Research Article

Fibers of cellulose sugarcane bagasse with bromelain enzyme immobilized to application in dressing

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Abstract
The enzymes have been gaining more importance in different fields, among the most important the pharmaceutical field. However, due to the protein nature of the enzymes, a significant part of them presents high instability under certain conditions of use. A great way to stabilize them is to use immobilization techniques. Bromelain from pineapple has shown potential to be used in the treatment of burn-like skin injuries and superficial injuries. Biocatalytic textile fibers from cellulose of sugarcane bagasse were prepared using surface functionalization with epichlorohydrin (4% v/v) and glutaraldehyde (0.5% v/v), and 1-ethyl- (3-dimethylaminopropyl) carbodiimide and bromelain immobilization by covalent bonding in the fibers. The best immobilization results for the bromelain enzyme immobilization were using the aminopropyltriethoxysilane and glutaraldehyde activating agent at pH 7 to values of 68.97% and 88.14% for total protein content and enzyme activity, respectively. Considering the approach described in this paper, others advanced materials from pulp fibers and bioprocesses might be developed using bromelain and other enzymes for the target applications.

Keywords Bromelain immobilization · Medical textile · Cellulose fibers · Activating agents

1 Introduction

Textile materials play an increasingly important role, and fibers, woven, non-woven, and knitwear can be used in a variety of applications in the medical field, especially with advances in nanotechnology [1]. For use in medical textiles, the materials must have specifications such as biocompatibility, sterilizable, non-toxic and exhibit excellent mechanical characteristics, especially strength and durability. These recommendations are necessary for the protection of the patient and the medical staff against infection and contamination. Others important properties for medical textiles are toughness, absorbability, and flexibility [2].

The materials used may be biodegradable, natural, synthetic, or non-biodegradable [3]. Medical textile materials can be classified as surgical textiles (implantable and non-implantable), textiles for extra-corporeal systems such as artificial organs, and hygiene and health products to bedding, uniforms and cleaning materials, among others [2].

Textile fibers for implantable application is a constant focus of studies and researches. Heart valve rings, vascular grafts, percutaneous devices, and hernia repair meshes can be found. Also, the fibers have been used in regenerative medicine [4, 5]. Use of fibers in bone tissue engineering offers clear advantages such as good porosity, lightness, and a better adjustable elastic modulus than a metallic product, which improves bone tissue regeneration [6]. About non-implantable materials, these are materials with applications outside the body, such as compresses, plasters, and gauzes.

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Wound care dressings are used in multiple applications, including protection against infection, absorption of blood and fluid exudation, scarring, and drug applications. They are usually formed by a contact layer with a wound, an absorbent layer, and the base material. A contact layer should not adhere to the wound. Collagen, alginate, and chitosan fibers are used as healing substances. The absorbent material may be from a cotton and viscose nonwoven. In the layer of contact with the skin are used knits, fabrics, and nonwoven of fibers of silk, polyamide, viscose, and polyethylene. In the base material, nonwoven fibers of cotton, viscose, and synthetic fibers are used [2].

Bandages are materials used in various applications including to hold the dressing over the wound. The bandages may be knitted, woven, and nonwoven from cotton fibers, viscose, polyamide, and elastane yarns. Elastane yarns provide comfort in the bandage structure [2, 7]. Besides, global trends for scientific and technological advancement in new materials highlight the importance of using industrial and agro-industrial waste as raw materials in production processes. Reuse and recycling of these wastes can minimize the environmental problems associated with the accumulation and reduce the use of noble raw materials [8, 9].

In Brazil, sugarcane is one of the largest monocultures, with an estimated yield of 615,839.9 million tons in 2018–2019. Most of the sugarcane produced is used to produce sugar and alcohol. Sugar production is estimated at 31,728.5 million tons, and ethanol is 32,314,145.9 million liters [10]. An amount of 1 ton of sugarcane used in the manufacture of sugar and ethanol generates, on average, 250 kg of bagasse and 200 kg of straw and tips [11].

