

INFLUENCE OF BUFFER SOLUTION ON TEMPO-MEDIATED OXIDATION

Mei Xu, Hongqi Dai,* Xuan Sun, Shumei Wang, and Weibing Wu

TEMPO-mediated oxidation has been reported to effectively convert C6 primary hydroxyl groups to carboxyl groups for better water-solubility. However, the pH decreases continuously during the oxidation process, and it is therefore difficult to maintain the stability of the reaction. The control of pH at a constant level throughout the oxidation process is a complicated task. The applicability of a carbonate buffer system and a borax buffer system with various continuous addition rate of sodium hypochlorite solution was considered. Carbonate buffer solution and borax buffer solution can efficiently buffer the pH. The results of carboxyl content and DP of celluloses proved that the activities of sodium hypochlorite solution can be maintained when sodium hypochlorite is added with controlled flow rates without adjusting pH by hydrochloric acid. Buffer solutions created a milder reaction environment in which the damage of celluloses would be buffered. The conclusion was consistent with DP tests of celluloses. Compared with carbonate buffer, the borax buffer with high ability of penetration could enhance the depth and width of oxidation, which was demonstrated by the results of X-ray diffraction patterns and carboxyl content of celluloses.

Keywords: TEMPO; Cellulose; Oxidation; Buffer solution; Drop rate; Carboxyl content

Contact information: Jiangsu Provincial Key Lab of Pulp and Paper Science and Technology, Nanjing Forestry University, Nanjing Jiangsu, 210037, P. R. China

**Corresponding author:* hgdhq@njfu.edu.cn

INTRODUCTION

Piperidine radicals are noted for their high stability. The 2,2,6,6-tetramethyl-1-piperidinyloxy radical (TEMPO)/NaBr/NaClO has been reported to effectively convert C6 primary hydroxyl groups of cellulose to carboxyl groups via a reactive aldehyde-intermediate (de Nooy et al. 1995). Regioselective oxidations of C6 primary hydroxyls of polysaccharides to carboxyl groups have been developed, in which 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO) and sodium bromide have been used as catalysts under aqueous conditions at pH from 10 to 11 (Isogai et al. 1998). Such modifications of cellulose are usually achieved by converting some of the surface OH moieties of cellulose into carboxyl groups (Saito et al. 2004). Neither the crystalline type of cellulose nor the width of the crystalline region is changed in the oxidation process. In addition, the oxidation process could increase the carboxyl content of cellulose, which as a consequence, would increase the hydrophilicity of cellulose (Saito et al. 2005).

The pH value is a key factor that affects the reaction of TEMPO/NaClO/NaBr, with the optimal value under aqueous conditions within the range 10 to 11. A stable pH ensures high quality of products. Recently it was reported that under acidic conditions

curdlan and other polysaccharides could be oxidized by 4-acetamide-TEMPO/NaClO/NaClO₂ with 100 mL of 0.2M acetate buffer at pH 4.7 (Tamura et al. 2010). In this process, the sodium hypochlorite solution was added all at once. Regioselective oxidation transforms C6 primary hydroxyls on cellulose to aldehyde groups at the beginning of the reaction under acidic conditions. Then the C6 primary aldehyde groups on cellulose are oxidized to carboxyl groups by NaClO₂. The Na₂CO₃-NaHCO₃ buffer system was also applied in the selective oxidation of ethanol by Liaigre (2005). It can be seen from the two examples above, alkaline buffer solution has the potential to be applied in the TEMPO/NaClO/NaBr aqueous system, because it does not affect the normal reaction and can maintain the reaction pH continuously.

A carbonate buffer system and borax buffer system can be used at pH 10.5. A carbonate buffer system is stable and does not react with cellulose. In the oxidation process, it only maintains a certain pH with a huge buffering capacity. In the borax-sodium hydroxide system, however, the outer electronic structure of boron atoms determined that it can form bonds with cellulose, and thus promoting the penetration of the reaction solution. The borax-sodium hydroxide buffer system also demonstrated a small buffering capacity. Thus, the sodium hypochlorite solution was not added all at once. Simultaneously, it can create a mild reaction environment when the TEMPO-mediated oxidation is started by trickling sodium hypochlorite with controlled flow rates.

