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# Biodegradation Behavior of Starch in Simulated White Water System of Old Corrugated Cardboard Pulping Process

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**Abstract:** Considering the serious barriers/issues induced by the accumulated starch generated in white water system of old corrugated cardboard (OCC) pulping process, large amounts of accumulated starch in white water would be decomposed by microorganisms and could not be utilized, thereby resulting in severe resource wastage and environmental pollution. This study mainly explored the effects of biodegradation/hydrolysis conditions of the two types of starch substrates (native starch and enzymatically ( $\alpha$ -amylase) hydrolyzed starch), which were treated via microorganism degradation within the simulated white water from OCC pulping system and their biodegradation products on the key properties were characterized via X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FT-IR), and gel permeation chromatography (GPC) technologies. The effects of system temperature, pH value, starch concentration, and biodegradation time on starch biodegradation ratio and the characteristics of obtained biodegraded products from the two

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types of starches were studied. In addition, the effect of  $\alpha$ -amylase dosage on the biodegradation ratio of enzymatically hydrolyzed starch and its properties was investigated. It was found that the native starch presented a maximal degradation ratio at a system temperature of 55 °C and pH value range of 5–7, respectively, the corresponding starch concentration within simulated white water system was 200 mg/L. Whereas the enzymatically hydrolyzed starch exhibited a highest degradation ratio at a system temperature of 50 °C and pH value of 5.5, respectively, and the corresponding starch concentration within simulated white water system was 100 mg/L. It was verified that native starch is more readily bio-hydrolyzed and biodegradation-susceptible by microorganisms in simulated white water system of OCC pulping process, while the enzymatically hydrolyzed starch exhibits better biodegradation/hydrolysis resistance to the microbial degradation than that of native starch. This study provides a practical and interesting approach to investigate the starch hydrolysis or biodegradation behaviors in white water system of OCC pulping process, which would greatly contribute to the full recycling and valorized application of starch as a versatile additive during paperboard production.

**Keywords:** starch; OCC pulping; white water; simulation; hydrolysis resistance; biodegradation behavior

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## 1 Introduction

Corrugated cardboard is one of the most important paper/paperboard products of pulp and paper industry and has been widely utilized in paperboard packaging industry owing to its various advantages, such as cost-effectiveness, low density, carbon neutrality, superior physical performance, and convenient recycling for fiber regeneration and reuse in pulp and paper industry <sup>[1]</sup>. The consumption demand for corrugated cardboard products in China and even all over the world has grown rapidly, which is mainly due to the fast development of logistics and transportation industry <sup>[2–3]</sup>. Owing to the critical shortage of forest resources in China, the available raw material for the production of corrugated cardboard is primarily old corrugated cardboard (OCC) <sup>[4]</sup>.

However, repeatedly recycling OCC leads to significant decrease of fiber length and strength, thus would degrade the physical properties of finished products prepared from recycled fibers <sup>[5]</sup>. Specifically, the wastepaper recycled from the domestic markets in China could produce degraded secondary pulp fibers with shorter fiber length, more fines, and serious fiber cornification phenomenon, which is not suitable for high-grade paperboard products <sup>[6]</sup>. Furthermore, by the

end of 2020, China have completely prohibited the import of solid waste, including foreign wastepaper that was usually used as raw fiber material for papermaking, which would further drive the overuse of low-quality wastepaper from domestic market and ultimately impair the physical properties of the paperboard products <sup>[7]</sup>.

Starch belongs to the abundant renewable polymers, it has been widely applied in pulp and paper industry in the processes of interlayer spraying, wet-end addition, paper surface sizing, and surface coating, to improve the physical performance of paper and paperboard <sup>[8]</sup>. Among all starch derivatives/species, enzyme hydrolyzed starch is especially and widely utilized for surface sizing during the production of corrugated cardboard <sup>[9]</sup>. Due to the serious restriction by the import ban initiated by China for foreign wastepapers, the paperboard manufacturers in China would prefer to harness more native starch or starch derivatives as additives/enhancers into pulp slurry or paper surface coating during paperboard production to improve the physical performance of paperboard products, thus compensating the degraded fiber strength and fiber shortages <sup>[10–11]</sup>.

As a result, paperboard manufacturers have reported that the usage of starch and starch derivatives in the

production of paperboard products has increased from 10 kg/t to 40–60 kg/t, which would induce the superfluous release of starch into the system and lead to serious threats during paperboard production<sup>[12]</sup>, such as (1) starch accumulation in the white water of OCC pulping process<sup>[13–14]</sup>; (2) explosion in microbial population within white water system<sup>[15–16]</sup>; (3) decrease in system pH value and deteriorated system stability, due to the presence of highly acidic hydrolysates produced by the degraded starch within white water<sup>[17–18]</sup>; and (4) most importantly, the serious starch biodegradation in white water system would result in unnecessary starch loss and increased COD/BOD level of wastewater for treatment<sup>[19–20]</sup>.

Several approaches have been proposed to address the potential threats. First, the starch substrates released in the white water system from OCC pulping process should be well prevented from microbial degradation hydrolysis and preserved in the white water system<sup>[21]</sup>. Second, the maintained starch substrates should be properly adsorbed or retained in pulp slurry during white water circulation, thus conserving the amount of fresh starch additives and improving the physical properties of final paperboard products<sup>[22–23]</sup>. The retained recycled starch in paperboard structure would improve the physical performance and save the fresh starch consumption, most importantly, reduce the pollution load of wastewater<sup>[24]</sup>. Therefore, studying the starch hydrolysis behavior in white water system of the OCC pulping process is a prerequisite or primary task to maintain and recycle the released starch substrates for their valorized applications.

In this study, microbial degradation process of starch was performed within simulated white water from the OCC pulping system to evaluate the biodegradation behavior of two types of starch substrates (native starch and enzymatically  $\alpha$ -amylase hydrolyzed starch) and further investigate the properties of their degradation products. In addition, the effect of  $\alpha$ -amylase dosage on the biodegradation ratio of enzymatically hydrolyzed starch and its properties was investigated. Specifically, the effects of system temperature, pH value, and

starch concentration on the degradation ratio and characteristics of biodegraded products from the two types of starch substrates were studied. The characterizations of X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FT-IR), and gel permeation chromatography (GPC) were carried out to research the properties of the two types of starch substrates and their biodegradation products.

