PRODUCTION AND APPLICATION OF GLUCOSE OXIDASE ENZYME IN TEXTILE TECHNOLOGY

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Abstract: Biotechnology is an ecological advantageous and moreover economically beneficial technology. The most established application of biotechnology in textiles has been in the field of enzymatic pretreatment. Glucose oxidase (GOD) 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 (HP). Glucose oxidases are commercially gaining a lot of attention in textile technology. In an enzymatic pretreatment, the textile substrate is less damaged when compared to a classical pretreatment. Enzymatic pre-treatments of cellulose fabrics often save large amounts of raw materials, chemicals, energy and water. 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 of glucose oxidases and their applications in textile technology.

Keywords: enzymes, eco-friendly characteristics, glucose oxidase, application, fermentation.

1. INTRODUCTION

Biotechnology is an ecologically advantageous and moreover economically beneficial technology. The most established application of biotechnology in textiles has been in the field of enzymatic pre-treatment [1, 2, 3, 4, 5]. Enzymes are biocatalysts with selective and specific activity, accelerating distinct reactions and remaining unchanged after the reaction. All enzymes are made of protein and because they are sensitive to heat, pH and heavy metal ions. Today enzymes are produced by biotechnological processes in great amounts of constant quality, and are therefore applicable to large-scale processes. Cur-
rently, enzymes are becoming increasingly important in sustainable technology and green chemistry. This has led to the tremendous interest among textile research community to explore exciting opportunities in industrial biotechnology which can offer new and transformative alternatives to conventional textile processing methods.

The application of enzymes has many advantages compared to conventional, non-enzymatic processes. Enzymes can be used in catalytic concentrations at low temperatures and at pH-values near to neutral. Today enzymes have become an integral part of the textile processing. There are two well established enzyme applications in the textile industry. Firstly, in the preparatory area amylases are commonly used for desizing process and secondly, in the finishing area cellulases are used for softening, bio-stoning and reducing of pilling propensity for cotton goods.

The most important natural cellulose fibre is cotton, whose use is constantly increasing. To prepare the fibres for further treatment and use, pretreatment processes are needed. Among the pretreatments, desizing, scouring and bleaching are of special interest for cellulosic fabrics [6, 7, 8, 9].

The conventional highly alkaline preparation of cotton can be an example. The traditional pretreatment is carried out with caustic soda at high temperature, which not only wastes energy and water, causes pollution, but also damage fabrics. Bio-preparation may be a valuable and environmentally friendly alternative to harsh alkaline chemicals for preparing cotton [5, 6, 8].

Especially in textile manufacturing the use of enzymes has a long tradition. Starch is widely used as a sizing agent. Using amylase enzymes for the removal of starch sizes is one of the oldest enzyme applications. Amylases are enzymes which hydrolyse starch molecules to give diverse products, including dextrans and smaller polymers composed of glucose units. Moreover, cellulases, pectinases, hemicellulases, lipases and catalases are used in different cotton pre-treatment and finishing processes [10, 11].

During the last years, intensive research of biotechnological processes involving enzymes and microorganisms has been made in the field of textile technology. The enzymes glucose oxidases are representatives of the oxidoreductases group, marked EC 1.1.3.4. 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 field of textile technology, glucose oxidases represent a method for the generation of hydrogen peroxide required for bleaching cellulose fibres [12]. Bleaching with glucose oxidase thus represents an economic and ecological potential when compared to the classical process with added hydrogen peroxide. Very little has been reported on enzymatically friendly wet processing of cotton fabric with glucose oxidases. A few Japanese publications and patents have claimed successful processes [13]. The most common microbial sources for the production are the Aspergillus, Penicillium and Saccharomyces species. The most common fungal sources of the enzyme GOD from the genus Aspergillus are A. niger, A. tubingensis, A. flavus, A. terreus, A. oryzae, A. carbonarius, and A. nidulans, while those from Penicillium are P. amagasakiense, P. variabile, P. chrysogenum, P. notatum, P. fumiculosum and P. ademetiaii [16].

Many other species of Penicillium have also been reported to produce GOD, such as P. pinophilum, P. canescens, P. fellutanum, P. glaucum, and Penicillium vitale. Other reported fungal species include Talaromyces flavus, Phanerochaete chrysosporium, Alternaria alternata, Pleurotus ostreatus, Pycnoporus cinnabarinus, Rhizopus stolonifer, and Flavodon flavus [16].

In the field of textile pretreatment and finishing processes these groups of enzymes are essential (catalases, laccases, peroxidases, glucose oxidases, amylases, cellulases, pectinases, proteases, lypases) [14].