In general, cotton fiber (Dai et al. 2011), softwood pulps (Dang et al. 2007), hardwood pulps (Fukuzumi et al. 2010, Mishra et al. 2011), lignocellulosic fibers (Hamid et al. 2011), and regenerated cellulose (Sun et al. 2005) can be oxidized by a TEMPO/NaClO/NaBr aqueous system. During the reaction, sodium hypochlorite solution is typically added all at once before the reaction (Qin et al. 2011). Sodium hydroxide solution was added continuously to maintain the pH (Saito et al. 2006). During oxidation of alcohols, da Silva Perez et al. (2003) controlled the reaction by adding sodium hypochlorite solution drop by drop. The problem of buffer capacity of the borax buffer was solved with the method of da Silva Perez. In this study, the applicability of the carbonate buffer system and the borax buffer system with various dropping rate of sodium hypochlorite solution was considered. This method further simplified the control of TEMPO oxidation, which has a potential to be applied in mass production.

EXPERIMENTAL

Materials

The softwood bleached kraft pulp used in this study was a commercial pulp supplied by Celgar Co., Ltd., Canada. TEMPO (2,2,6,6-tetramethyl piperidine-1-oxyl radical) was purchased from Johnson Matthey Co., UK. Sodium bromide and sodium hypochlorite solution (ca. 8.6% active chlorine) were supplied by Jiuyi Chemicals Reagent (Shanghai China). All other chemicals were analytically pure (Shanghai Chemicals Co., Ltd, China) and were used without further purification.

Carbonate buffer (pH 10.5) was prepared with 0.1 M sodium carbonate (500 mL) and 0.1 M sodium bicarbonate (250 mL) at room temperature. Borax buffer solution (pH 10.5) was prepared with 0.05 M borax (320 mL), 0.2 M sodium hydroxide (145 mL), and deionized water (285 mL) at room temperature.

Methods

TEMPO-mediated oxidation

The fibers (10 g) were suspended in buffer solution (750 mL, pH 10.5) containing TEMPO (0.025 g) and sodium bromide (0.25 g). The TEMPO-mediated oxidation was conducted in carbonate buffer solution (pH 10.5) and borax buffer solution (pH 10.5) at room temperature respectively for two hours with agitation. The TEMPO oxidation was started by trickling sodium hypochlorite at controlled flow rates. One gram of celluloses required 8 mmol of sodium hypochlorite solution. The solution of sodium hypochlorite was added, respectively, within 15 min, 30 min, 45 min, and 60 min to control the flow rates. The TEMPO-oxidized cellulose was thoroughly washed with water and stored at 4°C before further treatment or analysis.

Reaction rate

During the oxidation process, the generation of carboxyl groups was monitored by recording the pH value every 5 minutes. The slope of the time-pH curve was applied to reflect the generation rate of the carboxyl content.

Determination of carboxyl groups

The carboxyl content of the TEMPO-oxidized cellulose was determined by an electrical conductivity titration method (Saito et al. 2007). In the electrical conductivity titration method, sodium carboxyl groups in the TEMPO-oxidized cellulose were converted to free carboxyl groups by ion-exchange treatment. Fibers (3g) were suspended in water (450 mL). The slurry was adjusted to pH 2 to 3 with 0.1 M hydrochloric acid, and allowed to sit for 1 hr, followed by thorough washing with water. This process was repeated one more time (Mao et al. 2008).

Determination of degree of polymerization

The degree of polymerization of cellulose was estimated, since cellulose samples with different DP need to use different amounts in order to achieve a target viscosity during the test. Celluloses (0.15 g) before oxidation and TEMPO-mediated celluloses (1g) were dissolved respectively in copper ethylenediamine solution to determine the degree of polymerization of cellulose by capillary viscometer (Shi et al. 2010).

