# Physicochemical Characterization of the Yeast Cells and Lignocellulosic Waste Used in Cell Immobilization for Ethanol Production

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Additional information is available at the end of the chapter

### Abstract

Ethanol is one of the leading alternative fuels. Efforts have increased the development of technologies for producing ethanol efficiently and economically. The continuous fermentation using yeast cells immobilized in low-cost materials is presented as an excellent alternative. We used four lignocellulosic wastes for the inmobilization process. The materials were characterized physicochemically. The composition was determined by the Van Soest method. Zeta potential was measured to establish the hydrophobic or hydrophilic character of the material surfaces. The contact angles measurements were used to confirm the hydrophobic or hydrophilic character and the free energies interaction was established. Images were obtained by scanning electron microscope, and determination of surface areas and volumes was performed by adsorption and desorption isotherms. It was established that cell surface properties are modified by the immobilization process to which they are subjected. It was evident that cell immobilization depended on the properties of the carrier, as well as cell surface properties. Thus, in order to improve the process of cell immobilization, it is essential to understand the type of carrier-cell interactions that occur during the immobilization process, making necessary the knowledge of the main surface characteristics of both the media and of cells that can affect the process.

**Keywords:** ethanol, immobilization, yeast, lignocellulosic waste, continuos fermentation

## 1. Introduction

Global instability in the cost and supply of petroleum and the growing need in reducing the environmental impact of burning fossil fuels have renewed interest in alternative fuels from

renewable sources. Ethanol is one of the most important biofuels that can be implemented alone or in combination with gasoline in the current transport system. Because of this, efforts have increased the development and implementation of technologies for producing ethanol more efficient and economical.

One of the improvements proposed to the alcoholic fermentation is the realization of continuous process with immobilized cells. This implementation offers great advantages such as ease of product separation, reuse of biocatalysts, and high volumetric productivity [1]. The immobilization matrices studied have been hydrophilic polymer gels such as alginate, carrageenan, agarose, etc. In these matrices, the cells are immobilized by entrapment in the gel [2, 3]. However, this immobilization method is impractical on an industrial scale because of the cost of raw materials, the complexity of preparation of biocatalysts, and the short operational lifetime of these in fermentations [4].

In the last decade, raw materials for agro-industrial residues have been evaluated as promising carriers for the immobilization of cells. On these media the cells are immobilized mainly by adsorption, a simple and economical methodology. The cellulosic materials are re-generable, reusable, sterilizable heat, biologically and chemically stable under different fermentation conditions and with sufficient mechanical strength, key features for potential implementation at industrial level [5–8].

The study of the interactions cell-cell has been approached by many researches; nevertheless, these studies have been approximations and simplifications of the phenomenon, due to the fact that it is impossible bear in mind all the factors and forces that are involved in this type of interaction. A complete study should include factors such as the chemical composition and form of the cells, the positive and negative surface loads and his not homogeneous distribution, the permanent changes that happen in the components of the membrane cellular, the ionic permeability of the membrane, the modification of the cellular topology during the process, the active movement or locomotion that can present the cell, the movement random Brownian, the ionic force, the pH, the temperature, brownian, the ionic force, the pH, the temperature, the viscosity, etc. [9].

Physico-chemical treatment of the cellular adhesion problem can provide a thermodynamic description of the adhesion event by considering the cell as a colloidal particle with uniform surface properties, although the cell may modify their geometry and redistribute their membrane components. This approach generates useful information on the earliest stages of the adhesion process; in some cases, it fails to predict actual adhesion. From a physicochemical point of view, microbial adhesion is often seen as interplay of Van der Waals long-range forces, electrostatic forces, and various short-range interactions [10].

This study characterized physico-chemically four lignocellulosic residues—wood shavings, bagasse, corncobs, and corn leaves—which were used as carriers in the immobilization of yeast cells for ethanol production in packed bed bioreactors. The characterization was also performed to yeast cells that remained free and immobilized cells in the four lignocellulosic materials during the basal processes of immobilization. It is hypothesized that the modification of surface properties of yeast is caused by the specific conditions

of process, characteristics that modify the immobilization process on lignocellulosic substrates. To improve the process of cell immobilization, it is essential to understand the type of support-cell interactions that occur during the process, and it is essential to establish the main surface characteristics of both the media and of cells that affect the process of immobilization.

## 2. Methodology

### 2.1. The organism and culture media

In this work, we used the commercial yeast *Saccharomyces cerevisiae* Ethanol Red supplied by Fermentis. The strain was kept lyophilized and stored at 4°C. The medium for inoculum and fermentation is composed of: glucose 100 g/L, peptone 3.6 g/L, $(NH_4)_2SO_4$ 3 g/L, yeast extract 4 g /L, $KH_2PO_4$ 2 g/L, and $MgSO_4.7H_2O$ 1 g/L. The pH was adjusted to 5.0.

### 2.2. Composition material determination

To determine the content of the lignin, cellulose, hemicellulose, ash, and cell wall of lignocellulosic waste, we followed the methodology used by Van Soest [11–15]. This methodology consists primarily in the digestion of the materials in a sequence of detergent solutions, with changes in acidity to achieve solubilization of components due to their resistance or susceptibility to chemical reagents.

### 2.3. Surface area determination

The technique of Brunauer–Emmett–Teller (BET) adsorption isotherms was used for establishing the surface area for each material. It was also performed to determine the type of pore and pore-size distribution, using the methodology of adsorption isotherms barrett-joyner-halenda (BJH) desorption.

