Evaluation of Laser-Printed Paper Deinking Quality Facilitate By Lipase and Esterase Enzymes

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Abstract

The paper industry is one of the most developed, yet the most polluter industry. Recycling program is developed as one of the steps to counter with this environmental issue. The key of recycling process is the successfulness of the deinking process. Considering the concern to the environmental problem caused by the conventional deinking process, enzymes are applied. Enzymatic bio-deinking are proven to reduce the use of harmful chemicals and production of enormous wastewater. Research had been conducted to compare the deinkability and the quality of the secondary paper produced from enzymatic deinking and virgin pulp paper. The degree of ink removal is measured by the optical parameter improvements. Results indicate that enzymes facilitate ink removal successfully and the brightness increases very significantly among experimental trials. Lipase and esterase enzymes are applied in their optimum condition to facilitate deinking of laser-printed paper. The method applied is using GE brightness technique that commonly applied in United State. The methods also describe by TAPPI standards T 452. Lipase and esterase are applied individually and undergo concoction by a ratio of 100:100 and 100:50. The result indicates that brightness is varied. Individual lipase produced 48.48% paper brightness, whereby esterase is 39.47% compared to control. Concoction is done to optimize the result and the best result achieved is 71.62%, by concoction of lipase and esterase with ratio 100:100. The effect of the enzymes and enzymes concoctions on fiber morphological changes during the deinking process was studied by scanning electron microscopy (SEM).

*Keywords:*Laser-printed paper; lipase; esterase; enzymatic deinking, brightness

1.0 Introduction

The consumption of paper is substantially increasing in line with economic growth nowadays. The paper industry is the largest consumer of wood statically with boundless growth of demand in paper, thus causes any environmental problems that is collectively known as paper pollution. Based on the report prepared by The Star (2009), Malaysia consumes about 380,000 tonnes of paper annually and the amount of consumption had increased to approximately 1,200,000 tonnes in 2010. Everyday choices of paper can make a massive difference in the future of economic, as well as social benefits and global environmental health. The choices include a reduce in waste generation and the practice to use more green technology as well as managing the energy use of the office equipment. The demand of paper is increasing in line with pollution caused by industries. About 85 percent of water is consumed throughout the process which leads to enormous volumes of contaminated wastewater because of chemical application throughout the process of ink removal. Other than that, paper production also leads to deforestation, solid waste and air pollution.

At the national level, most countries have integrated waste management and recycling activities between their environmental concerns. The highest level contribution to waste paper collection and increasing recycling rates are from pulp and paper industries, print buyers and producers of graphic products, for example posters, labels and signboards. In order to counter with this problem, some countries decide to adopt laws and regulations, while others rely on voluntary agreements. Higher taxes or fees will be charged if the manufacturers of the waste fail to take responsibility for waste paper management. In Malaysia, waste from paper mill is categorized as municipal solid waste, which is trash or garbage that we use and throw away every day such as packaging and newspaper. Based on Environmental Quality (Scheduled Wastes) Regulations 2005, waste of paper is complying under Environmental Quality Act 1974 (Act 127). In order to comply with the government act and policies, as well as to satisfy requests from the customers, essential steps are taken to reduce the consumption of the virgin fibers in the industry. Recycling may help in conservation of land, but recycling cannot be done an indefinite number of times. It only can be recycled four to six times because the fibers will get shorter and weaker each time of the process. As a result, some of the virgin pulp needs to be added to maintain the quality and strength of the paper.
Paper recycling process will require an important step called the deinking process, which acts as the key process in

order to obtain brighter pulp so that the quality is comparable with a new product. It is the way to purify the pulp from the unwanted material by removing and detaching the contaminants and ink particles from the reusable paper fiber (H. Alzate Gil et al., 2013). However, the recycle fiber has poor optical properties and low quality competes with the virgin fiber that restricts their use in value-added papers (Leduc & Daneault, 2011). The Environmental Paper Network's Paper Calculator clearly shows that cycle paper is better to use than virgin paper, for all respective paper grades (Kinsella, 2012). In comparison, virgin paper will cut off trees in the making, consume more energy in the process and produce more solid waste and wastewater. This process constitutes towards major technical obstacles to achieve this rate of quality. Deinking is done by the combination of mechanical action and chemicals added throughout the process. Mechanical action consists of sorting and debalingprocesses. The procedure continues with a chemical added phase, which is pulping, cleaning and screening.

