the enzyme's native structure, but such covalent linkages provide strong and stable enzyme attachment and in some cases can reduce the enzyme deactivation rates. The physical immobilization methods more or less perturb the native structure of enzyme, but in this case the enzyme does not bind to the carrier. That is why low activity of the immobilized enzyme is observed [21].

Enzyme attachment to the matrix by covalent bonds is one of the most widely applied methods [29]. The bond is created through the reaction of reactive groups at the protein surface (e.g., NH_2 or OH groups). Via the immobilization by a covalent attachment, the stability of the enzyme caused by a strong interaction with the carrier is increased. But this strong interaction can limit the molecular flexibility of the enzyme and can have an effect on its activity [29].

Enzyme cross-linking by multifunctional reagents involves the attachment of molecules of the enzyme to each other via the covalent bonds. The immobilization of the enzyme is performed by the formation of intermolecular cross-linkages between the enzyme molecules by means of a bifunctional and multifunctional reagent such as glutaraldehyde, bisdiazobenzidine and hexamethylene diisocyanate [28]. This immobilization method is attractive due to its simplicity, but the cross-linking of the dissolved enzymes is hard to control.

The cross-linking between the enzyme and the carrier by a multifunctional reagent involves an attachment of molecules of the enzyme to the carrier. This

immobilization method is similar to the previous one, but with one exception: in this case the bi- or multi-functional compound perform a cross-linker's role and the intermolecular cross-linkages between the enzyme and the carrier are formed by this cross-linker.

The entrapping method is based on confirming enzymes in the lattice of a polymer matrix or enclosing enzymes in semipermeable membranes [28]. There is no chemical binding between an enzyme and a carrier, but a part of the enzymatic activity can be lost if a chemical polymerization is used. The entrapment in a polymer matrix can be accomplished by polymerizing or cross-linking. However, this method can be universal, but there is another disadvantage – the pore size – because a large pore size can cause enzyme leakage and a small pore size can prevent a diffusion of large substrate molecules into the matrix to reach the biocatalyst [29].

Microencapsulation is performed with semipermeable polymer membranes in such a way that enzymes are enclosed in microcapsules. The usual membrane pore size ranges from 1 to 100 nm, which is sufficient to prevent enzyme leakage and to allow the substrates to dialyse freely across the membrane [30]. Like in the entrapment case, the small pore size can be a disadvantage for high molecular weight substrates.

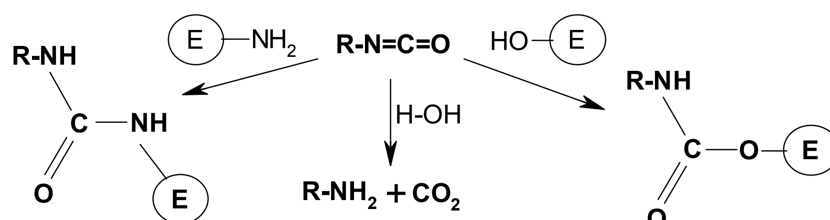
Adsorption on a water- insoluble matrix is one of simplest methods of immobilization, which is based on the physical interaction between the enzyme and the support surface. The bound is weak and affected by pH, temperature and a contact with salts and solvents [29].

Important applications of the immobilized enzymes in an analysis are their use in biosensors and diagnostic test strips. Those biosensors are constructed by integrating the immobilized enzymes with transducers. The enzymes are immobilized on a transducer's working tip or in/on a polymer membrane tightly wrapping it up. In principle, due to the enzyme specificity and sensitivity, biosensors can be tailored for nearly any target analyte, and these can be both enzyme substrates and enzyme inhibitors [31–33].

Application of polyurethanes for immobilization

PU foams, microspheres and microcapsules, and nanocomposites are used in biochemical areas.

PU can be considered as a suitable carrier for enzyme immobilization. A lot of scientists have noticed the advantage of PU foams for immobilization: easy control of the pore size, stable maintenance of the quantity of cells and large-scale application at low price. An enzyme can be attached to PU support covalently, by a physical adsorption, by an entrapment or by a coupled chemical and physical binding. During the covalent attachment of an enzyme to PU, the amino groups or/and hydroxyl groups of the enzyme react with the isocyanate groups of PU. As the immobilization procedure takes place in aqueous media, the water reacts with the isocyanate groups by forming CO_2 [9]. But this process is quite suitable because in this case no free NCO groups are left and they do not have any inactivation effect on the enzyme. The immobilization onto PU foams is presented in Scheme 1.



Scheme 1. Immobilization of enzymes onto PU foams (E-enzyme)

As immobilization carriers, PU-based materials are used in various forms: foam, microspheres and microparticles, powder, layer and coatings. The enzymes and cells immobilized onto/into PU are presented in Table 1

Table 1. **Immobilization of enzymes onto PU**

PU kind: isocyanate/diol (producer) if introduced	Enzyme (EC)	Application	Immobilization method, notes	References
Crystalline PU foam: TDI/propylene glycol	Amyloglucosidase (3.2.1.3)	Immobilization studies	Covalent attachment	[41]
Elastomeric PU foam: TDI/propylene glycol	Amyloglucosidase (3.2.1.3)	Immobilization studies	Covalent attachment	[41], [42]
HYPOL PU foam	Parathion hydrolase	Removal and detoxification of localized organophosphate pesticide spills	Covalent attachment	[85]

PU coatings: HMDI/polyol BAYHYDUR; polyisocyanate XP-7063, XP-7007, XP-7148 / BAYHYDUR polyol XP-7093 (Bayer Corp. Pittsburgh, PA)	Diisopropylfluoro-phosphatase (3.1.8.2)	Immobilization studies	Irreversible covalent incorporation of enzyme into water-borne PU coatings in single step protein-polymer synthesis	[53], [106]
PU foam	*Humicola lanuginosa* lipase (3.1.1.3)	Immobilization studies	Covalent attachment	[86]
PU foam (Safoam Company, Tehran, Iran)	Human butyrylcholinestarase (3.1.1.8)	Scavenger of organophosphorus pesticides and chemical warfare agent	Entrapment; starch was used as protective additive	[36]
PU foam (Vladimir, Russia)	Alkaline phosphatase (3.1.3.1)	Lead (II) determination	Entrapment; N-phtaloylchitosan was used as protective additive	[35]
	Horseradish peroxidase (1.11.1.7)	Mercury (II) determination	Entrapment; chitosan was used as protective additive	[34]
PU foam HYPOL 3000: TDI prepolymer (Hampshire Chemical GmbH, Germany)	Fetal bovine serum acetylcholinesterase (3.1.1.7), equine serum butyrylcholinesterase (3.1.1.8)	Detoxification and decontamination of organophosphates (OP) and as a biosensor to long-term OP determination	Covalent attachment	[43]
	Aspergillus ficuum phytase (3.1.3.8)	Immobilization studies	Covalent attachment	[9]
	Aspergillus melleus aminoacylase (3.5.1.14)	Production of L-amino acids	Covalent attachment	[9]
	Aspergillus niger glucose oxidase (1.1.3.4)	Biocatalyst in enantioselective sulfoxidation via *in situ* H_2O_2 formation from glucose and hydrogen	Entrapment and covalent attachment	[8]
	Aspergillus oryzae β-D-galactosidase (3.2.1.23)	Immobilization studies	Covalent attachment	[39]
	Caldaromyces fumago chlorperoxidase (1.11.1.10)	Catalyst for enantioselective oxygen transfer reactions	Covalent attachment	[9, 10]
	Caldaromyces fumago chlorperoxidase (1.11.1.10)	Biocatalyst in enantioselective sulfoxidation via *in situ* H_2O_2 formation from glucose and hydrogen	Entrapment and covalent attachment	[8]
	Escherichia coli phosphotriesterase (3.1.8.1)	Immobilization studies	Covalent attachment	[37]
PU foam HYPOL 5000: MDI (Hampshire Chemical GmbH, Germany)	*Escherichia coli* phosphotriesterase (3.1.8.1)	Immobilization studies	Covalent attachment	[37]
PU foam HYPOL FHP 2002 (Hampshire Chemical GmbH, Germany)	*Aspergillus niger* β-glucosidase (3.2.1.21)	Immobilization studies	Covalent attachment	[87]
	Cellulase (3.2.1.4) and β-glucosidase (3.2.1.21)	Immobilization studies	Covalent attachment, coimmobilization	[87]