### 2.4. Sedimentation test

The technique consists in the measurement of cell sedimentation after contact cell suspension with solution of $CaCl_2$, which promotes the aggregates cells formation. This methodology is used to establish the flocculating ability of cells. And their performance taking into account the work of Domingues et al. [16] and Branyik et al. [17].

### 2.5. Microbial Adhesion To Hydrocarbons test (MATH tests)

The technique consists of determining the ability of cell to adhere to several solvents and establish their electron-donor/electron acceptor or Lewis acid-base properties. This method is based on the comparison between microbial cell affinity to a polar solvent and to a non-polar solvent by simply measuring the fraction of cell removal from the aqueous phase in

the presence of these solvents. To perform the test MATH, we used two pairs of substances, chloroform is an electron acceptor solvent and hexadecane is a nonpolar solvent. We also used ethyl acetate as a solvent strongly electron donor and decane as a nonpolar solvent. Due to surface tension properties of these solvents, the differences between the results obtained with chloroform and hexadecane, and the results obtained with ethyl acetate and decane indicate that cell surface interactions are of type electron donor or electron acceptor and reveal hydrophobic and hydrophilic properties. Strains adhering well to the hydrocarbon are considered to be "hydrophobic" and strains adhering poorly are considered "hydrophilic." The removal is greatest when you have a pH value where the zeta potential of the organisms and/ or hydrocarbons is zero. Under this condition, there is no static repulsion [10]. This methodology was developed taking into account the work of Domingues et al. [16], Branyik et al. [17], and Bellon-Fontaine et al. [18].

### 2.6. Zeta potential measuresment

Zeta potential describes the static electric field strength of the double layer at the boundary between a particle and the fluid in which is immersed. Measurements are performed on equipment Zeta meter Malvern Instruments that employed folded capillary cells (zetasizer nano series Malvern).

#### 2.6.1. For cells

Cells recovered from fermentation are centrifuged and resuspended in solution 10 mM $KNO_3$. They are washed at least twice with this solution. Prepare a cell suspension of concentration 0.85 g/L dry cell weight with solution 10 mM $KNO_3$. Adjust the pH with $HNO_3$. We can prepare three different cells suspension with pH values of 3.0, 4.0, and 5.0. The cell suspension is filled into the electrophoresis cell, and after at least 40 electrophoretic mobility readings, the average zeta potential is calculated.

#### 2.6.2. For lignocellulosic materials

An amount of 0.5 g in dry state was triturated and then suspended in 100 mL of 10 mM $KNO_3$. The suspension of carrier particles was filtered through a polyester mesh (PE 15 mm Seidengazefabrik AG Thal, Switzerland) with mesh openings of 15 × 15 mm and the pH is adjusted with $HNO_3$. The cell suspension is filled into the electrophoresis cell and after at least 40 electrophoretic mobility readings the average zeta potential was calculated.

### 2.7. Contact angles

The technique consist in the measurement of contact angles, formed by sessile drops of three different liquids (two polar and one apolar), to enable the calculation of the surface free energy and the degree of hydrophobicity of cells and lignocellulosic materials. This methodology was developed taking into account the work of Henriques et al. [19] and Branyik et al. [17]. The apparatus used was a model OCA 15 PLUS, DATAPHYSICS.

*2.7.1. Contact angle measurement for cells*

Cells were harvested by centrifugation at 6000 g and 4°C for 10 min and washed with increasing concentrations of ethanol in water (10, 20, and 50% v/v). The resulting pellet was resuspended in 50% (v/v) ethanol. The final concentration was adjusted to 1.2 g/L dry cell weight. An aliquot of 1 mL of cell suspension is spread over the solidified agar layer in order to cover the entire surface. This layer is let to dry; this step is repeated four times. Contact angles are measured by the sessile drop technique at room temperature using water, formamide, and $\alpha$-bromonaphtalene. Each assay is performed in triplicate and at least 20 contact angles, per sample, are measured.

*2.7.2. Contact angle measurement for carriers*

To contact angle measurements, carrier particles are fixed on a microscopic slide by an adhesive tape. Contact angles are measured by the sessile drop technique, it is employed a drop volume of 3 μL and the same procedure for cells determination.

## 2.8. Scanning electron microscope (SEM)

For the realization of the images, we used the scanning electron microscope (FEG/SEM) FEI Nova 200 with EDAX, EDS/EBSD, and STEM. The images were performed on samples of materials before and after basal immobilization.

## 2.9. FT-IR and FT-Raman measurements

Fourier Transform Infrared Perkin Elmer, Spectrum one model, deuterated triglycine sulfate (DTGS) detector was employed to obtain the spectra. The samples were dried at 80°C for 30 min, dispersed in KBr to form a tablet, which is analyzed in the spectrometer. The analysis conditions were temperature 24°C, the number of sweeps of 8 with a resolution of 4 cm$^{-1}$ and a range of wavelengths (ν) 4000–400 cm$^{-1}$.

## 2.10. Basal immobilization process

We used pieces of each material: wood shavings, sugarcane bagasse, corncobs, and corn leaves. A cell suspension prepared in isotonic solution with 15 g/L of biomass was continuously recirculated. The process was realized by a period of 12 hours at 30°C, and we used column bioreactors with a volume of 150 mL.