X-ray diffraction patterns of oxidized celluloses

The softwood fiber samples were converted to powder substrates and then subjected to X-ray diffraction analysis from 5° to 60° at 5°/min of diffraction angle 2θ using the reflection method by means of a D/Max-3A (Japan) with a Nifiltered CuK α radiation ($\lambda=0.1548\text{nm}$) at 40 kV and 100 mA. The crystallinity indices based on 2θ scan from 5° to 60° were calculated according to Segal's method,

$$\text{Crystallinity} = (I_{002} - I_{AM}) \times 100\% / I_{002} \quad (1)$$

where I_{002} was identified with the intensity at 22.5, and I_{AM} was the intensity at 16.5.

RESULTS AND DISCUSSION

The Effects of Dropping Rate of Sodium Hypochlorite Solution on Oxidation

The first series of experiments involved the effect of adding sodium hypochlorite solution by different modes of oxidation. Aqueous systems, in the absence of buffer, were applied in the oxidation process. In the default method, sodium hypochlorite solution was pH-adjusted in advance and added at once. By contrast, sodium hypochlorite was added dropwise in a continuous manner. In Table 1, experimental conditions were designed to determine the effects of the dropping rate of sodium hypochlorite solution on oxidation.

Obviously, a mild reaction environment can be created when the TEMPO-mediated oxidation is started by used of a trickling sodium hypochlorite with controlled flow rates. The results of carboxyl content and DP reflected that the activities of sodium hypochlorite solution can be maintained without adjusting pH by hydrochloric acid. With other parameters held constant, the optimal flow rate was when the sodium hypochlorite was added gradually within 30 min. Under this condition, celluloses were protected and the oxidation reaction progressed extensively.

Table 1. Reaction Conditions in the Aqueous System

Reaction system	Add way of NaClO solution	pH	Reaction time	Amount of NaClO / mmol·g ⁻¹ fiber	Carboxyl content /mmol·g ⁻¹ fiber	DP
Aqueous media	add all at once	10.5	2hr	8	0.48	106
Aqueous media	add gradually within 15 min	10.5	2hr	8	0.5670	107
Aqueous media	add gradually within 30 min	10.5	2hr	8	0.6011	109
Aqueous media	add gradually within 45 min	10.5	2hr	8	0.5701	104
Aqueous media	add gradually within 60 min	10.5	2hr	8	0.5456	106

The Effect of Buffer System On TEMPO-Oxidized Celluloses

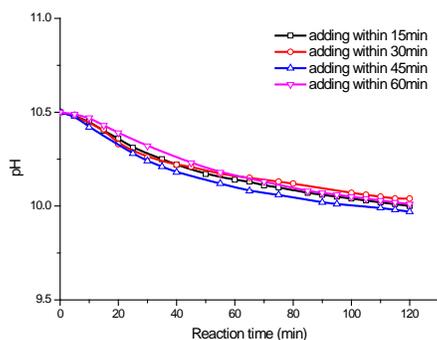
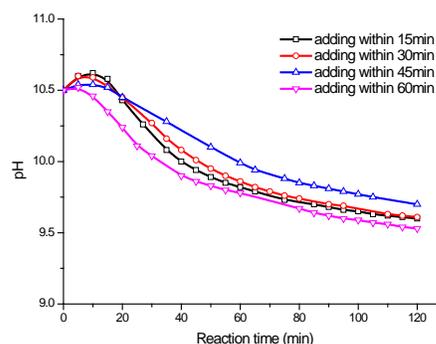
Fluctuations in pH inevitably resulted when sodium hypochlorite was controlled by flow rates without pH adjustment. Buffer solution used for this situation can moderate the impact of sodium hypochlorite (pH 12.6) and generation of carboxyl groups. The function of buffer was to keep the pH value of the solution in a proper rang. The pH value of buffer was maintained by the equilibrium state of conjugate acid-base pairs (Jia et al. 2002). It provided a milder environment for TEMPO-mediated oxidation.