Table 1 (continued)

	Glucose isomerase) (5.3.1.18), cellulose (3.2.1.4), β-glucosidase (3.2.1.21	Immobilization studies	Covalent attachment, coimmobilization	[88]
	Streptomyces rubiginosus glucose isomerase (5.3.1.18)	Immobilization studies	Covalent attachment	[88]
	Aspergillus niger amyloglucosidase (3.2.1.3)	Immobilization studies	Covalent attachment	[40]
	Candida rugosa lipase (3.1.1.3)	Oils and fats hydrolysis, esterification, interesterification	Entrapment and covalent attachment	[38]
PU foam HYPOL FHP 8190H (Hampshire Chemical GmbH, Germany)	*Aspergillus niger* amyloglucosidase (3.2.1.3)	Immobilization studies	Covalent attachment	[40]
PU foam HYPOL FHP X 4300: MDI prepolymer (Hampshire Chemical GmbH, Germany)	*Candida rugosa* lipase (3.1.1.3)	Oils and fats hydrolysis, esterification, interesterification	Entrapment and covalent attachment	[38]
PU foam: MDI/BD (HYPERLAST composition, Derbyshire,UK)	*Penicillium canescens* β-galactosidase (3.2.1.23)	Immobilization studies, lactose hydrolysis in whey	Covalent attachment	[6]
PU foam: TDI/EG	Cellulase (3.2.1.4)	Immobilization studies	Covalent attachment	[41, 42]
	Pectinase (3.2.1.15)	Immobilization studies	Covalent attachment	
PU foam: TDI/PEG (1000)	Lactase (3.2.1.23)	Immobilization studies	Covalent attachment	
	Trypsin (3.4.4.4)	Immobilization studies	Covalent attachment, glycerol or pentaerythritol as cross-linking agent	
	Urease (3.5.1.5)	Immobilization studies	Covalent attachment	
	Glucose isomerase (5.3.1.18)	Immobilization studies	Covalent attachment, glycerol as cross-linking agent and heat stability additive	
	Penicillin amidase (3.5.1.11)	Immobilization studies	Covalent attachment	
PU foam: TDI commercial polyisocyanate (9.5% free NCO groups /propylene glycol,)	Amyloglucosidase (3.2.1.3)	Immobilization studies	Covalent attachment	
PU prepolymer	Creatinine amidohydrolase (3.5.3.3)	Immobilization studies for amperometric biosensor construction, determination of creatinine in blood and urine	Covalent attachment	[109] [110]
	Sarcosine oxidase (1.5.3.1)	Immobilization studies for amperometric biosensor construction	Covalent attachment	[111]
PU microspheres: TDI/EG	Pepsin (3.4.4.1)	Immobilization studies	Pepsin-nano gold-PU conjugates	[50]
	Endoglucanase (3.2.1.4)	Immobilization studies	Endoglucanse-nano gold PU conjugates	[51]
PU powder: HMDI/BD	*Penicillium canescens* β-galactosidase (3.2.1.23)	Immobilization studies, lactose hydrolysis in whey	Covalent attachment	[6]

and Table 2. Polyurethane membranes are used in the biomedical and biosensor areas (Table 3).

PU foams for immobilization of enzymes

Various PU foams have been used to immobilize several enzymes (Table 1).

There are two methods of immobilization of enzymes onto/into PU foams:

1) Immobilization of enzymes onto PU foams, when the enzyme is immobilized onto solid PU foams by adsorption and entrapment [34–36];

2) Immobilization of enzymes could be performed during the synthesis of the carrier [37–42].

Table 2. **Immobilization of cells onto PU**

Immobilized cells	Method	Application	References
Rhizopus arrhizus NRRL 1526 fungus	Adsorption onto PU sponge	Fumaric acid production	[89]
Acetobacter aceti bacteria	Adsorption onto PU foam	Vinegar production	[62]
Rat parenchymal hepatocytes	Infusion of cell suspension on PU membrane	Immobilization studies	[76]
Pseudomonas sp. strain NGK1 (NCIM 5120)	Adsorption onto elastic PU foam	Naphthalene degradation	[66]
Phanerochaete chrysosporium mycelium	Adsorpton onto PU foam	Decolourization of a sugar refinery wastewater in a modified rotating biological contactor	[60]
Phanerochaete chrysosporium mycelia cells	Commercial PU foam	Lignine peroxidase production	[65]
Phanerochaete chrysosporium mycelia cells	Entrapment into PU foam	Manganese peroxidase production in a pulsed packed-bed bioreactor	[90]
Yarrowia lipolytica yeast cells	Freeze drying with PU foam, absorption	Absorption and degradation of oil on water surface	[72]
Oryza sativa L Rice callus	Entrapment into PU foam	*In Situ* Regeneration of Rice Callus	[91]
Oryza sativa L Rice callus	Entrapment into PU foam	Production of rice callus in turbine blade reactor	[75]
Oryza sativa L Rice callus	Entrapment into PU foam	Construction of suitable bioreactor for rice callus	[74]
Mycelia *Aspergillus niger*	Biofilm formation on PU surface	Citric acid production in a rotating biological contactor (RBC)	[92]
Phanetochaete chrizosporium cells	Adsorption onto PU foam	Biodegradation of chlorophenols	[70]
Phanerochaete chrysosporium (BKM-F-1767 (ATCC 24725)) cells	Immobilization onto PU foam	Manganese peroxidase production	[105]
Trametes versicolar	Adsorption onto PU foam	Biodegradation of pentachlorophenol in batch and continuous bioreactors	[73]
Trametes versicolar	Adsorption onto PU foam	Treatment of kraft bleach plant effluents	[93]
Aspergillus Niger cells	Adsorption onto PU foam	Production of gluconic acid from whey	[61]
Catharanthus roseus cells	Intrusion in PU foam	Fuzzy growth kinetics, immobilization studies	[54]
Bacillus pasteurii cells	Entrapment into PU foam	Calcite precipitation	[57]
Burkholderia cepacia cells	Entrapment into PU foam	Immobilization studies	[55]
Ascophyllum nodosum cells	Entrapment into PU foam	Removal of copper from aqueous solution	[58]
Prototheca zofii cells	Entrapment into PU foam	Immobilization studies	[56]
Two strains of P*rototheca zopfii*, i.e. thermotolerant RND16 and nonthermotolerant ATCC30253	Entrapment into oleophilic PU foam	Immobilization studies, biodegradation of mixed hydrocarbon substrate	[108]
Coffea arabica, Carthamus tinctorius and *Angelica sinensi* cells	Adsorption onto PU foam	Immobilization studies for comparison to immobilization of cells on loofa sponge	[94]
Pseudomonas sp. strain SY5	Adsorption onto PU foam	Degradation of polychlorinated biphenyls	[71]
Rhizopus oryzae strain IM 057412	Entrapment into PU foam	Removal of heavy metals	[59]
Saccharomyces cerevisiae	Entrapment into anionic polyurethane gel bead	Immobilization studies, ethanol production	[107]