## 2 Experimental

### 2.1 Materials

Native corn starch, starch derivatives, and OCC were kindly provided by Zhejiang Jingxing Paper Co., Ltd. OCC was recycled from domestic wastepaper in China. The enzyme  $\alpha$ -amylase was obtained from Shanghai Yi En Chemical Technology Co., Ltd. Iodine ( $I_2$ ) was purchased from Tianjin Guangfu Technology Development Co., Ltd. Potassium iodide (KI) was purchased from Tianjin Damao Chemical Reagent Factory. Hydrochloric acid (HCl) was obtained from Tianjin Sailing Chemical Reagent Technology Co., Ltd. Sodium hydroxide (NaOH) and dimethyl sulfoxide (DMSO) were obtained from Tianjin Jindong Tianzheng Fine Chemical Reagent Factory.

### 2.2 OCC pulping process for simulated white water system

The OCC sample (1000 g) was added to 10 L distilled water, followed by soaking for 4 h. Next, the soaked OCC sample was diluted with distilled water to produce a pulp slurry with 3% pulp concentration and subjected to a defibering process for 10000 rotations using a deflaker. The defibered OCC slurry was further diluted with distilled water to reach 1% pulp concentration and placed in a conditioned environment (30 °C and 50% humidity with direct sunlight) for 15 days to induce fully fermentation for further use<sup>[25–26]</sup>.

### 2.3 Preparation of $I_2$ -KI solution

A KI solution was prepared by uniformly mixing 400 mL of distilled water with the preweighted KI powder (20 g), followed by continuous magnetic stirring at 500 r/min. Once dissolved, 2 g of  $I_2$  was

added into KI solution under constant magnetic stirring at 500 r/min until complete dissolution, followed by transferring into a 1000-mL volumetric flask and stored in an amber glass bottle for further use.

## 2.4 $\alpha$ -Amylase hydrolysis treatment of starch

A dry corn starch sample (50 g) was placed in a three-necked flask, followed by adding 500 mL distilled water. The flask was placed in a water bath at 45 °C with magnetic stirring at 300 r/min for 5 min. A predesigned amount of  $\alpha$ -amylase solution was added into the flask, which was further subjected to incubation for 5 min under constant stirring at 500 r/min. Next, the water bath was gradually heated from room temperature and maintained at 90 °C for 30 min. After that, the system was cooled from 90 °C to 60 °C to obtain enzymatically hydrolyzed starch. The obtained product was oven-dried in a tin box at 105 °C for 8 h to obtain starch powder sample, which was further ground into fine starch powder with tiny particle size by using a mortar and stored in a plastic bag for further use<sup>[27–29]</sup>.

## 2.5 Full-wavelength UV scanning characterization

The fermented OCC pulp slurry was filtered to obtain the filtrate (white water). A certain amount of native starch was subsequently added to the above filtrate to obtain homogenous suspension, which was followed by adding 0.3 mL of I<sub>2</sub>-KI solution into 10 mL filtrate/starch suspension. After that, a centrifugation process was carried out to obtain the supernate solution. The obtained solution was analyzed using a full-wavelength UV spectrophotometer to measure its maximum absorption peak and referred as the background for the determination of starch concentration.

## 2.6 Determination of standard curves of starch solutions

The native starch and enzymatically hydrolyzed starch (1 g/L) were gelatinized at 90 °C for 30 min. Subsequently, a series of gelatinized starch solutions (10, 9, 8, 7, 6, 5, 4, 3, 2, 1, and 0 mL) were sucked out accordingly and added in a 10-mL volumetric flask to the constant volume by adding distilled water for precisely controlling the starch concentration. Next, 0.3 mL of I<sub>2</sub>-KI solution was added into the above

starch solutions to induce the color reaction of starch solution, followed by measuring their UV absorbance at 540 nm, to draw the standard curves for further use.

## 2.7 Effect of $\alpha$ -amylase dosage on starch biodegradation behavior

As for the effect of  $\alpha$ -amylase dosage on the starch biodegradation behavior, a series of  $\alpha$ -amylase dosages of 0.01%, 0.02%, 0.03%, and 0.04% were applied, the other procedures were followed as shown in Section 2.4 to obtain the fine enzymatically hydrolyzed starch powder treated with different  $\alpha$ -amylase dosages. Then the prepared fermented pulp slurry (500 mL) in Section 2.2 was taken out in a beaker, and added into 10 mL native starch solution with a concentration of 4%, followed by treating in a shaker at 45 °C and 150 r/min for 16 h. At the end of pulp/starch incubation process, the incubated pulp suspension was filtered to obtain the filtrate as white water system, followed by measuring its UV absorbance at 540 nm to calculate the starch concentration as background through the standard curves of starch solutions determined in Section 2.6. The filtrate was deemed as white water system from OCC pulping process and then divided evenly into four portions (70 mL of each portion). After that, the preweighted enzymatically hydrolyzed starch samples prepared with different  $\alpha$ -amylase dosages (0.01%, 0.02%, 0.03%, and 0.04%) above were added into the above four filtrate solutions, followed by measuring their UV absorbance at 540 nm. The four filtrate solutions were then shaken at 45 °C and 150 r/min for 4 h and 24 h reactions, followed by measuring their UV absorbance at 540 nm to determine the residual starch concentration, which can be applied to evaluate the starch biodegradation behavior within white water system of OCC pulping process.

## 2.8 Evaluation of the effects of starch concentration, system temperature, pH value, and biodegradation duration (0–24 h) on starch biodegradation behavior

To study the effect of starch concentration on starch biodegradation behavior within white water system, the prepared fermented pulp slurry (500 mL) in Section 2.2

was taken out in a beaker, and added into 10 mL native starch solution with a concentration of 4%, followed by treating in a shaker at 45 °C and 150 r/min for 16 h. At the end of pulp/starch incubation process, the incubated pulp suspension was filtered to obtain the filtrate as white water system, followed by measuring its UV absorbance at 540 nm to calculate the starch concentration as background. Subsequently, the filtrate was divided into 10 equal portions for further use. Then the native starch solution of 4% solid content with 0.5, 1, 1.5, 2, and 2.5 mL were added into the above five white water systems, respectively, the enzymatically hydrolyzed starch solution of 4% solid content with 1, 2, 3, 4, and 5 mL were added into the other five white water systems, respectively. Before reaction, the UV absorbances of the above ten white water systems were measured at 540 nm to record the reference point. Then, all of the above 10 solutions were uniformly mixed and shaken in a shaker at 45 °C and 150 r/min for 4 h and 24 h, followed by measuring their UV absorbance at 540 nm to calculate the residual starch concentration within white water systems, so as to evaluate the degradation behaviors of native starch and enzymatically hydrolyzed starch.

