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ALKALINE ENZYME ASSISTED DEINKING OF MIXED OFFICE WASTE PAPER WITH CELLULASE

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ABSTRACT

The enzymatic deinking process was developed in two stages, the first one was the enzyme pretreatment and the second one the subsequent flotation deinking, both conducted at pH = 5.5 and temperature = 55ºC. Other process parameters were: enzyme concentration = 1545.96 FPU, air flow rate of 0.5L/min, and Na_2SiO_3 concentration of 48.65 Be. The optimal deinking conditions were: Enzyme dosage of 0.15%, enzymatic action time of 20 min, flotation consistency of 0.5%, whereby the 70% of essays reached ISO brightness over 70%, with a maximum value of 80% and an average yield of 90%.

Keywords: deinking conditions, enzymatic pretreatment, ISO brightness, yield.

APPLICATION

This work gives information, which can be used to optimize the enzymatic deinking conditions of MOW papers with high yields, reducing costs and the amount of disposal charge in the process effluents.

INTRODUCTION

The paper world consumption indicators have been rising very fast and it is believed that they will continue increasing in the next two decades. Argentina Forestal says that this consumption at the beginning of the 21st century was calculated to be 300 million tons, in 2005 it had raised to 336 million and it is believed that in 2020 this figure will reach 566 million tons [1].

The bleached pulp world demand has grown on the market to about 45 million tons in 2005 and will grow to 74 million tons in 2020, which means a yearly growth of 1.9 million tons, increasing the South American environmentalists' concern. In view of that this region was selected for the industry to settle [2].

There are 13 million hectares of fast growth tree plantations with yields over 15 m^3 of wood per hectare per year. About 80 percent of these 13 millions are located in South America and Asia [3].

It is estimated that 24 trees can be saved per ton of recycled paper produced with 100% of recycled pulp. As Costa estimates, a ton of paper produced by recycled pulp flotation requires 60% less energy than one produced from virgin pulp. Additionally, recycled pulp production requires lower operating costs, fewer reagents, and generates fewer toxic effluents [4].

Other advantages of using recycled paper consist in no need to cut trees in order to obtain raw material, reduction of hazardous chemical compounds that are thrown into rivers, reduction of process costs in effluent water treatment, as well as energy reduction, because 2,400 kg of wood, 200,000 L of water, and about 7,000 kWh of energy are required to obtain a ton of paper from virgin pulp, whereas old paper, 100 times less water (2,000 L), and just one third of the energy (2,500 kWh) are required to produce the same ton of paper using recycled paper [3].

Thus, the world production increased from 26% to 41% of paper produced from recycled paper, from 1975 up to now. In South America, Argentina, Colombia, Venezuela, and Uruguay stand out as to their recycling processes, all of them presenting over 40% [5].

There are many well-known deinking processes in the world, like neutral and alkaline chemical deinking. In addition, the enzymatic deinking is used to improve some properties like drainage by 30%, which produces an increase in machine speed, which in turn means savings in energy and cost reduction, as well as a reduction by 50% in the amount of chlorine compounds. The paper production from recycled pulp is increasing due to the consumer demand [3].

Contrary to popular belief, the main purpose of recycling paper is not to find or to offer a solution for deforestation - which is a collateral consequence -, but to reduce the solids sewage, to make it easy to handle and to treat; in other words, it is to generate less disposal, which is both an environmental and an economic problem, and not only a big city problem [4].

The objective of this paper was to evaluate the deinking of MOW pulp with an enzymatic pretreatment and a subsequent flotation, the main parameters of evaluation having been ISO brightness and the number of specks. A screening design was made to evaluate the factors, and from these results to optimize the values, aiming for the parameters present in the best performance.

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ENZYMATIC DEINKING

Deinking is the process of removing undesirable particles (ink and filler load) from the reused paper [6]. The enzymatic deinking is highlighted in the deinking processes.

The enzymatic process has shown to be effective at a low production cost, because cost reduction is achieved in the water effluent treatment. Almost all mills agreed to keep secret the enzyme combinations, so that it is difficult to find the optimal formulation, even though there are some formulations available [6].