Bagasse is a residue rich in carbohydrate, being constituted by fibers and inner pith in proportions of approximately 65% and 35% respectively [12]. Like all other lignocellulosic material, the bagasse is constituted by three main macromolecular components: cellulose, hemicellulose, and lignin [13]. In this way, the composition of sugarcane bagasse has stimulated several research groups to develop technologies aimed at their use [14–19]. Sugarcane bagasse cellulose has been studied to produce derivatives such as acetate, paper, and textile fibers [18].

Many techniques have been used for enzyme immobilization, and the technique selection depends directly on the enzyme characteristics such as solubility, among the others [20]. In 1995, Kennedy proposed a method of classification for enzymes immobilization, which seeks to combine the nature of the interactions between the enzyme and the support responsible for immobilization (Fig. 1).

Bromelain is a mixture of proteolytic enzymes derived from the stem of the pineapple plant, (*Ananas comosus*) with a molar mass of 23.8 kDa In vitro and in vivo studies with bromelain revealed the properties, anti-inflammatory, antithrombolytic and fibrinolytic. Due to bromelain efficacy, safety, and lack of side effects after its oral administration, bromelain has gained growth, acceptance, and compliance among patients as a phytotherapeutic drug [22–24].

Another wide range of therapeutic benefits has been claimed for bromelain, such as oral treatment of diseases related to inflammation and blood coagulation, inhibition of platelet aggregation, angina pectoris, bronchitis, sinusitis, surgical trauma, thrombophlebitis, pyelonephritis and increased drug absorption, particularly antibiotics, modulation of tumor growth, third-degree burns and

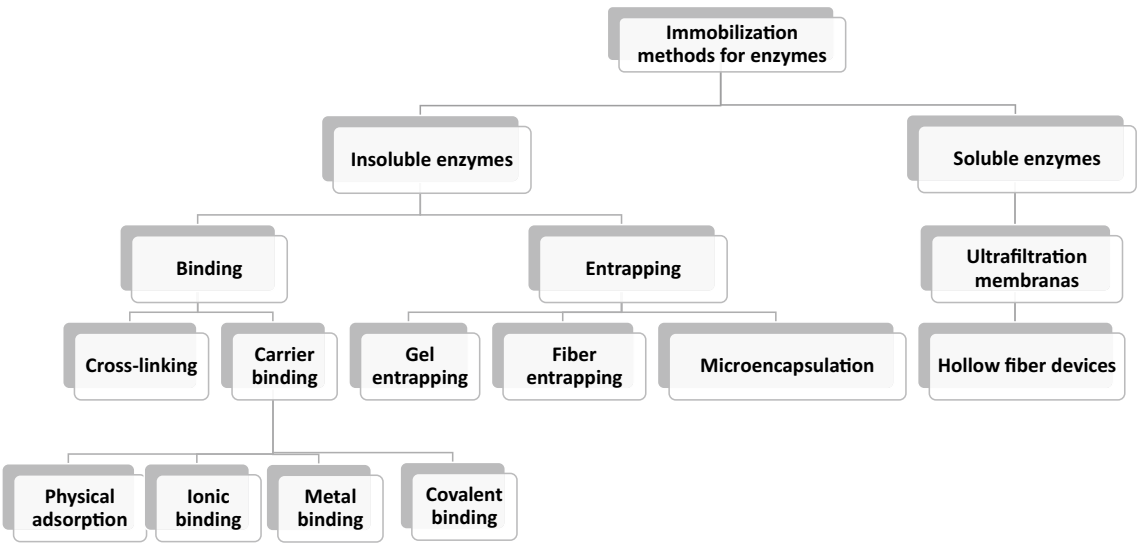

Fig. 1 Classification of enzyme immobilization methods [21]

improvement of antibiotic action [25–27]. Biochemical experiments indicate that these pharmacological properties depend only in part on proteolytic activity [27].

