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Study on saccharification process and strains filtration during the ethanol fermentation of office waste paper

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ABSTRACT

Office waste paper is a kind of renewable resource wich is high in cellulose and cheaper. In this paper, we selected the optimal conditions of the office waste paper atmospheric pressure two-stage acid hydrolysis by single-factor test, and choosing the optimal strains in ethanol fermentation between super saccharomyces cerevisiae、Zy-momonas mobile and candida shehatae. The result showed that the optimum conditions of hydrolysis technology for two-stage acid hydrolysis under normal pressure were as follows: in the stage of concentrated acid hydrolysis, acid concentration was 60%, hydrolysis temperature was 65°C, hydrolysis time was 120min and ratio of acid to solid was 18:1; in the stage of dilute acid hydrolysis, hydrolysis temperature was 100°C, hydrolysis time was 180min and ratio of acid to solid was 150:1. Super saccharomyces cerevisiae would be selected for the stage of ethanol fermentation. The optimized conditions were that inoculation amount was 400μl, the culture temperature was 34°C and the culture time was 60h.

Key words: Office waste paper; Two-stage acid hydrolysis; Microbe selected; Optimal condition.

INTRODUCTION

People need to develop new energy in order to adjust and improve the energy frame because of declining oil output, expanding need for energy and greenhouse-gas emissions. In addition to renewable such as wind, solar and hydro, people give more consideration to biofuels energy. But“the first generation of biofuels” focused on using corn for ethanol and soybeans of biodiesel, it consumed large amounts of agricultural and impacted on food security. So “the second generation of biofuels”- cellulosic material used into ethanol[1, 2]. The feedstock of cellulosic ethanol fuel is lignocellulose for biomass mainly.

The advantages of office waste paper were used as raw material for ethanol, such as: low prices, high-fiber and accessible[3]. Exploring process conditions of saccharification and fermentation for ethanol by office waste paper has the great practice meaning and long term effect. In this paper, we selected the optimal condition of the office waste paper atmospheric pressure two-stage acid hydrolysis, and filtrated microbe for the ethanol fermentation.

EXPERIMENTAL SECTION

Reagents & Solutions

H_2O_2, $NaSO_4$, NaOH, KH_2PO_4, MgSO, $C_2H_4O_2$, D(+)-Sucrose, $C_2H_3NaO_2 \cdot 3H_2ONH_4Cl$, analytical reagent grade and were purchased from local commercial supplies; NH4·H2O, concentrated sulfuric acid (mass fraction 98.3%). Agar, Peptone, Yeast extract, Nutrient agar, LB broth, MRS broth, malt extract medium. *Angel super saccharomyces cerevisiae* were purchased from local commercial supplies. The *Zy-momonas mobile* and the *Candida shehatae* were stored in the Laboratory. Pure water was prepared with Millipore purification system. Office waste paper was from school office. Office waste paper was broken to pieces by shredder, and then the alkaline

flotation deinking [4,5] of pre-processing methods was used. The content of cellulose that was measured by Van Soest method [6] was 88.7% in the pulps. pretreatment solution of office waste paper for consistency was 5%.

Apparatus & Measurements

JJ-1 electric mixer, Thermostat water bath cauldron, Centrifuge, KQ5200E Ultrasonic cleaner, BI-150A cooled incubator, MLS-3750 pressure vapour sterilizer, Millipore purification system, SW-CJ-2D cleaning bench, Adjustable miropipettor, BS224S precision scales, DA-130N Portable Alcohol Meter, 200μl, 1000μl pipettes gun Eppedendorf Reference. The content of reduced sugar (xylose and glucose)was measured by Agilent GC900A gas chromatograph in the stage of acid hydrolysis (HP-5 quartz capillary chromatographic column, the nitrogen as carrier gas, and with the split flow ratio being 60:1, the injector temperature 250°C, the detector temperature 270°C, the column temperature is programmed raised, the injection volume was 10μl.).The alcohol content[7]was measured by DA-130N Portable Alcohol Meter in the stage of fermentation for ethanol production.

