Research Article

Effect of Hemicellulase Enzyme in Flotation Deinking of Laser-printed Paper

Pravitra Chandranupap*
Department of Industrial Chemistry, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand

Panitnad Chandranupap
Department of Chemical Engineering, Faculty of Engineering, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand

* Corresponding author. E-mail: pravitra.c@sci.kmutnb.ac.th DOI: 10.14416/j.asep.2020.04.006
Received: 15 January 2020; Revised: 9 March 2020; Accepted: 7 April 2020; Published online: 27 April 2020

Abstract
Deinking is one of the most important steps in paper recycling process. Novel deinking method such as emzymatic deinking has grown more and more important in environmentally friendly paper recycling. The aim of this work was to investigate the effect of hemicellulase enzyme on the efficiency of ink removal from laser-printed paper in flotation deinking process. The influence on pulp and mechanical properties of deinked paper such as freeness, tensile index and tear index are also studied. The experiments performed at the dosages of hemicellulase enzyme from 0 to 0.15% with the concentration of nonionic surfactant (Tween-80) of 1.75% (Base on oven-dried weight of paper). The results obtained in this work revealed the enhancement of ink removal efficiency (11.3–95.3%), as indicated by the decreasing of the effective residual ink concentration (ERIC) as compared to non-deinked paper. Moreover, the results showed the improvement in freeness (3.7–13.2%), tensile index (5.2–18.1%), and tear index (19–25.6%) with respect to the control condition obtained with the absence of enzyme. These improvements were achieved considerably after flotation process but tended to drop down when the dosage of enzyme was too much. The maximum deinking efficiency (about 95%) was found at 0.1% hemicellulase enzyme (based on oven-dried weight of paper) which the mechanical properties after flotation deinking process increased about 25% and 18% in tear index and tensile index, respectively, as compared to control.

Keywords: Hemicellulase, Flotation, Deinking, Laser-printed paper

1 Introduction

Hemicellulose and cellulose are the most abundant organic natural resources in the world. In paper production, the pre-extraction of raw material usually leads to hemicellulose loss in pulp. Although hemicellulose is less important than the cellulose content in pulp, it still brings an important contribution to pulp quality. For example, hemicellulose can enhance beatability that improve the accessible to water molecules in pulp that is important for some paper products such as tissue and cleaning paper [1]. Moreover, hemicellulose in pulp serves as an inter-fiber binding agent that improves the strength properties of paper products, including tensile, tear, and burst [2].

Paper recycling is the process of turning waste paper into new paper products that can reduce demand of virgin pulp from deforestation. In recent fiscal year of 2019 (Oct. 2018–Sep. 2019), Thailand's domestic sale of printing paper only reached almost 700,000 ton [3].

Please cite this article as: P. Chandranupap and P. Chandranupap, "Effect of hemicellulase enzyme in flotation deinking of laser-printed paper," *Applied Science and Engineering Progress*, vol. 14, no. 3, pp. 370–377, Jul.–Sep. 2021, doi: 10.14416/j.asep.2020.04.006.

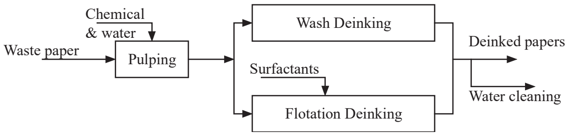

Figure 1: Recycle pulp process. (adapted from Behin and Vahid [6]).

One of major waste paper from this consumption is office paper (xerographic and laser-printed papers). Such waste paper has good fiber quality and relatively high brightness so it is preferably one of major sources for recycled paper. Deinking is the one of the most crucial steps in paper recycling. In order to develop new deinking technology, bio-deinking is one of newly methods to reveal a new way for paper recycling process [4].

There are generally two main deinking processes has been used in paper industry: wash deinking and flotation deinking. Wash deinking uses screen to retain wood fibers and the unwanted ink to pass through it. The advantage of wash deinking is the ability to separate inks from other chemicals because it is a size-dependent method. However, pulp loss is quite high. In contrast, flotation deinking selectively removes hydrophobic particles using surfactant and air bubbles from solution that produces less pulp loss [5]. Pulp recycling process can be simply expressed in Figure 1.