Anti-inflammatory effect of bromelain is also related to its proteolytic activity. Bromelain inhibits the synthesis of inflammatory mediators, prostaglandin E2, and thromboxane A2. It has also been reported to increase tissue permeability by fibrinolysis and promote fluid reabsorption of edema in the systemic circulation [28]. Anti-inflammatory activity of bromelain was studied in nanoparticles synthesized from katira gum loaded with this enzyme. The results showed that increasing the dose of bromelain from 20 to 40 mg/kg led to a significant inhibition in rat hind paw edema [29].

Bromelain has been used in several applications in the food and pharmaceutical industries. It can be used in the brewing of meat, in the clarification of beers, in the manufacture of cheeses, in the preparation of children's and dietetic foods, in the pretreatment of soybeans, in the treatment of leather, in the treatment of digestive disorders, wounds, and inflammations, preparation of hydrolyzed collagen. Bromelain can also be used to produce ointments, creams, lotions, and gels, precisely because of its proven therapeutic efficacy [25, 26, 30].

In the textile industry, bromelain at acidic pH can be used in the presence of sodium chloride for controlled surface hydrolysis of wool. Bromelain treated wool can be washed in the washing machine with minimum weight loss and tensile strength, at an industrial level [31].

The aim of this study is immobilized bromelain in fibers from sugarcane bagasse cellulose SH/H_2O_2 using three different activating agents (epichlorohydrin (4% v/v) + glutaraldehyde (0.5% v/v), 1-ethyl- (3-dimethylaminopropyl) carbodiimide (EDC) + universal buffer and γ-aminopropiltrietoxisilano (γ-APS) + (0.5%) glutaraldehyde) at pH 6 and 7 were tested.

2 Materials and methods

Sugarcane bagasse cellulose fibers (lyocell fibers) were obtained according to Costa et al. [18], bromelain from pineapple stem with 3–7 units mg^{-1} protein, of the Sigma-Aldrich, epichlorohydrin Sigma Aldrich, γ- aminopropyltriethoxysilane (γ-APS) Sigma Aldrich, 1-ethyl- (3-dimethylaminopropyl) carbodiimide (EDC) Sigma Aldrich and glutaraldehyde Sigma Aldrich. All reagents and solvents using were analytical grade.

2.1 Fiber activation

Sugarcane bagasse cellulose fibers were activating using epichlorohydrin (4% v/v) with glutaraldehyde (0.5% v/v), 1-ethyl- (3-dimethylaminopropyl) carbodiimide (EDC) and γ- aminopropyltriethoxysilane (γ-APS) with glutaraldehyde (0.5% v/v).

2.1.1 Fibers activation with epichlorohydrin (4% v/v) and glutaraldehyde (0.5% v/v)

Three samples of 0.2 g of fibers produced with bagasse cellulose (SH/H_2O_2) were weighed and treated with 25 mL of 4% (v/v) epichlorohydrin solution for 15 min at 50 °C and washed with distilled water. After, the fibers were held in a glutaraldehyde solution, 0.5% (v/v) for 2 h at room temperature. After this time, the samples were washed and dried for 2 h at 50 °C.

2.1.2 Fibers activation with 1-ethyl- (3-dimethylaminopropyl) carbodiimide (*EDC*)

Samples of 0.2 g of the fibers produced with bagasse cellulose (SH/H_2O_2) were placed in contact with a 0.75% (w/v) solution of 1-ethyl- (3-3-dimethylaminopropyl) carbodiimide (EDC) prepared in universal buffer solution at pH 7, for 60 min. After this period, the samples were vacuum filtered.

2.1.3 Fibers activation using *γ-aminopropyltriethoxysilane (γ-APS)* and glutaraldehyde (0.5% v/v)

Samples of 0.2 g of the fibers produced with bagasse cellulose (SH/H_2O_2) were placed in contact with a 1% (v/v) γ-aminopropyltriethoxysilane (γ-APS) solution prepared in universal buffer solution at pH 4, 6, 7 and 9 for a period of 24 h. Successive the samples were vacuum filtered and placed in a 0.5% (v/v) solution of glutaraldehyde for 1 h. The glutaraldehyde solution was removed.