As for the effect of system temperature on starch biodegradation behavior, the simulated white water system from fermented pulp slurry (500 mL) was first treated according to the aforementioned procedures. Subsequently, 5 mL of native starch solution with 4% solid content or 15 mL of enzymatically hydrolyzed starch solution with 4% solid content were added to the above fermented pulp systems and their starting UV absorbance at 540 nm was recorded, followed by the filtration treatment to obtain the white water filtrate after sufficient reaction process of 4 h and 24 h, which were treated in the water bath at 35, 40, 45, 55, and 65 °C, respectively. Then the obtained filtrates were characterized through the UV absorbance at 540 nm to determine the residual amount of native starch or enzymatically hydrolyzed starch treated at different system temperatures.

As for the effect of system pH value on starch

biodegradation behavior, similar procedures were followed as previously described. Subsequently, 1% dilute HCL and 1% NaOH were applied to adjust the pH value of white water systems to 4, 5, 5.5, 6, 6.5, 7, and 8. Then the native starch solution (4% solid content and 5 mL) or enzymatically hydrolyzed starch solution (4% solid content and 15 mL) were added into the above white water systems, respectively, and their starting UV absorbance at 540 nm was recorded. The above systems were treated in a shaker at 45 °C and 150 r/min for 4 h and 24 h, and the final UV absorbance at 540 nm was recorded to determine the residual starch concentration.

As for the effect of biodegradation duration (from 0 to 24 h) on starch degradation behavior within white water system, similar procedures were followed as previously described. A native starch solution (5 mL) with 4% solid content or 15 mL of enzymatically hydrolyzed starch solution with 4% solid content was added into the fermented pulp slurry, followed by a filtration process to obtain the white water filtrate and the starting UV absorbance at 540 nm was recorded. Then, the white water systems were treated in a shaker at 45 °C and 150 r/min for 24 h of biodegradation process, during which, 1 mL white water was taken out after each 1 h reaction for UV absorbance determination, finally, a series of UV absorbances at 540 nm of white water system at each reaction time period were recorded and the residual starch concentrations were calculated.

## 2.9 XRD analyses of starch samples

The freeze-dried starch samples were measured using XRD. The voltage of the X-ray emitter was 40 kV, and the current was 40 mA. The scanning range and rate were 4°–50° and 5°/min, respectively, with a continuous scanning mode. The obtained XRD patterns of starch samples were analyzed, and the relative crystallinity of the starch samples can be calculated as the following equation.

$$\text{Relative crystallinity (\%)} = \frac{\text{Crystal diffraction peak area}}{\text{Total area}} \times 100\%$$

### 2.10 FT-IR analyses of starch samples

The obtained dried starch samples were characterized using an infrared spectrometer. The scanning range of the spectrum was 0–4000  $\text{cm}^{-1}$ , with 64 scans and a resolution of 4  $\text{cm}^{-1}$ . Before the experiment, 100 mg of dried KBr tablets were scanned as a background. Thereafter, 1 mg starch sample and 100 mg KBr were homogeneously mixed and pressed to obtain the tablet sample for FT-IR testing.

The degree of order of the double helix (DO) and the degree of double helix (DD) results of the starch samples and their corresponding degradation products were calculated using OMNIC8.2. Herein, the values of 1047/1022  $\text{cm}^{-1}$  and 995/1022  $\text{cm}^{-1}$  were calculated to obtain DD and DO values, respectively.

### 2.11 GPC analyses of starch samples

Native starch, enzymatically hydrolyzed starch, and their hydrolyzed products (4 mg for each sample) were dispersed into 2 mL DMSO solution, followed by heating in a boiling water bath for 1 h. Once totally dissolved, the starch samples were further placed in a water bath at 80 °C for 12 h, followed by passing through a 0.45  $\mu\text{m}$  organic film. Finally, 1.5 mL treated starch samples were collected for GPC measurement.

## 3 Results and discussion

### 3.1 Effects of $\alpha$ -amylase dosage and biodegradation reaction duration on starch biodegradation behavior

Effect of  $\alpha$ -amylase dosage on enzymatically hydrolyzed

starch degradation behavior within simulated white water system is shown in Fig. 1(a). As shown in Fig. 1(a), the degradation ratio of enzymatically hydrolyzed starch within the OCC slurry system was distinctly decreased with the increase of  $\alpha$ -amylase dosage, and further plateaued when the  $\alpha$ -amylase dosage reached 0.03%. When the  $\alpha$ -amylase dosage was 0.04%, the degradation ratio of enzymatically hydrolyzed starch after 4 h degradation reaction was 28.3%, while that of its 24-h counterpart was 47.8%, indicating that higher  $\alpha$ -amylase dosage in the preparation of enzymatically hydrolyzed starch could enhance the hydrolysis resistance of starch against microbial degradation within white water system [30]. A large amount of native starch was first degraded into glucose, maltose, maltotriose, and  $\alpha$ -limit dextrin during the  $\alpha$ -amylase hydrolysis process to produce enzymatically hydrolyzed starch, which would be difficult to be hydrolyzed in the white water systems, implying that enzymatically hydrolyzed starch utilized for paper coating process possessed better stability and recyclability during white water circulation process of OCC pulping.

Effect of biodegradation time on the biodegradation behavior of native starch and enzymatically hydrolyzed starch is shown in Fig. 1(b). As shown in Fig. 1(b), the degradation ratios of native and enzymatically hydrolyzed starches were first clearly increased with biodegradation time from the starting 0–2 h in white water systems, and the degradation ratio of the native starch was larger than that of enzymatically hydrolyzed

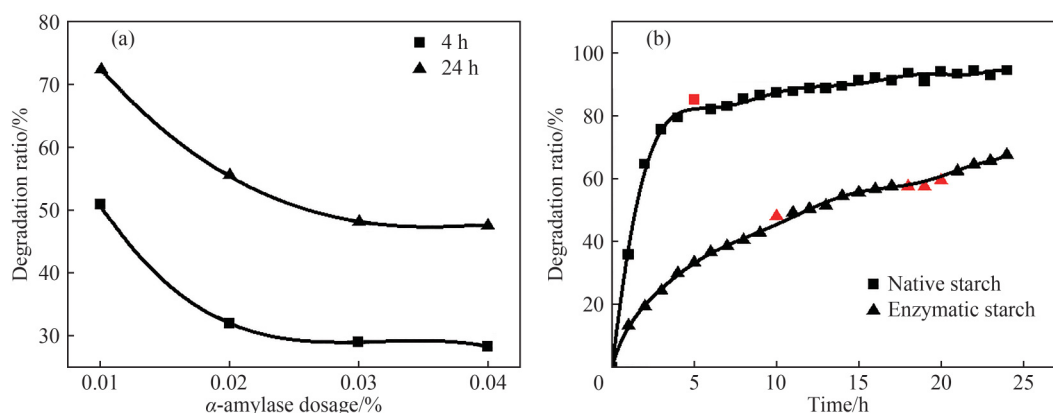


Fig. 1 Effects of (a)  $\alpha$ -amylase dosage on the degradation behavior of enzymatically hydrolyzed starch within simulated white water system; (b) biodegradation time on the biodegradation behavior of native starch and enzymatically hydrolyzed starch

