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Glukoza oksidaze – potencialni encimi za beljenje tekstilnih vlaken

Pregledni znanstveni članek

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Izvleček

Glukoza oksidaze katalizirajo oksidacijo β-D-glukoze v glukonsko kislino in z uporabo molekularnega kisika kot elektron akceptorja simultano proizvajajo vodikov peroksid. Glukoza oksidaze so zahvaljujoč vsestranski uporabi postale komercialno zelo pomembne v biotehnologiji, kemični, farmacevtski in prehranski industriji ter v zdravstvu. V zadnjih letih je povpraševanje po encimih glukoza oksidaze naraslo predvsem zaradi uporabe v biosenzorjih. V tekstilni tehnologiji so glukoza oksidaze tudi metoda za pridobivanje vodikovega peroksida za beljenje celuloznih vlaken. Za tvorbo vodikovega peroksida se lahko uporabi glukoza, nastala pri predhodnem razškrobljanju tkanin. Tako je postopek beljenja z encimi glukoza oksidaze ekonomski in ekološki potencial v primerjavi s klasičnim postopkom beljenja z dodanim vodikovim peroksidom. Prispevek opisuje osnovne značilnosti in postopke pridobivanja glukoza oksidaz ter podaja pregled uporabe v različnih tehnoloških segmentih s poudarkom na dosedanjih raziskavah pri beljenju bombažnih vlaken.

Ključne besede: encimi, glukoza oksidaze, beljenje, fermentacija, glukonska kislina

1 Uvod

Biotehnologija je aplikacija živih organizmov in njihovih komponent v industrijske postopke in proizvode. Leta 1981 je Evropska federacija za biotehnologijo definirala biotehnologijo kot integrirano uporabo biokemije, mikrobiologije in kemičnega inženirin-

Glucose Oxidases – Potential Enzymes for Bleaching Textile Fibres

Scientific Review

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Abstract

Glucose oxidases catalyse the oxidation of β-D-glucose into gluconic acid by utilizing molecular oxygen as an electron acceptor with a simultaneous production of hydrogen peroxide. Due to their versatility, glucose oxidases are commercially gaining a lot of attention in biotechnology, in the chemical, pharmaceutical and food industry, as well as in health care. The demand for the application in biosensors has increased recently. In the field of textile technology, glucose oxidases represent a method for the generation of hydrogen peroxide required for bleaching cellulose fibres. For the generation of hydrogen peroxide, the glucose gained during desizing can be used. Bleaching with glucose oxidase thus represents an economic and ecological potential when compared to the classical process with added hydrogen peroxide. This review represents the basic properties and production processes of glucose oxidases, reveals their multitudinous technological applications and emphasises recent research in the field of bleaching cotton fibres.

Keywords: enzymes, glucose oxidase, bleaching, fermentation, gluconic acid

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1 Introduction

Biotechnology is a field of applying living organisms and their components in the industrial processes and products. In 1981, the European Federation of Biotechnology defined biotechnology as an integrated use of biochemistry, microbiology and chemical engineering with the purpose of applying microorganisms [1]. Enzymes are biological catalysts which accelerate biochemical reactions. In the reactions, they modify the chemical bonding of other substances, at the same time not being consumed or changed during these reactions. Chemically, they are natural proteins composed of more than 100 different amino acids [2]. Regarding the specificity of a chemical reaction, enzymes are classified into the following six groups: oxidoreductases (EC 1), transferases (EC 2), hydrolases (EC 3), lyases (EC 4), isomerases (EC 5) and lygases (EC 6). In the field of textile pretreatment and finishing processes, EC1 (catalases, lacases, peroxidases, glucose oxidases) and EC 3 (amylases, cellulases, pectinases, proteases, lypases) groups of enzymes are essential [3].

During the last years, intensive research of biotechnological processes involving enzymes and microorganisms has been made in the field of textile technology. A proper use of enzymes which operate at milder pH and temperature conditions can strongly reduce water, energy, time and dangerous chemicals consumption in the textile pretreatment processes. Enzymes are distinguished according to their specific working activity, i.e. each enzyme performs a specific function in a specific part of the substrate. Therefore, in an enzymatic pretreatment, the textile substrate is less damaged when compared to a classical pretreatment. Being natural substances and biologically decomposable, enzymes do not present an obstacle during the wastewater treatment, which results in the wastewater being less polluted. Several processes, e.g. desizing with amylases or biopolishing of jeans with cellulases, are practically indispensable, while others, e.g. bioscouring of cotton with pectinases or enhancement of hydrophilicy of polyester with esterases, are still ploughing their way into industrial uses. Bleaching of natural cellulose fibres represents one of the pro-

ga z namenom izkoriščanja biokultur [1]. Encimi so biološki katalizatorji, ki pospešujejo hitrost biokemijskih reakcij. V reakciji spreminjajo kemične vezi drugih spojin, ne da bi se pri tem sami porabili ali spremenili. Kemično so naravni proteini, sestavljeni iz več sto različnih aminokislin [2]. Glede na specifičnost kemijske reakcije se encimi delijo na šest glavnih skupin: oksidoreduktaze (EC 1), transferaze (EC 2), hidrolaze (EC 3), liaze (EC 4), izomeraze (EC 5) in ligaze (EC 6). Na področju tekstilnega plemenitenja sta pomembni skupini EC 1 (katalaze, lakaze, peroksidaze, glukoza oksidaze) in EC 3 (amilaze, celulaze, pektinaze, proteaze, lipaze) [3].

Na področju tekstilne tehnologije se v zadnjih letih intenzivno proučujejo biotehnološki procesi, v katerih sodelujejo encimi in mikroorganizmi. S pravilno uporabo encimov v procesih plemenitenja tekstilij lahko močno zmanjšamo porabo vode, energije, časa in nevarnih kemikalij, saj encimi delujejo pri blagih pogojih pH in temperature. Za encime je značilno specifično delovanje. To pomeni, da vsak encim izvaja le določeno funkcijo na natančno določenem mestu substrata. Zato se praviloma tekstilni substrat med encimsko obdelavo manj poškoduje kot pri klasičnih obdelavah. Ker so encimi naravne snovi in so biološko razgradljivi, ne pomenijo ovire za čiščenje odpadnih voda, ki so praviloma tudi manj obremenjene. Nekateri postopki, kot je razškrobljenje z amilazami ali biopoliranje džinsa s celulazami, so tako rekoč nepogrešljivi, medtem ko si drugi, kot je izkuhavanje bombaža s pektinazami ali izboljšanje hidrofilnosti sintetičnih vlaken z esterazami, še utirajo pot v širšo industrijsko uporabo. Med postopke, za katere se še raziskujejo možnosti uporabe encimov, spada beljenje naravnih celuloznih vlaken. Bombaž se beli z vodikovim peroksidom, ki sam po sebi sicer ekološko ni sporna spojina. Vendar poteka beljenje z njim v močno alkalnih kopelih pri visoki temperaturi, za beljenje, izpiranje in nevtralizacijo se porabi veliko vode in energije, med postopkom pa se lahko poškodujejo vlakna [4]. Zaradi navedenih razlogov bi bil dobrodošel postopek, ki bi omogočal okolju prijaznejše beljenje in bi zaokrožil encimsko predobdelavo bombaža od razškrobljenja, prek izkuhavanja do beljenja.