The method of preparing a bound enzyme that prior to the subsequent foaming step includes the contact of an isocyanate with an aqueous enzyme solution under foam forming conditions, was described in [42]. In this case, some authors proposed the entrapment methods coupled with a chemical attachment during PU foam synthesis. Other authors predicated that the immobilization of enzymes on PU foams proceeds according to the covalent binding and adsorption on the surface, and the entrapment in PU foam pores practically has little influence [43]. The very fact that the mechanism of the covalent attachment of primary NH_2 groups of the enzyme and NCO groups of PU and especially low influence of the entrapment and adsorption during the immobilization was proven by scanning electron microscopy combined with immuno-gold labeling techniques [39].

HYPOL PU foam was used as a carrier for the immobilization of various kinds of enzymes [6–10, 38]. HYPOL 3000 is a prepolymer containing unreacted isocyanate moieties prepared from polyethylene glycol, trimethylolpropane, and an excess of toluene 2,6-diisocyanate [10]. HYPOL 3000 was used for a covalent immobilization of chloroperoxidase, phytase, aminoacylase [9], peroxidase, and coimmobilization of peroxidase with glucose oxidase [7] according to Scheme 1. The immobilization method was very efficient for the stability of the immobilized preparations, reusability and high activities of the enzymes. In the first three cases, the yield of the immobilization ranged from 100% at low loadings to 60% at high loadings [7].

The highest activity of the immobilized *Aspergillus oryzae* β-D-galactosidase was estimated using HYPOL 3000 PU prepolymer as a carrier. *In situ* copolymerization between the enzyme and prepolymer was used as an immobilization technique. It was determined that 63% of the enzyme activity was retained after immobilization [39].

As in the previous case, storage and thermal stability were increased for *Escherichia coli* phosphotriesterase, a nerve agent hydrolyzing enzyme, covalently immobilized within two types of PU foam, HYPOL 3000 and HYPOL 5000 (diphenylmethane-4,4'-diisocyanate based prepolymer), during the polymer synthesis using a prepolymer synthesis method. Besides an apparently little effect on the nature of enzyme, the catalytic function was estimated. Furthermore, more than 60% of the immobilized enzyme's initial activity was retained over 6 cycles of catalyzing paraoxon hydrolysis. The activity of the immobilized enzyme was more than 50%, however, the presented results showed that just 2.5 kg of the immobilized enzyme could be sufficient for a degradation of 30000 tons of nerve agents in just 1 year [37].

The relationship between some physico-chemical properties of relatively hydrophobic PU foams – HYPOL FHP 2002 and HYPOL X 4300 (hydrophilic prepolymers of TDI and diphenylmethane diisocyanate, respectively) and the activity and batch operational stability of the immobilized *Candida rugosa* lipase are investigated in [38]. No significant enzyme loss was observed along the ten successive batches due to a higher number of multi-point attachments between lipase and its support, HYPOL FHP 2002 foam. However, the efficiency of hydrolysis was considerably higher using lipase immobilized onto HYPOL FHP X 4300 foam as compared to the other counterpart [49]. It was observed that the use of PU foams with different aquaphilities leads to distinct microenvironmental conditions due to different partition coefficients between foams and substrates and products. A higher yield of the products was obtained when a less hydrophobic foam was used for enzyme immobilization [7].

PUs HYPOL FHP 2002 (produces foam) and HYPOL FHP 8190H (produces gel) were used for a covalent immobilization of *Aspergillus niger* amyloglucosidase. It was noticed that the foamable PU was a perfect support for amyloglucosidase (the activity of the immobilized enzyme was 25%) for the stability of the immobilized preparation in time (70% of the activity retained, while a free enzyme retained only 50%) and thermal stability at high temperatures (95 °C) [40].

Penicillum canescens β-galactosidase was immobilized into PU foams produced from different ratios of two commercial compositions: HYPERLAST ISOCYANATE 5003 and HYPERLAST 7982016 consisting of methylene diisocyanate (86%) and 1,4-butanediol (5-10%), respectively. The yield of immobilization was from 28% to 50%; and the efficiency of immobilization increased in parallel with the increasing content of active isocyanate groups on the carrier [6].

Patented works were intended for the immobilization of different kinds of enzymes on TDI-based PU foams. PU foams are the reaction products of TDI and polyhydroxy compounds of the group consisting of polyoxybutylene polyol polymer, ethylene glycol, diethylene glycol, polyoxyethylene polyol polymer, pentaerythritol, glycerol, trimethylol propane and polyoxypropylene polyol polymer. Those PU foams were used for the immobilization of urease, cellulase, pectinase, papain, bromelain, chimotripsin, trypsin, ficin, lysozyme, and glucose isomerase [41, 42].

The most sensitive and reproducible procedure among the known procedures for mercury (II) determination with visual detection based on PU foam-immobilized horseradish peroxidase, was presented in [34]. Different esters, ethers and their mixture types of PU foam were employed as supports for the immobilization of horseradish peroxidase. This enzyme was immobilized by transferring it into a water-soluble chitosan film on a tablet of PU foam. During this operation, the enzyme-natural structure remained stable. The stability of the immobilized enzyme was observed from 6 to 550 days depending on the type of PU foam. The use of a protective additive, a chitosan film, made the degree of sorption of the enzyme increase to 86.3%, in comparison with the absence of chitosan during immobilization (28.1%).

As in the previous case, the same types of PU foams were used for the immobilization of alkaline phosphatase. N-phtalylchitosan was tried as a protective additive. It was noticed, that enzyme sorption on PU foam in the presence and in the absence of chitosan was 90.4% and 32.8% respectively. Moreover, the immobilized enzyme keeps a catalytic activity for at least 1 year. Also, like in the previous work, this immobilized alkaline phosphatase finds its practical application in lead (II) determination in different types of soils by an inhibition of the enzyme [35].

Human butyrylcholinestarase entrapped in a commercial PU foam was employed as a good scavenger of organophosphorus pesticides and chemical warfare agents. PU support showed great properties for immobilization. During 1 year of storage at 5 °C, the activity of the entrapped enzyme did not change. More than 50% of the initial catalytic activity remained after 180 hours of storage of the immobilized enzyme at 55 °C, while the enzyme in solution kept only 0.25%. The enzyme was completely entrapped into the sponge and could be used repeatedly, because no significant decrease in the activity occurred after wash and assay cycles repeated for more than 10 times over 5 days. The immobilized enzyme could be applied in a respiratory filter, in this case the immobilized enzyme blocked blood and tissue cholinesterases with inhibition following parathion inhalation [36].

