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DECOMPOSITION OF HYDROGEN PEROXIDE BY CATALASE

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ABSTRACT

Catalase was the first oxidoreductase identified, characterized, and produced. It catalyses the decomposition of hydrogen peroxide into water and into oxygen in aerobic organisms. Catalase associated with superoxide dismutase eliminates free radicals (O_2^-, O_2^{-2}, and HO^-) inside the cells and fluids of living beings. Recently, it was shown that catalase may be anchored on the cytoplasmic membrane of tumor cells, playing a role in cancer development. Moreover, catalase showed significant performance in industry processes in which hydrogen peroxide is an intermediate, such as in the oxidation of glucose to gluconic acid by glucose oxidase. It was described that glucose oxidation in a continuous membrane reactor had conversion yields of 88% and 48% in the presence or absence of catalase, respectively.

KEYWORDS: Catalase, Hydrogen peroxide.

INTRODUCTION

Evidence on catalase activity was detected by Thénard in 1818 through the decomposition of hydrogen peroxide by the catalase of blood, becoming the first antioxidant enzyme to be discovered. Loew identified the catalyst in 1901, labeling it as catalase. In addition, the presence of the ferric protoporphyrin group into the active site of catalase was shown collaboratively by Warburg (1923) and Stern (1937). Finally, bovine liver catalase was purified and crystallized by Sumner and Dounce in 1937.[1]

Catalase (hydrogen peroxide oxidoreductase, EC. 1.11.1.6) is an enzyme found in all aerobic microorganisms, plants, and animals. There are several catalase isoenzymes, which are spread throughout aerobically respiring organisms. Based on their structure and function, the isoenzymes are divided into the following three groups: true catalases, catalase-peroxidases

(found in fungi, archaea, and bacteria, being sized 120–340 kDa), and manganese catalases (which do not have a heme group, being found only in bacteria, being sized 170–210 kDa). True catalases are homotetramers (200 – 340 kDa) containing four ferric protoporphyrin groups, being also be divided into the three following subfamilies: subfamily I (size between 55-69 kDa, being found in bacteria, algae, and plants); subfamily II (size between 75-84 kDa, having a flavodoxin-like domain and being found in some species of bacteria and fungi); and subfamily III (size over 100 kDa, being found in archaea, fungi, protists, plants, and animals). The human catalase belongs to this group, having a molecular weight (MW) of 248 kDa (four 62 kDa subunits) and requiring NADPH as cofactor.[1]

Catalase catalyzes preferentially the decomposition of hydrogen peroxide into oxygen and water according to reaction I (below). Under some conditions – mainly *in vivo* – it can oxidize low MW alcohols by using hydrogen peroxide generated from the action of an aerobic oxidase (glucose oxidase and xanthine oxidase, among others). This activity is called peroxidase activity (reaction II). The action of catalase associated with other enzymes is observed in a variety of processes, either in cell metabolism or in the industry.

(Catalase activity) $2\ H_2O_2 \rightarrow 2\ H_2O + O_2$ (I)

(Peroxidase activity) $RH_2 + H_2O_2 \rightarrow H_2O + R$ (II)

Where: RH_2 can be phenols, formic acid, formaldehyde, and alcohols.

In nature, reactions I and II are observed in organelles (peroxisomes and mitochondria, mainly) and erythrocytes. In mammalians, catalase is highly active in hepatic and renal tissues but low in connective tissues. Moreover, catalase in association with superoxide dismutase plays an important role in aerobic organisms by eliminating free radicals (O_2^-, O_2^{-2}, and HO^-) originated from incomplete oxidation of oxygen inside the cells. [2] Recently, it was shown that catalase may be anchored on the cytoplasmic membrane of tumor cells, playing a role in cancer development. [2]

The most accepted mechanism for catalase ($E–Fe^{+3}$) activity is:

1st step: $H_2O_2 + E–Fe^{+3} \rightarrow H_2O + O=Fe^{+4}–E$

2nd step: $H_2O_2 + O=Fe^{+4}–E \rightarrow H_2O + O_2 + E–Fe^{+3}$

The mechanism of catalase action shows that H_2O_2 acts simultaneously as oxidant (1st step: the oxidation number (NOx) of oxygen to H_2O_2 ranges from -1 to -2, whereas the iron ion in

catalase has its NOx changed from +3 to +4) and reducing agent (2nd step: NOx of oxygen to H_2O_2 ranges from -1 to 0, whereas the iron ion in the catalase intermediate has its NOx changed from +4 to +3). Depending on the H_2O_2 concentration and the duration of catalysis, catalase activity can decrease due to the formation of $E–[Fe^{+2}–O_2]$ as an inactive intermediate. [1]