Problems associated with textile industry are one of the major concerns of today’s green chemistry community. Global textile research arena is eager to find sustainable alternatives for existing environmental and economic constraints. Researchers are trying to find most suitable enzymes for various textile processing steps.

The present review represents glucose oxidase enzyme, their basic characteristics, production and application of glucose oxidase enzyme in textile technology.

2. REACTION MECHANISM OF GLUCOSE OXIDASE

Glucose oxidases (GOD) are flavoproteins with a flavin-adenine-dinucleotide (FAD) active site. The enzyme is highly specific for β-D-glucose and catalyzes the following reaction for hydrogen peroxide generation at pH 4.5–7 and temperatures of around 40°C (1):
In the presence of water, δ-D-gluconolactone forms D-gluconic acid, which serves as a sequestering agent during the bleaching process. If peroxide for textile bleaching is to be generated by glucose oxidases (GOD), slightly acidic to neutral conditions and far lower temperatures are required to avoid deactivation of the enzymes [13].

The inhibitors of glucose oxidases (GOD) include hydroxylamine, hydrazine, phenylhydrazine, sodium bisulphate, Ag+, Hg+, Cu2+ etc. Glucose oxidases produced from various microorganisms differ in their operating temperature, operating pH range and operating activity [12]. Enzymes are very sensitive to the changes in temperature. The optimum operating temperature of glucose oxidases from *Aspergillus niger* is 40-60°C and optimum pH at 4.5-7 [12].

An analytical method to determine the glucose oxidases activity is used based on the principle that glucose oxidases oxidize β-D-glucose into β-D-glucono-δ-lactone and hydrogen peroxide [12].

### 3. MICROBIAL PRODUCTION OF GLUCOSE OXIDASE

Commercial sources of enzymes are obtained from three primary sources, i.e., animal tissue, plants and microbes. These naturally occurring enzymes are quite often not readily available in sufficient quantities for food applications or industrial use. However, by isolating microbial strains that produce the desired enzyme and optimizing the conditions for growth, commercial quantities can be obtained. This technique, well known for more than 3,000 years, is called fermentation. Today, this fermentation process is carried out in a contained vessel. Once fermentation is completed, the microorganisms are destroyed, the enzymes are isolated, and further processed for commercial use [20].

There is a large number of microorganisms which produce a variety of enzymes [1]. Most of the industrial enzymes are produced by a relatively few microbial hosts like *Aspergillus* and *Trichoderma* fungi, *Streptomyces* fungi imperfecti and *Bacillus* bacteria. Yeasts are not good producers of extracellular enzymes and are rarely used for this purpose. Microorganisms that produce enzymes from textile importance are listed in Table 1.

Several methods, such as submerged fermentation (SmF), solid-state fermentation (SSF) and whole cell immobilization have been successfully used for enzyme production from various microorganisms [20].

| Microorganisms | Enzymes                           |
|----------------|-----------------------------------|
| Bacteria       |                                   |
| *Bacillus subtilis* | Amylase                        |
| *B. coagulans*   | α-amylase                        |
| *B. licheniformis* | α-amylase, protease              |
| Fungi          |                                   |
| *A. niger*      | Amylases, protease, pectinase, glucose oxidase |
| *A. oryzae*     | Amylases, lipase, protease        |
| *Candela lipolytica* | Lipase                      |
| *Penicillium purpurogenum* | Glucose oxidase             |
| *Penicillium amagasakiense* | Glucose oxidase            |
| *Penicillium chrysogenum* | Glucose oxidase          |
| *Penicillium notatum* | Glucose oxidase            |
| *Penicillium pinophilum* | Glucose oxidase            |
| *Penicillium variabile* | Glucose oxidase          |
| *Rhizopus sp.* | Lipase                           |
| *Trichoderma reesei* | Cellulase                     |
| *A. niger*      | Amylases, protease, pectinase, glucose oxidase |
| *A. oryzae*     | Amylases, lipase, protease        |
| *Candela lipolytica* | Lipase                      |

To produce glucose oxidase, the microorganisms of the *Aspergillus, Penicillium* or *Saccharomyces species* have to be grown with a fermentation process in a reactor containing a nutritive medium (various carbohydrate sources, peptone, water, inorganic salts, nitrogen compounds, calcium carbonate etc.) and under specific requirements at pH, temperature and pressure [15].

Microorganisms from the *Aspergillus niger species* successfully grow on each carbohydrate source, but higher growth values of glucose oxidases have been
obtained when using the glucose, sucrose and molasses media [15].