As an innovative way, enzymatic deinking is applied in the industry to decrease the impact of the mother earth. The enzymatic bio deinking has proven as an environmentally friendly solution for waste paper recycling. Enzymatic deinking uses enzymes to enhance the removal of inks from waste paper and can be more effective and less expensive than conventional deinking chemicals. Also, it tends to avoid some problems associated with alternative treatment technologies (Jeffries, Patel, Sykes, & Klungness, 1992)(Pala, Mota, & Gama, 2006). The traditional conventional deinking process usually requires large quantities of chemical in throughout the process (Lee, Darah, & Ibrahim, 2013). The process has become costly as well as damaging the environment by releasing the contaminants. Several enzymes such cellulases, hemicellulases, pectinase, lipase, esterase, α-amylase and lignolytic enzymes, or any of their combination, had been used for deinking of various waste papers (Pathak, Bhardwaj, & Singh, 2011). Previous research proves that enzymes can help to improve the paper properties such as the brightness of the paper and the resistance compared to paper deinked by the traditional conventional chemical methods (Lee et al., 2013).

The success of the enzyme application in the industry can be determined by comparing the effectiveness and the product produced from the secondary process and the new virgin pulp paper by the optical parameter improvements. International Association of the Deinking Industry (INGEDE) providing an assessment system, known as deinkability score that promotes eco-friendly print products. This protocol has also been adopted by the European Recycled Paper Council in 2008 (ERPC 2012) (INGEDE, 2012). The guideline assessed by three quality parameters of the deinked pulp (luminosity, color shade and dirt specks) and two process parameters (ink elimination and filtrate darkening). The luminosity of the paper can be easily identified by the brightness of the paper. Brightness is an important quality, primarily in white papers, as well as an important appearance property for an aesthetic value and its effect on legibility and difference between print and paper. The measure of brightness in paper is determined by the percentage of a wavelength of blue light (a specific spectral distribution with a principal wavelength of 457 nm) it reflected in the scale 1 to 100 with 100 being the brightest (Hubbe, Pawlak, & Koukoulas, 2008). Wavelength coincides with lignin absorption, which acts as the glue that grips fibers together and affect the paper quality by given a yellow tint to the paper. Bleaching quality, readability, the ink color perception and the contrast between light and dark hues are the aspects that affect by the brightness of the paper. If the brightness of paper increases, the letters, images and color will appears more bright and vivid.

Brightness quality can be control by the pretreatment of the paper samples before the samples go through deinking process. The purpose of this pretreatment is to remove impurities, remove stickies and loosen the bond between the applied ink and the paper fiber. The standard pretreatment applied by industrial field is using high molarity acidic agent such as 1 M hydrochloric acid (HCl) or sulfuric acid (H_2SO_4) and leave for a while. The acid will then will be rinsed off and buffer solution will be added, followed by the enzymatic deinking treatment. Majority, colloidal stick to the fiber after pretreatment and washing process if it is not properly manage. Micro and macro sticky will bind and form colloidal, together with some portion of unresolved ink and to produce spot (Sarja, 2007). Floatation process is essential after pretreatment and during treatment process.

Agglomerates start to contaminant the paper when sticky form. Once it produced and combined with each other, the bond linkage between sticky and ink becomes stronger. Hydrogen bond that formed is very strong and need enough optimum variable condition to counter with. So that, the ability to break the bond is relatively hard when agglomeration produce faster and makes the chances of brightness getting lower. Depolymerize of esterase molecules on sticky agglomerates, by which the bonds among sticky and ink weaken because of removal of hydrogen bond (Miao, Huang, & Lihui Chen, 2013). Other than that, enzymatic modification on surface of the stickies is also important (Bajpai, 2012). Air bubbles also source of agglomerate to accumulate. Means, bubble can be source of thin film production on paper and give hardness to esterase to attack because of recovered by film (Krolle & Du, 2012). Previous research proved that application of enzyme direct onto paper machine also reduce stickies deposition as long as efficiency of esterase to reduce is working (R. Jones, 2015).
In the other hand, shaking rate also plays an important role to control stickies in order to optimize brightness in the

