

Research about Component Enzyme Making Antibacterial Paper

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Abstract: The article researched a kind of green antibacterial paper based component enzyme. The glucose oxidase and lysozyme were added in paper by coating. And the article researched the antimicrobial effect through antibacterial annulus and SEM. The optimum antimicrobial effect that used glucose oxidase only was pH=5.5 and enzyme activity 4000-6000u/g (on even dry pulp). And then the optimum antimicrobial effect that used lysozyme only was pH=5.5 and enzyme activity 4000-6000u/g (on even dry pulp). Antibacterial annulus and TEM showed the antimicrobial effect that used component enzyme in paper surface was better. Furthermore, enzyme activity dosage of component enzyme was lower than single use. The optimum condition was enzyme activity of glucose oxidase 4000u/g (on even dry pulp), enzyme activity of lysozyme 20000u/g (on even dry pulp) and pH=6.0.

Introduction

Antibacterial paper research is a new field of antibacterial material in recent years. There are many ways give paper and paper products antibacterial function. Common method in the paper is join inorganic or organic antibacterial agents, it can give paper or paper products certain antibacterial properties to some extent. However, The traditional type of organic inorganic antibacterial agents has been unable to meet the requirements of people for green and harmless, Such as paper of newborn babies, female paper, medical paper, etc [1-8]. This article selection of glucose oxidase and lysozyme, is a kind of biological antibacterial agents which have catalytic ability of singleness. It is completely harmless to the human body and easy degradation in nature and does not produce any pollution to the environment. So using glucose oxidase and lysozyme as production of antibacterial paper, is really in the sense of pure green antibacterial paper. Two enzymes were added in paper by coating in this article. Study antibacterial effect when they are used alone and complex formulation. Then obtain the optimum process parameters of producing antibacterial paper by component enzyme.

Materials

(1)The raw material: Hardwood pulp board

(2)Chemicals: glucose oxidase: enzyme activity 50000U g⁻¹, lysozyme: enzyme activity 30000U mg⁻¹, Glucose solid (analytically pure), Acetic acid, sodium acetate (analytically pure)

(3)reagent: glucose solution(concentration 0.5mol/L);0.9% sodium chloride solution; pH=5.5

Acetic acid, sodium acetate buffer; pH=6.5 Acetic acid, sodium acetate buffer

(4)Bacterial species: E. coli

Equipment

(1)Paper making equipment

(2)Bacteria inoculation and incubation equipment

Flat dish, vaccination needle, High pressure sterilization pot, inoculation loops, constant temperature and humidity box, biological incubator

The results and Analysis

The antimicrobial effect research of antibacterial paper made by lysozymein

The experimental method[9-10]. (1)According to previous research of antimicrobial effect. The amount of lysozyme in 1000-5000 u/ml had good antibacterial effect. So the preparation of 1000, 2000, 3000, 4000, 5000 u/ml of lysozyme solution.

(2)According to per gram dry pulp enzyme dosage and concentration of the enzyme solution. A certain amount of enzyme solution evenly uniformed on manufacture paper with a glass rod, drying in 37 °C Air-blower-driven Drying Closet, Take out and use sterilized scissors to cut a diameter of 20 mm of paper and well marked.

(3)Vaccinated E. coli by spread plate method,pieces of paper put on a flat dish by using tweezers.

(4)Put a dish at 30 °C biochemical incubator for 48h.

The Antimicrobial Effect of Antibacterial Paper Made by Lysozymein. After 48h cultivate for E. Coli, the antimicrobial effect of antibacterial paper made by lysozymein (antibacterial annulus) shown in table 1, the diameter of antibacterial annulus has shown in table1, figure 2.

