Enzymatic Bleaching with Generated Hydrogen Peroxide by Glucose oxidation

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1. Introduction

Recently the bio-technology has been applied to the textiles industry in environmentally friendly view. It has been important in various fields of industry for the applied technology of enzyme reaction, which replace pollutive and highly energy consuming chemical process by the environmentally friendly and energy saving bio-process. In biotechnology-based low pollutive technology, enzymatic technology shows the possibility of various applications for scouring and processing of cotton fabrics about textile industry.

Especially, at the manufacturing or weaving process in textiles industry the starch can be hydrolyzed to the unit glucose by α-amylases or amyloglucosidases, glucose can be oxidased by glucose oxidase(GOD) for generating glucose oxidation substance and hydrogen peroxide in the presence of oxygen in aqueous solutions, which has been applied for the study of the bio-bleaching process.[3)]

Alternatively, hydrogen peroxide could be produced enzymatically by glucose oxidase catalyzed conversion of glucose in the presence of oxygen in aqueous solutions.

$$\text{Starch} \rightarrow \text{Glucose}$$

$$\text{Glucose} + H_2O + O_2 \rightarrow \text{Gluconic acid} + H_2O_2$$

The recent experimental study of the enzymatic bleaching process, glucose oxidation was performed without pH-titration and under aeration because of its economic condition. However these attempts were needed to addition of hydrogen peroxide for the bleaching of cotton fabrics, because of low concentration of generated hydrogen peroxide from the result of glucose oxidation. In this study, pH value was controlled by auto-stat-titration because of maintenance of the most suitable pH value for the optimum activity of glucose oxidase to obtain the sufficient concentration of generated hydrogen peroxide for the bleaching. Also the oxygen gas was used instead of the air for protecting the enzymes from the foaming caused by immoderate aeration. There are so many analyzing methods for amount of generated hydrogen, and usually the mathod by using potassium permanganate titration has been used. But this mathod has some problem in sample which contain organic materials, so the chromogenic method was used in this study.

2. Materials and Methods

2.1 Materials

Glucose oxidase (GOD; β-D-Glucose: oxgen 1-oxidoreductase; EC 1.1.3.4), glucose, gluconic

acid. Phenol, 4-aminoantipyrine, potassium phosphate (monobasic, dibasic), and peroxidase (from Horiseradish) were from Sigma, hydrogen peroxide (30w/w%) from Merck.

2.2. Estimate of optimum condition for glucose oxidation

Glucose oxidase has optimum value of activity between pH 4.5 and pH 6.0, and between 30℃ and 45℃ as based on reports. To obtain the optimum pH value, glucose oxidation was performed in the range of pH 5.0 - pH 6.0. 1.0M D(+)-Glucose dissolved in 0.05M potassium phosphate buffer was treated by glucose oxidase with constant aeration (0.2L/min) through glass tube introduced into the solution and stirring for 30min. The reaction solution was titrated with 0.01M NaOH by using auto-titrino for maintain the pH value. For the determination of optimum temperature glucose oxidation was performed at 32℃, 37℃, and 42℃ with same method for 25min. To estimate the effect of oxygen flow, glucose oxidation was done in various oxygen flow rates, 0.2L/min aeration, 0.2L/min and 1.0L/min oxygen flow.[1,2)]

2.3 Glucose oxidation and production of hydrogen peroxide

The reaction was proceeded at optimum condition, pH 5.7, 37℃, in 0.1M potassium phosphate buffer with constant aeration (0.2, 1.0L/min oxygen flow), stirring, and auto-titration by 0.05M NaOH.added for 6h. So the activity of glucose oxidase was determined by the amount of NaOH added.

2.4 Analysis of the amount of generated hydrogen peroxide

To estimate the amount of generated hydrogen peroxide, the chromogenic reaction was used.

$$\text{4-Aminoantipyrine} + \text{phenol} + 2H_2O_2 \rightarrow \text{Quinone dye} + 4H_2O$$

But this method has detection limit, so 1/10 diluted samples were analyzed by the chromogenic reagent.