Enzymatic deinking is the process with enzymes, which may act either on the fiber or the ink, depending on its function. The most used enzyme is Cellulase, which degrades the cellulose releasing ink particles attached to the paper to be easily removed. These enzymes may work helped by surfactants [5].

The enzymes most used in deinking are: lipases, esterases, pectinases, hemicellulases, cellulases, and lignolitic enzymes, the latter two acting on the fiber surface, while the other ones may degrade the vegetable oil based ink [5].

Depending on the function, type of ink, and the properties required by the pulp, it may be possible to establish a composition of the components, although it is remarkable that the deinking process is closely related to the type of printing, ink, and drying method [6].

Enzyme

Enzyme is a complex protein that produces a specific change in other substances without any change in itself, it acts as a reaction catalyst (reducing the activation energy). Enzymes may convert starch, proteins, and sugars into different substances. Blood coagulation is an example of enzymatic action.

Effects of enzymes used in deinking [8]

Korean researchers believe that enzymes hydrolyze themselves and depolymerize cellulose, releasing fibers. Ink particles are separated from the fibers in pulping. Some researchers believe that the enzymatic treatment weakens bonds because of the increment of fibrillation, due to the removal of surface layers of fibers. Other researchers believe that hydrolysis is not essential, due to the fact that enzymes can remove ink under not optimal conditions, i.e. they believe that the enzyme itself covers the fiber and breaks the surface, thus releasing the ink particles.

Enzymatic effects may be direct, removing microfibrils and fines and raising the release of ink, thus making flotation and washing easier, even though the fines content is not always reduced in the deinking process.

Welt and Dinus are sure that there is more than one mechanism and that the mechanical action is critical to enzymatic deinking. Nevertheless, the relative importance of each mechanism may depend on the fiber substrate, the composition of the ink, and the enzyme mixture.

Cellulase

Cellulase is the enzyme that degrades cellulose, the latter one being known as the largest proportion of homopolysaccharide in wood. It is a basic cellular structure of the plants and the most important substance produced by trees (Marx-Figini, M., 1964), being the main component of cell wood (Fengel, D., 1984).

Types and action [9]

There are five types of cellulase, classified taking into account the type of catalyzed reaction:

- Endo-cellulase: it breaks the inner bonds to disturb the crystalline structure of cellulose and to expose the individual bonds of cellulose polysaccharides.
- Exo-cellulase: it breaks between 2 and 4 units of glucose, attacking the exposed bonds left by the endo-cellulase, which results in tetrasaccharides of disaccharides, like cellobiose.
- Cellobiase or beta-glucosidase: it hydrolyzes the exo-cellulase products in individual monosaccharides.
- Oxidative cellulase: it depolymerizes the cellulose by drastic reactions.
- Cellulose phosphorilase: it depolymerizes the cellulose with phosphates exchanging water.

The most familiar case is the enzyme reducing the cellulose to betaglucose. This is produced in ruminants' stomachs, human beings do not produce this enzyme in their bodies, because they cannot use this energy.

Applications [9]

Cellulase is used for commercial stuff, like food processing, as e.g. coffee. It hydrolyzes the cellulose during the drying process of the grains. Besides, cellulases are widely known by their performance in the textile industry and in detergents for clothes. They are also used in pulp, for the paper manufacturing process, even in pharmaceutical applications. Cellulase is used in biomass fermentation for biofuels.

Enzyme kinetics [10]

Enzymes are protein catalysts that, like all catalysts, speed up the rate of a chemical reaction without being used up in the process. They achieve their effect by temporarily binding to the substrate and, in doing so, lowering the activation energy needed to convert it into a product. The rate at which an enzyme works is influenced by several factors, e.g.:

- The concentration of substrate molecules (the more of them available, the quicker the enzyme molecules collide and bind with them). The concentration of substrate is designated [S] and is expressed in units of molarity.
- The temperature. As the temperature rises, molecular motion - and hence collisions between enzyme and substrate - speeds up. But as enzymes are proteins, there is an upper limit beyond which the enzyme becomes denatured and ineffective.
- The presence of inhibitors.
  - Competitive inhibitors are molecules that bind to the same site as the substrate - preventing the substrate from binding as they do so - but are not changed by the enzyme.
  - Non-competitive inhibitors are molecules that bind to some other site on the enzyme, reducing its catalytic power.
- pH. The conformation of a protein is influenced by pH and as enzyme activity is crucially dependent on its conformation, its activity is likewise affected.

