Study on the Biological Oxidation Treatment of the Wastewater from the Production of Cellulosic Ethanol by Straw Residue

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Abstract
With the global energy crisis, food crisis and the environmental crisis, the straw as raw material of cellulosic ethanol caused great attention in the world. The waste water which containing some of cellulose, lignin and semi fiber is very difficult to be used in the production of cellulose. The condensed water is a kind of harmful wastewater that containing a large amount of volatile acid, furfural and volatile phenol in the production process. The water quality analysis and treatment method of cellulosic ethanol wastewater were carried out. The removal mechanism of white-rot fungi on cellulose, hemicellulose and lignin, and the removal effect of and the effect of residence time and pH value on the removal of COD and the change of lignin content in the treatment of wastewater were introduced. And the removal mechanism of rumen microorganism was introduced, and the degradation of cellulose and the inhibition of the degradation of and the relationship between the changes of COD and the biogas production were also tested in real time. After two kinds of different methods of biological treatment wastewater, in order to carry out the analysis and comparison, it is concluded that the degradation law of microbial treatment of wastewater in the production process of cellulosic ethanol wastewater can provide guidance for industrial wastewater treatment.

Keywords: COD, Cellulosic ethanol, biological oxidation, white-rot fungi; rumen microorganism.

Introduction

Cellulosic ethanol wastewater: In recent years, the society is facing a serious energy crisis, to develop fuel ethanol as a substitute for fossil fuels has become a broad consensus in the world[1]. In terms of raw materials for the production of fuel ethanol, ethanol production that based on corn, wheat and other grain crops will exist the problem of food, more and more people have begun to question the social benefits of ethanol. Therefore, from the long-term development perspective, the wood cellulose material which including wood waste, crop straw is the most promising fuel ethanol production of raw materials.[2]

Fig. 1: Corn straw

At present, the technology of producing fuel ethanol with cellulose as raw materials has been basically mature.[3] The principle is to use the raw materials like straw and other rich in cellulose by pretreatment to get the reducing simple sugars, such as glucose, xylose, etc. Fuel ethanol can be obtained from reducing simple sugars after fermentation, distillation. The remaining distillation of the waste water is the cellulose ethanol wastewater[4].

Analysis on the quality of cellulosic ethanol wastewater: Because the straw chemical structure of cellulose ethanol raw materials is complex, cellulose and hemicellulose are not only wrapped by lignin, and the combination of semi cellulose and lignin, cellulose with highly ordered crystal structure. Therefore, it must go through pretreatment, the destruction of the crystal structure, and the reduction of the degree of polymerization, the pretreatment process can be treated by steam explosion, in the temperature range of 170-220, the reaction time is 2-20min, the added water that before using a weak acid or weak alkaline solution soaking biomass is non-mandatory. After steam explosion treatment, the waste water contains cellulose and hemicellulose, acetic acid, 2 - sugar aldehyde and vanillin, vanillic acid, fragrant high oxalic acid, acetic acid clove ketone, syringic acid, syringaldehyde, eugenol and phenolic substances and so on.[5]

The composition of cellulose chemical The composition of is quite simple, chain macromolecular compound can be obtained by the connection between $\beta-1$ and 4-glycosidic bond linkage from D- glucose, the repeating unit of cellobiose is shown in fig. 2.

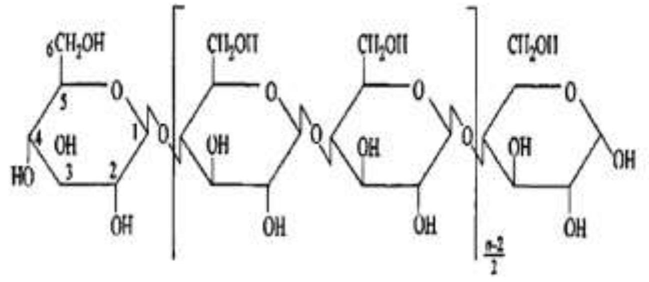

Fig. 2: Chemical structure of cellulose

Cellulose is a B-glucan that made by the dehydration of glucose units, which is the main structural component of the plant cell wall, generally about 35% to 60% of the weight of the wood cellulose material. Although the cellulose to glucose as the basic unit, but its nature is much different from starch, cellulose is a kind of structure that can not afford to be more nutritious. The degradation of cellulose was carried out under the catalysis of cellulase (cellulase). Some microorganisms, including fungi, actinomycetes, bacteria, and some bacteria in the rumen of the digestive system of ruminant can produce cellulase, so they can degrade and digest cellulose.[6]

Semi cellulose refers to the non - polysaccharide that connected with the cellulose in plant tissues, which is usually about 20% to 35% of the total of the cellulose material, the vast majority of the hemicellulose is mainly based on D. Arabinose. The composition of pentose (five-carbon) sugar polymer as the units of Xylose and D. Arabinose, but there are always very small amounts of hexose (Liu Tan) monomers, such as D glucose and D mannose. Many strains produce both cellulase and hemicellulase, and the change of the structure and composition of the semi cellulose is very large, which is generally composed of 200 short and highly branched polysaccharide chains. Such as xylan, grapes. Mannan, Arab - xylan, half milk - grapes – mannan and so on, Most of these are connected by $\beta-1$ and 4-glycosidic bond. Containing five carbon sugar (usually the D-xylose L. arabinose), six carbon sugar (D galactose, D-glucose and D. mannose) and uronic acid. [7]

Lignin is a cross-linking and complex polymer that uses variously substituted p-values as the basic unit, usually accounts for lignocellulosic material weight of 10% - 30%[8]. The function of lignin is considered to be a kind of pretreatment process of lignin (which may be very suitable for the wet oxidation process of the invention, while before the structure of the cellulose and semi cellulose in the cellulose material of the wood cellulose was destroyed by direct biological conversion (e.g., fermented bacteria), it is a natural obstacle. The lignin is a complex, which is composed as the units of benzene propane, and it is connected with a nonlinear and random way. They are connected with the complex and non - shaped three-dimensional structure by the ether bond and carbon-carbon bond and shown in fig. 3.