Decontamination and removal of organophosphate (OP) compounds from biological surfaces such as skin, and surfaces such as clothing or sensitive medical equipment, or an enviroment was carried out by cholinesterases (fetal bovine serum acetylcholinesterase, equine serum butyrylcholinestarase) immobilized on PU sponge. The preparation of cholinesterase to PU matrix retained a catalytic activity under such conditions of temperature, time and drying, where a free enzyme would rapidly denature. No decrease of the activity of the immobilized enzymes after the wash and assay cycles repeated for more than twenty times over three days was observed, and this fact indicated that the PU immobilized enzyme could be used repeatedly. Moreover, over 50% of initialy immobilized cholinesterases remained after 16 hours at 75 °C, while the free enzyme showed no activity at those conditions [43]. Also, OP degrading enzymes immobilized in this way could be used for OP sensitive and selective biosensors, for a long-term OP detection, biosensors retained 80% of their activity after 60 days of use with untreated natural fresh or salt water at room temperature [44].

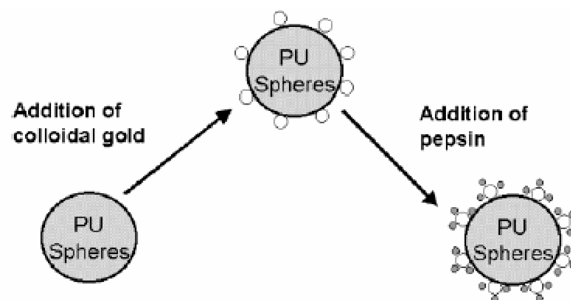
Polyurethane microparticles and polyurethane-containing nanomaterials for the immobilization of enzymes

Recently nanotechnologies with their synthesis and application of nanoparticles have occupied an important and promising place in many fields, and as it was noticed, nanotechnology has found its application in immobilization area. Colloidal gold was employed for immobilization. The interaction of colloidal gold particles with protein/enzymes has been investigated and studies of catalytic activity of the nano-bioconjugates have been carried out [45].

It is known that bulk gold is chemically inert. However, it was estimated that when it is in the form of particles with a diameter below 10 nm, it could become active [46].

Gole and associates in their articles presented aspartic protease from *Aspergillus Saitoi* [47], endoglucanase [48] and pepsin [45] bioconjugates with colloidal gold. Another scientist group presented a xantine oxidase immobilized on colloidal gold electrode for an amperometric biosensor employment [49].

PUs found an application in this field too. PU microspheres of 2 mm mean diameter were synthesized according to Shukla's patented work [14]. Those microspheres were used as a "core", and gold-nanoparticles were used as "shells". The enzyme pepsin was finally immobilized on the obtained composites [50]. The immobilization scheme is presented in Scheme 2.



Scheme 2. Immobilization of pepsin on the Gold-Nano-PU-Conjugated material [50]

Binding of the gold nanoparticles to the polymer surface occurs through nitrogen atoms in the PU bioconjugate. Besides, amine groups and cisteine residues in the proteins are known to bind strongly to gold colloids [45]. The percent coverage of the PU microspheres by the gold nanoparticles was estimated by using UV-VIS spectroscopy on a Schimadzu dual-beam spectrometer. The formation of PU-nanogold conjugates occurred by adding PU microsphere powder with colloidal gold solution dispersed in hexane. After shaking this system there was a loss in the intensity of the surface plasmon resonance due to a decrease in the concentration of gold nanoparticles in the aqueous solution. This is a supplementary improvement of binding of colloidal gold particles to the PU microspheres through nitrogen atoms in PU.

It was observed that the nanogold-PU conjugates as well as the enzyme-nanogold-PU bioconjugates showed an excellent catalytic activity and thermal stability, but there was one and major advantage of the latter: they showed a much more higher reusability and significant biocatalytic activity over 6 cycles of reuse. The

biocatalytic activity of PU-nanogold-pepsin bioconjugate was marginally higher than that of the free enzyme in solution: 13.2 IU/μg (International Unit per μg) and 11.2 IU/μg respectively, and significantly enhanced the temperature and pH stability [50]. The same technique was used for the immobilization of endoglucanase. This enzyme could thereafter be bound to the gold nanoparticles decorating the PU microspheres leading to a highly stable biocatalyst with excellent reuse characteristics. The high surface area of the host gold nanoparticles renders the immobilized enzyme "quasi free" at the same time retaining the advantages of immobilization such as the ease to reuse, an enhanced temporal and thermal stability, etc. [51].

Water-borne PU coatings result from the polymerization of aqueous polyester-based polyol dispersions and water dispersible aliphatic polyisocyanates. They are represented as a potentially ideal polymeric matrix for a multipoint and covalent immobilization of enzyme. Diisopropylfluorophosphatase that had catalyzed the hydrolysis of toxic organophosphorus nerve agents such as soman and diisopropylfluorophosphate, was immobilized into the water-borne PU coatings. The enzyme has been previously polymerized into monolytic PU foams with 67% of activity retention and an enhanced thermostability. It was determined that the immobilization efficiency approached 100%, and less than 4% of the protein was loaded to the enzyme-containing coating. For the determination of enzyme distribution in coatings, gold labeling has been used to localize the immobilized enzyme in PU monolith foams. Transmission electron microscopy showed that the gold/enzyme particles and clusters were randomly distributed at the microscale level. Moreover, at high temperature, diisopropylfluorophosphatase containing PU coatings lost 93% of its activity quickly, but after that became hyperstable [53, 53].

PU powder from hexamethylene diisocyanate and butanediol was used for the immobilization of *Penicillium canescens* β-galactosidase [6]. In this case only a covalent binding between the amino groups of enzyme and the isocyanate groups in PU powder took place, and this fact was a main reason of quite a low yield of immobilization (max 19.6%).

PU foams for immobilization of whole cells

Whole cells were immobilized into PU foams by adsorption and entrapment methods for different purposes (Table 3).

PU foams are perfect support for the cell growth.

Cell growth kinetics of the immobilized *Catharanthus roseus* was investigated in [54]. A PU foam obtained from commercially available thick sheets was a suitable support for the immobilization of those cells; their was faster growth than when they were freely suspended. PU foam had a high retention of plant cells and low phytotoxicity. *Burkholderia cepacia* cells were immobilized by an entrapment into hydrophilic PU foam BIPOL 6B (NCO = 6%) and three other prepolymers

Table 3. **PU as a component of biosensors**

PU kind	Biosensor kind	Application	References
Aliphatic acrylated urethane diacrylate membrane	Ion-sensitive field effect transistor	Urea biosensor	[95]
Aliphatic acrylated urethane diacrylate membrane	Amperometric	Glucose determination	[96]
Asymmetric PU/hydrophilic PU membrane	Potentiometric	Enzymes bio-selective sensors	[97]
Photocurable oligomer of urethane and Bisphenol A (epoxy) diacrylate based membrane	Ion-sensitive field effect transistor	K^+ sensitive ion sensor	[98]
Tecoflex (SG-80A) based membrane	Potentiometric	NH_4^+, K^+ ion-selective sensor	[82]
Aromatic diisocyanate and poly(tetramethylene ether glycol) based PU membrane	Potentiometric	Ion selective sensor	[99]
PU/poly(vinyl alcohol) based membrane	Potentiometric	NH_4^+ and proton selective electrode	[100]
Tecoflex PU	Electrochemical	Ion selective sensor	[101]
Photocurable urethane-acrylate membrane from urethane diacrylate oligomer	Potentiometric	pH sensor	[102]
Tecoflex PU	Potentiometric	pH sensor for biomedical application	[81]
Hydrophilic PU based membrane	Electrochemical	Blood gas determination	[103]
Teflon and Tecoflex (SG-85 A) PU films	Amperometric	Glucose sensor	[104]
Tecoflex (SG-80A) PU based membrane	Amperometric	Formaldehyde monitoring	[80]
Tecoflex (EG 80A) PU based membrane	Electrochemical transducer	Lactate and glucose biosensors	[79]

of lower NCO values (BIPOL 3, No. 350, No. 802) were used for comparison [55].