deinking process. In this research, shaking or agitation rates are regulated to control stickies as well as study the effect on the brightness of the paper product. Agitation is performed in order to disperse and mix the enzymes preparation in the reaction system, thus, enhance the enzymes surface contact with the fiber. Agitation also can prevent the accumulation of the toner on fiber paper surfaces (Lee, Darah, & Ibrahim, 2007). Previous research states that, continuous mixing at lower agitation rate improves the brightness of the pulp and in contrast, excessive agitation results to detrimental effect (Pala, Lemos, Mota, & Gama, 2001). This can achieved by removing the toner particles immediately as it is detached from the fiber surfaces as a result of enzymatic action (Lee, Darah, Che Omar, I, & Wan Daud, 2011). In most findings, continuous agitations at 60 rpm was adequate in facilitating the detachment or desorption of the enzyme or toner particles from the fiber surfaces and prevented toner from redeposition. Other previous findings stated that the shaking rate in the range of 50 rpm and 100 rpm is the best speed to avoid from other substance deposited.

Analogically, the distribution of enzymes in the reaction needs force to enhance the performance and it is achieved by the shaking rate of agitator. The distribution of enzyme is irregular to attach to ink with times and the average esterase enzyme attachment is low. This will results poor brightness on paper produced and if this happen, the retention time should be extend. Shear force or drag force is applied at the same time with the shaking process. Principle shear force is applied in container. The collision in the beaker between the paper sample, wall of the beaker and scrubber (if contain) will contribute in reduction of affinity ink attachment. Some modification is done in this research whereby scrubber was added to give more shear and drag force. Drag force on the surface paper and magnitude of this force is proportional to area of contact on paper. With nearer knowledge to the force related, drag force can remove particles such ink or toner molecules and should be increases drag force to overcome surface adhesion force between papers. Shear stress and force acting during rotation will not denature enzyme otherwise high speed rotational implement on the reaction (Mohamad Hekarl & Mashitah, 2007). Shaking rate of agitator at 150 rpm for 72 hours was the most optimum in the previous research.

As for the brightness evaluation, there are two methods that most widely accepted known as GE brightness and ISO brightness. GE brightness is commonly employed in the United States, while ISO brightness is widely applied in other countries, especially in Europe. The methods also describe by TAPPI standards, which are T 452 for GE brightness and T 525 for ISO brightness.The two methods use the application of their spectral characteristics, whereby both evaluate brightness at the same effective wavelength of 457 nm. The difference of these two methods is the optical geometry, thus lead to different brightness readings. According to TAPPI 452, GE brightness is measured with directional light incident at 45° with respect to the normal to the sample. The photodetector is mounted in the normal and receives light reflected along the normal-conditions sometimes expressed by the shorthand notation (45° illumination, 0° observation). ISO brightness, on the other hand, is measured by diffuse incident light. The detector is located on the normal, as in the case of GE brightness, and so ISO brightness can be described as (diffuse illumination, 0° observation). The different optical differ further with respect to brightness scales: GE brightness is measured relative to a magnesium oxide standard that is arbitrarily assigned the GE brightness 100 (all pulp and paper has GE brightness less than 100). ISO brightness, however, does not rely on an arbitrary standard, but is the absolute reflectivity of the sample. Therefore, ISO brightness is about one to two units lower than GE brightness

2.0 Methodology

2.1 Paper sample selection

A4 paper with a basis weight of 80 g/m^2 was printed with toner on a laser printer on 1.5" by 2" area. Only one side of each sheet of the paper was printed. The printed areas were cut and used in all the experiments. The printed papers were pre-treated with 0.5N HCl for 30 minutes before undergoing enzyme treatment.

2.2 Lipase and esterase assays

Two (2) commercial enzymes applied in the research are assayed using the different methods. Lipase was assayed by the olive oil emulsion method as described by Mustranta*et al*. (1993) with some modification. In the other hand, esterase activities was assayed as referred by the method described by Danielle, B., (2011). After the optimum conditions of enzymes were determined, the new environment is developed.