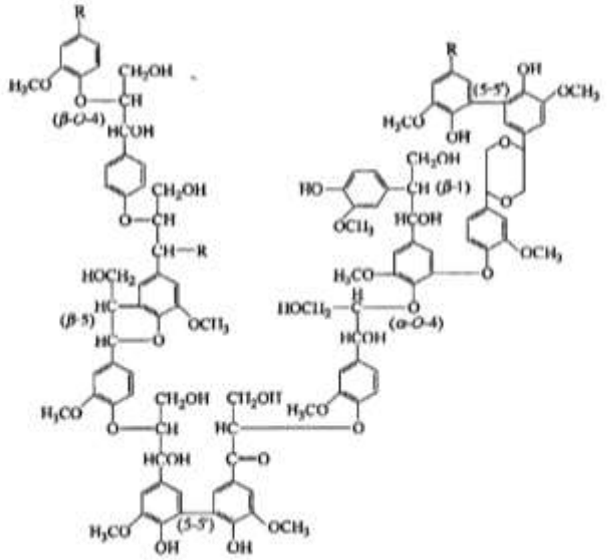

Fig. 3: Molecular structure of lignin

Treatment method of cellulosic ethanol wastewater: For organic wastewater, the domestic and international methods mainly include physical and chemical treatment, biological treatment etc.[9]

Physical and chemical treatment method is using physical and chemical synthesis to purify the wastewater. This is the wastewater treatment system that composed by physical and chemical method, or physical and chemical processes of a single processing method, such as flotation, adsorption, blowing off, crystallization, extraction, electrodialysis and electrolysis, reverse osmosis, ion exchange and so on. Chemical coagulation that used to remove the suspended and dissolved pollutants, it is a comparatively typical physical and chemical treatment system, which is used in the treatment of two stages of precipitation and activated carbon adsorption. [10]

Biological treatment process is mainly anaerobic biological treatment, aerobic biological treatment and anaerobic aerobic combination process. Anaerobic treatment is an effective technology to remove organic pollutants in the anoxic conditions. It can transform organic pollutants into methane and carbon dioxide and other gases (collectively referred to as methane). Anaerobic reactor can be divided into anaerobic suspended growth process and anaerobic contact growth process. The anaerobic reactor is divided into the following types: (1)common anaerobic digester; (2) anaerobic contact process; (3)liters flow anaerobic sludge bed (UASB) reactor; (4)anaerobic filter; (5): anaerobic granular sludge expansion bed (EGSB); (6)anaerobic fluidized bed reactor and anaerobic compound reaction. Aerobic biological treatment process is mainly the traditional activated sludge process and the 70's development of alternative processes, such as SBR, oxidation ditch, deep well aeration and biological contact oxidation process etc.[11]

This paper uses the following methods: white-rot fungi and rumen microbial treatment. White-rot fungus is a kind of large fungus which has strong ability to degrade lignin in nature, and it is the most found filamentous fungus with the ability to degrade. So the degradation ability of activated sludge by using white-rot fungus treatment is enhanced. At the same time, the real-time test of the rumen micro organism to deal with the cellulose material, the inhibition of the degradation of the material and the relationship between the COD changes and the gas. After two kinds of different methods of biological treatment wastewater, in order to carry out the analysis and comparison, it is concluded that the degradation law of microbial treatment of wastewater in the production process of cellulosic ethanol wastewater can provide guidance for industrial wastewater treatment.

Mechanism and experiment of the removal of cellulose, hemicellulose and lignin by white-rot fungi: White-rot fungus (White-rot fungus) is a kind of peculiar filamentous fungus, it can release the enzymes that degrade lignin and other components (cellulose, hemicellulose, pectin, etc.), so the lignin and cellulose, hemicellulose can be degraded. [12]

White-rot fungi can secrete ligninolytic enzymes, at the same time, it can also secrete cellulase, hemicellulase and pectinase enzyme, it can be completely degraded wood for CO_2 and water at the same time, and also can degrade cellulose and hemicellulose, so it is more suitable than wood mold, Aspergillus and other natural decomposition of lignocellulosic materials.White-rot fungi mycelium is multi nuclear, there are few diaphragm, no lock shaped joint, multi core of conidia is often the heteronuclear, but spore is homokaryon, there are two kinds of mating system for homothallism and heterothallism. White-rot fungus is a single electron oxidation, CO metabolism, and lipid peroxidation in order to carry out the degradation of white-rot fungus through the secretion of non specificity of the lignin degrading enzyme system. [13]

The products that the white-rot fungi degrades lignin contain carbon, hydrogen, and methoxy, they are less than the corresponding protolignin, while it contains more oxygen, carbonyl, the aromaticity decreased, $\alpha\beta$ unsaturated hydrocarbons increased, but the molecular weight and protolignin are almost the same. According to analyzing the lignin degradation products, it can be conluded that the lignin degradation consists of the following steps:(1) the demethylation and hydroxylation to form the oxygen adding polyphenol structure; (2) polyphenol ring cleavage, produce the hydrolysis of aliphatic hydrocarbon hydrocarbon; (3) Hydrolysis to shorten the fatty hydrocarbon. According to analyzing the lignin degradation products, it can be conluded that the lignin degradation consists of the following steps:(1) the demethylation and hydroxylation to form the oxygen adding polyphenol structure; (2) polyphenol ring cleavage, produce the hydrolysis of aliphatic hydrocarbon hydrocarbon; (3) Hydrolysis to shorten the fatty hydrocarbon. The enzyme system is in the cell or in the cell wall, including: (1) H_2O_2 generating enzymes system; (2) according to the 0_2, H_2O_2 lignin enzymes, to produce lignin free radical and quinone; (3) the lignin activated intermediates reduction to form a stable monomer quinone reductase system; (4) ring cleavage reaction occurs in the wood element in intracellular small molecule fragments, through the TCA loop, the $C0_2$ decomposition product is generated by decomposition product.[14]

Biodegradation of lignin by white-rot fungi: The degradation mechanism of white-rot fungi to lignin is as follows: the degradation of the starting conditions, the degradation of the main enzyme system, the degradation of the chain reaction to the free radical, and the degradation activity was carried out in the cell.

