CHEMISTRY JOURNAL OF MOLDOVA. General, Industrial and Ecological Chemistry. 2020, 15(2), 54-61
ISSN (p) 1857-1727 ISSN (e) 2345-1688
http://cjm.asm.md
http://dx.doi.org/10.19261/cjm.2020.775

DECOLOURISATION OF BEET SUGAR SYRUP USING ACTIVATED CARBON AND GLUCOSE OXIDASE ENZYME

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Abstract. The aim of this study was to develop and optimize a new approach for decolourisation of beet sugar syrup. In the proposed method, activated carbon (AC) was utilized to adsorb the organic colorants from the syrup. Meanwhile, the *in-situ* generation of hydrogen peroxide by the glucose oxidase (GOx) enzyme facilitated the decomposition of heavy-weight colorants to smaller molecules. Combining of the physical adsorption with the enzymatic reaction was allowed improving the decolourisation of beet sugar syrup from 35.29 to 83.68% compared to the basic adsorption by AC after 120 min of operation under the optimum conditions. The maximum decolourisation efficiency by the combined process was achieved at GOx dosage of 0.07 g, 20 mM glucose concentration, and solution pH 7, at the temperature of 30°C using 0.01 g of AC particles. Given the high effectiveness, reusability, and the eco-friendly nature of the process, the AC/GOx system can serve as an alternative to ordinary decolourisation techniques.

Keywords: beet sugar syrup, decolourisation, activated carbon, hydrogen peroxide, glucose oxidase.

Received: 12 November 2020/ Revised final: 19 December 2020/ Accepted: 22 December 2020

Introduction

Development of new methodologies and technologies seems necessary in order to manufacture high-quality sugar, yet in a cost-effective manner. Generally, the production line of high-quality sugar consists of several steps; firstly, the raw syrup undergoes liming, carbonation, and then heat treatment to eliminate some forms of impurities. The elevated temperature and pH during the initial treatments facilitate the Maillard and alkaline degradation reactions, leading to the formation of heavy-weight colourants [1]. Formed as a product of non-enzymatic browning reactions between reduced sugars and amino acids (Maillard reaction), melanoidins are polymeric compounds that affect the colour of the final product, reducing its quality [2]. The presence of organic colourants in the sugar syrups, such as melanoidins, alkaline degradation products, caramels, pectins, and melanins, requires a decolourisation process to be performed to attain the high-quality and healthy white sugar as the final product [3,4].

In this regard, researchers have been striving to improve syrup decolourisation efficiency through either chemical or physical techniques. Chemical decolourisation processes are mostly based on the oxidative potential of some chemical species, such as sulphur dioxide, hydrogen peroxide, ozone, and chlorinated compounds, which may pose health issues due to the residues of toxic compounds [5,6]. Moreover, these processes may require additional post-treatment steps to proceed the syrup to the next processing stages whilst process variables such as pH and temperature should be carefully maintained. On the other hand, physical decolourisation processes are based on techniques such as membrane filtration, ion exchange resins, adsorption and air flotation [7-12]. However, due to the diverse characteristics (chemical and physical properties such as

molecular structure, molecular weight, ionic charge and hydrophobicity) of the above-mentioned organic colourants, as well as limited selectivity or health problems associated with some of the above-mentioned methods, a combination of these techniques can be a more effective option [1,13]. Taking advantage of two or more decolourisation methods, combined methods can remove/degrade a greater number of organic colourants, leading to the obtaining of a high quality and safe product. Recently, Nguyen, D. *et al.* combined Fenton oxidation with chemical coagulation for decolourisation of synthetic and factory sugar cane juices [13]. The study revealed that the combination of the Fenton process with the chemical coagulation enhanced the decolourisation performance over the sole Fenton and coagulation processes. They have reported a maximum decolourisation of 42% after 2 min at the pH 9 using Fe^{2+} ions as the Fenton reagent, hydrogen peroxide and $AlCl_3$ as the coagulating agent [13].