starch at the same hydrolysis time, indicating that enzymatically hydrolyzed starch has a higher hydrolysis resistance against microorganisms within white water. In the first 5 h of biodegradation process, the native starch was biodegraded by 85.2%, indicating that most of the native starch can be biodegraded by the microorganisms within white water system in the first 5 h. While the enzymatically hydrolyzed starch underwent a rapid degradation stage in the first 4 h, with a degradation ratio of approximately 29.9%, which was much lower than that of the native starch. The enzymatically hydrolyzed starch was gradually biodegraded within white water system at a low biodegradation ratio of 67.6% during the following 20 h, indicating that the enzymatically hydrolyzed starch is more resistant to microbial decomposition, compared with native starch.

### 3.2 Effects of various experimental parameters/conditions on starch biodegradation behavior

Effects of native starch and enzymatically hydrolyzed starch concentrations on starch biodegradation behavior are shown in Fig. 2(a) and Fig. 2(b). As shown in Fig. 2(a) and Fig. 2(b), the degradation ratio of native starch

decreased with increasing starch concentration. When the native starch concentration was 1051 mg/L, the lowest degradation ratio was 58.7% at 4 h and 89.4% at 24 h. In addition, the influence of starch concentration on its degradation at 4 h was greater than that at 24 h. At 4 h, the difference between the maximum and minimum degradation ratio was 21.3 percentage points, and the difference at 24 h was 9.9 percentage points. As shown in Fig. 2(b), when the concentration of enzymatically hydrolyzed starch was 440 mg/L, the lowest degradation ratios were 33.3% at 4 h and 59.3% at 24 h, respectively. The differences between the maximum and minimum biodegradation amounts at 4 h and 24 h were 29.4 and 39.4 percentage points, respectively.

As previously stated, the native starch degraded rapidly in the first 4 h. Owing to the limited microbial content, the maximum concentration of starch biodegradation in the high degradation stage was approximately 600 mg/L. As a result, when the biodegradation duration increased to 24 h, most of the starch (native starch and enzymatically hydrolyzed starch) can be biodegraded, with the exception of a

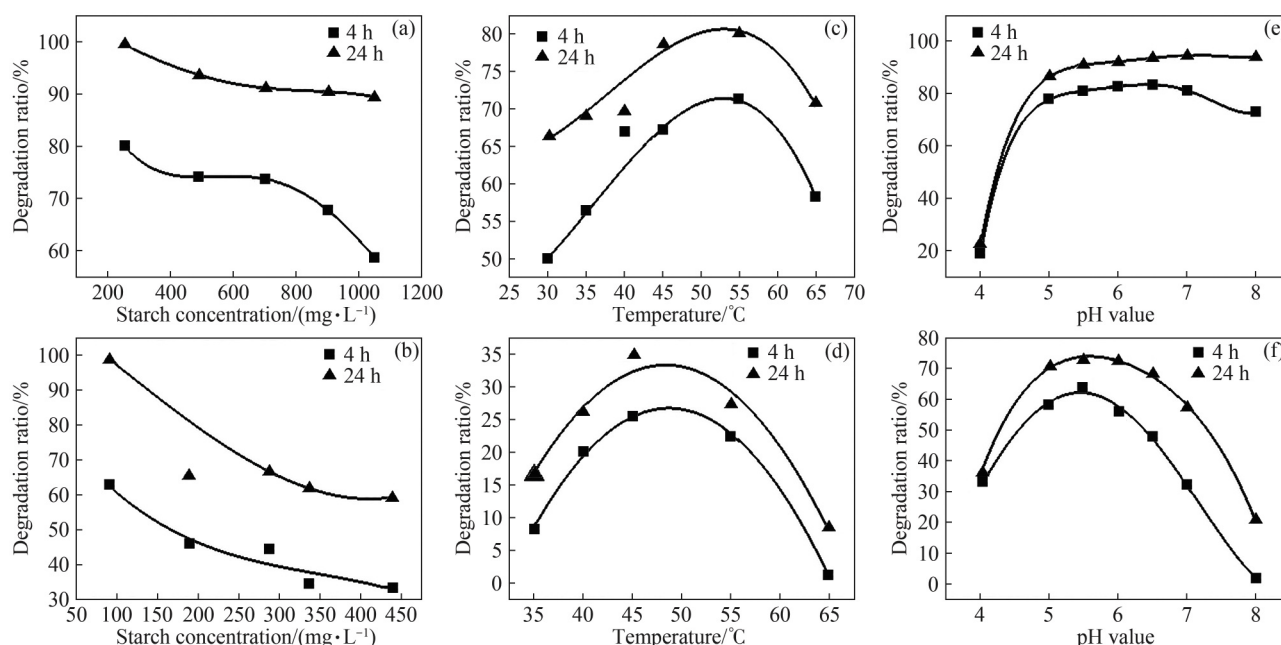


Fig. 2 Effects of (a) native starch and (b) enzymatically hydrolyzed starch concentrations on starch biodegradation behavior; effects of system temperature on the biodegradation behavior of (c) native starch and (d) enzymatically hydrolyzed starch; effects of system pH value on the biodegradation behavior of (e) native starch and (f) enzymatically hydrolyzed starch

small amount of non-degradable starch, which means that the biodegradation ratios for the two types of starches were marginally affected by starch concentration within white water system. It can be clearly noted from the above results that enzymatically hydrolyzed starch possesses a higher hydrolysis resistance to the microorganism biodegradation than the native starch. After 4 h or 24 h biodegradation process, the degradation ratio of enzymatically hydrolyzed starch was lower than that of the native starch.

