Some physico-chemical aspects related to the deinking process using xylanase as a surface modifier
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ABSTRACT
Physico-chemical properties of ink particles have an important role in deinking using either washing or flotation processes. To study such properties, turbidity, zeta potential and surface tension techniques were used in this research. In this work, oleic acid and xylanase are used as surface modifiers for the ink particles. The results show that addition of enzyme alone to the ink suspension and due to its adsorption on ink particles, increase ink dispersion. This will facilitate separation of ink using washing process. On the other hand, the addition of enzyme-oleic acid blend to ink suspension reduces the electronegativity of ink particles. This will make the ink particles to flocculate and facilitate its adhesion to the negatively charged air bubbles during the flotation process. It is expected that this fundamental knowledge will lead to process technology improvements in the deinking of office wastepaper.

Introduction
The deinking of fibers is an important step in waste paper recycling and the most common techniques used for deinking are washing and flotation. The wash deinking process is most efficient for removing the finer ink particles (<20 µm), where flotation deinking is usually more effective in removing larger particles (20-300 µm) from waste paper. During the recycling of waste paper, flotation deinking is the most widely used technique for deinking of variety of waste papers. [1-3]. In recent years, the growth in the use of recycled fiber has considerably increased the need for a more fundamental understanding of flotation deinking, [4]. In the deinking flotation process, where air and chemicals are added to the pulp, ink or toner particles, due to natural or induced hydrophobicity, exhibit attractive interactions with air bubbles. Ink particles attach themselves to the air bubbles and report to a froth phase which results in separation of these particles from the fibers, [5]. Deinking separation require the use of surfactants in order to improve efficient ink removal. A number of surfactants have been identified for use in paper recycling plants, [4]. Traditionally, fatty acids and their salts (soap) and sulfonated anionic surfactants were employed. During the past 20 years, a number of enzymes, including cellulase, xylanase, laccase and lipase, have been evaluated for their potential to replace hazardous chemicals in deinking recycled paper, [6-9]. It was demonstrated that enzyme deinking can reduce chemical cost, enhance ink and stickies removal, improve drainage and runnability and decrease COD and BOD content in process water and effluent, [10, 11].

Nature of enzyme
Enzyme is defined as a protein with catalytic properties due to its power of specific activation, [12]. Enzymes contain reactive groups such as free amino, carboxyl, hydroxyl, sulfo-hydroxyl and imidazole groups and frequently non-protein prosthetic groups. The presence of anionic and cationic groups gives the enzyme surface amphoteric properties which imply that, depending on the pH, the net charge of the enzyme surface can be either negative or positive or zero. The long peptide chain, of native protein molecules, is known to be folded and arranged in exact positions, [13]. So, in an enzyme, the combining sites are suitably located to make up active centers necessary for substrate binding and catalytic activity. Being proteins, enzymes are denatured and deactivated when subjected to heat, [14] or strong chemical conditions, [15]. Enzyme denaturation results in the unfolding and disorganization of the enzyme structure.

Materials and Methods
A commercial photocopy toner, 5052 from Xerox Corporation was used in this study. This toner consists of 85 to 90% styrene acrylate copolymer, 10 to 15% carbon black and minor components such as amorphous silica (<1%) and zinc stearate (<1%). Xylanase from Trichoderma Viride was supplied by Fluka Chemie GmbH. Oleic acid, KNO₃, NaOH and HCl are analytical grade.

The solution turbidity was measured using Turbidity Meter, HI 93703, Microprocessor, HANNA Instruments. A 0.5 gram of toner was stirred in 100 ml double distilled water with and without enzyme at a pulp density of 1% solid. After pH adjustment, the pulp was stirred for 20 minutes with magnetic stirrer. The suspension was, then transferred to the measuring cell. The suspension was inverted 10 times at 180º, left for settling and reading of turbidity are recorded with time.