Enzymatic deinking was given more consideration from pulp and paper industries for quite some time due to the industries' strategy in reducing hazadous chemicals in deinking process. Biological enzymes such as cellulase and hemicellulase have been used in recycled paper treatment in many mill practices [7]. Cellulases are components of many enzymes mainly produced from fungi or bacteria that catalyse the decomposition of cellulose and related polysaccarides. Hemicellulases are also comprised of various species that react many hemicellulose units (for example, xylanases hydrolyze xylan into xylose and mannanase hydrolyze mannan into mannose). The use of these enzymes leads to removal of the fibrils contained in the fiber surface, a process known as 'peeling', which also contributes to the detachment of ink from fiber surfaces [8]. Moreover, the separation of cellulose fibrils from the ink particles results in increasing the hydrophobic behaviour of the particles and thus enhancing the efficiency of the flotation [9]. The purposed mechanism for enzymetic deinking was illustrated in Figure 2.

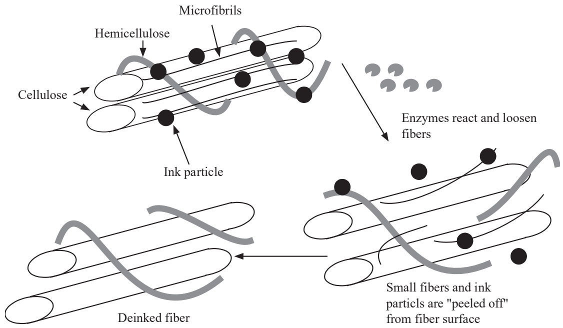

Figure 2: Proposed mechanism for enzymatic deinking.

Suspensed inks and fibrils after enzyme treatment are then remove from pulp slurry with flotation that use surfactant as ink removal. The chemical structure of surfactants used for flotation deinking may differ significantly. They can be cationic, anionic, or non-ionic [10]. Generally, anionic and non-ionic surfactant are commonly used. This is due to their ability to provide good ink detachment from fibers during deinking process. The critical micelle concentration (CMC) of surfactant also plays an important role in determining how much surfactant required in deinking [11].

The recent work of our research group investigated the enzymatic deinking of xerographic office paper by using cellulase enzyme in accordance with Triton X-100 as non-ionic surfactant [12]. Thus, effect of hemicellulase that reacted mainly onto hemicellulose part of deinked pulp was investigated in this work. In addition, This work used Tween-80 non-ionic surfactant in flotation process to remove ink particles after hemicellulose pretreatment. This is due to its low cytotoxicity (LC_{50} of 850.0 ± 26.0 μg mL^{-1}) in comparison with Triton X-100 (LC_{50} of 34.0 ± 2.0 μg mL^{-1}) [13]. Properties of deinked pulp were also tested in order to study the various parameters which had impact on deinking process.

2 Materials and Methods

2.1 *Chemicals, enzyme and raw materials*

The commercial hemicellulase enzyme (*Aspergillus niger*) used for deinking was purchased from Sigma-Aldrich (150 KU, H-2125 SIGMA, enzyme activity = 3.0 u/mg). The enzyme solution was prepared by dissolving 1 g of enzyme powder in 100 mL of distillated water and was stored at 4°C prior to use. Polysorbate 80 or Tween-80 (nonionic) surfactant, analytical grade, was obtained from Chemical Express Co., Ltd. Office papers (A4 size, basic weight 80 g/m^2) from Double A. (1991) Ltd. (Thailand) were printed with laser printer on one side of each sheet. The toner's brand is Samsung MLT-D104S. The percentage of uniform surface printed was about 50% of each sheet. This raw material was referenced as non-deinked paper.

2.2 *Repulping*

The laser printed papers were cut into shreds with paper shredder to the approximate size of 7×20 mm and soaked in tap water for 12 h at room temperature and then transferred into a mixer for disintegration. The paper slurry was disintegrated at 1500 rpm for 5 min under room temperature to 5% pulp consistency before deinking treatment.

