Chapter

Deinking of Mixed Office Waste (MOW) Paper Using Enzymes

G. Ramiro Escudero, González P. Jeovani and Perez S. Rosa Elena

Abstract

Deinking of Mixed Office Waste [MOW] paper was carried out by using a flotation device and adding enzymes as defibrillators. Employing the computational simulation of the molecular coupling between the cellulase enzyme obtained from *Trichoderma* sp. and cellulose, the enzyme-cellulose molecular complex and the main amino acids endo-β-1,4-D-glucanase of this molecule, responsible for the hydrolysis of cellulose, were obtained. Three of these amino acids were used for deinking. The deinked cellulose fibers were evaluated according to the standards of the paper industry (ISO whiteness [brightness], factor of reflectance, opacity, and tonality) to determine the efficiency of deinking. The experimental results show that the best optical characteristics of the sheets of deinked paper with amino acids are those where a mixture of aspartate, glutamate, and asparagine was applied, instead of their individual dosage. In addition to the aforesaid, the use of enzymes instead of the traditionally used reagent, such as sodium hydroxide, avoids the contamination of wastewater; additionally, the operation of the column is carried out easily, taking into consideration that the pH of the system goes from alkaline to neutral.

Keywords: paper deinking, flotation column, cellulose, aspartate, glutamate, asparagine

1. Introduction

The recycling of paper is a necessary option for the care of the environment and to comply with the environmental regulations established by governments toward the pulp and paper industry, and specifically, the industry that processes recycled paper fibers to obtain pulp free of ink and that includes in its process the passivation of colloidal particles [1]. Traditional deinking paper (e.g., office paper) is industrially "cleaned" by adding sodium hydroxide (defibrillator) and adding chemical reagents to improve the quality of recycled paper, such as whiteness and mechanical strength [2, 3]. This traditional process undoubtedly damages the environment and specifically urban water reservoirs due to the additional chemical agents contained in the recycled pulps [4–6].

The deinking process exposes the deinking equipment to highly alkaline conditions (pH greater than 10) because the addition of chemical reagents, so ideal conditions in conditions close to neutral pH are preferred, regardless of the chemical

reagents used to defribillate the cellulose, capture ink particles, and improve optical and mechanical properties of the recycled paper [5–9].

The conditioning of the cellulose pulp is essential to ensure the detachment and capture of the ink particles. The conditioning of the pulp by adding enzymes will promote the disintegration of the paper and the detachment of the ink, as the enzyme is introduced into the cellulose-ink interface. The catalytic action of the enzymes reduces the activation energy of the reactions that occur in the system, even at low concentrations of the reagent, which implies a low contamination of the conditioning medium. Like any chemical reagent, enzymes function within specific pH ranges [10–12]; for example, enzymes called cellulases hydrolyze cellulose fibers and enhance the detachment of ink particles [13–15], which will be available for capture, for example, through the use of devices that generate air dispersions, such as the case of flotation devices [6, 16].

This research work presents the results of the conditioning of recycled office paper pulp (Mixed Office Waste [MOW] type) using enzymes and capturing the ink particles through a flotation column, equipment that is commonly used for mineral processing. The variables that were quantified in the deinked paper to establish the feasibility of the use of enzymes are whiteness, reflectance, opacity, and tonality.

2. Materials and methods

2.1 Enzymatic deinking

Mixed Office Waste (MOW)-type paper was used. The sheets were cut and disintegrated in an industrial blender without chemical reagents. The amino acids such as aspartate, glutamate, and asparagine (Sigma-Aldrich) were added (individually and mixed of all three), as hydrolytic reagents to promote ink detachment.

Once the cellulose pulp had been conditioned for 30 minutes by adding the corresponding chemical reagents, it was fed to a laboratory flotation column made of transparent acrylic tubes. The device (gas disperser) responsible for the generation of bubbles was installed at the external base of the column (venturi-type disperser). The consistency of the pulp fed to the column was set at 4.0%. The experimental conditions are shown in **Table 1**.

In all experiments, pine oil was added as a surfactant to fix and maintain the surface tension at 0.63 N/m. **Figure 1** shows the experimental setup for paper deinking.