Effects of system temperature on the biodegradation behavior of native starch and enzymatically hydrolyzed starch are shown in Fig. 2(c) and Fig. 2(d). As shown in Fig. 2(c) and Fig. 2(d), the degradation ratios of the two types of starches showed similar biodegradation behaviors, the degradation ratios were first increased and then decreased along with the system temperature. The native starch illustrated a maximum degradation ratio at 55 °C. The degradation ratio of native starch was 71.3% and 79.8% after 4 h and 24 h degradation, respectively. However, the enzymatically hydrolyzed starch showed a highest degradation ratio at 50 °C. Meanwhile, the degradation ratios of enzymatically hydrolyzed starch were 25.5% and 35.0% after 4 h and 24 h duration, respectively.

As discussed above, 55 °C is the suitable degradation temperature for native starch substrate, while for enzymatically hydrolyzed starch this temperature is 45 °C. According to Ref. [31], the best growth environment for microorganisms in white water is in the 35 °C–55 °C range, during which, increasing temperature is conducive to the growth and explosion of microorganisms. The optimum biodegradation temperatures of the two types of starches in white water are similar (between 45 °C and 50 °C), which is similar to the optimum growth temperature of microorganisms in white water reported in Ref. [31].

Effects of system pH value on the biodegradation behavior of native starch and enzymatically hydrolyzed starch are shown in Fig. 2(e) and Fig. 2(f). As shown in Fig. 2(e) and Fig. 2(f), the degradation ratio of enzymatically hydrolyzed starch increased initially and

then decreased along with system pH value, while the degradation ratio of native starch increased to a certain level and then reached a plateau. The degradation ratio did not change markedly when system pH value of native starch was 5–7. After biodegradation treatment for 4 h as shown in Fig. 2(f), when system pH value increased to 6.5, enzymatically hydrolyzed starch showed a sharp decrease in degradation ratio, demonstrating enzymatically hydrolyzed starch exhibits improved hydrolysis resistance ability against microorganisms within white water system under neutral or weak alkaline condition. Similar results can be found after 24 h of biodegradation process, which indicated that the degradation ratio began to decrease as the pH value increased to 8, indicating that microorganisms in white water system have a high activity at pH value range of 5.5–7.0.

According to Ref. [32], the optimal pH value range for best growth of microorganisms in white water system is 4–7, which can be verified by Fig. 2(e). Native starch shows stable and high degradation ratio even at the pH value range of 5–8, due to the fact that native starch is more prone to be biodegraded in white water system and the variations of pH value hardly affect its degradation ratio. While enzymatically hydrolyzed starch has a better hydrolysis resistance ability against microorganisms compared with native starch. Furthermore, the biodegradation behavior of enzymatically hydrolyzed starch can be greatly restricted and decreased when adding alkaline solution into white water system to increase pH value, which is beneficial for maintaining starch substrate within white water system during OCC pulping process.

### 3.3 XRD analyses

XRD patterns of native and enzymatically hydrolyzed starch substrates are shown in Fig. 3(a). As shown in Fig. 3(a), the native starch has a sharp peak at  $2\theta = 15.12^\circ$ ,  $16.94^\circ$ ,  $17.98^\circ$ , and  $23.02^\circ$ , with a weak diffraction peak at  $2\theta = 20.26^\circ$  and an obvious type A crystal structure<sup>[33–34]</sup>. Enzymatically hydrolyzed starch only illustrates diffraction peaks at  $2\theta = 17.06^\circ$  and  $22.02^\circ$ , indicating that the crystal structure of native

starch has been destroyed during the hydrolysis process.

XRD patterns of OCC fiber and biodegradation products (24 h) from native starch and enzymatically hydrolyzed starch, respectively, are shown in Fig. 3(b). As shown in Fig. 3(b), the two types of starch substrates were respectively degraded by microorganisms within simulated white water system, therefore, the typical diffraction peaks of the native starch almost disappeared, while those of the fine fibers were remained, indicating that the native starch has been totally biodegraded by the microorganisms within white water system after 24 h reaction, which is consistent with the above data. The enzymatically hydrolyzed starch had a dispersion peak at  $2\theta = 21.86^\circ$  and retained a part of the starch diffraction peak, which confirmed that the degradation ratio of native starch was greater than that of enzymatically hydrolyzed starch following a 24 h microbial degradation, owing to the higher hydrolysis resistance of enzymatically hydrolyzed starch against the microorganism degradation.

### 3.4 FT-IR analyses

The DO and DD values in the crystalline region of starch samples can be analyzed and determined via FT-IR spectra. As shown in Fig. 4, after enzymatic hydrolysis, the obtained starch sample showed similar FT-IR spectra with that of native starch and did not generate new functional groups. However, both the degradation products from native starch and enzymatically hydrolyzed starch after 24 h biodegradation process

within white water system exhibited a new peak at  $1549\text{ cm}^{-1}$ , which showed that starch can be degraded in simulated white water system and that the cellulose fibers or fines derived from OCC pulping process could be mixed with starch samples during the separation process. After deconvolution of FT-IR spectra as shown in Fig. 4(b), the peaks at  $1047$ ,  $1015$ , and  $995\text{ cm}^{-1}$  were ascribed to the crystalline, amorphous, and ordered structures of starch substrate, respectively. After the deconvolution of FT-IR spectra from  $800\text{ cm}^{-1}$  to  $1200\text{ cm}^{-1}$ , the absorbance values of  $1047$ ,  $1015$ , and  $995\text{ cm}^{-1}$  were obtained, a ratio of  $1047/1022\text{ cm}^{-1}$  to  $995/1022\text{ cm}^{-1}$  was obtained, which represents DO and DD in the crystalline region, respectively<sup>[35–38]</sup>, as shown in Table 1.

Compared with the DO value of native starch (1.08), the enzymatically hydrolyzed starch showed a slightly decreased DO value of 0.92, which confirmed that  $\alpha$ -amylase can efficiently hydrolyze the crystalline lattice structure, thus affecting the order of the double helix within crystalline regions. After the microbial degradation treatment within white water system, the DO value of the native starch decreased to 0.52, which may be owing to the complete degradation of the native starch. Notably, compared with the DO value (0.92) of hydrolyzed starch without microbial degradation treatment, the DO value of enzymatically hydrolyzed starch after microbial degradation treatment within white water system was 0.71, which was lower than that of native starch, indicating that enzymatically hydrolyzed