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The affinity between the substrate and the enzyme molecules is measured as the inverse of Michaelis-Menten constant (K_m), the lower the K_m, the greater the affinity (thus, the lower the concentration of substrate needed to achieve a given rate).

If an experiment is set up in which an enzyme preparation can be added to different concentrations of substrate and the concentration of product formed is measured, if the product absorbs light – this can be easily done in a spectrophotometer- it can be seen that early in the run, when the amount of substrate is in substantial excess of the amount of enzyme, the rate observed is the initial velocity of V_i.

Plotting V_i as a function of [S], it is found that:

- At low values of [S], the initial velocity, V_i, rises almost linearly with increasing [S].
- But as [S] increases, the gains in V_i level off (forming a rectangular hyperbola).

The asymptote represents the maximum velocity of the reaction, designated $V_{max.}$

The substrate concentration that produces a V_i that is one-half of V_{max} is designated the Michaelis-Menten constant, K_m, as shown in **Figure 1**.

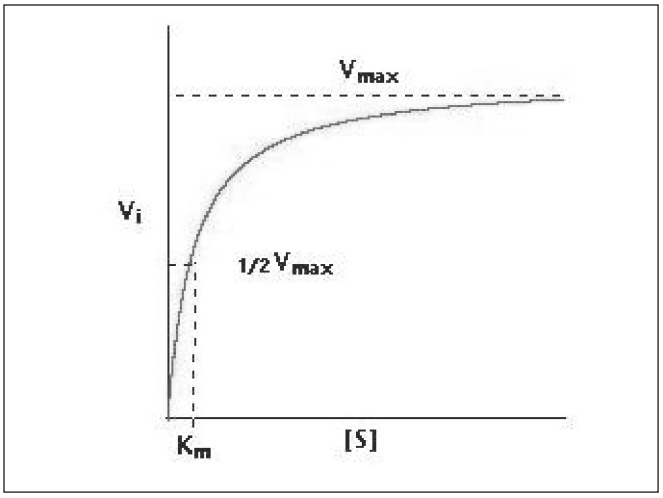

Figure 1. Graph showing V_i *vs.* [S]

Enzyme action mechanism [11]

The degradation of the polysaccharides is caused by the action of enzymes which are specialized for the different carbohydrates present in the cell wall of wood. Enzymes of the same type may have varying properties, depending on the fungal source.

The hydrolytic effect of individual enzymes is low, whereas the combination of exoenzymes (cellobiohydrolases) and endoenzymes increases the production of glucose. Thus, various enzymes are seen to cooperate in the degradation of cellulose.

Reese, in 1977, developed a scheme in which he differentiates between C1 and Cx enzymes, which is shown in **Figure 2**. The C1 enzymes are thought to destroy the surface of the crystalline structure of cellulose by some swelling and cleavage of some covalent linkages. According to this scheme, C1 enzymes prepare the substrate for the attack of Cx enzymes.

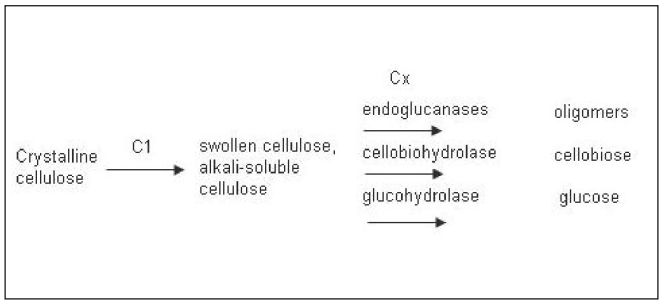

Figure 2. Enzyme action scheme

The Studies of Wood and McCrae (1979) resulted in a more complex action of C1 and Cx components. They assume a formation of enzyme-enzyme complexes on the surface of the cellulose. These complexes, consisting of endo and exoenzymes, solubilize the cellulose by a rapid sequential action.