Manganese catalases that do not have heme group but have two manganese ions in their active site decompose H_2O_2 as follows:

1st step: $H_2O_2 + E–[Mn^{+2}–Mn^{+2}\ (2H^+)] \rightarrow 2\ H_2O + E–[Mn^{+3}–Mn^{+3}]$

2nd step: $H_2O_2 + E–[Mn^{+3}–Mn^{+3}] \rightarrow O_2 + E–[Mn^{+2}–Mn^{+2}\ (2H^+)]$

Commercial catalase is obtained from bovine liver, filamentous fungi (*Aspergillus niger* and *Penicillium* vitale), yeast (*Saccharomyces* cerevisiae), and bacteria (*Micrococcus lysodeikticus, Micrococcus luteus,* and *Escherichia coli*).

Catalase is used in the industry, clinical analysis, therapeutics, and cosmetics.

Catalase is used in the industry for removing, namely: **a)** H_2O_2 added in crude milk by the farmer, in order to avoid microbial contamination during the transportation from the farm to the processing facility; **b)** H_2O_2 used in milk pasteurization. This procedure is valuable when milk is used in cured cheese production, since lactic acid bacteria and natural milk enzymes (lipase and proteases, for instance) are preserved; **c)** H_2O_2 used in bleaching of cotton fibers and other textiles; **d)** residual H_2O_2 in effluents generated by textile and cellulose/paper processing plants. Moreover, in the food industry, the H_2O_2-glucoseoxidase–catalase enzyme system is used in the desugarization of egg whites, yolks, or whole eggs aimed to pasta production, whereas the glucose oxidase–catalase enzyme system is used in the deoxygenation of beverages (soft drinks and wine).[3]

In clinical analysis, catalase associated with aldehyde dehydrogenase is used to determine uric acid in body fluids.[4]

In therapeutics, catalase can be used as medicine in the treatment of inflammation and oxidative stress. In addition, there is evidence that the catalase present in cancerous cells can be targeted by specific inhibitors.[1][4]

In cosmetics, catalase is used in dentifrices and mouthwashes. When associated with superoxide dismutase, it is used in preparations for skin protection against aging.[4]

This study evaluates the interference of catalase in the spectrophotometric determination of residual hydrogen peroxide at 240 nm. Moreover, the role played by catalase for improving the yield of continuous glucose/gluconic acid conversion by glucose oxidase in the membrane bioreactor was also evaluated.

MATERIAL AND METHODS

Material

Catalase from bovine liver (1mg of powder had 2,950U, where 1 U = 1 µmol of H_2O_2 consumed/min, measured at pH 4.6 and 37 ºC) and glucose oxidase from *Aspergillus niger* (1g of GO = 5,100 units. One unit (U_{GO}) oxidizes 1 µmol of β-D-glucose to gluconic acid and H_2O_2 per min at 35 ^{o}C and pH 5.1) were purchased from SIGMA®. The 100-kDa UF-membrane was purchased from Millipore. All other chemicals were of analytical grade.

Methods

Measurement of hydrogen peroxide

Hydrogen peroxide was determined by reading at 240 nm (Figure 1). The standardized equation to obtain the least square linear regression is represented by:

$y = 1.65x + 2.23x10^{-2}$ (r = 0.9990) (Eq.1)

Where: y = absorbance (λ = 240 nm) and x = H_2O_2 (mg).

The concentration of the standard solution used to obtain the standard plot ranged between 0.0608 and 0.608 mg of H_2O_2/mL (mother solution: H_2O_2 10V diluted 50-fold). The coefficient variation method was 4.7% ($\sigma = 1.365x10^{-2}$).