2.3 *Enzymetic flotation deinking process*

Enzyme dosages were used in the range of 0–0.15 wt% on oven-dried weight of paper pulp (g/100g of oven-dried paper pulp, % on o.d. pulp) [14]. The concentration of nonionic surfactant (Tween-80) was fixed at 1.75 wt% on oven-dry weight of paper pulp. The reaction of hemicellulase enzyme with pulp conducted at 50°C for 30 min under continuous slow mixing of pulp slurry with enzyme and nonionic surfactant. After that 800 mL of water was added into the slurry and heated up to 80°C for 10 min in order to deactivate the enzyme activity (no pH adjustment). After enzyme treatment, the slurry was then diluted to 0.3% pulp consistency by using tap water. The prepared pulp suspension was transferred into a 21 L custom-made laboratory scaled flotation cell (Figure 3). The flotation process was performed at 10 L air/min for 10 min at room temperature. Control pulp was processed as described above before flotation process with the absence of enzyme.

2.4 *Evaluation of deinked pulp*

Deinked pulp was taken before and after flotation process to prepare handsheets for determination of optical and mechanical properties of paper. Pulp properties such as residual ink and freeness of the pulp were also examined so as to evaluate the properties of the deinked pulp. The optical property of the handsheets such as brightness was determined. Mechanical properties of handsheets including tensile strength and tear resistance of paper were also determined.

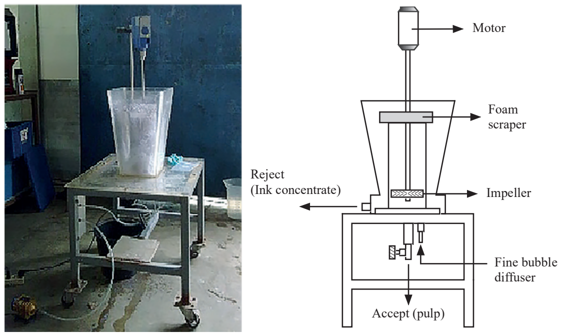

Figure 3: Custom-made laboratory scaled flotation cell.

2.4.1 Determination of pulp properties

The procedure used to measure the freeness of the pulp was conducted as described in TAPPI T227 om-04 (freeness of pulp, Canadian Standard Freeness) using the bursting freeness tester (LTDA, Regmed Industria Techica de Frecisao).

2.4.2 Determination of deinking efficiency

Deinking efficiency was indicated by the Effective residual ink concentration (ERIC) and brightness of the paper. Handsheets preparation was performed using TAPPI Test Method T218 om-91 for reflectance testing of pulp (Buchner Funnel Procedure). Handsheets were conditioned under controlled conditions as described in TAPPI Test Methods (TAPPI T402) before the deinked papers were evaluated for its ERIC and Brightness. The measurement of the paper brightness (% ISO) was carried out by the method of TAPPI T452 om-98 (brightness of pulp, paper and paper board) (directional reflectance at 457 nm) and ERIC of the paper (ppm) was examined using the method of TAPPI T567 om-04 (ERIC of pulp, paper and paper board) (directional reflectance at 950 nm) using Brightness and ERIC tester (Color-Touch PC, Technidyne Corporation).

2.4.3 Determination of mechanical properties of paper

The tensile index of the deinked papers was measured according to TAPPI T494 om-01 test method (Stograph E-S, Toyoseiki SHO). Meanwhile the method of TAPPI T414 om-98 was used to determine the tear index based on the internal resistance of the handsheet using the Electronic tearing tester (Model Protear, Thwing-Albert, Thwine-Albert Instrument).

2.4.4 Statistical method

The significance of difference between each test variables were determined using ANOVA analysis by SPSS® software. All tests were done with a confidence interval of 95%. Error bars shown in figures indicate means with standard error of five test specimens from each test unit of the sample.

3 Results and Disscussion

3.1 *Statistical analysis of deinking*

Statistical analysis using one-way ANOVA, as shown in Table 1, indicated significant difference (p-value < 0.05) in the results obtained in this work. It showed that the usage of hemicellulase enzyme along with nonionic surfactants (Tween-80) in flotation deinking process affected the deinked laser-printed paper properties (Freeness, ERIC, Brightness, Tear index and Tensile index).