## 2.11. Dry weight technique modified

Immobilized biomass was determined by the difference in dry weight of support before and after cell removal protocol. The removal was performed with a solution of NaOH 0.1% by mechanical agitation at 150 rpm for 24 hours at room temperature. The drying was performed at 105°C by 12 hours [20].

## 2.12. Free energy determinations

The total surface tension ($\gamma_{tot}$) and its components ($\gamma_{LW}$, $\gamma^+$, $\gamma^-$, $\gamma_{AB}$), the values of the free energy of interaction between cells and water and the components ($\Delta Gcwc_{tot}$) ($\Delta Gcwc_{LW}$, and $\Delta Gcwc_{AB}$) are calculated according to van Oss and co-workers [21].

# 3. Results and discussion

Table 1 shows the results for determining the composition of lignocellulosic waste. The content of lignin, cellulose, hemicellulose, ash, and cell wall presented significant differences for each material. Wood shavings, corn leaves, and bagasse have between 20 and 24% cellulose content more that lignin, however, the corncob has only 8%. The corn leaves in the material had higher content of cell wall and hemicellulose, whose main function is to provide the bond between the cellulose and lignin [22]. Lignin is the substance that gives structural rigidity to the material because it is responsible for holding the fibers harden and polysaccharide [23]. The corn leaf contains a low amount of lignin, a characteristic that may influence the ability of immobilization and the stability of the material in fermentation. We can experimentally verify that this material in the operation presents less stability. It tends to gather and pile up by the top of the bioreactor, resulting in not homogeneous distribution of the bed and its obstruction. The corn leaf was the material that showed less stability during cell removal protocol; it is easily disintegrated when placed in contact with NaOH at high concentration.

Natural materials typically have very low porosities and surfaces, so the amount of nitrogen adsorbed at 77 K was very low, this corresponds to materials with a poorly developed mesoporosity and no micropores. The size distributions of mesopores determined by the BJH method (desorption isotherm) resulted in none of the samples has a significant amount of mesopores than 20 nm; most of them are between 3 and 12 nm. The corn leaves and wood shaving materials presented mesopores between 3.1 and 3.4 nm. The values are summarized in Table 2. There is good agreement between the values of the areas calculated by the BET method and BJH method from the desorption isotherm, with the exception of wood shaving material. It is important that the BJH method counts only the pores with diameters greater than 3.1 and it is possible that this sample has a significant amount (in relative terms) of these pores. The corresponding size distribution suggests this. We can establish a small difference between the

|  | Lignin (%) | Cellulose (%) | Hemicellulose (%) | Ash (%) | Cell wall (%) | Total |
|---|---|---|---|---|---|---|
| Wood shavings | 27.09 | 46.79 | 16.62 | 0.45 | 9.05 | 100 |
| Bagasse | 29.68 | 52.18 | 7.24 | 0.01 | 10.89 | 100 |
| Corncobs | 38.15 | 45.95 | 9.95 | 0.34 | 5.61 | 100 |
| Corn leaves | 5.44 | 29.19 | 24.63 | 1.71 | 39.03 | 100 |

Table 1. Material's composition obtained using the Van Soest Method.

| Materials | $S_{BET}$ (m²/g)[a] | $S_{BJH}$ (m²/g)[b] | $S_{BJH}$ (m²/g)[c] | $V_P$ (cm³/g)[d] | $V_P$ (cm³/g)[b] | $V_P$ (cm³/g)[c] |
|---|---|---|---|---|---|---|
| Wood shavings | 2.6 | 2.5 | 2.1 | 0.005 | 0.005 | 0.005 |
| Corn leaves | 2.8 | 2.2 | 2.0 | 0.004 | 0.004 | 0.004 |
| Bagasse | 5.3 | 3.7 | 3.7 | 0.006 | 0.005 | 0.006 |
| Corncobs | 4.8 | 3.1 | 3.0 | 0.005 | 0.005 | 0.005 |

[a]Calculated by the BET method (adsorption isotherm).
[b]Calculated by the BJH (desorption isotherm).
[c]Calculated by the BJH (adsorption isotherm).
[d]Calculated by the maximum amount adsorbed (P/P0 = 0.99).

**Table 2.** Specific surface areas and volumes for lignocellulosic wastes, determined by the methods of BET and BJH.

samples of bagasse, with the corncob, bagasse can absorb a little more nitrogen than the cob, and this is consistent with the parameters. The bagasse has a higher adsorption at low relative pressure zone. Also size distribution of present mesopores is less than 20 nm, while most are distributed in sizes 3 to 12 nm. The top curves correspond to diameters between about 3.5 and 4.5 nm, values that do not differ significantly from the other samples. We can say with some certainty that all samples have a very low porosity, mainly relating to mesopores with diameters below 12 nm, with specific areas between 1 and 4 surface m²/g and pore specific volume between 0.002 and 0.006 cm³/g. The advantages of cell adhesion to nonporous carriers consist in lower mass transfer limitation of substrates and products due to direct contact between cells and bulk liquid and also in the simplicity of the immobilization. The main risk of this method is the biofilm detachment induced by changes in cell environment [24].