Conversion ratio of reducing sugar=Content of reduced sugar/ Quality of raw material

Experiment Procedure

The stage of hydrolysis process with sulphuric acid

In the stage of concentrated acid hydrolysis, a three-necked round-bottomed flask of 1000ml, was equipped with an electric mixer, a thermometer and a rubber plug. The complete setup was heated with thermostat water bath cauldron in order to reach constant temperature, while loaded the raw material which had definite ratio of acid to solid, putting the electric mixer to mix and beginning the experiment of concentrated acid hydrolysis. For the research achievements [8], we studied on the influence of acid concentration, hydrolysis temperature, hydrolysis time and ratio of acid to solid on the content of reduced sugar.

While the stage of concentrated acid hydrolysis was finished, added water to the three-necked round-bottomed flask of 1000ml in order to begin the stage of dilute acid hydrolysis. It's the stage of dilute acid hydrolysis. Studied on the influence of hydrolysis temperature, hydrolysis time and ratio of acid to solid on the content of reduced sugar. The stage of dilute acid hydrolysis had the same experiment-al procedure as the stage of concentrated acid hydrolysis.

The stage of microbe selected on the ethanol fermentation

Used the method of plate culture count in order to get microorganism counts [9]. Solution of the sample (*super saccharomyces cerevisiae*, *zy-momonas mobile* and *candida shehatae*) was extracted 1ml from 75ml/250ml culture flask, and continuously diluted the solution of inoculum solution to density to be 10^{-6} with aseptic water, then counted from plate. Therefore three parallel experiments were carried through to probe into the microorganism counts.

Activation & Amplification culture

Super Saccharomyces Cerevisiae

Angel *super saccharomyces cerevisiae* used 2% sucrose solution during resumption on 38°C last 15 min, and then activating the saccharomyces on 32°C 1.5h.The saccharomyces were vaccinated on slant of agar media and putted the slant on 37°C, 4 days in incubator until the white colony grown. Taking an ose saccharomyces which were mature on the slant transferred to liquid culture medium 37°C, 24 h, 50ml/150ml culture flask, in a shaker incubator set at 150 r/min[10].

Liquid medium of seed: MRS broth, natural PH. Fermentation medium: Solution of acid hydrolysis 75ml, peptone 5.0 g/L, beef extract 3.0 g/L, NaCl5g/L, PH 7.4[10, 11].

Zy-momonas Mobile

Got the strain from bevel culture medium overgrowing with *zy-momonas mobile* by inoculation loop to MRS broth 37°C, 24 h, 75ml/250ml culture flask, in a shaker incubator set at 150 r/min [11,12], and then transferred to liquid culture medium once more.

Liquid medium of seed: dextrose 50.0 g/L, peptone 5.0 g/L, yeast extract 5.0 g/L, KH2PO4 1g/L, MgSO4·7H2O, NH4Cl 2g/L, natural PH. Fermentation medium: Solution of acid hydrolysis 75mL, peptone 5.0 g/L, yeast extract 5.0 g/L, KH2PO4 1g/L, MgSO4·7H2O, NH4Cl 2g/L, PH 5.0[10, 14].

Candida Shehatae

Taking an ose saccharomyces which were growing *candida shehatae* on the slant transferred to liquid culture medium 28°C, 24 h, 75ml/150ml culture flask, in a shaker incubator set at 150 r/min[13], and then transferred to liquid culture medium once more.

Liquid medium of seed: malt extract medium, natural PH. Fermentation medium: Solution of acid hydrolysis75ml, peptone 20.0 g/L, yeast extract 10.0 g/L, CaCl2 0.25 g/L , MgSO4 0.25 g/L. PH 4.5[13].