A laser Zeta-meter, Malvern Instrument model Zeta Sizer 2000, was used for zeta potential measurements. In these measurements a 0.01 gm of toner was placed in 50 ml double distilled water with a known concentration of oleic acid and/or enzyme at ionic strength of 2 x10⁻² M KNO₃ as an indifferent electrolyte.
The surface tension measurements for surfactant solutions were performed at room temperature (20 – 22°C) using a pendant drop technique with the G10 Kruss instrument.

Results and Discussion
It has recently been demonstrated that the surface forces between toner particles are significantly controlled by the polymeric composition of the toner, [16].

Turbidity measurements
Toner -toner interaction
Turbidity is a parameter reflecting the degree of flocculation, a lower value of turbidity represents higher flocculation efficiency, [17]. The turbidity values for the toner suspension, without the addition of enzyme, as a function of pH are shown in figure (1). It is noticed that, for the toner used in this study, the polystyrene-methyl methacrylate, PMMA, segments of the polymeric toner have a profound effect on the interfacial properties of toner particles. It can be seen that such degree of turbidity varies with changing pH. The values of turbidity of ink in alkaline medium are always higher than its respective values in the acidic medium. It indicates that under acidic conditions the PMMA segments are coiled and toner particles coagulate due to hydrophobic interaction. On the other hand, in the alkaline solution, the PMMA segments are extended into the aqueous medium and the toner particles remain dispersed due to steric forces. However, it seems that the interaction force between the toner particles is going from attraction (coagulation) to repulsion (dispersion) as the alkalinity of the medium increases. The attraction and repulsion behavior appears to be due to the conformation of the toner polymeric-methylmethacrylate, (PMMA) segments.

Zeta Potential Measurements
Flotation deinking is affected by the surface charge of ink particles suspended in the pulp. Surface charge of ink particles can either enhance or hinder bubble-particle interactions and supply the driving force for the adsorption of surfactant, [18,19]. However, a number of tests were conducted to determine the effect of enzyme/oleic acid blend on the zeta potential of the toner particles.

Figure (3) shows the zeta potential of toner versus pH in 2 x10^-7 M KNO₃ solution. Experimental data shows that the toner carries a negative charge in water over a wide range of pH, namely 3-12. This becomes less negative with a corresponding decrease in the pH of the solution. The isolectric point is located at around pH 2.3. It can be said that anionic groups dominate over cationic groups found on ink surface.

Besides, Figure (3) presents the effect of enzyme treatment on zeta potential of toner particles. It is noticed that the presence of enzyme decreases the negativity of the ink zeta potential. This indicates that the enzyme adsorbs on the polymeric toner surface and there is a hydrophobic bond between the toner surface and the hydrophobic pocket found on the enzyme surface. The created new surface becomes hydrophilic due to the presence of hydrophilic groups on the enzyme surface. The hydrophilic groups of the enzyme contain proteinaceous amino group which carries a positive charge. This is the reason for decreasing the negativity of the zeta potential of the toner particles treated with enzyme as shown in figure (3).

