Size distribution analysis of microstickies treated by enzyme mixtures in papermaking whitewater

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ABSTRACT: Microstickies present a formidable challenge for papermakers. Many strategies have been explored to control them. Enzyme treatment is a promising technology, but the mechanism of its action has not been determined, thus inhibiting further application of this new technology. This study investigated characteristics and size distributions of microstickies treated by esterase-cellulase mixtures. Determination of particle size and number was accomplished using a modified flow cytometer, which combined streaming capillary flow, laser-based particle size analysis and fluorescent dye tracing. The results showed that treatment of samples with enzyme mixtures induced size reduction of the larger microstickies. This effect was most dramatic for 1:1 ratios of esterase to cellulase. The treated particles were more stable than untreated ones. The smaller microstickies treated with some ratios of esterase and cellulase tended to aggregate over time. The behaviors of microstickies treated by enzyme mixtures were only slightly affected by temperature and shearing action. The surface physicochemical characteristics of these particles indicated that changes of these basic properties affected the whole whitewater system and resulted in a new equilibrium among all the particles.

Application: This work provides a new method for controlling microstickies and could serve as a valuable reference for the future application of enzymes in papermaking.

The annual global production and consumption of paper and paperboard have recently reached almost 400 million tons, with the rapid development of paper recycling technologies at the end of the last century making waste paper an important fiber source. For example, consumption of pulp in China reached 97.97 million tons in 2016, accounting for almost a quarter of the world's total, with 63.29 million tons (65%) originating from recycled pulp [1,2]. The use of recovered paper reduces pollution and saves resources and energy [3,4]. However, the residual contaminants, especially sticky materials originating from ink carriers, adhesives, hot melts, waxes, and natural resins, seriously complicate the papermaking process and hinder the production of high-quality paper products [5-10].

The deposition of stickies from recovered pulp is mainly ascribed to the fact that paper subjected to recycling contains large amounts of synthetic adhesives from polymers, such as polyvinyl acetate, polyacrylate, ethylene vinyl acetate, and styrene acrylate [11-14]. During the pulping process, these sticky materials are transformed into particles of various sizes. Depending on their size, stickies are commonly classified as macrostickies (>100 μm) and microstickies (<100 μm) [15,16]. The larger stickies can be effectively removed by mechanical methods such as cleaning [17], screening [18,19], and flotation [20,21]. Removal of the smaller stickies (70%–90% of stickies) is challenging [8]. Moreover, the formation of microstickies is hard to control using chemical methods such as adsorption [22], chemical dispersion [23], and fixation [24].

Enzymes have been successfully applied in the pulp and paper industry to increase efficiency, cut costs, and improve the overall environmental effects and safety [25,26], as exemplified by the widespread use of esterases and cellulases in recent years. Several hydrophobic plant-derived compounds contain ester linkages (e.g., fatty acid esters and sugar ferulates), and are associated with microstickies. Cleaving these linkages with esterases reduces their molecular weight and hinders microstickies deposition, adhesion, and aggregation [27-29], whereas cellulases hydrolyze β-D-glucosidic linkages in cellulose to produce oligosaccharides, cellobiose, and (ultimately) glucose [30,31].

Microstickies treated by cellulase and esterase alone were previously studied. The results showed that the enzyme-treated microstickies disperse or agglomerate, depending on the dosage of cellulase and esterase [32,33]. For this study, microstickies treated by cellulase-esterase mixtures of different compositions were characterized using a modified flow cytometer to evaluate the effects of treatment duration, stirring speed, and temperature.

MATERIALS AND METHODS

Preparation of raw materials

Commercial A4 copy paper (80 g/m²) and label paper coated with a water-based acrylic adhesive were used in all experiments, in a mass ratio of 98:2. The release liner of label paper was removed and discarded, the label paper was attached to the surface of copy paper, and the obtained composite was kept in a reclosable plastic bag for at least 72 h [32].

Esterase, with optimal activity reported to reach 95.97 U/mL at pH 7–9 and 40°C–60°C [34,35], was supplied by Novozymes (Jinan, China). Cellulase was supplied by a local company (Guangzhou, China) and showed an optimal activity of 7.57 U/mL at pH 3–8 and 40°C–60°C [36].

The fluorescence tracer was Nile red, supplied by Partec Co. (Münster, Germany).

Experimental procedures

The sample papers were torn into 2 cm × 2 cm pieces, pulped at 50°C ± 1°C and 15% consistency in a Formax 450H laboratory pulper (Adirondack Machine Corporation; Queensbury, NY, USA) and screened using a Somerville screen (Testing Machine Inc.; New Castle, DE, USA) with a slot width of 0.10 mm. Then, 40 g of ovendry pulp was sampled, diluted into 2% consistency, and stirred at 370 rpm and 50°C ± 1°C for 1 h using an RW20 digital stirrer (IKA, Staufen, Germany). Next, 1000 mL of the pulp was filtered with a dynamic drainage jar (DDJ-0305, Cleveland Motion Controls, Ladson, SC, USA) with mesh openings of 75 μm and stirred at 800 rpm. The filtrate containing microstickies was used immediately for the subsequent steps.