In the stage of the microbes fermented to ethanol production, liquid seed of three strains were extracted by pipette gun into 250ml culture flask which had fermentative medium75mL, in a shaker incubator set at 150r/min. With these conditions, studied on the inoculation amount, cultured temperature and cultured time on the yield of the ethanol .

RESULTS AND DISCUSSION

The stage of concentrated acid hydrolysis

The influence of acid concentration: Under the conditions of acid concentration 40%, 50%, 60%, 70%, 80% and hydrolysis temperature 55°C, hydrolysis time 60min and ratio of acid to solid 15:1, the examination shown change of xylose and glucose yield with the acid concentration. Results are shown by Fig.1. The figure explains that the xylose and glucose yield increased at first, then decreased with the increase of acid concentration, the xylose and glucose yield were at most when acid concentration was 60%. It was assumed that the celluloses were hydrolyzed incompletely when the acid concentration was less than 60%, and the auxiliary reaction increased while the acid concentration was more than 60%.

The influence of hydrolysis temperature: Under the conditions of hydrolysis temperature 45°C, 55°C, 65°C, 75°C, 85°C, acid concentration60%, hydrolysis time 60min and ratio of acid to solid 15:1, it was found that the xylose and glucose yield changed with the hydrolysis temperature. Results are illustrated by Fig.2. The figure shows that the xylose and glucose yield increased at first, then decreased with the increase hydrolysis temperature , the xylose and glucose yield were at most when hydrolysis temperature was 65°C. It was speculated that the hydrolysis temperature could accelerate the rate of reaction when it was increased, and then auxiliary reaction increased while the hydrolysis temperature was more than 65°C.

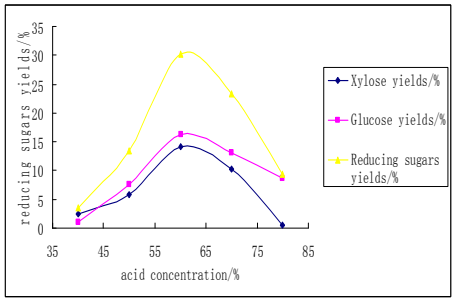

Fig.1. The acid concentration

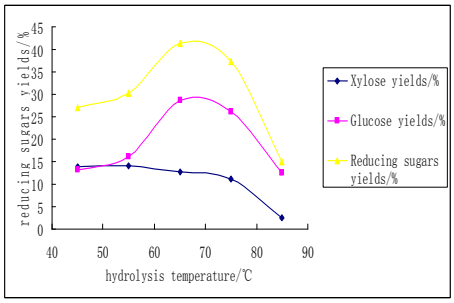

Fig.2. The hydrolysis temperature

The influence of hydrolysis time: The conditions under that hydrolysis time 30min, 60min, 90min, 120min, 150min, acid concentration 60%, hydrolysis temperature 65°C and ratio of acid to solid 15:1 were investigated for the yield of xylose and glucose. Results are shown on Fig.3. The figure showed that the xylose and glucose yield increased at first, then the xylose and glucose yield at most when hydrolysis time was 120min, the xylose and glucose yield were more steadily and changed little with the prolonging of time. Maybe the hydrolysis time prolonged could increase hydrolysis degree of celluloses, but the stability of reducing sugars were much affected by longer hydrolysis time and higher hydrolysis temperature.

The influence of ratio of acid to solid: Under the conditions that ratio of acid to solid 12:1, 15:1, 18:1, 21:1, 24:1, hydrolysis temperature 65°C, hydrolysis time 120min and acid concentration 60%, it was found that the xylose and glucose yield changed with the ratio of acid to solid. Results are shown on Fig.4. The figure shows that the xylose and glucose yield increased at first, then decreased with the increase of ratio of acid to solid. When ratio of acid to solid was 18:1 the xylose and glucose content were at most. It was assumed that the celluloses and acid weren't mixed incompletely when the acid to solid was less than 18:1, and the auxiliary reaction increased while the acid to solid was more than 18:1.