The aim of the work [56] was the optimization of PU-based formulations for an entrapment of bacteria using various surfactants. *Prototheca zopfii* cells were immobilized in 8 mm cube open-pore network PU foam pieces [56]. The volumetric biodegradation rate for hydrocarbons was estimated in the cells immobilized in PU cubes. The PU foam used was a hydrophobic one, and therefore, it might had the ability to trap hydrocarbons by a hydrophobic interaction. The activity of the immobilized cells was stable over three cycles of cultivation, and *Prototheca zopfii* immobilized in PU foam was incorporated into a bubble-column type bioreactor for degrading hydrocarbons and a potential efficiency of the immobilized cell system was confirmed.

As in the cases with enzymes, HYPOL PU prepolymers were widely used for the cell immobilization, and the application of the immobilized cells. In all the cases, HYPOL PU was a perfect support, for example, HYPOL 2000 (a water-based prepolymer consisting of a proprietary prepolymer, 97% (w/w) and toluene diisocyanate, 3% (w/w). The PU polymer matrix might be able to stabilize *Bacillus pasteurii* cells' microbial and enzymatic activities for a long period of time [57]. The cells immobilized into PU matrix were used for calcite precipitation. Scanning electron micrographs identified the cells embedded in the calcite crystals throughout PU matrices. PU matrix provided the microorganisms, which protected against an extremely alkaline environment, while serving as nucleation sites for the calcite crystals. *Ascophyllum nodosum* cells were immobilized into an open-pore hydrophilic HYPOL 2002 PU foam. A surfactant Pluronic 85 was used in producing a foam with interconnected pores. The immobilized cells were successfully used in copper removal from aqueous solutions [58]. The same type of PU foam and surfactant was used for the immobilization of *Rhizopus oryzae* strain IM 057412. Removal of heavy metals using a biomass immobilized in HYPOL PU foam proved to be successful, whereas a conventional PU foam proved to be unsuitable for this purpose [59].

Phanerochaete chrysosporium immobilized on a PU foam modified by a rotating biological contractor reactor was used for the decolouration of sugar refinery wastewater. It was estimated that during 40 days of a repeated batch test, the decolouration efficiency of 62% was achieved. The PU foam-immobilized cells not only removed the colour of the effluent by 55%, but also reduced total phenols and chemical oxygen demand by 63 and 48%, respectively [60].

A rotating biological contractor reactor with *Aspergillus niger* cells immobilized in PU foams was used for citric acid production. A biofilm of *Aspergillus niger*, which was active over 8 recycles without bioactivity loss, was formed on the PU foam surface [61]. A strong biomass retention of *Aspergillus niger* cells in an open porous PU foam (with a density of 40 kg/m^3, purchased from a local market) was observed. The immobilized cells were used for gluconic acid production from whey. It was estimated that the efficiency of glutonic acid production was higher (by 33%) as compared to the case when the free cells were used [61]. *Acetobacter aceti* cells, which were used for vinegar production, were immobilized on a commercial PU foam with an average pore size of 400 μm (density 0.02 g/ml, porosity 97%). PU foam allowed the immobilization of a large number of cells (about 10.5 millions/mg) in a short time (300 h). Therefore, because of a huge number of the immobilized cells in the shortest time and the highest acetification rate, this carrier was the most successful in comparison with the other two carriers (Siran and wood chips). The highly uniform porous structure in the foam provided a rapid and facilitated cellular adhesion. This profitable operation using the foam was by 70% faster, even when the quantity of the immobilized bacteria was by 20% less in the foam in comparison to the Siran carrier [62].

The production of an enzyme lignine peroxidase by immobilizing a white rot fungus *Phanerochaete chrysosporium* on a PU foam, was investigated by several authors [63–65]. A PU foam (Chiyoda Co., Ltd., Tokyo, Japan) of a cubic shape (10 mm × 10 mm) was used for the immobilization of the cells. Similarly to the case with *Acetobacter aceti* cells, PU foam produced a high biomass growth, but in this case foam affected lignine peroxidase activities, and as a result, exhibited enzyme productivity. The average activity of lignine peroxidase in PU foam was 2.9 IU/ml (Internetional Unit per ml), while in Biostage carrier based on polypropylene, the activity of enzyme was 12 IU/ml [65].

PU carrier was often used for the immobilization of cells that were used in the biodegradation of organic compounds. Elastic PU foam (Mukesh Foam Products, Bangalore, India) was an excellent carrier for the immobilization of *Pseudomonas* sp. strain NGK1 (NCIM 5120). The immobilized cells were used for the degradation of naphthalene, and even after 45 times over a period of 90 days the activity of the PU-immobilized cells was observed. The rate of naphthalene degradation using the PU foam-immobilized cells even at high loadings was much higher than that with free cells [66]. Pentachlorophenol was degraded by *Flavobacterium* cells immobilized on a PU foam [67]. *Phanerochaete chrysosporium* cells immobilized on PU foam, according to [68, 69], were also used for biodegradation of chlorophenols. It was noticed that the degradation efficiency of chlorinated phenol compounds with enzymes produced by the PU immobilized cells was higher than in the case with free cells [70]. The immobilization possibility and the use of *Pseudomonas sp.* strain SY5 bacteria immobilized on NCO-terminated, polypropylene glycol-based and MDI-based PU foam in degradation of polychlorinated biphenyls were studied. PU foams consisting of different amounts of NCO prepolymer were used; and it was estimated that the use of 10% of NCO

prepolymer was more suitable for the immobilization of microorganisms. The immobilized bacteria were more convenient for the degradation of these organic compounds (67.2% was degraded) in comparison with the non-immobilized ones (53%). This total difference between the immobilized and non-immobilized bacteria was due to a better oxygen transfer in the anaerobic batch flask. Thus, with the growth of the microorganisms, polychlorinated biphenyls, were initially adsorbed to the PU foams, and then were degraded by microorganisms, PU stabilized the quantity of bacteria required in a flask for cultivation. PU foams can be used as a carrier of microorganisms in the continuous wastewater treatment of polychorinated biphenyls and other pollutants. Moreover, the bacteria immobilized on PU can be used in the bioremediation of soils and rivers [71]. *Yarrowia lipolytica* yeast cells immobilized on a PU foam based on a polyether polyol mixture with carbodiimide-modified D-methyl diisocyanates with a ratio 10 : 2, also can be used in bioremediation as a hydrocarbon sorbent in open-water. In order to obtain the best results in maintaining the oil-degrading activity, the simplicity and cost of preparation, several techniques of immobilization were tested. Yeast cells were immobilized onto a PU foam by a freeze drying method, in addition to a direct immobilization, the cells were attached to chitin or a slow-release fertilizer (SRF), and after the immobilization on chitin or SRF, they were added to polyether polyol mixture for the preparation of PU foam. All the immobilized yeast cells in the PU foam showed the oil-degrading activity as free yeast cells. The obtained results showed that the chitin-immobilized cells and incorporated into PU foam became an excellent ability to absorb oil from the surface water and to degrade the absorbed oil. Moreover, the absorbance of oils increased with an increasing foam porosity (surface area proportion to weight), and it was 7–9 grams of oil per one gram of PU foam [72]. A white-rot fungus *Trametes versicolor* immobilized on a PU-foam was used for biodegradation of pentachlorophenols in batch and continuous bioreactors. The PU-foam-immobilized fungal yielded more than 99% of pentachlorophenols reduction with a residence time of 12 hours for the inlet pentachlorophenols concentrations from 20 to 25 mg/l [73].