Biodegradation mechanism of white-rot fungi on cellulose: White-rot fungi which can degrade the lignin can produce the related enzymes that degrade the cellulose when there has cellulose.

Mainly by $\beta-$ glucosaccharase, endo (circumscribed) -1, $4-\beta-$ glucanase and two cellobiohydrolases, the endo glucanase plays role within the cellulose polymer, the point of action is in the interior of the cellulose polymer, then cutting the non crystalline part of the cellulose , thus the new free end is generated, then by the hydrolysis of the external cutting type of the enzyme from the end of the fiber and using the fiber two sugar as the unit. In the end, the two sugar was hydrolyzed into two glucose, by the $\beta-$ glucosaccharase and the enzyme was degraded by the synergistic action. Its degradation process is as shown in fig. 4.[15]

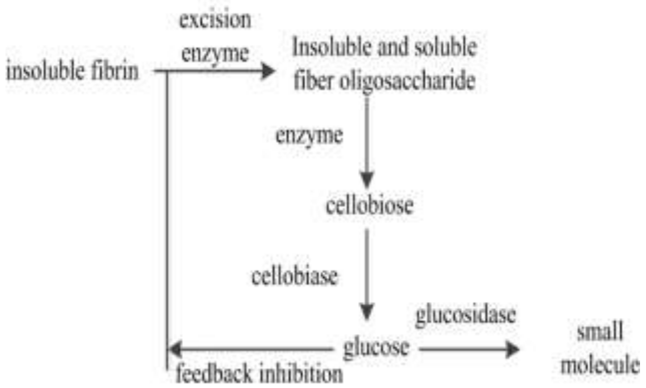

Fig. 4: the biodegradation of cellulose

The biodegradation mechanism of white-rot fungi on semi cellulose: Most of the white-rot fungi which have the ability of decomposing cellulose can decompose hemicellulose, and have a higher activity of extracellular enzymes. The semi cellulose macromolecule chain was first decomposed into a short chain of the enzyme (the Poly - enzyme and xylanase), and then was hydrolyzed to the sugar and by sugar Pu enzyme.[16]

Mechanism of hydroxyl radical and OH degradation of the wood cellulose: White-rot fungi in the degradation of the process of the production of hydroxyl free radicals ·OH has a very strong oxidation ability, can penetrate the cell wall, the division of cellulose and lignin, can make cellulose and phenol oxidase to enter and effect with cellulose and lignin directly. In addition, ·OH can change the lignin to produce new phenols, making it easier to be degraded by laccase or MnP. In addition, it can also destroy the crystalline structure of cellulose, make it more easy to be hydrolyzed. [17]

On the research of lignin model compounds shows that ·OH can attack the wood in the aromatic ring, various hydroxylated and ring opening reaction, but at room temperature can not obviously prolapse wood lignin. · OH to capture a hydrogen atom from a polysaccharide material, such as cellulose or hemicellulose, which has a very high and stable rate (about 109M-1SeC-1), these reactions produce a carbon centered transition state free radicals, they have a rapid reaction with O_2 to generate $ROO\cdot$ that is:[18]

$$Fe + H_2O_2 \rightarrow Fe^{3+} + OH^- + \cdot OH$$

$$RH + \cdot OH \rightarrow R\cdot + H_2O$$

$$R\cdot + O_2 \rightarrow ROZ\cdot$$

If the peroxide free radicals in the same carbon atoms to seize OH ,this will produce $\cdot OOH$: if it does not contain $a - OH$, in the role of the oxidation reduction, this will lead to some of the bond cleavage, the reaction will produce a mixture of. Natural cellulose by - OH attack will also occur the above reaction. In this way, the hydrogen bonds formed by the hydroxyl group will disappear, and the chain force becomes smaller and the structure becomes loose, so that the crystal of natural cellulose tends to be amorphous, and the catalytic degradation of other enzymes is easy.[19]

In summary, the hydroxyl radical generation system and its mechanism for the role of the mechanism of the wood cellulose: in the white-rot fungi, there is a mechanism for the production of reactive oxygen species (ROS) participation. When the cell wall was first degraded, the mycelium could secrete extracellular substances as electron donor. The material was very small, and could be infiltrated into the cell wall. The electron donor and the O_2 asked for catalytic oxidation and reduction reaction. O_2^- was generated by H_2O_2, and then H_2O_2 was reduced to $\cdot OH$, and $\cdot OH$ was degraded. The C-C bond, ester bond, hydrogen bond, etc., or the end of their oxidation, which can make the lignin polymer and cellulose polymer is further oxidized by other enzymes. [20]

White-rot fungi in the water purification process: White-rot fungi is seen as the most effective degradation for lignin, natural lignin is mainly completed the decomposition of white-rot fungi of basidiomycetes. [21]

In this project, learning from Cordon and others with alginate-embedded white-rot fungi ways to strengthen wastewater treatment.To mix a certain concentration of sterile sodium alginate solution with a white-rot fungus spore suspension, using a needle to the mixed liquid drop in a certain concentration of CaCl2 solution, crosslinking after a certain period of time. The prepared sodium alginate beads were washed with sterile distilled water, that is, the immobilized white-rot fungus was prepared.

The experiment of wastewater by immobilized white-rot fungi analysis method, COD was measured by dichromate method (GB11914-1989). The immobilized white-rot fungus was frozen at -10℃by 3h, and then dried at room temperature and then kept in a cool dry place. After saving 15d, the process of aeration was simulated in wastewater by 0.19/L. The activation temperature is 25℃.

