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Spécialité : Génie des Procédés

Présentée par Santi CHUETOR

Couplage de procédés de prétraitements chimio-mécaniques de la paille de riz en voie semi-humide : effets sur les propriétés physicochimiques, rhéologiques et réactivité

Soutenue le 20 Octobre 2015 devant le jury composé de

M. Henri Berthiaux, Professeur, Centre RAPSODEE Ecole des mines d'Albi	Rapporteur
Mme. Fabienne Guillon, Directrice de recherches, BIA INRA Nantes	Rapporteur
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ผมเอา ดร.ไปฝากครอบครัวเชื้อเต๊อะหละครับ

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Keywords:

Lignocellulose biorefinery

Lignocellulose (LC)

Lignocellulose biomass

Rice straw (RS)

Pretreatment

Combined pretreatment

Semi-humid media

Chemo-mechanical pretreatments

Grinding

Hydro-textural approach

Physicochemical properties

Mechanical properties

Rheological properties

Reactivity

Powder

Granular matter

Energy consumption

Bioconversion

List of abbreviations

LC: Lignocellulose

RS: Rice straw

EF-T: Electrostatic fractionation

TF-T: Turbo-separation technology

KM_6: RS cut with sieve 6mm by cutting mill

KM_2: RS cut with sieve 2mm by cutting mill

Remerciements

$IM_{0.5}$: Impact grinding with 0.5mm

$IM_{0.1}$: Impact grinding with 0.1mm

VBM_{20}: Vibro-ball milling 20min

VBM_{40}: Vibro-ball milling 40min

$CF_{0.25}$: Centrifugal milling 0.25mm

d_{50}: Median particle size

ϕ: Compactness

ρ: Density

S: Degree of saturation

W: Moisture content based dry basic

d_s^*: Real solid density

RS_0: RS non-treated

RS_{sh}: RS pretreated in semi-humid

RS_h: RS pretreated totally in liquid medium

E_M: Energy Consumption

Df: fractal dimension

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Chapter I:

General Introduction

Dans ce chapitre, nous avons présenté l'introduction générale du travail de thèse, les problématiques ainsi que les objectifs du travail.

I. Introduction générale

La Lignocellulose (LC) est une matière organique naturelle qui représente depuis fort longtemps une source alternative de carbone renouvelable qui peut être converties en bioénergie, biomolécules ainsi qu'en biomatériaux suivant un schéma de bioraffinerie (Taherzaeh, J. et al. 2004; Taherzadeh and Karimi 2008; Dwivedi, Alavalapati et al. 2009). La bioraffinerie de la matière LC constitue une filière technologique moderne qui permet de valoriser cette matière en passant plusieurs étapes dont des opérations de déconstruction.

Parmi les ressources agricoles, la paille de riz est considérée comme une source abondante de biomasse LC au niveau mondial, et présente une concentration importante en biomolécules d'intérêt (Binod, Sindhu et al. 2010). La bioraffinerie de paille de riz pour des applications énergies, biomolécules et biomatériaux jouera un rôle très important pour le développement socio-économique et environnemental des pays émergents ou en cours de développement.

L'établissement de filières durables se fonde en partie sur l'éco-efficience des procédés de traitements dont ceux dédiés à la déconstruction et que l'on compte chronologiquement parmi les premières opérations unitaires des filières de bioraffinage. En effet, afin de valoriser la matière LC, il est indispensable de recourir à un prétraitement qui permet de rendre la matière d'intérêt plus accessible ainsi que d'en augmenter la réactivité vis-à-vis de la déconstruction et des bioconversions, en assurant un gain global d'efficience. Le prétraitement est donc une étape incontournable destinée à fractionner autant que dissocier la LC.

La littérature relative aux prétraitements applicables à la LC, rapporte l'existence de nombreux procédés qui peuvent être divisés en quatre grandes catégories selon la nature des processus qu'ils mettent en œuvre : physique, chimique, physicochimique et biologique. Bien qu'efficaces à divers degrés, les prétraitements possèdent néanmoins des inconvénients que la recherche actuelle tente de surmonter. Les inconvénients majeurs reposent sur (i) une grande consommation énergétique, (ii) un coût de produits chimiques élevé (acides/bases, achat, recyclage, corrosion) ainsi que (iii) de forts impacts environnementaux.

Actuellement, le coût du prétraitement est en moyenne environ 40% du coût total de la bioraffinerie de la LC. Aussi, les recherches actuelles visent à trouver un point de fonctionnement optimal entre ces deux grands axes que sont consommation énergétique d'une part et impact environnemental d'autre part, tout en permettant d'améliorer significativement la réactivité de la LC. Notons en particulier les récents travaux de Barakat et al. (2013), qui ont montré qu'un procédé de prétraitement combiné chimio-mécanique a pu répondre en partie à ces exigences c'est-à-dire,

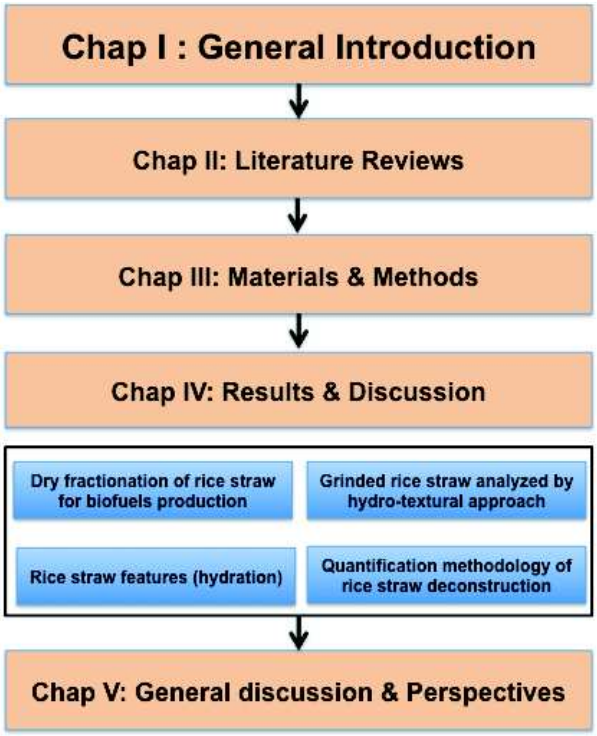
une minimisation de la consommation d'énergie et des quantités d'intrants chimiques conduisant à une réduction des impacts environnementaux néfastes.

La maîtrise des prétraitements applicables à la matière LC soulève de nombreuses questions scientifiques dont certaines touchent à des fronts de science relatifs à la physique de milieux granulaires partiellement saturé soumis à des sollicitations thermo-hydro-chimio-mécaniques, ou encore, la compréhension des processus de fragmentation. Ce travail de recherche s'intègre dans la thématique générale de la compréhension et de l'aide au développement des techniques modernes de bioraffinerie des agro-ressources, plus spécifiquement de la matière LC, pour des applications « bioénergies, biomolécules et biomatériaux ». Ce sont plus spécifiquement les prétraitements de broyage, fragmentation, dissolution partielle,…, qui permettent de faciliter la déstructuration de la matière de l'échelle de la paille jusqu'à quasiment l'échelle colloïdale, qui sont considérés.

Les travaux présentés dans ce manuscrit s'attachent plus spécifiquement à apporter une contribution à l'analyse des procédés combinés chimio-mécaniques pour le fractionnement en voie humide et semi-humide de la paille de riz. Le fractionnement en voie semi-humide constitue une originalité développée au sein de l'UMR IATE, qui consiste à fragmenter la paille de riz par un procédé de broyage assisté par des effecteurs chimiques liquides, simultanément pulvérisés dans le réacteur. Ces prétraitements mettent en jeu des processus multi-échelles qui couplent des transferts de masse, d'énergie et de quantité de mouvement sous des contraintes thermo-hydro-(bio)chimico-mécaniques imposées à l'échelle des réacteurs. L'étude de ces phénomènes procède de la mise en œuvre d'une pluridisciplinarité qui peut être appréhendée dans le cadre classique du Génie des Procédés tout en nécessitant cependant de prendre en compte la particularité discrète de la matière. En effet, parmi les différentes transformations qui lui sont appliquées, la matière LC occupe préférentiellement un état de poudre. Ces matières intermédiaires, sont des milieux granulaires difficilement assimilables à des fluides, même complexes, et il est nécessaire de considérer le comportement particulaire « du grain au lit » dans l'approche procédé.

Les objectifs majeurs de ce travail de thèse ont consisté à (i) à développer des méthodes originales de caractérisation de la paille de riz broyée en couplant les approches hydro-texturales et rhéologiques des milieux granulaires et (ii) mettre en place une méthodologie qui permette d'analyser les mécanismes de déconstruction de la paille de riz par un prétraitement semi-humide afin de comprendre et maîtriser le fractionnement. Le manuscrit est rédigé essentiellement en anglais. Cependant, quelques chapitres ont été directement écrits en français et ils feront l'objet d'une traduction ultérieure en vue de leur valorisation sous le format de publications. Le manuscrit est structuré en quatre chapitres et conclu par une discussion générale.

Ce manuscrit se divise en cinq chapitres :



Schématisation de la structure du manuscrit de thèse

Le **chapitre II** constitue une synthèse bibliographique où est notamment décrit le contexte général de la bioraffinerie de la lignocellulose. Les matériels et méthodes employés et développés dans ce travail expérimental sont présentés dans le **chapitre III**.

Le **chapitre IV** rapporte les résultats et discussions, nous divisons cette partie en quatre sous chapitres

IV-1 Le fractionnement par voie sèche de la paille de riz : combinaison du broyage ultrafin et de la séparation qui est une technique innovante afin d'obtenir des fractions d'intérêt avec différentes propriétés, pour la production biocarburant par exemple.

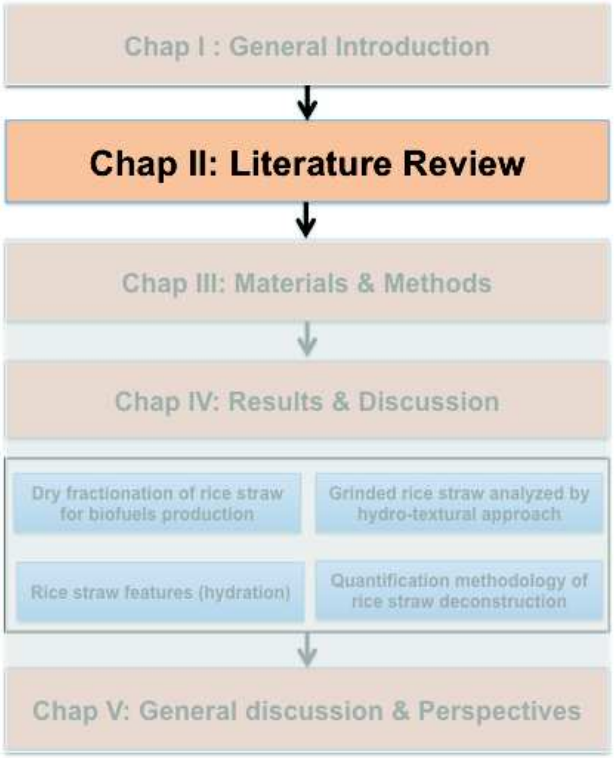
IV-2 Le développement d'une méthode de caractérisation de la paille de riz broyée afin de comprendre sa déstructuration par une approche hydro-texturale. Le suivi de l'évolution des paramètres hydro-texturaux permet de comprendre les mécanismes de déconstructions de la paille de riz lors du fractionnement.

IV-3 L'étude du comportement des fractions de la paille de riz vis-à-vis des transferts d'eau. Cette partie consiste à étudier le transfert du liquide dans la paille de riz afin de comprendre les cinétiques d'imbibition.

IV-4 La méthodologie de quantification des processus de déconstruction des pailles de riz

Chapter II:

Literature review



Ce chapitre est divisé en trois sous-chapitres rédigés en anglais ;

- LC biomass composition and characterization
- LC biorefinery and pretreatments of LC biomass
- Physical and mechanical properties of LC powder

Dans un premier temps, nous présentons le contexte général de la valorisation de la biomasse LC et spécifiquement la paille de riz avec une partie détaillée sur la micro- et macrostructure de la matière LC. La bioraffinerie a été décrite afin de comprendre les démarches de valorisation de cette matière. Le prétraitement étant une étape indispensable dans la bioraffinerie, dans ce sous-chapitre II, nous détaillons tous les procédés de prétraitement actuels qui permettent de déstructurer la matière LC. Dans lee dernier sous-chapitre, nous présentons toutes les propriétés physiques, mécaniques de la poudre de la matière LC ainsi que les méthodes de caractérisation qui permettent de bien comprendre la déconstruction de cette matière.

II. Literature review

II.1 Biochemical composition and macro- and microstructure of LC biomass

II.1.1 Structure and organization of LC matrix

Lignocellulosic (LC) biomasses are natural organic substances that include agricultural by-products, agricultural wastes as well as forestry residues (Schell and Harwood 1994; Balat, Balat et al. 2008; Dwivedi, Alavalapati et al. 2009). LC material is composed of three principle constituents: cellulose, hemicellulose and lignin, which are interlinked in complex matrix (Lee 1997; McMillan 1997; Limayem and Ricke 2012). These three major constituents are located in a plant cell wall. In this study, rice straw (RS) was studied as LC resource model. RS is a by-product of rice; it is left over during rice production.

In 2014, rice production was annual yield about 744.4 million tones (FA0 2014) which is equivalent to 1116.6 million tons of rice straw. Rice straw is one of the largest by-products of cereal crop in the world after maize and wheat.

Rice is a monocotyledon (monocots) in the Poaceae family (Oryzoideae). This species is one of the most important food crops that humans utilize, providing the primary source of starch for a large segment of the world's population.

Its anatomical structure contains broadly four major organs: leaf, spikelet, stem and root (Figure II-1). The leaf performs photosynthesis, the spikelets are the rice seeds, the stem provides physical support along with transport of water and nutrients, and the root anchors the plant while providing water and mineral adsorption. The stem structure has several vascular bundles scattered in a basic tissue of parenchyma storing cells, which is surrounded by a strong and dense epidermis (Figure II-1B).

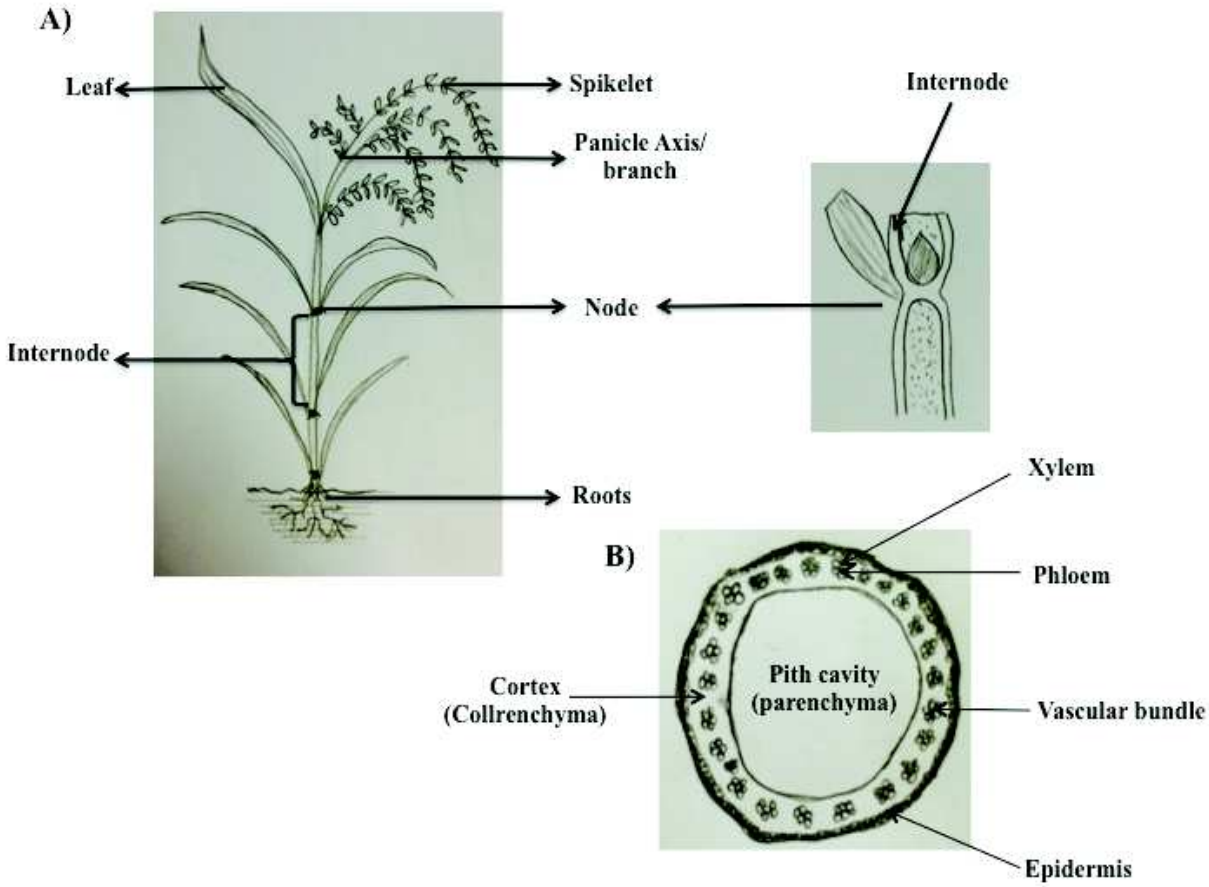


Figure II- 1 *Anatomical structure of rice A) Macroscopic structure of rice B) Stem of rice at maturity growth*

Histological of plant: Tissues

The plant cells include the elements belonging to various botanical elements, organs, tissues or cells. It contributes an essential function of plant life, maintain, and conduction. The plant cell wall is complex matrix of polymers that surrounds every plant cell. The walls provide the physical support required for plants to grow upright in terrestrial habits and serve many other important functions, such as being a barrier to attack by pathogens and insects. While all cell walls share some basic chemical characteristics, striking differences exist among plant tissues in cell wall concentration, composition, and structural organization. Cell walls of many tissues undergo dramatic shifts in chemical characteristics during maturation. Leaves and stems differ in the types and proportions of tissues they contain, and grasses and legumes differ in cell wall composition and tissue profiles.

At the microscopic level especially of plant each organ is generally made from several tissues such as dermal, ground and vascular tissues. In particular, the histology of rice straw contains normally an intact epidermis, a parenchyma, a sclerenchyma and vascular bundles (Figure II- 2).

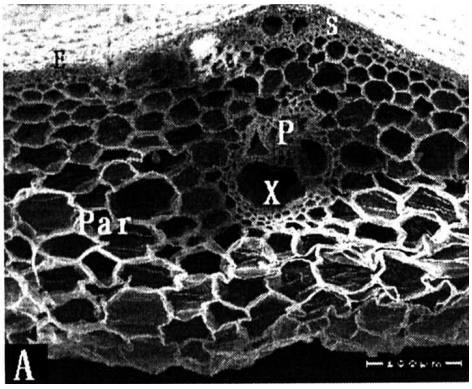


Figure II- 2 *Histology of rice straw A, Epidermis (E), Sclenchyma (S), Phloem (P), Xylem (X) and Parenchyma (Par)(Liu, Liu et al. 2005)*

The *dermal tissues* are the outer most part of monocotyledonous stem. These tissues are typically the epidermis, which formed as a thin but very dense layer made up by various thick-walled cells in a remarkably uniformed pattern.

There is a film of free cutin, the cuticle covers the outer wall of the epidermis cells. Wax is deposited in the cutinized layers, and makes them impermeable to water. The wax and cuticle of epidermis prevent bacterial access to the interior of plant and stem bacterial access to the interior of leaves and stems (Wilson and Mertens 1995; Chesson and Forsberg 1997). The dense layers of epidermal cells give additional mechanical strength to the stem and inhibit evaporation. The specialized cells, so called stomata, and make the gas penetration possible. The difference between the grasses and general plant is that grass epidermis is lignified whereas plant epidermis is non-lignified.

The *ground tissues* synthesize the organic compounds and support the plants by storing the produced products. The ground tissue in particular a grass stem is composed of different types of parenchyma cells. Primarily they store solutes and foodstuffs. Typically they have thin primary walls, but in some cases, e.g. storage parenchyma, the walls may become thicker due to deposition of carbohydrates, they may even become lignified. These tissues are comprised of parenchyma, collenchyma and sclerenchyma cells.

The **parenchyma** (Figure II- 3A) cells are normally the most abundant in the ground tissues and are cube shaped or elongate cells with a thick cell wall. The stem parenchyma are cubical and ~100 mm on side, with the cell wall thickness about ~1 μm (Wilson and Mertens 1995). The photosynthesis usually occurs in parenchyma cells and they stock all nutrients, which have been photosynthesized. These tissues are the most highly lignified.

The **collenchyma** (Figure II- 3B) cells are rectangular with unevenly thickened walls. The primary walls of the cells are composed of cellulose and pectin compounds and capable of stretching in

young organs. It functions as a supporting tissue and at maturity it becomes lignified and gives additional rigidity to the stem.

The **sclerenchyma cells** (Figure II- 3C) are the conductive and supporting tissues. According to the botanical need in each type of the grass plants, additional extra vascular strands of fibers are developed. In some plants the extra vascular fibers are in close connection with the original bundle. In other cases isolated strands of fibers can occur. Normally the sclerenchyma cells in the bundle itself have a small diameter and a thick fiber wall, whereas the extra vascular fibers often have a much larger diameter and a very variable wall thickness. The sclerenchyma wall are cylindrical in shape with dimension of ~7 *1000μm with the wall thickness about ~2-5μm (Wilson and Mertens 1995).

In rice straw these fibers occur as coarse bast fibers just inside the epidermal layer.

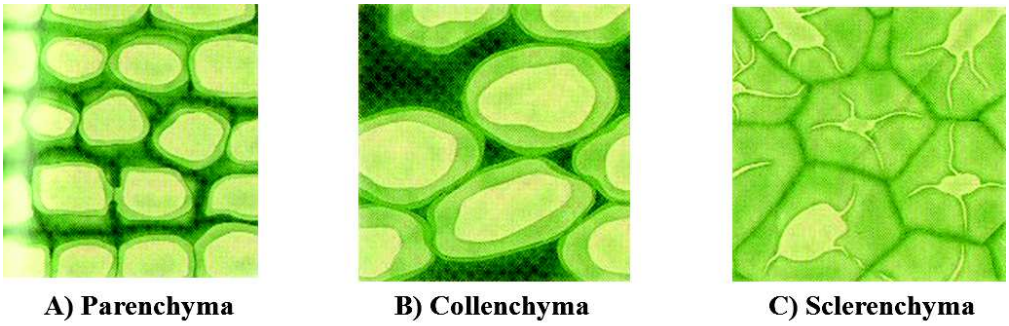


Figure II- 3 *Microscopic levels of plant tissues A) Parenchyma, B) Collenchyma and C) Sclerenchyma*

The *vascular tissues* are the conducting system in a plant stem, which are broadly specialized cells involved in the transportation of water and minerals. This are composed two major tissues: xylem and phloem. In the grass, these vascular bundles form parallel veins. In grass stem the vascular bundles are distributed throughout the stem cross-section, but the vascular bundles are more numerous and smaller in size in the exterior portion of the stem referred to as rind.

The **xylem** forms a continuous system that transports water and dissolved mineral nutrients throughout the plant from the roots. The xylem constitutes the lignified elements. The xylem tracheary elements consist of cells known as tracheid and vessel members. The tracheid consists of a single elongated cell with pointed ends and a secondary, cellulosic wall thickened with lignin (a chemical binding substance) containing numerous pits but having no perforations in the primary cell wall. The vessel consists of a vertical series of vessel members that vary from elongated to squat, drum-shaped cells the walls of which are secondarily thickened with rings, spirals, or networks of cellulose, that later become lignified.

The **phloem** transports sugars, proteins and minerals around the plant. Unlike the xylem where flow is only one way, from the roots up, phloem can move sugars both up and down the stem. Phloem is composed of various specialized cells called sieve tubes, companion cells, phloem fibers and phloem parenchyma cells.

Table II- 1: *Different tissues and their functions*

Tissues system	Functions	Behaviors
Dermal tissues • Epidermis	The single exterior layer that protects the stems, leaves, flowers, and roots. The outside surface of the epidermis tissue is usually covered with a waxy substance called cutin, which reduces water loss	Wax, and silica layers Hydrophobic structure
Ground tissues • Parenchyma • Collenchyma • Sclerenchyma	**Parenchyma:** Storage nutrients and photosynthesis occurred **Collenchyma:** Collenchyma provides structural support, particularly in growing shoots and leaves **Sclerenchyma:** Sclerenchyma tissue is a supporting tissue	Parenchyma: cell wall impregnated by lignin Collenchyma: fibrils cellulosic non lignified Sclerenchyma: cell wall impregnated by lignin
Vascular tissues • Xylem • Phloem	Xylem: transport of water and nutrients from the roots Phloem: transport of products elaborated from	Xylem: lignified cell walls Phloem: cellulosic walls

photosynthesis

The Table II- 1 shown each tissue has different functions and different behaviors. Due to these different behaviors it makes the plant structure be more complex.