Encimi glukoza oksidaze so predstavniki skupine encimov oksidoreduktaz z oznako EC 1.1.3.4. in katalizirajo oksidacijo β-D-glukoze v glukonsko kislino ter z uporabo molekularnega kisika kot elektron akceptorja simultano proizvajajo vodikov peroksid. Pridobivajo se z mikroorganizmi vrste *Aspergillus*, *Penicillium* ali *Saccharomyces*. Večina komercialno proizvedenih encimov glukoza oksidaze se pridobiva iz micelijev gliv *Aspergillus niger*, in sicer za proizvodnjo glukonske kisline ali njenih soli, natrijevega in kalcijevega glukonata [5].

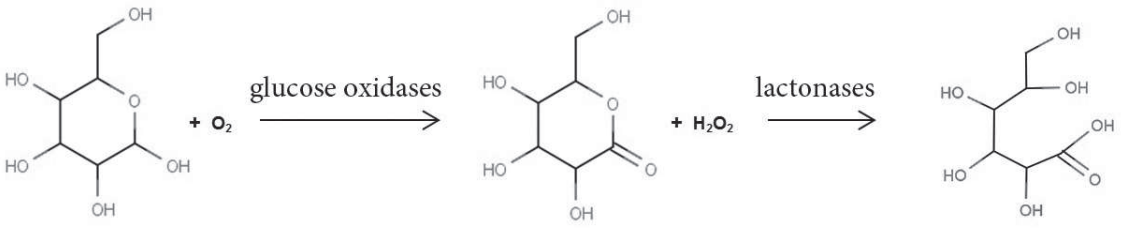
V prispevku so predstavljeni mehanizmi reakcije glukoza oksidaz, osnovne značilnosti, njihovo pridobivanje, industrijski pomen in raziskave, ki potekajo pri uporabi glukoza oksidaz v tekstilnem plemenitenju.

cesses for which the use of potential enzymes is still being investigated. Cotton is bleached with hydrogen peroxide, which is an ecologically undisputable substance. However, bleaching with hydrogen peroxide takes place in strong alkali bathes at high temperatures. Bleaching, rinsing and neutralisation require large amounts of water and energy; moreover, during the bleaching process, fibres might get damaged [4]. In consequence, an environment-friendly bleaching process would be essential and would supplement the enzymatic pretreatment of cotton, starting from desizing, through scouring and bleaching.

2 Značilnosti glukoza oksidaze

2.1 Mehanizem reakcije glukoza oksidaze

Glukoza oksidaze so flavoproteini, ki katalizirajo oksidacijo β-D-glukoze v D-glukon-δ-lakton in vodikov peroksid z uporabo molekularnega kisika kot elektron akceptorja. Reakcija je sestavljena iz redukcijske in oksidacijske stopnje. V redukcijski stopnji reakcije z oksidacijo β-D-glukoze v D-glukon-δ-lakton nastaja vodikov peroksid. Pri tem je flavo-adenin-dinukleotidna skupina - FAD, ki je aktivno mesto encima glukoza oksidaze, zreducirana v $FADH_2$. Sledi oksidacijska stopnja reakcije, v kateri encimi laktonaze (EC 3.1.1.17) katalizirajo hidrolizo D-glukon-δ-laktona v D-glukonsko kislino. Tukaj zreducirana $FADH_2$ skupina ponovno oksidira v FAD (slika 1) [5].



glucose oxidase – $FADH_2$ ↔ glucose oxidase – FAD

Figure 1: Reaction mechanism [5]

The enzymes glucose oxidases are representatives of the oxidoreductases group, marked EC 1.1.3.4. They catalyse the oxidation of β-D-glucose to gluconic acid by utilizing molecular oxygen as an electron acceptor with a simultaneous production of hydrogen peroxide. The most common microbial sources for the production are the Aspergillus, Penicillium and Saccharomyces species. Most of the commercially produced enzymes glucose oxidases are isolated from the mycelium of Aspergillus niger, grown principally for the production of gluconic acid or its salts, sodium or calcium gluconate [5].

The present review represents the reaction mechanisms of glucose oxidases, their basic characteristics, production, industrial importance and recent research in the field of textile pretreatment.

2.2 Pogoji delovanja glukoza oksidaze

Glukoza oksidaze so visokospecifične za pretvorbo β-izomerov D-glukoze, medtem ko α-izomeri niso primeren substrat. Zaviralci aktivnosti glukoza oksidaz so hidroksilamin, hidrazin, fenilhidrazin, natrijev bisulfat, Ag^+, Hg^+, Cu^{2+} itd. Glukoza oksidaze, pridobljene z različnimi mikroorganizmi, se razlikujejo v temperaturi delovanja, pH območju delovanja in aktivnosti delovanja. Glukoza oksidaze katalizirajo tvorjenje vodikovega peroksida pri pH vrednosti od 4.5 do 7. Optimalna pH vrednost območja delovanja glukoza oksidaz, proizvedenih z mikroorganizmi podvrste *Aspergillus niger,* je od 3.5 do 6.5 in z mikroorganizmi podvrste *Penicillium amagasakiense* od 4.0 do 5.5. Encimi so na splošno zelo občutljivi na spremembe v temperaturi. Stopnja reakcije in temperatura reakcije sta eksponentno povezani. Pri vsakem dvigu temperature za 10 °C se stopnja encimske reakcije podvoji. Optimalna temperatura delovanja glukoza oksidaz, proizvedenih z mikroorganizmi podvrste *Aspergillus niger,* je od 40 °C do 60 °C. Na splošno je aktivnost glukoza oksidaz, pridobljenih z mikroorganizmi vrste *Aspergillus,* nekoliko višja od aktivnosti glukozaoksidaz, proizvedenih z mikroorganizmi vrste *Penicillium* (tabela 1) [5].

2 Glucose oxidases characteristics

2.1 Glucose oxidases reaction mechanism

Glucose oxidases are flavoproteins which catalyse the oxidation of β-D-glucose into D-glucono-δ-lactone and hydrogen peroxide using molecular oxygen as an electron acceptor. The reaction is divided into a reductive and oxidative step. In the reductive step, a reaction during the oxidation of β-D-glucose into D-glucono-δ-lactone hydrogen peroxide is formed. Subsequently, the flavine adenine dinucleotide group (FAD), representing the active site of glucose oxidases, is reduced to $FADH_2$. In the following oxidation step, the reaction enzymes lactonases (EC 3.1.1.17) catalyse hydrolysis of D-glucono-δ-lactone to D-gluconic acid. Here, the reduced $FADH_2$ group is once more oxidized to FAD (cf. Figure 1) [5].

2.2 Glucose oxidases operating activity

Glucose oxidases are highly specific in converting the β-anomers of D-glucose, while α-anomers do not appear to be a suitable substrate. The inhibitors of glucose oxidases include hydroxylamine, hydrazine, phenylhydrazine, sodium bisulphate, Ag^+, Hg^+, Cu^{2+} etc. Glucose oxidases produced from various microorganisms differ in their operating temperature, operating pH range and operating activity. Glucose oxidases catalyse the hydrogen peroxide generation at the pH range 4.5–7. The optimum pH range for glucose oxidases from the Aspergillus niger and Penicillium amagasakiense species was shown to be 3.5–6.5 and 4.0–5.5, respectively. Enzymes are very sensitive to the changes in temperature. The relationship between the reaction rate and temperature is exponential.