Mesoporous activated carbon (AC) is widely utilized in many industrial processes such as separation, purification, catalysis and electrolysis [14-18]. In recent years, it has been employed as an efficient adsorbent material in the process of sugar syrup decolourisation given its high internal surface area and surface functionalized groups [19]. Mudoga, H.L. *et al.* investigated commercial and sugar beet pulp-based AC for decolourisation of industrial sugar syrup and reported optimum decolourisation performance of up to 80%, using 0.2–1 g of commercial carbon per 100 g of sugar syrup [3]. The decolourisation performance of AC can be enhanced by combining the physical adsorption process with chemical oxidation. Hydrogen peroxide is a biodegradable oxidant that produces active oxygen radicals as a result of its decomposition. These active oxygen species such as hydroxyl radical, can degrade a vast array of organic compounds to minerals. Hydrogen peroxide decomposition can proceed in the presence of AC in a similar pathway to the Fenton process, according to Eqs.(1,2) [20].

$$AC + H_2O_2 \rightarrow AC^+ + OH^- + OH^\bullet \quad (1)$$

$$AC^+ + H_2O_2 \rightarrow AC + H^+ + HO_2^\bullet \quad (2)$$

Due to the safety risks associated with the storage and transportation of hydrogen peroxide, *in-situ* generation of this oxidant can improve the safety of the process [21]. Glucose oxidase (GOx) enzyme (EC 1.1.3.4) can be employed for *in-situ* production of hydrogen peroxide in the presence of glucose as its substrate in an eco-friendly manner, as presented in Eq.(3) [22].

$$C_6H_{12}O_6 + H_2O + O_2 \rightarrow C_6H_{12}O_7 + H_2O_2 \quad (3)$$

The aim of this study was to present a novel combined method for the decolourisation of the beet sugar syrup. In this regard, a combination of physical adsorption (using AC) and chemical oxidation (*in-situ* generated hydrogen peroxide by GOx enzyme) was utilized for simultaneous adsorption and degradation of colour precursors produced during the initial treatment stages of the raw syrup. In this process, AC acts as an adsorbent and simultaneously helps decompose hydrogen peroxide generated by the enzymatic reaction of GOx and glucose to produce high-potential hydroxyl radicals to degrade a vast array of organic colourants. In addition, the effects of various parameters such as pH, temperature, enzyme and substrate concentration on the decolourisation performance were investigated and the optimum point of operation was obtained.

Experimental

Materials

Beet sugar syrup (66 °Brix) was collected from a local sugar mill and stored in a refrigerator at the temperature of 4°C during the experiments. Industrial grade GOx enzyme was supplied by Kimia Enzyme Co., Iran. Glucose and granulated AC (Norit GAC 1240W, steam activated, average diameter of 1 mm) were purchased from Sigma Aldrich Co., The United States. All chemicals were of analytical grade and obtained from Merck Co., Germany.

Experimental procedure of beet sugar syrup decolourisation

Initially, the pH of the obtained beet sugar syrup was adjusted to 7 using the 0.1 mM NaOH solution. Afterward, the sample was heated to reach 80°C in a water bath for 10 min to simulate the pre-treatment stage of the raw syrup and form the organic colourants [3]. The obtained solution was cooled down to reach 20°C. In the combined AC/GOx process, the GOx enzyme solution, glucose and AC were added to 100 mL of the processed syrup at the determined concentrations and placed in a shaker incubator with a rotation rate of 170 rpm for 2 h. For comparison purposes, the process was also carried out using AC only for colourant adsorption. Additionally, the effectiveness of GOx-immobilized on AC particles on sugar solution decolourisation was studied. During the reaction, the samples were

collected from the reaction medium and filtered through Whatman 110 filter paper. The second filtration was carried out using a ceramic filter to remove fine carbon particles.

Syrup spectrophotometric determination

The syrup concentration was determined by measuring its absorbance using a spectrophotometer (UV- 2100PC UV/Vis spectrometer Shimadzu, Kyoto, Japan) at the wavelength of 420 nm in accordance with the international commission for uniform methods of sugar analysis (ICUMSA) method, which recommends this specific wavelength for determining the sugar colour content [23]. The decolourisation efficiency was calculated using Eq.(4) [22].

$$Decolourisation(\%) = 100 \times (1 - \frac{A_t}{A_0}) \quad (4)$$

where, A_0- initial absorbance of the beet sugar syrup;

A_t- absorbance of syrup at the given reaction time (min).