The endoglucanases act more randomly on the cellulose chains, producing new reaction places for the endwise-acting cellobiohydrolases. This synergism seems to be a very important mechanism, particularly for the highly ordered regions of cellulose, as there is no possibility for the large macromolecules of the enzymes to penetrate the cellulose structure. In this connection, we should note the important observation that the endoglucanase with the lowest molecular weight (13,000 Dalton) is the one acting randomly. It also explains the need for at least two enzymes of the same type of glycosidic linkages, which differ with regard to steric conditions.

There is evidence that the enzymatic degradation of cellulose is influenced by additional factors, such as the presence of monosaccharides, of polyoses, or of an H_2O_2 – Fe system, which may act as a starter substance or aid in decomposing the crystalline system.

METHOD

Experimental design

The experimental design was made in Statgraphics, with the purpose of planning the number of essays and repetitions. The design chosen was a screening design 2k fraction. The lower and higher levels of each factor are shown in **Table 1,** and the responding variables were number of specks and ISO brightness.

The variables selected for the enzyme were pretreatment consistency, enzyme dosage, enzymatic action time, and chelate concentration, and the constant parameters were T=50°C and pH=5. These variables are taken into account in the enzymatic pretreatment of the pulp.

In the deinking process, carried out in a flotation cell, after pretreatment the variables selected were: consistency, time, collector concentration, surfactant concentration, peroxide concentration and silicate concentration.

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Table 1. Rate of values for variables

Factors	Low level	High level	Units
(D_E)	0.05	0.15	%
(C_E)	3	6	%
(t_E)	20	100	min
(C)	0.5	1.5	%
(t)	6	12	min
(S)	0	400	mg/L
(CC)	0	400	mg/L
(CP)	0.5	1.5	%
(CS)	1.5	4.5	%
(Q)	0.5	1	%

Notes: 1) E means enzymatic stage. 2) The percentage is based on dry oven mass of pulp.

Deinking procedure

The procedure begins with the calculation of the amount of pulp required to be used, and checking the consistency needed in the flotation cell. The pulp used was supplied by Familia Sancela S.A. The pulp undergoes a pretreatment, where the EDTA is added to, the pH and the temperature are controlled because of the optimal conditions for the addition of the enzyme, the chemicals acting during the settled time of the experimental design. After the enzymatic pretreatment, deinking is performed in a Denver flotation cell, where the pH and the temperature are checked and controlled and then the surfactant, sodium silicate, calcium chloride and hydrogen peroxide are added thereto. When the process is finished, the pulp is gathered and directed to a handsheet former. After drying, the number of specks and ISO brightness are measured. There are also some process blanks that are made without the enzymatic pretreatment. The scheme process is shown in **Figure 3**.

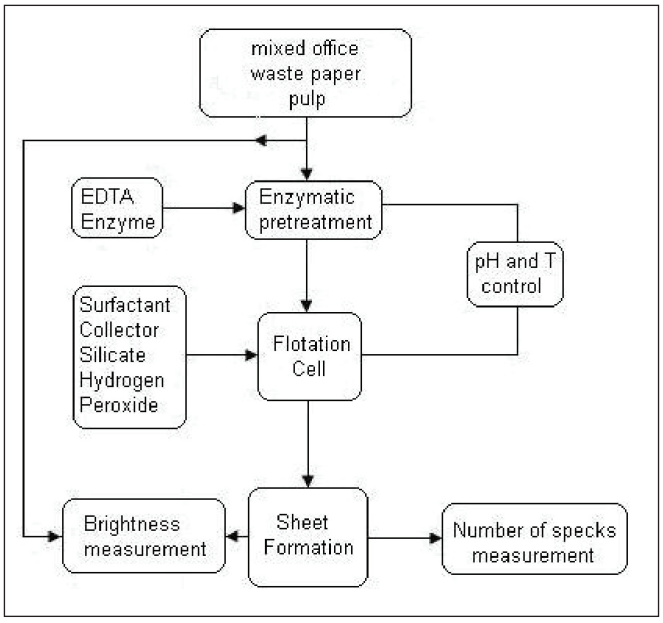

Figure 3. Procedure diagram

Cellulase activity measurement [12]

The cellulase activity was determined taking into account the IUPAC standards in FPU (filter paper units) per milliliters of the original enzyme solution.