2.3 Analiza aktivnosti glukoza oksidaze

Za analizo aktivnosti glukoza oksidaz se uporablja analitična metoda, ki temelji na dejstvu, da glukoza oksidaze ob prisotnosti kisika oksidirajo β-D-glukozo v β-D-glukon-δ-lakton in vodikov peroksid. Peroksidaze nato na osnovi vodikovega peroksida katalizirajo oksidacijo obarvanega substrata. Za analizo aktivnosti se uporabljata substrata 2,2-azino-di-(3-etilbenztiazolin-sulfonat) (ABTS) ali o-dianizidin (slika 2). ABTS oksidiran substrat tvori zeleno-modro obarvan produkt z maksimumom absorpcije pri 420 nm valovne dolžine, oksidiran o-dianizidin pa tvori kinoniminsko barvilo z maksimalno absorpcijo pri 500 nm. Sprememba barve oksidiranega substrata se izmeri spektrofotometrično. Za proučevanje kinetičnih lastnosti glukoza oksidaze se lahko uporabi metoda FT-IR, ki temelji na absorpciji substrata in produkta pri različnih valovnih dolžinah. Prednosti metode FT-IR so v hitrejši analizi, potrebi po manjši količini substrata in encima ter možnosti določanja količine proizvedenega D-glukon- δ-laktona [5].

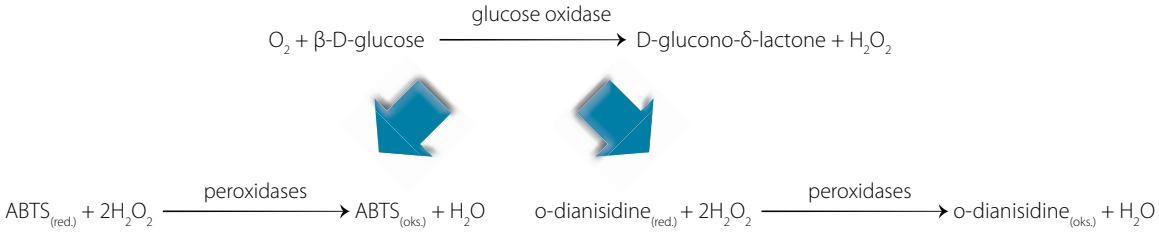


Figure 2: Analyses of glucose oxidases activity with ABTS (left) and o-dianisidine (right) chromogenic dyes [5]

2.4 Imobilizacija glukoza oksidaze

Pri klasičnih postopkih se encimi po uporabi zavržejo. Z metodo imobilizacije se encimi adsorpcijsko, ionsko in kovalentno vežejo na poljubne nosilce. Pri imobilizaciji se v primerjavi s klasičnimi postopki porabi manjša količina encima. Poleg tega so imobilizirani encimi večkrat uporabni. Glukoza oksidaze so bile v preteklosti imobilizirane že na različne materiale, kot so kopolimerne membrane iz polietilen akrilne kisline, silikonske nosilce, svilene fibroinske membrane, aktiviran ogljik, steklo, kolagen, polikarbonat, poliuretan, polipirolne filme, celulozo itd. Porozno steklo in celuloza sta najpogosteje uporabljena nosilca zaradi velikega volumna notranjih por, ki omogočajo višjo katalitično aktivnost encima. Na anorganskih in poroznih magnezijevih silikatnih nosilcih je mogoče sočasno imobilizirati encima glukozo oksidazo in katalazo. Visoke koncentracije vodikovega peroksida lahko povzročijo deaktivacijo encimov glukoza oksidaze. Katalaze v nosilcu varujejo glukoze oksidaze tako, da nastali vodikov peroksid razgradijo v vodo in kisik. Sočasna imobilizacija tako izboljša aktivnost, stabilnost in ponovno uporabo encimov glukoza oksidaze. Imobilizirani encimi so v večjem obsegu že zamenjali klasične postopke s prostimi encimi v prehranski industriji. Prav tako se uporabljajo za pridobivanje optično čistih spojin v kemični industriji in farmaciji [5, 6].

For each 10 °C rise in temperature, the rate of the enzyme reaction doubles. The optimum operating temperature of glucose oxidases from Aspergillus niger is 40–60 °C. The operating activity of glucose oxidases from the Aspergillus species is slightly higher compared to the operating activity of glucose oxidase from the Penicillium species (cf. Table 1) [5].

2.3 Analyses of glucose oxidases activity

To determine the glucose oxidases activity, an analytical method based on the principle that glucose oxidases oxidize β-D-glucose into β-D-glucono-δ-lactone and hydrogen peroxide is used. Hydrogen peroxide is then utilized to oxidise the substrate with peroxidases. 2,2-azino-di-(3-ethylbenzthiazolin-sulphonate) (ABTS) or o-dianisidine substrates are used to determine the glucose oxidases activity (cf. Figure 2). ABTS forms a greenish-blue oxidized product measured at 420 nm, while the oxidized o-dianisidine forms a quinoneimine dye measured at 500 nm. The resultant colour change of the substrate is monitored spectrophotometrically. To study the kinetic properties of glucose oxidases, the FT-IR method, which is based on the absorption of the substrate and the product at different frequencies, can be used. The advantages of using the FT-IR method are the

3 Postopek pridobivanja glukoza oksidaz

Za pridobivanje encimov glukoza oksidaze je treba najprej s fermentacijo vzgojiti mikroorganizme vrste *Aspergillus*, *Penicillium* ali *Saccharomyces* [5]. Pridobivanje poteka v reaktorju brez svetlobe, z medijem hranilnih snovi pod natančno določenimi pogoji; pH, temperaturo in tlakom [2].

Medij, s katerim mikroorganizmi proizvedejo glukoza oksidaze, vsebuje različne vire ogljikovih hidratov (glukozo, sukrozo, galaktozo …), pepton, vodo, anorganske soli, dušikove spojine, kalcijev karbonat itd. Različni tipi ogljikovih hidratov vplivajo na rast in končno aktivnost glukoza oksidaze (tabela 1). Čeprav mikroorganizmi podvrste *Aspergillus niger* uspevajo na vseh virih ogljikovih hidratov, so bile višje vrednosti aktivnosti pridobljene glukoza oksidaze zaznane na mediju z viri glukoze, sukroze in molaze. Tudi poznejše dodajanje deleža vira ogljikovih hidratov pripomore, da se pridobi več glukoza oksidaze [5].