A carbonate buffer system and borax buffer system are two kinds of common alkali buffer solution that can be used at pH 10.5. In the following experiments, a carbonate buffer system and a borax buffer system were applied in the oxidation process. Experiments in Table 2 were designed to determine the effects of drop rate of sodium hypochlorite solution on oxidation in the buffer system.

Table 2. Reaction Conditions in the Buffer System

Reaction system	Add way of NaClO solution	pH	Reaction time	Amount of NaClO /mmol·g ⁻¹	Carboxyl content /mmol·g ⁻¹ fiber	DP
Carbonate buffer system	add gradually at 15min	10.5	2hr	8	0.5917	110
Carbonate buffer system	add gradually at 30min	10.5	2hr	8	0.6068	110
Carbonate buffer system	add gradually at 45min	10.5	2hr	8	0.5968	108
Carbonate buffer system	add gradually at 60min	10.5	2hr	8	0.5567	110
Borax buffer system	add gradually at 15min	10.5	2hr	8	0.6126	116
Borax buffer system	add gradually at 30min	10.5	2hr	8	0.6076	116
Borax buffer system	add gradually at 45min	10.5	2hr	8	0.6133	113
Borax buffer system	add gradually at 60min	10.5	2hr	8	0.6369	116

In the buffer, pH was not entirely the same. This was due both to the generation of carboxyl groups during the reaction and a variety of other chemical reactions that changed the ratio of conjugate acid-base pairs. During the oxidation process, the generation of carboxyl groups was monitored by recording the pH value every 5 minutes. Figures 1 and 2 show the changes in pH value in the carbonate buffer and borax buffer, respectively, during the reaction.

**Fig. 1.** Variation of pH under different sodium hypochlorite drop rate in the carbonate buffer**Fig. 2.** Variation of pH under different sodium hypochlorite drop rate in the borax buffer

According to time-pH curve, the relationship between time and reaction rate can be obtained. The slope of the time-pH curve was applied to reflect the rate of generation of the carboxyl content. Figures 3 and 4 reflect the relationship between time and reaction rate.

In the control group, the pH of the system was stable with carbonate buffer when fiber was not added during the reaction, while other conditions were not changed. As shown in Fig. 1, the pH of the system was stable between the pH of 10.5 to 10 with

carbonate buffer. From Fig. 3, it can be seen that sodium hypochlorite concentration had little effect on the reaction. The optimal addition rate of sodium hypochlorite was adding it gradually within 30 min. This was due to the fact that the reaction was finished at 2 h. Under other addition rates, time needs to be extended, because the reactions did not end with a low reaction rates. In addition, comparing Table 1 with Table 2, it can be seen that the oxidation regulation of carbonate buffer solution was the same as that of the water system. With the increasing flow rate of sodium hypochlorite, the carboxyl content of the products increased then decreased. The carboxyl content and DP were all optimal when the sodium hypochlorite was added gradually within 30 min. Reaction was rapid under high flow rate. Cellulose macromolecules were degraded under high sodium hypochlorite concentration. At reduced flow rate the cellulose can be protected. But the reaction activities were also low. In addition, cellulose molecules would be cleaved when the flow of sodium hypochlorite was added continuously at the end of the reaction. Therefore, satisfactory results could not be achieved if the dropping rate of sodium hypochlorite was too fast or too slow. Experimental results shown that a milder reaction environment can be provided by a moderate dropping rate.