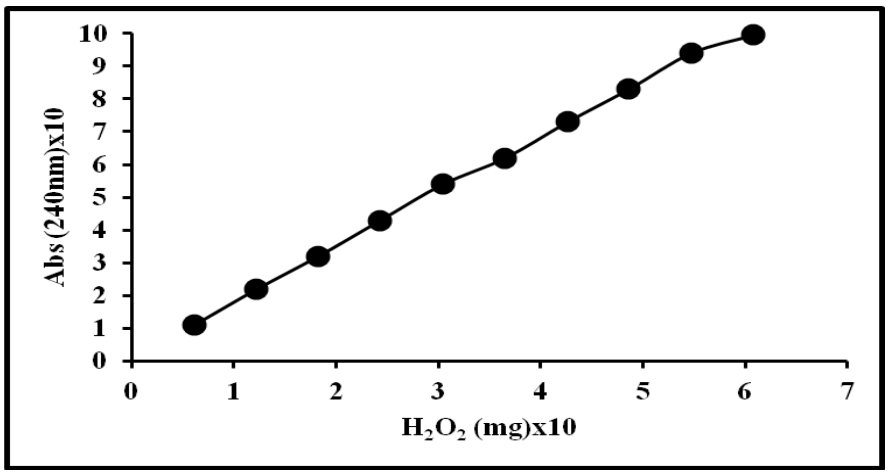

Figure 1: Standard curve for hydrogen peroxide determination.

Effect of catalase on the determination of residual hydrogen peroxide

In a 250mL-beaker, 100 mL of 0.01M acetate buffer (pH 4.5) were added and placed into a water-bath at 37 °C for 15 min. A 1 mL aliquot was taken to be used as blank. Then, 30 mL of H_2O_2 (6.12 g/L) was added into the beaker. After 5 min, a 1 mL aliquot was taken for determining the initial H_2O_2 concentration. The reaction was started by the addition of 20 mL of catalase solution (2,950 U, 5,900 U or 29,500 U). Aliquots of 1 mL were taken every 5 min and immersed in boiling water for 3 min. Then, the sample was introduced in a 1.5-mL quartz cuvette and the residual amount of H_2O_2 was determined at 240 nm. The total reaction time was 30 minutes.

Effect of catalase on the determination of residual hydrogen peroxide (Modified procedure)

In a 250-mL beaker, 100 mL of 0.01 M acetate buffer (pH 4.5) were added and placed into a water-bath at 37 ^oC for 15 min. A 1 mL aliquot was removed to be used as a blank. A volume of 6 mL was taken from a 30 mL H_2O_2 solution (6.2 g/L) and poured into the beaker. After mixing, 1 mL of the solution was taken for measuring the initial H_2O_2 concentration. Then, the reaction was started by adding 4 mL of a 20 mL buffered catalase solution (29,500U). Every 5 minutes, 6 mL and 4 mL aliquots of H_2O_2 and catalase solutions, respectively, were added into the beaker until all the volumes of both solutions have been added after 20 min from the beginning of reaction. The residual H_2O_2 concentration was measured in 1 mL aliquots taken before every addition of H_2O_2 and catalase volumes. The total reaction time was 30 minutes. Every sample was immersed in boiling water and, after cooling, the total absorption of the sample was determined at 240nm. A parallel test was made under the same conditions without H_2O_2 (blank assay).

Glucose oxidation by glucose oxidase in a continuous membrane bioreactor[5]

A 10-mL enzyme membrane bioreactor (Bioengineering® AG, Wald, Germany) previously described[5] was used in two continuous tests. **Test 1:** ten milliliters of buffered GO solution (10 UGO/mL in 0.01 M acetic acid/acetate buffer, pH 5.5) was introduced inside the bioreactor, which had a 100 kDa UF-membrane. The bioreactor was fed continuously with 5.0 mM buffered glucose solution at a feeding rate of 3.0 h^{-1}. The reaction was carried out for 24 h at 30 ^oC and 100 rpm. Pure oxygen was bubbled into the reservoir of the glucose solution, so that the dissolved oxygen (DO) concentration in the inlet solution remained at

approximately 16.0 mg/mL (DO was measured using an oximeter, DM40, Digimed®). Aliquots from the outlet solution were measured for obtaining the concentration of glucose and H_2O_2; **Test 2:** identical to Test 1, except for introducing catalase (100 U/mL) into the bioreactor. The glucose concentration was determined as described previously.[5]

The yield of the processes was calculated by

$Y = [G_{cons} \div G_0].100$ (Eq. 2)

Where: Y = yield (%), G_{cons} = glucose consumed ($G_0 - G_{outlet}$), G_0 = inlet glucose concentration (mM), and G_{outlet} = residual glucose concentration (mM).