Plant cell walls: Ultrastructure of plant

The cell wall of a plant cell is generally made up of middle lamella, primary and secondary cell walls respectively with different distribution according to the plant origin (Figure II- 4).

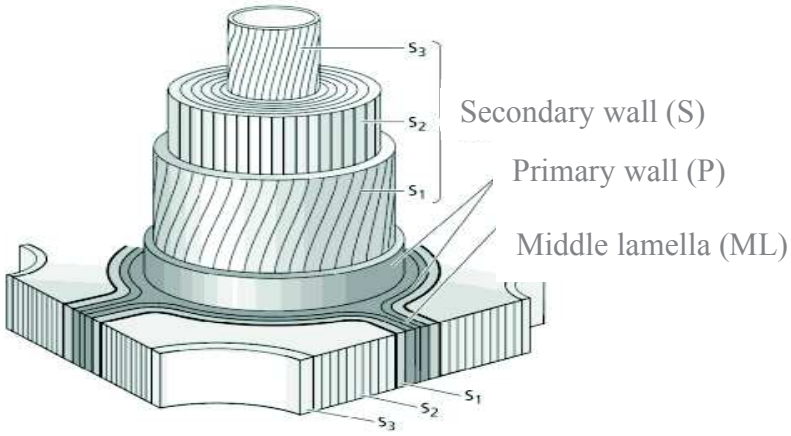


Figure II- 4 *Ultrastructure of plant cell wall*

Of the total cell wall in plant, the secondary wall is clearly the major contributor to volume and mass; it is about 82% of total wall volume.

Regarding the ultrastructure of the rice straw, the focus is on the plant cell level which made up of three layers, namely the middle lamella (ML), the primary wall (P) and the secondary wall (S)(Xu, Zhong et al. 2006).

- **Middle lamella (ML)** is an adjoining pectic primitive membrane between two cells (units), which surround the primary and then the secondary wall. It is drilled at the level of the punctuations by the plasmodesmes, which allows the intercellular exchanges. In case of rice straw, this layer contains high lignin content than the primary and the secondary walls.
- **Primary wall (P)** is situated between the small strip and the secondary wall. It composes predominantly high cellulose and it has a relatively random cellulose microfibril orientation and contains hemicellulose, pectin and protein, all completely embedded in lignin.
- **Secondary wall (S)** is located between the cytoplasmic membrane and the primary wall. It has the same biochemical composition as the primary wall but with a different distribution. It is made by laying down successive layers of cellulose microfibrils and lignins. In this

layer, there is less hydrated network, less matrix substance and more cellulose content. In rice straw cell, the secondary wall is lignified as the primary wall and the middle lamella. The secondary wall is broadly sub-divided into three layers from the outside of the cell wall to the lumen. These are known as: out layer (S1), middle layer (S2) and inner layer (S3).

Within plant cell walls, there are the microfibrils that are cross-linked and stabilized by shorter molecules that make the cellulose microfibrils into a network. The microfibrils vary in different cell wall layers. In the primary wall (P), the microfibrils are oriented randomly (longitudinal and transversal). The cellulose microfibrils are more numerous in the secondary wall (S) and are organized and laid generally parallel to one another within these layers. Moreover, due to the organized and parallel arrangement of cellulose microfibrils, it is a highly crystalline region of cellulose (Xu 2010). In fact, the variation of microfibrils within LC material depends on the species, morphological regions and environmental condition.

The matrix components also have an important role in forming the cell wall. They consist of lignin, hemicellulose and others polymers, which are surrounded by the cellulose microfibrils. The linkages of this structure are based on natural liaisons (covalent, ionic, hydrogen etc.). In addition to cross-linking individual microfibrils, the hemicellulose in the secondary cell walls is made of covalent associations. This is especially true in cereal straw (rice and wheat straw). This association is made by phenolic acids, which make the covalent linkages in cell wall. Furthermore, there are ferulic and p-coumaric acids that also make the link with arabinoxylan and xylan-lignin (Sun, Xu et al. 2005; Kristensen, Börjesson et al. 2007).

A good understanding of the cell wall architecture of LC biomass is important for both research and for the development of effective pretreatment and biorefinery technologies. The understanding of LC supramolecular organization is very important and necessary in order to deconstruct more efficacy this complex and heterogeneous structure, to for example fermentable sugars and into biofuels and other bio-products.

II.1.2 Biochemical composition of LC biomass

It is noticeable to utilize rice straw were used in the entire form rather than in as separate components. Rice straw composes mainly of three groups of organic compounds cellulose, hemicelluloses and lignin

Cellulose

Cellulose is the most abundant polymer available in the world and it constitutes 30-60% of dry matter (Sun and Tomkinson 2000). The cellulose content is normally higher in the secondary walls

than the primary walls and the middle lamella. The cellulose is normally a linear homopolymer composed of D-glucopyronose units linked by β→ 1, 4 glycosidic bonds (Figure II- 5)(Lee 1997; Himmel, Ding et al. 2007; Limayem and Ricke 2012). Glucose is a monomer of cellulose, which is linked together to form cellulose. The cellulose is normally organized from a crystalline structural form due to the multiple hydroxyl groups (-OH) (Alvira, Tomas-Pejo et al. 2010; Xu 2010). These groups form many intra- and intermolecular hydrogen bonds; the chains pack regularly in places to form hard stable crystalline regions. Furthermore, the cellulose has also high degree of polymerization compared to others a major constituent in plant cell wall and it is organized in parallel, which forms microfibrils. Through the cellulose hydrolysis, glucose is a potential sugar, which could be converted into biofuels via fermentation process. Due to its crystalline structure, cellulose hydrolysis becomes difficult. Moreover, X-ray analysis has been used to determinate the crystallography of cellulose by using X-Ray diffraction. The crystallography of cellulose could be normally divided into five stages; cellulose I, II, III, IV and X. Native cellulose corresponds to the cellulose I form. In order to change the cellulose form for example from cellulose I to cellulose II, the chemical hydrolysis in alkaline medium would be a favorable process (Isogai, Usuda et al. 1989).

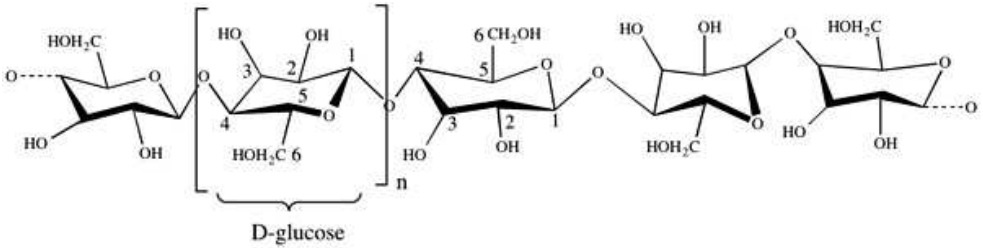


Figure II- 5 *Cellulose structure (β-1,4-glucosidic bonds)*

Hemicelluloses

Hemicelluloses are considered as second plant cell wall polysaccharides consisting of 20-33% of dry matter of biomass (Sun and Tomkinson 2000). They form hydrogen bonds with cellulose and covalent bonds (mainly alpha-benzyl ether linkages) with lignin and ester linkages with acetyl units and hydroxycinnamic acids. The hemicelluloses are heterogeneous polymers of pentoses (xylose, arabinose), hexoses (mannose, galactose) and sugar acids (Saha 2003; Ji, Huang et al. 2012). Their general formulate are $(C_5H_8O_4)_n$ and $(C_6H_{10}O_5)_n$. Besides, the hemicelluloses consist of various different sugars units that are arranged in different substituents, in cereal straw the xylose is a major constituent, then arabinose, glucose, galactose, mannose, glururonic acid respectively. The distribution of each component depends on the original plant. For example, rice straw has xylose as principle sugar. Typically, the backbone of hemicelluloses is (1->4)-linked B-D-xylopyranosyl units and similar backbone for cereal straw from Gramineae family (Limayem and Ricke 2012).

Xylan is the main polysaccharide made from xylose monomer and it is characterized by b-> 1-4 linkages between xylose monomers (Xu 2010). It normally contains 20-30% of biomass. In Poaceae family as rice straw, there is a substitution of arabinoses in C2 and C3 (arabinoxylanes) that have a role to make a linkage with lignin by ester and ether bridges (Kristensen, Börjesson et al. 2007). Unlike cellulose, the hemicelluloses are not chemically homogeneous which are not unique molecules differing only in degree of polymerization and crystallinity. The degree of polymerization of hemicelluloses is less than in the cellulose and almost hemicelluloses structure are amorphous which could be easier hydrolyzed to monomer sugars than cellulose at low temperature. The hemicelluloses are generally found in the primary walls and the middle lamella. Through efficient enzymes technology, the fermentable sugars of hemicellulose especially pentose (xylose, arabinose etc.) could also be converted into biofuels *via* fermentation process.

Lignin

Lignin is the third constituent that is present in plant cell wall especially in the primary walls and the middle lamella, which composes mostly 8-18% of dry matter in biomass. The lignin is a family of branched non-carbohydrate polymers and a main component of straw (Lee 1997; Sun and Tomkinson 2000). Lignin is a polymer composed of phenylpropanoid monolignol units (Lapierre, 1993). The lignin is apathetic three-dimensional phenolic polymers resulting from the radical copolymerization of three phenylpropanoid monomers: coumaryl alcohol, coniferyl and sinapyl (Taherzadeh and Karimi 2008; Limayem and Ricke 2012). Each monomer corresponds to lignin structural; coumaryl alcohol (hydroxyphenyl unit H), coniferyl (guaiacyl unit G) and sinapyl (syringyl unit S) (Figure II- 6). The concentration is distributed according to the original plant. In cereal straw case, the lignin has been justified as GSH-lignin, which is known to be different from softwood (G) or hardwood (GH). Furthermore, hydroxycinnamic acids found in cereal cell wall, mainly coumaric and ferulic acid have been investigated as cross-links between lignin and carbohydrates (Fidalgo, Terron et al. 1993; Boerjan, Ralph et al. 2003; Xu 2010). The lignin is generally distributed with hemicelluloses in the spaces of intercellulose microfibrils. The lignin has a role to giving mechanical resistance to plants, also allowing them to resist the biological attacks, in particular by making barrier for enzymes cellulolytic, chemical or physical by its antioxidant and hydrophobic properties. Through its rigid potential properties, the lignin is a major component to be considered as using in the bio-based product (biomaterial).

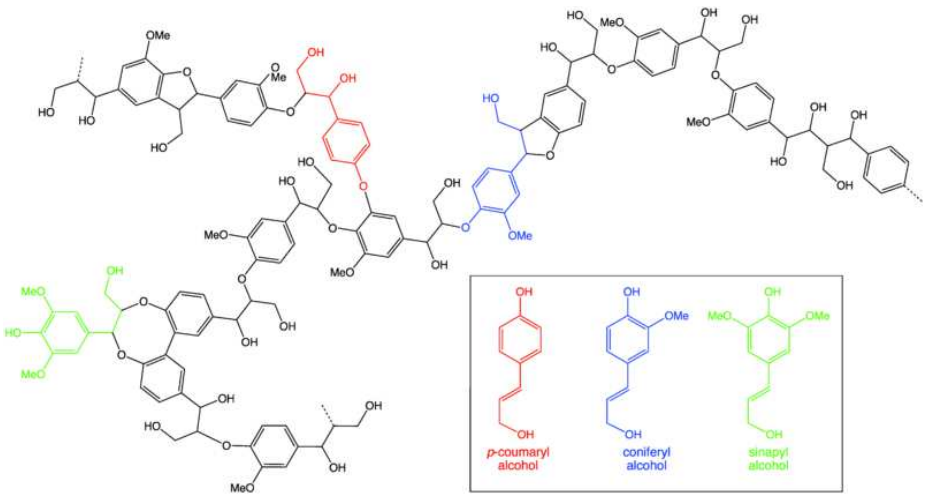
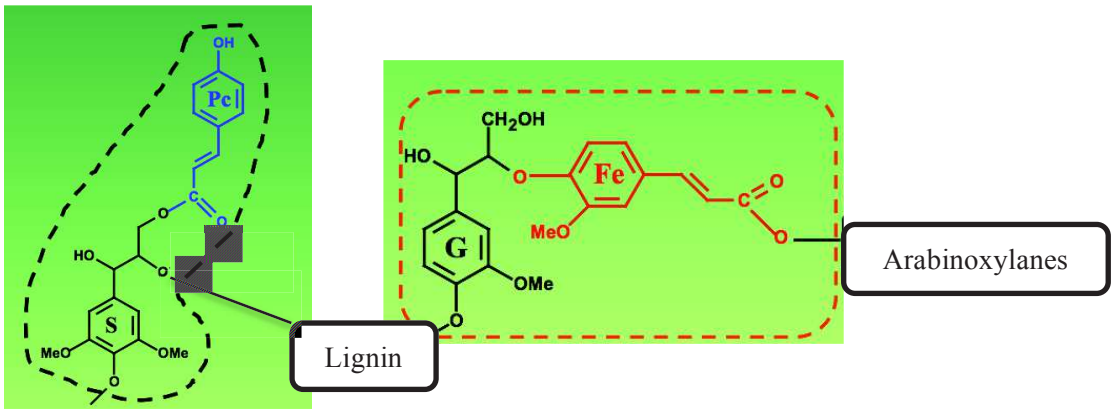


Figure II- 6 *Lignin structure (Smokefoot)*

Lignocellulosic networks

The microfibrils of cellulose are covered with a non-cellulosic matrix. According to the tissues/ cell wall, this matrix is contained hemicelluloses or lignin. The cohesion is assured by the different natural bonds (covalent, non-covalent, Ionic). In particular, the graminae are contained the phenolic acids which represent a small proportion (less than 2 % of the dried-basic mass). The phenolic acids have an important role to establish the covalent liaisons in cell walls. The hydroxycinnamic acids ferulic and *p*-coumaric, are the other phenylpropanoid components of grass cell walls but are virtually absent from legume cell walls (Hartley and Ford, 1989). Ferulic acid is esterified to arabinose molecules of arabinoxylans. Some *p*-coumaric acid is similarly esterified to arabinoxylan, but most is esterified to syringyl-type lignin. Arabinoxylan polymers are cross-linked through diferulates in grasses (Ralph et al., 1994).

In graminaes especially in cereal straw, the ferulic acid is linked to the arbionoxylandes by ester and also associated to the coniferyl (guaiacyl unit G) of lignin. The *p*-coumaric acid, are associated to sinapyl (syringyl unit S) by ester bonds (Figure II- 7). These two units (G and S) represent the free phenolic acids which could solubilize in the alkaline medium.



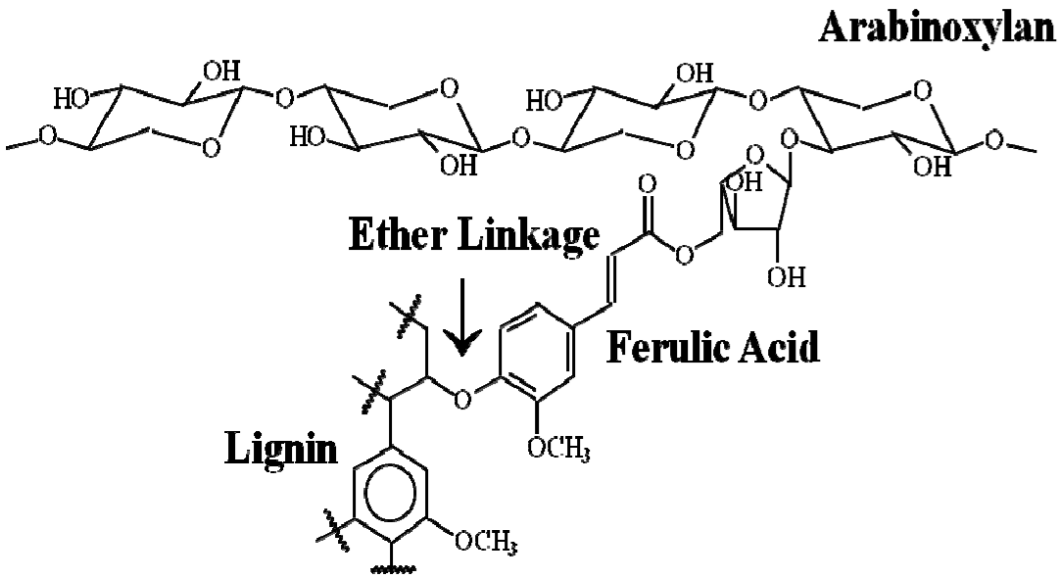


Figure II- 7 *Furulate cross-linking of lignin to hemicellulose in cereal straw Jung, H.G.2012*

Other components

Other components such proteins and ashes were also investigated as components in plant cell wall (Xu 2010; Van Dyk and Pletschke 2012). The protein is likely to have diverse roles in relation to the formation of plant cell wall and it was found in the range 2-10% in primary cell wall. Furthermore, the ashes contents generally vary depending on the agronomical factor and the amount of contaminated soil. In general, the ash content of fibrous is between 1-20%. Almost the agricultural fibers are rich in mineral, which is 65-70% SiO_2 of total mineral(Xu 2010). Moreover, the silicium has a role to increase mechanical resistance of plant cell wall as well as contributes to the plant resistance against insects and fungi. On the other hand, the pectic substances have been considered as minor component in plant cell wall that was found especially in primary cell wall.

Table II- 2 showed the concentration of different components in several plants. The cellulose is the major component in LC material, which varies between (30-50%), hemicelluloses (18-30%) and lignin (6-40%) respectively (l and Howard 2003) (Figure II- 8). In case of wood, the lignin concentration distribution is higher than another type of plant.

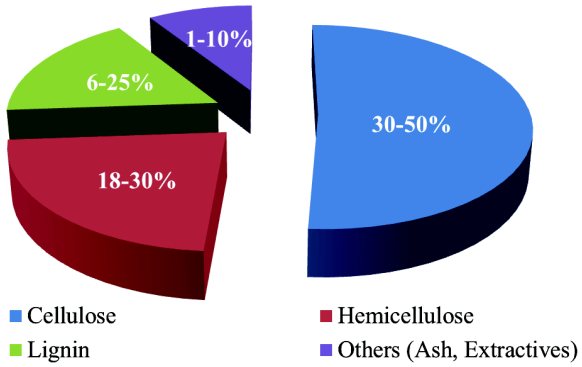


Figure II- 8 *Lignocellulose compositions distribution*

Table II- 2: *Biochemical compositions of different lignocellulose materials*

Raw material	Chemical composition %(w/w)				Reference
	Cellulose	Hemicellulose	Lignin	Ash	
Rice straw	49,8	22,5	13,8	13,8	(Chuetor, Luque et al. 2015)
	40,33	28,6	6,97	18,03	(Narra, Dixit et al. 2012)
	39	23	14	18	(Cabrera, Muñoz et al. 2014)
	35,45	23,78	12,56	13,45	(Oberoi, Babbar et al. 2012)
	40,7	24,8	15,3	N/A	(Ai, Li et al. 2014)
	38,3	28	14,9	18,8	(Zhang and Cai 2008)
	39,89	23,21	20,65	17,25	(Suriyachai, Weerasaia et al. 2013)
Wheat straw	36	26	7,6	N/A	(Vancov and McIntosh 2011)
	45,3	26,22	22,4	N/A	(Barakat, Chuetor et al. 2014)
	41,8	28,6	N/A	N/A	(Persson, Ren et al. 2009)
	32,6	24,7	20,6	N/A	(Govumoni, Koti et al. 2013)
Corn stover	38	20,2	19,5	N/A	(Chen, Stevens et al. 2013)
	36,2	24,55	21,2	1,56	(Xu, Zhang et al. 2012)
Sorghum	32,4	27	7	N/A	(Vancov and McIntosh 2011)
	45	28	22	5	(Chen, Boldor et al. 2012)
	49,78	27,72	10,83	N/A	(Cao, Sun et al. 2012)
Sugarcane	34,4	15,4	29,1	10,1	(Sakdaronnarong, Srimarut et al. 2014)
	38,9	21,3	20	1,8	(Wu, Li et al. 2011)
Wood (eucalyptus)	41,8	18,7	36,1	N/A	(Park and Kim 2012)

As seen in the Table II- 2, rice straw seems having large carbohydrates (cellulose and hemicellulose) content, which contains typically molecules that could be converted into biofuels. In addition, rice straw is rich in ash content that is mostly silica, (60-70% total ash content). For this reason, the rice straw has been chosen in term of potential biochemical compositions for its valorization compared to other cereal straws or woods.

II.1.3 Valorization of LC biomass: Biorefinery

Biorefinery is defined as a facility to convert whatever biomass into different products presenting multifunctional particularly such as fuels, power, heat and value-added chemical (Kamm and Kamm 2004; Octave and Thomas 2009). The biorefinery is an analog of petroleum refinery that means biomass was transformed into different products. The biorefinery is considered as a promising alternative technique to petrochemistry. Almost all of biomass feedstock can be converted to different classes of biofuels, biochemicals through several processes that maximize economic and environmental benefits(Laurent, Roiz et al. 2011). The biorefinery could be classified in different types of raw material as first generation from food products and second generation from LC material. At present, the second generation of bioenergy called lignocellulosic fuels has been more interesting than the first one because of non-direct competition with food products. The LC can produce several products via two different techniques; biochemical and thermochemical pathway (Figure II- 9).

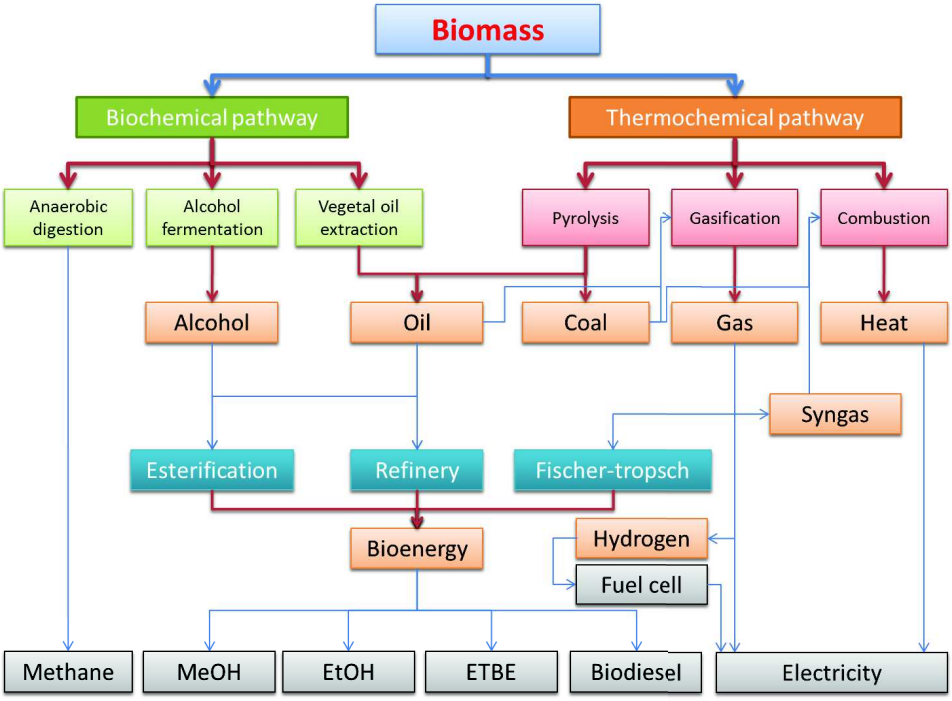


Figure II- 9 *Schematic of Biorefinery*

For example in order to obtain the biofuel such ethanol, the biomass generally was treated via biochemical pathway. The biochemical pathway could divide into three major techniques; anaerobic digestion to produce methane (CH_4), alcohol fermentation to produce all alcohol (methanol, ethanol, ethyl tert-butyl ether (ETBE), biodiesel etc…) and vegetal oil extraction. Major products obtained from biochemical pathway are mostly bioenergy. The common process used to produce bioenergy from LC material was described in many steps (pretreatment, enzymatic hydrolysis, fermentation, distillation). On the other side, thermochemical pathway implies gasification process in which the natural gas is produced; combustion process is applied for heating. Almost thermochemical pathway allows producing most products for electricity discipline. The biochemical pathway presents more advantages by being carried out at low temperature while the thermochemical requires a higher temperature. Nevertheless, various steps are necessary for LC conversion for example to bioethanol/bio-product:

- Pretreatment which has a role of a preparation step of raw material by disruption of polymers, deconstruction of structure to making more available accessibility of constituents (cellulose, hemicellulose and lignin)
- Enzymatic hydrolysis which permits the release of sugars contained in LC material
- Fermentation, released sugars from enzymatic hydrolysis can be converted into bioethanol
- Extraction/separation and fractionation of polysaccharides or lignin

A pretreatment is considered as a crucial step and it is unavoidable process in LC biorefinery. The pretreatment has an objective to alter or modify LC structure and release more available sugars for subsequent enzymatic hydrolysis. Various pretreatments have been investigated in literature, which can be divided into four categories: physical, chemical, physic-chemical and biological.