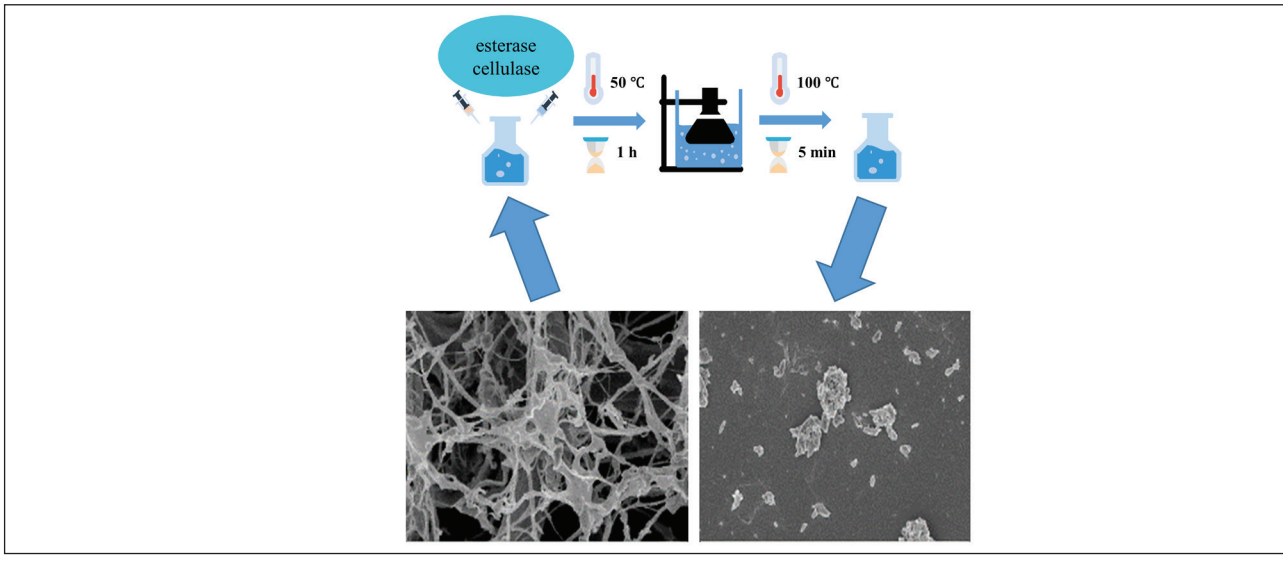
As shown in **Fig. 1**, a 200 mL sample of the filtrate was put in glass flasks, treated with enzyme mixtures (80 U/L, esterase:cellulase at ratios of 1:1, 1:4, 1:3, 3:1, and 4:1), held at 50°C, and agitated at 200 rpm for 1 h on a shaking table (KS 4000i, IKA, Staufen, Germany). The enzyme activity was stopped by placing the flasks in boiling water for 5 min and cooling to room temperature with ice. The stickies size distribution was not affected in this heating cycle procedure (**Fig. 2B**). All the experiments were fully replicated three times. Figure 1 shows this procedure and the corresponding scanning electron microscopy (SEM) images of solid matter deposited from whitewater before and after enzyme treatment. From Fig. 1, it can be found that the dispersion of microstickies was induced and divided into smaller ones that no longer attached to the surface of fibers. The samples from whitewater observed by SEM were dropped onto the mica plate, air-dried, and then coated. The image displayed was magnified 30000 times with 5.0 kV of electron high tension.

We investigated the effects of time by sampling the enzyme-treated filtrate after 0, 15, 30, 45, and 60 min of stirring with an IKA RET basic magnetic stirrer (IKA; Guangzhou, China) at 200 rpm and 50°C. The influence of stirring speeds of 200, 400, 600, 800, and 1000 rpm at 25°C was investigated. As for the effect of temperature, the samples were heated separately at 20°C, 30°C, 40°C, 50°C, 60°C, and 70°C and stirred at 200 rpm.

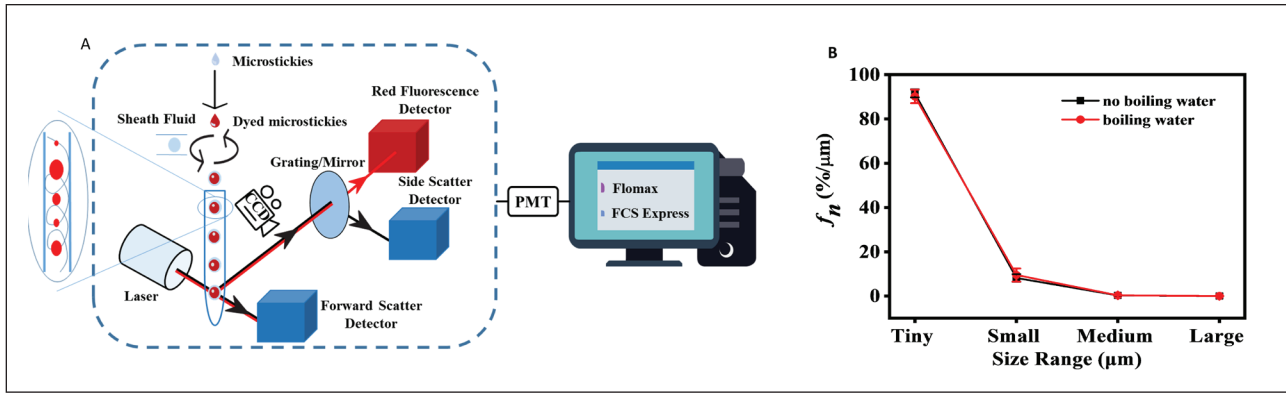
Whitewater containing microstickies was analyzed using a microstickies detector [37], a DDS-307A conductivity meter (INESA Analytical Instrument Co. Ltd.; Shanghai, China), a PCD 03 pH particle charge analyzer (Mütek Analytic GmBH; Herrsching, China), and a 2100N turbidity meter (HACH LANGE GmBH; Dusseldorf, Germany).

Microstickies detection

Microstickies were detected using a particle-sorting microfluidic pulse spectrophotometer system consisting fluid pumps, a laser light source, and signal analysis systems. The diluted sample was pumped through the center of the vortex formed by the sheath fluid and passed through the center of the observation chamber (Fig. 2A). The particles with adsorbed fluorescent tracer were excited by the laser, and their fluorescence intensities were recorded and analyzed with the signal



1. Treatment method for microstickies-containing whitewater with mixed enzymes.



2. Principle of microstickies detection and analysis (A) and effects of boiling on size distribution of microstickies (B).

acquisition system. A linear relationship was obtained between particle fluorescence intensity and size after calibration using standard microspheres (**Fig. 3**) [36]. This calibration was used in subsequent particle size analysis.

The test for differences between three duplicate samples distributions is the chi-square test. The chi-square statistic is as follows:

$$\chi^2 = \sum_i \frac{(R_i - S_i)^2}{R_i + S_i} \quad (1)$$

where R_i is the number of events in bin i for the first data set and S_i is the number of events in the same bin i for the second data set.