Variables	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Amino acid	**ASP**	**ASP**	**GLU**	**GLU**	**ASN**	**ASN**	**ASP:GLU: ASN**	**ASP:GLU: ASN**
Concentration, %	0.1	0.2	0.1	0.2	0.1	0.2	0.1:0.1:0.1	0.2:0.2:0.2
Consistency, %	4	4	4	4	4	4	4	4
pH	7	7	7	7	7	7	7	7
Temperature, °C	25	25	25	25	25	25	25	25
Surfactant addition, ppm	100	100	100	100	100	100	100	100

ASP, aspartate; GLU, glutamate; ASN, asparagine. The % is calculated based on the dry paper mass.

Table 1.
Experimental conditions for deinking with amino acids.

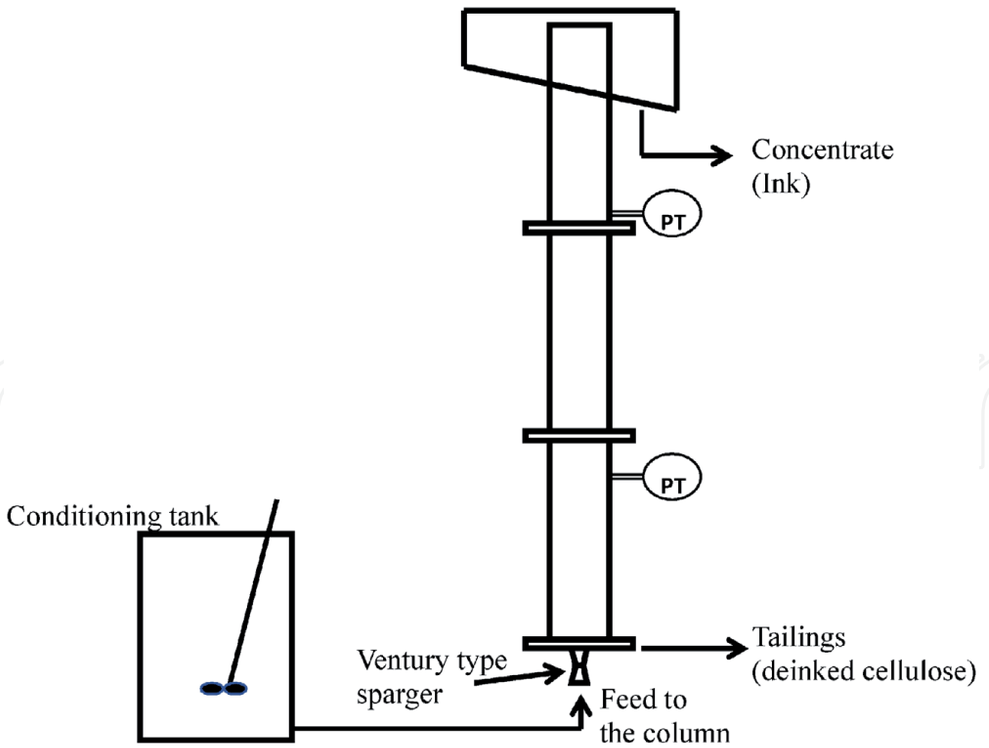


Figure 1.
Experimental setup for deinking of recycled paper. PT = pressure transducer devices.

The gas holdup in the column was estimated through the pressure drop values from pressure transducers installed in two points along the column. The mean bubble diameter of a swarm was calculated by solving the Drift Flux Analysis model [17].

2.2 Evaluation of deinking

The TAPPI T-205-OM-81 standard was applied to the samples collected in the tailings stream (deinked fiber), for each experimental variation of **Table 1**. According to the mentioned standard, the quantified characteristics are whiteness, reflectance, opacity, and tonality.

2.3 Molecular coupling

The method of genetic algorithms coupled to local search or Lamarckian (AUTODOCK 3.0) was used to identify the geometry between the cellulose fragments (ligand) and the cellulase enzyme (substrate).

3. Results and discussion

3.1 Molecular coupling between cellulose and enzyme cellulase

The molecular model representing five cellulose monomer units was taken as a basis. **Figure 2** shows the theoretical model on which this analysis is based.

From the database of the simulation program AutoDockTools, the cellulase enzyme is extracted into its endo-β-1,4-D-glucanase (rigid substrate), as shown in **Figure 3**.

The results from the simulation were analyzed using the AutoDockTools suite with the option of analyzing the conformational space of the ligand substrate. The initial geometry of the studied complex is shown in **Figure 4**.

Figure 2.
Molecular model of cellulose (ligand) of five monomeric units of cellulose.