Table 1: Examples of some microorganisms and media composition to produce glucose oxidases [5]

Microorganism	Media composition [gl^{-1}]	Activity [Uml^{-1}]
Penicillium variabile P16	glucose (80) , $NaNO_3$ (5), KCl (0.5), KH_2PO_4 (1), $FeSO_4 \cdot 7H_2O$ (0.01), peptone (1), $CaCO_3$ (35)	5.52
Penicillium pinophilum DSM 11428	sucrose (40), $Na_2PO_4 \cdot 2H_2O$ (4.45), KH_2PO_4 (1.5), $NaNO_3$ (1.9), $MgSO_4 \cdot 7H_2O$ (0.02), $CaCl_2 \cdot 2H_2O$ (0.02), malt extract (10), yeast extract (5), vitamins (10)	1.9
Aspergillus niger – glucose oxidases genetic modification of *Saccharomyces cerevisiae* microorganisms	glucose (20), yeast extract (10), peptone (20)	125
Aspergillus niger BTL	sucrose (70), $(NH_4)_2HPO_4$ (0.4), KH_2PO_4(0.2), $MgSO_4 \cdot 7H_2O$ (0.02), peptone (10), $CaCO_3$ (35)	7.5
Aspergillus niger AM111	glucose (80), peptone (30), $NaNO_3$ (0.5), KH_2PO_4 (1), $CaCO_3$ (35)	2.5

Temperatura in pH vrednost vplivata na rast in na fiziologijo mikroorganizmov. Vplivata na topnost, navzemanje surovin, aktivnost encima, morfologijo celične membrane, pridobivanje stranskih produktov in reakcije oksidacije ter redukcije. Optimalna pH vrednost rasti in pridobivanja encimov je med 6 in 7. Kalcijev karbonat in fosfati uravnavajo pH vrednost. Optimalen medij za pridobivanje glukoza oksidaze naj bi vseboval 3 % kalcijevega karbonata. Optimalna temperatura pridobivanja se razlikuje glede na vrsto mikroorganizmov. Optimalna temperatura pridobivanja glukoza oksidaze z mikroorganizmi podvrste *Aspergillus niger* je od 27 do 37 °C. Ko je proces fermentacije končan, ostanejo v reaktorju velika količina neporabljenih hranilnih snovi, voda, mikroorganizmi in še

speed of assay, the need for smaller amounts of the substrate and enzyme, and the feasibility of the quantifying D-glucono-δ-lactone formation [5].

2.4 Immobilization of glucose oxidases

In classical processes, enzymes are disposed after the usage. The methods of immobilization involve adsorption, ionic and covalent binding on optional carriers. When compared to the classical process, the immobilization process enables lower enzyme consumption. Furthermore, the immobilized enzymes can be used in repeated applications. Glucose oxidases have been immobilized on various materials, e.g. polyethylene acrylic acids copolymer membranes, silicone supports, silk fibroin membranes, activated carbon, glass, collagen, polycarbonate, polyurethane, polypyrrole films, cellulose etc. Since high surface area materials enable a higher catalytic enzyme activity, the porous glass and cellulose represent the most popular supports. The co-immobilization of glucose oxidases and catalases can be performed on inorganic and porous magnesium silicate supports. High hydrogen peroxide concentrations can deactivate glucose oxidases. Catalases present in the support protect glucose oxidases in decomposing the generated hydrogen peroxide into water and oxygen. The co-immobilization improves the operating activity, stability and enables a repeated application of glucose oxidases. The immobilized enzymes have been implemented on a larger scale in the food industry, where they have replaced the free enzyme processes. In addition, they are used to produce optically pure substances in the chemical and pharmaceutical industry [5, 6].

3 Glucose oxidases production process

To produce glucose oxidases, the microorganisms of the Aspergillus, Penicillium or Saccharomyces species have to be grown with a fermentation process [5]. The production takes place in a reactor containing a nutritive medium, without light and under specific requirements, i.e. pH range, temperature and pressure [2].

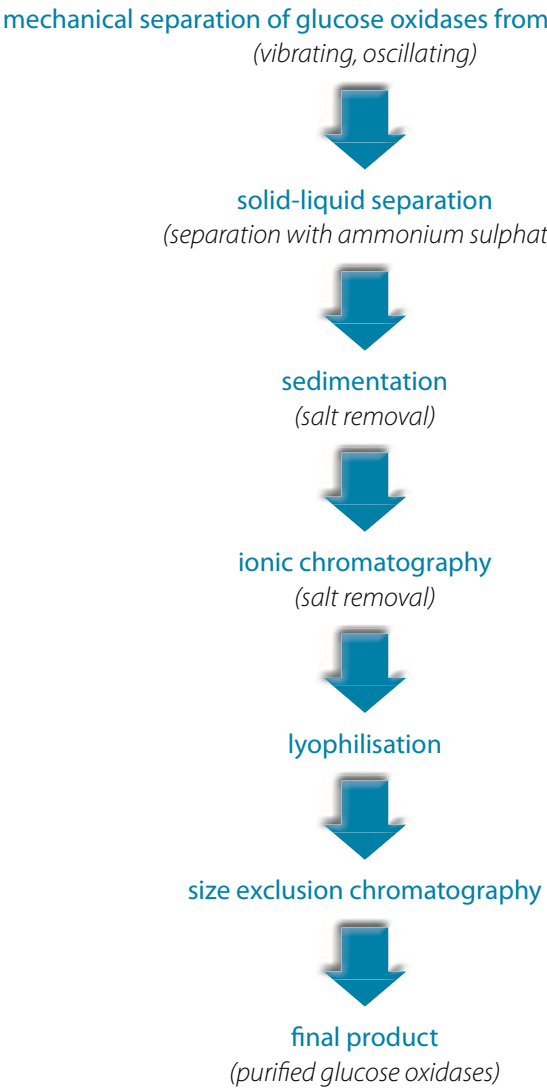


Figure 3: General set of processes to produce pure glucose oxidases [5]

uporabni encimi. Zato je ključnega pomena uspešno ločevanje glukoza oksidaz iz reaktorja (slika 3). Glukoza oksidaze se lahko proizvedejo znotraj- ali zunajcelično in v obliki micelijev. Ločevanje glukoza oksidaze iz celic ali micelijev poteka z uporabo mehanskih sil (tresenjem, nihanjem), centrifugiranjem ali filtracijo. Za popolno ločitev encimov je potrebna vrsta različnih procesov (slika 3) [5].

Težavi, s katerima se sooča proizvodnja encimov glukoza oksidaze, sta nizka produktivnost in sočasno pridobivanje drugih encimov, kot so katalaze. Katalaze katalizirajo razpad vodikovega peroksida v molekularni kisik in vodo. Reakcija poteka pri temperaturi od 20 do 50 °C ter v alkalnem pH območju (pH 6-10) [7]. Katalaze, prisotne v pripravkih z glukoza oksidazami, lahko neželeno razgrajujejo encimsko proizvedeni vodikov peroksid. Metode kromato-

The medium required for the microorganisms to produce glucose oxidases contains various carbohydrate sources (glucose, sucrose, galactose etc), peptone, water, inorganic salts, nitrogen compounds, calcium carbonate etc. Various carbohydrate sources influence the growth and operating activity of glucose oxidases (cf. Table 1). Although microorganisms from the Aspergillus niger species successfully grow on each carbohydrate source, higher growth values of glucose oxidases have been obtained when using the glucose, sucrose and molasse media. Higher glucose oxidases growth quantity can be achieved by adding carbohydrates in smaller portions at a later stage [5].

The temperature and pH range influence the growth and physiology of microorganisms. They affect the nutritive solubility and uptake, enzyme activity, cell-membrane morphology, by-product formation, and oxidation and reduction reactions. The optimum pH range for the growth and enzyme production is between 6 and 7. Calcium carbonate and other phosphates are used to regulate the optimum pH range. The optimum medium for the glucose oxidases production should contain 3% of calcium carbonate. The optimum temperature for the production differs from one microorganism species to another. The optimum production temperature for glucose oxidases from Aspergillus niger is 27–37 °C.

After the fermentation process is completed, the reactor still contains large amounts of useless nutritive sources, water, microorganisms and useable enzymes. Hence, a crucial step after the completion of the fermentation process is recovery of glucose oxidases from the reactor (cf. Figure 3). Glucose oxidases can be produced intracellularly or extracellularly, or in the form of mycelia. The separation of glucose oxidases from cells or mycelia can be facilitated by using mechanical forces (i.e. vibrating, oscillating), centrifugation and filtration. For a complete separation of enzymes, a set of various processes is required (cf. Figure 3) [5].