Table 1: ANOVA results of hemicellulase enzyme on deinked pulp

Properties of Deinked Pulp	Variable's p-values	R^2
	Enzyme (% on o.d.pulp)	
Freeness	< 0.001	0.999
ERIC	< 0.001	0.979
Brightness	< 0.001	0.895
Tensile index	< 0.001	0.901
Tear index	< 0.001	0.810

3.2 *Freeness (Canadian Standard Freeness)*

Freeness is a measure of an ability of water to drain through the pulp. A high drainage rate means a high freeness. Freeness is an important factor in the paper mill on account of an influence on the operating condition of a paper machine. The physical testing of pulp freeness was done according to TAPPI T227 om-09 [15]. As shown in Figure 4, pulp freeness after the

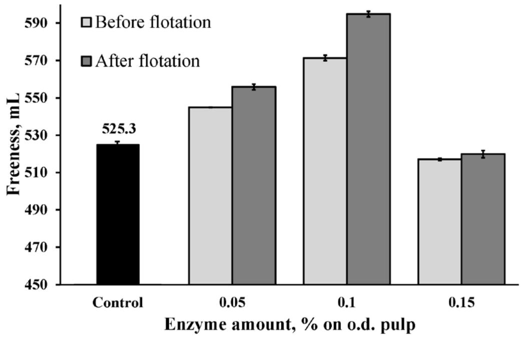

Figure 4: The effect of dosage of enzyme on freeness.

enzymatic deinking process increased with the dosage of enzyme and had a significant higher increase after flotation process. This may be due to enzymatic hydrolysis that selectively remove fine fibers [16]. The maximum freeness of the pulp before and after flotation were 571.30 and 594.8 mL at 0.1% enzyme (based on oven-dried weight of paper), respectively. The maximum freeness of the pulps before and after flotation increased by 8.8% and 13.2%, respectively as compared with the control pulp. This can be attributed to the stability of air bubbles as a result of surfactant leading to higher removal of fines and short fibers in flotation process. Consequently, pulp freeness after flotation increased. Then the pulp freeness decreased when increasing the dosage of enzyme. It can be attributed to much more broken short fibers due to the reaction of hemicellulase enzyme and fibers when the enzyme dosage is too much resulting in the reduction of drainage rate.

3.3 *Deinking efficiency*

3.3.1 Effective residual ink concentration (ERIC)

ERIC is the value of the amount of remaining ink which refers to the total ink particle area (mm^2) per sheet area (m^2) in the paper before and after deinking process. The efficiency of ink removal as indicated by ERIC was shown in Figure 5. It was found that ERIC decreased as increasing the dosage of enzyme. However, the ink removal as indicated by ERIC before flotation decreased even though adding more enzyme up to 0.1%, but the ERIC values were still high. Because the detached ink particles are still

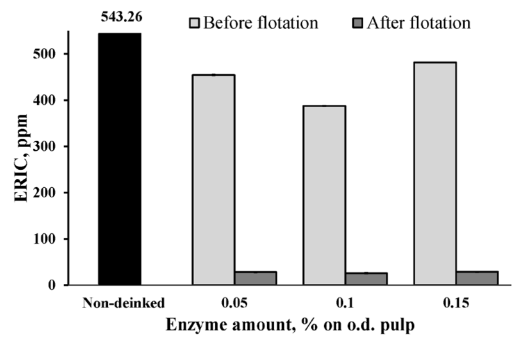

Figure 5: The effect of dosage of enzyme on ERIC.

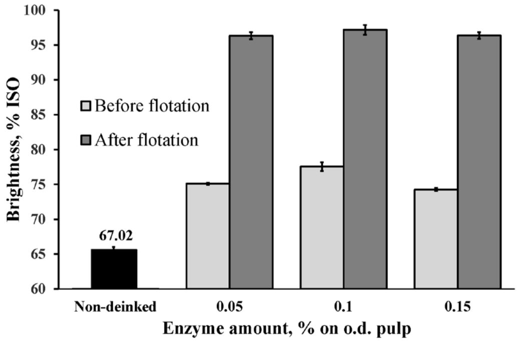

Figure 6: The effect of dosage of enzyme on brightness.

in pulp slurry and may be re-deposited onto pulp. The improvement of ink removal was achieved considerably after flotation process. This is due to the interaction of hemicellulase with fibers causing the better detachment of ink particles from fibers. In addition, the flotation process can also enhance the deinking efficiency because surfactant molecules can bind with ink particles and accumulate ink particles to the suitable size for flotation (10–100 µm) [10]. Then the suitable size of aggregation of ink particles which attached to air bubbles float to the top surface of pulp slurry of the flotation cell are removed. As compared to non-deinked paper, the maximum deinking efficiency was found at 0.1% enzyme (based on oven-dried weight of paper) by 28.7 and 95.3% before and after flotation, respectively.