2.2 Bromelain immobilization

Solutions of bromelain (0.025 g in 25 mL phosphate buffer) at pH 6 and 7 were prepared, and 20 mL of each enzymatic solution was added to the treated fiber samples to contact time of 3 h [34, 35]. After this time, the enzyme solutions were removed, and the volume measured to check for loss of volume by absorption. The samples of the enzyme solutions 0 h, 3 h, 1st, 2nd, and 3rd wash, were used to measure activity. Determination of the proteolytic activity was carried out according to modifications of the methodologies proposed by Kunitz and Walter [32, 33].

Soluble protein concentration was determined by the colorimetric method of Bradford [36] which is based on the color change of the acidic solution of Coomassie Brilliant Blue G 250, when binding to a protein the maximum

absorbance changes from 465 to 595 nm, and calculations were performed by Eq. 1 [5]:

$$RP = \left(\frac{P_{ads}}{P_0}\right) \times 100 \quad (1)$$

RP = Protein yield (%); P0 = Protein available for immobilization (mg), and Pads = ($P_0 - P_s - P_f$) the amount of protein adsorbed to the support was determined as the difference between P_0 the proteins and remaining in the supernatant P_s in the filtered and P_f at the end of the immobilization process (mg).The yield of the immobilization process was calculated by Eq. 2:

$$RI = \left(\frac{U_{act}}{U_{ads}}\right) \quad (2)$$

$$U_{ads} = U_{act} - U_s - U_f \quad (3)$$

In which RI = Immobilization yield (%), Uact = enzyme activity units provided for immobilization; Uads = overall units of enzyme absorbed by the support is given by the difference between the given units for the immobilization (Uact), waste units present in the supernatant (Us) and filtered at the end of the immobilization process (Uf).

2.3 pH stability of bromelain immobilized in cellulose fibers

25 mg of fibers content bromelain and 25 mL of universal buffer solutions at pH 2–12 were placed in Falcon tubes and the samples incubated to 37 °C for 60 min, and the proteolytic activity was measurement [32, 33].

Fig. 2 Fibers from cellulose of sugarcane bagasse. *Source*: author

Table 1 Results of immobilization of bromelain in lyocell fibers produced with cellulose of sugarcane bagasse SH/H_2O_2 using different activating agents

IM[a]	pH	Protein yield (%)	Enzymatic activity yield (%)
1a	6	44.8	50.5
1b	7	52.3	55.6
2a	6	49.6	49.04
2b	7	80.0	77.0
3a	6	43.43	41.11
3b	7	68.97	88.14

[a]Immobilization methods (IM): 1- Treatment with (4%) epichlorohydrin + (0.5%) glutaraldehyde: (a) buffer solution pH 6 and (b) buffer solution pH 7. 2- Treatment with EDC + universal buffer: (a) buffer solution pH 6 and (b) buffer solution pH 7. 3- Treatment with (1%) γ-aminopropyltriethoxysilane (γ-APS) + (0.5%) glutaraldehyde (a) buffer solution pH 6 and (b) buffer solution pH 7

3 Results and discussion

By the immobilization process, there are several factors such as pH, temperature, ionic strength of the medium, enzyme concentration, type of support and even the reagents used during the immobilization process can influence the yields obtained [34].

The immobilization studies were performed using the lyocell fibers produced in the laboratory from the cellulose of the sugarcane bagasse SH/H_2O_2 and epichlorohydrin (4% v/v) with glutaraldehyde (0.5% v/v), 1-ethyl-(3-dimethylaminopropyl) carbodiimide (EDC) and γ-aminopropyltriethoxysilane (γ-APS) with glutaraldehyde (0.5% v/v) as the activating agents.

Based on Fig. 2, no visual differences in the fibers activated using different activating agents were observed.