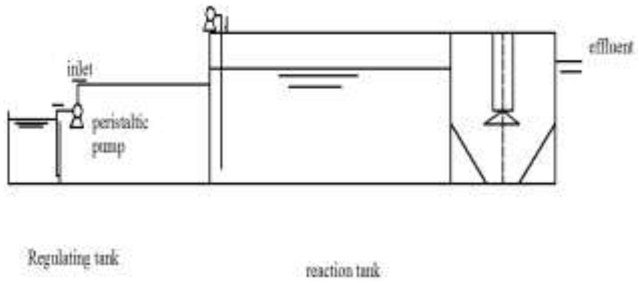

Fig. 5: Schematic diagram of experimental apparatus

Experiment process: wastewater is treated by the filter to remove coarse debris, in the regulation of water, the raw water can be diluted six times, the concentration of COD is about around 2400 mg/L, by a peristaltic pump for metering and upgraded to a reaction tank of aerobic reaction, setting aeration pump. Gas water mixture into two sedimentation tank sedimentation separation, supernatant night discharge. In the aerobic reactor, the immobilized white-rot fungus and the white-rot fungus were suspended in the control, and a good oxygen reactor was added to the immobilized white-rot fungus, and the other aerobic reactor was added to the white-rot fungus. The dosage of 50mg / L as a suspended state. The size of reactor if 1000mm*500mm*400mm, effective volume is 1.50L.

Test items and methods: CODcr selects chromium determination method CODer; the method of turbidity measurement is WGZ-200 type photoelectric turbidity meter; the method of pH value determination is PHS - 25 type digital acidity meter.

Lignin was determined according to the linear relationship between the concentration of lignin and the absorbance of lignin, and the absorbance was determined by UV spectrophotometry at 340nm wavelength. The concentration of lignin can be used to quantify the degradation ability of lignin by white-rot fungi through comparing the changes of COD value to check standard curve.

The production of standard curve is to use 0.05g crude alkali lignin, soluble in 1000 mL distilled water. Then configuring different gradient concentration of the solution. The concentrations were 20%, 40%, 60%, 80%, 100% respectively, and the absorbance of the lignin at different concentrations was determined by 340 nm.

The test results of the white-rot fungus: (1)Adding the immobilized and suspended white-rot fungi on the removal of raw water.

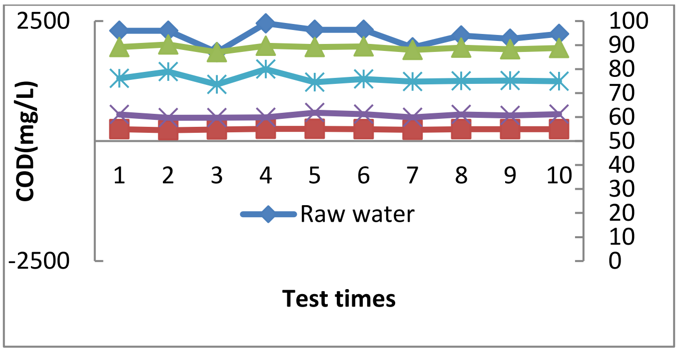

Fig. 6: The effect of COD removal by suspended and immobilized white-rot fungus on raw water

From this graph, it is can be seen that maintaining water quantity 6.0L/h, when the concentration of COD was fluctuating between 1895-2462 mg/L, The COD concentration in the effluent of the white-rot fungus was 490-590 mg/L, and the removal rate of COD was about 76%. Immobilized white-rot fungus was added at 0.19 to 1000 mL ratio, and the effluent concentration of COD was 201 ~ 254 mg/L, the removal rate of COD was about 90%. It can be seen that the addition of immobilized white-rot fungus, COD removal rate can be increased by about 15%.

(2)The effect of residence time on treatment.

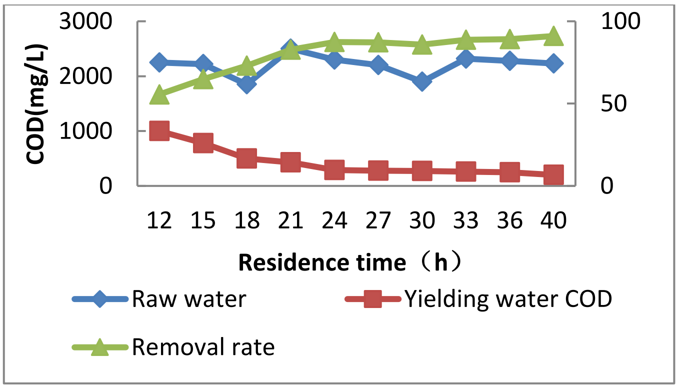

Fig. 7: Residence time on the removal of raw water COD

From Fig.7, in the case of maintaining the amount of the amount of bacteria. It increased with the retention time of the immobilized white-rot fungus, when the white-rot fungus immobilized residence time (HRT) is at 24hours, COD removal efficiency reached 91.5%. Increase the time of immobilized white-rot fungi, the increase of COD removal rate is not significant, stabilized at a certain value， if to continue to increase the immobilized white-rot fungi residence time, the total removal of the effluent CODcr not make much sense. For the device, the extension of HRT is to reduce the amount of equipment, increase the volume of equipment, increase the one-time investment cost, so the immobilized white-rot fungus residence time (HRT) is 24h, the effect is better.

(3) Effect of pH on treatment

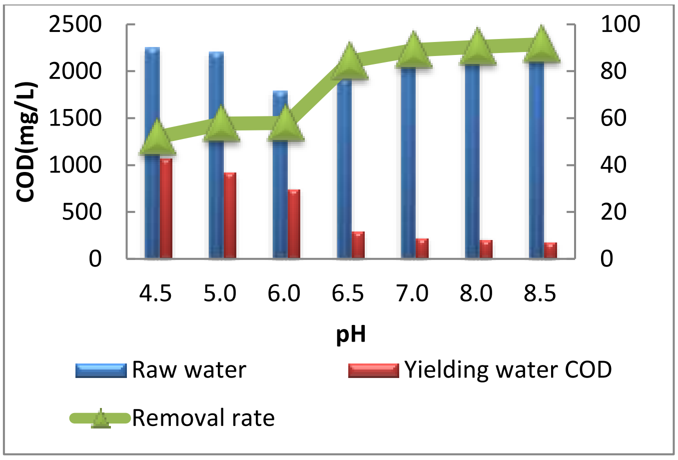

Fig. 8: pH of immobilized white-rot fungi COD removal efficiency

From this graph, it can be seen that when the pH is 8.5, immobilized white-rot fungi in the COD removal rate was the highest, reached 92.6%, the effluent of COD is 160 mg / L.