3.3.2. Brightness

Brightness is a measure of the amount of reflectance of a specific wavelength of blue light (457 nm). Figure 6 showed that the brightness increased with the dosage of enzyme, but it had a tendency to drop at 0.15% of hemicellulase enzyme. This finding was consistent with previous literature [17] that described a reduction in brightness at high enzyme dosage due to the accumulation of the enzyme particles on the fiber surfaces. The maximum Brightness of the pulp before and after flotation were 77.55 and 97.19% ISO at 0.1% enzyme, respectively, which were 10.5 and 30.2 units higher relative to non-deinked paper. The improvement in brightness obtained after enzymatic flotation deinking was a consequence of the reduction of residual ink particle size due to the mechanism of enzyme resulted in suitable size for flotation step. The result of brightness is opposite to the result of ERIC because of higher brightness resulting from lower ERIC.

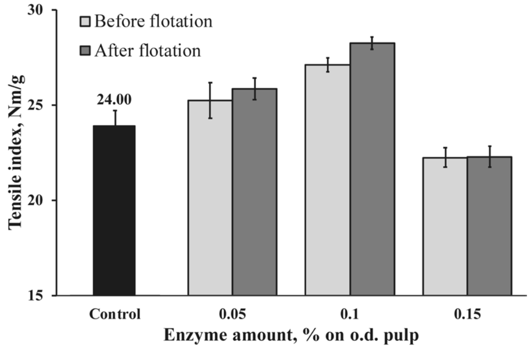

Figure 7: The effect of dosage of enzyme on tensile index.

3.4 *Tensile index*

Tensile index is the value of tensile strength in N/m divided by grammage of paper. Tensile index usually reflects fiber bonding ability in paper. Figure 7 showed that tensile index increased with dosage of enzyme. It can be attributed to the fibrillation of the pulp fibers due to the hydrolysis of hemicellulase enzyme at the surface resulting in an increase of fiber bonding. In addition, the mechanism of enzyme with microfibrils reaching out from the fiber surface may result in better tensile strength despite observed increased freeness according to the improvement of interfibrillar bonding [16], [18]. However, tensile index tended to decrease

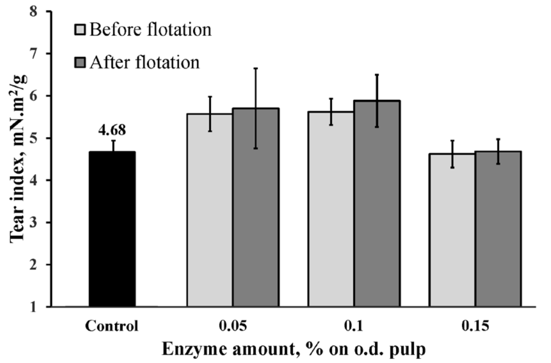

Figure 8: The effect of dosage of enzyme on tear index.

when the dosage of enzyme approached 0.15%. This is caused by too much hydrolysis reaction of enzyme with fibers resulting in shorter fibers. Thus, the strength of paper decreased. The maximum tensile index of the pulp before and after flotation were 27.12 and 28.24 nm/g at 0.1% enzyme (based on oven-dried weight of paper), respectively. The maximum tensile index of the pulps before and after flotation increased by 13.0 and 18.1%, respectively as compared with the control pulp.

3.5 *Tear index*

Tear index is the force required to tear a sheet of paper under standard conditions. Fiber length has a strong influence on tear index. As shown in Figure 8, the effect of enzyme loading on tear index had the same characteristic as tensile index. It can be explained that excessive enzyme dosage would reduce the fiber length as a result of the action of enzyme on the fiber surface in combination with the shear forces of mixing in flotation process [18]. The maximum tear index of the pulp before and after flotation were 5.62 and 5.88 mN·m^2/g at 0.1% enzyme (based on oven-dried weight of paper), respectively. The maximum tear index of the pulps before and after flotation increased by 20.1% and 25.6%, respectively as compared with the control pulp.

The summary of the results obtained was shown in Table 2.