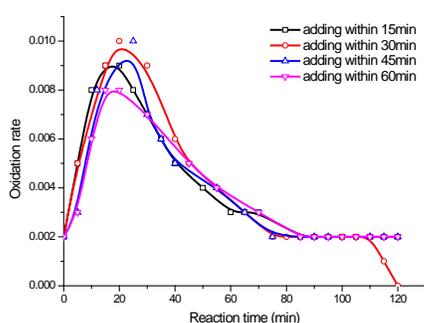


Fig. 3. The relationship between time and reaction rate in the carbonate buffer

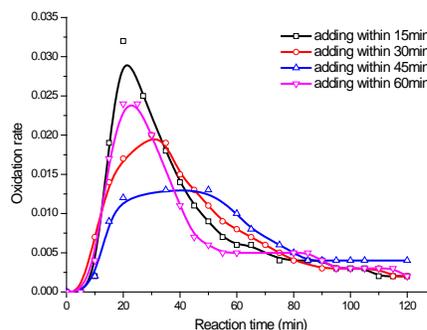


Fig. 4. The relationship between time and reaction rate in the borax buffer

As shown in Fig. 2, the buffering capacity of the borax buffer was smaller compared with the carbonate buffer. Therefore, the dropping rate of sodium hypochlorite was a key factor in the borax buffer. As shown in Fig. 4, both high dropping rate and a slow dropping rate were beneficial to the reaction rate. Obviously, borax buffer was different from carbonate buffer in oxidation regulation. The sodium hypochlorite concentration was not the only influencing factor. The penetration of the reaction solution can be promoted in a borax buffer. For this reason, the penetration time was also an important influencing factor. The carboxyl content was high when the reaction rate was fast under high flow rate of sodium hypochlorite. The carbonate buffer system itself did not play a role in cellulose. In the borax buffer, the buffer had a swelling effect on celluloses. Because the structure of B atom is $2s^22p^1$, and the main oxidation number is 3. It could form a 4- or 6-coordination compound. Since the B atom has a small atomic radius, it usually forms a 4-coordination structure (Zhang et al. 2010). Because of the structure of borax, it could form chemical bonds with the hydroxyl groups of cellulose (Zhang et al. 1998). Thus, the reaction solutions were promoted to penetrate into cellulose with the increase of soaking time. If a steady stream of sodium hypochlorite

was added continuously with the increase of soaking time, a higher carboxyl group content was obtained with borax buffer. According to its small capacity and its infiltration, the experiment condition was optimal when the sodium hypochlorite was being added gradually within 60 min. The conclusion was consistent with Table 2.

Structural Analyses of the Oxidized Celluloses with Different Conditions

X-ray diffraction was used to further understand the structure of celluloses after being subjected to different conditions. Five reaction conditions of cellulose and their X-ray results are shown in Fig. 5. The crystallinity of celluloses is shown in Table 3.

Table 3. Results of X-ray Diffraction Patterns

Reaction Number	Reaction Condition	DP	Crystallinity /%
0	Fibers before oxidation	1100	77.43%
1	Fibers were oxidized by adding sodium hypochlorite at once.	106	77.30%
2	Fibers were oxidized by adding sodium hypochlorite gradually within 30min in water system.	109	77.37%
3	Fibers were oxidized by adding sodium hypochlorite gradually within 30min in carbonate system.	110	78.05%
4	Fibers were oxidized by adding sodium hypochlorite gradually within 30min in borax system.	116	91.12%

As shown in Fig. 5, the crystal types of celluloses were not changed after oxidation. In the buffer-free system, the pH value of reaction was not stable and celluloses would be damaged in that changing oxidation environment.