All the spectra show similar absorption features, although the intensities of the absorption bands are different. The assignment of the following absorbance bands is in accordance with the literature [25]. The methylene ($CH_2$) structural unit has symmetric and asymmetric vibrations. The asymmetric C–H stretching vibrations for $CH_2$ involve one C–H bond contracting, while the other bond is lengthening. This vibration is observed at $2926 \pm 10$ cm⁻¹. The symmetric methylene stretch involves both the C–H bonds lengthening or contracting at the same time. This band typically appears at $2855 \pm 10$. These bands are present in both blank cells and immobilized cells. The presence of band at $1375 \pm 10$ cm⁻¹ is a strong indication of the presence of a metyl group in a sample. These bands correspond to the symmetric C–H bending vibrations of the methyl ($CH_3$) group and are observed in the wood shaving sample. The spectral signature bands due to the C=O stretching vibration, which appears as an intense band between 1800 and 1600 cm⁻¹. This vibration is observed in both blank cells and immobilized cells. The overriding spectral feature of a carboxylic acid is the broad, intense O–H stretching band typically found from 3500 to 2500 cm⁻¹, and often centered around 3000 cm⁻¹. This band almost by itself, tells you that sample contains a carboxylic acid. The sharper C–H stretching vibrations are superimposed upon the broad O–H stretching band. However, some times the O–H stretch masks the C–H stretching bands. Note that on the low wavenumber side of the O–H stretching band in the blank and immobilized cells of both samples, between 2500 and 2800 cm⁻¹, there are some broad features of medium intensity. The in-plane O–H bending band is found from 1440 to 1395 cm⁻¹. In the spectrum, this band is found at 1400 cm⁻¹. Carboxylic acids contain a C–O

bond, and the C–O stretch of carboxylic acids appears between 1320 and 1210 cm⁻¹. The C=O stretch of carboxylic acids is in the same regions as the C=O stretch of ketones, aldehydes and esters. The C=O stretching band by itself is not sufficient evidence to identify a carboxylic acid. Other bands, such as the O–H stretch must be found to confirm the assignment.

The $NH_2$ group in a primary amide can bend in addition to stretching. The in-plane bending vibration involves the H–N–H bond angle getting bigger and smaller, like the opening and closing of a pair of scissors. This vibration is called the $NH_2$ scissors mode, and is found from 1650 to 1620 cm⁻¹. Note that the wavenumber range for the C=O stretch and the $NH_2$ scissors overlap. The $NH_2$ part of a primary amide can also bend out of the plane defined by the functional group. This out-of-plane bending band is typically very broad and is found from 750 to 600 cm⁻¹. This band is observed in all spectra for the materials. Primary amides contain one C–N bond, and the stretching of this bond gives rise to a band from 1430 to 1390 cm⁻¹. It is found well-defined in the spectrums of corncobs, corn leaves, and wood shaving, and it is less evident in the bagasse spectrum. The secondary amides are probably the most common and important type of amide. Proteins, nylons, and other polymers contain secondary amide linkages. This band appears between 3370 and 3170 cm⁻¹. In the cells spectrum, this band is overlapped by the O–H stretching band. The carbonyl stretch of secondary amides appears in the same range as other amides, from 1680 to 1630 cm⁻¹. The N–H moiety can bend as well as stretch and, like many other functional groups, there is an in-plane and out-of-plane bending vibration in secondary amides. The in-plane N–H bending vibration usually appears from 1570 to 1515 cm⁻¹. It is found in the spectrum of both cells samples in 1542 cm⁻¹. The primary amine C–N stretching vibration for saturated molecules occurs from 1250 and 1020 cm⁻¹. It is observed at 1088 cm⁻¹ for cells spectrum and 1062 cm⁻¹ for the materials spectrum. In summary, the FT-IR spectra obtained showed the presence of CH, $CH_2$, $CH_3$, NH, $NH_2$, $NH_3$, COOH, and CONH groups on the surface of all samples cells and materials as shown in **Figure 1**).

Images obtained by SEM for the materials before and after basal immobilization are presented in **Figure 2(a)** and **(b)**. In **Figure 2(a)**, we can see the superficial differences between the materials prior to immobilization. The corncob is the material that has a rough, irregular surface, and multiple pores are observed, corresponding to embryo sacs that make up the fruit of the corn. The wood shaving has a surface with two types of arrays, one consisting of a flat, smooth region, equivalent to the wood fibers and is the main constituent. The other region is composed of pores corresponding to the tracheae in the body secondary woody plants. The corn leaf is the material having a smooth and homogeneous surface; there is complete cellular structure and the presence
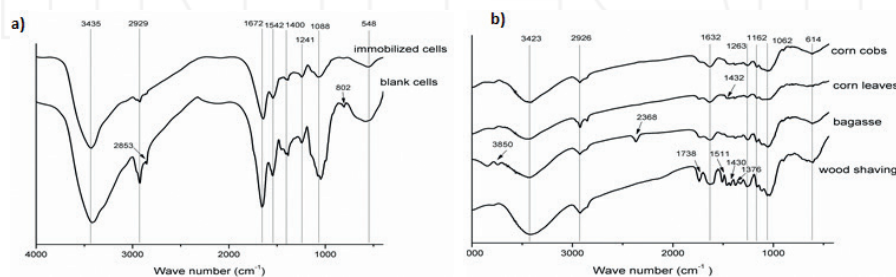