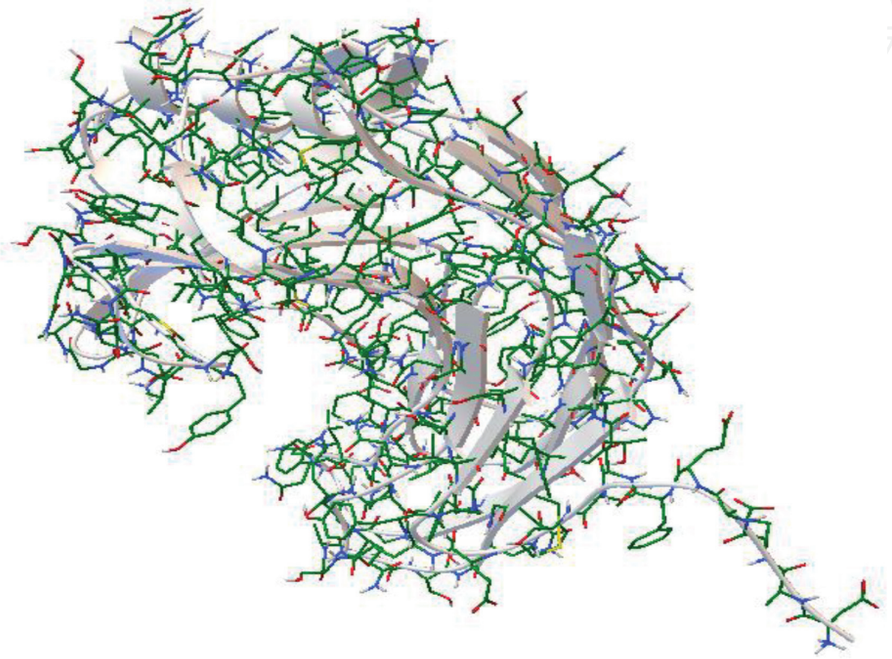


Figure 3.
Schematic representation of the endo-β-1,4-D-glucanase enzyme molecule.

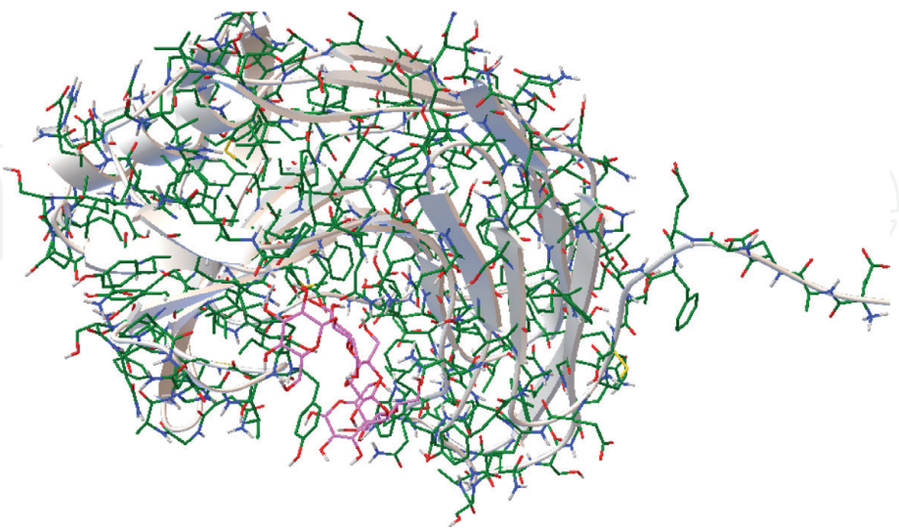


Figure 4.
Initial geometry of the complex enzyme-cellulose.

From the results, the crystallographic coordinates of the crystallized ligand are taken as a reference, grouping the 100 conformations evaluated and obtained in clusters. Each docking is validated after running the same evaluation five times. In the conformational search process, the Lamarckian genetic algorithm (LGA) is

applied. The Intelec parameter is activated, so that the internal electrostatic of the ligand affects the energy of docking (docked energy) that guides the optimization process, but not to the binding energy of the ligand (binding free energy). The algorithm analyzes 100 possible poses of the cellulose in the enzyme, adopting the flexibility property for the cellulose molecule, so as not to rule out this interaction phenomenon between ligand and substrate.