The size distribution of microstickies was characterized in terms of weight-averaged particle size (d), the number density distribution per unit of volume (f_n), and the volume density distribution per unit of volume (f_V), which were calculated as follows:

$$d = \sqrt[3]{\frac{\sum n_i d_i^3}{\sum n_i}} \quad (2)$$

where d_i is the particle (equal volume) diameter and n_i is the numbers of particles with diameter d_i:

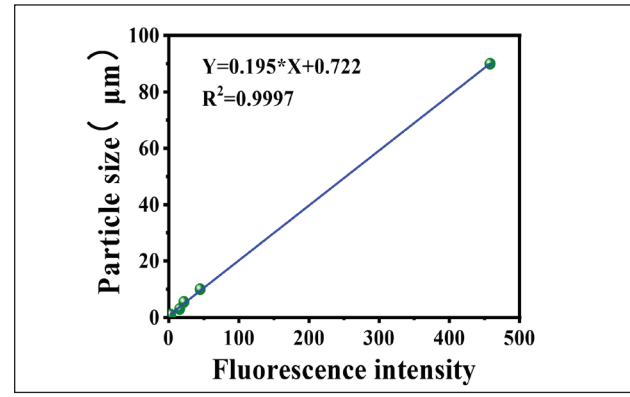
$$f_n = \frac{g}{\Delta d_p} \quad (3)$$

where g is the quantitative frequency distribution calculated as:

$$g = \frac{\Delta n}{n_0} \times 100\% \quad (4)$$

where Δn is the numbers of particles in the unit interval, n_o is the total numbers of particles, and Δd_p is the width of the unit interval.

The volume density distributions per unit of volume can be calculated by the following formula:



3. Relationship between fluorescence intensity and particle size [36].

$$f_V = \frac{V_i}{\triangle d_p} \quad (5)$$

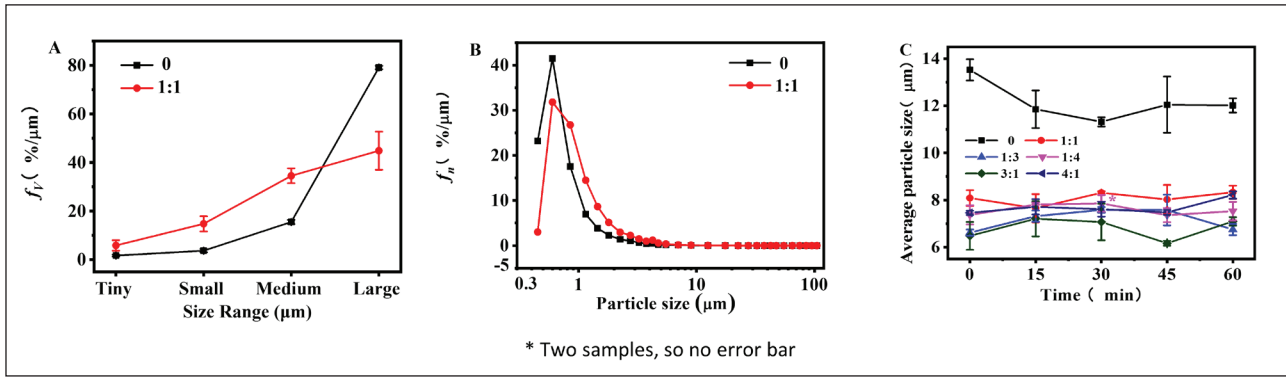
where V_i is the quantitative frequency distribution, calculated by the following formula:

$$V_i = \frac{\frac{4}{3}\pi d_i^3 n_i}{V_0} \times 100\% \quad (6)$$

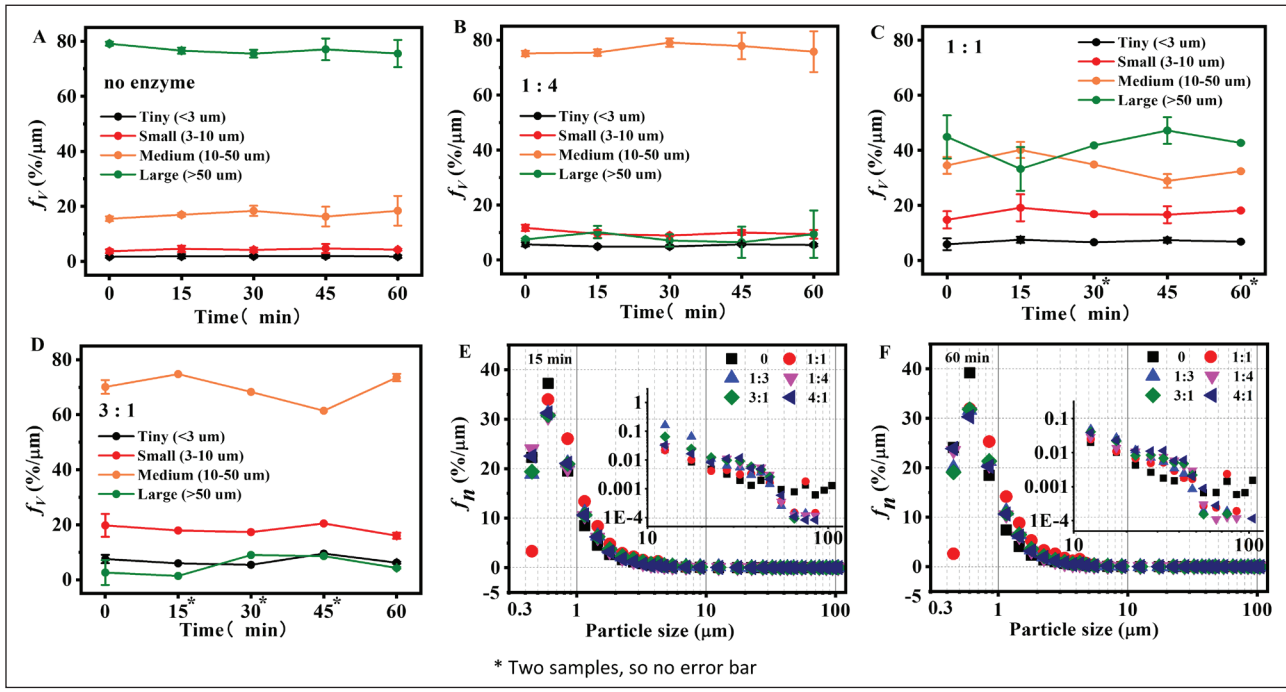
V_0 is the total volume of particles.