The results showed that the optimum conditions on the stage of concentrated acid hydrolysis were acid concentration was 60%，hydrolysis temperature was 65°C, hydrolysis time was 120min and ratio of acid to solid was 18:1.

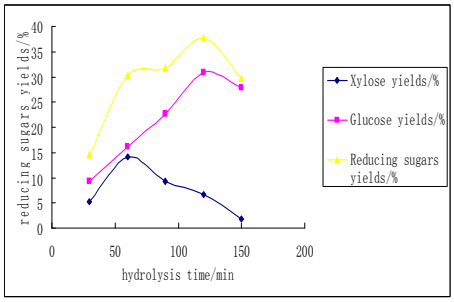

Fig.3. The hydrolysis time

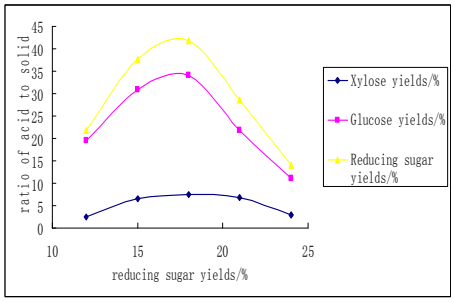

Fig.4. The ratio of acid to solid

The stage of dilute acid hydrolysis

While the stage of concentrated acid hydrolysis was finished, infused water to the three-necked round-bottomed flask of 1000ml and then beginning to the next stage.

The influence of hydrolysis temperature: While conditions on hydrolysis temperature 70°C, 80°C, 90°C, 100°C, 110°C, hydrolysis time120min and ratio of acid to solid 150:1, discussed the yield of the xylose and glucose. Results are shown on Fig.5. The figure explains that the xylose and glucose yield increased with elevated the hydrolysis temperature, and the xylose and glucose yield were more steadily and changed little with the temperature more than 100°C. Choosing the hydrolysis temperature was 100°C in order to reduce cost and get less by-products.

The influence of hydrolysis time: Under the conditions of hydrolysis time 60min, 90min, 120min, 150min, 180min, hydrolysis temperature100°C and ratio of acid to solid 150:1, it was found that the xylose and glucose yield changed with the hydrolysis time. Results are shown on Fig.6. The figure shows that the xylose and glucose yield increased with the hydrolysis time growth. Maybe the hydrolysis time prolonged could increase production of reducing sugars, we got the hydrolysis time 180min because of longer hydrolysis time would get more by-products.