Immobilization of GOx on AC particles

In order to immobilize GOx on granulated AC, a quantity of 0.05 g of AC was dispersed in 12.5 mL of GOx solution in a phosphate buffer with the pH 6.15 and placed in a shaker incubator for 150 min at the temperature of 25°C. Afterwards, the obtained suspension was separated through centrifugation [24].

Results and discussion

Enzymatic syrup decolourisation process

The efficiency of the proposed AC/GOx method for decolourisation of beet sugar syrup was evaluated in comparison to the decolourisation performance attained by AC individually. The optimal conditions determined from preliminary experiments were: 0.7 g GOx dosage and 20 mM glucose, pH 7 and temperature of 30°C. The process was monitored and the obtained results were presented in Figure 1. The AC particles managed to decolourise the beet sugar syrup by 35.29% after 120 min through adsorption process. On the other hand, the combination of AC with the enzymatic reaction improved decolourisation of the syrup from 10.13 to 68.27% after 10 min of the operation, proving the effectiveness of the chemical oxidation reaction in degrading the organic compounds present in the solution. After 120 min of operation, the colour removal effectiveness was found to be 83.68% using AC/GOx combined process. The increase in the decolourisation of the beet sugar syrup by addition of GOx and glucose can be assigned to the degradation of organic colourants by active oxygen species produced by the enzymatic reaction. On the other hand, the adsorption of the colourants on AC was limited and the decolourisation results were comparatively lower.

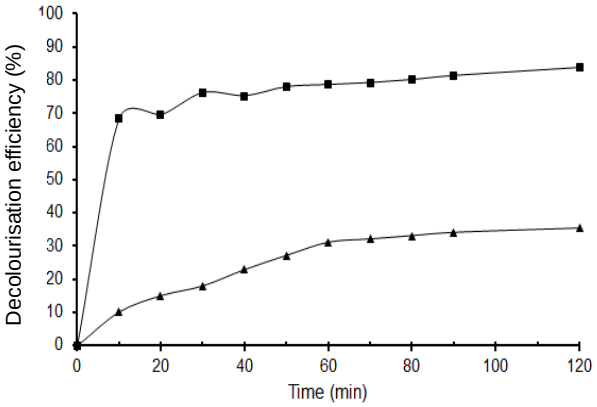

Figure 1. Comparison of beet sugar syrup decolourisation efficiency by AC (▲) adsorption and the proposed AC/GOx (■) process.

Effect of glucose concentration

In this study, H_2O_2 was produced using a redox reaction between glucose and oxygen, catalysed by GOx. The enzymatic activity of GOx depends on many parameters, such as substrate concentration, temperature, and the pH of the medium [25,26]. To study the effect of glucose concentration, beet syrup decolourisation experiments were conducted at several concentrations of glucose (15, 20, 25, and 30 mM), where the temperature was adjusted to 30°C and the AC dosage was 0.1 g. Figure 2 demonstrates the decolourisation efficiency of the syrup during 120 min of operation.

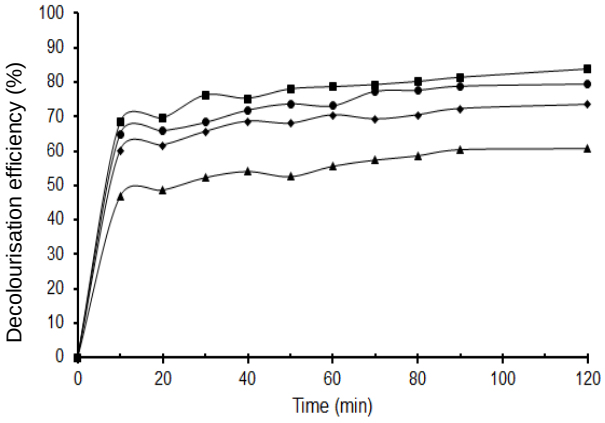

Figure 2. Decolourisation efficiency of beet sugar syrup at various concentrations of glucose (15 mM (▲); 20 mM (■), 25 mM (●), 30 mM (♦)) 0.07 g GOx dosage, pH 7, and T= 30°C.