1mL of a sodium citrate buffer solution with pH = 5 is added to a test tube. Then, 0.5mL of enzymatic solution previously diluted with citrate buffer is added to the same test tube.

The samples were taken to a water bath at 50°C, where the filter paper strip (substrate) of 1x6 cm is added to and stirred. The blank reagent is prepared by mixing 1mL of buffer with 0.5mL of the enzyme diluted solution used.

The samples and the blanks are incubated at 50°C during 60 minutes, while a series of standard solutions of a dehydrated glucose buffer of 10mg/mL concentration is prepared.

0.5mL of each dilution, 1mL of sodium citrate buffer, and 3mL of DNS (dinitrosalicylic acid) are mixed together. The blank reagent was prepared by mixing 1.5mL of buffer solution with 3mL of DNS. The test tubes in the bath at 50°C were isolated and 3mL of DNS was added thereto.

The test tubes were taken to a boiling water bath and were kept there during 5min. All samples, standards, and blanks were put together in this bath. Then the reaction was stopped and the test tubes taken to an ice bath.

20mL of distilled water is added thereto and completely mixed. After about 20 minutes, when the substrate leftovers had sedimented, the color of the samples was checked in a spectrophotometer at 540 nm of wavelength, using the correct blanks. Then the calibration curve was plotted by using the absolute amounts of glucose in the standards and the respective absorbency.

The amount of glucose released in the samples was calculated like the absolute glucose. Then the enzyme concentration was calculated, which is represented by the portion of the volume of the initial enzymatic solution that there is in the solution of the essays used.

Finally, the enzyme concentration released by exactly 2mg of glucose was determined, plotting the amount of released glucose

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versus the enzyme concentration on a semilogarithmic paper and the FPU was calculated by means of Equation 1.

$$FPU = \frac{0.37}{Enzyme\ concentration\ released\ by\ 2mg\ of\ glucose} \qquad Equation\ 1.$$

Gloves were used in the experiment, because DNS is carcinogenic and mutagenic.

RESULTS

Enzymatic activity of cellulase

The amount of glucose released in the samples shown in **Table 2** is calculated by means of the calibration curve equation.

Table 2. Calculation of the released cellulose (mg/mL)

A=abC + intercept		
Abs	**a [=] mL/mg*cm**	**Released Glucose (mg/mL)**
0.543	0.441	1.303628118
0.715	**Intercept**	1.693650794
0.999	-0.0319	2.337641723
1.087		2.537188209
1.125		2.623356009

The enzyme concentration versus the amount of glucose released was plotted. The semilogarithmic paper is used to make the measure easier, so that the plot shown in **Figure 4** was not possible and it was adjusted in the scale of the axes.

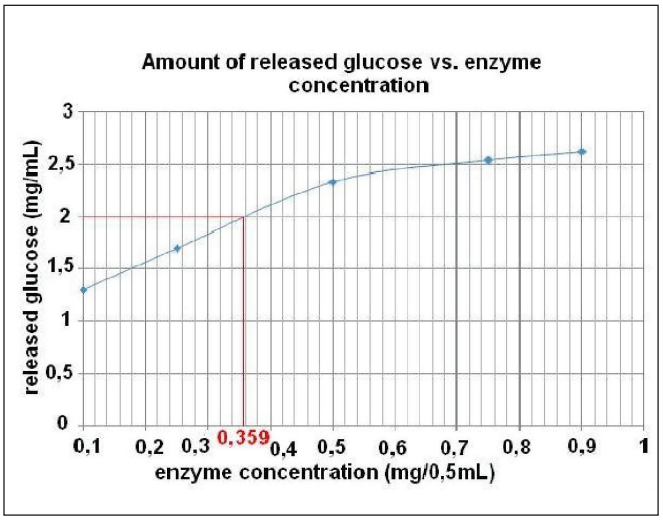

Figure 4. Enzyme concentration *vs.* amount of released glucose in the samples

The enzyme concentration is calculated from the dilutions chosen to guarantee values of released glucose of about 2mg under reaction conditions. The dilutions chosen were: 10%, 25%, 50%, 75%, and 90%.