RESULTS AND DISCUSSION

Three tests to the decomposition of hydrogen peroxide were performed by using catalase at 2,950U, 5,900U, and 29,500U (Figure 2).

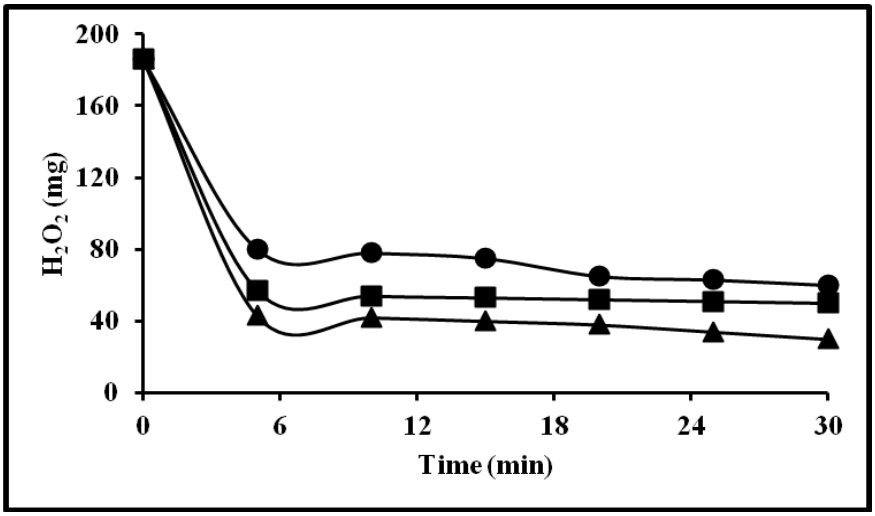

Figure 2: Decomposition of hydrogen peroxide by catalase at 2,950U (●), 5,900U (■), and 29,500U (▲).

Figure 2 show that H_2O_2 is not completely decomposed even when using high catalase activity (29,500U). This unexpected result was probably caused by the presence of the ferric protoporphyrin group into the molecular structure of the enzyme, which can absorb at λ = 240 nm. The hypothesis was checked by following the catalase absorption at 240 nm for 30 min (Figure 3).

Figure 3 show that catalase (29,500U) presents higher absorption at pH 4.5 than at pH 7.0. The latter pH is recommended for measuring catalase activity.[6] However, when catalase is used in a multienzyme system, it must act at the pH of the system. For example, when

catalase is associated with glucose oxidase to convert glucose into gluconic acid, the pH required by the system is between 4.5 and 5.1.[5]

Thereby, the procedure for determining the residual hydrogen peroxide concentration must be modified taking into account the catalase absorption, as presented in the item "effect of catalase on the determination of residual hydrogen peroxide (modified procedure)". The main modification consisted in the addition of H_2O_2 and catalase volumes – 6 mL and 4 mL, respectively– into the beaker according to a fed-batch mode. In this study, the substrate addition followed a constant addition law, although other addition laws (linear, exponential etc.) can also be used, since the blank assay is made in the same feeding mode.[7]

The residual amount of H_2O_2 (x_r) must be calculated by

$A_{HP} = A_T - A_C$ (Eq. 3)

Where: A_{HP} = absorbance of residual H_2O_2; A_T = total absorbance; and A_C = catalase absorbance.

Combining Equations 1 and 3 resulted:

$x_r = [(A_T - A_C) - (2.23x10^{-2})] \div 1.65]$ (Eq.4)