To aid interpretation, the microstickies were grouped by size: tiny (<3 μm), small (3-10 μm), medium (10-50 μm), and large (>50 μm). The volume percentages of different classes were calculated with the following formulas:

$$\begin{aligned} f_{VT} &= \sum_0^{d_i} V_i \ (d_i < 3 \text{ um}) \\ f_{VS} &= \sum_0^{a_i} V_i \ (3 \le d_i < 10 \text{ um}) \\ f_{VM} &= \sum_0^{d_i} V_i \ (10 \le d_i < 50 \text{ um}) \\ f_{VL} &= \sum_0^{d_i} V_i \ (d_i \ge 50 \text{ um}) \end{aligned} \quad (7)$$



4. Effects of mixed enzyme treatment on microstickies: A) volume density distribution, B) number density distribution, and C) average particle size versus time.



5. Effects of time on the volume density distribution: A) no enzyme, B) 1:4, C) 1:1, and D) 3:1; and number density distribution of microstickies treated by different esterase:cellulase mixtures at E) 15 min and F) 60 min.

RESULTS AND DISCUSSION

Effects of enzyme treatment time

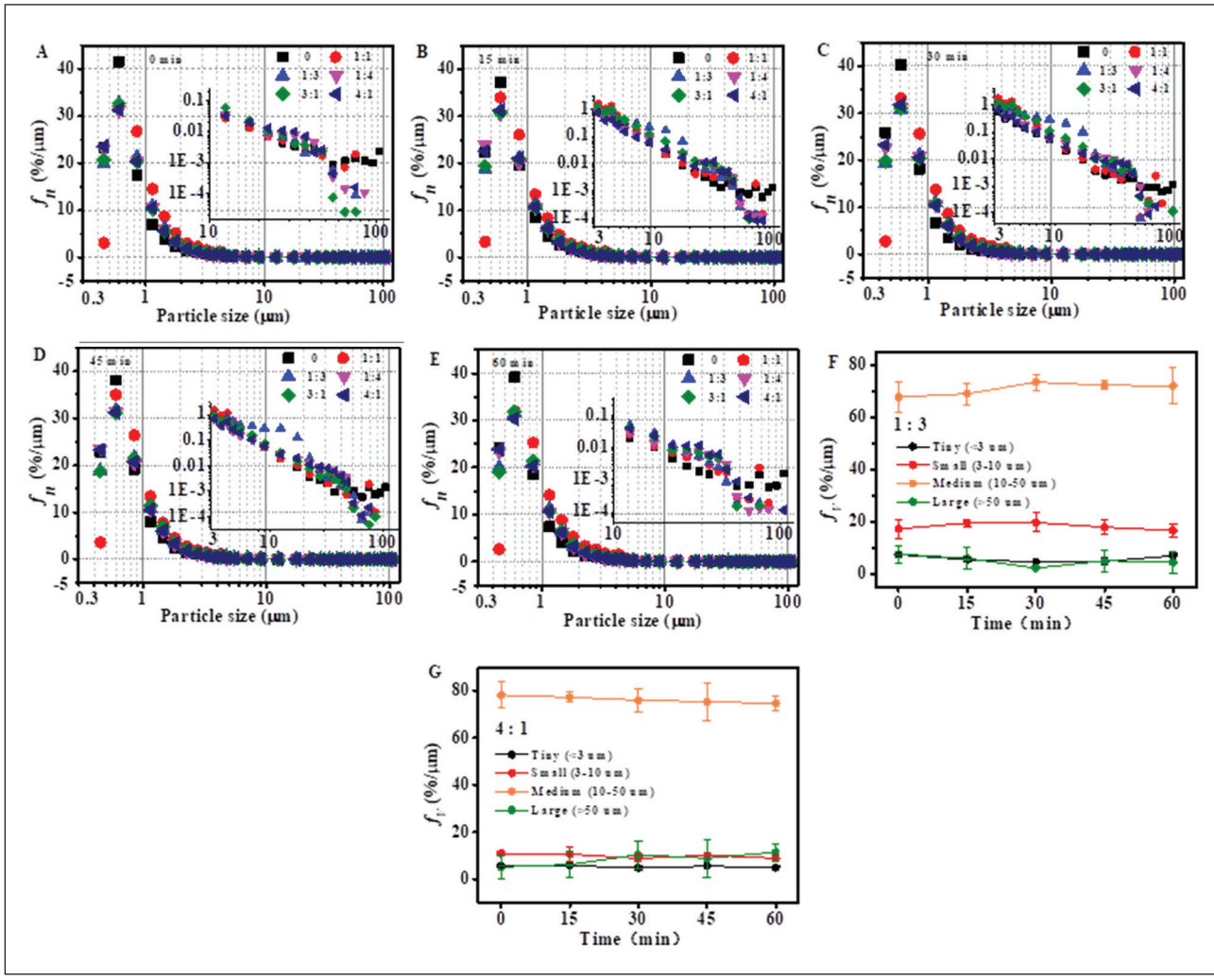
Figures 4A and **4B** are the volume density distribution and number density distribution of enzyme treated and untreated microstickies, respectively. Figures 4A-C indicate the dispersion and agglomeration of mixed-enzyme-treated microstickies.

As shown in Fig. 4C, the average particle size of mixed-enzyme-treated microstickies was smaller than those of untreated microstickies and varied with time, with the exact behavior being dependent on the enzyme ratio.

The detailed size distribution of microstickies (**Figs. 5B-F**) revealed that the enzyme ratio did not affect the observed patterns and showed that the particle size varied slightly upon enzyme treatment.