With this figure, the enzyme concentration that released exactly 2mg of glucose was determined, and with this value equation 1 is used. The value in FPU was 1.030640669, expressed in grams, but it was needed in milligrams, which is 1030.640669. Then, with the dilutions (1.5 factor), 1545.961003 is the enzyme concentration.

ISO brightness and number of specks

ISO brightness

The results of the measurement of ISO brightness are shown in **Figures 5** and **6**.

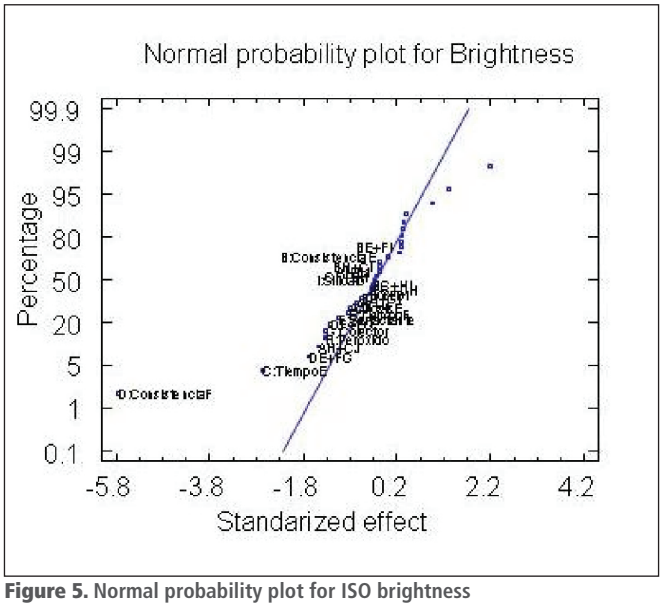

Figure 5. Normal probability plot for ISO brightness

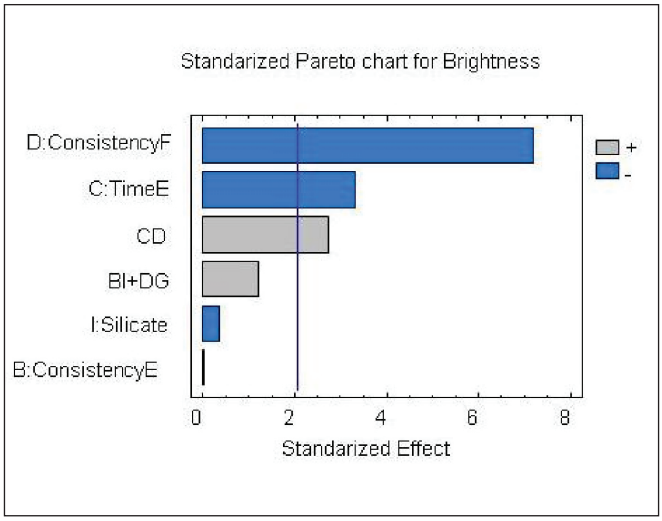

Figure 6. Standardized Pareto chart for ISO brightness

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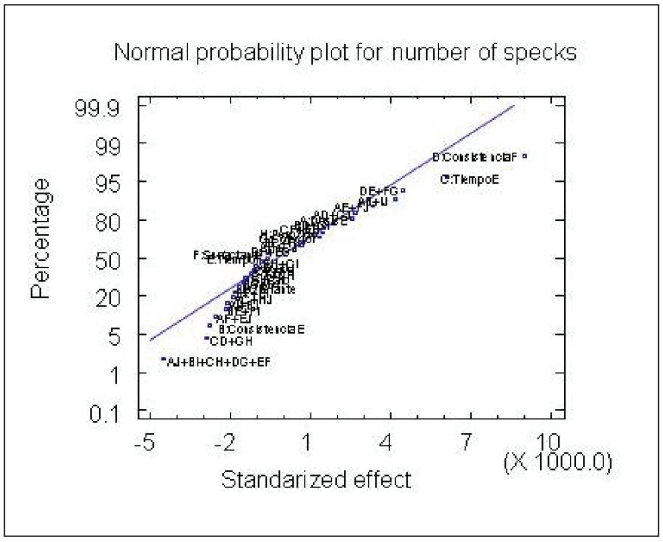