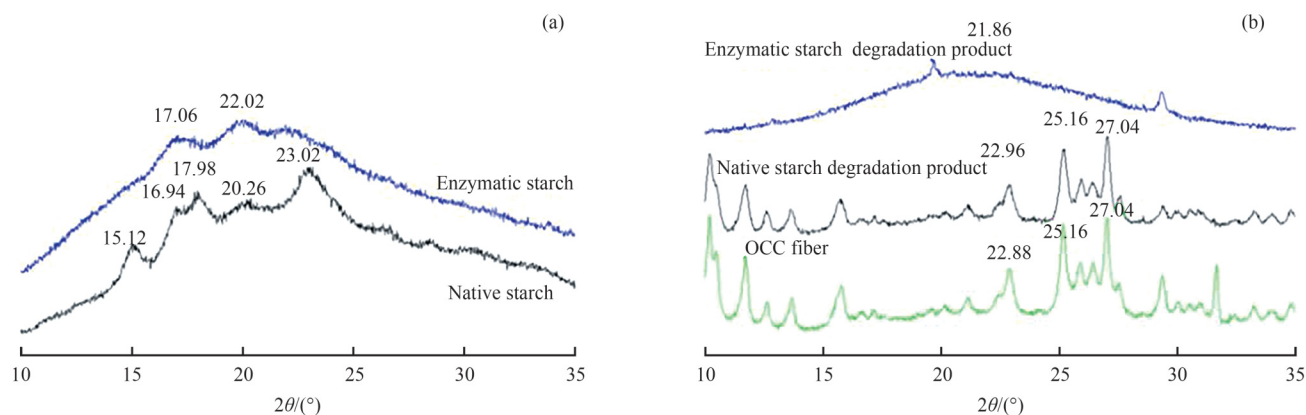


Fig. 3 XRD patterns of (a) native and enzymatically hydrolyzed starch substrates; OCC fiber and biodegradation products (24 h) from native starch and enzymatically hydrolyzed starch, respectively

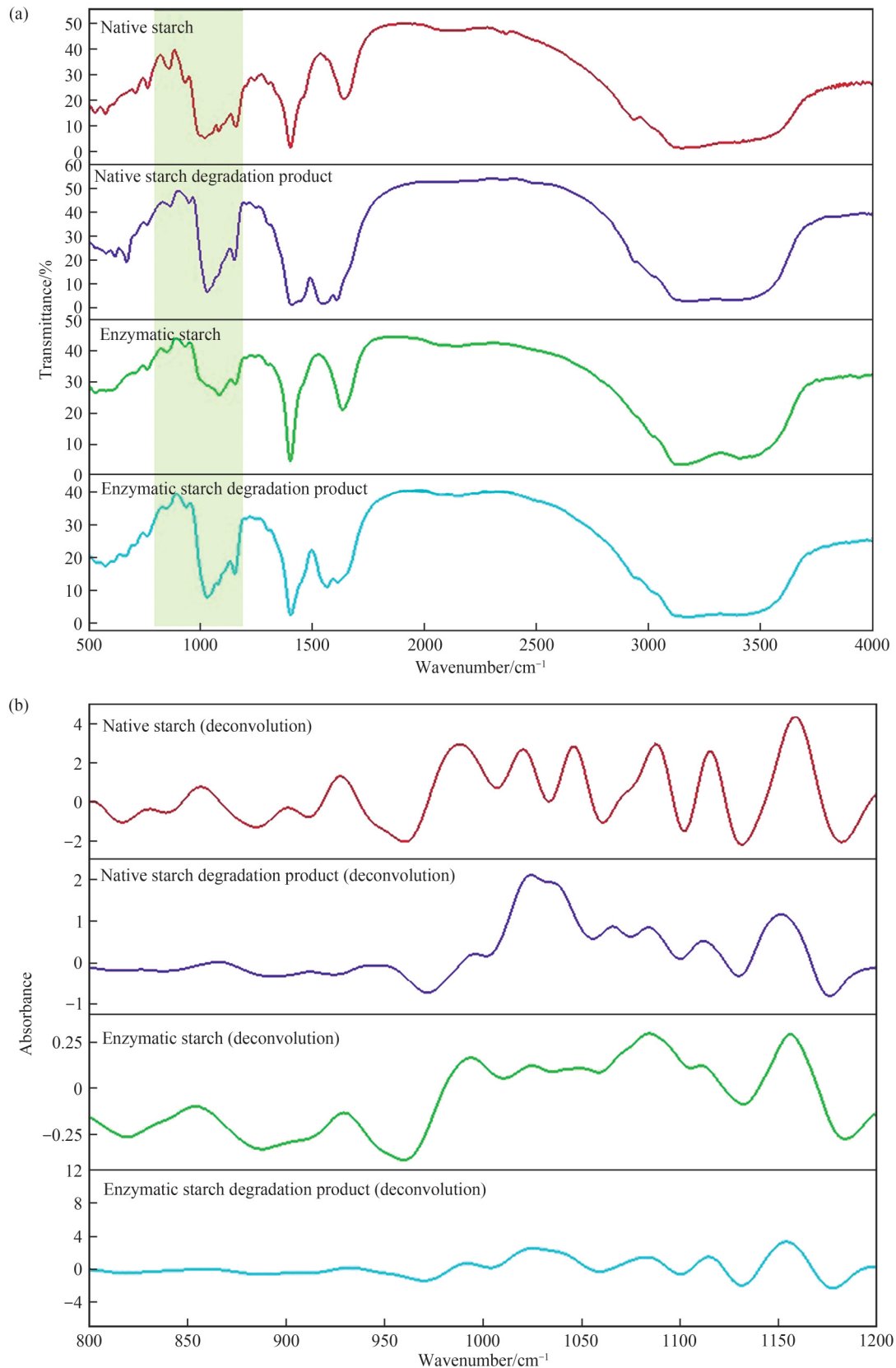


Fig. 4 (a) FT-IR spectra of native starch and enzymatically hydrolyzed starch and their corresponding biodegradation products (24 h) from white water systems; (b) deconvoluted FT-IR spectra of native starch and enzymatically hydrolyzed starch and their corresponding biodegradation products derived from Fig. 4(a) at the wavenumber range of 800–1200 cm<sup>-1</sup>

Table 1 DO and DD values of two types of starches and their biodegradation products

| Sample                                  | DO   | DD   |
|-----------------------------------------|------|------|
| Native starch                           | 1.08 | 0.94 |
| Native starch biodegradation product    | 0.52 | 0.10 |
| Enzymatic starch                        | 0.92 | 1.41 |
| Enzymatic starch biodegradation product | 0.71 | 0.33 |

starch possessed a higher hydrolysis resistance against microorganisms in white water system.