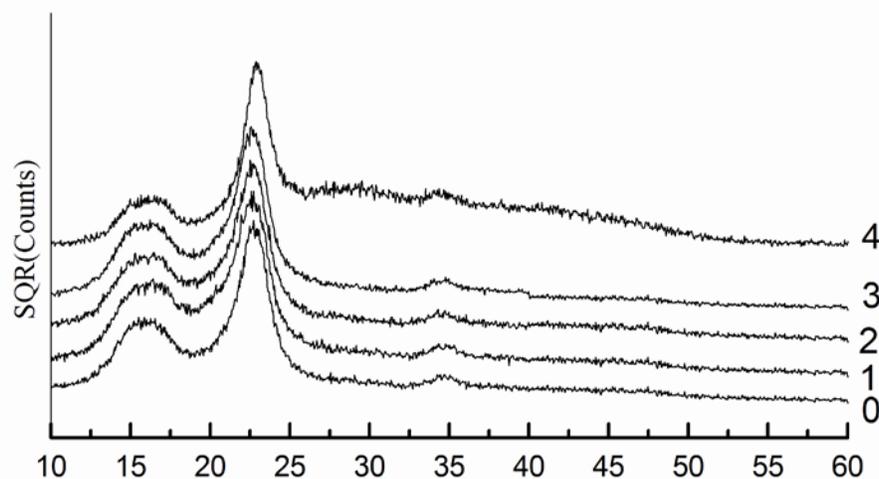


Fig. 5. X-ray diffraction patterns of the original (0), after the TEMPO-mediated oxidation with adding sodium hypochlorite at once (1), after the TEMPO-mediated oxidation with adding sodium hypochlorite gradually within 30 min in water system (2), after the TEMPO-mediated oxidation with adding sodium hypochlorite gradually within 30 min in carbonate system (3), after the TEMPO-mediated oxidation with adding sodium hypochlorite gradually within 30min in borax system (4) at room temperature.

According to the results, the crystallinity degrees of cellulose were not changed in the buffer-free system and carbonate system. It can be concluded that the carboxyl groups were not formed inside crystallites during the TEMPO-mediated oxidation; the significant amounts of carboxyl groups formed in the water-insoluble fractions were mostly present on the crystal surfaces and in amorphous regions.

In borax system, the crystallinity of cellulose increased markedly. Most part of the amorphous region of the cellulose tended to become ordered in the borax buffer, and the crystallinity of cellulose increased. The borax solution could form chemical bonds with the hydroxyl groups of cellulose. In the process of washing celluloses, some borax solution was removed by water, and a small amount of borax solution was locked in the new crystalline region of cellulose. This was why a water peak appeared in the X-ray map of borax buffer solution (Chen et al. 1990). The results further indicated that the borax solution could form chemical bonds with the hydroxyl groups of cellulose. Therefore, the borax buffer solution could enhance the depth and width of oxidation in the TEMPO-mediated system.

CONCLUSIONS

1. In a buffer-free aqueous system, the results for carboxyl content and DP of cellulose demonstrated that the activities of sodium hypochlorite solution can be maintained when sodium hypochlorite is added with controlled flow rates without use of hydrochloric acid to adjust the pH.
2. Buffer solutions created a milder reaction environment in which the damage of celluloses would be minimized. This conclusion was supported by observations of the DP of cellulose. Compared with carbonate buffer, the borax buffer showed a high ability to penetrate into the fibers, and it was able to enhance the depth and width of oxidation, which was shown by X-ray diffraction patterns and carboxyl content of celluloses.
3. The oxidation regulation of carbonate buffer solution was same as that of the buffer-free system. The sodium hypochlorite concentration was the key factor. The optimum condition involved adding sodium hypochlorite gradually within 30 min.
4. A borax buffer was different from the carbonate buffer in oxidation regulation. The penetration time was another important factor. Therefore, the optimum condition involved adding sodium hypochlorite gradually within 60 min.

ACKNOWLEDGMENTS

The research has been financially supported by the Priority Academic Program Development of Jiangsu Higher Education Institutions, China.