Figure 7. Normal probability plot for the number of specks

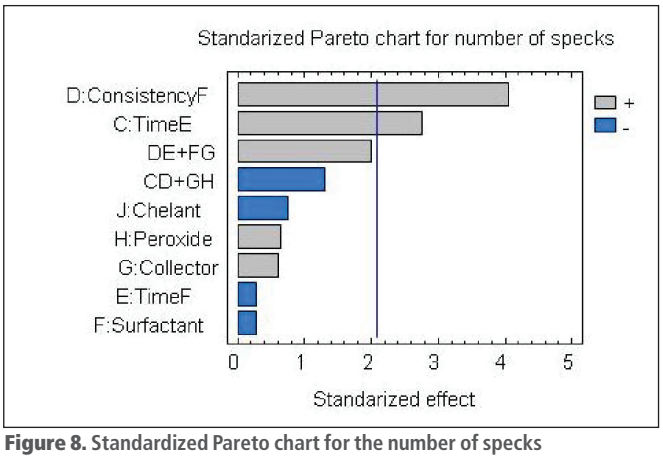

Figure 8. Standardized Pareto chart for the number of specks

Number of specks

The results of the measurement of the number of specks are shown in **Figures 7** and **8**.

Yield was calculated with the foam, which is the material rejected in the flotation cell, and with the grams of pulp prior to flotation, which will indicate the rejection percentage, so that yield can be calculated.

DISCUSSION

Evaluation of process parameters before deinking

The evaluation procedure of the peroxide concentration was carried out to check the value indicated on the flask, due to the fact that peroxide is unstable, and may turn into H_2O and O_2. The value was 29. 325%, which represents a 2.25% deviation from the one reported by the manufacturer (30%).

The enzyme activity was developed in order to prove the one reported, looping forward the enzyme has the same ability of action despite of time, even though in general this factor is reduced with the storage time. The value obtained by the FPU method was 1545.961003, which means a 3.01% deviation from the original value reported by the manufacturer, which was 1,500 FPU. This means that the value reported was not the actual one, it should be about 1,600 FPU.

Airflow is an important factor in the flotation cell of the deinking process, due to the fact that a high value of airflow means that there will be bubbles of big size, which may drag not only ink particles, but also pulp, and if the value is very low the ink particles may not be dragged.

The ashes of the raw material must be of a low value, because most of the material is cellulose, and the ashes of the process output have to be of a high value, because all wanted there is ink, as otherwise pulp fibers would be lost. The average of the ashes in the process output was 39.77%, which means an ash elimination of about 40%.

This value is variable according to the amount of metals added to the other reactant used in the flotation cell. Ashes are made up of ink, filler minerals used in paper manufacturing, and metals in the reactant added. The ash content in the raw material amounts to 11.01%.

The percentage of yield was calculated to be 1.5% of the consistency, which is the high level of this variable. This high level was chosen because there are more possibilities of losing pulp at this level than at the lower one. The yield values are shown in **Table 3**, and range from 86% to 96%, which means that high yield values were obtained in almost all the experiments. Due to the fact that there was no statistical difference between dirt count and ISO brightness, yield cannot be a parameter defining the deinking process in this case, in which most of the yield values are around 90%.

The specific gravity of Na_2SiO_3 was 48.65 Baumé (Be) degree,

Table 3. Yield percentage

Essay	Foam (g)	Ash (g)	g of pulp	dots/m²	ISO brightness	% Rejection	% Yield
2	5.6427	2.1543	45.0000	36752	69395	12.5393	87.4607
11	1.3536	0.6923	45.0000	38042	69.65	3.0080	96.9920
14	4.3668	1.8020	45.0000	30710.5	70.88	9.7040	90.2960
17	3.6692	1.1838	45.0000	30270	69.575	8.1538	91.8462
20	5.3593	1.9606	45.0000	48079.5	66.14	11.9096	88.0904
22	5.9082	2.3162	45.0000	39458	65.175	13.1293	86.8707
Raw Material	1.4913	0.1655	11.0977	**Ash percentage of undeinked pulp**			

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which is a typical value for solution preparations ranging from 47 to 52.8 Be degrees.