The chromogenic reagent solution was a 1:1:3 (by volume) mixture of 4-aminoantipyrine (1mmole), phenol (1mmole) and potassium phosphate buffer. The hydrogen peroxidase (25mg) was dissolved in potassium phosphate buffer (20mL).

The chromogenic reagent solution (4.0mL) added to a 1/10 diluted sample (0.5mL) containing hydrogen peroxide and hydrogen peroxidase solution (0.5mL). The mixture was stirred at 37℃ for 10min. The absorbance of the supernatant liquid was measured at 505nm against the reagent blank. The latter was negligible.[5)]

3. Results and discussion

3.1. Estimate of optimum condition for glucose oxidation

The optimum condition of glucose oxidation was estimated by comparing with the amount of NaOH added among those reactions during initial reaction time.

In the glucose oxidation, the activity of glucose oxidase was given at pH 5.7 with aeration. The activity of glucose oxidase was little different within pH 5.5 ~ pH5.7. But the activity of glucose oxidase was reduced below pH 5.0 and above pH 6.0 (Fig.1). The optimum temperature of glucose oxidation was 37℃ (Fig.2). The optimum pH value is good to maintain

generated hydrogen peroxide, but the optimum reaction temperature is not good, because hydrogen peroxide is stable in the present of weak acid and at low temperature.

3.2. Glucose oxidation and production of hydrogen peroxide

Through the results of reaction with aeration, we knew that oxygen limitation was the most important factor of glucose oxidation, so pure oxygen gas was used with various flow rates to obtain sufficient level of hydrogen peroxide concentration for bleaching. Through the amount of NaOH added to maintain the optimum pH value from the reaction-time curve, the activity of glucose oxidase began to be decreased after 2hours and reached the equilibrium point after 6hours passed (Fig.3).[4)]

Probably reduction of the activity of glucose oxidase was caused by inactivation of the enzyme during the reaction time. Inactivation of glucose oxidase could be caused by forming in the eruption of oxygen gas to the reaction solution or by oxygen limitation or by high concentration of hydrogen peroxide formed in the reaction or by shear forces during stirring. In the glucose oxidation with 0.2L/min oxygen flow rate, the concentration of oxygen gas, which was poured sufficiently to solve oxygen limitation before the reaction was started, was decreased fast to 0 approximately within 1min, and was increased very slowly with about 0.01mg/L min during reaction time. In case of the glucose oxidation with 1.0L/min oxygen flow, the concentration of oxygen could not be measured because of excessive forming. Also the result of glucose oxidation without titration was compared with same experiment with titration. The reaction was equilibrated at pH 4.0 approximately within 3hours.

3.3. Analysis of amount of generated hydrogen peroxide

Hydrogen peroxide is not stable due to its decomposition, and the rate of decomposition is increased in solution which contains other substance, i.e., organic materials, metal ions, and salts or under strong base solution. To obtain and maintain sufficient concentration of hydrogen peroxide for bleaching, the reaction has to be done in weak-acidic solution at low temperature because of its decomposition reaction. In fig.4, the concentration of generated hydrogen peroxide reached between about 0.07 w/w% and 0.10 w/w% within an hour and was maintained at 0.10w/w% approximately which is enough hydrogen peroxide concentration to bleaching.

Comparing with glucose oxidation graphs, while the reaction went forward continuously, the concentration of generated hydrogen peroxide was maintained regularly concentration at about 0.1 w/w%. This fact was caused by the instability of hydrogen peroxide. The instability of hydrogen peroxide could be increased by containing other substances in solution and in temperature above 4℃. Also decomposition of hydrogen peroxide probably due to supplyment of demanded oxygen concentration for glucose oxidation and self-decompose for the further oxidation of organic acid. Actually, considering the effect of evaporation, concentration of generated hydrogen peroxide decreased slowly as reaction time passed in the reaction with 1.0L/min oxygen flow rate, but concentration of generated hydrogen peroxide increased slowly in the reaction with 0.2L/min oxygen flow rate.