### 3.5 GPC analyses

Fig. 5 showed the GPC spectra of native starch and enzymatically hydrolyzed starch and their corresponding biodegradation products (24 h) after microorganism biodegradation treatment in the simulated white water systems. The corresponding molecular weight of the two types of starches and their biodegradation products are listed in Table 2. It was clearly noted that the enzymatic hydrolysis of starch produced short-chain molecular peaks compared with the control starch sample. After microbial biodegradation within simulated white water system, the starch substrates showed two distinct long- and short-chain starch molecular peaks (the red lines

illustrated in Fig. 5(a) and Fig. 5(b)). From the number-average molecular weight ( $M_n$ ) and weight-average molecular weight ( $M_w$ ) values shown in Table 2, it can be seen that the microorganism degradation treatment within the simulated white water system of native starch can induce a significant decrease of two-order of magnitude in both  $M_n$  and  $M_w$  (e.g.,  $M_n=1.554\times 10^8$  vs.  $M_n=1.844\times 10^6$ ), while the decline in the biodegradation products of enzymatically hydrolyzed starch illustrated a same magnitude (e.g.,  $M_n=2.039\times 10^5$  vs.  $M_n=1.735\times 10^5$ ). The above results verified that enzymatically hydrolyzed starch had a higher hydrolysis resistance against microorganisms in simulated white water system, which is well consistent with the above conclusions.

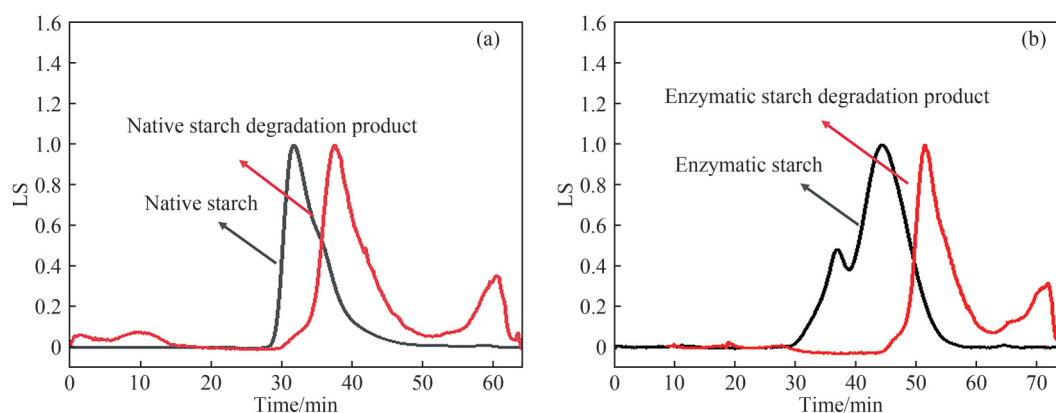


Fig. 5 GPC spectra of (a) native starch and (b) enzymatically hydrolyzed starch and their corresponding biodegradation products (24 h) after treatment in white water systems

Table 2 Molecular weight of two types of starches and their biodegradation products

| Sample                                  | $M_n$              | $M_w$              | Area/% |
|-----------------------------------------|--------------------|--------------------|--------|
| Native starch                           | $1.554\times 10^8$ | $1.922\times 10^8$ | 100    |
| Native starch biodegradation product    | $1.844\times 10^6$ | $2.594\times 10^6$ | 0      |
| Enzymatic starch                        | $2.039\times 10^5$ | $3.587\times 10^5$ | 95.2   |
| Enzymatic starch biodegradation product | $1.735\times 10^5$ | $2.051\times 10^5$ | 0      |

## 4 Conclusions

In summary, the microorganism degradation treatment of two types of starch substrates, i.e., native starch and enzymatically ( $\alpha$ -amylase) hydrolyzed starch, within simulated white water of the old corrugated cardboard (OCC) pulping system was performed, as well as the analysis of the degradation products to explore their hydrolysis behaviors. The degradation ratio of native starch was greatest when the temperature was 55 °C, at a starch concentration of 200 mg/L and the pH value of 5–7. While enzymatically hydrolyzed starch had the highest degradation ratio at a temperature of 50 °C, starch concentration of 100 mg/L, and pH value of 5.5. Enzymatically hydrolyzed starch was seriously affected by temperature, pH value, starch concentration, and biodegradation duration (0–24 h). The characterizations of X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FT-IR), and gel permeation chromatography (GPC) were also carried out to verify the conclusions that enzymatically hydrolyzed starch had a higher hydrolysis resistance against microorganisms in simulated white water system. This study provides a practical and interesting approach to investigate starch hydrolysis or biodegradation behaviors within the white water system of OCC pulping process, which would greatly contribute to the full recycling and valorized application of starch as a versatile additive during paperboard production.

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## References

- [1] Campean T, Grad F, Gradinariu C. Eco-friendly corrugated board and sustainable packaging manufacturing. *Environmental Engineering and Management Journal*, 2017, 16(3), 705-714.
- [2] Kreplin F, Putz H J, Schabel S. Multiple recycling of paperboard: Paperboard characteristics and maximum number of recycling cycles-Part I: Multiple recycling of corrugated base paper. *TAPPI Journal*, 2019, 18(11), 631-638.
- [3] Borghi A D, Parodi S, Moreschi L. Sustainable packaging: an evaluation of crates for food through a life cycle approach. *The International Journal of Life Cycle Assessment*, 2020, 26(4), 753-766.
- [4] Chibani N, Djidjelli H, Dufresne A. Study of effect of old corrugated cardboard in properties of polypropylene composites: Study of mechanical properties, thermal behavior, and morphological properties. *Journal of Vinyl and Additive Technology*, 2016, 22(3), 231-238.
- [5] Zhang X L, Di J J. Morphological and Structural Characteristics of Different Types of Wastepaper Fibers. *Packaging Engineering*, 2021, 42(3), 47-53.
- [6] Lee J H, Seo Y B, Jeon Y. Strength property improvement of OCC-based paper by chemical and mechanical treatments (I). *Journal of Korea Technical Association of The Pulp and Paper Industry*, 2000, 32(1), 10-18.
- [7] Wang X C. "Foreign Garbage" banned for one year, China's decision affects global solid waste disposal system. *Resource Recycling*, 2018, 3(9), 12-15.
- [8] Chemelli A, Gomernik F, Thaler F, Huber A, Hirn U, Bauer W, Spirk S. Cationic starches in paper-based applications—A review on analytical methods. *Carbohydrate Polymers*, 2020, DOI: 10.1016/j.carbpol.2020.115964.
- [9] Brenner T, Kiessler B, Radosta S. Processing surface sizing starch using oxidation, enzymatic hydrolysis and ultrasonic treatment methods—Preparation and application. *Carbohydrate Polymers*, 2016, DOI: 10.1016/j.carbpol.2015.10.086.
- [10] CHEN H, LUAN D. Study on existing problems and solutions of secondary fiber in papermaking. *East China Pulp and Paper Industry*, 2021(1), 1-4.
- [11] MIAO Y J, LIAN M L, JIA Q M, MA N, SU W D, YANG Y H. Progress on the research of hornification restoration of recycled fiber. *Applied Chemical Industry*, 2019(2): 438-443.
- [12] ZHANG X H. Preparation System and Technology Optimization of Surface Sizing Starch. *Paper and Paper Making*, 2018, 37(5), 44-47.
- [13] Hubbe M A. Dissolved and colloidal substances (DCS) and the charge demand of papermaking process waters and suspensions: A review. *BioResources*, 2012, 7(4), 6109-6193.