Low productivity and a simultaneous production of other enzymes as catalases represent the basic difficulties that burden the glucose oxidases production. Catalases catalyse the decomposition of hydrogen peroxide into molecular ox-

grafije omogočajo uspešno ločevanje encimov glukoza oksidaze in katalaze ter ločevanje glukoza oksidaze z večjo od glukoza oksidaze z manjšo aktivnostjo.

Posamezni postopki pridobivanja čistih encimov so izredno zahtevni. Zato se znanstveniki intenzivno ukvarjajo z raziskavo predhodne genske modifikacije mikroorganizmov, ki so nato sposobni proizvesti veliko količino le izbranega encima. Uporaba genskega inženiringa tako omogoča proizvajanje encimov z višjo stopnjo čistosti in kvalitativno spremenjenimi lastnostmi (boljšo učinkovitostjo, večjo toplotno stabilnostjo, večjo aktivnostjo pri višjih pH vrednostih, večjo stabilnostjo do težkih kovin in oksidacijskih sredstev, daljšo časovno stabilnostjo) [5].

Končna oblika glukoza oksidaze, pridobljena z mikroorganizmi *Aspergillus niger,* ostane stabilna najmanj šest mesecev pri temperaturi okoli 20 °C. Dobri stabilizatorji končnega produkta glukoza oksidaze so različni sladkorni alkoholi (etilen glikol, glicerol, eritirol, ksilitol, sorbitol, polietilen glikol) [5].

4 Dosedanje raziskave na področju encimskega beljenja tkanin

Razškrobljanje, klasično izkuhavanje z natrijevim hidroksidom in beljenje z dodanim vodikovim peroksidom v močno alkalnem mediju ob visoki temperaturi so postopki predobdelave celuloznih tkanin. Ti postopki porabijo neznansko veliko energije, vode in kemikalij, povzročajo pa tudi poškodbe celuloznih vlaken [6]. Encimi amilaze se kot sredstvo za razškrobljanje uporabljajo že skoraj sto let. V zadnjih letih se za bioizkuhavanje bombaža uveljavljajo encimi pektinaze. Pektinaze razgradijo pektin v povrhnjici bombažnega vlakna, s čimer omogočijo tudi odstranjevanje drugih hidrofobnih snovi (voskov) s površine vlakna, ki tako postane hidrofilno [4]. Glukoza oksidaze, ki katalizirajo tvorbo vodikovega peroksida iz zračnega kisika in glukoze, pa so nova alternativa beljenja celuloznih tkanin z encimi. Za tvorbo vodikovega peroksida se lahko uporabi glukoza, nastala pri predhodnem razškrobljanju tkanin. Encimi amilaze se delijo na α-amilaze, β-amilaze, α-1,6-glukozidaze in amiloglukozidaze. Danes se za razškrobljanje tkanin uporabljajo α-amilaze, ki razgradijo škrob na delno razgrajene oligosaharide.

Anis in sodelavci [8, 9] so raziskali možnost beljenja z encimi glukoza oksidaze. Belilna kopel za uspešno beljenje z glukoza oksidazami naj bi vsebovala okoli 800 mg l^{-1} glukoze. Ugotovili so, da α-amilaze ne proizvedejo zadostne količine glukoze, le okoli 200 mg l^{-1}, medtem ko amiloglukozidaze pri enakih pogojih proizvedejo kar 4000 mg l^{-1} glukoze. Le amiloglukozidaze so sposobne cepiti β-D-glukozne enote amilopektina, amiloze ali glikogena in so tako končni produkt razgradnje škroba le posamezne enote glukoze. Kot optimalne pogoje pridobivanja glukoze v razškrobilni kopeli so določili ob pH vrednosti 4.1 z dodatkom ocetne kisline,

ygen and water. The reaction takes place at the temperature 20–50 °C and pH range 6–10 [7]. Catalases which are present in the glucose oxidases preparations can undesirably decompose the enzymatically generated hydrogen peroxide. The chromatography methods enable a successful separation of glucose oxidases and catalases, and a separation of glucose oxidases with a higher operating activity from glucose oxidases with a lower operating activity.

Individual production processes to gain pure enzymes are very demanding. Therefore, scientists vigorously research preliminary genetic modifications of microorganisms that enable a higher quantity production of a specific enzyme. The use of genetic engineering enables the enzyme production of higher purity levels and qualitative modified properties (e.g. higher efficiency, higher thermal stability, higher operating activity with a higher pH range, higher stability towards hard metals and oxidation agents, longer time stability) [5].

The final glucose oxidases preparation from Aspergillus niger remains stable for minimum 6 months at the temperature around 20 °C. Polyhydric alcohols (i.e. ethylene glycol, glycerol, erythritol, xylitol, sorbitol, polyethylene glycol) have shown stabilizing effects of glucose oxidases end product [5].

4 Previous research in field of fabric pretreatment in textile technology

Desizing, conventional scouring with sodium hydroxide and bleaching with added hydrogen peroxide in a strong alkaline pH and at a high temperature are pretreatment processes of cellulose fabrics. These processes consume a lot of energy, water and chemicals, and cause damage to cellulose fibres [6]. Enzymes amylases have been used in desizing for almost as long as a century. During the last few years, enzymes pectinases have gained importance in the bioscouring of cotton. Pectinases decompose the pectin inside the epidermis of cotton fibres and consequently remove other hydrophobic substances (waxes) from the fibre surfaces, making them hydrophilic [4]. Glucose oxidases which catalyse the generation of hydrogen peroxide by

temperaturo 62 °C in čas 45 minut. Uspešnemu razškrobljanju z amiloglukozidazami je sledilo beljenje bombaža z encimsko proizvedenim vodikovim peroksidom. Kot optimalne pogoje pridobivanja vodikovega peroksida z glukoza oksidazami so določili temperaturo 55 °C in čas 45 minut v kislem pH mediju. Pri teh pogojih je proizvedeni vodikov peroksid neaktiven za beljenje, saj le-ta uspešno beli pri pH vrednosti od 10.5 do 10.8 in temperaturi od 90 do 120 °C. Za beljenje proizvedeni vodikov peroksid je zatorej treba aktivirati. Preizkusili so beljenje pri temperaturi okoli 90 °C v različnih pH medijih. Beljenje v kislem in nevtralnem mediju je potekalo z aktivatorji vodikovega peroksida. Rezultati so pokazali, da vzorci, beljeni v kislem in nevtralnem mediju, zaradi nezadostnega aktiviranja vodikovega peroksida niso dosegali zadostne beline. Stopnja beline vzorcev, beljenih z encimsko pridobljenim vodikovim peroksidom v alkalnem mediju pri temperaturi 90 °C, je bila le za 7 % nižja od vzorcev, beljenih po klasičnem postopku z dodanim vodikovim peroksidom.

Opwis in sodelavci [10] so ugotovili, da se količina proizvedenega vodikovega peroksida s pomočjo encimov glukoza oksidaze lahko poveča z doziranjem encima v manjših odmerkih v celotnem času belilnega procesa.