Toner and enzyme interaction
The results of ink turbidity, using different doses of enzyme at pH 6, are presented in figure (2). It can be observed that enzyme, significantly, changes the interaction force between the toner surfaces. Xylanase can completely reverse the interaction from attraction to repulsion. This occurs with increasing the enzyme concentration from 0.01% to 0.1%. These results indicate that, in fact, xylanase has a tendency to adsorb at the polymeric toner surface. This is due to the interaction of the toner particles with the xylanase hydrocarbon chain, where the driving force for this event is an attractive interaction between hydrophobic surfaces. On the other hand, the hydrophilic portions of xylanase extend from the toner surface and support the dispersion power of enzyme onto the toner particles. It is known that washing is a process in which the cellulose fibers are separated from the pulp by screening while the ink particles are removed with the water, [1, 2]. Hence, the efficiency of washing systems relies on the dispersion of the toner particles. Therefore, enzyme addition is useful for ink separation using washing process where it creates a stable emulsion of ink particles that does not readily redeposit onto the fiber.
carboxylic groups, is protruding into the aqueous phase. It can be seen that zeta potential of toner particles become more negative with increasing oleic acid concentration, figure (4). In this case, the negatively charged ink particles are difficult to negatively with increasing oleic acid concentration, figure (4). In this case, the negatively charged ink particles are difficult to attach to the air bubbles, carrying negative charge, [20], during deinking flotation. This indicates that the electrostatic forces between an air bubble and ink particles are repulsive and that enzymes play an important role in reducing this force. However, the addition of enzyme-oleic acid blend to the toner particles resulted in a reduction of the negativity of ink zeta potential. Hence, enzyme will be adsorbed on the polymeric toner surface and there is a hydrophobic bond between the toner surface and the hydrophobic pocket found on the enzyme surface. In this case amino group present on the enzyme surface, which carries a positive charge, interacts with the negativity charged carboxylic group of oleic acid through electrostatic interaction. Accordingly, the electrostatic repulsion between ink particles and air-bubbles is reduced and contact can be established. The hydrocarbon chain of oleic acid species, which protruding into the aqueous phase, form hydrophobic aggregates onto ink particles that stick to the bubbles during deinking flotation.

Probable Mechanism
In spite of the fact that enzyme molecules are coated mostly with hydrophilic functional groups, it is understood that they have hydrophobic pockets on their surfaces, [21]. These pockets are available for interaction with appropriately sized hydrocarbon chains, implanted on an inert matrix, forming hydrophobic bonds, [22]. Besides, it was reported that, forces within the enzyme active site, such as hydrogen bonding, electrostatic, hydrophobic and van der Waals interactions, can align the substrate and enzyme reactive groups into proper orientation for reaction, [13]. So, it is suggested that the interaction of enzyme-oleic acid blend with ink particles may involve three mechanisms:

i- The enzyme acts as a bridge between the fatty acid and the ink particles, carried negative charge, forming superficial hydrophobic aggregates that attach to bubbles; 
ii- The enzyme-oleic acid complex hetero-coagulates with the ink particles (microencapsulation), forming superficial hydrophobic aggregates that attach to bubbles; and 
iii- The enzyme destabilizes the ink particles, causing coagulation, with the polar part of the tensoactive agent interacting with the flocs to form hydrophobic aggregates that stick to the bubbles.

Interaction at the air-water interface
Surface Tension of oleic acid

The variation of surface tension with concentration of oleic acid is shown in figure (5). Clearly, the surface tension decrease with increase of surfactant concentration. The change is much sharper at higher surfactant concentration. This indicates the adsorption of oleic acid at the solution-air interface where the surface tension levels off at a limiting value of about 35.52 dyne/cm. The concentration at which γ approaches the limiting value coincides with the reported value of the critical micelle concentration (CMC) of oleic acid which is \( \approx 10^{-5} \) M [23]. From the surface tension γ-pH graphs, figure (6), it is found that maximum lowering of γ occurs at pH 8. It was reported that, lower surface tension is obtained in the pH range of extensive dissociation, [24]. This extra lowering of γ at such pH of dissociation is due to the formation of intermediate species, an acid soap (RCOOH. RCOO), containing a 1:1 complex of ionized and an unionized carboxylate, [24]. This lowering of γ is caused by co-adsorption of these two surfactant species at the air/water interface.
group to water sub-phase would account to the observed low surface tension.

Figure (7) Variation of surface tension with xylanase addition at different solution pH's

Besides, each enzyme has a characteristic pH at which its activity is highest. For some enzyme the optima are quite sharp, for others there are rather broad optimum pH ranges. Hence, it seems that xylanase is active at acidic pH, figure (7), where the surface tension is leveled off at 48 mN/m. On the other hand, denaturation of xylanase occurs in alkaline medium where surface tension was not much affected at pH 10 and leveled off at 58 mN/m.