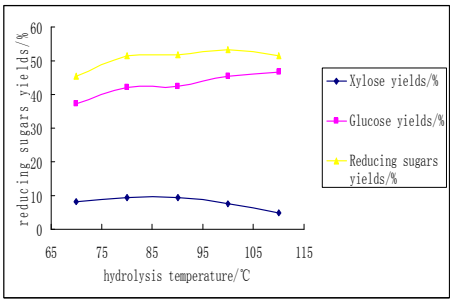

Fig.5. The hydrolysis temperature

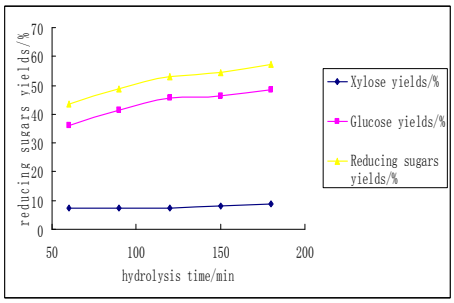

Fig.6. The hydrolysis time

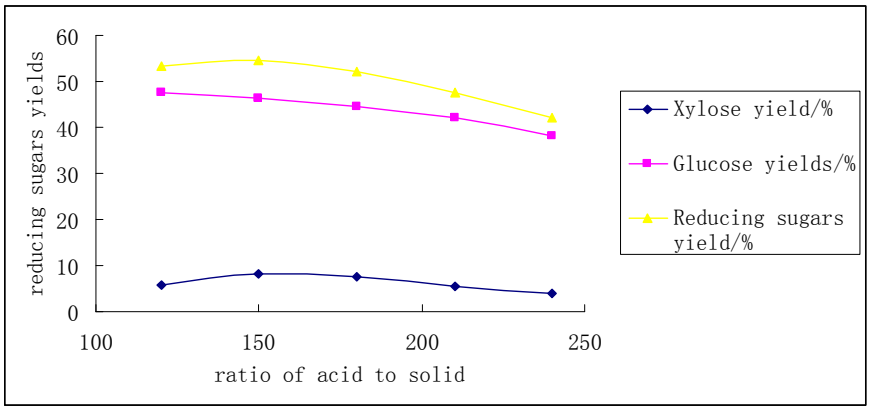

Fig.7. The influence of ratio of acid to solid

The influence of ratio of acid to solid: Under the conditions of ratio of acid to solid 120:1, 150:1, 180:1, 210:1, 240:1, hydrolysis temperature 100°C and hydrolysis time 180min, the examination of the effects shown change of xylose and glucose yield with the ratio of acid to solid. Results are shown on Fig.7. The xylose and glucose yield

increased at first, then decreased with the increase of ratio of acid to solid, the yield of xylose and glucose were at most when ratio of acid to solid was 150:1. It was assumed that the celluloses and acid weren't mixed incompletely when the acid to solid was less than 150:1, and the reducing sugars would be hydrolyzed and oxidized while the acid to solid was more than 150:1.

The resulted showed that the optimum conditions on the stage of dilute acid hydrolysis were hydrolysis temperature was 100°C, hydrolysis time was 180min and ratio of acid to solid was 150:1.

The stage of microbe selected on the ethanol fermentation

The inoculation amount of *Super Saccharomyces Cerevisiae:* Discussed the yield of the ethanol under the effect about inoculation amount was 200μl, 400μl, 600μl, 800μl, 1000μl (microorganism counts: 2.31×10^8/ml), cultured temperature was 37°C, cultured time was 24h and in a shaker incubator set at 150 r/min. Results are shown on Fig.8.

The inoculation amount of *Zy-momonas Mobile:* Studied on the yield of the ethanol under these circumstances: the cultured temperature was 37°C, cultured time was 24h, the inoculation amount was 200μl, 400μl, 600μl, 800μl, 1000μl (microorganism counts: 2.45×10^8/ml) and in a shaker incubator set at 150 r/min. Results are shown on Fig.8.

The inoculation amount of *Candida Shehatae:* Discussed the yield of the ethanol under the inoculation amount was 200μl, 400μl, 600μl, 800μl, 1000μl (microorganism counts： 1.98×10^8/ml), cultured time 24h, the cultured temperature 34°C and in a shaker incubator set at 150 r/min. Results are shown on Fig.8.

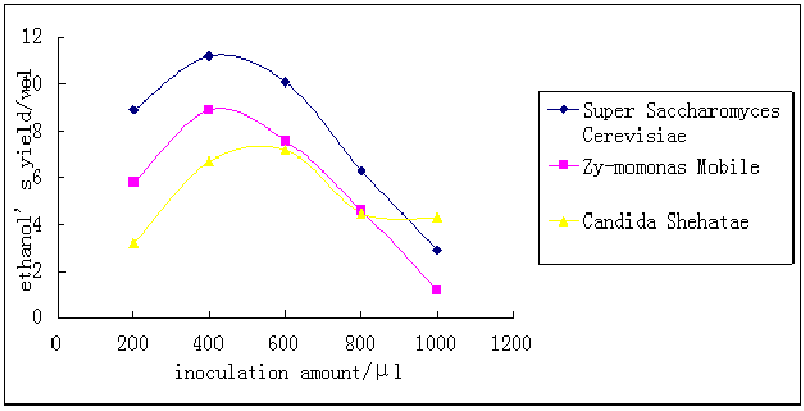

Fig.8. The effect of ethanol's yield on the inoculation amount

The figure shows that the yield of the ethanol increased at first, then decreased with the increase of inoculation amount, maybe the reducing sugars were fermented incompletely when the inoculation amount was too little, and the nutrition was inadequate while the inoculation amount was too much. The optimized conditions of inoculation amount was *super saccharomyces cerevisiae* 400μl, *zy-momonas mobile* 400μl , *candida shehatae* 600μl.