However, the LC biomass valorization is still limited by economic, techniques and also environmental impacts. Recently issue concerned its valorization of this material, the research tend to find an environmentally friendly process, economical and beneficial techniques. The biorefinery plays an important role to convert renewable raw material like LC biomass into bioenergy, material and value-added chemical products in considering the environmental impacts.

II.1.4 Lignocellulose bioconversion

In the previous section, the LC material presents its abilities and its potential in term of renewable resource. In order to valorize LC material, several processes are implied to accomplish the LC bioconversion. The LC bioconversions involve firstly the pretreatments, which allow the LC material being more accessible to microorganisms or enzymes. Afterward, the enzymatic hydrolysis, during the hydrolysis, the macromolecules such as cellulose, hemicelluloses are broken

down under enzymes actions into monomers or dimers. By size reduction, the microorganisms or enzymes could access through the cell membrane of plant and then promote the simple molecules using subsequent for energy sources. Interestingly, in case of bioenergy second generation especially bioethanol, the hydrolysis could be realized separately or simultaneously by combining enzymatic hydrolysis and fermentation together.

Enzymatic hydrolysis consists of breaking down the macromolecules (cellulose, hemicellulose) into simple molecules. Cellulose and hemicelluloses can be hydrolyzed to fermentable sugars (glucose, xylose) and microbially fermented into various bio-products such as ethanol or chemically converted into other products. In order to degrade the cellulose and hemicellulose, most of microorganisms or enzymes are quite specific and selective (Saha and Cotta 2006). For example, cellulase from *Trichoderma reesei* is commercially usually used to be enzymes for breaking down the cellulose structure through several steps. Firstly, enzymes attack the crystalline regions in order to disorder and swell the cellulose fibers. Afterward, specific enzymes break down almost β-1, 4-linked obtaining at least 6 molecules of glucoses. After that the cellulose is hydrolyzed into cellobiose and the last step, the enzymes β-glucosidase attack in order to liberate glucose. Several specific enzymes using to hydrolyze the cellulose are endo-glucanase; exo-glucanase and β-D-glucosidase (Arantes and Saddler 2010). These enzymes normally come from both bacteria and fungi. Each enzyme has a specific role to break down specific linkage in cellulose. Many studies shown that the structural features of LC material affect directly cellulase action for example crystallinity of cellulose, hemicellulose content as well as lignin content.

On the other hand, hemicelluloses hydrolysis has been traditionally studied. Many bacteria and fungi produce many hemicellulases. Furthermore, the hemicellulases commercially prepared via genetic modification of *Trichoderma* or *Aspergillus*. As hemicelluloses are composed typically of many polysaccharides, in order to liberate monosaccharides the hydrolysis needs specific enzymes to break down such beta-1,4-glucosidic linked (Saha 2003), separation of arabinose and xylose as well as glucuronic acid. Therefore, the degradation of LC, which not only liberates glucose from cellulose but also converts hemicellulose into valuable saccharides, is carried out mainly by an array of independent and synergistic hemicellulases.

The recent challenge in LC degradation is to degrade lignin. The degradation of lignin is imperative for industrial enzymatic biomass-conversion because it not only increases cellulose and hemicelluloses accessibility but also decreases cellulosic enzymes inactivation caused by lignin adsorption.

Several type of enzymes are summarized in the Table II- 3(Zhang, Himmel et al. 2006), many LC-degrading enzymes employ hydrolytic reaction mainly acting on cellulose and hemicelluloses, whereas others employ oxidoreductive ones mainly acting on lignin to convert LC.

Table II- 3: *Cellulosic enzymes using to degrade LC material*

Enzymes catalytic	Origin	Functionality
Cellulases	Trichoderma reesei fungi	
celloboihydrolase	Trichoderma reesei fungi	Degradation crystalline cellulose
Endo-1,4-beta-D-glucanase	Trichoderma reesei fungi	Degradation amorphous cellulose
Beta-d-glucosidase	Aspergillus Niger	Degradation celloboise
Hemicellulases	Aspergillus Niger	
endo-beta-xylanase	Aspergillus Niger	Degradation of glucuro (arabinoxylan)
Acetyl xyland esterase	Aspergillus Niger	Degradation of ester bond
glucanase/manase etc..	Aspergillus Niger	Degradation of mono-and disaccharides
LC oxidoreductases	White-rot fungi	Degradation of lignin via oxidoreduction reaction

Enzymatic hydrolysis is a preliminary step before the valorization of potential molecules. As mentioned in the previous section LC material has a recalcitrant complexity structure. In order to break down the LC networks for liberating potential molecules, there are a lot of specific enzymes that are responsible to specifically crack the linkages in which the interested molecules were located. For the isolation of each molecule such as glucose, there is many kind of enzymes which play a role to break down many types of linkages in cellulose. Even though, cellulase could broadly break down almost cellulose linkages, it is still inadequate because of its specific linkages in cellulose structure. Another way, the hydrolytic enzymes could be more effective by synergistic of cellulases and hemicellulases.

Ethanol fermentation/biogas production conventionally ethanol fermentation consists of using microorganisms mostly yeasts to degrade glucose (C6) into ethanol (Mosier, Wyman et al. 2005; Margeot, Hahn-Hagerdal et al. 2009). The ethanol fermentation could be done separately or simultaneously with enzymatic hydrolysis (Hahn-Hagerdal, Galbe et al. 2006). The ethanol production from LC material has at least four steps: pretreatment process, enzymatic hydrolysis, fermentation and distillation. In addition, the enzymatic digestion and fermentation could be performed together in order to eliminate some excess steps. Numerous studies have recently focused on the possibility to produce an ideal microorganism, which would be able to convert all kind of carbohydrates into ethanol. Moreover, this microorganism has to be capable to produce

ethanol despite the presence of inhibitors. Many studies recently showed that it is possible to convert pentose (C5) into ethanol especially in saccharification with simultaneous fermentation because of the weak glucose concentration.

On the other hand, biogas could also be produced from LC biomass via biologically anaerobic digestion. In this process, the organic carbon is converted by oxidation and reduction reaction normally into methane (CH_4)(Taherzadeh and Karimi 2008; Kaparaju, Serrano et al. 2009). The LC material is interesting in term of biochemical composition, which could be converted directly into biogas without enzymatic hydrolysis while the ethanol fermentation needs this process. The enzymatic hydrolysis is unnecessary in biogas production because the biogas digestion does not need the specific molecules. In addition, the biogas digestion of LC material could be produced from the whole LC material. However, in order to valorize this material into biogas, the presence of some chemical constituents hinder the biogas production yields such lignin content (Taherzadeh and Karimi 2008; Kratky and Jirout 2011). It would mean therefore that the pretreatment is still necessary for removing or solubilizing lignin before the conversion process.

The ethanol and biogas productions yields are still insufficient because the digestibility of potential molecules (carbohydrates) is still inadequate. The improvement of production yields has been considered being a recent issue of LC biomass pretreatment for biofuels production research. In fact, the researches focus on the improvement of bioconversion yields by developing the pretreatment processes, characterization methods in order to respond the technical and economic conditions.

II.1.5 Factors influencing lignocellulose bioconversion

The LC biomass is typically a recalcitrant structure and it is constructed by its chemical compositions, which build a spatial network as a protective support. In order to convert LC material into various products via bioconversion, there are many factors influencing the bioconversion capacity. The factors affecting biomass accessibility could be divided into two categories direct and indirect factors. The direct factor is related to physical characteristics of material such pore size, volume, particle size, and specific surface area. Whereas the indirect factors are especially related to structure-relevant such the chemical compositions (lignin, cellulose, hemicelluloses etc.) (Zhao, Zhang et al. 2012). In order to enhance the accessibility of potential molecules, the pretreatment is responsible to modify both previous factors. Due to the complex matrix and rigid structure of LC, the release of components for bioconversion becomes difficult. The presences of broadly some chemical compositions break down the efficiency of its valorization. In the literature reviewed the natural factors, which limit the valorization include:(Lee 1997; l and Howard 2003; Pan, Arato et al.

2005; Arantes and Saddler 2010; Limayem and Ricke 2012; Van Dyk and Pletschke 2012; Belal 2013; Behera, Arora et al. 2014; Peng, Chen et al. 2014; Zhang, You et al. 2014)

- The lignin content
- The cellulose crystallinity
- The hemicellulose content especially the linkages with lignin
- The heterogeneous and complex structure of cell wall
- The challenges for enzymes acting on an insoluble substrate
- The formation of inhibitors during conversion process

The LC complex matrix contributes to the recalcitrance of biomass, the natural factors are just minor contributions compared to the chemical compositions and physical features of LC (Table II-4) (Lee 1997). In addition, there are also many mechanical and rheological properties, which can influence especially the flowability of products.

Table II- 4: *Influenced factors of lignocellulose bioconversion*

Physical structures	**Effects**
Cellulose crystallinity	High crystalline region, difficult to attack by enzymes
Specific surface area	More specific surface area, increase in enzymatic rate
Particle size	Decrease in particle size, enhance enzymatic efficiency
Particle shape	Physical and mechanical properties
Particle size distribution	Poly-diversity makes different rate of reaction, mixing
Pore volume	Increase in rate of enzymatic hydrolysis
Degree of polymerization (DP)	Faster the cellulose degradation by enzymes
Density	Physical properties
Chemical composition	**Effects**
Lignin	Hinders enzymes attacked
Hemicellulose	Limits the accessibility of cellulose when it was linked to cellulose
Acetyl group	Decrease the enzymatic rate
Cell wall protein	Can be negligible in enzymatic hydrolysis
Mechanical/Rheological properties	**Effects**
Flow behavior	Difficult to mix, transporting
Compressibility	Influence the rheology properties
Frictional behavior	Influence the flow behavior
Cohesive strength	Influence the flow behavior

Physical features of LC biomass have been obviously considered as the first barrier of LC bioconversion. The particle size is one of the most important characteristics that define the accessibility of material(Mosier, Wyman et al. 2005). The LC material must firstly be reduced to

have more specific surface area in order to enhance the enzymatic digestibility. In addition, the particle size reduction process makes directly and indirectly some physical features change for being more accessible in subsequent process. In the particle size reduction process, the cellulose crystallinity is reduced and the intra-pore volume of material is increased (NIKLAS 1998). Moreover, the reduction in cellulose crystallinity is indicative of greater disruption of inter- and intra –chain hydrogen bonds resulting in greater surface area accessibility of cellulose to enzymes. All physical features are related one to another feature.

There are not only physical characteristics that hinder the LC bioconversion yields, but also the chemical compositions in micro-molecules level. As shown in micro-molecules scale of LC material, the associations in parietal ultrastructure between constituents make the enzymes attacks being difficult. Furthermore, some componential group could affect the bioconversion such acetyl group (Sun, Tomkinson et al. 2001; Kumar, Barrett et al. 2009; She, Sun et al. 2010; Liang, Cheng et al. 2014). In addition, others compositions can have an effect in enzymatic hydrolysis even these are minor quantities such as protein content.

On the other, in order to form/ elaborate the products for valorizing the LC material; some mechanical and rheological properties become an important factor. The physical features and chemical composition have been considered as major obstacles hindering the accessibility of enzymes. In order to improve the efficiency of enzymatic accessible yields, a pretreatment has been mentioned as a preliminary step for liberating and exposing the LC networks. However, the complex structure of biomass makes it difficult to isolate, how pretreatment influence cellulose digestion and understanding the relationship of cellulose digestibility by enzymes to pretreated biomass characteristics is challenging research.

II.2 Pretreatments of LC biomass

Pretreatment process is considered as preliminary step of LC biorefinery. The pretreatment is indispensable step in biorefinery for valorizing LC constituents to value-added products for example cellulose. The cellulose is the most available polymer in nature and it often as a source of fuels, also a principle substrate for biomolecules by using glucose. Moreover, the LC material has a complex structure that hinders the bioconversion. In order to make more accessible molecules and increase the bioconversion yields, the pretreatment is therefore a responsible step to decompose/disrupt the complex matrix. In addition, the aim of pretreatment is broadly to make LC material more reactive as well as accessible. The pretreatment can be normally categorized according to the nature of processes such as physical, chemical, physic-chemical and biological.

II.2.1 Chemical

Acid pretreatment uses concentrated or dilute acids, which lead to break down an inflexible structure of LC material. The concentrated acid such sulphuric H_2SO_4 (65-85% w/v) or phosphoric H_2PO_4 (85% (w/v) acids were used to pretreat with dried material (5-10% moisture content) at low temperature (30-60°C) and pressure. The dilute acid is commonly 0.2-2.5% (w/w) concentration and it is used to pretreat at high temperature (120-210°C) and pressure(Bensah and Mensah 2013). The utilization of dilute acid leads to solubilize hemicelluloses, while using concentrated acid could hydrolyze the cellulose and the remaining hemicellulose(Talebnia, Karakashev et al. 2010). In case of concentrated acid pretreatment, Romero et al.,(Romero, Ruiz et al. 2010) reported that using 31.8% (w/w) of H_2SO_4 could increase reducing sugar in olive tree. On the other hand, the dilute acid pretreatment, Aguilar et al., (Aguilar, Ramírez et al. 2002) reported that the 90% of the hemicelluloses was hydrolyzed in sugarcane bagasse with 2% of H_2SO_4. Furthermore, Kim et al., 2013 showed that pretreated rice straw using nitric acid (HNO_3) 0.65% (w/v) could achieved 83% of enzymatic hydrolysis and 87% of released xylose. In addition, the nitric acid pretreatment can also increase ethanol production 33% compared to the non-treated rice straw (Kim, Lee et al. 2014). The dilute acid has been commonly used to pretreat LC material instead of using the concentrated acid because of its chemical costs. Many studies reviewed that we can decrease the amorphous portion and enhance enzymatic hydrolysis, using dilute acid (H_2SO_4) 1.2% to pretreat rice straw (Kim et al., 2011). The obtained results showed that more than 90% of glucose was recovered and there was a decrease in cellulose crystallinity (Kim, Lee et al. 2012). In addition, numerous studies have reviewed the development of acid pretreatment of LC biomass; they highlighted the advantages and drawbacks of this technique. A main advantage of acid pretreatment is to convert more fermentable sugars for a subsequent enzymatic hydrolysis. Moreover, the hemicelluloses are solubilized and could be degraded into xylose, mannose, acetic acid etc. (Behera, Arora et al. 2014). However, the acid pretreatment have also some drawbacks, is its requirement of special corrosion-resistant reactors that are normally expensive both in investment and operation costs. In addition, it needs a washing step that is required to remove the acid before the ethanol fermentation step in order to adjust the suitable pH in fermentation. Beside, other drawback is the formation of fermentation inhibitors that could reduce the effectiveness of the fermentation (Talebnia, Karakashev et al. 2010; Gary, Elizabeth et al. 2011). The acid pretreatment is therefore less attractive for LC materials due to the inhibitors formation (HMF), corrosion problems as well as high operation costs.

Alkaline pretreatment consists generally of using bases catalysts such as sodium hydroxide (NaOH), potassium hydroxide (KOH), calcium hydroxide ($Ca(OH)_2$) and ammonia (Zhang and Cai 2008; Kim, Lee et al. 2014; Singh, Shukla et al. 2014). The alkaline pretreatment leads to remove

acetyl groups and uronic acids (Chen, Chen et al. 2012; Chen, Stevens et al. 2013; Behera, Arora et al. 2014). Moreover, the alkaline pretreatment involves the saponification of intermolecular ester bonds linking hemicelluloses and other constituents especially lignin in rice straw (Behera, Arora et al. 2014; Singh, Shukla et al. 2014). The saponification reaction leads to swell and decrease in the polymerization degree of cellulose. Among existing chemical pretreatments, the alkaline pretreatment provides the most effective technique for breaking down the ester bond between carbohydrates and lignin (Gáspár, Kálmán et al. 2007). The sodium hydroxide (NaOH) has been commonly used for alkaline pretreatment. Zhang et al.,(Zhang and Cai 2008) reported that rice straw pretreated with 2% of NaOH could increase 54.83% of cellulose and decrease 61.07% of hemicellulose. Moreover, NaOH pretreatment could affect the histological changes in the transvers section of rice straw (Figure II- 10). The alkaline pretreatment using NaOH seems to be effective in term of releasing the glucose and solubilizing the hemicellulose. Barakat et al., (Barakat, Chuetor et al. 2014) reported that alkaline pretreatment by using 5% (w/w) NaOH could achieve 330gkg^{-1} of glucose yield extracted in wheat straw. The alkaline pretreatment by using NaOH not only increases the released glucose but also increases in the biogas yield (He, Pang et al. 2008).

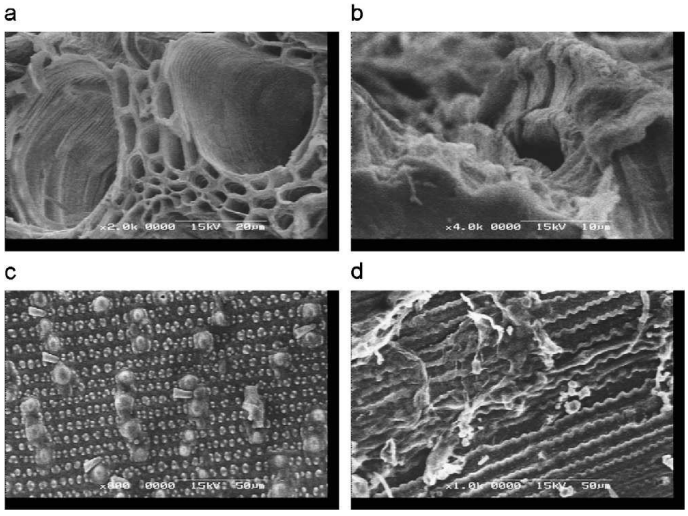


Figure II- 10 *SEM micrographs of rice straw before and after 2% NaOH pretreatment. (a) Transverse section of rice straw stem before pretreatment. (b) Transverse section of rice straw stem after NaOH pretreatment. (c) Longitudinal section of rice straw stem before pretreatment. (d) Longitudinal section of rice straw stem after NaOH pretreatment (Zhang and Cai 2008)*

Another type of alkali catalysts has been used to pretreat LC material such as ammonium carbonate. The rice straw pretreated by ammonium carbonate (20%) resulted in an increase of 72% the enzymatic digestibility (Kim, Lee et al. 2014). The optimized condition for alkaline pretreatment are usually performed at ambient temperature, but it take a little longer time than high temperature

(Gary, Elizabeth et al. 2011). On the other hand, many studies had shown the combination of alkaline with other pretreatment processes such mechanical, wet oxidation, oxidizing agents and other chemical agents like hydrogen peroxide, providing a great efficiency for enzymatic hydrolysis (Barakat, de Vries et al. 2013; Barakat, Chuetor et al. 2014). For example the combination of NaOH and hydrogen peroxide, favors enzymatic hydrolysis via removing lignin and no inhibitors formation(Cao, Sun et al. 2012). Interestingly, Barakat et al., 2014 showed that the association of two techniques pretreatments: alkaline and mechanical pretreatment (grinding) (Barakat, Chuetor et al. 2014) provides a decrease of energy consumption. It could be hypothesized that the combination of alkaline and mechanical could not only improves enzymatic digestibility but also decreases the operation costs. However, alkaline pretreatment is normally unsuitable for wood feedstock because of high lignin content. Other drawbacks are the solubilized hemicellulose as well as the inhibitors formation.

Wet oxidation consists of using the oxidation reaction in an aqueous solution such acidic, neutral, alkaline conditions with oxygen in air. The separation liquid/solid step is necessary before enzymatic hydrolysis. The suspended biomass is filtered to separate the cellulose-rich solid and the solid component is washed with deionized water (Brodeur, Yau et al. 2011). This technique leads to oxidize the hemicelluloses, to cleave hemicelluloses chains as well as to peel the phenolic structure of lignin (Iyer, Wu et al. 1996; Martín, Klinke et al. 2007; Banerjee, Sen et al. 2009). The satisfied condition for wet oxidation pretreatment is normally carried out at high temperatures, pressures, pH and catalysts loading(Kim and Lee 2005). The combination of wet oxidation with other pretreatment could reduce the formation of inhibitors (Martin, Gonzalez et al. 2006; Martín, Klinke et al. 2007).

Organosolv pretreatment involve the utilization of a mixture of organic solvents and inorganic acid catalyst in aqueous form. The pretreatment leads to break down an internal lignin structure and also hemicellulose bonds (Zhao, Cheng et al. 2009; Behera, Arora et al. 2014). Moreover, the removal of lignin and the hemicellulose solubilization take place during this pretreatment by hydrolyzing the internal lignin bond and ester bonds between hemicelluloses and lignin (Zhao, Cheng et al. 2009). The use of acid in the organosolv technique improves the lignin removal yield (Huijgen, Telysheva et al. 2014). For example, Snelders et al., reported that 96% of lignin was solubilized by using acetic and formic acid based organosolv technique (Snelders, Dornez et al. 2014). Furthermore, Amiri et al., 2014, reported that in rice straw pretreated by using organosolv, the cellulose content increased 26% compared to untreated rice straw (Amiri, Karimi et al. 2014). In addition, the organosolv pretreatment can generally remove lignin and partially hemicelluloses with resulting increase of specific surface area and pore volume. The optimized operation condition is

recommended at high temperature which could decrease of cellulose crystallinity as well as cellulose degree polymerization (Zhao, Zhang et al. 2012). However, the organosolv pretreatment needs a lot of unit operations such a separation, recycle solvent etc. which cause making a cost-effective improvement to this technique.

Ionic liquids (ILs) uses salts solution, typically composed of ions (cations and anions). The common forms contain the imidazolium cation which can pair with anions such as chloride, bromide, acetate, sulphate etc.(Bensah and Mensah 2013). The ILs lead to transform carbohydrates into fermentable sugars by two pathways: one is to improve enzymatic hydrolysis efficiency, and the other one is the transformation of the hydrolysis process from a heterogeneous to a homogenous reaction by dissolution in the solvent (Wang, Wu et al. 2011). Moreover, the ILs are able to decrease significantly the cellulose crystallinity by breaking up the inter- and intra-molecular hydrogen bonds networks, resulting in the increase of cellulose accessible(Zhao, Zhang et al. 2012). Zhao et al. reported that more than 96% of released glucose yields by using ILs. (Zhao, Baker et al. 2010). Furthermore, Sun et al., demonstrated also that ILs is an effective process for rice straw pretreatment. It achieved more 70% of reducing sugars yields (Sun, Ye et al. 2013). Beside, Hou et al., indicated that using 20% of cholinium lysine ionic liquid-water mixtures to pretreat rice straw, resulting 81% of glucose yields (Hou, Li et al. 2013). In addition, Gao et al., mentioned that using $[C_4mim]Cl$ as ILs to pretreat four different biomass could remove 23.7%-64.8% of lignin and also decrease of cellulose crystallinity, with resulting increase of specific surface area (Gao, Chen et al. 2013). Through the component ions in ILs, the hydrogen bonds of cellulose break down (Xie, Liu et al. 2012) and the cellulose is solubilized, thus the crystallinity is reduced (Tian, Fang et al. 2011).

Oxidizing agents consists of using of oxidizing agents such as ozone and hydrogen peroxide under mild alkaline or neutral conditions. This technique causes an oxidative delignification which lead to improve enzymatic digestibility (Bensah and Mensah 2013). The ozonolysis leads to degrade lignin and hemicelluloses. Panneerselvam et al. investigated that the ozone pretreatment has been effective to remove more than 60% of lignin and achieved more than 58% of cellulose recovery per liter of ozone (Panneerselvam, Sharma-Shivappa et al. 2013). Furthermore, the combination with other techniques such extrusion technique demonstrated more 66% of recovered glucose and 70% of recovered sugar in switchgrass (Karunanithy, Muthukumarappan et al. 2014). Other association, the wet disk milling followed by ozone in bagasse reached to 89.7% of glucose yield in enzymatic hydrolysis(de Barros, Paredes et al. 2013). However, ozone pretreatment requires a large quantity of ozone that causes effective operational costs. The ozone pretreatment could be associated with other pretreatment such as extrusion, wet disk milling (de Barros, Paredes et al. 2013; Karunanithy, Muthukumarappan et al. 2014).

On the other hand, the hydrogen peroxide (H_2O_2) has been widely studied to pretreat of LC material. The H_2O_2 pretreatment could solubilize of hemicelluloses and lignin in case of using low concentration (Ayeni, Omoleye et al. 2014). Normally, the H_2O_2 used to be associated with alkaline solution such sodium hydroxide, ammonia in order to enhance the enzymatic digestibility. For example, Sun et al. reported that the combination of H_2O_2 with NaOH resulting more 95% of lignin dissolution (Sun, Tomkinson et al. 2000; Sun, Tomkinson et al. 2001). Moreover, Barakat et al. reported that more 75% of glucan and xylan was released after applying alkaline peroxide pretreatment in wheat straw(Barakat, Chuetor et al. 2014). Furthermore, the combination of alkaline and H_2O_2 provide an increasing in enzymes accessibility as well as bringing out a delignification and also a solubilization of hemicelluloses. Zhang et al. reported also that a combination of alkaline peroxide pretreatment of poplar achieved 64.9% of delignification. Moreover, the semi-simultaneous saccharification and fermentation improved of ethanol yield production from 12.8% to 63.1% (Zhang, You et al. 2014). Therefore, the association of alkaline and H_2O_2 seems to be effective pretreatment process to enhance biomass conversion into bioethanol or biogas.