The 100 poses analyzed are grouped into the so-called clusters in such a way that they are ordered from the highest to lowest docking energy (binding free energy). This binding free energy is the ligand-substrate coupling with the highest affinity as it has the most negative or lowest bond free energy. It is worth emphasizing that AUTODOCK returns two energies: the so-called docked energy, used to guide the docking, and the binding free energy, the latter allows us to define the ligand-substrate coupling with the highest affinity, that is to say, with the more negative bond free energy. For the case of endo-β-1,4-D-glucanase and cellulose, the pose with the highest affinity drawn the following parameters: binding energy −0.57 kcal/mol, inhibition constant 379.8 mM, intermolecular energy −9.52 kcal/mol, internal energy −14 Kcal/mol, and torsional energy of 8.95 Kcal/mol.

Figure 5 shows the amino acids with the most negative enzyme-cellulose bonds, according to the model: glutamate (GLU, A:207), asparagine (ASN, A:27), tyrosine (TYR, A:118), aspartate (ASP, A:106), tryptophan (TRP, A:29), and phenylalanine (PHE, A:108), whereas **Table 2** shows, according to Morrison and Neilson, the structures of amino acids that interact with cellulose molecules of the endo-β-1,4-D-glucanase.

The experimental results are shown in **Table 3**. A relevant characteristic to determine the efficiency of the deinking process is whiteness, and it is determined through the measurement of the brightness. Brightness ISO is determined using standard sensitivity spectrum centered at a wavelength 457 nm according to ISO 2470 or TAPPI T525 standard and is represented in percentage points. In tests 1 and 2, an ISO whiteness of 85 and 86%, respectively, with 445.2 and 412.5 ppm of black points were obtained. For the L * tonality, there are values of 93.05 in test 1, and 94.25, in test 2. The a * and b * tones manifested with practically equal values, 1.6

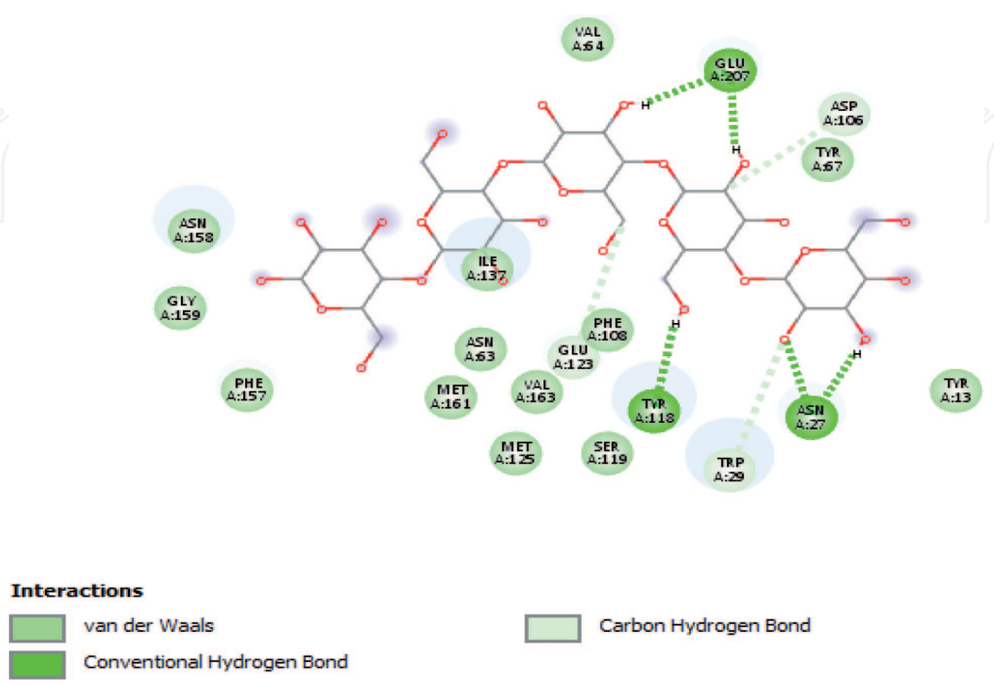


Figure 5.
Linking amino acids in the best enzyme-cellulose according to the modeling.