According to Figure 2, increasing the glucose concentration from 15 mM to 20 mM enhanced the decolourisation of sugar syrup, but any further increment of the glucose concentration reduced the decolourisation rate. As an enzymatic reaction, the *in-situ* production of hydrogen peroxide using GOx enzyme follows the Michaelis–Menten model [22]. According to this kinetics model, the enzymatic reaction rate is limited to the maximum rate (r_{max}) and any further increase in the substrate concentration above the optimum value is ineffective towards the reaction rate. The maximum sugar decolourisation was achieved at the glucose concentration of 20 mM, which is in agreement with the optimum glucose concentration reported for this enzyme by Elhami, V. *et al.* [26].

Effect of GOx dosage

In order to investigate the effect of enzyme dosage on the decolourisation process, the experiments were conducted at 20 mM glucose, 0.01 g of granulated activated carbon, and various dosages of GOx (0.05, 0.07, 0.1, 0.3 g). As shown in Figure 3, the decolourisation rate was elevated by increasing the GOx dosage from 0.05 to 0.07 g.

However, in the presence of higher concentrations of GOx, the decolourisation rate of the beet syrup was diminished. The *in-situ* production rate of hydrogen peroxide is directly proportional to the GOx dosage. By increasing the GOx concentration, the hydrogen peroxide generation was enhanced, thus resulting in the enhancement of the decolourisation efficiency by increasing the number of available active oxygen species. However, the overwhelming presence of hydrogen peroxide is detrimental to hydroxyl radicals, as shown in the reactions Eqs.(5,6) [27].

$$H_2O_2 + OH^{\cdot} \rightarrow H_2O + OH_2^{\cdot} \quad (5)$$

$$HO_2^{\cdot} + OH^{\cdot} \rightarrow H_2O + O_2 \quad (6)$$

At the higher concentrations, hydrogen peroxide would act as a scavenger of hydroxyl radicals, inhibiting the oxidative performance required to degrade organic compounds [28].

Effect of temperature

The effects of operating temperature on decolourisation efficiency of beet sugar syrup using AC (0.01 g) and GOx were investigated by performing the experiments at different temperature values of 15, 25, 30, and 35°C. During these experiments, the initial levels of GOx and glucose were set at 0.07 g and 20 mM, respectively. The decolourisation performance of the process at various temperature values is depicted in Figure 4, showing that by increasing the temperature from 15 up to 30°C, the decolourisation rate improved, which can be attributed to the enhanced enzymatic activity at higher temperature values. Increasing the reaction temperature to 35°C promoted enzyme deactivation, which resulted in a lower production yield of hydrogen peroxide. As comparison, it should be mentioned that the reported conventional sulphitation method for syrup decolourisation should be performed at a temperature ranging from 100 to 105°C, while the carbonation process demonstrated its highest efficiency at 80°C [29].

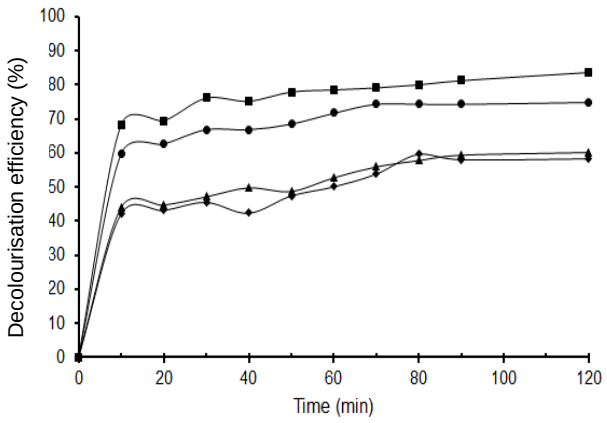

Figure 3. Effect of GOx dosage (0.05 g (▲); 0.07 g (■), 0.1 g (●), 0.3 g (♦)) on decolourisation of beet sugar syrup at 20 mM glucose, pH 7, and T= 30°C.