Table 2: Summary of pulp/paper properties after deinking process

Pulp/Paper Properties	Repulping + Enzyme Treatment + Flotation (Enzyme, % on o.d. pulp)		
	0.05	**0.10**	**0.15**
Freeness[a]	+5.8% (+3.7)	+13.2% (+8.8)	–1.0% (–1.6)
ERIC[b]	–94.8% (–16.3)	–95.3% (–28.7)	–94.7% (–11.3)
Brightness[b]	+29.3 units (+8.1)	+30.2 units (+10.5)	+29.4 units (+7.2)
Tensile index[a]	+7.75% (+5.2)	+18.1% (+13.0)	–7.2% (–7.3)
Tear index[a]	+21.8% (+19.0)	+25.6% (+20.1)	0.0% (–1.3)

The sign +/– indicates the increase/decrease that compared to [a] or [b]
[a] Compared to the pulp slurry after repulping without performing the flotation process with the absence of enzyme.
[b] Compared to non-deinked paper (raw materials).
The number in parentheses indicate the treatment before flotation (only repulping and enzyme treatment).

3.6 *Comparison of enzymatic floation deinking and chemical flotation deinking*

The enzymatic flotation deinking (using cellulase or hemicellulase) were compared with traditional (chemical) flotation deinking process that used mixture of naphthalene and amyl acetate (20 : 80 weight ratio) as a deinking agent. All results were compared at the same experimental condition. Except cellulase and chemical treatments used Triton X-100 as nonionic surfactant while Tween-80 was used as nonionic surfactant in hemicellulase treatment. But both nonionic surfactants (Triton X-100 and Tween-80) were used at the value of 0.9 CMC of each surfactant. Deinking efficiency, as indicated by ERIC and brightness, tensile index and tear index were shown in Table 3.

Table 3: Comparison of pulp properties among enzymatic flotation deinking and chemical flotation deinking

Properties	Enzyme Treatment		Chemical Treatment [19]
	This Work	**Cellulase [12]**	
ERIC, ppm	25.8	30.35	29.9
Brightness, %ISO	97.19	98.50	84.86
Tensile index, nm/g	28.24	31.31	0.58
Tear index, mN·m^2/g	5.88	5.11	8.1

It is apparently seen that the quality of pulp properties obtained from an enzymatic flotation deinking was better, in terms of deinking efficiency (ERIC and brightness), and tensile index, compared to a chemical flotation deinking process. However, deinked pulp obtained from enzyme treatment has lower tear index than chemical treatment. This may due to the shortening of fiber length according to enzyme mechanism. In addition, the influence of hemicellulase and cellulase on deinking efficiency and mechanical properties (tensile index and tear index) of pulp was rather insignificant difference.

4 Conclusions

The obtained experimental results brought about to the following conclusions: The flotation process enhanced the removal of ink particles from laser-printed paper. The flotation process showed better result than non-flotation. About 95% of ink removal efficiency was obtained after flotation process. The optimal condition of enzymatic flotation deinking was at 0.1% enzyme (hemicellulase) dosage conjunction with 1.75% surfactant (Tween-80). The properties of deinked laser-printed paper after enzyme treatment and flotation process were 594.8 ± 1.46 mL Freeness, 25.80 ± 0.62 ppm ERIC, 97.19 ± 0.69% ISO Brightness, 28.24 ± 0.33 nm/g Tensile index and 5.88±0.62 mN·m^2/g Tear index. At this optimal condition, about 13, 25 and 18% increase in freeness, tear index and tensile index, respectively, as compared to control.

Acknowledgments

The authors are grateful for the support of King Mongkut's University of Technology North Bangkok (University Grant/KMUTNB-GEN-56-03, budget year of 2012). The authors also thank Faculty of chemical engineering, King Mongkut's University of Technology North Bangkok and Department of Imaging and Printing Technology, Faculty of Science, Chulalongkorn University for supporting research equipment and properties analysis of paper.

References

[1] S. H. Yoon and A. van Heiningen, "Kraft pulping and papermaking properties of hot-water pre-extracted loblolly pine in a integrated forest products biorefinery," *Tappi Journal*, vol. 7, no. 7, pp. 22–27, Jul. 2008.

[2] D. U. Lima, R. C. Oliviera, and M. S. Buckeridge, "Seed storage hemicelluloses as wet-end additives in papermaking," *Carbohydrate Polymers*, vol. 52, no. 4, pp. 367–373, Jun. 2003.