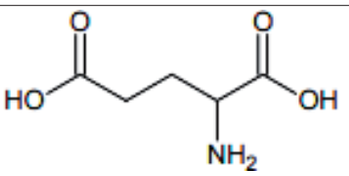
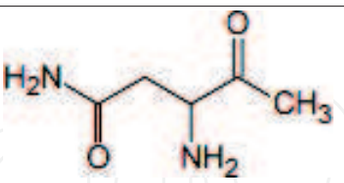
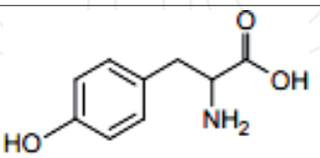
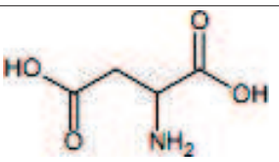
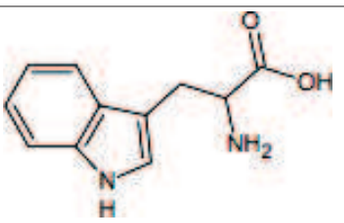
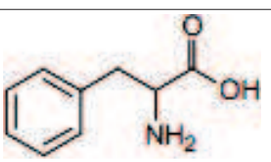
Amino acid	Structure
Glutamate (GLU)	
Asparagine (ASN)	
Tyrosine (TYR)	
Aspartate (ASP)	
Tryptophan (TRP)	
Phenylalanine (PHE)	

Table 2.
Structures of the amino acids that interact with the cellulose molecule [18].

Variables	1	2	3	4	5	6	7	8
	ASP	ASP	GLU	GLU	ASN	ASN	ASP:GLU: ASN	ASP:GLU: ASN
%	0.1	0.2	0.1	0.2	0.1	0.2	0.1:0.1:0.1	0.2:0.2:0.2
Brightness (ISO), (%)	85	86	84.9	86.9	81.5	85.8	89.1	90.8
Black points, (ppm)	445.2	412.5	455.3	377.1	550.8	423.3	333.0	303.4
L*	93.05	94.25	93.8	94.44	91.6	94.6	94.7	95.3
a*	1.6	1.6	1.67	1.27	1.5	1.3	1.65	1.7
b*	−3.3	−3.36	−2.55	−2.1	−2.8	−2.5	−4.3	−4.52
Reflectance (ISO, %)	41.5	42.7	39.57	47.15	40.2	39.32	35.9	34.7
Opacity (%)	88.3	89.5	88.9	89.9	88	89.5	89.6	89.9

Table 3.
Results of the optical characteristics were measured to the deinked sheets utilizing the use of amino acids.

and − 3.3 for both cases; the same occurs in reflectance and opacity, with very close values, 41.5 and 42.7% of reflectance, and 88.3 and 89.5%, of opacity.

For glutamate, increasing the dose from 0.1 to 0.2% gives an increase in the whiteness of 2%, that is, from 84.9% it rises to 86.9%. Black spots are a direct reflection of inking quality and flotation effectiveness, and this number also decreases with increasing GLU dosage from 455.3 to 377.1 ppm. The tonality variables L *, a *, and b *, such as opacity, change slightly, and reflectance, on the other hand, is modified to a great extent from 39.57 to 47.15%, as the dose of the amino acid in question increases.

When treating the MOW paper with asparagine 0.1%, important changes are noted concerning the tests with aspartate and glutamate. When 0.1% ASN is dosed, the ISO whiteness obtained is 81.5%, the black points 550.8 ppm, and the L * of 91.6, these being much lower than those obtained in the rest of the amino acids. For this case, the variables a *, b *, reflectance, and opacity are practically similar to what was observed with the rest of the amino acids.

Mixing the three amino acids (test 7), adding 0.1% of each depending on the weight of the dry paper to be inked, a result of whiteness much higher than that obtained in the other tests is obtained. The resulting whiteness was 89.1% and the black points 333.0 ppm. The variable b * changes to a lesser extent to −4.3. The parameters L *, a *, and opacity remain above the average of those obtained in the first tests; however, the reflectance decreases considerably to 35.9%, this being inversely proportional to the whiteness.

In test 8, MOW paper deinked with 0.2% of each amino acid obtained the best quality results, ISO whiteness of 90.8% and black points of 303.4 ppm, values much higher than expected, taking into account that the MOW paper is not being treated with any chemical bleach and is only working with three process stages: pulp, chemical treatment, and flotation. The tonality parameters achieved: L * = 95.3, a * = 1.7, and b * = −4.52, oscillate in the values obtained in the previous tests and fall within the expected, according to the values established by the standard, which are L *, from 92.5 to 95.5; a * from 1.3 to 2.3; b * from −9 to −8, reflectance from 32 to 40% and minimum accepted opacity of 87%.