2.3 Enzymatic hydrolysis

The pretreated paper from HCl solution was rinsed using distilled water until the sample become odorless. The sample was then leaved for a while. 10 ml of sodium phosphate buffer solution was mixed with 10ml of enzymes

assay into 50ml beaker. Three (3) grams sieve sand added into the solution and the stir smoothly. Four (4) piece of paper were added into the solution. The paper need to be fully dipped into the prepared solution. The tip of the beaker was covered with aluminium foil to avoid contamination. The beakers were placed in incubator with shaking rate of 200 rpm for 60 minutes at 30°C. Next, flotation process was done. The paper samples were rinsed using distilled water for three (3) times carefully without tear up the wet paper samples. After that, the treated paper samples were put in incubator with the temperature of 50°C for 20 minutes for drying process.

2.4 Enzyme treatment

Enzymatic hydrolysis was performed at pH 5.5 and temperature 40°C. 4 pieces of 80g papers were dipped into 0.2 M citric-NaOH buffer for 10 minutes and shake at 60 rpm prior to enzyme addition (Lee et al., 2013). In order to make sure that the distribution of enzymes is even, lipase (L) and esterase (E) was previously diluted to 10 U/ml. The dosage chosen was based on prelude experiments makes to decide the suitable amount of enzymes necessary to fabricate reasonable deinking level (Lee et al., 2013). As for concoction, the second enzymes were added at 10 minutes interval. Next, the mixtures were incubated for 60 minutes. Control was run simultaneously using thermally inactivated enzyme (Gübitz, Hayn, Urbanz, & Steiner, 1996). The reactions were stopped by boiling for 10 minutes and the solutions were used to assay for reducing sugar (Lee et al., 2013). The sample papers were rinsed thoroughly three times using distilled water before applying to the flotation process. The initial conditions for a flotation process were set at pH 6.0, 0.5% (w/w) of Tween 20, 2 L/min of air flow rate, room temperature and 30 minutes of flotation time (Lee et al., 2013). Subsequent to the process, brightness analysis is conducted.

2.5 Brightness analysis

Figure 1 below is the setup of the brightness test that was conducted by illuminating the product with a fixed angle of 45° relative to the angle of observation. In TAPPI Method T 452 the incident light is 45° from the normal to the plane of the paper.

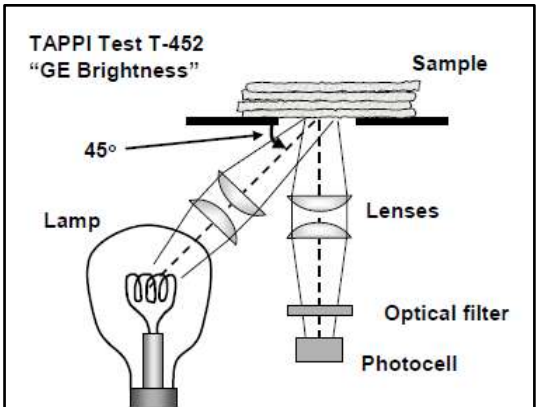

Figure 1: Basic Configuration for Test of Paper brightness, when using directional illumination (45/0°), as in TAPPI Method T 452(Hubbe et al., 2008)

2.6 GE Brightness Measurement using Brightimeter

The brightimeter is on and allowed to warm up for 30 minutes. The calibration of the instrument is checked by placing the clean working standard over the sample specimen. Samples are cut in the dimension of 1.5" by 2" and placed in a pile with felt side up. Such a pile is called opaque. Opaque was set in pile squarely over the sample opening (felt side down and machine direction parallel to the long axis of the instrument) and 1 kg weight is placed on top of the stack. The brightness value is read off from the instrument panel and recorded. Lower sample is moved to the top of the pile and the second sample is measured. The procedure is repeated until six (6) samples have been measured. The brightness increase (%) was determined by subtracting brightness after pulping from brightness after flotation as in equation 1 below (Dutt, Tyagi, Singh, & Kumar, 2012).

$$D_B = \frac{B_F - B_p}{B_B - B_P} \times 100\% \quad (1)$$

Where
B_P = Brightness after pulping, % (ISO)
B_F = Brightness after flotation, % (ISO)

B_B = Brightness of the sample paper without the presence of ink particles (blank), % (ISO)
D_B = Deinkability factor based on brightness, % (ISO)