The results of the immobilization of bromelain in the lyocell fibers in terms of protein and enzymatic activity yield are shown in Table 1. It was observed that for the three different activating agents, the best results were obtained using pH 7 for both protein and enzymatic activity yield, similar results were observed in other studies [37]. Although, the highest results of enzyme activity recovered were obtained using with γ-aminopropyltriethoxysilane (γ-APS) + (0.5%) glutaraldehyde and pH 7 (88.14%) and to protein yield (80%) to EDC + universal buffer and pH 7. To immobilization using the fiber activation treatment with (4%) epichlorohydrin + (0.5%), glutaraldehyde no stronger influence of pH was observed. Increase of 10% to enzymatic activity and 21% to protein yield. Similar activities recovery to pH 7–8 were obtained by other authors using many different supports such as chitosan [37–39], electrospun regenerated cellulose ultrafine fibers [40].

The strongest pH influence was observed in the treatment with (1%) γ-aminopropyltriethoxysilane (γ-APS) + (0.5%) glutaraldehyde, obtaining an increase

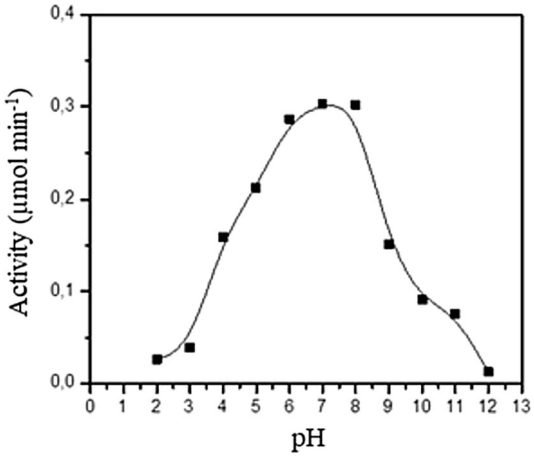

Fig. 3 Stability profile to immobilized bromelain to pH 2–12 at 37 °C and 60 min

of 114% in the enzymatic activity and 58% in the protein yield.

Comparing the difference between protein yield and enzymatic activity yield, (4%) epichlorohydrin + (0.5%) glutaraldehyde treatment, values of 12.7 and 6.3% to pH 6 and pH 7 respectively, were obtained, these values showing that not all immobilized proteins remained active. Often, until the immobilization processes, the active site of the enzymes can be blocked, generating these differences. To bromelain immobilization process using EDC and (1%) γ-APS + (0.5%) glutaraldehyde, the differences indicated that the total immobilized protein in the fiber has catalytic activity and efficient recovery of enzymatic activity of free bromelain was obtained.

The effects of pH on the stability of soluble and immobilized enzymes are shown in Fig. 3. Proteolytic activity of bromelain to pH values between 2 and 12, at 37 °C and 60 min was tested.

A peak of activity at pH 7 (0.3031 µmol mL^{-1} min^{-1}) was observed and the bromelain immobilized enzyme was highly stable at pH range 6-8, in this pH range the enzyme may be in charged or neutral form. The equilibrium of the loads is an essential factor for the bond of the catalyst to the support to occur [41]. The enzyme remained active during the immobilization process, and the ideal pH value for the immobilization process was 7.0, which also shows that it is within the stability of the enzyme.

4 Conclusion

Use the sugarcane bagasse fibers as a biomaterial for enzymes immobilization has been evaluated in this work and immobilization yield and protein retention were evaluated as parameters to determinate the ability of sugarcane bagasse fibers to immobilize bromelain to be implemented in medical applications. The best results of immobilization of the bromelain enzyme in the fibers were obtained with the activating agent aminopropyltriethoxysilane and glutaraldehyde at pH 7 with values in terms of total proteins of 68.97% and activity of 88.14%. The efficiency of immobilization depends on many parameters as the properties of the enzymes, supports, immobilization method, among the others. However, previous and present studies of immobilization indicated the suitability of use different activating agents for bromelain immobilization in sugarcane bagasse fibers.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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