II.2.2 Physicochemical

Steam explosion is commonly used to break down LC via the combination of both chemical and physical techniques. This pretreatment typically changes biochemical structure and physical features. The principle of this pretreatment is to remove hemicelluloses, thus enhancing the enzymatic hydrolysis. The steam explosions perform normally at high temperature and pressure for a short period time. The mechanism disruption of material fibrous takes place after the depressurization. The reduction of particle size is a major relevant factor to be effective of the process. However, steam explosion is hardly to remove lignin but it is redistributed on the fiber surface (Li, Henriksson et al. 2007). On the other hand, using the combination of dilute-acid and steam explosion to decompose the LC structural have been largely studied. For example, Chen et al., 2011 showed that two steam explosion steps was used to pretreat rice straw, the first step is to use the 2% of sulfuric acid with saturation steam at 165°c for 2min and then the second steam explosion at 180°c for 20min. This gave a high glucose yield about 240g glucose/kg rice straw (Chen, Pen et al. 2011). Furthermore, the combination of steam explosion and superfine grinding improved the enzymatic hydrolysis efficiency. This combination process gave about 370 g glucose/kg rice straw via the steam explosion at 179.9°c for 5min and then using FJM-200 fluidized bed opposed jet mill for 25min (Jin and Chen 2006) (Figure II- 11). Moreover, Kaar et al., 1998 mentioned that the sugarcane bagasse was pretreated by steam explosion at 216°C achieved the glucose conversion yields between 41-67% and xylose conversion yields between 17-85% (Kaar, Gutierrez et al. 1998). In term of accessible surface area, the steam explosion technique leads to reduce the particle size, resulting in the increase of accessible surface area. However, the cellulose

crystallinity has not been affected after the steam explosion pretreatment. Besides, the steam explosion processes produce slurry that can be separated to solid and liquid fraction. With separation step requires more energy and more steps in the processes what makes the processes be high cost effective.

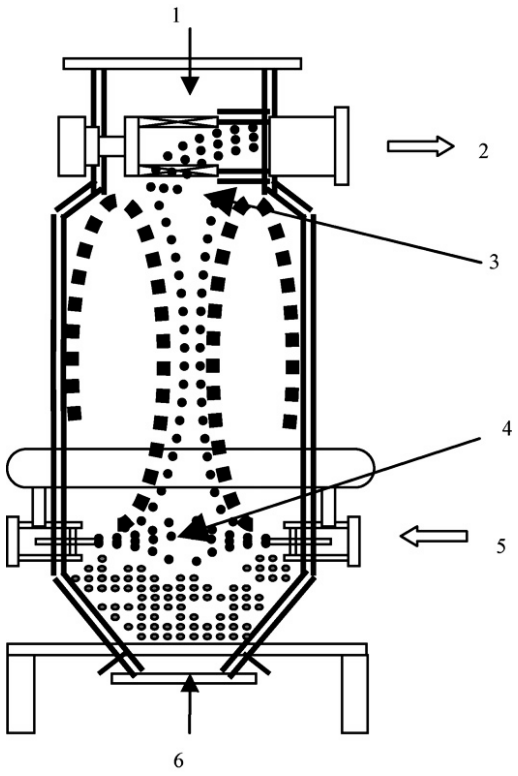


Figure II- 11 *Schematic diagram of the FJM-200 fluidized bed opposed jet mill. 1, Infeed; 2, collection; 3, classification section; 4, grinding section; 5, compressedair; 6, discharge opening(Jin and Chen 2006)*

Ammonia fiber explosion (AFEX) is an alkaline physicochemical pretreatment process. The pretreatment consists in using the explosion of biomass to liquid ammonia at high pressure (250-300psi) and high temperature (60-100°C) for a time about 30min. In this pretreatment, ammonia has been suitable to swelling and disrupting of biomass fibers, partial decreasing in cellulose crystallinity, breaking down of lignin-carbohydrate linkages and also altering lignin structure (Taherzadeh and Karimi 2008; Behera, Arora et al. 2014). The pretreatment has many advantages such as no inhibitor formation, high surface area and improvement of enzymatic digestibility (Taherzadeh and Karimi 2008; Singh, Shukla et al. 2014). However, the AFEX has also limitation in term of phenolic fragments and extractives might be remained at the cellulosic surface. It might need a washing step to remove it before. Furthermore, the pretreatment has been more effective with the LC in which contains less of lignin content. In addition, it cannot solubilize hemicellulose

compared to other pretreatment. Moreover, the AFEX needs recycling of ammonia to reduce and decrease cost effective and environmental impact.

II.2.3 Biological

Another technique of pretreatment of LC material, the biological pretreatment, typically involves using the microorganisms or enzymes to enhance the enzymatic hydrolysis. The main occupation of microorganisms during pretreatment is usually to degrade lignin and hemicellulose (Taherzadeh and Karimi 2008). Various fungi have been investigated to pretreat LC material. The white-rot fungi is commonly one the most effective microorganisms for biological pretreatment of LC biomass(Sun and Cheng 2002). For example, Taniguchi et al. mentioned that the rice straw pretreated by using *Pleurotus ostreatus* of white-rot fungi was suitable for biological pretreatment and this fungus could improve an enzymatic hydrolysis (Taniguchi, Suzuki et al. 2005; Taniguchi, Takahashi et al. 2010). Moreover, the biological pretreatment does not only improve the bioethanol production but also the digestion in biogas production. Furthermore, Khoung et al., 2014 indicated that the sugarcane bagasse was pretreated by using white-rot fungi could reduce the lignin content from 23.4% to 17.1% after 4 weeks incubation times with resulting increase ethanol production 20% compared to untreated bagasse (Khuong, Kondo et al. 2014). In addition, Zhang et al., 2007, supported that the biological pretreatment improved the lignin removal, >20% of lignin was removed after four weeks biodegradation. Additionally, the enzymatic hydrolysis yields increased with increasing time (Zhang, Yu et al. 2007). On the other hand, Zhi et al., 2014, reported that the wheat straw pretreated by P. Chrysosporium kind of white -rot fungi, could improve the biohydrogen production and it could remove 28.5% of lignin fraction. Moreover, the wheat straw structure was dramatically destroyed to form a loose and porous surface. It would have meant that the white-rot fungi pretreatment influenced not only biochemical compositions but also the structure of wheat straw(Zhi and Wang 2014). However, the biological pretreatment have some advantages such as no chemical, low requirement energy but the kinetic pretreatment need quite long time in most of existing pretreatment. In addition, the enzymatic hydrolysis yields increase with the increase in time. Mostly the biological pretreatment using white-rot fungi need more than at least four weeks biodegradation in order to remove lignin content and the high moisture content was also considered (50-80%). Furthermore, the novel discover is to use oxidative enzymes in order to cleave cellulose structure especially to decrystallinizecellulose by oxidizing the C1 and C4 in cellulose structure.

II.2.4 Physical / Mechanical

The physical pretreatment essentially refers to mechanical size reduction. The process of mechanical comminution is a standard operation in order to reduce the particle size. The reduction of particle size affects susceptibly a specific surface area for enzymatic hydrolysis (Singh, Shukla et

al. 2014). The mechanical pretreatment such as milling or grinding, allows directly reducing the particle size and also decrease the crystallinity of cellulose, which could enhance the enzymatic hydrolysis and biodegradability of LC material. The biomass size reduction leads to disrupt of plant cell wall and dissociation of tissues (Barakat, de Vries et al. 2013). Many studies reported that mechanical pretreatment provided the particle size reduction enhancing the enzymatic hydrolysis. For example, Silva et al. mentioned that using ball milling could reach the particle size of about 10μm and could improve glucose yield and also enhance the wheat straw degradability. In addition, the ball milling could decrease the crystallinity of cellulose from 22 to 13% (Silva, Couturier et al. 2012). The mechanical pretreatment such as communition, milling, grinding provided the increase in the specific surface area, which leads to improve the enzymatic accessibility. Moreover, the mechanical size reduction has been investigated to be effective to size reduction, decrease in cellulose crystallinity. A lot of dry ball milling, Hot-compressed water and wet disk milling were largely performed and these technique achieved 89.4%, 70.3% and 78.5% of glucose yield. This experiment is to compare the energy consumption in which the wet disk milling showed an economical process for the enzymatic hydrolysis of rice straw (Hideno, Inoue et al. 2009). On the other hand, the combination of hot compressed water and wet disk milling could achieve 94.5% of glucose yield after a pretreatment with 135°C for 60min of hot compressed water and 15cycles of wet disk milling(Hideno, Inoue et al. 2012). The physical/mechanical pretreatment seems providing many advantages such as increasing in internal surface, decreasing in crystallinity of cellulose and reducing of polymerization degree. However, the process has some drawbacks as requirement of high-energy consumption due to grinding/milling operation. Moreover, the mechanical size reduction could reduce particle size without changing the surface chemistry (Dibble, Shatova et al. 2011). The mechanical pretreatment therefore has been suggested to combine with another pretreatment in order to save and decrease of cost effective(Barakat, de Vries et al. 2013).

On the other hand, physical pretreatment is also the utilization of irradiation techniques that involve using for example gamma rays. It is responsible to cleave the cellulose liaisons, thus giving a larger surface area and decreasing cellulose crystallinity. This technique is, however, highly effective costs to industrial scale up. It would be suggested to use in combination with another pretreatment technologies supposed to be environmentally friendly as well as saved effective costs.

II.2.5 Innovative processes

Most of existing pretreatments have shown their own advantages which to be related to the functionalities of LC material application. It provides reducing cellulose crystallinity, solubilizing of hemicelluloses and lignin, breaking down of lignin-carbohydrate linkages etc. (Barakat, de Vries et al. 2013). However, almost all pretreatments provide some drawbacks such high cost effective,

high-energy consumption, the formation of inhibitors as well as providing environmental impacts. Presently, the development of combination of pretreatments seems to be interesting in order to overcome these problems as well as to eliminate some obstacles.

The actual development of pretreatment technologies aims to come across and find the work point under two constraints: consumption of energy and environmental impacts. The question supposed is how to develop the pretreatment process which could reduce in water consumption, decrease in chemical catalyst as well as decrease in energy consumption Figure II- 12.

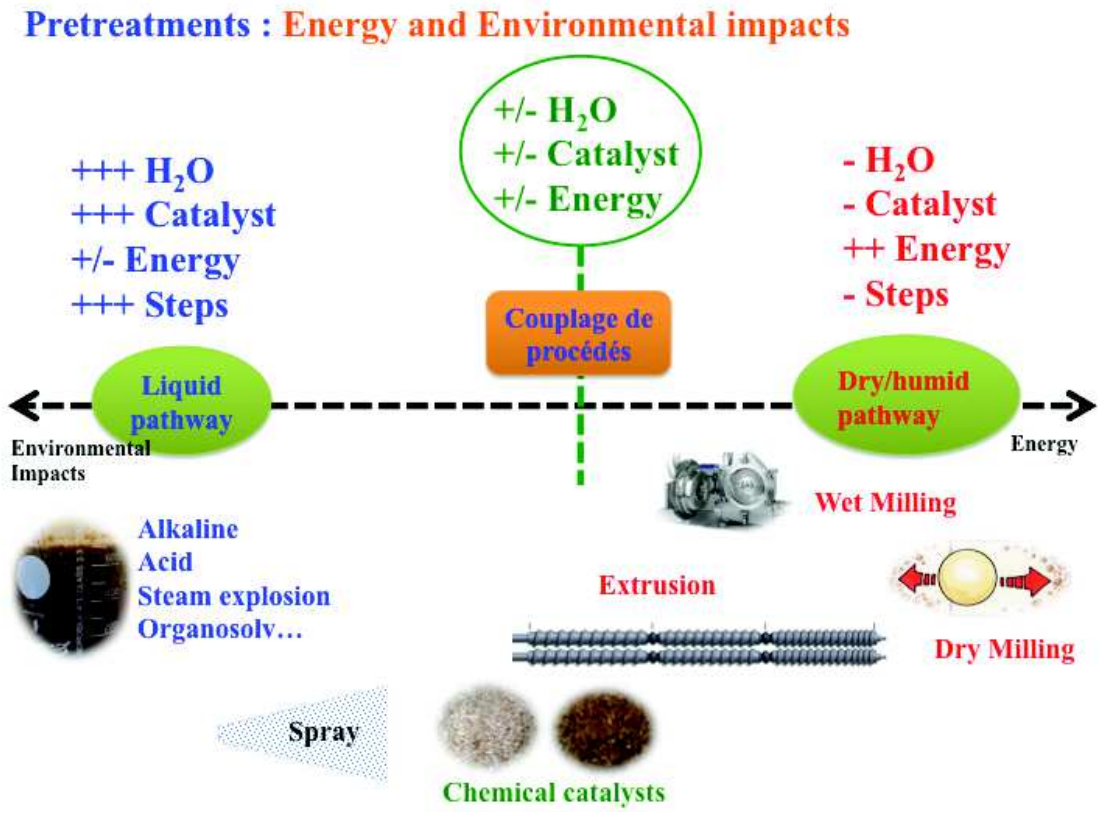


Figure II- 12 *Innovation pretreatment process*

The recent point of deconstruction of LC via eco-conception fractionation is one of the new conceptions for LC biorefinery. This fractionation technique defines the dissociation of molecules, the disruption of tissues as well as the separation/isolation of molecules for further applications. By word eco-conception, it mentions about the way to be economically as well as ecologically for LC biorefinery.

In many instances, the final biomass application requires small particle size (>20µm) in order to enhance its performance (Silva, Couturier et al. 2012). For example, LC biomass needs a small particle size to enhance enzymatic accessibility via increasing specific surface area. The particle size reduction via communition, grinding, and milling are often used to define "reduction in particle

size". This unit operation is generally applied in chemical, food, mineral, pharmaceutical as well as agriculture industries.

The dry fractionation defined by the disruption of structures especially all tissues, organs etc. The mechanical size reduction is considered as dry fractionation, which permits increasing the bulk density and improving flowability as well as digestibility (Miao, Grift et al. 2011). Various mechanical techniques could be found in the literature, aiming to reduce directly a particle size. The reduction of particle size consists in fragmentation, deconstruction by using a mechanical constraint. However, several factors affect the efficiency of mechanical process such as moisture content, mechanical behavior, particle form, temperature (Barakat, de Vries et al. 2013). Many properties have an effect on the desired particle size in this process.

The crystallinity of cellulose, the degree of polymerization and specific surface area are in particular the primary factors that hinder the enzymatic accessibility. To modify these factors, the grinding, milling operations alter the structure of biomass. The mechanical pretreatment presents many advantages in term of increasing specific surface area, decreasing crystallinity of cellulose and consequently increasing enzymatic accessibility yield (Silva, Couturier et al. 2012). However, the mechanical technique still presents important drawbacks such as cost effective due to high-energy consumption. The energy requirement due to grinding or milling processes remains an important parameter in evaluating mechanical size reduction processes. Moreover, the literature reported that the energy requirement of mechanical size reduction mainly depends on many factors such material characteristics, temperature, chemical composition, machine parameter as well as final particle size requirement (Mani, Tabil et al. 2004; Bitra, Womac et al. 2009; Miao, Grift et al. 2011). The LC properties and moisture content are primary important factors to be considered as the main factors for mechanical size reduction. In order to minimize cost effective, energy requirement, the mechanical pretreatment could be combined with other pretreatment that could be economic and increase enzymatic hydrolysis yields.

Barakat et al. 2013, (Barakat, Chuetor et al. 2014) reported that the combination of chemical and mechanical pretreatments in spray system provided a decrease of energy consumption due to milling process compared to non-treated wheat straw. Moreover, they could eliminate some steps in pretreatment process especially solid/liquid separation step by using spray system for pulverizing chemical catalyst on biomass in which the process does not generate effluents (Figure II- 12). Mostly the combined pretreatments studies are the combination between mechanical and chemical pretreatments because the mechanical pretreatment consists of altering the LC structure especially physical features, which hinder the efficiency of enzymatic digestibility (Barakat, Chuetor et al. 2014), whereas chemical pretreatment allows modifying especially the intra/inter structure.

Interestingly, the combinations of mechanical and chemical pretreatments have been shown to reduce in cost effective especially in process due to milling energy requirement. Conventionally, the chemical pretreatment consists of fragilizing the LC structure and expose inter/intra molecules in order to prepare readily biomolecules for its further applications. Once, the biomass has been pretreated by chemical catalyst, the biomass characteristics have been changed during pretreatment. In addition, these characteristics changes, which consequently make material, be easier to fractionate or isolate. Consequently, the particle size reduction needs less energy demand compared to the non-treated biomass (Barakat, Chuetor et al. 2014). It seems that the combinations of chemical and mechanical pretreatments have many advantages in term of reducing total process costs, no generation of effluent as well as improvement of bioconversion (Barakat, Chuetor et al. 2014).

In addition, using milling combined with separation technique in biorefinery has recently developed being the new dry fractionation technology for environmentally friendly process. Barakat et al., 2014 and Chuetor et al., 2015 reported that the new dry technology of biomass refinery by associating milling and separating to separate fractions that are enriched in cellulose or lignin-hemicellulose in order to produce biofuels (Barakat and Rouau 2014; Chuetor, Luque et al. 2015).

The Table II- 5 summarizes the different pretreatments and different conditions in comparing the cellulose recovery as well as glucose yields from enzymatic hydrolysis.

Table II- 5: *Different pretreatment techniques*

Pretreatment conditions	Chopping technologies	Particle size (mm)	Enzymatic hydrolysis and fermentation	Cellulose (g/gbiomass)	Glucose (g/kg biomass)	Chemical/water usage	Ref
Extrusion/extraction +sulfuric acid (3%w/w at 130°C for 20min) extrusion screw 40rpm at 120°C	sherdder	<10	cellulase 20FPU/g glucose at 50°c 100rpm for 72h	0,383	316,68	N/A	Chen, Xu et al. 2011)
Dry ball milling(DBM) for 60min 1700rpm	ball milling TI-300 system	0,125-0,250	cellulase 10FPU/g substrate + b-glucosidase 6,7mg/g substrate at 45°c for 72h	0,333	331	N/A	Hideno, Inoue et al. 2009)
Hot-compressed water (HCWT) at 180°c for 30min	N/A	N/A	cellulase 10FPU/g substrate + b-glucosidase 6,7mg/g substrate at 45°c for 72h	0,333	313	N/A	Hideno, Inoue et al. 2009)
Wet disk milling (WDM) for 10 repeated milling	Super masscolloider MKZA10	N/A	cellulase 10FPU/g substrate + b-glucosidase 6,7mg/g substrate at 45°c for 72h	0,333	291	N/A	Hideno, Inoue et al. 2009)
Sulfuric acid 2%(w/w for 4h) saturation steam at 165°C for 2min +steam explosion 180°c for 20min	milling	0,233	cellulase 20 FPU/ g cellulose at 50°c 100rpm for 72h	0,311	240	N/A	Chen, Pen et al. 2011)
Steam explosion + jet mill (179,9°c for 5min and 4544rpm 25min)	jet mill (FJM-200, power 1,5kW)	0,06	cellulase 0,55FPA/mg at 50°c 160rpm for 48h	0,365	372,86	N/A	Jin and Chen 2006)
Ionic liquid 3% rice straw at 110°c for 1h	cutting	0,250-0,420	cellulase 33U/ml at 50°c for 58h	0,362	374,28	N/A	Feng and Shifen 2011
hot compressed water (135°C for 60min) + wet disk milling ratio	cutter milling + supermasscolloider	3	cocktail enzymes (20FPU/g rice straw)	0,312	294,84	20L water	(Hideno, Inoue et al.

1:20 for 15 cycles	MKZA10		at 45°C for 72h				2012).
HNO3 pretreatment ratio 1:10(w:v)158.8°c for 5.86min	cutting	2	cellulase 30FPU/g cellulose at 50°c 170rpm for 72h	0,417	220		(Kim, Lee et al. 2014)
Torrefaction 220°c for 40min	cutting+ torrefaction +blender	<0,065	cocktail enzymes (cellulase+xylanase+b-glucosidase) at 50°C 150rpm for 72h	0,3625	201	N/A	(Sheikh, Kim et al. 2013)
Cholinium lysine ionic liquid 20%wt-water mixture at 90°C for 1h	cutting	0,150-0,200	cellulase 35U/ml at 50°c 200rpm for 72h	0,377	305,37	N/A	(Hou, Li et al. 2013)
1-buthyl-3-methylimidazolium acetate([BMIM]5OAc]) ionic liquid at 120°c for 3h with 5% solid loading	ball-milled	0,1 and 0,8	cellulase 20FPU/g + b-glucosidase30IU/g at 45°C for 72h	0,454	499,35	N/A	(Poornejad, Karimi et al. 2013)
N-methyl morpholine N-oxide (NMMO) ionic liquid at 120°c for 3h with 5% solid loading	ball-milled	0,1 and 0,9	cellulase 20FPU/g + b-glucosidase30IU/g at 45°C for 72h	0,449	481,8	N/A	(Poornejad, Karimi et al. 2013)
dilute sulfuric acid 1%	grinding	0,2-0,4	cellulase 1,93kU +himicellulase 1,93+ cellobiase 4,97kU at 50°C for 96h	0,38	580	N/A	(Hsu, Guo et al. 2010)
Dilute sulfuric acid 1% (w/w) 5min at 160°c	milling	3	cellulase 10FPU/g dry matter +b-glucosidase 15IU/g at 50°c 100rpm for 72h	0,366	500	N/A	(Chen, Chen et al. 2014)
H_2SO_4 pretreatment ratio 1:10(w:v)158.8°c for 5.86min	cutting	2	cellulase 30FPU/g cellulose at 50°c 170rpm for 72h	0,417	200	N/A	(Kim, Lee et al. 2014)

II.2.6 Advantages and drawbacks of pretreatments

All existing pretreatments have not only advantages but also provide some drawbacks, which depend on the final functionality of products (Table II- 6). Regardless of LC biorefinery, whatever the biological or thermo-chemical pathways, the pretreatment is necessary before subsequent steps in LC valorization. The pretreatments allow preparing the readily materials for next steps. In order to make the material more be reactive, the pretreatments have an important role to alter or modify the LC structure.

Table II- 6: *Advantages and drawbacks of different pretreatments*

Pretreatment	**Advantages**	**Drawbacks**
Mechanical	Reduce particle size as well as crystallinity of cellulose No water consumption	Very high energy consumption due to grinding/milling operation
Biological	Hemicellulose and lignin degradation Less of energy consumption	Low hydrolysis yield; long time High cost- effective
Physico-chemical	Lignin transformation Hemicellulose solubilization High glucose and xylose yields	High cost-effective Toxic waste Partially hemicellulose degradation
Chemical **Concentrated acid** **Dilute acid**	High glucose conversion At room temperature	High cost-effective ; corrosion Inhibitors formation
	Less of corrosion Less of for inhibitors formation	Degradation product Less of sugar concentration
Alkaline	Lignin and sugar solubilization Low cost-effective No inhibitors formation	Necessary to adjust pH Long time

The mechanical pretreatment via grinding/milling process, allows dissociating macro- and microstructure such tissues, organs and cells. The particle size reduction by mechanical

grinding process leads to decrease particle size that consequently increases specific surface area. This process could also reduce the cellulose crystallinity that hinders the accessibility of enzymes to cellulose. The efficiency of enzymatic hydrolysis in bioconversion can actually improve by reducing the particle size that makes more available surface area for enzymes or microorganisms. Furthermore, the particle size reduction via mechanical grinding has non-environmental impacts (no water consumption). However, this operation is considered highly energy consumption. The energy requirement depends on the final particle size as well as the some properties of material(Vidal, Dien et al. 2011) (Table II- 6). Barakat et al, 2013(Barakat, de Vries et al. 2013) reported that mechanical pretreatment is considered as dry fractionation process, which does not generate effluents and no water consumption. As mentioned that this processes is highly energy consumption, the mechanical pretreatment should be combined with other pretreatments in order to save cost and improve enzymatic hydrolysis yield.

The biological pretreatment is one of the most promising processes in term of low energy consumption. This process leads especially to degrade hemicellulose and lignin. However, the biological pretreatment needs long duration; it could be reached in 2 days to 1 month and low hydrolysis yields. Moreover, using enzymes or microorganisms have high cost (Table II- 6).

The physicochemical pretreatment such steam explosion leads to degrade lignin and solubilize hemicellulose, which enhances the hydrolysis yields. Steam explosion can be improved by combining with acid catalyst in order to increase the recovery of hemicellulose sugars. No matter how, the steam explosion is normally performed at high temperature which makes a high economic cost. In addition, it generates also toxic waste in residues (Table II- 6).