(4) Changes in lignin content
The standard curve was made with the ultraviolet spectrophotometer to determine the absorbance of lignin solution in 340nm wavelength, the correlation coefficient of the curve was 0.991. And according to the standard equation: $y=0.0235x+0.0045$ and from two different systems regularly measured OD, the two systems in different time of lignin concentration changes can be calculated.

In the determination of the change of absorbance: in different amount of white-rot fungus inoculation, the changes of lignin in the two systems of the immobilized and suspended state are shown in Figure 9 and figure 10. From Figure 9, it can be seen that the absorbance value of the two system have been decreased, which show that the lignin content of the two systems are not stopped. However, from the point of view of the change of the numerical value, the suspended state system treatment effect should be inferior to the immobilized system which also reflects the advantages of immobilized in the treatment of fiber ethanol wastewater.

It can be seen from the Table.1 and Figure.10, the lignin concentration of the two systems has decreased gradually until almost to be stable, while the immobilized lignin concentration system decreased rapidly and greatly.

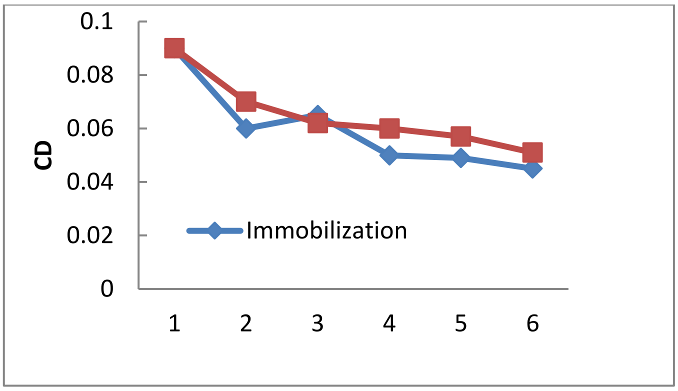

Fig. 9: The change of OD over time

Table 1
Immobilized lignin concentration changes and suspended state system (× 10^3g / L)

Time (d)	**1**	**2**	**3**	**4**	**5**	**6**
stationary state	6.61	5.92	6.01	5.65	5.62	5.54
suspended state	6.61	6.12	5.94	5.90	5.80	5.70

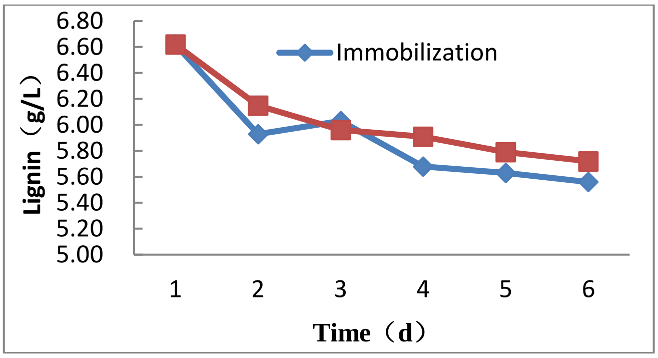

Fig. 10: Immobilized lignin concentration changes and suspended state system (× 10^3g / L)

Removal mechanism and experimental results of cellulose, hemicellulose and lignin in rumen microorganisms

Rumen bacteria species and their relationship with each other: In 1850, Mitcherlich was observed in 1945, and Seilliere was found in 1906 in the digestive juice of snail. And cellulase was found in natural cellulose in 1945.After a few years, it was noted that rumen microorganisms could secrete cellulase, and have a very strong ability to degrade cellulose.

The rumen microorganism is based on heterotrophic anaerobic bacteria, from the point of view of Microbiology, rumen microbes include bacteria, fungi, and bacteria, and ancient bacteria. The microbial types in the three stage of anaerobic digestion of organic wastes were found in rumen fluid, which was found in the rumen of bacteria, acid bacteria, acetic acid bacteria and methane producing bacteria. Microorganisms in the rumen is a very complete nature and organic substance transformation ecosystems. General anaerobic digestion has the problem of product inhibition, but because the rumen microorganism is a compound bacteria group, the product of one kind of reaction is often the other kind of reaction, the substrate inhibition phenomenon is eliminated. [22]

In the whole ecosystem of rumen, the dominant place is the rumen bacteria in cellulose digestion. Cellulose digestion by rumen fungi although is better than that of fine pour, but due to its content that in the rumen is low, so the cellulose digestion total effectiveness is quite low. [23]

The interaction of cellulase and enzyme reaction: Cellulase is generally divided into three categories, endo cellulase, cellobiohydrolase enzyme and cellobiase. The interaction between the various enzymes in the rumen. The composite of rumen microbial cellulase enzymes, including intracellular and extracellular enzymes. These enzymes have different activities for different substrates. According to Wilson (1992) published in the Neocallimastix frontali.s, RK21 filtrated out at least 3 different enzymes. The activity of cotton fiber is mainly composed of a kind of high molecular compound, which has the activity of glucose enzyme activity, High molecular compounds of $\beta-$ glucosidase activity, and the other enzymes. In the process of dissolution of crystalline cellulose, there is a synergistic effect between the components of the high molecular compound and the low molecular weight of the glucose enzyme and $\beta-$ glucosidase. In the condition of no influence on the activity of the glucose enzyme and the activity of the $\beta-$ glucosidase, the crude filtrate of the feed solution with chitosan was completely destroyed by the activity of crystalline cellulose. An enzyme involved in the degradation of crystalline cellulose than glucose enzyme or B. Glucosidase more thermal instability, it has the vitality stronger when the PH value is 6.0 and the temperature is 40 °C. [24]

The interaction of cellulase and enzyme reaction: The degradation rate constant was positively correlated with the specific surface area. Some main degradation are related to the total surface area and has less influence on the area of capillary table, digestibility and retention time. The reaction rate of the substrate is slightly faster than that of the substrate after the expansion of the micro crystalline fiber by 72%-77%. Although the crystallinity of the treated fibers decreased and the void fraction increased, the reaction rate was slower with higher concentration of phosphoric acid. The decline of the fermentation rate is obviously because the type of fiber is converted from type I to homogeneous II, because the fermentation rate of type II cellulose is also significantly slower, and also to experience a longer retention time. And the rumen microorganisms in the fiber cell structure is not easy to adapt to the transformation. The speed of alkali cellulose (II) fermentation is slow. [25]

Net water test of rumen microorganisms: Ruminants can use fibrous plant nutrition elements effectively, which rely on a large number of microorganisms in the rumen, including bacteria, archaea and bacteria, protozoa and fungi. The rumen of the ruminants can provide a suitable environment to maintain the number of different kinds of microorganisms and the activity of various microbial enzymes. The internal environmental factors of maintaining the number of microorganisms in the rumen, including constant temperature, constant pH value and hypoxia condition. The temperature range is 38-42, and the oxidation reduction potential (Eh) is 250-450mv.