**Figure 1.** FT-IR results for (a) yeast cells and (b) lignocellulosic wastes.
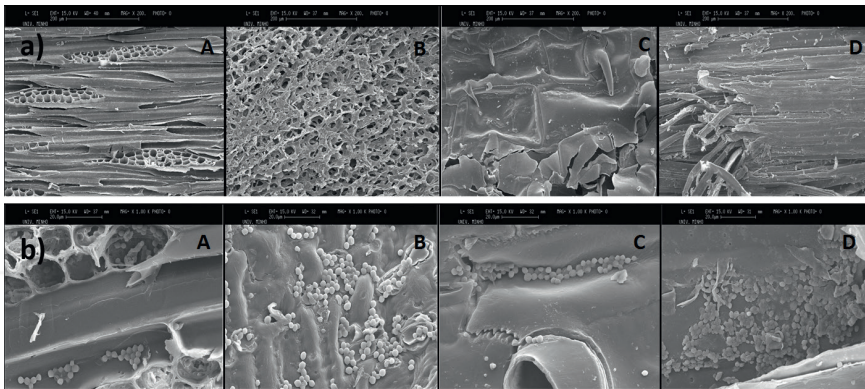
**Figure 2.** (a) Scanning Electronic Micrograph of lignocellulosic wasted, (b) Scanning Electronic Micrograph of lignocellulosic wasted after basal immobilization; (A) wood shavings, (B) corncobs, (C) corn leaves and (D) bagasse.

of trichomes or hairinesses characteristic of this tissue. The bagasse has an ordered structure in the form of overlapping plates; equivalent to longitudinal segment of cane stalk and due to mechanical treatment for the extraction of juice in sugar cane cannot be seen defined cellular structure.

**Figure 2(b)** shows the images of the material after basal immobilization. We can see that the yeast cells adhered to all surfaces of different materials. In the case of the wood shaving, immobilization was performed in both the pore region and the smooth region. In the corncob, the cells display more uniform adhesion across the surface of the material; however, in the corn leaf, the cells were immobilized preferentially in regions where there were creases. The bagasse laminar surface was covered uniformly by the cells. It can be seen that the cells are attached to chains and forming group. The highest number of immobilized cells was bagasse and corncob.

The superficial characteristic of cells before basal immobilization process, as shown in **Figure 3**, indicate an affinity for chloroform greater than hexadecane. The differences in affinity were due to interactions of Lewis acid-base, i.e., interactions donor / acceptor of electrons
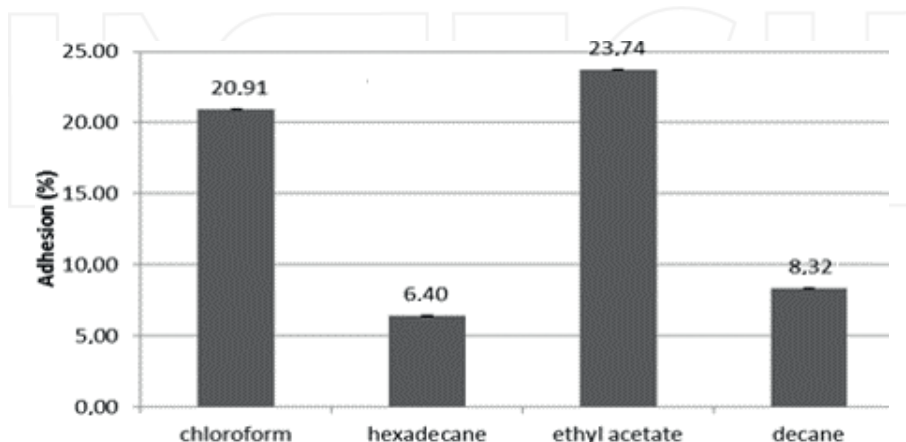


**Figure 3.** Test MATHS for free cells.

resulting from the electron donor nature of the yeast. These results are consistent with those obtained by Mercier-Bonin et al. [26]. From the results obtained with the other pair of solvents, we can establish that there is a higher affinity of the cells by the ethyl acetate solvent than the nonpolar solvent decane. It reveals an electron acceptor nature even slightly higher than its electron donor nature. The low affinity of cells to the nonpolar solvents indicates hydrophilic properties [10, 27]. We can conclude that yeast cells used in this study have an electron acceptor nature and hydrophilic properties.

Adhesion analysis for cellular samples obtained from the basal immobilization process on wood shaving, corncobs, and corn leaves carriers revealed an increase in the adhesion of immobilized cells over no immobilizes cells. Both free and immobilizes cells exhibited an electron donor nature, a result contrary to what was obtained with blank cells (free cells suspended in isotonic solution not subjected to immobilization). However, the results of adhesion to cells in the process with bagasse like carrier showed differences in this trend, i.e., retained their electron acceptor nature. It is important to note that all cell samples retained the hydrophilic character; it was evidenced by the poor adherence to the nonpolar solvents. These results suggest that the immobilization process modifies the surface characteristics of the cell and the immobilization by adhesion is influenced also by the surface characteristics of the carrier used. The results of cell adhesion after the basal immobilization process with each of the carriers are presented in **Figure 4**.