Concerning the chemical pretreatment, in this case could be divided into 2 kinds of techniques; acid and base (Table II- 6). About acid pretreatment, LC material could be pretreated with both concentrated and diluted acid. The use of concentrated acid seems the most promising technique in term of high glucose yields and it can be performed at room temperature. But it provides high cost and also the corrosive problems. Furthermore, this process leads to inhibitors formation that consequently hinders the enzymatic hydrolysis and fermentation. On the other hand, using dilute acid to pretreat LC material makes material more readily for hydrolysis and less cost effective compared to concentrated acid. The dilute acid has still limitation in term of sugar concentration. Some sugars have been partly solubilized during the pretreatment that causes the bioconversion yields being inadequate.

Another chemical pretreatment using alkaline catalyst, it mostly uses to pretreat cereal straw because it contains less of lignin compared to wood or others LC material (Table II- 6). The

alkaline catalyst could solubilize both lignin and hemicelluloses (partly) that are associated with cellulose, consequently more liberated cellulose to facilitate to hydrolysis. In addition, the alkaline catalyst has no inhibitor formation and also is cheaper than acids. However, the alkaline pretreatment uses a long reaction time to attain the desired bioconversion yields and it is necessary to adjust pH in case of ethanol fermentation.

As mentioned previously, all pretreatments have specific functions for altering or disrupting the LC structure. Now the best pretreatment processes can be only one process to pretreat LC material, which lead to obtain the best bioconversion yields. Because, there is numerous constraints such economic cost, technical constraint as well as environmental impact etc. It is still not clear that all pretreatment could pay off whatever it is deserved. Furthermore, it is important to note that no such processes are yet in industrial operation. In addition, the important cost performance assumptions for cost projections have not been verified at a commercial scale.

The recent LC pretreatment researches perspectives face to obtain the desirable pretreatment attributions as following conditions:

- Cost of chemical for pretreatment and subsequent neutralization and pre-bioconversion conditioning should be kept low
- Generation of wastes in pretreatment should be minimized
- Limited size reduction should be need prior to pretreat because biomass milling is energy intensive and costly
- Pretreatment should preserve hemicellulose sugars and make them more accessible for fermentation
- Pretreatment must promote high products yields in subsequent enzymatic hydrolysis or fermentation operation with minimal conditioning costs and little loss of sugar during processing
- Low enzymes loading should be adequate to realize greater than 90% digestibility of pretreatment of cellulosic material
- Pretreatment should facilitate recovering of lignin and others constituents for conversion to valuable co-products and to simplify downstream process

The effects of each pretreatment on the LC structure are summarized in Table II- 7 (Mosier, Wyman et al. 2005; Brodeur, Yau et al. 2011). The majority of these pretreatments aim

mostly to increase accessible surface area that consequently more available specific surface for enzymes attack(Margeot, Hahn-Hagerdal et al. 2009).

Table II- 7: *Effects of different pretreatments*

	Physical pretreatment		**Chemical pretreatment**					**Physicochemical pretreatment**		**Biological pretreatment**
	Milling/grinding	**Irradation**	**Acid**	**Alkaline**	**Wet oxidation**	**Organosolv**	**Ils**	**Steam Exploision**	**AFEX**	**White-rot fungi**
Accessible surface area	++	++	++	++	ND	++	++	++	++	++
Pore volume	++	++	+	++	0	++	+	ND	ND	++
Degree of polymerization	++	0	++	+	0	0	+	+	ND	0
Cellulose crystallinity	++	++	++	++	0	++	++	ND	+	0
Hemicellulose solubilization	ND	+	++	+	++	+	++	++	++	+
Lignin solubilization	ND	++	+	++	++	++	++	+	+	++
Inhibitors formation	++	ND	++	+	++	0	ND	++	+	0
Altering lignin structure	ND	++	++	++	ND	++	++	++	++	++

II.2.7 Assessment of pretreatment

The primary economic barrier of LC bioconversion is the cost of converting LC materials into valuable products in particular, the hydrolysis of cellulose and hemicelluloses into fermentable sugars. In order to obtain accessible LC material being readily to convert, the pretreatment belongs to one of the most important steps in LC biorefinery. In fact the pretreatments are about 40% of total cost in LC bioconversion. Whatever LC bio-based products, biofuels, biomaterials these need the pretreatment to alter or modify its complex structure. The LC bioconversion will pay off that we deserve or not, it depends on many factors and even the used technologies (Galbe and Zacchi 2007). The challenge hereby is to improve the technology sufficiently to realize these costs.

The recent effort in pretreatment processes is how to develop the pretreatment techniques in order to realize the total cost in LC conversion process.

In spite of almost pretreatment has its advantages but it also provides drawbacks. In the meantime recent discussed issue in LC pretreatment is how to reduce the intensive costs and to get more its bioconversion yields. The question has been proposed that could be beneficially pathway converting LC in all bio-based products. The LC biorefinery is considered as an analog petroleum refinery but the last one has an important cost investment and even the limitation of sources (Figure II- 13)(Hendriks and Zeeman 2009). For this reason LC material has been developed to be competitive to petroleum source.

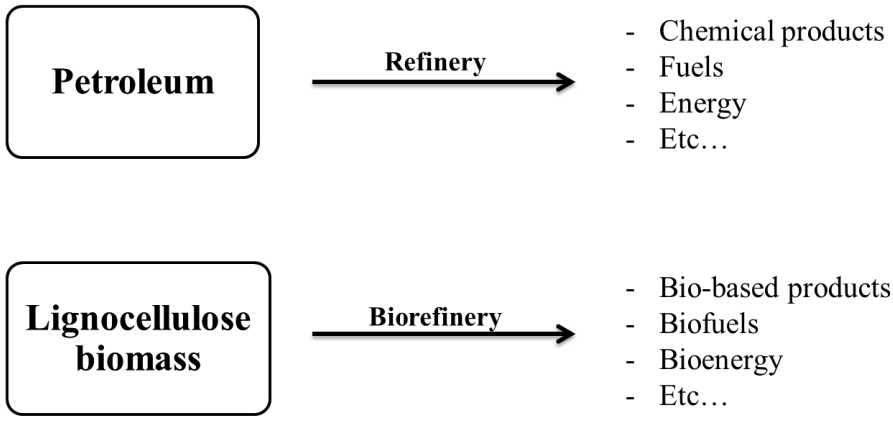


Figure II- 13 *Petroleum refinery and LC biorefinery*

To be competitive to petroleum refinery, the LC biorefinery must be more economical as well as less of environmental impacts compared to petroleum refinery.

The eco-conception in LC biorefinery has been recently developed in particular, the pretreatment process. Some drawbacks of pretreatment making LC bioconversion are inadequate in term of costs, environmental impacts.

Unavoidable step in pretreatment is particle size reduction process which consists of milling or grinding material to get particle size being more accessible to enzymes. This technique provokes high-energy consumption due to the grinding/milling process. High-energy requirement is an obstacle for biomass conversion transformation (Shadbahr, Zhang et al. 2015). The energy consumption depends on deeply the final desired particle size and some material features. Whatever grinding technology provides highly mechanical energy consumption. The appreciated challenge is how to decrease the costs by reducing energy consumption. As mechanical size reduction allows just only decrease in particle size and tissue dissociation, it is still insufficient to make biomolecules be more available and even more accessible to enzymes. The chemical pretreatments take place in order to modify structure and molecules. Furthermore, using chemical catalysts to pretreat LC material is still likely problematic in term of effective costs. Conventionally the chemical pretreatments consist of soaking the solid biomass material directly in the chemical solution and the separation liquid/solid step is necessary (Figure II- 14). From here, the chemical pretreatment by soaking generates some toxic waste/effluents that could be an environmental impact. In addition, ethanol fermentation from LC the pretreated biomass has to be neutralized in order to facilitate the enzymatic hydrolysis and fermentation. The washing by water is therefore to be applied. The water consumption is a problem in order to adjust the pH for the mild condition in subsequent step after pretreatment.

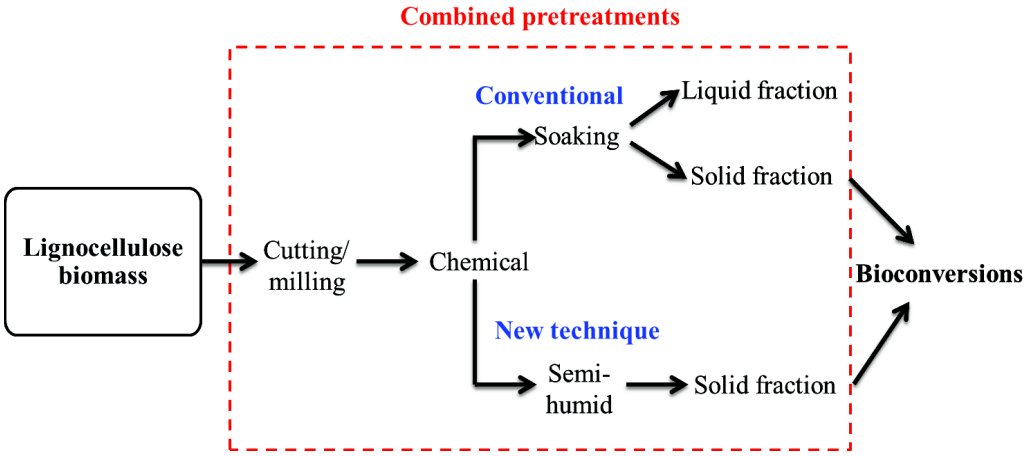


Figure II- 14 *Combined pretreatments of LC in biorefinery*

Nowadays, the development of pretreatment technologies has been brought up to realize in order to be an eco-conception of LC biorefinery. Numerous researches in LC pretreatment have shown that combination of pretreatments could respond to economical constraint and technical tasks. Barakat et al., 2014 (Barakat, Chuetor et al. 2014) reported that combination of chemical and mechanical size reduction allows decreasing in specific energy consumption. Using alkaline catalysts allowed obtaining the pretreated wheat straw more soft and consequently the energy consumption due to

grinding process has been reduced. In their study, instead of using soaking technique, they sprayed alkaline catalysts on biomass in order to eliminate the separation step. In addition, the semi-humid alkaline chemo-mechanical could decrease about 40% in total energy requirement and increase about 75% in total reducing sugars.

Ideal LC biorefinery is to save intensive cost, decrease energy consumption, non-effluent generation and improve bioconversion yields. Nevertheless, the economical, technical limitations have been presented as special obstacles and it brings up to realize again that the applied process will pay off what it deserves.

Dry fractionation technology is novel innovative technique that could get rid of some obstacles in LC biorefinery (Barakat, de Vries et al. 2013). Ultrafine grinding and separation technique have been considered as dry fractionation (Barakat, Mayer-Laigle et al. 2014; Barakat and Rouau 2014; Chuetor, Luque et al. 2015). In which the combination of two techniques allows preparing the fraction prior to biofuels as bioethanol. This technique has use of no water, chemical catalyst neither. In addition, this technique allows enrich/isolate constituents for further application. Recently the development of LC pretreatment must pay attention to be applicable to high loading solid and less chemical catalysts loading as well as less of hydrolyzed enzymes.

Assessment of pretreatment theorethically produces a disrupted, pretreated substrate that is easily hydrolyzed but avoids the formation of sugars degradation products and fermentation inhibitors. Assessment of biomass pretreatment processes depends on a parameter, which is defined as the combined effect of temperature, acidity and duration of pretreatment. Studies on biomass pretreatment have used the several parameters for comparing efficiency of pretreatment. LC pretreatment, for the purpose of improving the bioconversion of LC materials, has been studies for years. Although potential research has been developed various effective pretreatment on all kind of LC biomass feedstock, none pretreatment has not been industrialized for LC production due economic feasibility. The development of pretreatment has shown methods in collecting process performance date, and evaluating their economic feasibility. Currently, the research of LC pretreatment is facing several technic and economic challenges, including environmental impact, delignification, fermentation of hexose into ethanol, hemicelluloses and cellulose separation, energy requirement and intensive processing costs (Gnansounou, Dauriat et al. 2009; Singh, Pant et al. 2010; Limayem and Ricke 2012; Shadbahr, Zhang et al. 2015).

The research has been done on the development of advanced pretreatment technologies to prepare more accessible LC substrate to increase bioconversion yields. An ideal cost-effective pretreatment process might have been considered several following characteristics:

(i) Maximum fermentable sugars recovery;

(ii) Minimum inhibitors produced form LC degradation during pretreatment;

(iii) Low environmental impact;

(iv) Low requirement of post-pretreatment processes such as washing, neutralization and detoxification;

(v) Minimum usage of water and chemicals;

(vi) Low energy requirement;

(vii) Relatively high pretreatment rate;

(viii) Production of high value-added by-products

There is no doubt that the future research on LC pretreatment would pay in particular attention on the following points. Firstly, the reduction of water and chemical usage; secondly, improvement of recovery of carbohydrate and also value-added by-products, thirdly, development of delignification resulting the benefits of fermentation of pentose sugars with improved economic of pretreatment; fourth, understanding the fundamental pretreatment mechanism and the relationship between the biomass structure behaviors and bioconversion and the last, reduction of the formation of inhibitors which could significantly inhibit bioconversion (Gnansounou, Dauriat et al. 2009; Singh, Pant et al. 2010; Limayem and Ricke 2012; Shadbahr, Zhang et al. 2015).

II.3 Physical and Mechanical properties of lignocellulose powders

The LC deconstruction process could be analyzed as the combination of two aspects: product aspect and process aspect. The deconstruction is responsible to dissociate or alter the macro until microstructure. The fragmented LC here is constituted between product scale, which correspond to the granular media (powder) and microstructure scale: tissues and cell scale. There are many methods to characterize the evolution of pretreatment such macrostructure process scale (energy consumption) and microstructure (biochemical characterization). However, in order to understand the mechanisms of deconstruction, there is a lack of characterization in the intermediate scale between the macro and microstructure (Figure II- 15).

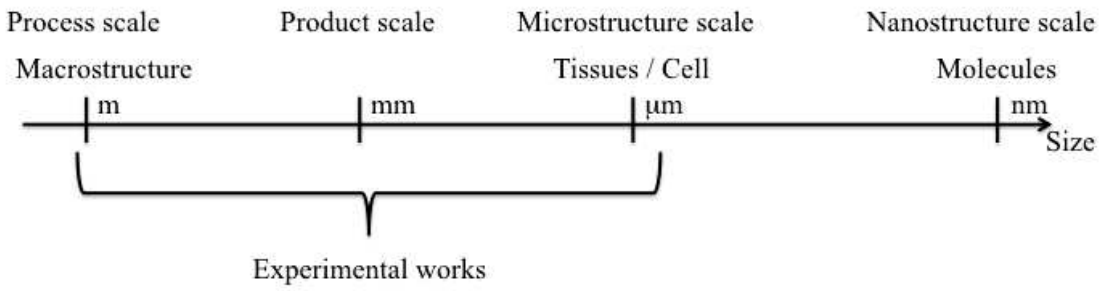


Figure II- 15 *Schema of the scale problem*

This work concerned the development of methods characterization, which is necessary in order to understand what happens during the transformation process as well as the evolution of deconstruction of LC material. Several characterization methods have been discussed in this following section.

II.3.1 Mechanical/Rheological properties

Lignocellulose pretreatment processes are considered as transformation or elaboration processes of LC substrate for subsequent step in biorefinery in particular bioconversion. These transformations represent the combination of two twin-aspects: process and product. This combination (process/product) represents the continuous in-dissociable in order to define a physical state and behavior presentation resulting in elaboration operation. In order to facilitate the LC bioconversion, the LC substrate has first of all to be reduced in particle size. Due to the mechanical particle size reduction process, the milled substrate has to be a small particle size that represents highly specific surface area and more accessible surface area to enzymes attacks.

Most LC particle size after pretreatment prior to bioconversion commonly comprises millimeter to micrometers. These intervals of particle size are sufficient to be reached the biodegradability ability. Because mechanical particle size reduction has a limitation in term of intensive costs and also technical limitations. As know that the potential LC biomolecules are located almost in plant cell wall that is about 1-10µm, the mechanical particle size reduction could not reach to rupture this layer. Various factors affect the dissociation of LC structure such as mechanical properties (hardness), size, shape as well as physicochemical characteristics (Annoussamy, Richard et al. 2000). One of the most important factors is mechanical property in particular hardness, which affect directly the disruption of tissues or organs. On the other hand, moisture content is also an important factor that affects the LC material disruption.

Concerning the eco-concept of LC biorefinery, the mechanical size reduction seems to be highly effective-costs that due to final desired particle size and mechanical properties of materials.

LC is considered as viscoelastic material that means the combination of both elasticity and viscosity of material. In order to use the LC material as renewable sources, it has to be ruptured and dissociated its macro and microstructure before using it as raw material in biorefinery. The dissociation or disruption of LC material, the mechanical properties is implied that include hardness, form, size etc (Annoussamy, Richard et al. 2000; Muller 2003).

The fragmentation of LC material therefore needs two operated parameters in order to understand the fragmentation mechanism. As mentioned, LC material is considered as viscoelastic material; it exists a limitation of deformation point during applying force, which corresponds to the rupture or

breaking down point of material. The mechanical properties knowledge could be relevant information in order to control the final products with improved functionalities. The functional products improvement could carry out via various processes such milling, mixing etc. In case of LC material, the biomass needs to be reduced in particle size before operating in subsequent step in order to increase the accessible specific surface for enzymes attacks. Once, LC material has been reduced in size via mechanical process, first information can evaluate the transformation process are the particle size and the shape of milled materials. These would be significant information in order to choose the operation technique corresponding to the final products. The mechanical characterization of LC particle is the way to study their structural organization. In addition, it will be the relevant information for engineer in order to model, establish as well as design equipment using in transformation process.

Many literatures data shown that the mechanical size reduction technique has a limitation in term of final particle size, which corresponds to 4-5µm. This particle size corresponds to the thickness of plant cell wall (http://www.ncbi.nlm.nih.gov/books/NBK21709/) (Dupuy, Mackenzie et al. 2010). The mechanical particle size reduction leads to destroy mostly the macro structure and the void of material. In order to alter or modify microstructure of LC material, the chemical pretreatment could take place before or after mechanical pretreatment. The combination of chemical and mechanical pretreatments would reach to modify both the microstructural level of material and increase in enzymatic accessibility (Barakat, Chuetor et al. 2014).

Concerning the rheological properties of the powder, it can be defined by Coulomb's law (Rondet, Ruiz et al. 2013). This law represents the "flowing" threshold of a granular media under applied shear and normal stress. The characterization of the coulombian behavior, leads to determine the friction and cohesion of material (Eq.II-1). As we known the transformation of material can modify the rheological properties such as flowability of product. The coulomb's law can describe in following equation:

$$\boldsymbol{\tau = \mu\sigma + C} \qquad \textit{(Eq. II- 1)}$$

Where: τ = applied shear (kPa), µ = coefficient of friction (kPa), = normal stress (kPa) and C = apparent cohesion (kPa)

The coefficient of friction is linked to the frictional internal angle: $\mu = \tan(\theta)$. Cohesion is dependent on the type of the material, size of the material grains and the packing of the grains and on the humidity of the granular material.

II.3.2 Kinetic of densification: the major models

The description of the relaxation kinetics of granular media submitted to collapses of fixed intensity is based, mostly the models on arguments of free volume and steric exclusion. These models have been present briefly chronological apparition. Kawakita model. This model allows describing the volume variation of powder submitted to the collapse system.

The description of the relaxation kinetics of granular media subject to tapping experiments is based, for most models of the literature, on arguments of free volume and size exclusion. The next paragraphs consist in a chronological order description of these models.

Kawakita model. This model leads to describe the variation of the powder volume according to tapping stresses (Kawakita and Lüdde 1971). For normalized tapping stresses, the volume reduction: $C = (V_0 - V_N)/V_0$ where V_0 is the initial volume of the bulk product and V_N its volume after *N* taps, is given by: $N/C = N/a + 1/ab$. Parameters *a* and *b* are associated to many properties of the product (Yamashiro *et al.*, 1983). According to (Rough, Wilson et al. 2003) *a* could be associated to intergranular friction and cohesion and also correlated with the Hausner index, while *b* would rather be related to the compressibility of the packed material. This relationship can be rewritten according to solid volume fraction (ϕ) as follows:

$$\boldsymbol{\phi = \frac{\phi_0(1+bN)}{1+bN(1-a)}} \qquad \textbf{(Eq. II- 2)}$$

Rough (Rough, 2003) experiments are closely fitted by this equation except when the number of taps is lower than 10. It has also been shown (Knight, Fandrich et al. 1995; Nowak, Knight et al. 1997; Philippe and Bideau 2002) that for a same raw material, very different behaviors are observed depending on the intensity of the taps and the initial solid volume fraction. *a* and *b* values are consequently highly variable. The "physical" interpretation of these parameters is only based on correlations.

Kohlrausch-Williams-Watts model (KWW model). KWW model also called modified Heckel model (Yu and Hall 1994) is a famous empirical law largely used in physics (Eq. II-3). It does not have any physical justification at the local level but it can describe correctly the densification under tapping of a granular medium:

$$\boldsymbol{\phi = \phi_\infty - \Delta\phi exp\left(-\left(\frac{t}{\tau}\right)^{\beta}\right)} \qquad \textbf{(Eq. II- 3)}$$

In Eq. II-3, *t* is the time in a process of densification under tapping at regular intervals ($t \propto N$), ϕ_∞ is the compactness at the end of experiment with: $\Delta\phi = \phi_0 - \phi_\infty$; ϕ_0 is the initial compactness of the bed. The parameter τ is a characteristic relaxation time, quantifying the capacity of progressive

arrangement of grains until final steady-state, while β is an adjustment parameter. The monitoring of the evolution of compactness according to the number of taps by capacitive or gammametric measurements, as well as numerical simulations by Philippe and Bideau, 2002 (Philippe and Bideau 2002), showed that this model fits experimental results for a wide range of accelerations. This confirms the robustness of this exponential model that can be used in a broad range of situation. There is Arrhenius interdependency between the characteristic relaxation time and acceleration, which plays here the role of granular temperature. This confirms the analogy between weakly solicited granular medium and glassy systems within the meaning of the "jamming systems" (D'Anna, Mayor et al. 2003; Richard, Nicodemi et al. 2005)

« Chicago » model. The works carried out by the team of the James Frank Institute of Chicago (Knight, Fandrich et al. 1995) are the most famous in the field of the densification under vibration of a granular media. After they highlight the bad fitting of the different types of relaxation model (Hong, Yue et al. 1994; Knight, Fandrich et al. 1995) to their capacitive measures monitoring the evolution of the compactness of a monodisperse glass bead bed according to the number of vertical taps, these authors developed a three parameters heuristic law (Eq. II-4):

$$\boldsymbol{\phi = \phi_\infty - \frac{\Delta\phi}{1+k\ln\left(\frac{t}{\tau}\right)} = \phi_\infty - \frac{\Delta\phi}{1+A\ln(1+N)}} \qquad \textbf{\textit{(Eq. II- 4)}}$$

In Eq. II-4 k is a fitting parameter and τ a relaxation time related to the rearrangements in compact granular media. Philippe and Bideau, 2002 (Philippe and Bideau 2002) have found that relaxation time was related to the intensity of the tapping according to a relation implying the existence of an intensity threshold playing the role of "potential barrier" regarding the granular mobility in compact systems. For constant vibration frequencies: $t \propto N$, the relation can be rewritten in a way that explicitly makes appear the number of taps (Eq. II-4), where A is a fitting parameter that integrates k and τ. It is this last form, which is the most used. Mathematically, this equation expresses the succession two phases. The first one is characterized by a rapid rearrangement of the medium. The second one is characterized by the fact that the steady state is reached asymptotically. This equation has been experimentally validated on a population of monodisperse glass beads, on a wide range of tapping conditions. It has also been successfully used to fit experiments carried out on other granular systems (rough surface powders) and other geometrical conditions of experimentation. The confrontation between experiments and simulations (Remond 2003) also strengthens the robustness of this model. Philippe and Bideau, 2002 (Philippe and Bideau 2002) showed however that, in the specific case of dry granular media, the experimental data was however best fitted by a stretched exponential equation as for example the Kohlrausch-Williams-Watts (Eq. II-3) model. Furthermore, experimental results of Rosato and Yacoub, 2000 (Rosato and Yacoub 2000) highlight the relative

independence of the model regarding the way vibrations are applied. However they show that the final steady state density obtained thanks to the "Chicago model" is always higher than that obtained experimentally. It is the major drawback of this model for which ϕ_∞ becomes a fitting parameter.

Despite these unsatisfactory experimental confrontations, this heuristic model was theoretically explained thanks to a local description at the grain scale. Boutreux and de Gennes, 1997 (Boutreux and deGennes 1997) have developed a model at the grain scale, which is based on a free volume approach linking compactness to the grain mobility. This model makes it possible to find an equation, which is mathematically really close to that of the Chicago team. The work of Fiscina *et al.*, 2010 (Fiscina, Lumay et al. 2010) can also be mentioned in this context. They extended the scope of this equation to wet and cohesive granular media by linking the fitting parameters to the capillary properties of the liquid-solid interaction.