The variable b * is controlled with a pigment from aniline, and the negative of its value indicates the color shift toward blue. The addition of such pigment in the manufacture of paper is directly proportional to the negativity of this parameter, that is, the higher the aniline, the more negative the b *. In deinking, these pigments responsible for the color are detached from the vicinity of the fiber and removed by flotation, so a decrease in this parameter is to be expected after deinking. Opacity is required on all printing papers; it should be sufficient to prevent the printing on the reverse side of the paper from negatively affecting the appearance of a print, so the higher the opacity, the higher the print quality. The result in this test was 89.9%.

The optical properties evaluated in this work are higher than those obtained under similar experimental conditions by using NaOH and the enzyme cellulase *Thricodema* Sp., as defibrillators [19].

3.2 Morphology of the deinked fibers

Through scanning electronic microscope (SEM), there is possible to appreciate the morphology of the deinked fibers, to appreciate the degree of fibrillation, because its intensity affects the mechanical properties of the fiber. In the micrographs of the pulp deinked with aspartate, glutamate, asparagine (**Figures 6**–**10**), and a mixture of them, it is observed that the depolymerization induced by amino acids does not represent excessive fibrillation that affects the internal morphology of the cellulose fiber and therefore its mechanical properties. No particles of toner

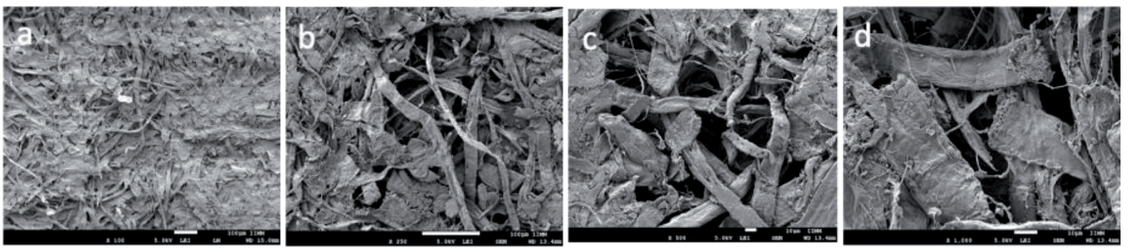


Figure 6.
Micrographs of the deinked fibers. Aspartate case. Test 4: (a) 100X, (b) 250X, (c) 500X, (d) 1000X.

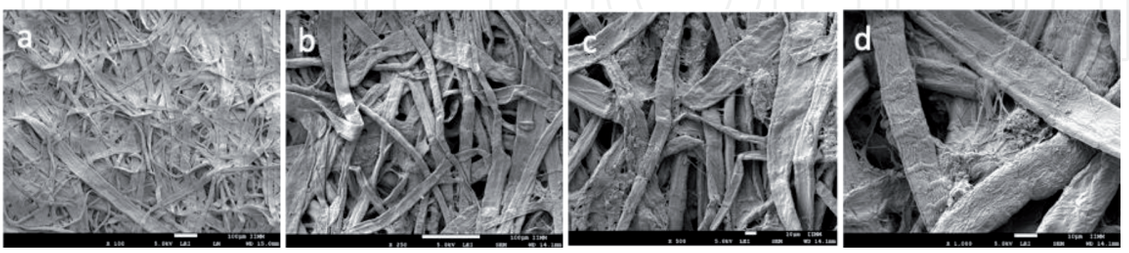


Figure 7.
Micrographs of the deinked fibers. Glutamate case. Test 5: (a) 100X, (b) 250X, (c) 500X, (d) 1000X.

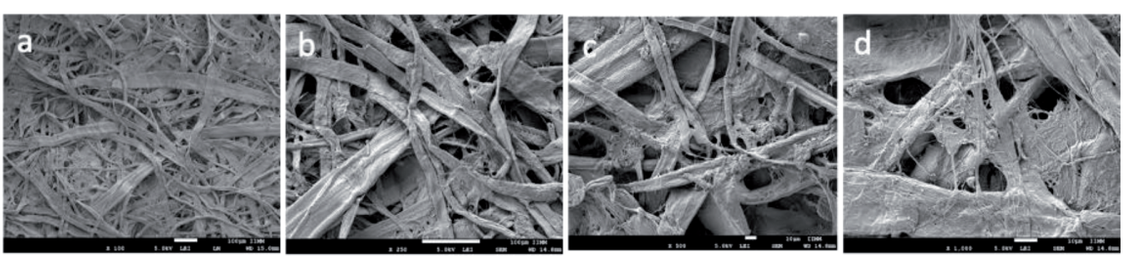


Figure 8.
Micrographs of the deinked fibers. Aspargine case. Test 6: (a) 100X, (b) 250X, (c) 500X, (d) 1000X.