REFERENCES CITED

- Chen, J. (1990). *Pulping Chemistry*, M. Light Industry Press, 36-37.
- Dai, L., Dai, H., Yuan, Y., Sun X., and Zhu Z. (2011). "Effect of TEMPO oxidation system on kinetic constants of cotton fibers," *BioResources* 6(3), 2619-2631.
- Dang, Z., Zhang, J., and Ragauskas, A. J. (2007). "Characterizing TEMPO-mediated oxidation of ECF bleached softwood kraft pulps," *Carbohydrate Polymers*. 70, 310-317.
- da Silva Perez, D., Montanari, S., and Vignon, M. R. (2003). "TEMPO-mediated oxidation of cellulose III," *Biomacromolecules* 4(5), 1417-1425.
- De Nooy, A. E. J., Besemer, A. C., and van Bekkum, H. (1995). "Highly selective nitroxyl radical-mediated oxidation of primary alcohol groups in water-soluble glucans," *Carbohydrate Research* 269, 89-98.
- Fukuzumi, H., Saito, T., Okita, Y., and Isogai, A. (2010). "Thermal stabilization of TEMPO-oxidized cellulose," *J. Polymer Degradation and Stability* 95, 1502-1508.
- Kaddami, H., Sbiai, A., Sautereau, H., Maazouz, A., and Fleury, E. (2011). "TEMPO-mediated oxidation of lignocellulosic fibers from date palm leaves," *Carbohydrate Polymers* 86(4), 1445-1450.
- Isogai, A., and Kato, Y. (1998). "Preparation of polyuronic acid from cellulose by TEMPO-mediated oxidation," *Cellulose* 5, 153-164.
- Jia, Z., Zhang, S., He, Q., and Xuan, D. (2002). *Inorganic and Analytical Chemistry*, M. Higher Education Press, 128-129.
- Liaigre, D., Breton, T., and Belgsir, E. I. M. (2005). "Kinetic and selectivity control of TEMPO electro-mediated oxidation of alcohols," *Electrochemistry Communications* 7, 312-316.
- Mao, L. S., Law, K. N., Brouillette, F., and Daneault, C. (2008). "Effect of 4-acetamido-TEMPO-mediated oxidation conditions on the formation of carboxylic acid groups on mechanical pulp fibers," *Journal of Pulp and Paper Science* 34(4), 198-202.
- Mishra, S. P., Thirree, J., Manent, A.-S., Chabot, B., and Daneault, C. (2011). "Ultrasound-catalyzed TEMPO-mediated oxidation of native cellulose for the production of nanocellulose: Effect of process variables," *BioResources* 6(1), 121-143.
- Qin, Z.-Y., Tong, G.-L., Chin, Y. C. F., and Zhou, J.-C. (2011). "Preparation of ultrasonic-assisted high carboxylate content cellulose nanocrystals by TEMPO oxidation," *BioResources* 6(2), 1136-1146.
- Saito, T., and Isogai, A. (2004). "TEMPO-mediated oxidation of native cellulose. The effect of oxidation conditions on chemical and crystal structures of the water - Insoluble fractions," *Biomacromolecules* 5, 1983-1989.
- Saito, T., Kimura, S., Nishiyama, Y., and Isogai, A. (2007). "Cellulose nanofibers prepared by TEMPO-mediated oxidation of native cellulose," *Biomacromolecules* 8, 2485-2491.
- Saito, T., Okita, Y., Nge, T. T., Sugiyama, J., and Isogai, A. (2006). "TEMPO-mediated oxidation of native cellulose: Microscopic analysis of fibrous fractions in the oxidized products," *Carbohydrate Polymers* 65, 435-440.
- Saito, T., Shibata, I., Isogai, A., Suguri, N., and Sumikawa N. (2005). "Distribution of carboxylate groups introduced into cotton linters by the TEMPO-mediated oxidation," *Carbohydrate Polymers*. 61, 414-419.

- Shi, J., He, F. (2010). *Analysis and Detection of Pulp and Paper*, M. Light Industry Press, 67-71.
- Sun, B., Gu, C., Ma, J., and Liang, B. (2005). "Kinetic study on TEMPO-mediated selective oxidation of regenerated cellulose," *Cellulose* 12, 59-66.
- Tamura, N., Hirota, M., Saito, T., and Isogai, A. (2010). "Oxidation of curdlan and other polysaccharides by 4-acetamide-TEMPO/NaClO/NaClO₂ under acid conditions," *Carbohydrate Polymers*. 81, 592-598.
- Zhang, D., Xi, P., and Wang, Q. (1998). "The application of borax in konjac production," *Science and Technology of Food Industry*. 3, 37-38.
- Zhang, X., Li, J., and Li, C. (2010). "The research of IR and crystal structure in borax," *J. Guangdongchemical* 37(8), 77-79.

Article submitted: October 30, 2011; Peer review completed: December 10, 2011;
Revised version received: January 5, 2012; Second revision received and accepted:
February 11, 2012; Published: February 14, 2012.