After the fermentation process is completed, the reactor still contains large amounts of useless nutritive sources, water, microorganisms and useable enzymes. After the completion of the fermentation process is recovery of glucose oxidase from the reactor. Glucose oxidase can be produced intracellularly, extracellularly, or in the form of mycelia. The separation of glucose oxidase from cells or mycelia can be facilitated by using mechanical forces, centrifugation and filtration [12, 15].

Productivity of extracellular glucose oxidase was examined for various microorganisms and it was found in strains belonging to genus *Penicillium*. As the best glucose oxidase producer, *Penicillium purpurogenum* No.778 was isolated from natural source. This microorganism produced glucose oxidase in a simple medium containing beet molasses, NaNO₃ and KH₂PO₄ by submerged culture for 3 days. That value was about 10-times of that of *Penicillium amagasakiense* which has been known as an excellent glucose oxidase producer. Culture conditions for glucose oxidase production were examined, which were extremely different among microbial species. In the case of *Penicillium chrysogenum* AJ7007 and *Penicillium purpurogenum* No.778, the effects of aeration and carbon sources were remarkably different from each other [16]. It has also been reported that *Penicillium notatum* and *Penicillium chrysogenum* produce glucose oxidase in surface culture but not in submerged culture [16].

Glucose oxidase was purified about 25-fold from culture supernatants of *Penicillium purpurogenum* No.778, and some properties of the enzyme were examined. The optimum temperature and pH for the activity were 35°C and 5.0, respectively. The enzyme was stable at pH 5.0 to 7.0 when it was incubated at 40°C for 2 h, while it was stable at temperature lower than 50°C when incubated at pH 5.6 for 15 min [16].

Production of GOD by fungi is commonly performed by solid-state fermentation (SSF) and submerged fermentation (SmF). SmF has been found to be more effective at producing GOD because it is easier to control the environmental factors involved in this technique when compared to SSF. The studies also explain an SSF process for the low-cost production of important enzymes with attractive properties for commercial applications. These research findings confirmed that *A. tubingensis* was able to exhibit remarkable GOD activity when culture conditions were optimum as compared to other producer strains. However, these traditional methods for the production of GOD have reached their limit. Accordingly, different strategies to overcome these limitations and enhance their production have been explored, including recombination, immobilization, mutagenesis and screening [17]. For practical applications, immobilization of microorganisms on solid materials offers several advantages, including repeated usage of enzyme, ease of product separation and improvement of enzyme stability [23].

A number of nutritional factors influencing glucose oxidase (EC 1.1.3.4) production by *Aspergillus niger* NCIM 545 were studied. Considerable amount of glucose oxidase was produced from *A. niger* species with sucrose as the carbon source, sodium nitrate as the inorganic nitrogen source, and peptone as the organic nitrogen source. Glucose oxidase activity increased remarkably by 28.93 fold (from 0.00993 to 0.29 U ml⁻¹ ) with CaCO₃-supplemented media. The outcome of Plackett–Burman design showed CaCO₃, peptone, and MgSO₄ as significant parameters. Further optimization using a threefactor central composite design with 20 experiments increased yield of glucose oxidase from 0.29 to 2.05 U ml⁻¹ (sevenfold) with a decrease in cultivation time from 96 to 72 h [18].

Finally, GOD production was investigated in a semi-continuous system for 7 days. The most frequent isolates isolated from soil samples belonged to the genera *Aspergillus*, *Penicillium* and *Trichoderma*. *Aspergillus niger* LMM01 was the best GOD producer. Glucose, peptone and KH₂PO₄ were demonstrated to be the optimal carbon, nitrogen and phosphorus sources, respectively. Multivariate experiments demonstrated that the parameters with the greatest effect on GOD production were pH and agitation. Stable expression results for GOD (7.74 U/ml) were obtained over 7 days in a semi-continuous process [19].

4. APPLICATIONS OF GLUCOSE OXIDASES

Due to constantly increasing level of pollutants governments of many countries imposing stricter limitations on release of pollutants. Therefore, there is ever increasing demand for clean processes i.e., processes which either cause no pollution or less pollution. Textile industry particularly the chemical processing sector always has a major share in the global pollution. Enzymes can often replace chemicals or processes that present safety on environmental issues. Enzymes play key role in such alternative processes [20].
The application of enzymes has many advantages compared to conventional, non-enzymatic processes. Enzymes can be used in catalytic concentrations at low temperatures and at pH-values near to neutral. Moreover, enzymes are biologically degradable and can be handled without risk. The conventional highly alkaline preparation of cotton can be an example. The traditional pretreatment is carried out with caustic soda at high temperature, which not only wastes energy and water, causes pollution, but also damage fabrics. Bio-preparation may be a valuable and environmentally friendly alternative to harsh alkaline chemicals for preparing cotton [21].