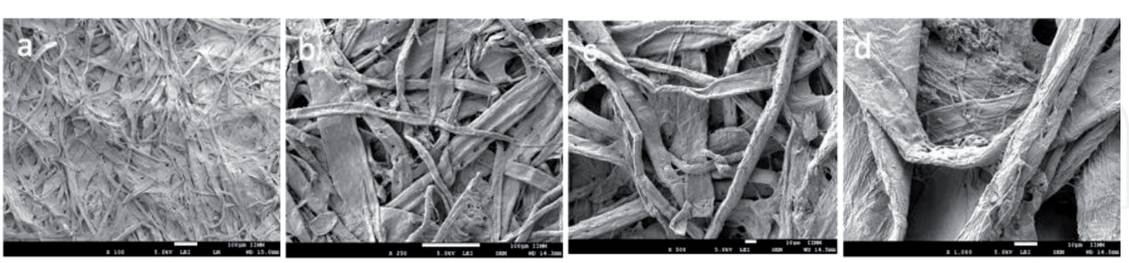


Figure 9.
Micrographs of the deinked fibers. 0.1:0.1:0.1 mixture. Test 7: (a) 100X, (b) 250X, (c) 500X, (d) 1000X.

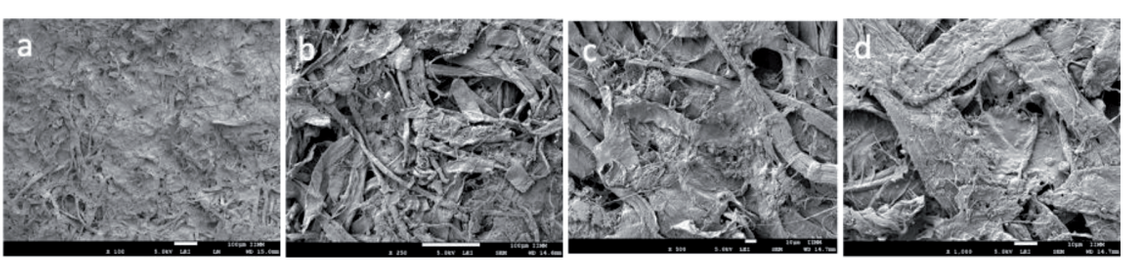


Figure 10.
Micrographs of the deinked fibers. 0.2:0.2:0.2 mixture. Test 8: (a) 100X, (b) 250X, (c) 500X, (d) 1000X.

or printing ink are seen, only small agglomerations, possibly of starch, precipitated calcium carbonate, and/or residues of other additives typical of the manufacture of paper.

Figure 10 shows the micrograph of fibers from test 8, which obtained the best results of the optical characteristics, from it can be seen that the morphological structure of the fiber retains its robustness, and some fibers look flatter than others, as a result of forming the sheets of paper; however, there is no fibrillation or excessive fine formation. The fragmentation of inks is another phenomenon that occurs in deinking processes, which is desirable up to a certain point so that the particles formed from these acquire the appropriate size to be floated more efficiently. When this happens, papers with superior brightness and whiteness are obtained, without considerable repercussions on the mechanical properties of the deinked fiber.

4. Conclusions

In the present work, through the mathematical simulation and analysis of the molecular coupling of the enzyme to cellulose, by the docking technique, the enzymes with the best coupling between the enzyme-cellulose complexes were aspartate, glutamate, and asparagine.

From the paper deinking experiments using these enzymes, the chemical mixture of the three amino acids results in the highest values of optical properties of the paper sheets formed with the deinked fibers: ISO whiteness or brightness (90.8), reflectance (34.7), opacity (89.9), and tonality ($L^* = 95,3$, $a^* = 1.7$, and $b^* = -4.52$), being these values higher than those obtained through conventional methods.

It is worth mentioning the importance of the flotation column during the deinking, due to the fact of the number of bubbles available for capturing the ink particles.

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