4. Conclusions

The concentration of hydrogen peroxide generated by glucose oxidation was about 0.1 w/w%. According to the literature, the concentration of hydrogen peroxide for the bleaching is 0.1 w/w%, so this study shows the possibility that the bleaching process can be done by generated hydrogen peroxide. Also waste water and raw materials can be reduced in the textile process by the application of this study. Though enzymatic process is uneconomical, it can be solved by immobilization. Also gluconate which is another end-product can be applied to a filed of medical science.

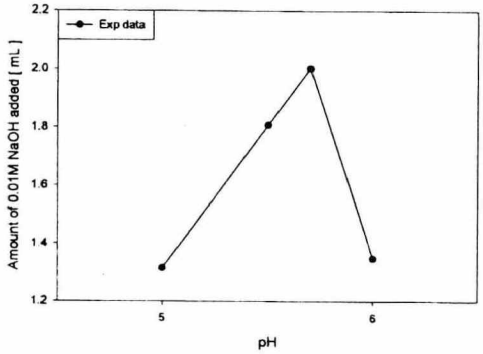

Fig 1. Optimum pH value of Glucose oxidation

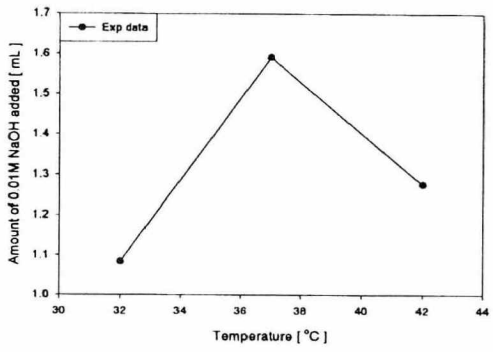

Fig 2. The optimum temperature for glucose oxidation

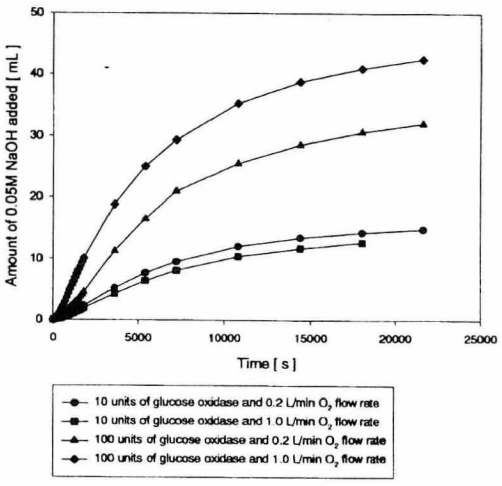

Fig 3. Glucose oxidation with different conditions

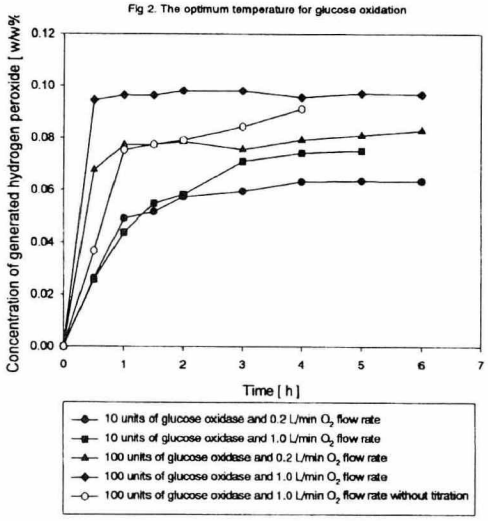

Fig 4. The concentarion of generated hydrogen peroxide from glucose oxidation with different condition

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