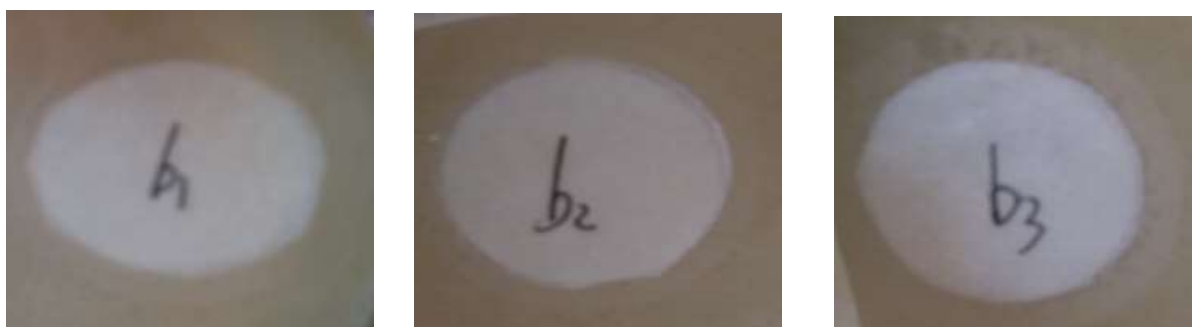




Fig. 1: antibacterial annulus photograph of antibacterial paper made by lysozyme

Table1 antibacterial annulus diameter of antibacterial paper made by lysozyme

Sample name	b1	b2	b3	b4	b5
Lysozyme dosage(on even dry pulp)	5000	10000	15000	20000	25000
diameter(mm)	23.1	24.2	24.4	25.2	25.2

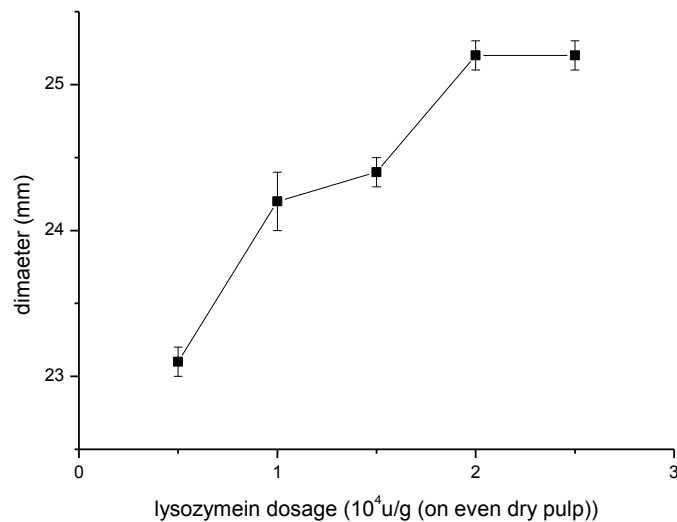


Fig. 2: The change of antibacterial annulus diameter with lysozyme dosage of antibacterial paper made by lysozyme

As shown in figure 2-1, on the edge of the b1 - b5 paper have formed the antibacterial annulus of light color, the growth of antibacterial annulus and *e. coli* on paper was obviously inhibited. It has shown some antimicrobial effect of antibacterial paper made by lysozyme. Table 1, figure 2 shown antibacterial annulus diameters of paper made by different lysozyme dosage. From table 1, figure 2 can be seen, lysozyme dosage in 5000-20000 (u/g on even dry pulp) range. The antimicrobial effect of paper have more obvious improve when increase the lysozyme dosage. When lysozyme dosage has exceeded 20000 (u/g on even dry pulp). The antimicrobial effect of paper have no obvious change when increase the lysozyme dosage. Considering the antimicrobial effect of antibacterial paper and the cost of antibacterial, the lysozyme dosage is better not over 25000 (u/g on even dry pulp).

The Antimicrobial Effect Research of Antibacterial Paper Made by Glucose Oxidase

The Experimental Method. (1)According to previous research of antimicrobial effect. The amount of glucose oxidase in 400-1200 u/ml had good antibacterial effect. So the preparation of 400, 600, 3000, 800, 1000, 1200u/ml of glucose oxidase solution.

(2)According to per gram dry pulp enzyme dosage and concentration of the enzyme solution. A certain amount of enzyme solution evenly uniformed on manufacture paper with a glass rod, drying in 37°C Air-blower-driven Drying Closet, take out and use sterilized scissors to cut a diameter of 20 mm of paper and well marked.