The effects of agitation of rice calli from *Oryza sativa* L cultured in a jar-fermentor with disk turbine impellers, an air-lift reactor and a turbine-blade reactor were investigated in [74]. The latter reactor with *Oryza sativa* L rice callus immobilized on PU foam (INOAC Corp., Japan) showed the best results for the construction of bioreactor for mass propagation of embryonic rice calli. Four kinds of PU foam with different average pore size (3.1, 1.9, 1.3, 0.9 mm) were used for the immobilization of cells. It was noticed, that a higher ratio of the immobilized cells was 33% with an average pore size of 1.3 mm. The efficiency of immobilization of *Oryza sativa* L rice callus, the effects of a support volume, and the turbine-blade bioreactor operation and modification were analyzed in [75].

For immobilization studies, rat parenchymal hepatocytes were immobilized into a PU sponge-like porous structure membrane, with micropores (pore size <100 μm) and macropores (pore size >100 μm). The porosity of PU membrane was approximately 90% and immobilization efficiency was more than 99% of hepacytes at high cell densities. According to the author's predication, high efficiency of immobilization was because of a unique structure of the PU sponge: the macropores served as channels for cell loading during immobilization and for medium-feeding perfusion culture [76].

Polyurethane membranes in biomedical and biosensor use

A biosensor is a compact analytical device incorporating a biological or biologically-derived sensing element (enzyme, antibody, etc.) either integrated within or closely associated with a physicochemical transducer. The usual aim of a biosensor is to produce either discrete or continuous digital electronic signals which are proportional to a single analyte or a related group of analytes [77].

R. The´venot and associates [78] in their article proposed a classification of electrochemical biosensors by a measurement type: potentiometric, amperometric, conductometric, impedimetric, ion-charge or field effect. Non-electrochemical transducers are also used within biosensors: piezoelectric, calorimetric, optical.

PU has found quite a wide application in this field, and most current researches are dedicated to electrochemical biosensors. The biosensors where PUs are used as a different kind of membranes are presented in Table 3. It was noticed that PUs are suitable materials for this purpose.

For example, Tecoflex, a commercial aliphatic polyether based PU, is widely used in biosensor constructions as a plasticized membrane (lactate and glucose sensor [79], formaldehyde [80], pH sensor for medical application [81], etc.). It was estimated that Tecoflex SG-80A, a PU-based membrane, had better adhesion to sillicone nitride in comparison with a polyvinyl chloride membrane and exhibited less overall nonspecific protein adsorption than PVC [82]. This kind of aliphatic PU contains mobile and fixed residual sites which are responsible for H^+ and M^+ potentiometric response of the plasticized membrane [81]. Moreover, the entrapment of enzymes and co-factor behind this membrane with anionic sites enhanced the sensitivity and stability of the sensor [80].

Another side of the biochemical application of PU was presented by Gao and co-workers [83]. As it is known, PU has been widely used due to its perfect properties and blood biocompatibility. This is a main reason to use it in blood-containing areas, such as vascular grafts, clambers for artificial hearts, catheters and pacemaker insulation [84]. It was noticed that cytocompatibility is necessary in tissue engineering scaffolds and

implanted devices. For these purposes, PU membrane was modified by grafting of methacrylic acid (MAA) by UV irradiation, and then gelatin and arginine-glycine-aspartic peptide (RGD) was covalently immobilized on 1-ethyl-3-(3-dimethylaminopropyl) carbodiimine hydrochloride-activated PU-*graft*-MAA membrane surface. Immobilization of such substances as gelatin or RGD on the photoinduced grafting PU-membrane is a perspective method to make cytocompatible polymers for implanted devices.

FUTURE TRENDS OR FINAL REMARKS

PU is a suitable support material for the immobilization of enzymes, whole cells and as a constructive material in a biomedical membrane and biosensors, as it has been proven by several applications in different industrial processes. A lot of enzymes were immobilized onto different kinds of PU foams. Almost all the preparations immobilized onto PU showed a good stability in time and possibility to reuse. There are some patented works on the synthesis of PU microspheres with a controlled particle size, however, only few publications are devoted to the application of PUs microspheres for immobilization purposes. It is a quite new and perspective area of PU application.

ACKNOWLEDGEMENTS

The work was supported by the Marie Curie Fellowship of the European Community programme "Improving the Human Research Potential and Socio-Economic Knowledge Base" under Contract No. HPMT-CT-2001-00379.

Received 19 October 2006
Accepted 21 October 2006

References

1. www.polyurethane.org
2. D. Randall and S. Lee, *The Polyurethane Book*, Wiley, NY (2002).
3. M. Szycher, Szycher's Handbook of Polyurethane, CRC Press LLC, Florida (1999).
4. C. J. Benning, Plastic Foams: the Physics and Chemistry of Product Performance and Process Technology, Vol. 1, Wiley, NY (1969).
5. W. Wirpsza, Polyurethane: Chemistry, Technology and Application, Wydawnictwa Naukowo-Techniczne, Warsaw, 1991 (in Polish).
6. S. Budriene, N. Gorochovceva, T. Romaskevic, L. V. Yugova, A. Miezeliene, G. Dienys and A. Zubriene, *Centr. Eur. J. Chem.*, **3**, 95 (2005)
7. S. Ferreira-Dias, A. C. Correia and M. M. R. da Fonseca, *J. Mol. Catal. B: Enzym.*, **21**, 71 (2003).
8. F. Van de Velde, N. D. Lourenco, M. Bakker, F. van Rantwijk and R. A. Sheldon, *Biotechnol. Bioeng.*, **69**, 286 (2000).
9. M. Bakker, F. van de Velde, F. van Rantwijk and R. A. Sheldon, *Biotechnol. Bioeng.*, **70**, 342 (2000).
10. F. Van de Velde, M. Bakker, F. van Rantwijk and R. A. Sheldon, *Biotechnol. Bioeng.*, **72**, 523 (2001).
11. F. Van de Velde, M. Bakker, F. van Rantwijk, G. P. Rai, L. P. Hager and R. A. Sheldon, *J. Mol. Catal. B: Enzym.*, **11**, 765 (2001)
12. L. S. Ramanathan and S. Sivaram, US 6,123,988 (2000).
13. L. S. Ramanathan, P. G. Shukla and S. Sivaram, *Pure Appl. Chem.*, **70**, 1295 (1998).
14. P. G. Shukla and S. Sivaram, US 5,859,075 (1999).
15. B. Radhakrishnan, E. Cloutet and H. Cramail, *Colloid. Polym. Sci.*, **280**, 1122 (2002).
16. T. M. Santosusso, U.S. 4,083,831, (1978).
17. JP 04-161416. In: P. G. Shukla and S. Sivaram U.S. 5,859,075 (1999).
18. E. Jabbari and M. Khakpour, *Biomater.*, **21**, 2073 (2000).
19. J. H. Sounders and K. A. Pigott, U.S. 3,214,411 (1965).
20. B. Krajewska, *Enzyme Microb. Technol.*, **35,** 126 (2004).
21. N. Durán, M. A. Rosa, A. D'Annibale and L. Gianfreda, *Enzyme Microb. Technol.*, **31**, 907 (2002).
22. C. Bullock, *Sci. Prog.*, **7**, 119 (1995).
23. J. M. Woodley, Immobilized biocatalysts. In *K. Smith, Solid supports and catalysts in organic synthesis. Ellis, Horwood* (1992).
24. M. F. Chaplin and C. Bucke, Enzyme Technology. Cambridge University Press, Cambridge, (1990).
25. J. F. Kennedy and J. M. S. Cabral, Immobilized enzymes. *In: W. H. Scouten, Solid phase biochemistry. Analytical and Synthetic Aspects,* Wiley, NY, (1983).
26. W. Tischer and F. Wedekind, *Top. Curr. Chem.*, **200**, 95 (1999).
27. W. H. Scouten, J. H. T. Luong and R. S. Brown, *Trends Biotechnol*., **13**, 78 (1995).
28. M. C. Flickinger and S. Drew, *Encyclopedia of Bioprocess Technology – Fermentation, Biocatalysis, and Bioseparation*, Wiley, NY (1999).
29. F. Van de Velde, N. D. Lourenço, H. M. Pinheiro and M. Bakker, *Adv. Synth. Catal.*, **344**, 815 (2002).
30. R. Angold, G. Beech and J. Taggart, Food Biotechnology. Cambridge University Press, Cambridge, 1989. In *F. Van de Velde, N. D. Lourenēo, H. M. Pinheiro and M. Bakker, Adv. Synth. Catal.* **344**, 815 (2002).
31. G. G. Guilbault, Immobilized enzyme electrode probes. In: *W. H. Scouten, Solid phase biochemistry. Analytical and synthetic aspects,* Wiley, NY (1983).
32. J. Davis, D. H. Vaughan and M. F. Cardosi, *Enzyme Microb. Technol.*, **17**, 1030 (1995).
33. G. S. Wilson and Y. Hu, *Chem. Rev.*, **100**, 2693 (2000).
34. I. A. Veselova and T. N. Shekhovtsova, *Anal. Chim. Acta.*, **392**, 151 (1999).
35. I. A. Veselova and T. N. Shekhovtsova, *Anal. Chim. Acta.*, **413**, 95 (2000).
36. H. Mehrani, *Environ. Toxicol. Pharmacol.*, **16**, 179 (2004).
37. K. E. LeJeune, A. J. Mesiano, S. B. Bower, J. K. Grimsley, J. R. Wild and J. Russell, *Biotechnol. Bioeng.*, **54**, 105 (1997).