These bibliographical elements lead to analyze the following conclusions. First of all, non-proposed laws are not really a recognized authority. Each of may correlate suitably the experimental results under certain conditions without being able to be applicable in a universal way. Furthermore, it is specified that these laws are empirical. The laws bring in, for the most part of between them, 2 or 3 parameters of wedging the physical meaning of which is either disputed, or rest to be demonstrated. We try to qualify the evolution of the properties of densification and indirectly the properties of flowing, according to the grinding, also, we choose to confront the experimental results with these models and to hold only one which can great extrapolate.

II.3.3 Lignocellulose particle considered as unsaturated wet granular matter

Wet granular medium regroup an abundant diversity both in the mineral and in the reigns of the alive. LC biomass is considered as raw material in transformation is realized in order to elaborate product more highly value-added. In order to valorize the LC material especially in bioconversion step, that has to be typically a powder form, resulting an increased specific surface area. LC substrate particle contains broadly three dispersed phases: liquid, solid and gas, which are in interaction via interface issues of LC behaviors. During the transformation or elaboration processes, the LC particle properties have been relatively changed, depending on its features.

Application of transformation, which under effect of mechanical, thermal constraint and/or hydric, contribute to the evolution that we deserve to control the granular material towards usage function of final product. This transformation represents the combination of both aspects creation/texture of products and performance operated processing. Understanding of perspective aspects (product and process) is an analysis some descriptive parameters of granular matter during the transformation process.

The humid granular media consists of specific class of materials, simply characterized by: (i) discrete complexion according to cohesion, (ii) particle size and (iii) its features as flowability Figure II- 16.

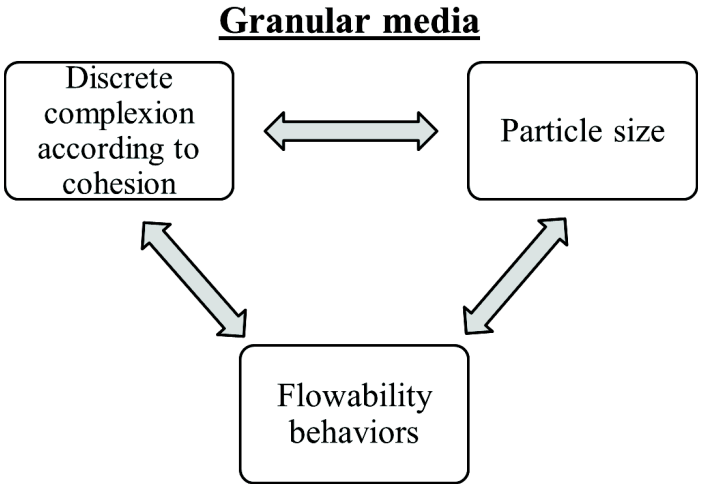


Figure II- 16 *Specific class of wet granular matter*

LC material is considered as unsaturated wet granular media, which contains broadly three dispersed phases: solid, liquid and gas. LC is also considered as heterogeneous form with a mixture of fibrous form and sphere form. An unsaturated heterogeneous media is constituted by the superposition of three different and scattered phases: solid, liquid and gas (Figure II- 17). This phenomenological approach, which is based on the theory of fields applied to mixtures, allows defining the densities state parameters on the scale of elementary volume. Once defined, these densities state parameters are treated as continuous fields. For this thermodynamics three-phase system, we adopt the definitions of the usual state parameters in wet granular media. As three-phases are deformable, six densities parameters (three masses and three volumes) are necessary to put into the mass transfer. These six densities are established by using these based quantities (Table II- 8).

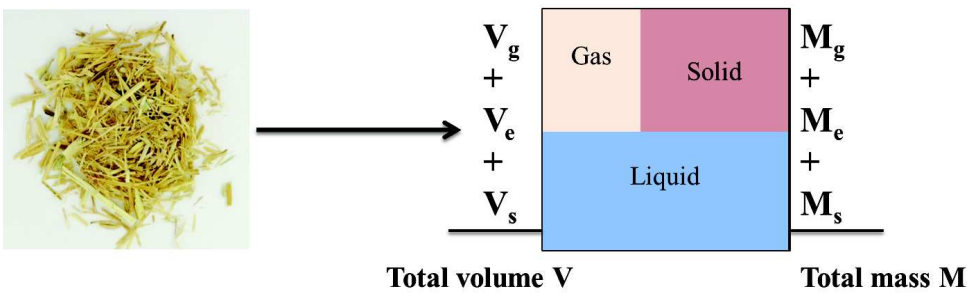


Figure II- 17 *Wet granular media schematic and state parameters of wet granular media*

Table II- 8: *Densities state parameters of wet granular media*

Real densities	Apparent densities
$\boldsymbol{\rho_s^* = \frac{m_s}{V_s}}$	$\boldsymbol{\rho_s = \frac{m_s}{V}}$
$\boldsymbol{\rho_e^* = \frac{m_e}{V_e}}$	$\boldsymbol{\rho_e = \frac{m_e}{V}}$
$\boldsymbol{\rho_g^* = \frac{m_g}{V_g}}$	$\boldsymbol{\rho_g = \frac{m_s}{V}}$

Three real densities are measurable by using Nitrogen pycnometer and mass and volume of gas are generally negligible. These grandeurs allow evaluating three standard variables as following table (Table II- 9):

Table II- 9: *Standard variables of unsaturated wet granular media*

Solid real density ($\boldsymbol{d_s^*}$)	$\boldsymbol{d_s^* = \frac{\rho_s^*}{\rho_e^*}}$
Moisture content based dry basic (*W*)	$\boldsymbol{w = m_e/m_s}$
Degree of saturation *(S)*	$\boldsymbol{S = V_e/(V - V_s)}$
Compactness *(ϕ)*	$\boldsymbol{\phi = V_s/V}$

Unsaturated wet granular media has to be considered under following assumptions:

(i) Solid phase which corresponds to the solid matter defined by nitrogen pycnometer, is notdeformable

(ii) Liquid phase is incompressible

(iii) Gas phase is thermodynamically equilibrium with liquid phase (dissolution and vaporization of liquid negligible)

In order to follow all relevant parameters (ϕ, w) during transformation process, a hydro-textural diagram has been established. This diagram is limited in upper part by saturation curve: $\phi_{sat}^{-1} = 1 + d_s^* w$ (where d_s^* represents real density solid). Beyond this curve, the system loses physical realism as mixture state. The saturation curve represents the maximum moisture content that all of existent pores could be filled up by liquid phase (*i.e.* solid « saturated » by liquid).

II.3.4 Hydro-textural approach

The presentation of multiphase heterogeneous physical state of wet granular media could represent in hydro-textural diagram. This hydro-textural diagram is a phase diagram that represents three sufficient parameters descriptive of physical state of wet granular media. The description of macroscopic state results the connectivity transition as well as consistent rheological transition. The hydro-textural diagram of wet granular media is a graph representing compactness (ϕ) and moisture content (w).

Saturation state

The simple hydro-textural state description corresponds to the saturation in liquid. Under the hypothesis of incompressibility of these phases, the total volume is then the sum of the solid volume and the liquid volume. This volume addition is translated a relation between the moisture content and the compactness as following:

$$\boldsymbol{\phi_{sat} = \frac{V_s}{V_s + V_e} = \frac{1}{1 + d_s^* W}} \qquad \textbf{\textit{(Eq. II- 5)}}$$

The ratio of the particles densities and liquid is the only parameter, which controls the saturation state of wet granular media. The saturation curve is the first one information that is drawn on the hydro-textural diagram (Figure II- 18). The hydro-textural zone is located under the saturation curve that corresponds to the unsaturated states. The states situated above the saturation curve are not susceptible to the mixtures. They correspond to a separation of phase (exuded liquid, sedimentation).

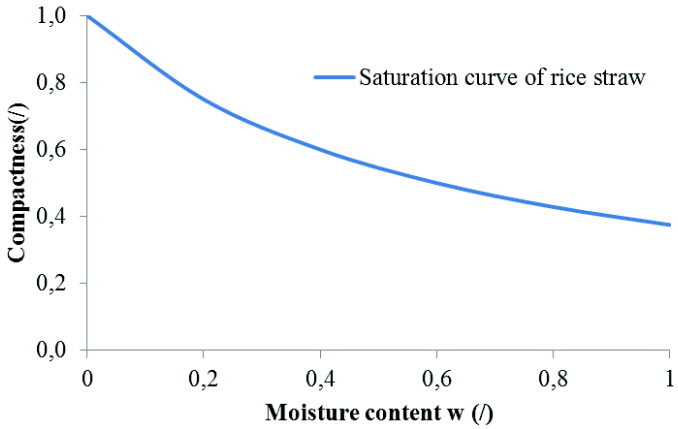


Figure II- 18 *Saturation curve of rice straw with* $\boldsymbol{d_s^* = 1.67}$

The deformation or the swelling of particles due to absorption of water can vary the density of particles. Moreover, the swelling some biopolymers operates an intrinsic variation of density. Furthermore, the fermentation of a biological granular matrix can also lead to vary density of the dry materials. The solid density might change not only with the hydration but also with the constraints of implementation of the wet granular; provoke a particle fragmentation constituted by various agglomerated raw materials. It is therefore necessary to consider a real solid density (d_s^*) as moisture content or operating parameters. For each case, a mass variation is concomitant in the variation of volume. In addition, it can also imagine that a thermal expansion or that an intrinsic removal of liquid can be a variation of real solid density. But dissolution or precipitations of the particle is unchanged the real solid density.

The hydro-textural diagram reveals transitions of phase essentially due to the loss or the gain of constituent's connectivity as well as to the rheological consistency of the mixture. The capacities of the wet granular media in state's transformations are thus represented in the same coherent. It is suitable to introduce the influences of the transformation processing applied to the system, by integrating their specific operating capacities. One is the mixture and other is applied process; this principal division is resembled the diagram of the phases. Indeed, considering in particular transformation as a texturing by collapse or compaction or fluidization, either a transfer as a drying, a filtration as well as an imbibition; it is possible to analyze the influence of one or several representative operating parameters, on the state hydro-textural.

II.3.5 Hydration or imbibition methods of LC material

Concerning chemical pretreatment process in particular semi-humid system; the chemical catalysts have been used to spray on the LC material. The liquid absorbed ability of LC material is one of the most important parameters, which could define the performance of this technique. As known that

LC material is a hydrophobic material that resists the liquid or water. It may eventually hinder the liquid penetration into microstructure of material and consequently the efficiency of pretreatment decreases. Many studies reported that porous material can store significant amount of liquid because of its available pore in material, while Coppens et al., 2007 showed that the water storage capacity of rye and rape residues was modified during their decomposition (Coppens, Garnier et al. 2007; Beaugrand and Berzin 2013; Iqbal, Beaugrand et al. 2013). However, there are few studies addressing the relationship between the maximum liquid content and LC material characteristics in agricultural systems. Various studied domains are used to explain the adsorption phenomena of this material, it is well understood that plant cells are composed of hydrophilic (cellulose and hemicellulose) and hydrophobic (lignin) constituents combined in a cohesive network that affects liquid penetration. At the plant cell wall level, liquid retention can be influenced by the presence of micro- and macropores that probably open or close and become saturated with water. Changes in water storage characteristics might be resulting both from structural and chemical transformation that occurs during decomposition. The hypothesis of imbibition study is that either physical properties (morphology, size, and density) or chemical behaviors (biochemical composition), or both, would evaluate water storage abilities. The study of hydration or imbibition methods allows understanding the mechanism of liquid penetration as well as optimizing the semi-humid system.

II.3.6 Physical and physicochemical properties of LC material

As mentioned that the utilization all potential constituents is hindered by its complex recalcitrant structure such as inter, intra molecular associations, interaction between components as well as all linkages.

Recent research on LC bioconversion development efforts indicates that the path forward should focus on overcoming recalcitrance based on the chemical and structural nature of biomass. Generally, the chemical properties of biomass and its deconstruction tent to be correlated with overall conversion yields, while physical biomass properties usually affect processing costs through cutting/milling energy requirements. However, the physical and the chemical changes in biomass are usually closely related, to the extent that they are often difficult to manipulate independently, and therefore, the distinction and their relative importance remain poorly understood. In addition, these characteristics of LC make the hard way to transform biomass into product. The biorefinery normally requires several steps (mechanical, chemical and thermal) for increasing the conversion yields. Each step requires an efficient method of conveying material.

The first transformation of LC material is pretreatment. The pretreatment step makes in particular physical LC substrate structure change in order to be more accessible and be readily for subsequent

steps. In literature, several physical features of LC biomass affects its transformation such particle size, specific surface area, cellulose crystallinity, pore volume, degree of polymerization and other measures of accessibility.

These physical and physicochemical characteristics could change during the pretreatment process, it is common and unavoidable that processes that change one property also affect other (Vidal, Dien et al. 2011). In addition, the studies of mechanical properties are required for optimum design of equipment used in transporting, processing and store the material (Ghorbani, Hemmat et al. 2012). Physically, pretreatment processes aim to rupture, fracture and alter the complex structural organization of biomass. During these processes, there are changes of its properties in order to enhance biological degradation processes. The changes in properties of material are significant, which might be improving any characteristics of biomass. The Table II- 10 summarizes the desired properties of LC substrate in order to improve the bioconversion yields. For example, mechanical pretreatment (grinding/milling) could change a morphological behavior as well as some physical properties such particle form, fibers or sphere form.

Table II- 10: *Physical and physicochemical properties enhancing the LC bioconversion*

	Desired functional LC substrate
Physical properties	
Particle size	Decrease to increase accessible surface area
Form (elongated/sphere)	Produce identical elongated particle with large surface area
Cellulose crystallinity	Reduce to liberate more cellulose to enzymes attacks
Depolymerization	Decrease to facilitate enzymatic hydrolysis
Intra-pore volume	Increase to have more accessible zone for enzymes attacks
Physicochemical/chemical properties	
Lignin content	Lignin solubilization enhancing enzymatic hydrolysis
Hemicellulose degradation	Isolate hemicellulose in order to convert in fermentation
Linkages between compositions	Break down the linkages resulting increase the enzymatic degradation

Concerning the physical characteristics, the particle morphology (size and shape) during pretreatment is at the intersection between chemical and physical changes (Dibble, Shatova et al. 2011). Beside, experimental and theoretical investigations indicate that physical properties influence biomass dynamic including drying, heating and reaction rate (Lu, Ip et al. 2010). Particle size reduction is a primary task that is possibly important for the following reasons. Frist, particle

size reduction makes it convenient for incorporation into harvest and postharvest. Second, particle size reduction is an energy intensive process, with potentially significant feedstock cost implication. Last, particle size influences on biomass storage and transportation (bulk density)(Vidal, Dien et al. 2011). Mani et al., 2006 reported that different LC material has a different capability to rupture mechanically which resulting the different mean particle diameter. Lu et al., 2010, reported also that the particle size and shape of wood biomass reacted differently during enzymatic hydrolysis according to its shape and particle size (Lu, Ip et al. 2010). Guo et al., 2011,(Guo, Chen et al. 2012) noted that particle shape and size distribution influenced the flow behavior of biomass particle another example, corn stover was the finest compared to another biomass (wheat, barley straw) when using the same mechanical technique. This difference might be due to the difference in mechanical properties. In addition, they also reported that the moisture content influenced the mean particle size (Mani, Tabil et al. 2006). Ghorbani et al., 2012, reported that the bulk density increased with a decrease in mean particle diameter of alfalfa grinding by hammer mill. Additionally, the bulk density also increased when the moisture content increase(Ghorbani, Hemmat et al. 2012). Another example, separation technique can fractionate or alter the particle size, shape, and chemical composition (Barakat and Rouau 2014; Chuetor, Luque et al. 2015). The understanding of the physical changes the biomass may be undergoing can be obtained from studying its rheological changes during all step involved (pretreatment, saccharification, fermentation, separation). The physical properties of cellulosic material cause cellulosic slurries to be very viscous at high solids loadings, which make mixing difficult in reactor. This cause a difficult process, since the quality of mixing between enzymes and substrate is drop and provoked an insufficient rapid reaction rates. Long fibers, large particle size, can entangle and resist flow properties.

On the other hand, the chemical pretreatment could also change especially the biochemical composition as well as physicochemical properties. The associations between compositions make the enzymes attacks difficult. In order to dissociate or rupture the linkages, chemical pretreatment could take place. For example, acid pretreatment could break down all liaisons in LC networks in particular solubilize lignin and partially hemicellulose. Alkaline pretreatment commonly using NaOH, is responsible to degrade the ester linkages between hemicelluloses and lignin.

Interestingly an important proposed question is how to identify and follow the mechanisms involved during pretreatment processes. The studies of mechanisms involved during biomass transformation, allowed to understand how the biomass structure was gotten organized. The physical, mechanical and rheological characteristics are represented to be efficient relevant parameters to describe the structure of material.

The method characteristics of biomass throughout various stages of pretreatments would greatly facilitate the design, implementation and characterization of industrial biorefining operations. The transformation process of biomass generally need many operations such as chopping, grinding, thermodynamic, chemical which contribute to the evolution of raw material toward its final use properties. This transformation couples the product properties and the process capability. In order to identify the impact of the product and process aspects in the transformation, the follow-up of relevant description parameters of raw material throughout the transformation process has been concerned. So far, the properties concerning the changes of LC material during the transformation processes could be divided into two categories: physical and mechanical/rheological properties.

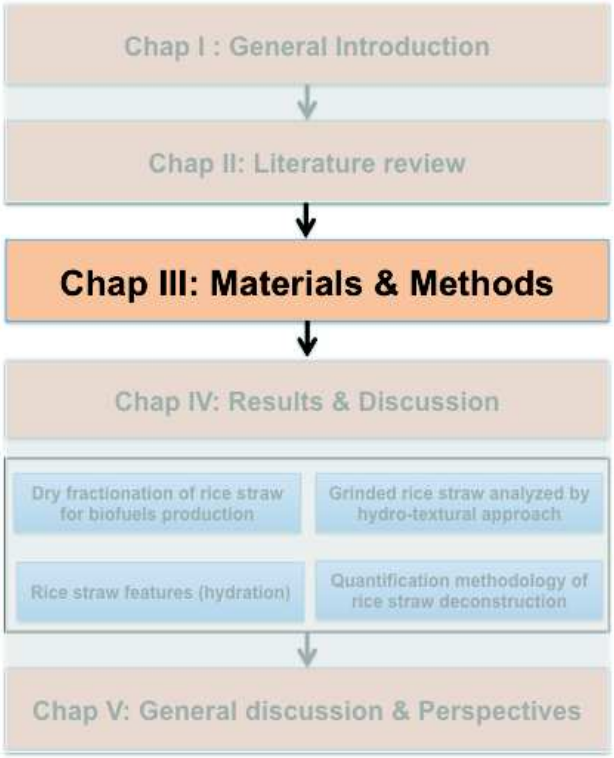
The proposed hypothesis at the present is that the pretreatments have an effect on rheology and the heat and mass transfer of the reacting LC substrates (Dibble, Shatova et al. 2011). Practically, rheology gives us insights into biomass slurry handling parameters, like pumping requirements and mixing feasibility. Fundamentally, the rheology can be a macroscopic measure of changes to constituents' particle including solids loading, particle size and size distribution, and surface chemistry. For non-colloidal spheres and fibers forms, the breadth of the distribution primarily causes the effect on rheology, which is rather than the median particle size. In addition, the mechanical properties are also important to describe the transformation of material. The need of knowledge in frictional behavior is key point that could be efficient parameters to design of equipment and to predict of flow behavior. The Flowability is a measure of quality of powdered materials that influences the final products in chemical, mineral, and food industries. There are three key parameters that are described the flowability: cohesive strength, friction and compressibility (Knowlton, Carson et al. 1994). These three keywords could be affected by physical properties such as moisture content, particle size, particle size distribution and shapes.

Rondet et al., 2013, (Rondet, Ruiz et al. 2013) reported that the moisture content affected the rheological and mechanical characterization of wheat semolina by increasing the coefficient of friction and the cohesion of agglomerates powders. Mani et al., 2004, reported that the moisture content of corn stover grind makes the increase of friction coefficient. Moreover, the compressibility of corn stover grind may depend on the particle size distribution. If more fine particles are present in the sample, they will fill in the void spaces, resulting in higher compressibility (Mani, Tabil et al. 2004). Similarly to the results from Ghorbani et al., 2012 with alfalfa grind (Ghorbani, Hemmat et al. 2012). Regardless of material, the moisture content is one of important parameter, which could describe the structural material. Mostly, the increase in friction coefficient with moisture content may be explained by the increase in adhesion between the grinds and the surface at higher moisture contents.

At the moment, the physical, physicochemical and mechanical properties affect the LC deconstruction. In order to facilitate the enzymes attacks and to make more accessible substrate, the applied pretreatment processes have to improve in term of further applications and the desired functional substrates.

Chapter III:

Materials & Methods



Ce chapitre consiste à présenter les matériels ainsi que les méthodes utilisés afin de réaliser ce travail de thèse. Nous divisons ce chapitre en quatre sous-chapitres qui sont rédigé en anglais ; mechanical size reduction, separation technologies, chemical pretreatment et enzymatic hydrolysis and fermentation. Dans un premier temps, nous présentons les techniques de broyages qui permettent de réduire la taille de la matière lignocellulosique aux différentes tailles caractéristiques selon les modes de sollicitation. Nous présentons ensuite les techniques de séparation, dans ce sous-chapitre nous nous intéressons à produire des fractions de paille de riz avec différentes propriétés afin de produire des molécules plateforme pour la bioconversion. Troisièmement, nous présentons les méthodes de prétraitement chimique, spécifiquement par une solution alkaline (soude) en différentes modalités : semi-humide et humide. Enfin, dans le dernier sous-chapitre, nous détaillons les méthodes de bioconversion : hydrolyse enzymatique et fermentation.

III. Materials & Methods

III.1 Mechanical size reduction

The size reduction process is indispensable in LC biorefineries. In order to produce the different particle sizes, the size reduction process by milling/grinding is necessary. After literature reviews, four types of milling; cutting mill, impact grinding, centrifugal grinding and vibro-ball milling were used in this study to produce different particle size (Figure III- 1). Each technology was performed in order to produce different particle size such coarse particle (300-1000µm) which was obtained by using cutting mill. To obtain intermediate particle (100-300µm), there is two grinding technology (impact and centrifugal grinding). For fine and ultrafine particle, the sample was obtained by using vibro-ball milling according to grinding time.

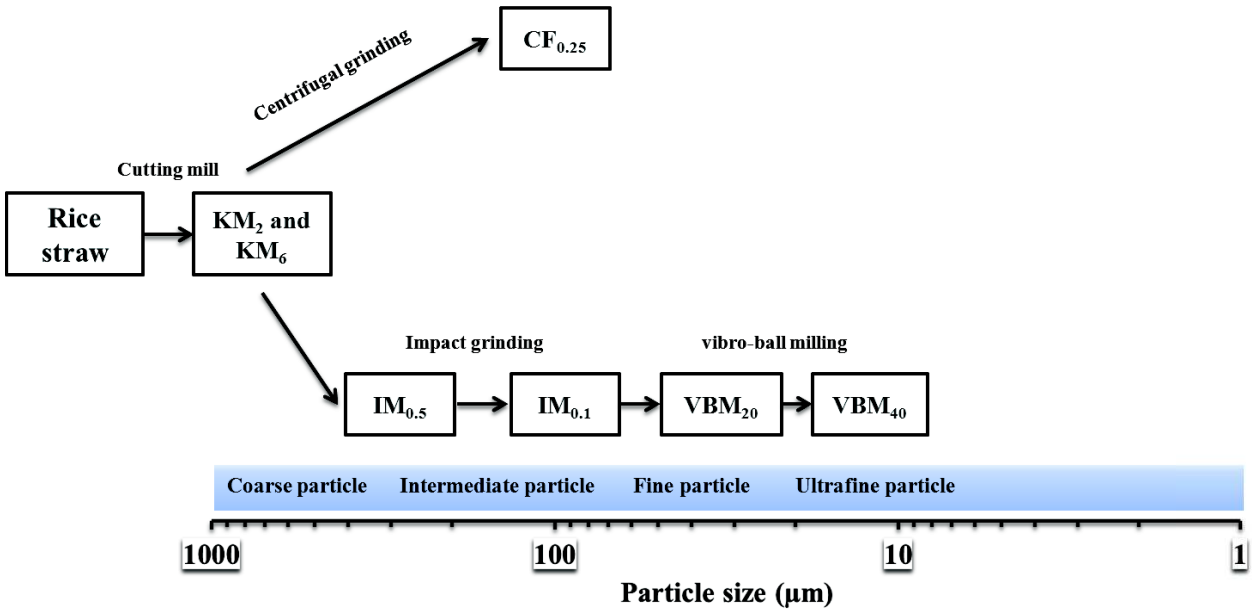


Figure III- 1 *Diagram of rice straw reduction and sample names*

III.1.1 Raw material & Starting material (Coarse particle)

Rice straw (RS) is obtained from a local farm in Languedoc-Roussillon region, south of France. The straw is beforehand dried and its moisture content corresponds to the hygroscopic state at the conditions of temperature and relative humidity of the laboratory. The material is constituted the fragments of dry straw with the size of some centimeter. For starting material, the RS was first cut by mill equipped with 6 mm and then 2mm sieve (SM200, Retsch, GE) (Figure III- 2).