Figures 5A and 5B show that larger microstickies were likely to contribute to the formation of smaller particles with different enzyme ratios (1:4, 1:3, 3:1, 4:1), and similar trends in those ratios were observed with time (Figs. 5B and 5D). Meanwhile, the volume of >50 μm particles at an enzyme ratio of 1:1 exceeded that observed for other particle sizes (Fig. 5C). However, the proportion of smaller particles (<3 μm) observed after a treatment time (t) of 60 min was lower than that observed at t = 15-45 min. Simultaneously, the total amount of particles at t = 60 min exceeded that at t = 15-45 min (Figs. 5B and 5D). Thus, it can be concluded that smaller particles aggregated to form larger particles (i.e., the enzymatic cleavage of glycosidic and ester bonds resulted in the initial dispersion of microstickies followed by re-aggregation). The detailed distributions of original and enzyme-treated microstickies are shown in **Fig. 6**.

Table I shows that whitewater properties on the whole



6. Effects of time on the number density distribution of microstickies treated by different esterase:cellulase mixtures at A) 0 min, B) 15 min, C) 30 min, D) 45 min, and E) 60 min, and at volume density distribution of F) 1:3 and G) 4:1.

were largely unaffected by the esterase:cellulase ratio, with turbidity showing initial variations followed by a steady decrease with time. This behavior confirmed the initial dispersion and subsequent aggregation of microstickies.

Effects of stirring speed

Figure 7 shows the effects of stirring speed on the average size of microstickies, revealing remarkable variations for untreated microstickies. With increasing stirring speed, the average size initially increased, sharply decreased, and finally exhibited a slight increase. Although a similar trend was seen for enzyme-treated samples (except for the esterase:cellulase ratio of 3:1), their particle size was more stable than that of untreated ones, as shown in **Fig. 8**.

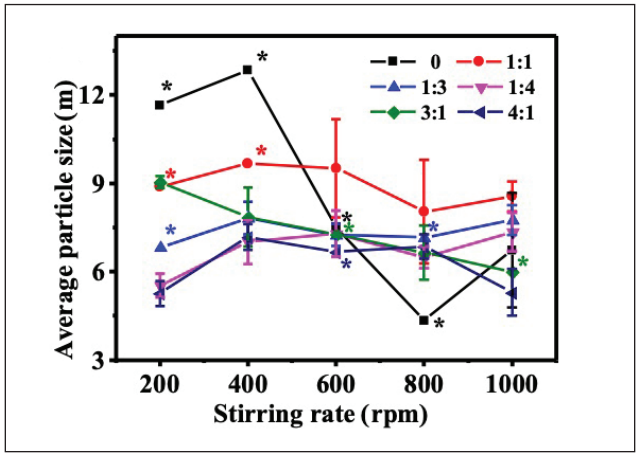
The esterase:cellulase ratio had little effect on the particle size distribution of microstickies, with fairly similar amounts

Time, min	Cationic Demand, meq/L						Turbidity, NTU					
	0	1: 4	1: 3	1: 1	3: 1	4: 1	0	1: 4	1: 3	1: 1	3: 1	4: 1
0	0.068	0.164	0.123	0.096	0.151	0.164	112	502	357	76	378	525
15	0.068	0.164	0.137	0.096	0.123	0.151	114	506	351	74	357	522
30	0.096	0.164	0.137	0.109	0.109	0.151	122	509	343	75	351	517
45	0.096	0.164	0.137	0.109	0.109	0.151	113	506	339	74	308	523
60	0.068	0.164	0.137	0.109	0.109	0.151	112	513	322	74	302	514

I. Effects of time on the properties of mixed-enzyme-treated whitewater.

of small particles (<3 μm) seen for different ratios. However, for enzyme ratios of 4:1, 3:1, 1:3, and 1:4, the amount of 10-50 μm particles exceeded that seen for untreated samples, with a greater fraction of larger particles observed for ratios of 1:4, 1:3, and 3:1 compared to that of untreated samples (Fig. 8A). Thus, smaller particles in enzyme-treated samples (esterase:cellulase ratios of 1:4, 1:3, and 3:1) showed enhanced aggregation at a lower stirring speed.

At an increased stirring speed, the particle size distributions of enzymatically treated microstickies were similar, which, in combination with Fig. 8A, shows that a higher fraction of 10-50 μm particles was observed for non-treated samples. Thus, increasing the stirring speed to 800 rpm induced the dispersion of microstickies into smaller particles. When the stirring speed was increased to >800 rpm, the volume percentage of particles in the range of 10-50 μm was highest for samples treated by enzyme, exceeding that obtained at a 1:1 ratio or for untreated samples, with an opposite trend observed for a size range of >50 μm. Notably, medium particles occupied a larger proportion and bigger particles tended to become a lower proportion on the whole. Thus, enzyme-treated microstickies initially underwent aggregation, followed by fragmentation at an increased stirring speed, with particularly pronounced aggregation seen for esterase:cellulase ratios of 1:4, 1:3, 3:1, and 4:1. The detailed distributions of original and enzyme-treated microstickies are shown in **Fig. 9**.