38. S. Ferreira-Dias, A. C. Correia and F. O. Baptista, *Bioprocess. Eng.,* **21**, 517 (1999).
39. Z. -C. Hu, R. A. Korus and K. E. Stormo, *Appl. Microb. Biotechnol.* **39**, 289 (1993).
40. K. B. Storey, J. A. Duncan and A. C. Chakrabarti, *Appl. Biochem. Biotechnol.,* **23,** 221 (1990).
41. L. L. Wood, F. J. Hartdegen and P. A. Hahn, US 4,342,834, (1982).
42. L. L. Wood, F. J. Hartdegen and P. A. Hahn, U.S. 4,312,946, (1982).
43. R. K. Gordon, S. R. Feaster, A. J. Russell, K. E. LeJeune, D. M. Maxwell, D. E. Lenz, M. Ross and B. P. Doctor, *Chem. Biol. Interact.,* **119–120**, 463 (1999).
44. R. Gordon, Polyurethane Foam Linked Mammalian Cholinesterase for Decontamination and Detection of OP Nerve Agents. *Chemical and Biological Medical Treatment Symposia* III, Conference proceedings, Spiez, Switzerland, (2000).
45. A. Gole, C. Dash, V. Ramakrishnan, S. R. Sainkar, A. B. Mandale, M. Rao and M. Sastry, *Langmuir*, **17**, 1674 (2001).
46. M. Haruta, *Chem. Rec.,* **3**, 75 (2003).
47. A. Gole, C. Dash, C. Soman, S. R. Sainkar, M. Rao and M. Sastry, *Bioconjugate Chem.,* **12**, 684 (2001).
48. A. Gole, S. Vyas, S. Phadtare, A. Lachke and M. Sastry, *Colloids Surf. B,* **25**, 129 (2002).
49. J. Zhao, J. P. O'Daly, R. W. Henkens, J. Stonehuerner and A. L. Crumbliss, *Biosens. Bioelectron.,* **11**, 493 (1996).
50. S. Phadtare, A. Kumar, V. P. Vinod, C. Dash, D. V. Palaskar, M. Rao, P. G. Shukla, S. Sivaram and M. Sastry, *Chem. Mater.,* **15**, 1944 (2003).
51. S. Phadtare, S. Vyas, D. V. Palaskar, A. Lachke, P. G. Shukla, S. Sivaram and M. Sastry, *Biotechnol. Prog.,* **20**, 1840 (2004).
52. G. F. Drevon, K. Danielmeier, W. Federspiel, D. B. Stolz, D. A. Wicks, P. C. Yu and A. J. Russel, *Biotechnol. Bioeng.,* **79**, 785 (2002).
53. G. F. Drevon and A. J. Russell, *Biomacromol.,* **1,** 571 (2000).
54. Z. D. Hu and Y. J. Yuan, *Chem. Eng. Sci.,* **50**, 3297 (1995).
55. J. W. Santo Domingo, J. C. Radway, E. W. Wilde, P. Hermann and T. C. Hazen, *J. Ind. Microbiol. Biotechnol.,* **18**, 389 (1997).
56. T. Yamaguchi, M. Ishida, T. Suzuki, *Process Biochem.,* **34**, 167 (1999).
57. S. S. Bang, J. K. Galinat and V. Ramakrishnan, *Enzyme Microb. Technol.,* **28**, 404 (2001).
58. M. S. Alhakawati and C. J. Banks, *J. Environ. Manage.,* **72**, 195 (2004).
59. M. S. Alhakawati, C. J. Banks and D. Smallman, *Water Sci. Technol.,* **47**, 143 (2003).
60. C. Guimarães, P. Porto, R. Oliveira and M. Mota, *Process Biochem.*, **40**, 535 (2005).
61. R. Mukhopadhyay, S. Chatterjee, B. P. Chatterjee, P. C. Banerjee and A. K. Guha, *Int. Dairy J.,* **15**, 299 (2005).
62. I. De Ory, L. E. Romero and D. Cantero, *Process Biochem.,* **39**, 547 (2004).
63. C. Capdevila, G. Corrieu and M.Asther, *J. Ferment. Bioeng.,* **68**, 60 (1989).
64. P. Bonnarme, M. Delattre, G. Corrieu and M. Asther, *Enzyme Microb. Technol.,* **13**, 727 (1991).
65. S.-S. Shim and K. Kawamoto, *Water Res.,* **36**, 4445 (2002).
66. S. Manohar, C. K. Kim and T. B. Karegoudar, *Appl. Microbiol. Biotechnol.,* **55**, 311 (2001).
67. K. T. O'Reilly and R. L.Crawford, *Appl. Environ. Microbiol.,* **55**, 2113 (1989).
68. S. H. Choi, E. Song, M. B. Gu and S.-H.Moon, *Korean J. Biotechnol. Bioeng.*, **13**, 223 (1998).
69. S. H. Choi, E. Song, K. W. Lee, S. Moon and M. B. Gu, Pentachlorophenol degradation using lignin peroxidase produced from the *Phanerochaete chrysosporium* immobilized on polyurethane foam. In*: G. B. Wickramanayake and R. B. Hinchee, Bioremediation and Phytoremediation. The first international conference on Remediation of Chlorinated and Recalcitrant compounds.* Battle Press, Columbus, 161 (1998).
70. S. H. Choi, S. H. Moon and M. B. Gu, *J. Chem. Technol. Biotechnol.*, **77**, 999 (2002).
71. K. Na, Y. Lee, W. Lee, Y. Huh, J. Lee, M. Kubo and S. Chung, *J. Biosci. Bioeng.,* **90**, 368 (2000).
72. Y.-S. Oh, J. Maeng and S.-J. Kim, *Appl. Microb. Biotechnol.,* **54**, 418 (2000).
73. S. Pallerla and R. P. Chambers, *Catal. Today.,* **40**, 103 (1998).
74. K. H. Moon, H. Honda and T. Kobayashi, *J. Biosci. Bioeng.,* **87**, 661 (1999).
75. C. Liu, K. H. Moon, H. Honda and T. Kobayashi, *Biochem. Eng. J.,* **4**, 169 (2000).
76. H. Kurosawa, K. Yasumoto, T. Kimura and Y. Amano, *Biotechnol. Bioeng.,* **70**, 160 (2000).
77. A. P. F. Turner, I. Karube and G. S. Wilson, *Biosensors: Fundamentals and Applications*. Oxford University Press, Oxford (1987).
78. D. R. Thévenot, K. Toth, R. A. Durst and G. S. Wilson, *Biosens. Bioelectron.*, **16**, 121 (2001).
79. P. A. Abel, T. Von Woedtke, B. Schulz, T. Bergann and A. Schwock, *J. Mol. Catal. B: Enzym.,* **7**, 93 (1999).
80. R. Kataky, M. R. Bryce, L. Goldenberg, S. Hayes and A. Nowak, *Talanta*, **56**, 451 (2002).
81. V. V. Cosofret, M. Erdosy, J. S. Raleigh, A. T. Johnson, M. R. Neuman and R. P. Buck, *Talanta,* **43**, 143 (1996).
82. G. S. Cha, D. Liu, M. E. Meyerhoff, H. C. Cantor, A. R. Midgley, H. D. Goldberg and R. B. Brown, *Anal. Chem.*, **63**, 1666 (1991).
83. C. Gao, J. Guan, Y. Zhu and J. Shen, *Macromol. Biosci.,* **3**, 157 (2003).
84. G. M. Bernacca, I. Straub and D. J. Wheatley, *J. Biomed. Mater. Res.,* **61**, 138 (2002).
85. P. L. Havens and H. F. Rase, *Ind. Eng. Chem. Res.*, **32**, 2254 (1993).
86. F. van Rantwijk, A. C. Kock-van Dalen and R. A. Sheldon, *Prog. Biotechnol.,* **15,** 447 (1998).
87. A. C. Chakrabarti and K. B. Storey, *Appl. Biochem. Biotechnol.,* **22**, 263 (1989).