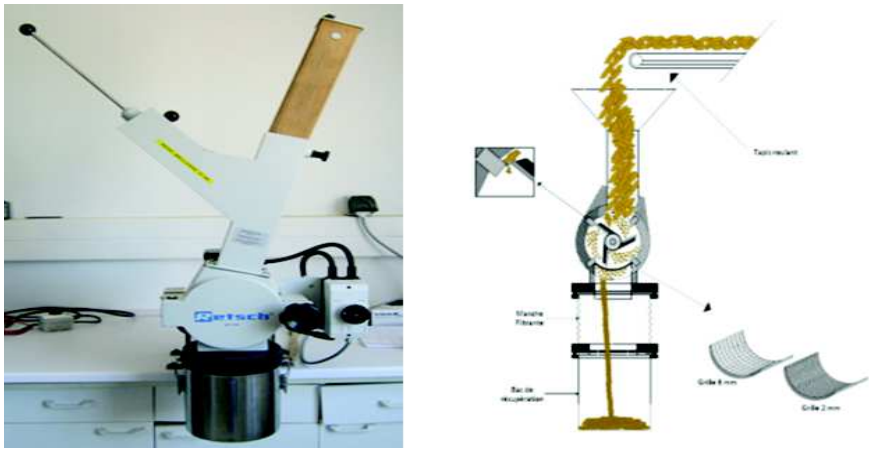


Figure III- 2 *Cutting Mill equipped 6 and 2mm sieve (SM200, Retsch, GE)*

III.1.2 Intermediate particle

In this part using an impact grinding experiment, KM_2 was successively ground in an impact mill type 100 UPZ Pallet (Hosokawa Alpine, GE) (Figure III- 3). The milling was operated at 18000rpm and equipped with a selection sieve, 8-teeth rotor and output recovery by cyclone associated with an aspirator. This type of milling uses a hammer rotor and a screen sieve, which induces impact between particles and rotor in high-speed rotation. The particle passed a sieve when their size was smaller than sieve size, 0.1 and 0.5mm in this study. The obtained samples were called $IM_{0.5,}$ $IM_{0.1}$ and they were ground at room temperature.

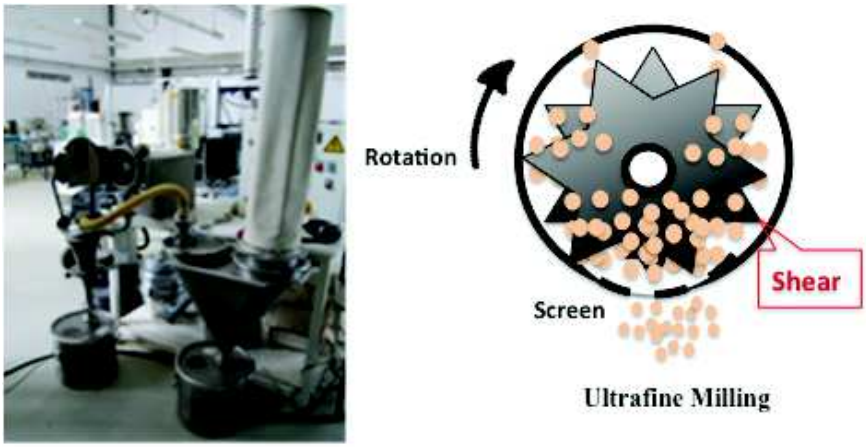


Figure III- 3 *Impact milling type 100 UPZ pallets (Hosokawa Alpine)*

Another type of milling technology in order to make an intermediate particle is centrifugal milling. The centrifugal milling was carried out with a centrifugal grinder type ZM200 (Retsch, GE) equipped with a 0.25mm sieve, a rotor with 12-teeth, and output recovery by cyclone equipment (Figure III- 4). The optimization conditions to operate and avoid the heating effects were obtained at 12000rmp rotation speed at room temperature. The obtained sample was called $CF_{0.25}$.

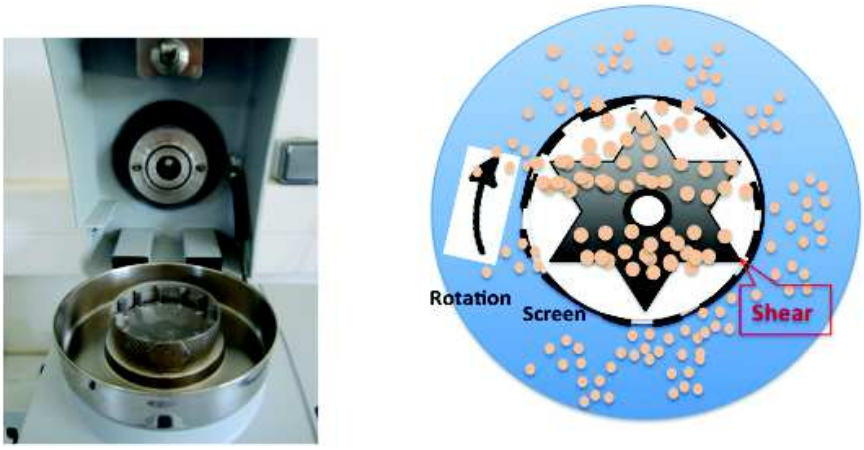


Figure III- 4 *Centrifugal milling type ZM200 (Retsch, GE)*

III.1.3 Fine and ultrafine particle

The $IM_{0.1}$ was ground by vibro-ball milling type MM400 (Retsch, GE) to obtain an ultrafine fraction with particle size less than 100µm (Figure III- 5). The vibro-ball milling consists of grinding jars, which perform oscillations in a horizontal position. The grinding ball causes them to impact with high energy on the particle. Also, the movement of the grinding jars combined with the movement of the balls result in the intensive mixing of the particle. In this case, grinding was performed at room temperature for 20min or 40 min. The respective obtained fractions were centered on 50µm and 25µm, and were called VBM_{20} and VBM_{40}.

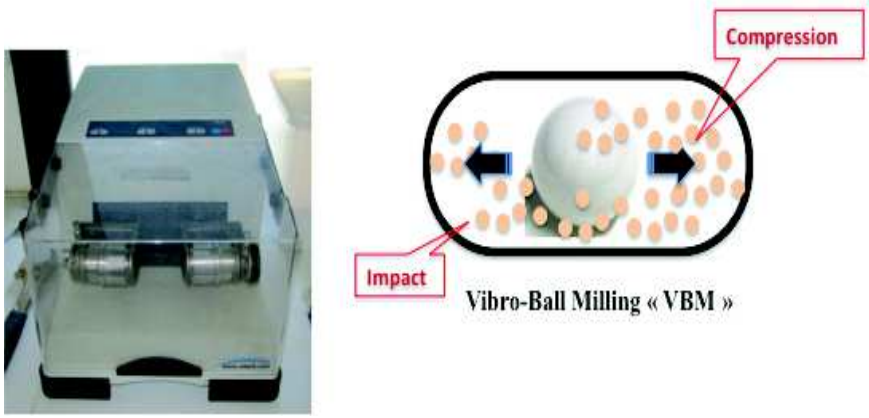


Figure III- 5 *Vibro-ball milling type MM400 (Retsch, GE)*

III.2 Separation technologies

In this study, the dry fractionation has been developed in order to obtain the different physicochemical properties as well as controlled fractions for its further applications. The dry fractionation is a combination of fine grinding and separation techniques. The separation

technologies used in this study are electrostatic fractionation (EF-T) and turbo-fractionation technology (TF-T), which were described in a following part.

III.2.1 Electrostatic Fractionation Technology (EF-T)

A pilot electrostatic separator (TEP System, Tribo Flow Separations, Lexington, USA) was used for the production of various fractions displaying different compositions, using ultrafine particles as starting material. This electrostatic separator is illustrated in Figure III- 6. The feeding system of the separator was operated at 150rpm; the particles were then conveyed by compressed air in a charging line where they were charged by tribo-electricity, by impacting each other and impacting against the walls of the charging line. The charged particles were then put in a separation chamber containing two high voltage electrodes (10,000 V), where the positively charged particles are attracted by the negative electrode and the negatively charged particles are attracted by the positive electrode. A particle recovery system equipped with two cyclones allowed to separately recover two fractions (one containing the positively charged particles and the other, the negatively charged particles). These two separated fractions underwent a second separation step, giving four different fractions. When the starting material was “F0”, only two separation steps were carried out: the fractions “F1A-” and “F1B+” were obtained from F0 as a result of the first separation step, while the fractions “F2A-” and “F2A+” were obtained from “F1A-”, and the fractions “F2B-” and “F2B+” were obtained from “F1B+” as a result of the second separation step. It should be noted that, in this process, the “F2A-” and “F2B+” fractions are the ones issued from the most direct lineage (2 negative steps for “F2A-” and 2 positive steps for “F2B+”.

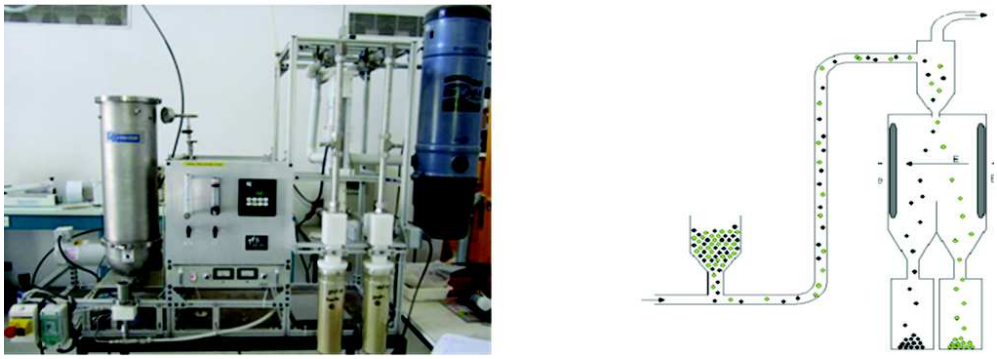


Figure III- 6 *Electrostatic fractionation technology (EF-T)*

III.2.2 Turbo-Fractionation Technology (TF-T)

An air classification or turbo-fractionation technology (TF-T) consists of a pilot (Hosokawa alpine Japan), which was used to produce coarse and fine particles displaying different densities with an adjustable screen size limit (Figure III- 7). This separator works by air aspiration through a selector

and collection organ. The core element of the fine sizing plant is the 50 ATP Turbo-separators, a bucket-wheel classifier with a classifier wheel of 50 mm diameter. The classifier blows air through the classifier wheel from the exterior into the interior and discharges the fine particles. At the same time, the coarse particles are rejected by the classifier wheel and fall into the coarse particle receptacle. The rotational speed and classifier airflow must be set according to the desired screen size limit. The particle feeding was operated at 1kg/h with different rotational speeds between 3000-12000rpm. At each operated speed, the particles were separated into two fractions; the coarsed particle fraction was denoted as "Cf" with respect to the fine particle fraction ("Ff").

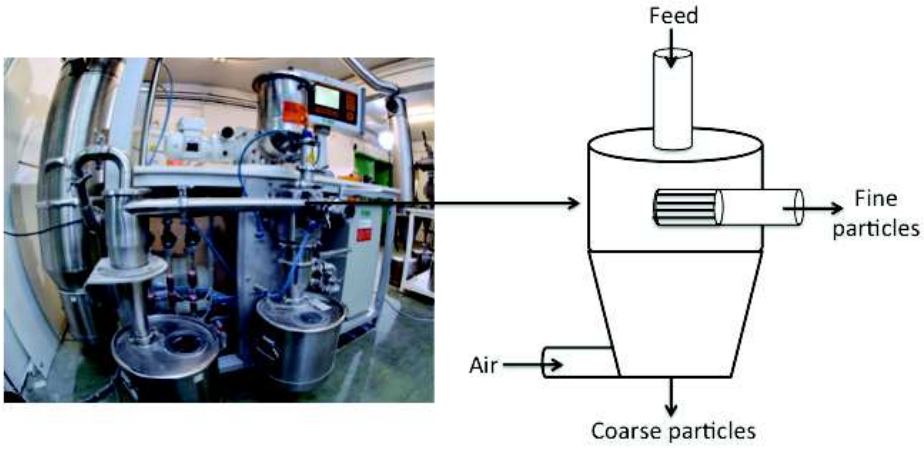


***Figure III-** 7 Turbo-fractionation technology (TF-T)*

III.3 Characterizations of Lignocellulose particle

III.3.1 Scan electron microscopy (SEM)

Scanning electron microscopy analysis (SEM) was conducted to determine the structural change, morphological structure and surface characteristics of grinded rice straw. A grinded rice straw was prepared by attaching it on specimen stub. The sample was then heated with methane to eliminate the electrostatic effect prior to imaging with scanning electron microscope (SEM Hitachi S4800). The converted SEM images were then treated by Image J® software to deduce the powder sizes of a representative population of particles (~30). The aspect ratio was than determined by calculating ratio of maximum length and minimum of each sample. For each grinded powder, distribution of aspect ratios is represents by the mean value and the standard deviation (σ).

III.3.2 Particle size and surface area

Particle size measurement

The particle size measurement is performed using laser granulometer (Malvern Instruments Ltd., United Kingdom). The particle size distribution was determined from 0.02µm to 2000µm. The samples were suspended in ethanol solution (essai paillet de l'éthanol). The principle of measurement consists of diffraction phenomena of bundle laser; more the particle is small, more the diffraction angle is large (Figure III- 8A). The first obtained results providing by granulometer is the particle size distribution in volume in the population (sample), that is the distribution of the equivalent spheres in which the same occupies volume as by the measured sample, leading to the same specter.

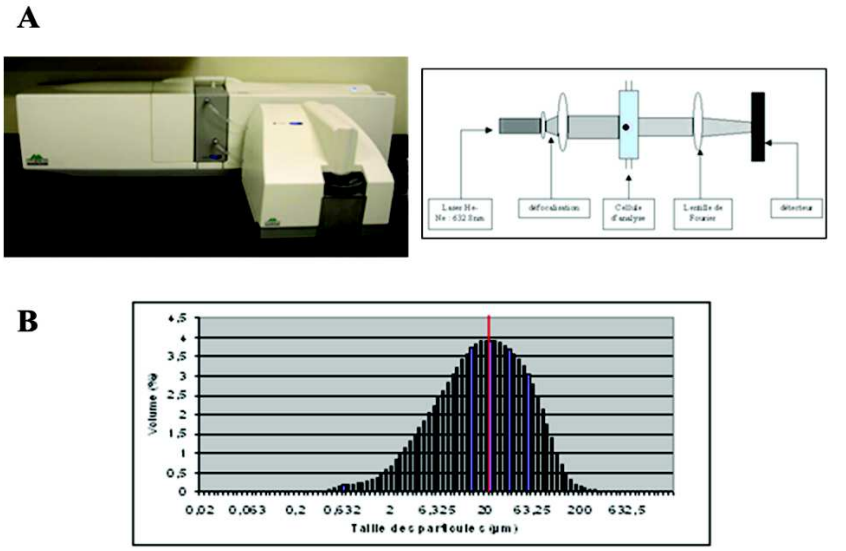


Figure III- 8 *A) Laser granulometer (Malvern Instruments Ltd., United Kingdom), B) Particle size distribution in histogram form*

The results providing have been shown in an accumulated histogram form (Figure III- 8B). The results in histogram could be divided statically into three important data as following description.

- D_{10} = expressed in µm, it represent the particle size diameter below within 10% (in volume) of sample.
- D_{50} = it represent the particle size diameter below within 50% (in volume) of sample.
- D_{90} = it represent the particle size diameter below within 90% (in volume) of sample.

In this study, D_{50} was used to be a median diameter for all particle size of sample and the span was also used to be a distribution of particle size, which was calculated by the following equation;

$$Span = \frac{D_{50}}{D_{90} - D_{10}} \qquad \textit{(Eq. III- 1)}$$

In case of biomass as rice straw, biomass particles are known to be elongated (not spherical), this technique allows an acceptable comparison between samples.

Specific surface area

In this study the method determining the specific surface was calculated by using the following equation;

$$S_a = \left[\left(\frac{S_p}{V_p}\right) * \frac{1}{\rho_s}\right] \qquad \textit{(Eq. III- 2)}$$

where $S_p = 4\pi\left(\frac{Z_p}{2}\right)2$ is surface of particle (m^2), Z_p the particle size (m), $V_p = \frac{4}{3} * \pi\left(\frac{Z_p}{2}\right)^3$ the volume of particle (m^3) and ; ρ_s the density of the solid.

On the other hand, the determination of specific surface area by BET method used to be performed, which was based on the BET theory that consists of the gas adsorption phenomena. The physical adsorption of is gas on the external and internal surface of porous material.

III.3.3 Biochemical compositions analysis

Carbohydrate analysis

The carbohydrate and lignin composition of LC samples was measured after concentrated acid hydrolysis. The lignin content in samples was determined by the Klason method. 100 mg of dried samples were treated with 72% H_2SO_4 at ambient temperature for 2hrs. The solutions were diluted with water to 12% H_2SO_4 and autoclaved at 100°C for 3hrs. The hydrolysates were filtered (10 µm). Klason lignin content was determined as the weight of the residue retained on the filter after drying at 105°C for 24h. The filtrates were analyzed for sugars using a high-pressure liquid chromatography (HPLC). HPLC analysis enabled to quantify monosaccharides (glucose, xylose, and arabinose). The analysis was done with a combined HPLC Water system, using a BioRad HPX-87H column at 50°C. The solvent was 0.005 M H_2SO_4 with a flow rate 0.3 mL/min. The recovery of monosaccharides was determined by standard addition (D-fucose) to the samples. A refractive index (RI) detector (Waters) was used to quantify carbohydrates. The system was calibrated with glucose, xylose and arabinose standards (Sigma–Aldrich). Before measurements, all samples (1 mL) were filtered through 0.22 µm nylon filters. All determinations reported here were duplicated results.

Ashes content

Ashes content was measured by using a muffle furnace (HERAUS) at 900°C for 2 hours. The sample was weighted approximately 1g in crucible (M_1) and subsequently placed in a muffle furnace 900°C for 2 hours. After 2 hours of burning, the sample was removed from the furnace and placed directly into desiccator; cool for a specific period time. The sample was reweighted (M_2).

The ashes content have been performed in duplicate experiments. The determination of ashes content was calculated by the following equation.

$$\boldsymbol{Ash\ content = \frac{M_2}{M_1} * 100} \quad \textit{(Eq. III- 3)}$$

III.3.4 Measurement of specific energy

The total energy ($E_{T)}$ consumed during the fractionation process is defined as the sum of energy required for milling (E_M) plus electrostatic or turbo-fractionation (E_{EF} and E_{TF}). The total energy consumption (E_T in Wh Kg^{-1} WS) was measured according to equation 1: $(\boldsymbol{E_T = E_M + E_{EF}})$ for EF technology and $(\boldsymbol{E_T = E_M + E_{TF}})$ for TF technology combined with ultrafine milling. The milling energy (E_M) using a screen size of 0.1mm, $E_{TF\text{-}T}$ and $E_{EF\text{-}T}$ are measured using a wattmeter. The power active, active electric energy (Wh), frequency hertz and time were logged into a PC card at 1-s intervals. The energy consumption ($E_{EF\text{-}T}$ for example) was calculated according to the following equation;

$$\boldsymbol{E_{EF-T} = \frac{\int_0^t (P_t - P_0)dt}{m} = \frac{\int_0^t \Delta P_t dt}{m}} \qquad \textit{(Eq. III- 4)}$$

Where Pt is the power (Watts) consumed at a time t, P_0 is the average power consumption (Watts) under idle conditions (without biomass), and *m* is the mass in kg of biomass to be ground. Three repetitions of power (P_0 and P_t) were conducted for each sample.

The *E*-factor calculation is defined by the ratio of the mass of waste per unit of product.

E-factor = Total waste (kg) / Product (kg)

Total amount of reactants (Kg) = biomass + chemical catalyst + water

Amount of product (Kg) = glucose

Amount of waste = total amount of reactants – glucose

III.3.5 Mechanical and Rheological characterizations

Interparticulate interactions essentially control the rheological properties of the powder, like the flowability. Studies of mechanical behaviour of the rice straw powders could make better understanding the structural surface organization of material, and also give abilities of flowing or compacting.

Mechanical properties trials

Compression tests and shearing tests of the rice straw powders were measured using the FT4 powder rheometer (Freeman Technology, Worcestershire, UK) (Figure III- 9 ()). The measurement were carried out in a cylindrical glass cell (diameter =50mm; height = 50mm). The rice straw sample is then carefully introduced into the measuring cell with a broad spatula and then submitted to a specific conditioning procedure that aims to ensure an initial uniform packing state from one test to another. After this pre-conditioning step, the rice straw sample was subjected for 60s to 15kPa pre-consolidation step carried out using a 48mm micro-perforated piston moving vertically. The top glass cell was then withdrawn together with the powder excess in order to level the pre-consolidated sample surface. After the rice straw pre-conditioning step, shear stress is applied at increasing normal stress. Mohr circles are than drawn and according to Coulomb law (Eq. III-1), involve determining the apparent friction coefficient and the apparent cohesion for each powder

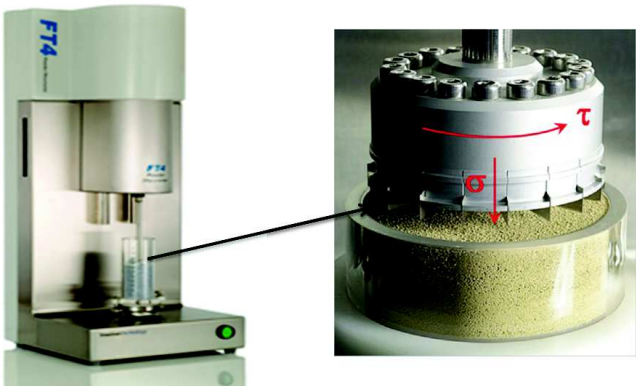


Figure III- 9 *FT4 Rheometer (http://www.pharmaceuticalonline.com/doc/powder-tester-ft4-powder-rheometer-0001)*

Tapped density

Tapped density (Figure III- 10) is a method to test flowability and compressibility of granular material under cyclic acceleration. That consists to measure the volume decreasing, which corresponds to an increasing of the compactness. The tapped density is a bulk density after mechanically tapping the powder sample. It is obtained by cycling tapping a graduated measuring cylinder containing the powder sample. After observing the initial powder volume, the cylinder is tapped under a gravitational falling acceleration, and volume readings are taken until little further volume change is observed. The sample powder was subjected into 100ml-graduated cylinder with a mass of 80% of total cylinder volume and was then set at a settling apparatus. The cylinder was hold on and carried out 100, 500 and 10000 taps on the same power sample and read the corresponding volume V_{100}, V_{500} and V_{10000}.

Figure III- 10 *Auto-tap machine (http://www.quantachrome.com/density/autotap.html)*

III.3.6 Textural characterization

Through the biomass transformation, the main objective is to change some properties of powders in order to facilitate the subsequent processes such bioconversions. In spite of several studies was reviewed that the pretreatment process is the most important point that LC material properties has been changed both macro- and microstructure. But the characterization methods are still inadequate. For this reason, this study aims to develop the characterization methods, in order to follow the intra- and inter-change in structure of LC material. Almost parameters defined in this study were hereby related especially the texture of material.

Real and Apparent Densities

Real solid density (ρ_s^*) was determined by using gas pycnometer type ULTRAPYC 1200e (Quatachrom) that measures the volume of a sample of powder. The measurement principle consists

of measuring the difference of pressure between a known reference volume (V_R) and the volume of the instrument's sample cell (V_c). Nitrogen is used as the gas to fill the reference and sample cells. The pressure is set at around 40 kPa (near maximum as specified by the instrument specifications). The pycnometer volume of the sample (V_{pvc}) is calculated by the following equation;

$$V_{pvc} = V_c - V_R * (\frac{P_1}{P_2} - 1) \quad \textit{(Eq. III- 5)}$$

Where P_1 is the pressure reading after pressurizing the reference cell (kPa), P_2 is the pressure after connecting the reference cell to the sample cell. The particle density $\rho_s^*$of the sample is its mass m_p divided by the pycnometer particle volume (V_{pvc}), the real density could be calculated by using the following expression;

$$\rho_s^* = \frac{m_p}{V_{pvc}} \quad \textit{(Eq. III- 6)}$$

Apparent density (ρ_s) was calculated by using a hydrostatic balance **(**Figure III- 11**).** The principle of measurement is based on the buoyancy method; measuring weight of sample in liquid (paraffin oil). The apparent weight of sample in liquid; this weight is typically reduced by buoyancy force. This value is used to combine between the weight in air (m_a) and the weight in liquid (m_{sl}).