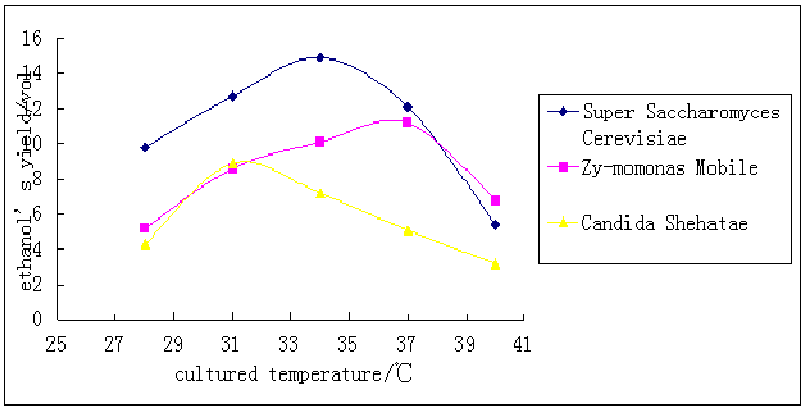

Fig.9. The effect of ethanol's yield on the cultured temperature

The effect of ethanol's yield on the cultured temperature

The cultured temperature of *Super Saccharomyces Cerevisiae:* Discussed the yield of the ethanol under the effect about the cultured temperature was 28°C, 31°C, 34°C, 37°C,40°C, the inoculation amount was 400μl, cultured time 24h and in a shaker incubator set at 150 r/min. Results are shown on Fig.9

The cultured temperature of *Zy-momonas Mobile:* Discussed the yield of the ethanol under the cultured temperature was 28°C, 31°C, 34°C, 37°C, 40°C, the inoculation amount was 400μl, cultured time 24h and in a shaker incubator set at 150 r/min. Results are shown on Fig.9.

The cultured temperature of *Candida Shehatae:* Discussed the yield of the ethanol under the effect about the cultured temperature was 28°C, 31°C, 34°C, 37°C, 40°C, the inoculation amount was 600μl, cultured time 24h and in a shaker incubator set at 150 r/min. Results are shown on Fig.9.

It was assumed that the cultured temperature could accelerate the rate of growth when the was increased in the beginning, but the microbe couldn't grow well while cultured temperature was too hot. The figure shows that optimized conditions of the cultured temperature was *super saccharomyces cerevisiae* 34°C, *zy-momonas mobile* 37°C, *candida shehatae* 31°C.

The effect of ethanol's yield on the cultured time

The cultured time of *Super Saccharomyces Cerevisiae:* Discussed yield of the ethanol under the effect about the cultured time was 24h, 36h, 48h, 60h, 72h, the cultured temperature 34°C, the inoculation amount was 400μl and in a shaker incubator set at 150 r/min. Results are shown on Fig.10.

The cultured time of *Zy-momonas Mobile:* Studied on the yield of the ethanol under these circumstances: the cultured temperature was 37°C, the inoculation amount was 400μl, cultured time was 24h, 36h, 48h, 60h, 72h and in a shaker incubator set at 150 r/min. Results are shown on Fig.10.

The cultured time of *Candida Shehatae:* Studied on the yield of the ethanol under these circumstances: the cultured temperature was 31°C, the inoculation amount was 600μl, cultured time was 24h, 36h, 48h, 60h, 72h and in a shaker incubator set at 150 r/min. Results are shown on Fig.10.