(3)Vaccinated E. coli by spread plate method, pieces of paper put on a flat dish by using tweezers.

(4)Put a dish at 30°C biochemical incubator for 48h.

The Antimicrobial Effect of Antibacterial Paper Made by Lysozymein. After 48h cultivate for E. Coli, the antimicrobial effect of antibacterial paper made by glucose oxidase (antibacterial annulus) shown in figure 2-3, the diameter of antibacterial annulus shown in table 2-2, figure 2-4.



Figure 3 antibacterial annulus photograph of antibacterial paper made by glucose oxidase

As shown in figure 3, on the edge of the p1 - p5 paper have formed the antibacterial annulus of light color. But compared to figure 1 it seems no matter significance level or size of antibacterial annulus are not better than lysozymein. The antimicrobial effect that separate use of glucose oxidase as antimicrobial agent to make paper is no better than lysozymein. Analysis the reason, may due to the main antibacterial principle of glucose oxidase is decomposition of oxygen around the paper. This is a kind of indirect antimicrobial by excludes oxygen .And the antimicrobial effect of the

lysozyme embodied in direct antimicrobial of bacteria as the role of the substrate. Table 2, figure 4 shown antibacterial annulus diameters of paper made by different glucose oxidase dosage. From table 2, figure 4 can be seen the improve of its antimicrobial effect with the increase of glucose oxidase dosage. But the extend of increase will be down when glucose oxidase dosage over 5000(u/g on even dry pulp). So Considering the antimicrobial effect of antibacterial paper and the cost of antibacterial, the glucose oxidase dosage is better not over 6000 (u/g on even dry pulp).

Table 2. Antibacterial annulus diameter of antibacterial paper made by glucose oxidase

Sample name	p1	p2	p3	p4	p5
oxidase dosage (on even dry oulp)	2000	3000	4000	5000	6000
dimaeter(mm)	20.8	21.5	22.3	23.0	23.2

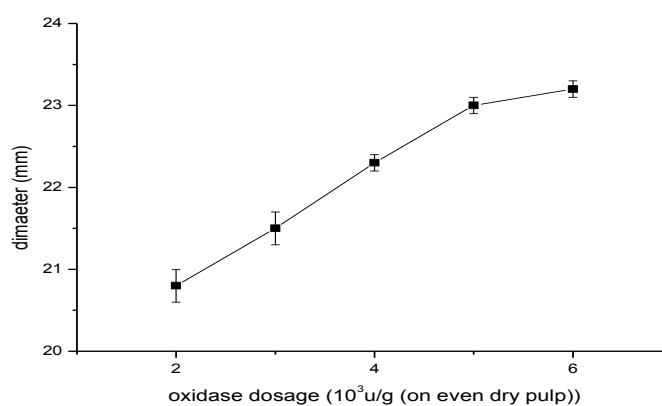


Fig. 4: The change of antibacterial annulus diameter with oxidase dosage of antibacterial paper made by glucose oxidase

The Antimicrobial Effect Research of Antibacterial Paper Made by Component Enzyme. In order to increase the antimicrobial effect of antibacterial paper and reduce the dosage of antimicrobial enzymes. The article makes paper by component glucose oxidase and lysozyme. Play a direct antimicrobial effects of lysozyme and indirect antimicrobial effects of glucose oxidase at the same time.

The Experimental Method. (1)Preparation of 2000, 3000, 4000 u/ml lysozyme solution with pH 5.5, 6, 6.5 and 1600, 2000, 2400u/ml glucose oxidase solution.

(2)According to figure 2-3,mixed the dosage of 1:1 and evenly uniformed on manufacture paper with a glass rod, drying in 37°C Air-blower-driven Drying Closet. Take out and use sterilized scissors to cut a diameter of 20 mm of paper and well marked.

(3)Vaccinated E. coli by spread plate method, pieces of paper put on a flat dish by using tweezers.

(4)Place upside down the dish and put it at 30°C biochemical incubator for 48h.

The Antimicrobial Effect Research of Antibacterial Paper Made by Component Enzyme. After manufacture paper, composite enzyme as an antimicrobial agent, inoculated the e. coli and biochemical incubator for 48h. The antimicrobial effect shown in figure 5, the diameter of

antibacterial annulus has shown in table 4, figure6.