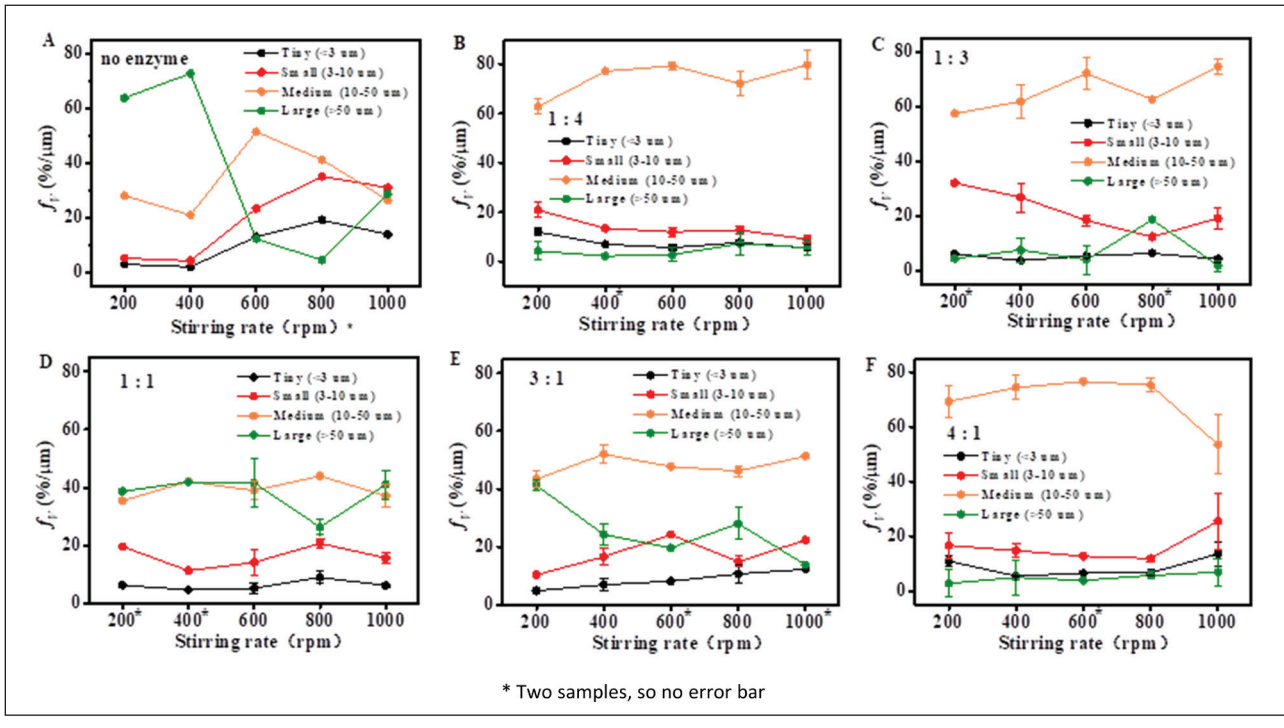


7. Effects of stirring speed on the average particle size of mixed-enzyme-treated microstickies.

Table II indicates that turbidity and cationic demand first increased, then decreased, as stirring speed increased. Changes in the specific surface areas of particles affected their surface charge densities and thus influenced the properties of whitewater, implying that the initial aggregation of microstickies was followed by their subsequent dispersion, in agreement with the size distribution results.

Effects of temperature

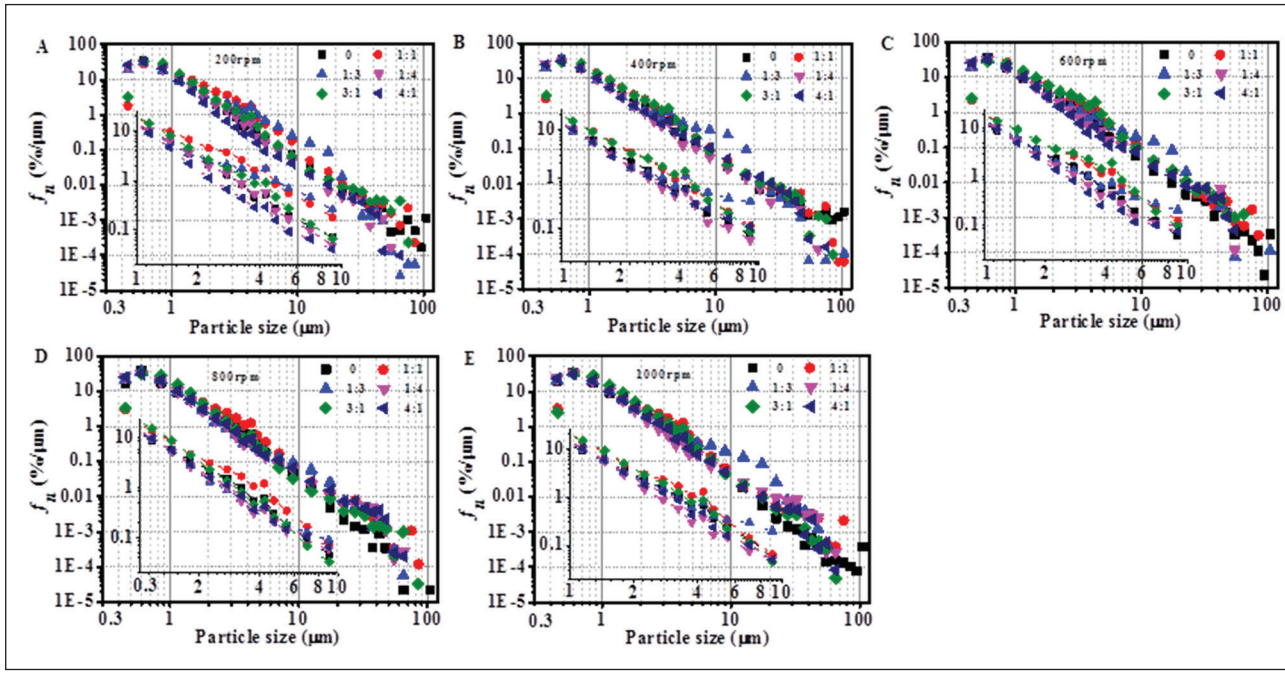
Figure 10 shows that the average particle size of enzyme-treated microstickies decreased with increasing temperature, except for the esterase:cellulase ratio of 1:1, with slight variations observed for different enzyme ratios. The largest average particle size was seen at a ratio of 1:1, at which the particles size was relatively unchanged at the lower temperature, almost largest at 50°C, and then slightly smaller at higher temperatures. Moreover, the particles of untreated microstickies



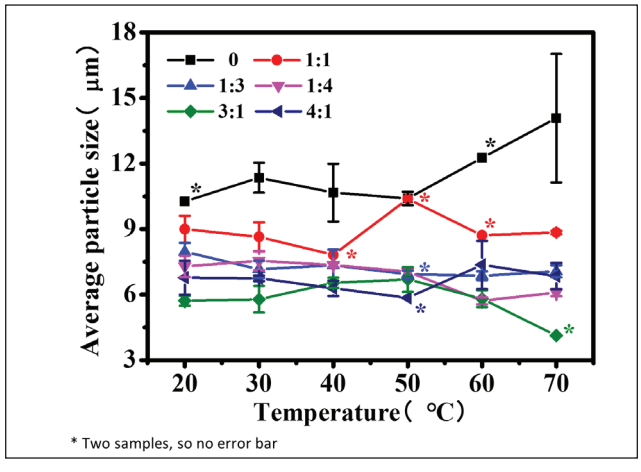
8. Effects of the stirring speed on the particle size distribution of mixed-enzyme-treated microstickies: A) no enzyme, B) 1:4, C) 1:3, D) 1:1, E) 3:1, and F) 4:1.