The results obtained for the sedimentation test performed on the cells used as blank and the free cells taken from the basal immobilization process with bagasse are presented in **Figure 5**. As shown no increased capacity flocculating or settling of the cells after being subjected to the immobilization process conducted by 12 hours was observed. It is possible that a change occurs with the time in cell physiology, allowing an increase in the capacity of sedimentation. This behavior has been reported by other researchers [17]; however, the period of time of immobilization process that the cells were subjected in these studies was superior to 100 hours; it was much higher than the evaluated in this study.
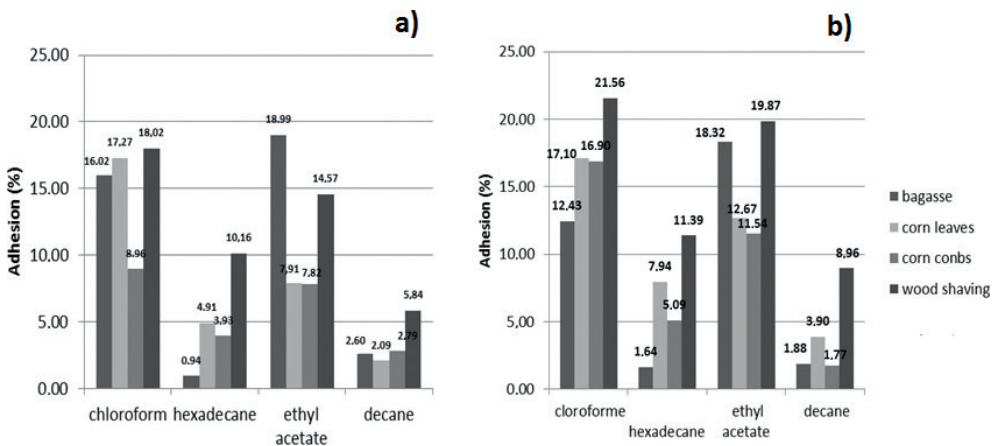


**Figure 4.** Test MATH for cells in basal immobilization process. (a) Free cells and (b) immobilized cells.
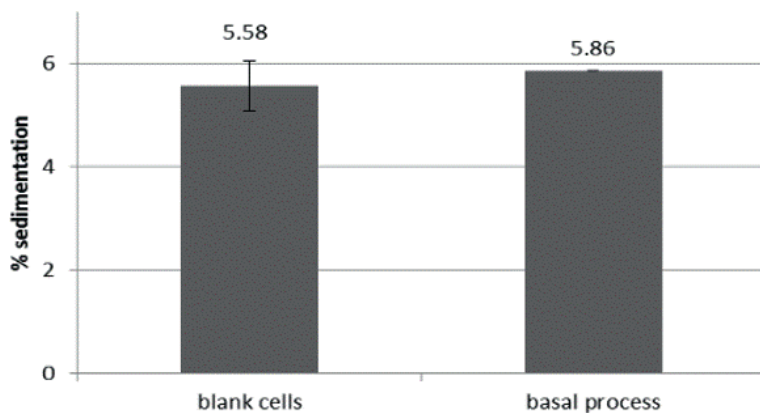
**Figure 5.** Sedimentation test for free cells obtained in different process.

An approach based on a balance of free energy of interaction between cell-liquid, liquid-medium, and medium-cell interface was used to estimate the physicochemical properties of the surface of cells and media, which could lead to adhesion. Electrodynamic forces (Lifshitz-Van der Waals) and hydrophobic forces (acids/Lewis bases) were determined using contact angle measurements and tests of electrophoretic mobility (zeta potential). The contact angle measurements were obtained from the formation of sessile drops of three different liquids; water and formamide as polar liquids and liquid $\alpha$-bromonaphthalene as apolar. The calculation of surface free energy and the degree of hydrophobicity can be defined in terms of the change in free energy of interaction between two particles of the same material immersed in water (w). The free energy comprises a polar component (AB) and an apolar component (LW), $\Delta G_{1w1TOT} = \Delta G_{1w1AB} + \Delta G_{1w1LW}$. When the value of de $\Delta G_{1w1TOT}$ is negative, the interaction energy between molecules is attractive, which means that cells have less affinity for water than for themselves giving them a hydrophobic character. On the other hand, the cells are hydrophilic when this value is positive [27].

The value of contact angle with water can provide preliminary information on the hydrophobicity of the cells. If the value is greater than 50°, the surface is considered hydrophobic; if the value is less than 50°, the surface is hydrophilic [28]. In the results presented in **Table 3**, we can see values of contact angles above 50° for all lignocellulosic waste, indicating their

| | Wood savings | Corncobs | Corn leaves | Bagasse | Free cells |
|---|---|---|---|---|---|
| Water ($\theta_w$ (°)) | 84.50 ± 06.40 | 84.40 ± 06.10 | 115.67 ± 15.28 | 104.20 ± 11.10 | 19.88 ± 1.87 |
| $\alpha$-Bromonaphthalene ($\theta_B$ (°)) | 32.27 ± 07.84 | 33.19 ± 09.63 | 44.63 ± 11.49 | 28.60 ± 10.00 | 76.50 ± 2.67 |
| Formamide ($\theta_F$ (°)) | 74.25 ± 11.53 | 67.57 ± 10.15 | 75.72 ± 12.47 | 75.11 ± 11.37 | 13.80 ± 1.04 |

**Table 3.** Contact angle for lignocellulosic waste materials and yeast cells.

hydrophobic character. In the case of blank cells, the value of contact angle with water is less than 50°, indicating a hydrophilic character. These results are consistent with those previously obtained in the MATH test.

The cell surface is the site of the physicochemical interactions with the support leading to adhesion. Previous studies have suggested the relationship between the ability of adhesion and cell surface hydrophobicity [29]. The hydrophobicity of yeast cells is affected by temperature, nutrition, and growth phase [19]. While the nature of the substrate surface can be considered temporarily unchanging, the nature of the yeast surface is a function of their physiological state. In the **Table 4**, we shows the values of contact angles obtained for cell samples of immobilized process carried out with the four materials. The properties of the cell surface have varied. There is an increase in the value of contact angle with water for all samples of cells that were immobilized; this indicates an increase in cell surface hydrophobicity generated by changing the physiological state of the cell and the process conditions. The only material that did not show this behavior was the corn leaf. In this material, the contact angle value increased for the cells that remained free during the process, however, the immobilized cells showed a similar value to the blank cells.