As shown in figure 5, the Composite enzyme as an antimicrobial agent make the paper and antibacterial annulus effect of antimicrobial paper is obvious. Antibacterial annulus edge can be clearly seen. the e. Coli hardly to see inside the antibacterial annulus or on the paper. Even the density of e. coli around antibacterial annulus is low. It is to say use composite enzyme make paper is better than single enzyme. In combination with figure 2-6, table 2-4, it can be seen that the best antibacterial effect of NO.9 can reach 26.2 mm. The process parameters is pH=6.lysozymein dosage is 10000(u/g on even dry pulp).glucose oxidase dosage is 6000(u/g on even dry pulp).To reach the best antibacterial effect, the dosage of the two enzymes are lower than when used alone. Thus it can be seen, using glucose oxidase and lysozyme as component enzyme will not produce mutual inhibition, but also promote each other. Play a direct antimicrobial effects of lysozyme and indirect antimicrobial effects of glucose oxidase at the same time. In order to prove both promote action of antibacterial, the experiment take the e. Coli which around the antibacterial annulus to make TEM.As shown in figure 2-7. Figure 2-7 illustrates that E. coli on the edge of antibacterial annulus lost most of the cell wall. Part of the e. coli cells structure is not complete. This degree of cell damage is usually caused by oxygen pressure and the cells erosion of the outsider [11-13]. So on the surface or around the antibacterial paper made by component enzyme, two enzymes both played a antibacterial effect itself and have the effect of the mutual promotion.

Table 2-3. The table of orthogonal experiment.

NO.	oxidase dosage(on even dry oulp)	Lysozymein dosage(on even dry oulp)	pH
1	4000	5000	5.5
2	4000	7500	6
3	4000	10000	6.5
4	5000	5000	6
5	5000	7500	6.5
6	5000	10000	5.5
7	6000	5000	6.5
8	6000	7500	5.5
9	6000	10000	6

Table 4. Antibacterial annulus diameter of antibacterial paper made by component enzyme.

Sample name	1	2	3	4	5	6	7	8	9
dimaeter (mm)	22.2	25.0	25.5	22.5	22.8	21.5	22.6	22.0	26.2

Summary

According to the research, no matter antibacterial paper made by glucose oxidase and lysozymein alone or by component enzyme both have a good and real green antibacterial effect. The conclusion is as follows:

Lysozyme dosage in 5000-20000 (u/g on even dry pulp) range, antibacterial effect increased

significantly, the largest diameter of antibacterial annulus is 25.2mm, When lysozyme dosage has exceed 20000 (u/g on even dry pulp) ,The antimicrobial effect of paper have no obvious change when increase the lysozyme dosage. Considering the antimicrobial effect of antibacterial paper and the cost of antibacterial, the lysozyme dosage is better not over 25000 (u/g on even dry pulp).

The antimicrobial effect that separate use of glucose oxidase as antimicrobial agent to make paper is not better than lysozyme. The largest diameter of antibacterial annulus is 23.2mm. The improve of its antimicrobial effect with the increase of glucose oxidase dosage. But the extend of increase will be down when glucose oxidase dosage over 5000(u/g on even dry pulp). Considering the antimicrobial effect of antibacterial paper and the cost of antibacterial, the glucose oxidase dosage is better not over 6000 (u/g on even dry pulp).

The Composite enzyme as an antimicrobial agent make the paper and antibacterial annulus effect of antimicrobial paper is well. pH=6, Lysozyme dosage is 10000 (u/g on even dry pulp), glucose oxidase dosage is 6000 (u/g on even dry pulp), the largest diameter of antibacterial annulus is 26.2mm. To reach the best antibacterial effect, the dosage of the two enzymes are lower than when used alone.

TEM show that the E. Coli cells damage is usually caused by oxygen pressure and the cells erosion of the outsider. On the surface or around the antibacterial paper made by component enzyme. The lysozyme that bacteria as the substrate happened the directly antibacterial effect and the glucose oxidase that oxygen as the substrate happened the indirectly antibacterial effect .Both of them have the effect of the mutual promotion.