Stirring Rate, rpm	Cationic Demand, meq/L						Turbidity, NTU					
	0	1: 4	1: 3	1: 1	3: 1	4: 1	0	1: 4	1: 3	1: 1	3: 1	4: 1
200	0.082	0.123	0.137	0.068	0.137	0.137	176	327	340	68	300	362
400	0.096	0.137	0.137	0.096	0.140	0.151	226	325	374	94	302	349
600	0.082	0.151	0.137	0.109	0.137	0.137	216	335	374	104	295	346
800	0.068	0.151	0.137	0.109	0.123	0.151	166	341	369	155	583	344
1000	0.068	0.164	0.151	0.082	0.137	0.151	161	512	369	74	545	340

II. Effects of stirring speed on the properties of mixed-enzyme-treated whitewater.



9. Effects of stiring on the particle size distributions of mixed-enzyme-treated microstickies: A) 200 rpm, B) 400 rpm, C) 600 rpm, D) 800 rpm, and E) 1000 rpm.

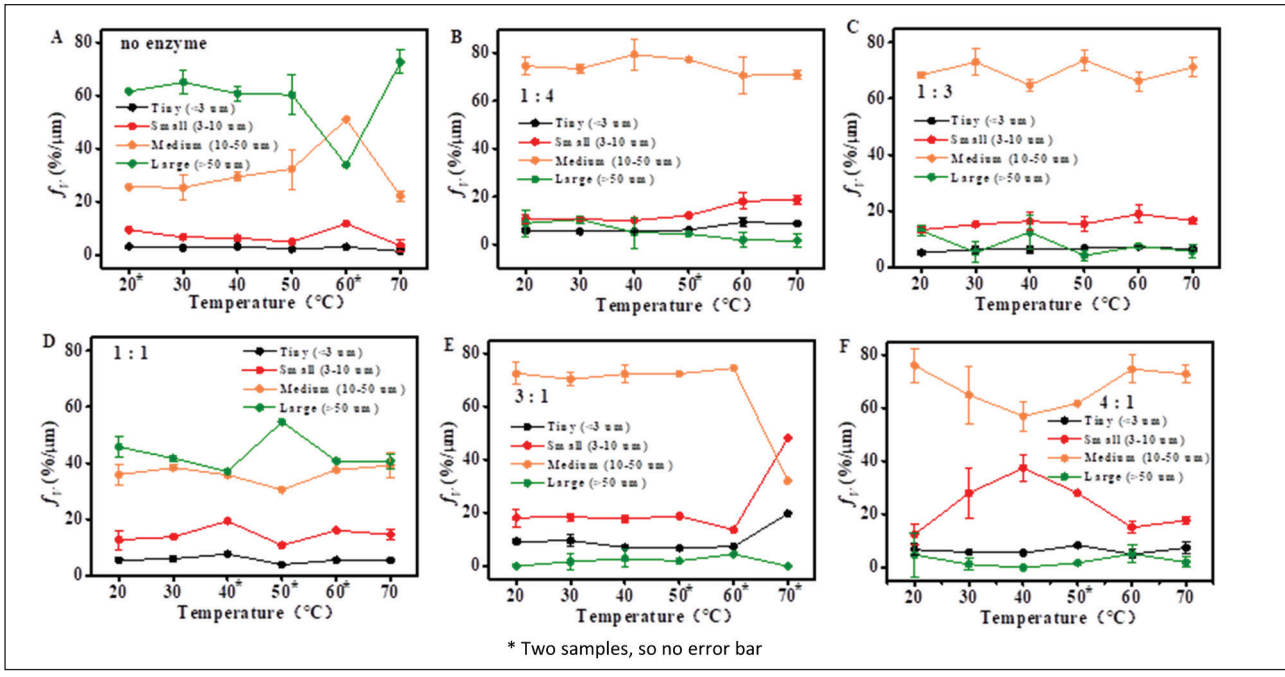


10. Effects of the temperature on the average particle size of mixed-enzyme-treated microstickies.

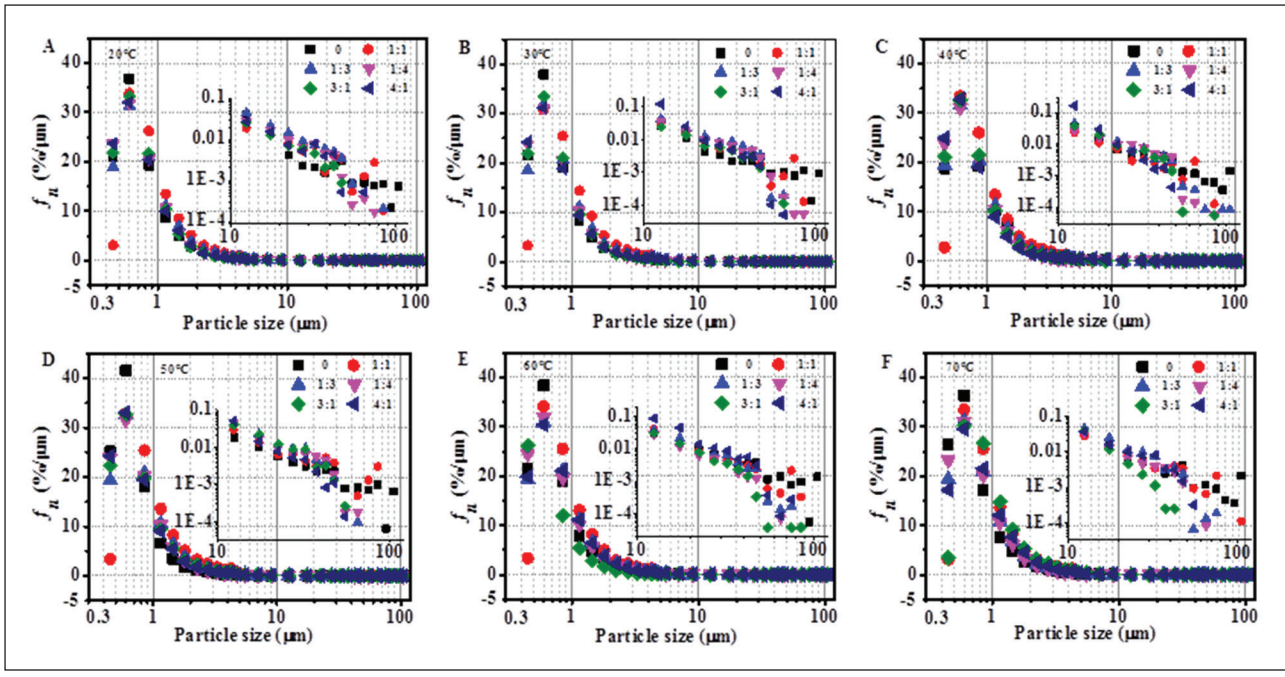
clearly tended to aggregate with increased temperature.

Figure 11 shows the effect of temperature on the particle size distribution of microstickies treated by different esterase:cellulase ratios. The size distribution changes of untreated microstickies were more obvious than those of treated microstickies with changes in temperature. A larger fraction of large particles (>50 μm) was observed for untreated microstickies than for treated ones with increased temperature. The small particle sizes of microstickies (<10 μm) were always of smaller proportion, whether those microstickies were treated by enzymes or not. The fraction of medium and large microstickies (10-50 μm; >50 μm) treated by a ratio of 1:1 was smaller than the same level particles applied with other enzyme proportions (Figs. 11A-C,E,F), with no apparent differences observed for patterns obtained at different esterase:cellulase ratios.

Untreated microstickies tended to disperse at lower temperature and tended to undergo agglomeration at elevated



11. Effects of temperature on the particle size distributions of mixed-enzyme-treated microstickies: A) no enzyme treated, B) 1:4, C) 1:3, D) 1:1, E) 3:1, and F) 4:1.



12. Effects of temperature on the particle size distributions of mixed-enzyme-treated microstickies: A) 20°C, B) 30°C, C) 40°C, D) 50°C, E) 60°C, and F) 70°C.

temperatures. However, the mixed-enzyme-treated microstickies, which have broken ester bonds, were less sticky and tended to disperse with increasing temperature. The detailed distributions of original and enzyme-treated microstickies are shown **Fig. 12**.

Table III shows that characteristics of the whitewater system had no significant changes after treatment with different ratios of esterase:cellulose. In addition, an initial increase in turbidity followed by a decrease was seen with increasing temperature, reflecting that the number of particles in the system first increased and then decreased. Therefore, the microstickies particle size changes resulting with enzyme treatment were also indirectly reflected by changes in other parameters, showing that high temperature did not decrease the strength of enzyme-treated microstickies and the enzyme treatment of microstickies induced some dispersion.