88. K. B. Storey and A. C. Chakrabarti, *Appl. Biochem. Biotechnol.,* **23,** 139 (1990).
89. M. Petruccioli, E. Angiani and F. Federici, *Process Biochem.*, **31,** 463 (1996)
90. M. T. Moreira, G. Feijoo, C. Palma and J. M. Lema, *Biotechnol. Bioeng.,* **56**, 130 (1997).
91. Ch. Liu, K. Moon, H. Honda and T. Kobayashi, *J. Biosci. Bioeng.,* **91**, 76 (2001).
92. W. Jianlong, *Bioresour. Technol.*, **75**, 245 (2000).
93. S. Pallerla and R. P. Chambers, *Tappi J.,* **79**, 156 (1996).
94. Y-K. Liu, M. Seki, H. Tanaka and S.Furusaki, *J. Ferment. Bioeng.*, **85**, 416 (1998).
95. C. Puig-Lleixà, C. Jiménez, J. Alonso and J. Bartrolí, *Anal. Chim. Acta*, **389**, 179 (1999).
96. C. Puig-Lleixà, C. Jiménez and J. Bartrolí, *Sens. Actuators, B*, **72**, 56 (2001).
97. J. H. Shin, S. Y. Yoon, I. J. Yoon, S. H. Choi, S. D. Lee, H. Nam and G. S. Cha, *Sens. Actuators, B.,* **50**, 19 (1998).
98. A. Bratov, N. Abramova, J. Munoz, C. Dominguez, S. Alegret and J. Bartroli, *Anal. Chem.*, **67**, 3589 (1995).
99. S. Y. Yun, J. K. Hong, B. K. Oh, G. S. Cha, H. Nam, S. B. Lee and Y. I. Jin, *Anal. Chem.,* **69**, 868 (1997).
100. D. Liu, M. E. Meyerhoff, H. D. Goldberg and R. B. Brown, *Anal. Chim. Acta*, **274**, 37 (1993).
101. E. Linder, V. V. Cosofret, R. P. Buck, J. W. Kao, M. R. Neuman and J. M. Anderson,. *J. Biomed. Mater. Res.,* **28**, 591 (1994).
102. C. Puig-Lleixà, C. Jiménez, E. Fàbregas and J. Bartrolí, *Sens. Actuators, B,* **49**, 211 (1998).
103. W. D. Potter, US 4,534,355 (1985).
104. H. Yang, T. D. Chung, Y. T. Kim, C. A. Choi, C. H. Jun and H. C. Kim, *Biosens. Bioelectron.,* **17**, 251 (2002).
105. H. Chung, J. Oh, S. Hwang, I. Ahn and Y. J. Yoon, *Biotechnol. Let.*, **27**, 477 (2005).
106. A. J. Russell, K. Danielmeier, D. Wicks, and G. F. Drevon, US 6,905,733, (2005).
107. Y. H. Lin, Sz.-C. John Hwang, W-C. Shih and K-C. Chen, *J. Appl. Polym. Sci.*, **99**, 738 (2006)
108. R. Ueno, S. Wada and N. Urano, *Fisheries Science*, **72**, 1027 (2006).
109. J. A. Berberich, L. W. Yang, I. Bahar and A. J. Russell, *Acta Biomater.,* **1**, 183 (2005).
110. J. A. Berberich, A. Chan, M. Boden and A. J. Russell, *Acta Biomater.,* **1,** 193 (2005).
111. J. A. Berberich, L. Wei Yang, J. Madura, I. Bahar and A. J. Russell, *Acta Biomater.,* **1,** 173 (2005).

Tatjana Romaškevič, Saulutė Budrienė, Krzysztof Pielichowski, Jan Pielichowski

POLIURETANŲ PRITAIKYMAS FERMENTŲ BEI LĄSTELIŲ IMOBILIZAVIMUI: APŽVALGA

S a n t r a u k a

Poliuretanas, kaip funkcinė medžiaga, plačiai naudojama įvairiose srityse. Poliuretanai yra biosuderinami ir stabilūs junginiai, todėl naudojami biocheminiuose bei biotechnologiniuose procesuose. Šiame straipsnyje apžvelgiama pastarųjų 15 metų literatūra apie įvairių poliuretanų sintezę ir savybes, jų pritaikymą fermentų bei ląstelių imobilizavimui, taip pat panaudojimą kuriant biosensorius. Apžvelgiamos poliuretanų panaudojimo perspektyvos.