The fabric should be free from natural and added impurities before it goes colouration. Some of the chemicals like caustic soda, soda ash, hydrogen peroxide, hydrochloric acid, detergent and auxiliaries that are used at different stages preparatory process to remove such impurities are found to be harmful to the environment. Modern wet processing industries are followed the enzymes in the preparatory process instead of using harmful chemical because enzymes are more convenient, effective and environment friendly. In textile finishing processes the application of enzymes is emerging and, in some cases, conventional chemical finishing processes are replaced by enzymatic finishing [24].

Alternative eco-friendly desizing agents are available in the market in the form of enzymes. Today enzymes have become an integral part of the textile processing. There are two well-established enzyme applications in the textile industry. Firstly, in the preparatory finishing area amylases are commonly used for desizing process and secondly, in the finishing area cellulases are used for softening, bio-stoning and reducing of pilling propensity for cotton goods. Enzyme desizing is the most widely practiced method of desizing starch. In the textile industry amylases are used to remove starch-based size for improved and uniform wet processing.

The application of enzymes has many advantages compared to conventional, non-enzymatic processes. Moreover, enzymes are biologically degradable and can be handled without risk [22]. Especially in textile manufacturing the use of enzymes has a long tradition.

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 [12].

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, textile industry etc. Glucose oxidases which catalyse the generation of hydrogen peroxide by 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 [12]. After a successful desizing with amylloglucosidases, bleaching of cotton with enzymatically generated hydrogen peroxide took place.

Glucose oxidase enzyme was used for bleaching when the whiteness index was improved with lower strength loss [25]. In the presence of molecular oxygen, glucose is oxidized by the enzyme glucose oxidase to gluconic acid and hydrogen peroxide. D-Gluconic acid acts as a sequestering agent during bleaching. Amyloglucosidases, pectinases and glucose oxidases are compatible concerning their active pH and temperature range, and were selected.

A combination of two or all three preparation steps with minimal amounts of treatment baths and rinse water showed compatible results in whiteness, absorbency, dyeability and tensile properties of the treated fabrics. Studies were done of biobleaching of wool under both oxidative and reductive conditions. The studies showed that hydrogen peroxide bleaching in the presence of protease preparation, Bactosol SI, considerably improved whiteness and hydrophilicity [25].

Peroxidases (POD) are used in textile decoloration and bleaching processes, but these enzymes are unfortunately inactivated rapidly at high hydrogen peroxide concentrations. A new concept has therefore been developed, which is based on a simultaneous application of glucose oxidase and peroxidase. Starting with glucose as a substrate for glucose oxidase (GOD), hydrogen peroxide was generated in situ. The freshly formed substrate H$_2$O$_2$ was immediately used by the POD oxidizing colored compounds in dyeing baths. Moreover, experiments were carried out to check if this combined system with GOD, glucose and POD could be used even in heterogeneous systems such as the textile bleaching of natural cotton fibers. Starting from 55, a significant higher degree of whiteness (according to Berger) up to 66 could be obtained [26].
4. CONCLUSIONS

Pollution free processes are gaining ground all over the world. In this scenario, enzymes emerging as the best alternative to the polluting textile processing methods. Enzymes are not only beneficial from ecological point of view but they are also saving lot of money by reducing water and energy consumption which ultimately reduce the cost of production. It seems that in the future it will be possible to do every process using enzymes. Biotechnology offers a wide range of alternative environmentally-friendly processes for the textile industry to complement or improve the conventional technologies. The use of various enzyme is in the early stages of development but their innovative applications are increasing and spreading rapidly into all areas of textile processing. The textile industry was identified as a key sector where opportunities available from adapting biotechnology are high but current awareness of biotechnology is low. In textile processing the enzyme can be successfully used for preparatory process like desizing, scouring and bleaching. These enzymatic processes give the similar results as that of conventional methods. Though these enzymatic processes we can reduce the water consumption, power energy, pollution, time, and increasing quality. These are just a few applications of Biotechnology; however, many such potentials are yet to be explored. The textile industry can greatly benefit from the expanded use of these enzymes as highly specific and efficient, non-toxic, environmentally friendly compounds, work under mild conditions (pH, temperature) with low water consumption that results in reduced the use of harsh chemicals in the textile industry, process times, energy and water savings and improved product quality. Advances in enzymology, molecular biology and screening techniques provide possibilities for the development of new enzyme-based processes for a more environmentally friendly approach in textile industry. It seems that in the future it will be possible to do every process using enzymes.

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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