Temperature, °C	Cationic Demand, meq/L						Turbidity, NTU					
	0	1: 4	1: 3	1: 1	3: 1	4: 1	0	1: 4	1: 3	1: 1	3: 1	4: 1
20	0.082	0.178	0.137	0.137	0.137	0.164	183	501	384	63	391	343
30	0.137	0.178	0.113	0.137	0.137	0.151	138	499	389	66	388	357
40	0.068	0.151	0.137	0.123	0.123	0.151	136	499	384	67	365	352
50	0.068	0.151	0.151	0.137	0.137	0.123	126	303	389	66	501	367
60	0.082	0.151	0.151	0.096	0.164	0.123	116	326	220	71	362	386
70	0.082	0.164	0.153	0.137	0.137	0.123	11	301	352	68	414	349

III. Effects of the temperature on the properties of mixed enzyme-treated whitewater.

CONCLUSIONS

This work determined the effect of esterase/cellulase treatment on the properties of microstickies and provides a valuable reference for effective future applications of enzymes in papermaking.

Time had almost no effect on the size of mixed-enzyme-treated microstickies. Increasing the stirring speed induced significant particle size variations for untreated microstickies. However, the average particle size of samples treated with esterase:cellulase in a ratio of 3:1 clearly decreased, with only slight variations observed for other enzyme ratios.

The particle size of untreated microstickies generally increased with increasing temperature. The microstickies treated by mixed enzymes with different proportions slightly dispersed, except for the samples with a 1:1 ratio, which dispersed at 40°C, aggregated at 50°C, and remained almost constant at other temperatures.

The physicochemical characteristics of the surface of particles, especially the microstickies, affected the whole system and were induced to establish a new balance. The pH of the system was not affected. **TJ**

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ABOUT THE AUTHORS

Microstickies are among the most serious barriers to papermaking. We researched macrostickies in the procedure of pulping, which supplied the measure, method, and idea for this study of microstickies.

The most difficult aspect of this study was the sensitivity of particles of microstickies, which easily gather and disperse. We had to measure the microstickies accurately and uniformly in a short time.

One of our discoveries was that the role of the enzymes mixture depends on the proportions of the enzymes used. Surprisingly, the microstickies particles were more sensitive when the enzymes were applied.

The findings from our study can help mill technical staff know the role of enzymes used in pulp and white water and help them use this technology rationally.

As a further extension of this work, our next step is to investigate microstickies removal from white water.

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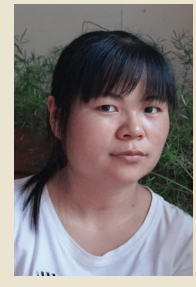
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