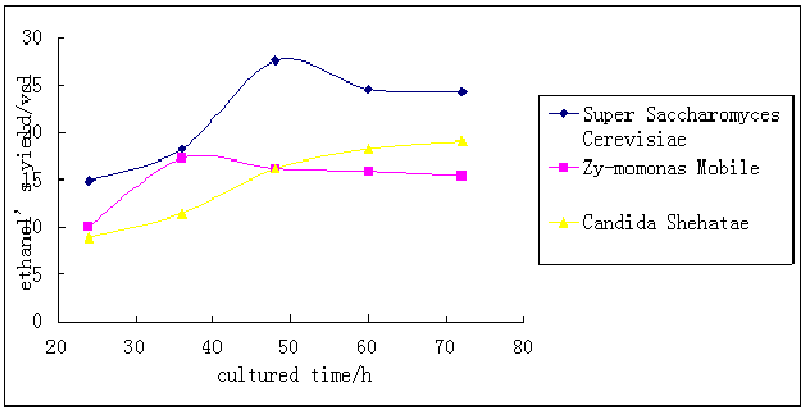

Fig.10. The effect of ethanol's yield on the cultured time

The figure shows that optimized conditions of the cultured time were *super saccharomyces cerevisiae* 48h, *zy-momonas mobile* 36h, *candida shehatae* 72h. Maybe the cultured time could accelerate the rate of growth, and then the microbe would die by the incubation time raised.

CONCLUSION

In the stage of concentrated acid hydrolysis, acid concentration was 60%， hydrolysis temperature was 65°C, hydrolysis time was 120min and ratio of acid to solid was 18:1; in the stage of dilute acid hydrolysis, hydrolysis temperature was 100°C, hydrolysis time was 180min and ratio of acid to solid was 150:1. We can use orthogonal design in order to optimize process of acid hydrolysis for the future.

Then the stage of ethanol fermentation showed that the inoculation amount of *super saccharomyces cerevisiae* was less than *candida shehatae*, cultured time was moderate and could grow on the higher temperature, so the *super saccharomyces cerevisiae* will be chose for ethanol fermentation. We will ferment with mixed bacteria next step.

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REFERENCES

[1] Marcelo S. Sthel, José G. R. Tostes, Juliana R. Tavares. *Natural Science*., **2013**, 5 (02), 244-252
[2] Naik, S.N. Goud, Vaibhav V. Rout; Prasant K. Dalai, Ajay K. Elsevier in its journal *Renewable and Sustainable Energy Reviews.*, **2010**, 14, 578-597.
[3] Yazuo. People's Daily., **2013**.08.05.
[4] S. Chaiarrekij , H. Dhingra , B.V. Ramarao. *Resources, Conservation & Recycling*., **2000**, 28 (03), 219-226.
[5] Peng Yunyun, Wu Shubin. *Paper Science & Technology* **2011**., 30(05), 31-35.
[6] Van Soest P J. *Association of Official Agricultural Chemists*., **1963**, 46, 825
[7] Tianxiang Wu. *Liquor-Making Science & Technology*., **1998**, 88, 37-38.
[8] Demirbas AH. *Applied Biochemistry and Biotechnology*., **1999**, 77, 77 -79.
[9] GB_4789.2-**2010** Aerobic plate count.
[10] Lihong Yuan. Laboratory Experiments in Microbiology, 1st Edition, Chemical Industry Press, Beijing, **2010**, 211-217.
[11] Rogers PL, Lee KJ, Skotnicki ML. *Advances in Biochemical Engineering*., **1982**, Vol.23, 27-84.
[12] Chen Jinggang, Zhang Baoshan. *Liquor Making Science &Technology*., **2009**, 178, 91-100. [in Chine-se].
[13]M. A. Alexander, T. W. Chapman, and T. W. Jeffries. *Applied Microbiology and Biology*., **1988**, 28, 478-486
[14] Olena B R,Oksana M C, RII S. Fems *Yeast Research*., **2003**, 4, 157-164