2.7 Observation of Enzymatic Treated Papers under Scanning Electron Microscope (SEM)

The ink removal of the treated paper can be observed using the imaging technology of SEM. The image produced was the detailed morphology of pulp samples pertaining to changes in the surface of the fiber texture before and after enzymatic treatment were carried out. After the flotation process, the deinked papers samples were rinsed thoroughly three times with distilled water and air-dried under room temperature (Lee et al., 2013). The deinked paper was cut and mounted onto a scanning electron microscope specimen stud with a double-sided sticky tape and the papers were then coated with a layer of approximately 20 nm thick gold using sputter coater (POLARON SC515) (Lee et al., 2013). The coated papers were examined under SEM with 500 times magnification.

3.0 Result and discussion

3.1 Effect of enzyme concoctions on bio-deinking

Two (2) commercially available enzymes were used in the study and both were purchased from Sigma (USA). The paper sample had undergo the pulping process which was treated with these two (2) enzymes prior to ink flotation, its brightness, D_B improve compared to the control. The enzyme was assayed to obtain the optimum condition and new environment was developed accordingly. The enzymes then combined with each other in two different ratios as stated in Table 1 below. The tabulated data of result from experiment conducted are recorded and the brightness increases are calculated. From the data in Table 1, esterase produced brighter pulp after pulping process, but lipase produced a brighter pulp after floatation. The brightness increase (%) is the greatest in the concoction of lipase and esterase, in a ratio of 100:100 by 71.62%. Figure 2 below is the graphical presentation of the effect of enzymes on the percentage of brightness increase. The trend shows that a combination of enzymes, lipase and esterase in ratio in the same ratio produces brighter paper compared to other trials.

Table 1:Effect of different enzyme combinations during enzymatic deinking of laser-printed paper

Control	Enzymes	Bb	Bp	Bf	Ratio	D_B	D_B (%)
	Lipase	100	35	60		0.38	38.46
		100	34	66		0.48	48.48
		100	33	64		0.46	46.27
	Esterase	100	29	55		0.37	36.62
		100	28	56		0.39	38.89
		100	24	54		0.39	39.47
	L + E	100	25	75		0.67	66.67
		100	23	77		0.70	70.13
		100	26	79	Ratio 100:100	0.72	71.62
	E + L	100	26	59		0.45	44.59
		100	25	58		0.44	44.00
		100	26	58	Ratio 100:100	0.43	43.24
	L + E	100	33	60		0.40	40.30
		100	34	66		0.48	48.48
		100	33	64	Ratio 100:50	0.46	46.27
	E + L	100	23	55		0.42	41.56
		100	22	53		0.40	39.74
		100	24	52	Ratio 100:50	0.37	36.84

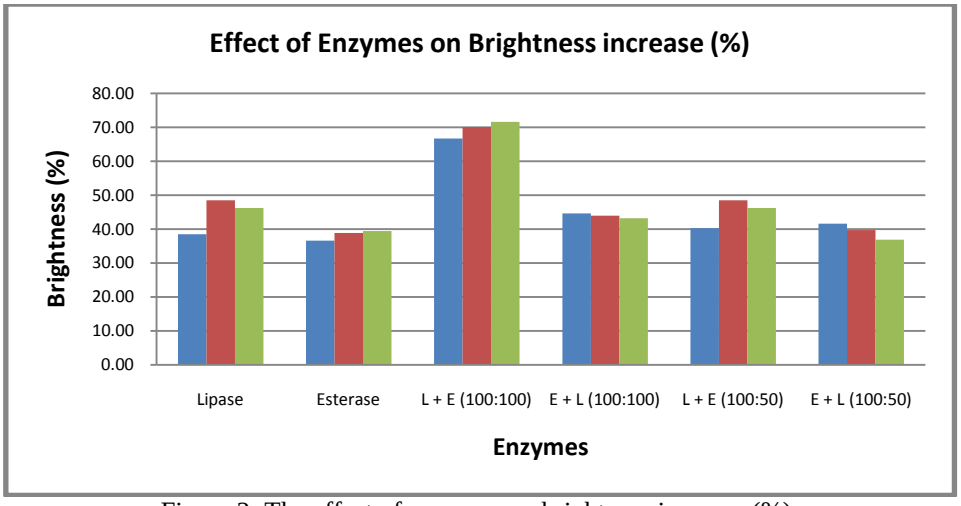

Figure 2: The effect of enzymes on brightness increase (%).