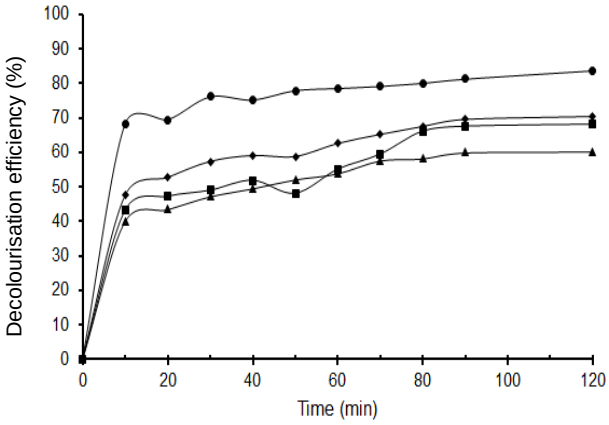

Figure 4. Effect of operating temperature (15°C (▲); 25°C (■), 30°C (●), 35°C (♦)) on decolourisation of beet sugar syrup, at 0.07 g GOx dosage, 20 mM glucose, and pH 7.

Campiol, J.L.M. *et al.* employed artificial neural networks for optimization of sugar juice using commercial hydrogen peroxide and reached the optimum effectiveness at the temperature of 50°C [6]. The proposed AC/GOx process reached the optimum beet sugar syrup decolourisation efficiency at the temperature of 30°C, increasing cost and energy efficiency of the process.

Effect of solution pH

The pH of the solution highly affects the activity of the enzyme, GOx exhibits highest enzymatic activity at pH 5.5 [30]. However, the adsorption capacity of organic substances on AC highly depends on the pH of the solution [31,32]. Additionally, the adsorption of hydrogen peroxide on AC is necessary for its decomposition and formation of hydroxyl radicals, which depend on the pH. Hence, the determination of optimum pH is necessary to attain higher decolourisation efficiency. The decolourisation experiments were carried out in the pH range from 5 to 8, the values of substrate, GOx, and AC dosages were adjusted to 20 mM, 0.07 g, and 0.01 g, respectively, and the process was carried out at the temperature of 30°C. According to Figure 5, the system pH 7 was found to be the optimum value for the decolourisation process, offering the highest combination of adsorption of colourants on AC's surface and their oxidation by active oxygen species produced by decomposition of hydrogen peroxide. The favouriting of processes involved in the AC/GOx at neutral pH excludes the neutralization process, which makes this method even more attractive compared to conventional techniques. The ordinary sulphitation and carbonation are performed at pH ranging from 3.8–4.2 and 8–9.5, respectively, necessitating further treatments in the technological scheme [29].

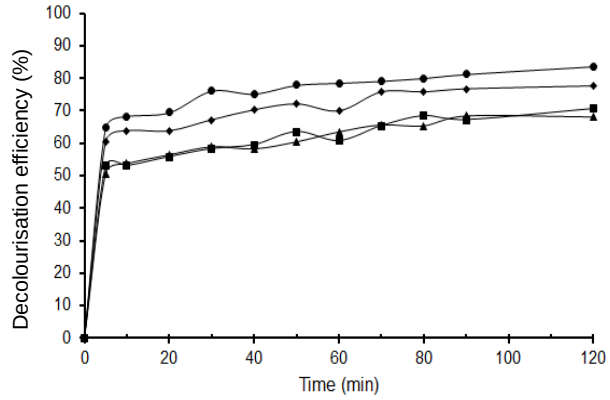

Figure 5. Decolourisation of beet sugar syrup by the combined AC/GOx process at various solution pH (pH 5 (▲); pH 6 (■),pH 7 (●), pH 8 (♦)) , 0.07 g GOx dosage, 20 mM glucose, and T= 30°C.