**Surface tension of enzyme-oleic acid blend**

Ionic surfactants are known to show strong association behavior with globular proteins in aqueous solutions [25]. The charged head group of a surfactant is electrostatically attracted to an oppositely charged amino acid residue of the protein. Additionally, the alkyl chain of the surfactant is hydrophobically attracted to non-polar regions on the surface as well in the interior of the globular protein.

Figure (8) shows the surface tension versus oleic acid with and without xylanase. It is noticed that the addition of oleic acid resulted in a larger decrease in the surface tension of the solution demonstrating that oleic acid species exhibit a higher degree of stabilization of the air-water interface than xylanase. The curve for oleic acid shows a dip at a concentration just around the CMC. The oleic acid - xylanase blend curves are different from the curve for oleic acid. The presence of xylanase with oleic acid reduces the surface tension to values lower than those obtained by oleic acid.

Figure (8) Effect of oleic acid concentration on surface tension in the presence of xylanase

Under the used conditions, oleic acid micelles and xylanase-oleic acid complexes coexist. It is assumed that two different complexes coexist: complex 1 is similar to that obtained at low oleic acid concentrations, (<10^{-5}M) and has a compact structure; and complex 2 is a larger complex obtained at higher oleic acid concentrations, (>10^{-3}M) in which xylanase probably has a more open, expanded structure, presumably caused by the binding of a greater amount of oleic acid species. The hypothesis of a more expanded xylanase molecule corresponds well with the change in the observed surface tension values. In addition, xylanase-oleic acid system was studied at two different pH values, (6 and 8). It is seen that weak interaction between the enzyme and oleic acid was noticed at high pH values where surface tension was not much affected. It can be said that there is a pronounced interaction between xylanase and oleic acid at low pH where surface tension has a lower values than at high pH. At low pH, xylanase causes oleic acid to aggregate, cooperatively, at a concentration lower than the CMC of oleic acid in pure water. This behavior is indicative of an entropic-ally driven aggregation taking place at sub-CMC concentration and formation of oleic acid- xylanase complex. This, strongly, suggesting the preferential localization of the xylanase-oleic acid particles species at the air-water interface. On increasing the oleic acid concentration, the complex (xylanase+oleic acid) is gradually replaced by oleic acid and the surface tension eventually approaches the value obtained with oleic acid only.

**Probable mechanism for the interaction between oleic acid and xylanase at the air-water interface:**

It was assumed that an electrostatic binding of oleic acid species to the oppositely charged sites on the enzyme surface at low oleic acid concentration would correspond to, [26]. After saturation of the charged sites and at higher dosage of oleic acid, a co-operative association between hydrocarbon tail of oleic acid and the hydrocarbon portion of the enzyme take place that is driven by hydrophobic interaction. This means that with increasing oleic acid concentration, oleic acid molecules do not only bind to the cationic sites of the enzyme, but also, hydrophobic interactions become increasingly more dominant. Also, the hydrophobic tail of oleic acid will penetrate into the hydrophobic domains of the enzyme globular molecule in order to reduce their contact with water.

**Conclusions**

The results from the different techniques used to study the interaction between xylanase, oleic acid and ink particles can be summarized as follows:

- Turbidity measurements indicate interaction between toner-particles. Flocculation was noticed at low pH and dispersion at alkaline solution. Dispersion of the ink particles is the key to efficient washing processes. Hence, the addition of enzyme causes the ink particles to be dispersed where washing process can be used for ink separation.

- Zeta potential results suggest strong interaction between enzyme-oleic acid blend with ink particles. It has been concluded that the main mechanism involves formation of enzyme-oleic acid complex followed by micro-encapsulation of ink particles through a hetero-coagulation mechanism. This will facilitate the bubble/ink particles capture during deinking using flotation process.

- Surface tension data of the enzyme - oleic acid blend show a pronounced lowering of surface tension, at low pH. This indicates a strong interaction between enzyme and oleic acid at air-water interface.

**References**


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