3.2 Observation of enzymatic treated papers under Scanning Electron Microscope (SEM)

The structures of the paper's fiber on the surface changes were analyzed concomitantly with the action of the enzymes and enzyme combination using an electron microscope. Electron Microscopes are scientific instruments which apply the beam of highly energetic electrons to study and examine objects on a very well fine scale (Ma, Shieh, & Qiao, 2006).Scanning Electron Microscope (SEM) can produce an image of surface features and its texture down to a few nanometers by electron illumination from reflected electrons, with high magnification and high resolution. This imaging technique is very flexible and can view almost all kinds of samples, conducting or non-conducting, based on surface interaction in all directions through x-y-z (3D) rotation of the sample.

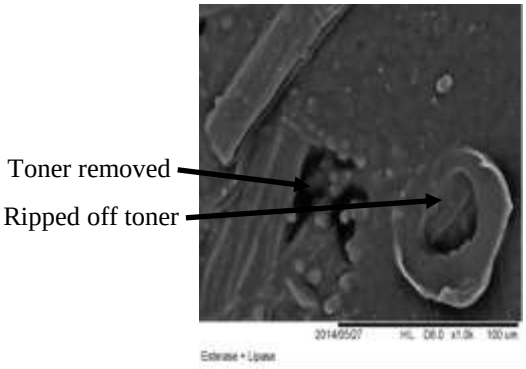

Figure 3: Removal of ink toner by scanning electron micrograph using esterase and lipase.

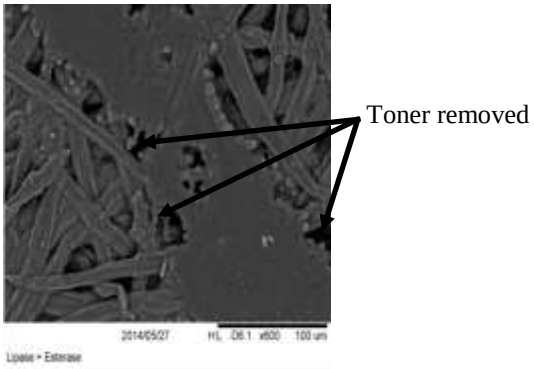

Figure 4: Removal of ink toner by scanning electron micrograph using lipase and esterase.

The image produced from this microscopy imaging will demonstrate the removal of ink from the paper fiber. The toner was in flat, plate like particle which was homogeneous dispersed onto a paper fiber. After undergoing the enzymatic hydrolysis, the applied enzyme sequence will result to toner ripped off as indicated in the figure above. Figure 3 shows the scanning electron micrograph of ink removal the laser printed-paper after enzymatic hydrolysis using esterase, followed by lipase. The hydrolysis were performed at pH 5.5; 40°C for 60 minutes. On the other hand, Figure 4 shows the reverse sequence of ink concoction. Lipase is applied before esterase in condition of pH 5.5; 40°C for 60 minutes. Both figures clearly show the ink is being ripped off and removed. This approved the application of enzymes in the pulp and paper industry for deinking purposes. Therefore, to enhance the brightness of the paper, the optimization of the enzymatic reaction process with continuous removal of hydrolyzed ink must be carried out.

4.0 Conclusion

The development of enzymatic deinking shows the effectiveness of the enzyme application in the paper industry. These environmental friendly technologies help in reducing the rate of pollution nowadays. The paper produce from the process also can be compared with new pulp paper. The comparison is based on the optical properties which is the brightness of the paper. However, development of the optimum condition is needed as enzymes itself

have their own specificity. Lipase and esterase enzymes can facilitate deinking successfully in their optimized condition. The concoction of the enzymes increased the brightness of the pulp by 71.62% compared to the control during biodeinking of laser-printed paper. The imaging techniques of SEM are use to study the ink removal of the fiber surface and it showed the ripped off and removed toner. As a conclusion, enzymatic deinking has high potential to be widely developed as an alternative method to current chemical thinking process which is not environmentally friendly and costly.

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