Glukonska kislina je končni produkt encimske obdelave glukoze z glukoza oksidazo. V belilni kopeli proizvedena glukonska kislina je odlično kompleksirno sredstvo, ki kompleksira za beljenje nevarne težke kovinske ione. Dodatki stabilizatorjev, kot so magnezijeve soli in vodno steklo, niso potrebni. Dodatek ciklodekstrinov lahko varuje glukoza oksidaze in druge encime pred njihovo deaktivacijo, ki jo povzročajo anionski kompleksanti (disperzijska, omakalna sredstva, sredstva proti penjenju,…) [10, 11].

Tzanov in sodelavci [6] so raziskali beljenje z glukoza oksidazami, imobiliziranimi na silanizirane porozne nosilce, aluminijev oksid in steklo z uporabo veznega sredstva glutaralaldehida. Stekleni nosilec je vezal več odstotkov encima in stopnja nastajanja vodikovega peroksida je bila hitrejša kot pri aluminijevem oksidu. Vendar se je aluminijev oksidni nosilec izkazal za primernejšega pri ponavljajoči se uporabi. Le-tega je mogoče ponovno uporabiti v najmanj treh ciklih. Tkanine, beljene z imobiliziranimi encimi, so imele višjo stopnjo beline kot tkanine, beljene s prostimi encimi.

Opwis in sodelavci [12] so za razbarvanje barvalne kopeli uporabili nov postopek s sočasno uporabo glukoza oksidaz in peroksidaz. Peroksidaze na osnovi vodikovega peroksida katalizirajo oksidacijo obarvanih primesi v barvalni kopeli. Vendar te pri visoki koncentraciji vodikovega peroksida postanejo neaktivne. Tako so glukoza oksidaze proizvajale vodikov peroksid, ki se je sočasno prek peroksidaz porabljal za razbarvanje obarvanih primesi.

Raziskave encimske predobdelave celuloznih tkanin so usmerjene v optimiziranje posameznih postopkov in v združevanje posameznih postopkov v enokopelni proces. Z združevanjem postopkov predobdelave se prihranijo pomožna sredstva, energija in izpiralna

utilizing molecular oxygen and glucose represent a new alternative of bleaching fabrics with enzymes. For the generation of hydrogen peroxide, the glucose gained during fabric desizing can be used. Enzymes amylases are classified into α-amylases, β-amylases, α-1,6-glucosidases and amyloglucosidases. Today, desizing with α-amylases is used, the latter decomposing starch into partly degraded oligosaccharides.

Anis et al [8, 9] researched the ability of bleaching with enzymes glucose oxidases. For efficient bleaching, the bleaching bath should contain around 800 mgl^{-1} of glucose. They concluded that α-amylases generate an insufficient amount of glucose, only 200 mgl^{-1}, while at the same conditions amyloglucosidases generate 4000 mgl^{-1}, respectively. Only amyloglucosidases are capable of dividing the β-D-glucose units of amylopectin, amylose or glycogen. Subsequently, the end product of desized starch is represented only by single units of glucose. The optimal conditions of producing glucose in desizing liquor were determined at the pH value 4.1 with added acetic acid, temperature 62 °C and time 45 minutes. After a successful desizing with amyloglucosidases, bleaching of cotton with enzymatically generated hydrogen peroxide took place. The optimal conditions for generating hydrogen peroxide with glucose oxidases were determined at the temperature 55 °C and time 45 minutes in an acidic pH. At these conditions, the generated hydrogen peroxide is inactive for bleaching, since it effectively bleaches at the pH range 10.5–10.8 and temperature 90–120 °C. Therefore, the generated hydrogen peroxide has to be activated for bleaching. They tested bleaching at the temperature of around 90 °C in various pH media. Bleaching in acid and neutral media took place with hydrogen peroxide activators. The results showed that the samples bleached in acid and neutral media did not reach an adequate whitening index due to the insufficient activation of hydrogen peroxide. The whitening index of the samples bleached with enzymatically gained hydrogen peroxide in alkaline media at the temperature 90 °C was only by 7% lower compared to the samples bleached with a traditional process with added hydrogen peroxide.

Opwis et al [10] established that the amount of hydrogen peroxide generated with enzymes

voda. Pomanjkljivost ponovne uporabe kopeli za razškrobljanje in izkuhavanje za procese beljenja so iz vlaken odstranjene nečistoče in druge nevlaknate primesi. Te se odlagajo na materialu, med beljenjem pa se določena količina vodikovega peroksida porabi tudi za njihovo beljenje [13]. Eren in sodelavci [14] so razškrobljanje z amiloglukozidazami, beljenje z encimsko proizvedenim vodikovim peroksidom z glukoza oksidazami, razgrajevanje preostalega vodikovega peroksida po beljenju s katalazami in barvanje z izbranim reaktivnim barvilom združili v enokopelni postopek. Stopnja beline encimsko obdelanih vzorcev je bila 11 % nižja od stopnje beline vzorcev obdelanih po klasičnem postopku. Ugotovili so, da imajo vzorci, obdelani po enokopelnem encimskem postopku, višje pretržne trdnosti kot vzorci, beljeni po klasičnem postopku. Razlog je v nižji koncentraciji vodikovega peroksida med procesom beljenja. Klasični postopek v primerjavi z encimskim vsebuje tudi do dvakratno količino vodikovega peroksida, in sicer okoli 1500 mgl^{-1}. Ugotovili so tudi, da ima encimski enokopelni postopek manjši vpliv na okolje, KPK je bil nižji kar za 42 %, BPK pa za 21 %, saj pri klasičnem postopku škrob konča v odplakah in tako veliko prispeva k obremenjevanju okolja.

Kot smo že omenili, danes najpogostejša oblika beljenja tkanin z vodikom peroksidom zahteva veliko energije in povzroča poškodbe bombažnih vlaken. Ti negativni vplivi beljenja z vodikovim peroksidom so lahko zmanjšani z uporabo aktivatorjev beljenja, ki vodikov peroksid pretvarjajo v perocetno kislino, ki ima večjo oksidacijsko moč kot vodikov peroksid. Perocetna kislina omogoča beljenje pri nižji temperaturi in v nevtralnem mediju, med beljenjem pa ostanejo vlakna nepoškodovana. Najpogosteje uporabljena belilna aktivatorja v industriji detergentov sta tetraacetiletilendiamin (TAED) in nonanoiloksibenzen sulfonat (NOBS) [15]. Različni tipi belilnih aktivatorjev in njihovo optimalno delovanje v procesu beljenja se raziskujejo že vrsto let [16–24].

Na Oddelku za tekstilstvo Naravoslovnotehniške fakultete Univerze v Ljubljani se že vrsto let ukvarjamo z encimskimi predobdelavami [25–31]. Trenutne raziskave so usmerjene v beljenje bombažnih tkanin z encimi glukoza oksidaze. Za beljenje so bili preizkušeni nekateri aktivatorji beljenja (TAED, TBBC in NOBS). Obetavne rezultate dajeta aktivatorja TAED in TBBC, čeprav stopnja beline še ni primerljiva s stopnjo beline tkanin, beljenih po klasičnem postopku. V teku so optimiziranje postopkov ter preizkušanje drugih aktivatorjev beljenja in združevanje posameznih encimskih postopkov predobdelave bombažnih tkanin v enokopelni proces.

5 Uveljavljena področja uporabe glukoza oksidaz

Glukoza oksidaze so v zadnjih letih, zahvaljujoč njihovi vsestranski uporabi, postale komercialno zelo pomembne na področju ke-

glucose oxidases increases when adding the enzyme in small doses during the whole bleaching process.