Evaluation of process parameters after deinking

From Figure 5 it can be observed that the effects having relevant influence on brightness are: consistency in the flotation cell, action time of the enzyme, and the interaction between them.

From Figure 6 it can be observed that the behavior of the effects having actual influence on the improvement in brightness results from a short enzyme action time, as well as a low flotation cell consistency, the interaction between which is due to the higher change in brightness at low levels of enzymatic action time than at high levels of the same factor. Thus, the positive effect shown in Pareto diagram is caused when the interaction produces the higher brightness: low flotation cell consistency and short enzymatic action time. These results are similar to those reached by M. Pelach and others, also by C. Lee, I. Darah, and C. Ibrahim [13, 14], who also used cellulase and determined that at these factor levels the efficiency of flotation increased.

The results achieved in brightness due to high levels of enzymatic action time are explained as reported by Pala, Mota, and Gama (2006), who stress that if there is a long time of enzymatic action there will be an excessive fibrillation and fragmentation of ink particles and, therefore, there will be more chance of the inks staying in the pulp fibers, reducing the efficiency of ink removal and decreasing pulp brightness as a consequence.

The high levels of brightness caused by low levels of consistency are explained by a better dispersion of chemicals and enzymes in the pulp, because the pulp is more diluted, so that there is an increase of the superficial contact area, as shown by C. Lee and others (2007), and due to the fact that the pulp in the cell is more fluid there are stronger impact forces with the impeller and with the cell walls.

In Figure 7, the effects having influence on the resulting number of specks are the consistency in the flotation cell and the enzymatic action time, which allows concluding that the other effects only produce noise in the model and that there are some factors missing in the analysis.

From Figure 8 it can be observed that the behaviors of the effects having an actual influence on the reduction of the number of specks are a short enzymatic action time and a low consistency in the flotation cell. The interaction effects in this case are not significant.

The analysis of the numbers of specks is analogous to that of brightness; the variables behave so as to favor the elimination of specks. Such an increase in the superficial contact area of the reactant and a value of mechanical stresses in the flotation cell aim to improve the optical properties of the pulp. The values obtained are

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comparable with those obtained by Vargas and Vélez [6] and lower than those obtained by Magnin and Lantto [15]

The results are not that bad, the amount of the number of specks is explained by a poor disintegration of the pulp used to make this paper.

CONCLUSIONS

The value of airflow measured is 7.7697mL/s or 0.46L/min, which is lower than the one used by Zöllner and Schröeder, which was 5L/min [16]. This is the reason why it is important to evaluate this parameter, the value of which may be explained by the frequency and consistency of the pulp in the flotation cell.

The optimal values to get higher ISO brightness (80%) are: enzyme dosage of 0.15%, enzymatic action time of 20 min, flotation consistency of 0.5%, chelate of 1%, a low level of collector, surfactant of 400mg/L, pH of 5, and a temperature of 50°C.

The optimal values to obtain a lower number of specks (9,849 dots/m^2) are: enzyme dosage of 0.15%, enzymatic action time of 20 min, flotation consistency of 0.5%, pH of 5 and a temperature of 50°C; as to chelate, collector, and surfactant, it is not possible to reach correct conclusions.

The model for the number of specks is not the best one, which may be explained by the fact that there are many variables producing much noise in the model and that there are some important parameters which have not been taken into account.

As to brightness, the model is acceptable, because it explains nearly 80% of the behavior. 66% of the brightness results indicate over 70% in ISO brightness.

47% of the results in the number of specks are below 30,000.

The highest yield value obtained was 96% and the lowest one was 86%.

The higher brightness (80%) shows a 25% improvement in virgin pulp brightness (55%). With regard to the lower number of specks (9,849 dots/m^2), a 66.48% improvement can be observed, in comparison to the optimal value (14,806 dots/m^2) obtained from the model. ■

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