- [14] Li H M, Qi Y G. Starch and its derivatives for paper coatings: A review. *Progress in Organic Coatings*, 2019, 135(5), 213-227.
- [15] Lin L, Yang J, Ni S. Resource utilization and ionization modification of waste starch from the recycling process of old corrugated cardboard paper. *Journal of Environmental Management*, 2020, DOI: 10.1016/j.jenvman.2020.111031.
- [16] Lin L, Yang J, Qu Y F. Physicochemical properties of secondary starch from OCC waste pulp based on resource reuse. *Transactions of China Pulp and Paper*, 2020, 35(1), 47-53.
- [17] Lu X Q, Zhen G Y, Estrada A L, Chen M, Nic J L, Hojo T, Kubota K, Li Y Y. Operation performance and granule characterization of upflow anaerobic sludge blanket (UASB) reactor treating wastewater with starch as the sole carbon source. *Bioresour Technol*, 2015, DOI: 10.1016/j.biortech.2015.01.010.
- [18] Badshah M, Parawira W, Bo M. Anaerobic treatment of methanol condensate from pulp mill compared with anaerobic treatment of methanol using mesophilic UASB reactors. *Bioresource Technology*, 2012, DOI: 10.1016/j.biortech.2012.08.109.
- [19] Radosta S, Kiessler B, Vorwerg W. Molecular composition of surface sizing starch prepared using oxidation, enzymatic hydrolysis and ultrasonic treatment methods. *Starch-Strke*, 2016, 68(5), 541-548.
- [20] YIN Y Q, SHAN W P, JI X, XU P. Review on the treatment of starch wastewater. *Environmental Pollution & Control*, 2005(8), 625-629.
- [21] Zhang H, Li Y X, Wang P L. Synthesis of  $\beta$ -Cyclodextrin Immobilized Starch and Its Application for the Removal of Dyestuff from Waste-water. *Journal of Polymers and the Environment*, 2019, 27(5), 929-941.
- [22] Paul K. New Technology aimed at Improving the Sustainability of Paper. *Paper Technology*, 2014, 55(4), 12-15.
- [23] Ekman J, Hietaniemi M, Kolari M. Novel starch saving program improves sustainability in board manufacturing through the reuse of recycled fiber starch. *China Pulp & Paper Industry*, 2018, 39(2), 66-70.
- [24] HE W W, HUO R F. Research progress of high concentration starch wastewater treatment. *Industrial Water Treatment*, 2022, 42(4), 16-23.
- [25] Liu L, An X, Zhang H. Ball milling pretreatment facilitating  $\alpha$ -amylase hydrolysis for production of starch-based bio-latex with high performance. *Carbohydrate Polymers*, 2020, DOI: 10.1016/j.carbpol.2020.116384.
- [26] Zhang F, Wang L, An X, Liu H. Improving sizing performance of middle layer of liquid packaging board containing high-yield pulp. *Cellulose*, 2020, DOI: 10.1007/s10570-020-03081-1.
- [27] Duan C, Tian C, Feng X. Ultrafast process of microwave-assisted deep eutectic solvent to improve properties of bamboo dissolving pulp. *Bioresource Technology*, 2022, DOI: 10.1016/j.biortech.2022.128543.
- [28] Duan C, Tian C, Tian G. Simultaneous microwave-assisted phosphotungstic acid catalysis for rapid improvements on the accessibility and reactivity of kraft-based dissolving pulp. *International Journal of Biological Macromolecules*, 2022, DOI: 10.1016/j.ijbiomac.2022.12.182.
- [29] Guan M, An X, Zhang H. Lignin emulsifying rosin for improved sizing performance and mechanical properties of liquid packaging board. *Industrial Crops and Products*, 2022, DOI: 10.1016/j.indcrop.2022.115276.
- [30] Schulz A G M, Van A J M M, Beynen A C. Dietary native resistant starch but not retrograded resistant starch raises magnesium and calcium absorption in rats. *Journal of Nutrition*, 1993, DOI: 10.1111/j.1365-277X.1993.tb00390.x.
- [31] Englyst H N, Kingman S M, Cummings J H. Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*, 1992, 46(2), 33-50.
- [32] Tung T Q, Miyata N, Iwahori K. Cassava starch processing wastewater: Potential pollution and role of microorganisms. *Japanese Journal of Water Treatment Biology*, 2010, 38(3), 117-135.
- [33] Wang S, Copeland L. Effect of Acid Hydrolysis on Starch Structure and Functionality: A Review. *Critical Reviews in Food Technology*, 2015, 55(8), 1081-1097.
- [34] Hizukuri S. Relationship between the distribution of the chain length of amylopectin and the crystalline structure of starch granules. *Carbohydrate Research*, 1985, 141(2), 295-306.
- [35] Li A, Xa A, Hao Z A. Ball milling pretreatment facilitating  $\alpha$ -amylase hydrolysis for production of starch-based bio-latex with high performance. *Carbohydrate Polymers*, 2020, DOI: 10.1016/j.carbpol.2020.116384.
- [36] Sevenou O, Hill S E, Farhat I A. Organisation of the external region of the starch granule as determined by infrared spectroscopy. *International Journal of Biological Macromolecules*, 2002, 31(1), 79-85.
- [37] Shingel K I. Determination of structural peculiarities of dextran, pullulan and  $\gamma$ -irradiated pullulan by Fourier-transform IR spectroscopy. *Carbohydrate Research*, 2002, 337(16), 1445-1451.
- [38] Smits A L M, Ruhnau F C, Vliegthart J F G. Ageing of starch based systems as observed with FT-IR and solid state NMR spectroscopy. *Starch-Strke*, 2010, 50(11), 478-483. **PBM**