This indicates that the oxygen content in the rumen is lower. PH influences the rumen ecosystem by affecting the growth efficiency of bacteria and several main protozoas in the rumen. The pH value of rumen is greatly affected by food ingredients. The pH value of rumen is generally 6.2-7.0, foraging fine feed as food for the pH value is between 5.5-6.5, and some cases are also lower than 5.5. It can be seen from the process of the decomposition and using fiber material, bacteria and fungi play about 80% of the role, protozoa plays an important role balance control. For ruminants that with low-quality roughage-eating, rumen cellulose decomposing bacteria play a very important role in the nutrition of the host animal.

The anaerobic reaction is carried out in the reaction tank, the pollutants are removed by anaerobic digestion, the sludge is separated and the supernatant is discharged. To the rumen microorganism in the anaerobic reactor with the blank control, and one of the anaerobic reactors were inoculated with the sludge, as a blank; another anaerobic reactor inoculated with sludge and to add the rumen microorganisms.

The reactor size 400mm*450mm*900mm, which is made of organic glass, the effective volume of 120L. In the test items and methods, the CODcr selects chromium determination method CODer, and the turbidity was determined by the WGZ-200 type photoelectric turbidity meter. The PHS-25 type digital pH meter was used in the determination of pH.

Test results of rumen microorganisms

(1) the effect of adding rumen microorganisms on the removal of raw water.

From this graph, it is can be seen that keeping the influent water as 2.5L/h, the concentration of influent COD fluctuated between the 7810-8620 mg/L ,the blank effluent COD is between 3052-3845 mg/L, removal rate is between the 54%-63%, When the COD was added to the rumen microorganism, the water was fluctuated between 2001-2662mg/L, removal rate is about 70%, thus can be seen that adding rumen microbes can improve the COD removal rate, Mainly due to the degradation rate of cellulose was improved by the microbial enzyme system in the rumen.

(2) The effect of temperature on removal
The effect of temperature on the removal of COD is shown in fig.13.

From Fig.13, it can be seen that the removal rate of 71%, the COD concentration of the treated effluent is 2436mg/L. The removal rate decreased with the decrease of temperature.

(3) The effect of pH on removal

From fig.14, it can be seen that when the PH is 8.5, the removal rate of rumen microbial anaerobic bacteria COD is the highest, reaching 73.5%, the COD concentration of the treated effluent is 2240mg/L.

(4)The effect of residence time on the removal of COD

From Fig.15, it can be seen that anaerobic removal rate increases with the growth of rumen microbial residence time, when the microbial residence time (HRT) is 48h, the removal rate of COD reaches 72%, increasing the retention time of rumen microorganisms, the removal rate of COD is not very large, and to be stable in a value, if continuing to increase the residence time of rumen microbial, the total removal rate of CODer is not much significance. For anaerobic device, the extension of HRT is to reduce the amount of equipment, to increase the volume of equipment and the one-time investment cost, so when the microbial residence time (HRT) is 48h, the effect of test device is better.