In **Tables 5** and **6**, the results for surface tension and interaction energies are calculated. The high values obtained for $Ys^-$ are consistent with measurements of zeta potential, and also showed a negative surface charge to the cells (see results in **Figure 6(a)**. The result showed a pH of 3.0–5.0 and an ionic strength of 10 mM; the zeta potential has a range of −13 to 16 mV. These values reveal an electron donor nature of the yeast cell surface. Some authors suggest that it may be due to presence of carboxyl and phosphate groups [17]. **Figure 6(b)** shows that the zeta potential is negative for all lignocellulosic materials and there is a marked increase in negativity with the pH increase. For a pH range of 3.0 –5.0, the zeta potential range is between −7 and −21 mV.

Positive values obtained for $\Delta G_{1w1IF}$ (interaction free energy of cells) are positive, indicating the hydrophilic character of the cells. There were no changes to this feature during the process of cell immobilization, although there were differences between cells that were free and those that were immobilized. Cells that were immobilized had a value less hydrophilic, indicating a variation in the surface properties of yeast cells. For lignocellulosic material was obtained $\Delta G_{2w2IF}$ negative value, indicating its hydrophobicity. Also the high value of $Ys_{LW}$ confirms its apolar character. The interaction energy between the carrier and the cell when immersed in water is presented at the end of the table ($\Delta G_{1w2IF}$ ), this value reveal the possibility of cell immobilization. Positive values of $\Delta G_{1w1AB}$ involve repulsion between the particles or molecules of the material 1 immersed or dissolved in the liquid (w). It is also a major factor in the stability of particles in suspensions.

It is theoretically possible for a given material to only have a value of $\gamma s+$ or presence of only one value for $\gamma s^-$. So your $\gamma s_{AB}$ is zero and its surface tension ($\gamma s$) is equal to total $\gamma s_{LW}$. However, such substances, which are designated as single-pole, can interact strongly with bipolar materials and mono-polar materials of opposite polarity, despite the apparently non-polar nature of its surface tension [27]. This feature shows the lignocellulosic materials.

| | Cells blank | Wood shaving | | Corncobs | | Corn leaf | | Bagasse | |
|---|---|---|---|---|---|---|---|---|---|
| | | Free cells | immobilized cells | Free cells | Inmobilized cells | Free cells | inmobilized cells | Free cells | Inmobilized cells |
| $\theta_w$ (°) | $19.88 \pm 1.9$ | $22.7 \pm 3.4$ | $27.3 \pm 2.7$ | $23.9 \pm 3.7$ | $19.5 \pm 1.5$ | $19.4 \pm 1.7$ | $29.5 \pm 3.0$ | $22.8 \pm 2.0$ | $29.0 \pm 2.6$ |
| $\theta_B$ (°) | $76.50 \pm 2.7$ | $75.6 \pm 5.2$ | $81.0 \pm 2.9$ | $80.1 \pm 1.0$ | $85.4 \pm 1.3$ | $85.6 \pm 2.3$ | $86.8 \pm 2.1$ | $84.5 \pm 2.3$ | $86.5 \pm 3.2$ |
| $\theta_F$ (°) | $13.80 \pm 1.0$ | $27.9 \pm 4.0$ | $29.4 \pm 1.7$ | $24.5 \pm 2.6$ | $22.6 \pm 2.8$ | $25.7 \pm 1.9$ | $27.2 \pm 2.7$ | $25.5 \pm 2.1$ | $27.1 \pm 2.9$ |

**Table 4.** Contact angles obtained for free cells and immobilized cells in basal immobilization process with each carried.

| | Cells blank | Wood shaving | | Corncobs | | Corn leaf | | Bagasse | |
|---|---|---|---|---|---|---|---|---|---|
| | | Free cells | immobilized cells | Free cells | Inmobilized cells | Free cells | Inmobilized cells | Free cells | inmobilized cells |
| $Ys^{LW}$ (mJ/m²) | 16.898 | 17.345 | 14.860 | 15.247 | 12.955 | 12.880 | 12.383 | 13.341 | 12.518 |
| $Ys^{+}$ (mJ/m²) | 11.368 | 7.992 | 9.826 | 10.656 | 12.865 | 12.014 | 13.183 | 11.965 | 13.017 |
| $Ys^{-}$ (mJ/m²) | 46.504 | 51.619 | 47.823 | 47.869 | 50.270 | 52.211 | 43.519 | 49.271 | 44.039 |
| $s^{AB}$ (mJ/m²) | 45.985 | 40.622 | 43.355 | 45.170 | 50.862 | 50.090 | 47.905 | 48.560 | 47.886 |
| $s$ (mJ/m²) | 62.883 | 57.967 | 58.215 | 60.417 | 63.817 | 62.970 | 60.288 | 61.901 | 60.404 |
| $\Delta G_{1w1}^{AB}$ (mJ/m²) | 11.929 | 19.022 | 14.292 | 13.343 | 11.942 | 13.791 | 8.795 | 12.543 | 9.175 |
| $\Delta G_{1w1}^{LW}$ (mJ/m²) | -0.658 | -0.637 | -1.371 | -1.174 | -2.298 | -2.366 | -2.672 | -2.097 | -2.619 |
| $\Delta G_{1w1}^{IF}$ (mJ/m²) | 11.271 | 18.386 | 12.921 | 12.169 | 9.643 | 11.426 | 6.124 | 10.456 | 6.557 |
| $\Delta G_{1w2}^{AB}$ (mJ/m²) | -11.842 | -13.574 | -11.960 | -10.128 | -13.439 | -3.734 | 0.304 | -5.319 | -2.813 |
| $\Delta G_{1w2}^{LW}$ (mJ/m²) | 1.636 | 1.478 | 2.386 | 2.185 | 3.059 | 2.192 | 2.334 | 3.176 | 3.534 |
| $\Delta G_{1w2}^{IF}$ (mJ/m²) | -10.206 | -12.096 | -9.574 | -7.943 | -10.380 | -1.541 | 2.638 | -2.143 | 0.721 |