Study of AC reusability with free GOx enzyme

Sustainability of a chemical process is a significant parameter for its possible real-world applications. The reusability of a catalyst is also an essential factor affecting the sustainability and cost-effectiveness of the process. Catalysts tend to lose their catalytic activity due to the alteration of their physiochemical properties and physical adsorption of chemical species over their surface, compromising their overall performance. In order to assess the reusability of AC in the combined enzymatic process, decolourisation of the syrup was performed at the optimum conditions of the process. Afterward, the AC particles were extracted from the reaction medium and dried in the oven at 40°C for 30 min. The recycled AC particles were used in two additional 120 min decolourisation cycles in conjunction with fresh GOx enzyme. The decolourisation performance of the recycled AC was almost stable throughout the three successive cycles, dropping from 83.68% to 82.06% and to 80.22% after the second and third runs, respectively; showing a neglectable decline of 3.46% in the last run. The promising reusability of AC can be explained by the presence of free radicals in the medium which were able to degrade the adsorbed organic compounds by AC.

Effect of GOx immobilization

The syrup decolourisation process was investigated using immobilized GOx enzyme on AC particles. The experiments were performed under optimum conditions determined in the previous sections. The results of this experiment are illustrated in Figure 6. The maximum colour removal efficiency using immobilized enzyme decreased in comparison with free enzyme from 83.63 to 67.34%.

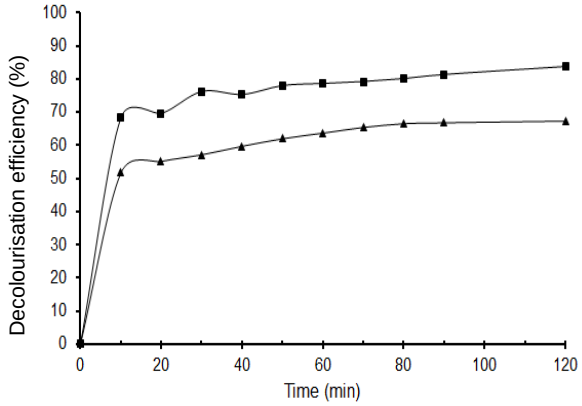

Figure 6. Decolourisation of beet sugar syrup with GOx-immobilized AC particles (■) and the combined AC/GOx (▲), 20 mM glucose, pH 7, and T= 30°C.

The decolourisation performance decrease can be attributed to a drop in GOx activity in response to immobilization process. Meanwhile, immobilization is vital for industrial applications due to the advantages associated with the implementation of immobilized enzymes, such as reusability, easier separation and higher stability. The immobilized AC/GOx catalysts produced acceptable decolourisation efficiency, which would broaden its application.

Conclusions

In this study, a novel combined method for the decolourisation of the beet sugar syrup was proposed by making use of activated carbon (AC) granules and glucose oxidase (GOx).

The simultaneous presence of GOx and adsorption of organic colourants on the surface of AC particles led to the rapid decolourisation of the beet sugar syrup. The determined optimum conditions (GOx dosage of 0.7 g, glucose concentration of 20 mM, pH 7 and temperature of 30°C), gave a decolourisation performance of 83.68% achieved after 120 min, which was significantly higher than through physical adsorption by AC.

Furthermore, syrup decolourisation performance was found to be 67.34% using GOx-immobilized AC particles, which would promote the sustainability of the process. The proposed decolourisation method achieved its maximum performance at neutral pH at the temperature of 30°C, which can be considered as an ideal condition from economical and industrial points of view in comparison to more traditional methods such as the sulphitation process performed in an acidic environment (pH 4) at a temperature of 100°C. In addition, hydrogen peroxide was produced in an eco-friendly manner through a biological process, improving the process safety.

The reusability of the AC particles during the syrup decolourisation process was examined after three successive cycles, showing a neglectable decline of 3.46% in the last run. Ultimately, the promising reusability of AC particles and the possibility of enzyme immobilization, combined with the above-mentioned advantages, made the AC/GOx combined process a notable replacement for the ordinary sugar decolourisation techniques.

Acknowledgments

The authors truly appreciate University of Tabriz and Sharif University of Technology for providing all the supports for this research.

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