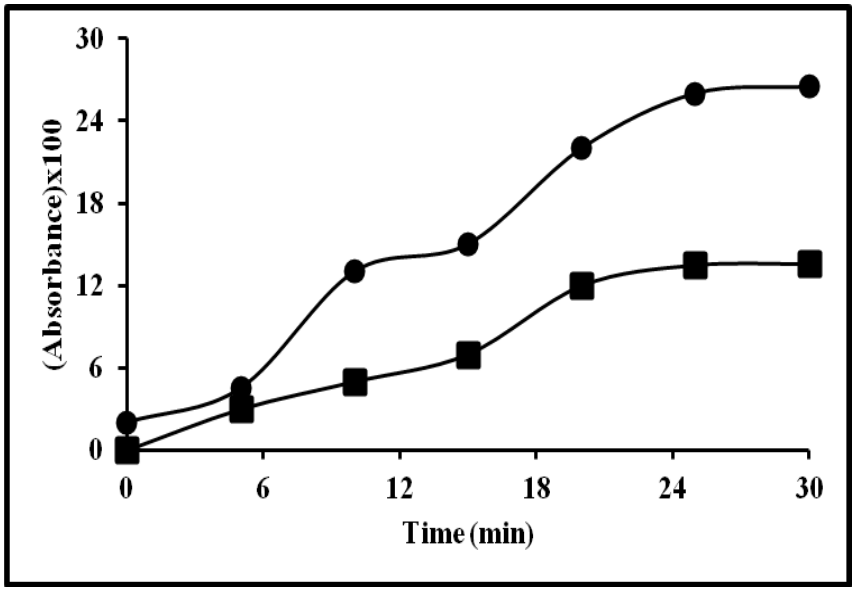

Figure 3: Catalase absorbance at 37 °C and pH 4.5 (●) and pH 7.0 (■).

Thereby, repeating the test in which H_2O_2 was decomposed by catalase (29,500U) (Figure 2), but subtracting the absorption due to catalase, we obtain a complete decomposition of H_2O_2 after 20 min of reaction (Figure 4).

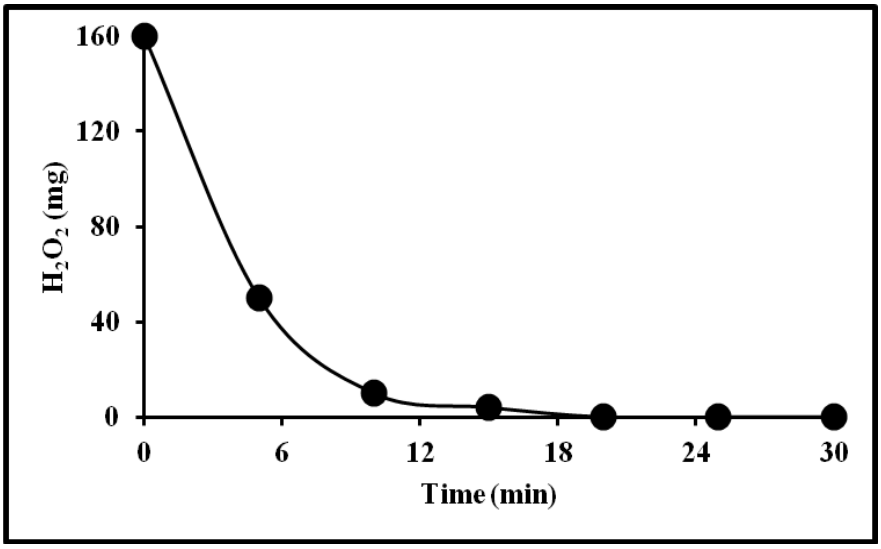

Figure 4: Decomposition of hydrogen peroxide by catalase (29,500U) at pH 4.5 and 37 ^{o}C after subtracting the absorption due to catalase.

To enhance the role of catalase in the multienzyme system, we carried out two tests using a continuous membrane reactor with and without addition of catalase (Figure 5). The tests were focused on the glucose/gluconic acid conversion by glucose oxidase.[5]