**Table 5.** Surface tensions and free energies of interaction between cells and water (1w1). Free energy of interaction between cells and substrates in water (1w2).

|  | Wood shaving | Corncobs | Corn leaves | Bagasse |
|---|---|---|---|---|
| $Ys^{LW}$ (mJ/m$^2$) | 37.631 | 37.195 | 32.305 | 38.829 |
| $Ys^+$ (mJ/m$^2$) | 0.000 | 0.000 | 0.267 | 0.000 |
| $Ys^-$ (mJ/m$^2$) | 10.582 | 6.760 | 0.000 | 0.220 |
| $s^{AB}$ (mJ/m$^2$) | 0.000 | 0.000 | 0.000 | 0.000 |
| s (mJ/m$^2$) | 37.631 | 37.195 | 32.305 | 38.829 |
| $\Delta G_{2w2}{}^{AB}$ (mJ/m$^2$) | −36.315 | −49.113 | −92.455 | −95.837 |
| $\Delta G_{2w2}{}^{LW}$ (mJ/m$^2$) | −4.354 | −4.184 | −2.299 | −4.959 |
| $\Delta G_{2w2}{}^{IF}$ (mJ/m$^2$) | −40.666 | −53.297 | −94.754 | −100.796 |

**Table 6.** Superficial tensions and free energy of interaction between the carriers and water (2w2).
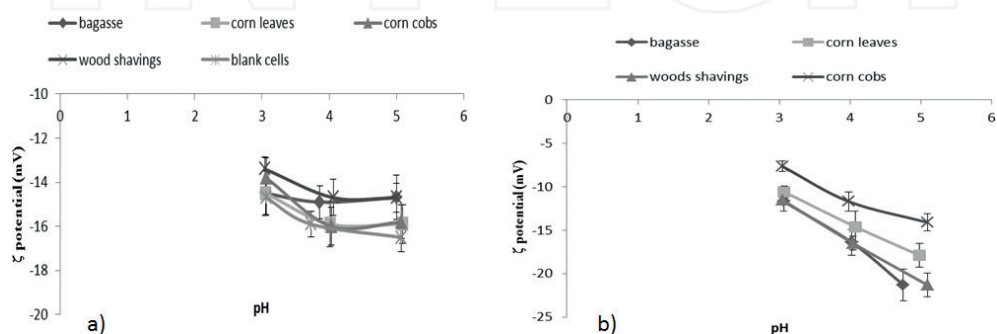


**Figure 6.** Zeta pontential (a) immobilized cells on basal process and (b) lignocellulosic wasted.

The implications when the interfacial free energy of interaction $\Delta G_{1w2}$ is greater than zero, a net repulsion must occur between particles or molecules of materials 1 and 2 immersed or dissolved in the liquid w (always that there is not a cancellation of the electrostatic attraction on materials 1 and 2), otherwise, i.e., $\Delta G_{1w2}$ less than zero, if there is an attraction. The energy of interaction between the carriers and cells is attractive for both wood shaving and corncobs free cells as the immobilized. Bagasse and the corn leaves have negative interaction energy (attraction) between the free cells and carriers, however, positive values are obtained for the energy of interaction with immobilized cells. Despite these results, the cell immobilization occurs on the supports, as shown in the images achieved in the SEM.

## 4. Conclusions

The characterization performed both lignocellulosic materials used as carriers, and yeast cells, gives relevant information about the nature of the surfaces. There are differences in the

surfaces of four lignocellulosic wastes, as well as differences in their compositions. These variations can significantly influence cell adhesion and stability of biocatalysts in fermentation conditions used in ethanol production. It highlights the fact that the cells was immobilized on each of the four carrier evaluated. Yeast cells suspended in isotonic media was adhered to support, when they are placed into contact during 12 hours, corresponding to basal immobilization process. It was established that the yeast cells used in this study initially presented a hydrophilic surface and have electron acceptor nature.

After the basal immobilization process, the cell surface properties are modified. The cells have a less hydrophilic character and change their electron acceptor to electron donor character. Also, there are differences between free cells and the cells that adhere to the surface of the supports. These results confirm the variation of surface properties of cells, depending on their physiological state and the conditions under which the immobilization process was performed. To establish the potential presented by these materials in the development of functional biocatalysts for ethanol production in continuous packed-bed reactors, it is necessary to evaluate its performance under fermentative conditions, and it is necessary to establish that the yeast cells remain attached to the carriers during the process. It is necessary to establish the variations that exist in their surface properties. Nowadays, these studies are being developed in the group.

## Acknowledgements

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