[3] The Office of Industrial Economics, "e-Statistic," 2019. [Online]. Available: https://indexes.oie.go.th/industrialStatistics1.aspx

[4] S. Zhenying, S. Dong, X. Cui, Y. Gao, J. Li, H. Wang, and S. N. Zhang, "Combined de-inking technology applied on laser printed paper," *Chemical Engineering and Processing: Process Intensification*, vol. 48, no. 2, pp. 587–591, Feb. 2009.

[5] P. Bajpai, P. K. Bajpai, and R. Kondo, "Biotechnology for environmental protection in the pulp and paper industry," *Biofuture*, vol. 2000, no. 196, p. 45, Jan. 2000.

[6] J. Behin and Sh. Vahed, "Effect of alkyl chain in alcohol deinking of recycled fibers by flotation process," *Colloids and Surfaces A: Physicochemical Engineering Aspects*, vol. 297, no. 1, pp. 131–141, Apr. 2007.

[7] O. U. Heise, J. P. Unwin, J. H. Klungness, W. G. Fineran, M. Sykes, and S. Abubakr, "Industrial scaleup of enzyme-enhanced deinking of nonimpact printed toners," *Tappi Journal*, vol. 79, no. 3, pp. 207–212, Mar. 1996.

[8] D. E. Tsatsis, D. K. Papachristos, K. A. Valta, A. G. Vlyssides, and D. G. Economides, "Enzymatic deinking for recycling of office waste paper," *Journal of Environmental Chemical Engineering*, vol. 5, no. 2, pp. 1744–1753, Apr. 2017.

[9] H. Pala, M. Mota, and F. M. Gama, "Enzymatic versus chemical deinking of non-impact ink printed paper," *Journal of Biotechnology*, vol. 108, no. 1, pp. 79–89, Feb. 2004.

[10] Y. Zhao, Y. Deng, and J. Y. Zhu, "Roles of surfactants in flotation deinking," *Progress in Paper Recycling*, vol. 14, no. 1, pp. 41–45, Nov. 2004.

[11] J. K. Borchardt, "The use of surfactants in deinking paper for paper recycling," *Current Opinion in Colloid & Interface Science*, vol. 2, no. 4, pp. 402–408, Aug. 1997.

[12] P. Chandranupap and P. Chandranupap, "Enzymatic deinking of xerographic waste paper with non-ionic surfactant," *Applied Science and Engineering Progress*, vol. 13, no. 2, pp. 136–145, 2020.

[13] B. Arechabala, C. Coiffard, P. Rivalland, L. J. M. Coiffard, and Y. D. Roeck-Holtzhauzer, "Comparison

of cytotoxicity of various surfactants tested on normal human fibroblast cultures using the neutral red test, MTT assay and LDH release," *Journal of Applied Toxicology*, vol. 19, no. 3, pp. 163–165, Apr. 1999.

[14] K. Somsri, P. Chandranupap, and P. Chandranupap, "Environmentally friendly deinking of xerographic wastepaper," presented at TIChE International Conference, Nakonratchasima, Thailand, October 25–26, 2012.

[15] *Freeness of pulp (Canadian standard method*, TAPPI Standard T 227 om-09, 2009.

[16] J. Park and K. Park, "Improvement of the physical properties of reprocessed paper by using biological treatment with modified cellulase," *Bioresource Technology*, vol. 79, no. 1, pp. 91–94, Aug. 2001.

[17] A. Saxena and P. Chauhan, "Role of various enzymes for deinking paper: A review," *Critical Reviews in Biotechnology*, vol. 37, pp. 1–15, Jul. 2016.

[18] T. W. Jeffries, J. H. Klungness, M. S. Sykes, and K. R. Rutledge-Cropsey, "Comparison of enzyme–enhanced with conventional deinking of xerographic and laser-printed paper," *Tappi Journal*, vol. 77, no. 4, pp. 173–179, Apr. 1994.

[19] P. Chandranupap, P. Chandranupap, and P. Kongsat, "Flotation deinking of xerographic and laser-printed paper: Influence of surface-active substances," *Advance Material Research*, vol. 287–290, pp. 3028–3031, Jul. 2011.