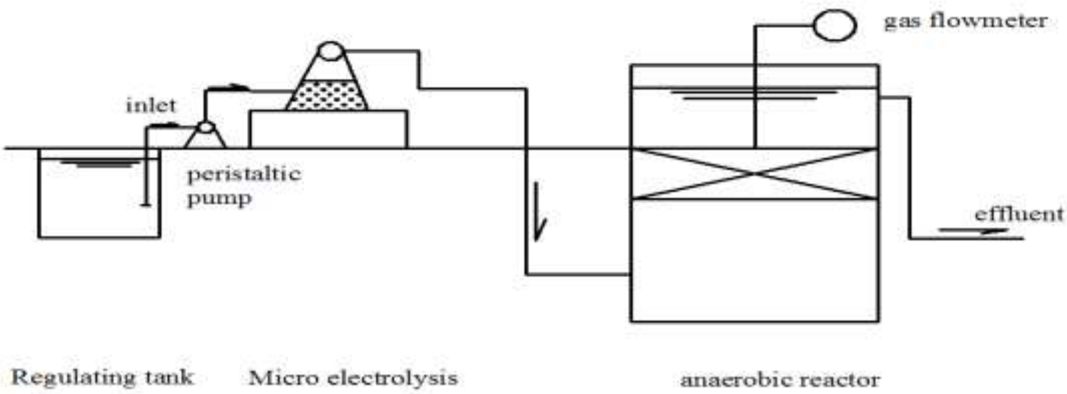

Fig. 11: Test flow chart

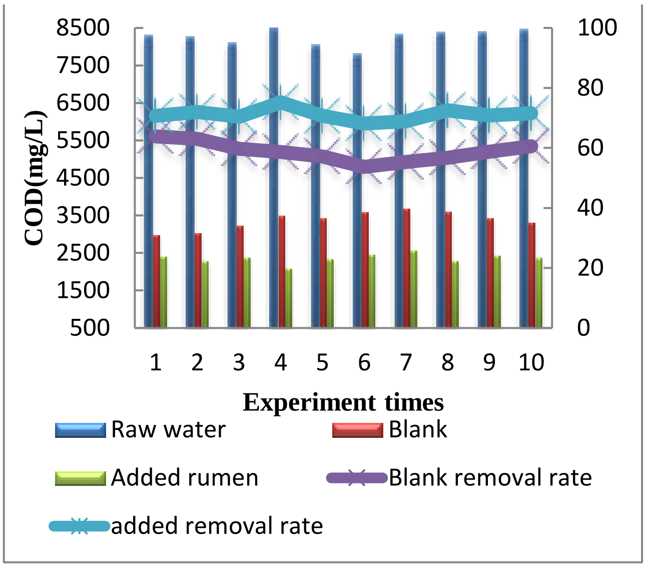

Fig. 12: The effect of COD removal by rumen microorganisms on the raw water

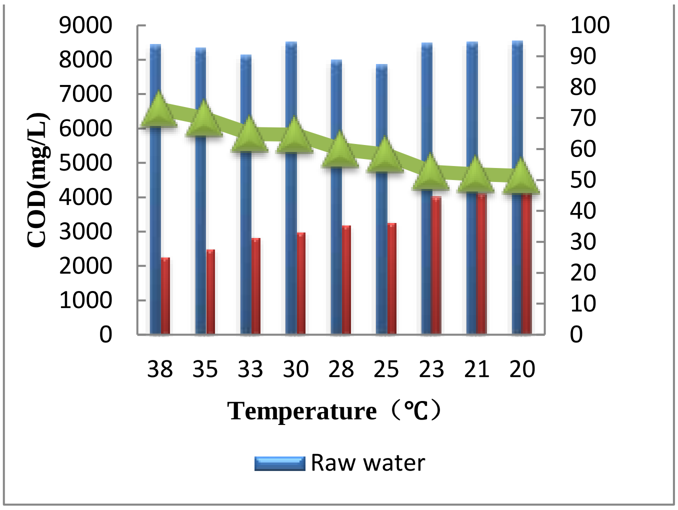

Fig. 13: The effect of temperature on removal effect

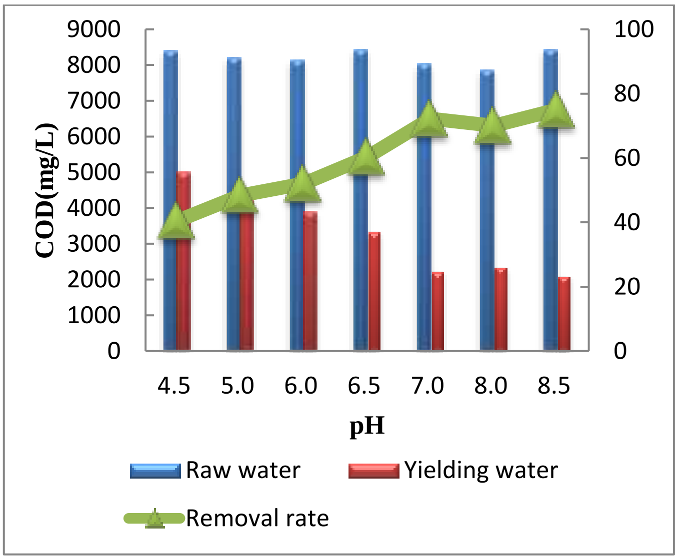

Fig. 14: Effect of pH on the removal of rumen microorganisms

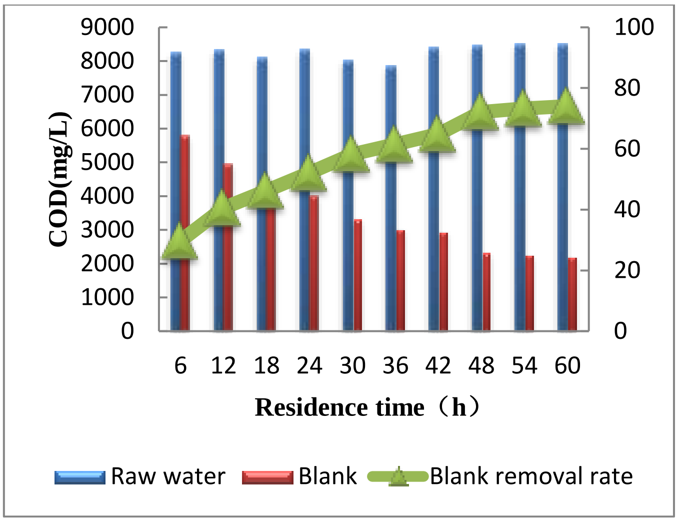

Fig. 15: The effect of rumen microbial residence time on the removal of COD.