Gluconic acid is an end product of the enzymatic treatment of glucose with glucose oxidases. The gluconic acid generated in the bleaching bath presents an excellent complexing agent, which complexes the heavy metal ions which are disturbing for the bleach. The addition of stabilizing agents as magnesium salts and water glass is not necessary. The addition of cyclodextrins protects glucose oxidases and other enzymes from their deactivation, which is caused by anionic surfactants (e.g. dispersing agents, wetting agents, anti-foams etc) [10, 11].

Tzanov et al [6] researched bleaching with glucose oxidases immobilised on silanized porous supports, alumina oxide and glass, using glutaraldehyde as the crosslinking agent. The glass support bound higher percentage of enzymes and the rate of hydrogen peroxide generation was faster than with alumina oxide. However, the alumina oxide support appeared to be more appropriate for a repeated application. It can be reused for at least three assays. The fabrics bleached with immobilised enzymes had a higher whiteness degree than the fabrics bleached with free enzymes.

Opwis et al [12] used for the decolouration of the dyeing bath a new method with a simultaneous application of glucose oxidases and peroxidases. Peroxidases catalyse on the basis of hydrogen peroxide the oxidation of coloured compounds left in the dyeing bath. Nevertheless, they become inactive at a higher hydrogen peroxide concentration. Consequently, the glucose oxidases generated hydrogen peroxide which was simultaneously used by peroxidases for the decolouring of coloured compounds.

The research of the enzymatic pretreatment of cellulose fabrics focuses on the optimization of individual processes and on joining individual processes into a one-bath treatment. With joined pretreatment processes, the auxiliary agents, energy and rinse water are spared. The negative feature of reusing the desizing and bioscouring treatment baths are the impurities and other non-fibre contaminants removed from the fibres. They are deposited on the fabric and during the bleaching process, a certain

mične, farmacevtske in prehranske industrije, v zdravstvu, v biotehnologiji in drugod. Nizka cena in relativno dobra stabilnost sta razloga, da so glukoza oksidaze najpogosteje uporabljeni encimi kot analitični reagent določanja glukoze [5].

Biosenzorji glukoze za diabetike

Glukoza oksidaze so primerne za uporabo v biosenzorjih za določanje stopnje glukoze v krvi. Biosenzorji merijo število elektronov in njihov naboj, ko le-ti prehajajo čez encim in se vežejo na elektrodo. Nekateri biosenzorji delujejo tudi po principu merjenja sprememb v fluorescenci aktivnega mesta (FAD) glukoza oksidaz [5].

Biogorivne celice

Bioelektronske naprave za svoje delovanje potrebujejo malo energije. V procesu biokatalize pretvarjajo biogorivne celice biokemično energijo v električno. Eden izmed tipov biogorivnih celic kot katalizator uporablja tudi encime. Te biogorivne celice so sestavljene iz seta dveh elektrod, modificiranih z biokatalitičnimi encimi, ki specifično oksidirajo ali reducirajo substrate. Na primer, encimi glukoza oksidaze ali glukoza dehidrogenaze katalizirajo oksidacijo glukoze na anodi, medtem ko encimi lakaze ali bilirubin oksidaze katalizirajo redukcijo kisika na katodi [5].

Dodatki v prehrani in pijači

Glukoze oksidaze z odstranjevanjem ostankov glukoze in kisika ohranjajo barvo, okus in čistost konzerviranih izdelkov in gaziranih pijač. Proizvedeni vodikov peroksid je dobro antibakterijsko sredstvo, ki se lahko s pretvorbo v vodo in kisik odstrani z dodatkom encimov katalaze. Na primer, kombinacija encimov glukoza oksidaze in katalaze se uporablja za preprečevanje dehidracije jajčnega prahu med njegovo proizvodnjo. V pšenično testo dodana glukoza oksidaza prav tako pripomore k boljši kakovosti kruha [5].

Vina z nizko vsebnostjo alkohola

Glukoza oksidaze s pretvarjanjem deleža glukoze v D-glukon-δ-lakton znižujejo vsebnost alkohola v vinu tudi do 2 %. Med fermentacijo encimi prav tako antibakterijsko delujejo proti bakterijam ocetne in mlečne kisline. Posledično se zmanjša potreba po dodatku konzervansov [5].

Ustna higiena

Glukoza oksidaze in laktoperoksidaze so lahko tudi antibakterijska sredstva izdelkov za ustno higieno. V ustni votlini nahajajoče se bakterijske vrste *Streptococci* povzročajo razkrajanje zobne sklenine. Le-te uspešno uničuje z encimi proizvedeni vodikov peroksid [5].

Glukonska kislina

Encimi glukoza oksidaze so pomemben vir proizvodnje glukonske kisline, ki je produkt hidrolize D-glukon-δ-laktona. Glukon-

amount of hydrogen peroxide is lost for their bleaching [13]. Eren et al [14] combined into a one-bath treatment the desizing with amyloglucosidases, bleaching with enzymatically generated hydrogen peroxide with glucose oxidases, the decomposition of the remained hydrogen peroxide after the bleaching with catalases and the dyeing with a selected reactive dye. The whiteness degree of enzymatically treated samples was by 11% lower compared to the whiteness degree of conventionally treated samples. They discovered that the samples treated in a one-bath enzymatic treatment had higher tensile strength compared to the samples bleached with a conventional treatment. The reason lies in a lower hydrogen peroxide concentration during the bleaching treatment. The conventional treatment compared to the enzymatic one can contain even more than a double amount of hydrogen peroxide, i.e. around 1500 mgl^{-1}. They also concluded that the enzymatic one-bath treatment has less impact on the environment, the COD was lower by 42%, BOD by 21%, while at the conventional treatment, starch ends in the wastewater and consequently contributes to the pollution of the environment.

As mentioned earlier, bleaching with hydrogen peroxide is the most frequent way of bleaching fabrics and takes place in strong alkaline media at high temperatures. This process demands a lot of energy and damages cotton fibres. The negative aspects of bleaching with hydrogen peroxide can be reduced by using bleaching activators, which convert hydrogen peroxide into peracetic acid. The generated peracetic acid has a higher oxidative power compared to hydrogen peroxide and therefore enables bleaching at a lower temperature in neutral media. The most frequently used bleaching activators in the detergent industry are tetraacetylethylenediamine (TAED) and nonanoyloxybenzene sulphonate (NOBS) [15]. Various types of bleaching activators and their optimal activity in the bleaching process have been the subject of research for many years now [16–24].

At the Department of Textiles, Faculty of Natural Sciences and Engineering, University of Ljubljana, the enzymatic pretreatments have been researched for several years [25–31]. Currently, the research is focused on the bleaching of cot-

ska kislina je nekorozivna, nehlapna, nestrupena in blaga organska kislina, odporna proti oksidaciji in redukciji pri visokih temperaturah. Kot kompleksirno sredstvo v alkalnem mediju kompleksira ione težkih kovin (Ca^{2+}, Fe^{2+}, Al^{3+},…), njeno delovanje je boljše od EDTA in drugih kompleksantov. Obstajajo različni pristopi pridobivanja glukonske kisline, in sicer so ti kemični, elektrokemični, biokemični in bioelektrokemični proces. Zaradi nižjih stroškov pridobivanja in večje učinkovitosti se glukonska kislina komercialno še vedno pridobiva v procesu fermentacije z mikroorganizmi podvrste *Aspergillus niger* in glukozo kot glavnim virom ogljikovih hidratov. Koncentracija glukoze v mediju znaša od 10 do 15 % in je v obliki glukoznih monokristalov ali v obliki sadnega ali grozdnega sirupa z 98-odstotnim donosom glukonske kisline. Zaradi visoke tržne cene glukonske kisline in njenih derivatov se intenzivno iščejo cenejši viri ogljikovih hidratov. Glukonska kislina se proizvaja za potrebe prehranske (34,5 %), farmacevtske (9,2 %) in gradbene industrije (46 %). Kot prehransko dopolnilo uravnava kislost, sterilizira in beli prehranske izdelke, kot sol je v uporabi v zdravilih. V gradbeništvu se uporablja kot ojačevalno sredstvo cementa za večjo odpornost na ekstremne vremenske pojave. Prav tako pa se kot blaga kislina uporablja v kovinski in usnjarski industriji. V naravi se nahaja v medu, sadju in vinu [5, 11, 32].