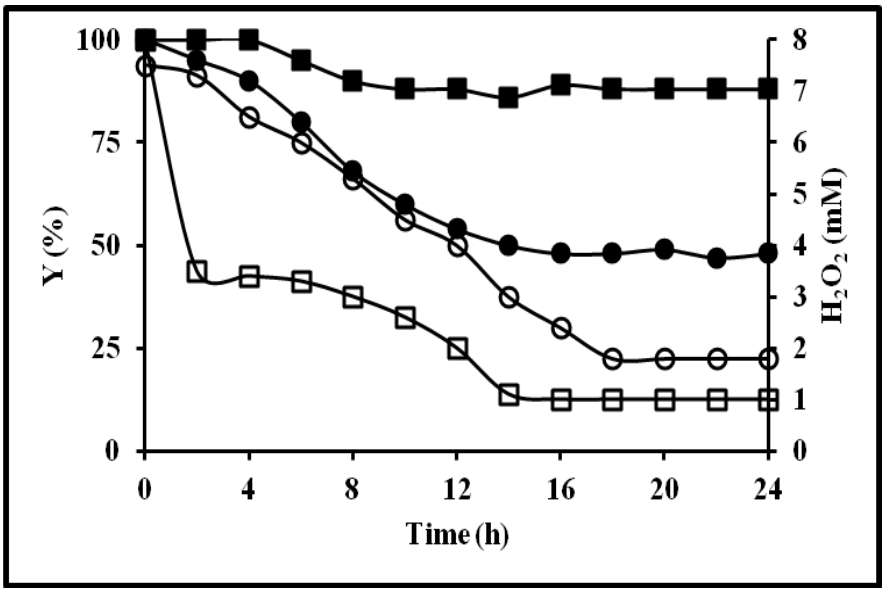

Figure 5: Variation of yield (black filled symbols) and hydrogen peroxide concentration (unfilled symbols) as a function of time for continuous glucose/gluconic acid conversion by glucose oxidase in presence (■;□) or absence (●;o) of catalase.

In Figure 5, we observe that after 24 h of continuous process, Tests 1 and 2 presented a yield of 88% and 48%, respectively, enhancing the importance of catalase in this process. Moreover, reaching a 100% conversion yield by a continuous procedure is practically

impossible, since less active forms of both glucose oxidase (GO-Fe^{+3}) and catalase (E-Fe^{+2}) accumulate into the reaction medium during the process.[1][5] Although both enzymes have an iron ion in their molecules, fully active glucose oxidase and catalase require, respectively, ferrous and ferric ions. To simultaneously ensure such N_{ox} for iron atoms in presence of hydrogen peroxide is impossible due to its oxidizing/reducing character. Nonetheless, carrying out the conversion into a reactor operated at fed-batch mode could overcome such handicap, insofar as the amount of hydrogen peroxide in the reaction medium could be controlled.[8]

CONCLUSION

The data presented allowed to conclude that catalase – which decompose hydrogen peroxide into water and oxygen – plays an important role in aerobic organisms by eliminating free radicals originated from incomplete oxidation of oxygen inside the cells. Moreover, evidence on the role of catalase in several types of cancer has been enhanced. There is several industry processes in which catalase is used, such as in the removal of hydrogen peroxide from milk, bleached cotton fibers, and effluents generated by textile and cellulose/paper processing plants; it is also used in the food industry for the desugarization of egg whites, yolks, or whole eggs and in the deoxygenation of beverages (soft drinks and wine). There are several catalase isoenzymes that differ in molecular weight and type of prosthetic group (manganese or iron ions) inserted into the active site domain. Thus, attention must be taken in order to select the most adequate catalase for a process. The role of catalase in multienzyme systems must also be enhanced, since its presence can increase the overall yield of industry processes, such as increasing the glucose/gluconic acid conversion by glucose oxidase using a membrane bioreactor from 48% (without catalase) to 88%.

ACKNOWLEDGMENT

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REFERENCES

1. Glorieux C, Calderon PB. Catalase, a remarkable enzyme: targeting the oldest antioxidant enzyme to find a new cancer treatment approach. Biological chemistry, 2017; 398(10): 1095-1108.

2. Vitolo M. Overview on the biological role of free radicals. World Journal of Pharmaceutical Research, 2019; 8(13): 23-46.
3. Szalkucki T. Applications of oxidoreductases. In: Enzymes in Food Processing. Nagodawithana T, Reed G (Editors). Academic Press Inc., San Diego, 1993; 279-291.
4. Bon EPS, Ferrara MA, Corvo ML. Enzymes in Biotechnology. Editora Interciência, Rio de Janeiro, 2008.
5. Martins LC, Vitolo M. Use of glucose oxidase in a membrane reactor for gluconic acid production. Applied Biochemistry and Biotechnology, 2007; 140: 161-170.
6. Banerjee D, Kumar PA, Kumar B, Madhusoodanan UK, Nayak S, Jacob J. Determination of absolute hydrogen peroxide concentration by spectrophotometric method. Current Science, 2002; 83(10): 1193-1194.
7. Vitolo M. Membrane reactor applied to enzyme conversion of sucrose and glucose. World Journal of Pharmacy and Pharmaceutical Sciences, 2020; 9(9): 43-70.