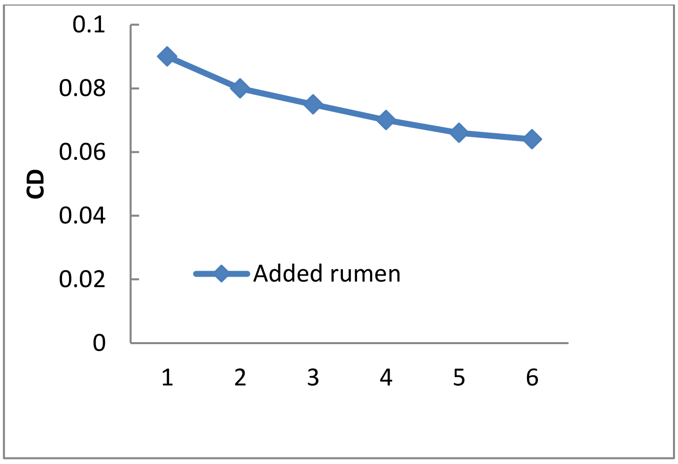

Fig. 16: OD changes with time

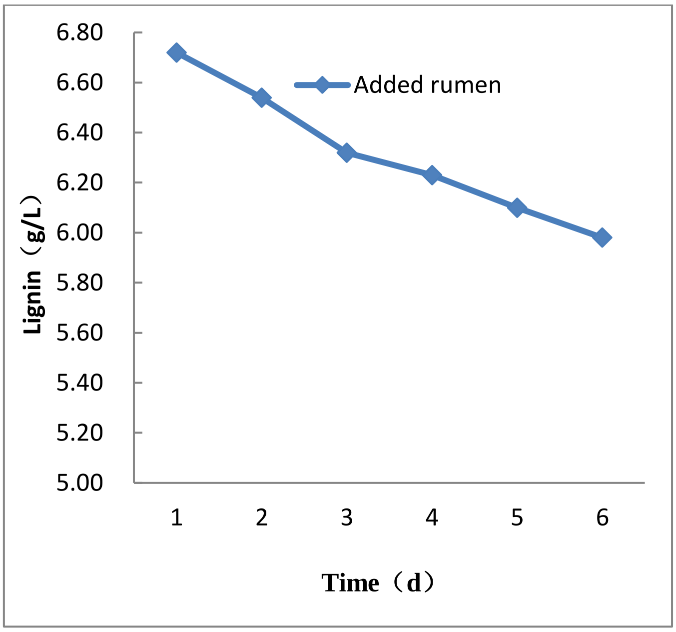

Fig. 17: Change in quality (*10^{-3}/L)

(5)Changes in lignin content

The absorbance of lignin solution in 340nm wavelength was determined by UV spectrophotometer, and the standard curve was obtained, and the correlation coefficient was 0.991. The change of absorbance was determined: both of the lignin content of the two is kept decreasing. However, the decline of lignin is not too large, which indicates that the degradation of the rumen microorganisms in the low efficiency of lignin.

Test result analysis

Comparison and analysis of raw water removal: In the experiment of removing the raw water from rumen microorganism, the blank removal rate of COD was between 54%-63%; while adding rumen microbial, the removal rate of COD was about 70%; in the experiment of the removal of raw water with suspended state and immobilized white-rot fungus. the rate of the added suspended white-rot fungus COD removal is around 76%, the rate of immobilized white-rot fungi COD removal is around 90%. As can be seen from Table 3, the rate of immobilized white-rot fungi COD removal is highest.

Table 3
Comparison and analysis of COD removal effect of raw water

Type	COD removal rates
blank	54%-63%
rumen microorganism	70%
Suspended white-rot fungus	76%
Immobilized white-rot fungus	90%

The reason for the difference of COD removal rate of suspended state and immobilized white-rot fungus is that the wastewater contains some cellulose, hemicellulose, lignin, and is not easy to be degraded, and the immobilized white-rot fungus can be adsorbed on the carrier. It is not easy to lose, the degradation of the waste water, the suspended state in the process of degradation is easy to lose, so that the number of white-rot fungi and the removal rate decreased.

Effect of pH on removal of COD: In the experiment of COD removal efficiency of rumen microbial pH, pH was 8.5, and the removal rate of COD was the highest, reaching 73.5%. In the test of pH on COD removal effect of immobilized white-rot fungus, when pH was 8.5, the removal rate of iimmobilized white-rot fungus COD was the highest, reaching 92.6%. This indicated that the highest COD removal rate was achieved at the time of pH=8.5, but the removal rate of COD was significantly higher than that of the former.

Effect of residence time on removal of COD: In the experiment of COD removal efficiency, when the rumen microbial residence time (HRT) was 24h, the removal rate of COD was 56%, and the removal rate of COD reached 72% when the rumen microbial residence time (HRT) was 48h. The removal rate of COD increased with the increase of rumen microbial residence time. When the immobilized white-rot fungus residence time (HRT) was 24h, the removal rate of COD reached 91.5%. and the removal rate of COD increased by a little, and was stable at a certain value. Thus, the fixes white-rot fungus was more efficient in the removal of COD.

Changes in lignin content

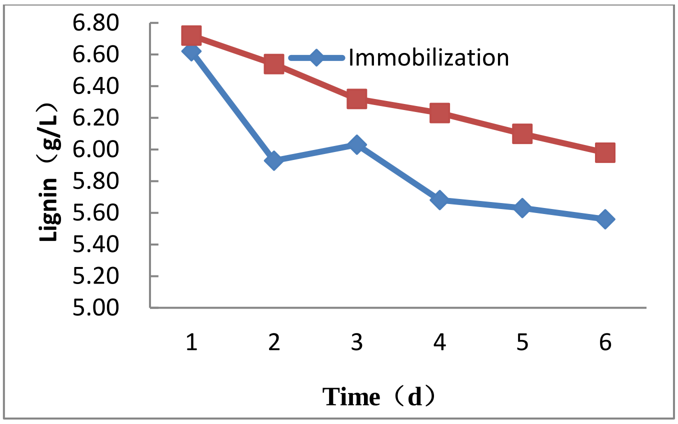

Fig. 18: The degradation of lignin by white-rot fungi and rumen microorganisms

From fig.18, it is obvious that the efficiency of the immobilized white-rot fungi is higher than that of the rumen microorganisms.

Conclusions

The composition and characteristics of the fiber ethanol wastewater were analyzed, and the laboratory tests were carried out to get the following conclusions:

(1)Using immobilized white-rot fungi to cellulosic ethanol steam blasting plate and frame filter liquid to carry on experiment, pH 8.5, residence time (HRT) 24 h, the rate of COD removal reached 91.5%, the effluent COD is 160 mg / L. At the same time, comparing the effect of whether adding the white-rot fungi, it can be seen that the addition of a certain amount of white-rot fungus, the removal rate increased by 15%, the COD concentration of 210-254 mg/L, the removal rate was about 90%.

(2)In the experiment of the addition of rumen microorganisms to the waste water, the removal rate reached 71% when temperature was 38, and the effluent COD concentration was 2436mg/L. At the same time, compared with the rumen microbial test, to maintain the influent water to 2.5L/h, COD concentration was between 7810 ~ 8620mg/L. when COD was added to rumen microorganisms, the removal rate was between 54%-63%, when the COD was added to the rumen microorganism, the effluent fluctuated between 2001-2662mg/L, and the removal rate was about 70%. The removal of rumen microorganisms by white-rot fungi was compared. The removal rate of immobilized white-rot fungus in COD and lignin was obviously higher than that of the rumen microorganisms. The results of this paper can be used in the process of industrial wastewater treatment process in the process of the production of cellulosic materials.

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