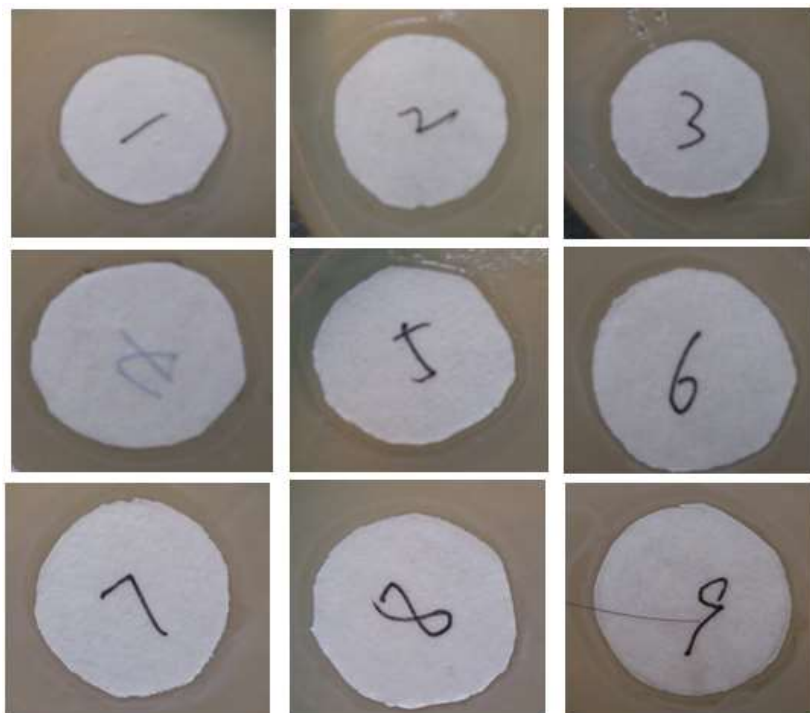


Fig. 2-5. Antibacterial annulus photograph of antibacterial paper made by component enzyme

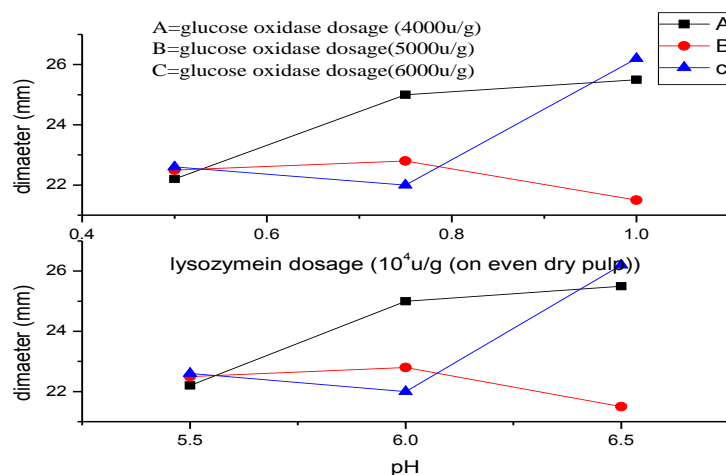


Fig. 6. the change of antibacterial annulus diameter with enzyme dosage and pH of antibacterial paper made by component enzyme

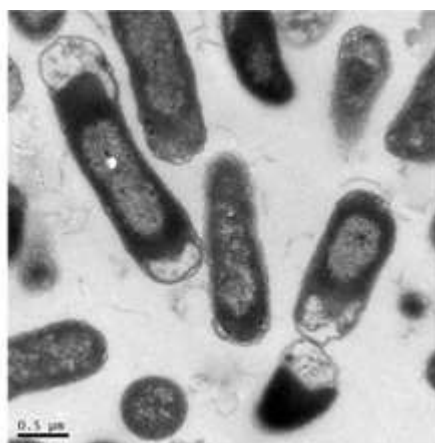


Fig. 7. Photograph of TEM ---E. coli on the edge of antibacterial annulus

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