6 Sklepi

Na podlagi prispevka lahko povzamemo naslednje:

- Glukoza oksidaze so pomembni encimi v različnih industrijskih postopkih. Danes so glukoza oksidaze nepogrešljive v prehranski in farmacevtski industriji ter na področju biotehnologije.
- Glukoza oksidaze so sposobni proizvesti različni mikroorganizmi, vendar so se do zdaj komercialno uveljavili le nekateri. Glukoza oksidaze, proizvedene z mikroorganizmi podvrste *Aspergillus niger,* v primerjavi z drugimi odlikujejo širše pH območje delovanja, delovanje pri višjih temperaturah in višja aktivnost delovanja.
- Kemična modifikacija obstoječih encimov in genski inženiring omogočata pridobivanje učinkovitejše glukoza oksidaze.
- Raziskave postopka imobilizacije encimov glukoza oksidaze so pokazale dobro stabilnost le-teh in možnost večkratne uporabe.
- Glukoza oksidaze uspešno proizvajajo vodikov peroksid, potreben za beljenje celuloznih tkanin. Beljenje z glukoza oksidazami je ekonomski in ekološki potencial nasproti klasičnemu postopku beljenja z dodanim vodikovim peroksidom. Nadaljnje raziskave so usmerjene v doseganje stopnje beline, ki bi bila primerljiva s stopnjo beline tkanin, beljenih po klasičnem postopku.

ton fabrics with enzymes glucose oxidases. For the bleaching, some bleaching activators have been tested (i.e. TAED, TBBC and NOBS). Promising results are shown by TAED and TBBC; however, the whiteness degree is still not comparable with the whiteness degree of traditionally bleached fabrics. Further work involves the process optimisation and testing of other bleaching activators, and combining individual enzyme pretreatment processes of cotton fabrics into a one-bath treatment.

5 Applications of glucose oxidases

Glucose oxidases have gained considerable commercial importance in the last few years due to their multitudinous applications in the chemical, pharmaceutical and food industry, health care, biotechnology etc. Glucose oxidases are the most widely used enzymes as analytical reagents for the determination of glucose due to their relatively low cost and good stability [5].

Glucose biosensors for diabetics

Glucose oxidases are one of the possible enzymes that can be used in biosensors to measure blood glucose levels. Biosensors work by keeping a track of electrons and their resultant charge when they pass through enzymes and get connected to an electrode. Some biosensors work in monitoring the changes of fluorescence in the active site (FAD) of glucose oxidases [5].

Biofuel cells

Bioelectronic devices require a small power source to sustain operations. During the biocatalysing process, biocells convert the biochemical energy into electrical energy. One type of biofuel cells uses enzymes as biocatalysts. These biofuel cells consist of a two-electrode set modified with biocatalysed enzymes that specifically oxidise or reduce substrates. For example, enzymes glucose oxidases or glucose dehydrogenases can catalyse glucose oxidation on the anode, while enzymes lacases or bilirubin oxidases catalyse oxygen reduction on the cathode [5].

Food and beverage additive

Glucose oxidases successfully remove the residual glucose and oxygen in order to preserve the

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colour, taste and purity of tinned products and gassed beverages. The generated hydrogen peroxide is an excellent antibacterial source that can be converted to water and oxygen with the addition of enzyme catalases. For example, a combination of glucose oxidases and catalases is used to prevent the dehydration of egg powder during the manufacture. The addition of glucose oxidases into wheat dough improves bread quality [5].

Low-alcohol wine

Glucose oxidases reduce the potential alcohol content of wine by about 2% through a partial conversion of glucose into D-glucono-δ-lactone. During the fermentation process, enzymes work antibacterially against the acetic and lactic acid bacteria. Consequently, fewer preservatives need to be added [5].

Oral hygiene

Glucose oxidases and lactoperoxidases can also be used as antimicrobial agents in the oral-care products. The bacteria species Streptococci, which is housed in the oral cavity and causes tooth decay, can be successfully exterminated through the enzymatically generated hydrogen peroxide [5].

Gluconic acid

The enzymes glucose oxidases are an important source of the gluconic acid production, which is the end product of D-glucono-δ-lactone hydrolysis. Gluconic acid is noncorrosive, non-volatile, nontoxic, mild organic acid resistant to oxidation and reduction at high temperatures. As a chelating agent in an alkaline pH, it chelates heavy metal ions (Ca^{2+}, Fe^{2+}, Al^{3+} etc), its action is better than that of EDTA and other chelators. There are different approaches available for the production of gluconic acid, namely a chemical, electrochemical, biochemical and bioelectrochemical approach. Due to the low production costs and higher efficiency, gluconic acid is still commercially obtained through the fermentation process from the Aspergillus niger microorganisms and glucose as the main carbon hydrate source. The glucose concentration in the media is 10–15% either in the form of glucose monohydrate crystals or fructose or dextrose syr-

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up with a 98% yield of gluconic acid. Since the commercial prices of the obtained gluconic acid and its derivatives are very high, cheaper sources of carbon hydrates are intensively searched for. Gluconic acid is produced for the use in food (34.5%), pharmaceutical (9.2%) and construction (46%) industry. As a food additive, it regulates the food product acidity, sterilizes and bleaches food products, and as salt, it is used in medicine products. In the construction industry, it is used as a concrete reinforcement for a higher resistance towards extreme weather conditions. It is also used as mild acid in the metal and leather industry. In nature, it can be found in honey, fruit and wine [5, 11, 32].

6 Conclusions

Regarding the present review, the following can be concluded:

- *Glucose oxidases are very important enzymes present in many industrial processes. Today, glucose oxidases are indispensable in the food and pharmaceutical industry, and in the field of biotechnology.*
- *Glucose oxidases can be produced from various microorganisms; however, until today, only few enzymes have gained commercial importance. Glucose oxidases produced from the microorganisms Aspergillus niger are in comparison to others distinguished by their wider operating pH range, operating activity at higher temperatures and higher operating activity.*
- *The chemical modification of recent enzymes and genetic engineering enables the production of more efficient glucose oxidases.*
- *A research in the immobilization processes of glucose oxidases showed their good stability and ability of repeated applications.*
- *Glucose oxidases successfully generate hydrogen peroxide required for bleaching cellulose fabrics. Therefore, bleaching with glucose oxidases, compared to the classical process with added hydrogen peroxide, represents an economic and ecological potential. Further investigations focus on attaining the whiteness index comparable to the whiteness index of conventionally bleached fabrics.*

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