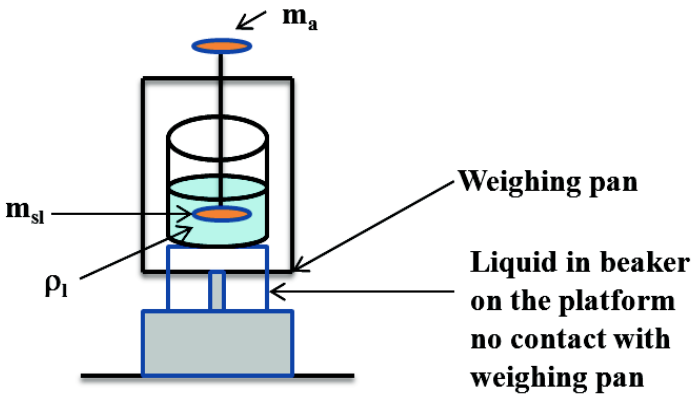


Figure III- 11 *Sketch of the hydrostatic balance for the buoyancy method with a frame for hanging the plummet and a bridge for holding the container for liquid*

According to buoyancy method, the density of solid (sample) is unknown (ρ_s) which could be calculated by the following expression;

$$\rho_s = \rho_l * \frac{m_a}{m_l} \; where \; m_a = \; m_s, m_p; \; m_l = (m_a - m_{sl}) \; and \; \rho_l = parafin \; oil \; density.$$

$$So, \rho_s = \rho_l * \left(\frac{m_a}{m_a - m_{sl}}\right) \quad \textit{(Eq. III- 7)}$$

On the other hand, in this study the apparent volume of solid (*Vs*) has been used to calculate the compactness of sample, the *Vs* could be calculated by the following equation;

$$V_s = \left(\frac{m_a - m_{sl}}{\rho_l}\right) \; where \; V_s = \frac{m_a}{\rho_s} \qquad (Eq.\ III\text{-}\ 8)$$

Moisture Content based dry basic matter (W)

Moisture content was determined by using a drying oven at 104°C for 2 hours. The samples were weighed about 1.5-2g in copal (M_1) in duplicate experiments and they were then placed into the drying oven for 2hours. After 2 hours of drying, they were removed directly into desiccator and cooled for a specific period time. The samples were reweighed (M_2). The moisture content was calculated by the following equation;

$$W(/) = \frac{(M_1 - M_2)}{M_2} * 100 \qquad (Eq.\ III\text{-}\ 9)$$

III.3.7 Hydro-textural approach

Grinding process performed lead to not only many changes in external properties especially morphology and particle size (particle size distribution and form) (Schell and Harwood 1994; Mani, Tabil et al. 2004; Silva, Couturier et al. 2012; Yang, Ji et al. 2014) but also the internal properties, which was called hydro-textural. Ruiz et al. (2011) have been developed an approach to describe all internal and external properties changes of wet granular material due to transformation process (particle size, compactness and moisture content)(Ruiz, Rondet et al. 2011). This approach consists of the construction of hydro-textural diagram, which represents the evolution of compactness and moisture content. In addition, Ruiz et al. (2005) and Rondet et al. (2008), have been defined some hypothesis and some details about this approach that has been applied in the current study (Ruiz, Delalonde et al. 2005; Rondet, Delalonde et al. 2008), specifically effect of grinding process on powder characteristics.

A wet granular such rice straw is typically in solid-liquid-gas three-phase system. Each phase could generally define by its real density that called ρ^*. Real density for solid phase is $\rho_s^* = \frac{m_s}{V_s}$, measured by using a Nitrogen Pycnometer. All relevant parameters to describe macroscopic state of material in porous granular media could subtract by its real density and three measured parameters: granular media volume (V), internal water mass (m_h) and dry mass (m_s). The humid and dry masses were measured respectively by a precision balance before and after drying in an oven at temperature 104°C during 2h. Volume of the powders was measured by using a hydrostatic balance with paraffin oil, which ensures the wetting of powders without penetrating there. Moisture content $w = \frac{m_h}{m_s}$, compactness $\phi = \frac{V_s}{V}$ and degree of saturation $S = V_e/(V - V_s)$, are calculated from experimental

measured parameters. In order to describe the macroscopic characteristic system, these three parameters are normally necessary and sufficient (Ruiz, Delalonde et al. 2005; Rondet, Delalonde et al. 2008).

In addition, in order to follow all relevant parameters (ϕ, w) during grinding process, a hydro-textural diagram has been established. This diagram is limited in upper part by saturation curve:$\phi_{sat}^{-1} = 1 + d_s^* w$ (where d_s^* represents dry density solid). Beyond this curve, the system loses physical realism as mixture state. The saturation curve represents the maximum moisture content that all of existent pores could be filled up by liquid phase (*i.e.* solid « saturated » by liquid). The experimental applied approach in this current study will use this hydro-textural approach about grinding process of rice straw.

III.3.7 Imbibition kinetic / liquid transfer

The transfer of liquid test to LC material consists of using hydrostatic balance in order to measure the weights of rice straw. Weights are measured in "air" and in "water" at different times. Kinetics of water content and volume changes are established. Apparent weight of the straw is measured by immersion in the water (Figure III- 11). The apparent density of rice straws had a magnitude less important than water density. So, it is necessary to confine the straws in a metal cage, which ensure the immersion. Kinetics is treated by the hydro-textural approach to observe the quantity of water, which flow in the straw porosity and quantify the swelling of the texture.

III.4 Chemical pretreatments (alkaline pretreatment)

Chemical pretreatment consists typically of breaking down the LC structure that has a heterogeneous complex network. Chemical catalysts have a role to interrupt the inter- and intra-linkages between hemicellulose and lignin (ester bonds). In this study, sodium hydroxide (NaOH) was used as catalyst to treat the grinded RS cutting 2mm (KM_2). The sodium hydroxide was dissolved in distilled water to adjust the alkaline concentration at 10% w/w (10g of catalyst/100g of KM_2). The sodium hydroxide solution was also made with amounts of water required to adjust the moisture content of RS to 40% (dry basis). The three sample were pretreated (Figure III- 12); the RS non-pretreated (RS_0), the RS pretreated in semi-humid system with different reaction times (1, 2, 4, 6 and 8hours) at room temperature, which were called dry chemical treatment "RS_{sh}" and the RS pretreated in a liquid medium with a biomass/liquid ratio of 1:4 with 24hours of reaction time at room temperature, which celled "KM_2 + NaOH". The pretreated RS samples were dried at 40°C for 24hours in order to obtain the initial moisture content (10-12%) and then the non-pretreated and chemical pretreated cut milled RS sample were subjected to centrifugal milling (Retsch, ZM 200)

using 0.25mm sieve which called $CF_{0.25}$ and the energy consumption of RS grinding and milling were measured using a watt meter (E_M).

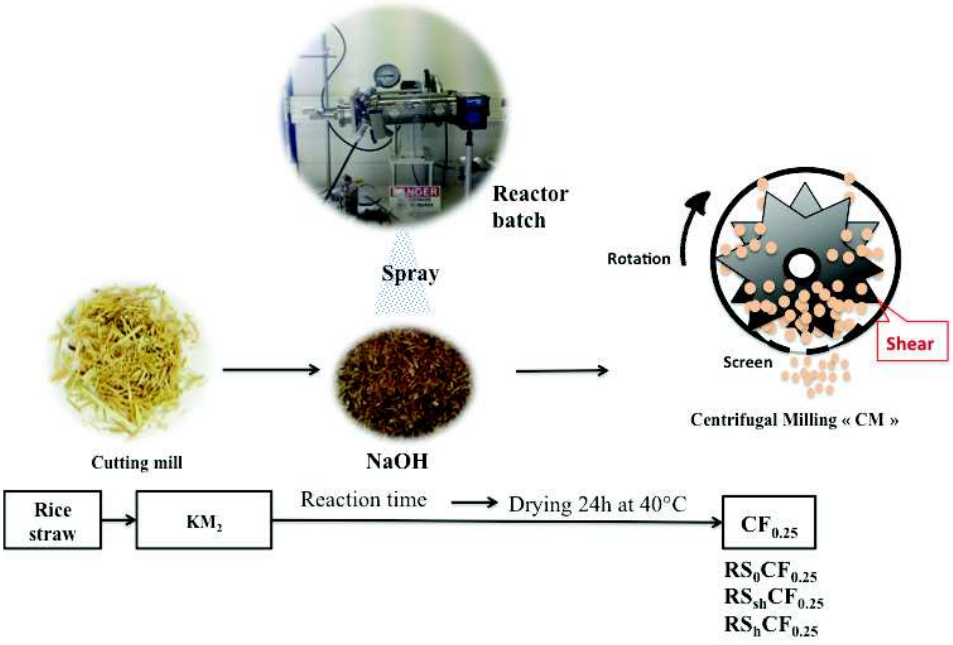


Figure III- 12 *Chemo-mechanical pretreatments of rice straw*

III.4 Enzymatic hydrolysis and Ethanol fermentation

Enzymatic hydrolysis

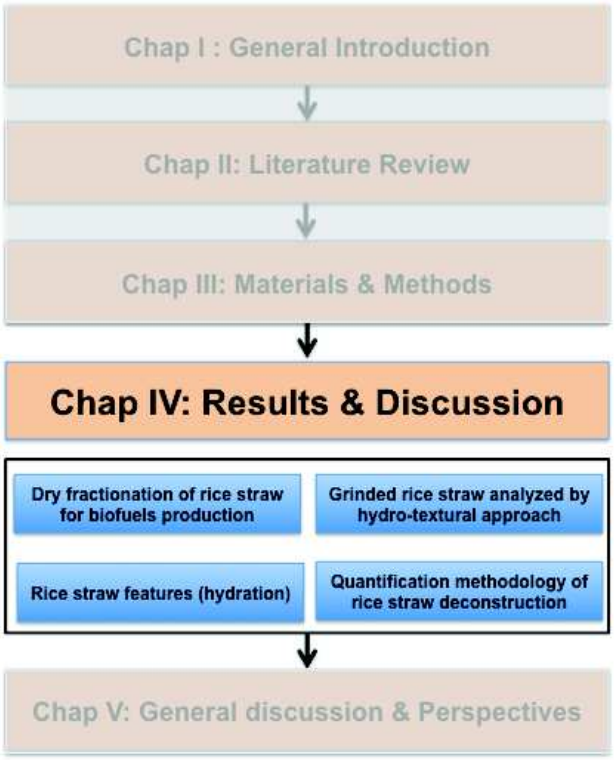
Enzymatic hydrolysis of rice straw was performed using an enzyme cocktail (*Trichoderma Longibrachiatum* C9748) obtained from Sigma Aldrich (20 FPU g^{-1} biomass). In general enzymatic hydrolysis was conducted at a solid concentration of 10% (w/v) in a 50 mM sodium acetate buffer (pH 5.0) at 37°C for 72h with agitation. Sodium azid was added at the end of the experiment to inhibit microbial growth. The experiment was performed in triplicate. The enzymatic digestibility was assessed by the obtained soluble sugars (mg g^{-1} biomass) determined by HPLC analysis (Barakat, Chuetor et al. 2014).

SSF (Simultaneous Saccharification and Fermentation) for ethanol production

SSF experiments were carried out in 2 mL serum bottles, each containing 7.5% (w/v) dry RS in potassium phthalate buffer (50 mM, pH5.5), 0.1 mL (20U/g) of *Trichoderma Longibrachiatum* C9748 enzymes and 0.9 mL of nutrients containing: 9 g L^{-1} yeast extract, 5 g L^{-1} urea, 0.5 g L^{-1} $MgSO_4 \cdot 7H_2O$ and 1 g L^{-1} KH_2PO_4. Flasks were closed and incubated at 30°C for 72 hrs. Samples were withdrawn at 0, 24, 48, and 72hrs. The experiment was performed in triplicate.

Chapter IV:

Results & Discussion



Ce chapitre contient les résultats expérimentaux obtenus. Nous le divisons en quatre sous-chapitres rédigés en anglais et en français. Ceux en anglais sont sous forme de publications. Dans un premier temps, nous présentons le fractionnement par voie sèche de la paille de riz pour la production de bioéthanol. Cette partie est un article qui est publié dans le journal « Green Chemistry ». Deuxièmement, une partie sur l'analyse des poudres de pailles de riz par une approche hydro-texturale qui permet une nouvelle compréhension de la déconstruction de la paille de riz au cours du broyage. Cette partie est rédigée en anglais et a fait l'objet d'une communication au colloque STPMF 2015 à Nancy. Ces résultats sont par ailleurs été soumis sous forme d'article dans la revue Powder Technology. Troisièmement, nous présentons les comportements de la paille de riz sous sollicitations hydriques. Dans cette partie nous testons les aptitudes aux transferts du liquide au sein de la paille de riz afin de bien maîtriser le prétraitement chimique. Enfin, dans le dernier sous-chapitre, nous présentons la méthodologie de quantification des processus de déconstruction des pailles de riz.

IV.1 Innovative combined dry fractionation technologies for rice straw valorization to biofuels

Une partie de ce chapitre a fait l'objet d'une publication dans le journal « Green Chemistry ». Nous proposons une version intégrale en anglais

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Abstract

The separation of lignocellulose into its major components (cellulose, hemicelluloses and lignin) is a key step in lignocellulosic biorefineries. Most pretreatments of lignocellulosic biomass into chemicals or biofuels are currently based on expensive chemical and energy consuming processes, which entail significant resource consumption (e.g. water) and generate a number of residual streams. In this work, two innovative dry fractionation technologies (Physical fractionation: turbo- and electrostatic separation of lignocellulose particles) have been developed for rice straw "RS" fractionation and bioconversion to sugars and biofuels. Turbo- fractionation technology (TF-T) comprises particle separation according to their size and density, whereas electrostatic fractionation (EF-T) is based on the separation of particles according to their surface properties (chemical composition and charges). TF-T and EF-T are suitable to produce lignocellulose fractions displaying very different structures, biochemical compositions and reactive surface without extensively damaging the raw fibers as well as minimizing waste generation (*E*-factor 0.7-0.75). The produced fractions could be hydrolyzed, being able produced large quantities of glucose (250-280 g kg^{-1} RS) after 72h of hydrolysis and subsequently ethanol (130-150 g kg^{-1} RS) after fermentation. TF-T and EF-T can therefore improve the economic feasibility by low energy consumption and produce reactive lignocelluloses particles with different physicochemical structures in a short time, which can be easily converted to biofuels minimizing waste (no effluent generation).

***Keywords*:** Lignocellulose biomass, Biorefinery, Dry mechanical-physical fractionation, Electrostatic and Turbo- separation, Enzymatic hydrolysis, Biofuels.

***Abbreviation*:** RS: Rice Straw, EF-T: Electrostatic Fractionation Technology, TF-T: Turbo-Fractionation Technology, SSF: Simultaneous Saccharification and Fermentation

IV.1.1 Introduction

Biofuels production from agricultural residues has been recently investigated as a renewable energy alternative to current fossil fuels. LC biomass is one of the most important carbon sources, with a remarkable potential as raw material for the production of several valuable products (e.g. chemicals, materials and biofuels)(Barakat, de Vries et al. 2013), (Antonio Melero 2012; Galbe and Zacchi 2012). Polysaccharides account for over 70% in LC feedstocks, which can be biologically converted into biofuels. Rice straw "RS" is one of the most important by-products of rice cultivation and processing. With a global rice production increasing at an average of 16 million tons per year (FAO, http://www.fao.org/economic/est/publications/ricepublications/rice-market-monitor-rmm/en/, 2011), RS can potentially produce yearly over 200 billion liters of bioethanol. RS composition typically comprises cellulose (35-40%), hemicelluloses (25-30%), lignin (10-15%) and ash (8-15%). The interest of RS for biofuel production relates to its high carbohydrate and low lignin content as compared to others LC feedstocks and residues. Ethanol production from LC feedstocks comprises two key steps, namely i) (enzymatic or chemical hydrolysis of cellulose/hemicellulose to C6 and C5 sugars and ii) microorganism-mediated fermentation of sugars to bioethanol. Hydrolysis of polysaccharides to monomers and issues with C5 sugars from hemicellulose is known to be the rate-limiting steps in biofuels fermentation of most LCs substrates (Hendriks and Zeeman 2009; Kumar, Barrett et al. 2009). The structural heterogeneity and complexity of the LC matrix is in fact one of the obstacles to enhance hydrolysis efficiency and subsequent bioconversion. In a perspective of LC biorefinery, the separation/fractionation of LC into its major components (cellulose, hemicelluloses and lignin) constitutes the first step of its refining into high value added products. A number of pretreatment or fractionation processes have consequently been developed to improve the accessibility of fermentable sugars in LC biomass aiming to maximize ethanol production (Hendriks and Zeeman 2009; Kumar, Barrett et al. 2009; Galbe and Zacchi 2012; Barakat, de Vries et al. 2013). These include mechanical, chemical, physical-chemical and biological methods or a combination of them (Barakat, de Vries et al. 2013). Most effective pretreatments achieve a crystallinity reduction (cellulose fraction), a decrease of particle size and an increase of reactive surface area, which improved the hydrolysis step and subsequent fermentation to biofuels production. Currently, most LC biomass pretreatments into chemicals or biofuels are based on expensive chemical/enzymatic processes, which in some cases cannot valorize all major components from the feedstock. Furthermore, many of these processes consume large water quantities and generate significant waste (e.g. effluents). Dry fractionation processes and separation technologies (air classification, turbo-separation, electrostatic separation, sieving, etc.) have attracted a great deal of attention due to their possibilities in various fields including electronic

waste valorization, food industry, pharmaceuticals, ceramics and materials science, etc.). Such dry fractionation processes have been reported to efficiently decrease particle size and improving reactive surface area, being comparably energy-consuming to conventional mechanical and physicochemical pretreatments (Jin and Chen 2006; Barakat, de Vries et al. 2013). As example, Papatheofanous *et al*.(Papatheofanous 1998) developed a dry fractionation methodology for wheat straw, initially milled by a disc mill and separated into two fractions (a) chips, containing mostly internodes and (b) meal, consisting mainly of ground leaves and nodes(Papatheofanous 1998). The internode fraction (63% of the whole straw) contained 8% more cellulose, 9% more lignin and 10% less ash compared with the unfractionated material. Corn stover fractions with high corn leaf content were also found to be more susceptible to enzymatic hydrolysis (Chundawat, Venkatesh et al. 2007). Interestingly, samples with different particles sizes obtained via dry fractionation protocols were reported to have remarkably different chemical compositions (Zhu, Wang et al. 2009). A very finely divided sample (<0.127 mm) contained about 40% more lignin and 33% less cellulose as compared to other fractions, together with a much larger surface area. These studies suggested that increasing mean specific surface area by size reduction could have a significant effect on fractionated substrates with respect to non-pretreated LCs(Zhu, Wang et al. 2009). Wheat bran has also been fractionated using a combination of ultrafine milling and electrostatic separation in an electrical field (positively "+" and negatively "-" charged electrodes)(Hemery, Rouau et al. 2009; Hemery, Holopainen et al. 2011). The objective was to break down bran tissues in order to individually isolate their sub-cellular constituents (cell walls rich in fiber versus cell content rich in micronutrients). This type of separation was successfully conducted to prepare fractions concentrated in aleurone and pericarp from wheat bran (Hemery, Rouau et al. 2009; Hemery, Holopainen et al. 2011). The authors show that fiber-rich particles of pericarp were more abundant in the fractions of negatively charged particles and aleurone cell walls (β-glucans, arabinoxylans, ferulic acid). Comparatively, loose protein containing material from aleurone and endosperm were more abundant in positively charged particles. In a recent study, the fractionation of the fibrous fraction from steam-exploded rice straw (SERS) with high moisture content was performed with respect to the separation degree of fibrous versus non-fibrous tissue using a fluidized bed opposed jet mill(Jin and Chen 2007). The fluidized bed opposed jet mill method was found to be suitable to produce high fiber tissue content fractions without extensively damaging the raw fibers(Jin and Chen 2007).

In this work, novel fractionation technologies, namely ultrafine milling combined with turbo-air (TF-T) and electrostatic (EF-T) classification have been developed with the aim to produce relevant fractions from rice straw valorization. The influence of the fractionation processes on the

biochemical composition and structure; reactive surface area, glucose and bioethanol yields after enzymatic hydrolysis and fermentation were studied and compared to conventional RS pretreatments.

IV.1.2 Fractionation of RS combining ultrafine milling with electrostatic separation technology (EF-T)

The electrostatic fractionation technology (EF-T) concept is depicted in Figure IV- 1. EF-T is based on conveying of LC particles in a charging line where they are charged by tribo-electricity. The charged particles are subsequently moved to a separation chamber containing two high voltage electrodes, where positively charged particles "F+" are attracted by the negative electrode and negatively charged particles "F−" are attracted by the positive electrode. Two successive steps of electrostatic fractionation or separation (EF) were carried out using the sample "F0" (powder produced with 0.1 mm particle size) as the starting material (see Figure IV- 1B). Samples were collected after each separation step yielding two sets of three fractions (F1A−, F2A−, F2A+, F1B+, F2B+ and F2B+).

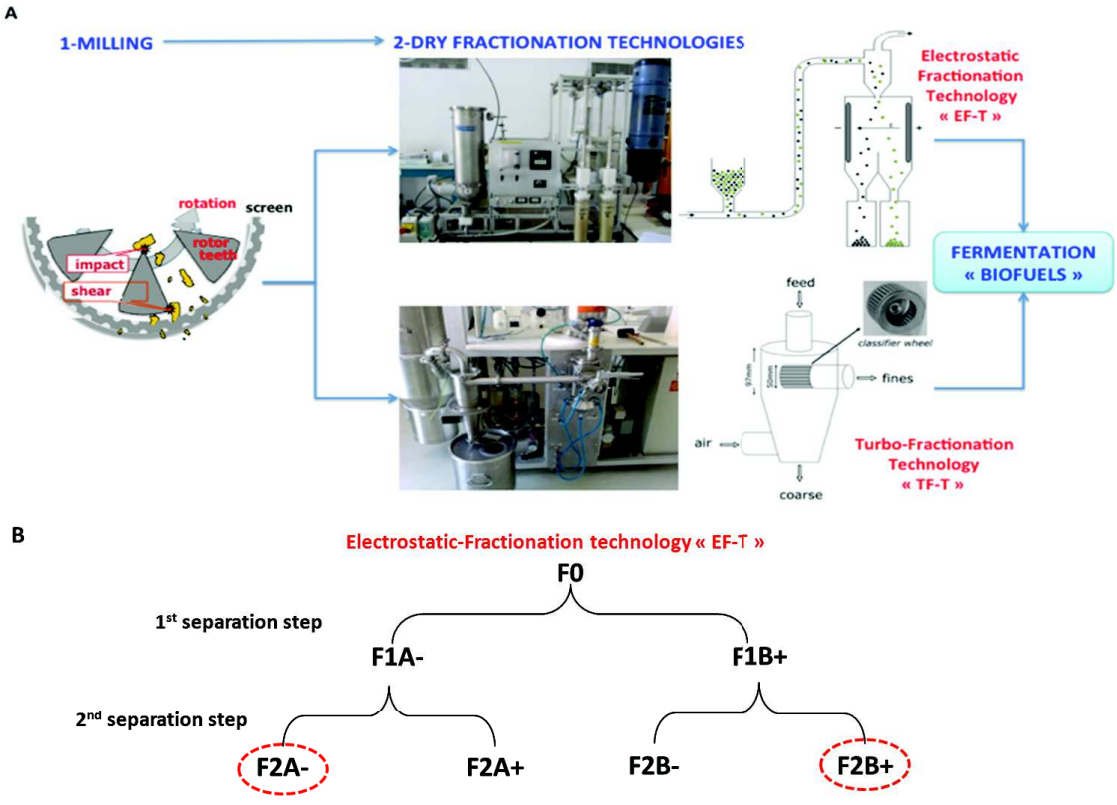

Figure IV- 1 *Electrostatic (EF-T) and Turbo (TF-T) Fractionation technologies route developed for rice straw (RS) in this study (A) Fractions prepared by electrostatic fractionation (B).*

Table IV- 1 summarizes recovery yields and particle sizes of different fractions prepared via EF-T. Results clearly pointed out that EF-T could have a significant influence on particle size diameter

(D50). Positively charged "F+" fractions possessed reduced sizes with respect to negatively charged fractions "F−" and untreated starting materials "F0" (ca. 10 µm smaller, Table IV- 1). Positively and negatively charged fractions were also characterized in terms of particle surface, biochemical composition, crystallinity and enzymatic accessibility. The general characteristics and physicochemical properties of the studied LC fractions indicated that a number of changes were induced to samples upon EF-T treatment, these being dependent on the charge of particles in the fractions and the number of separation steps. Surface areas (SA) also varied according to charged particles, with positively charged fractions exhibiting slightly superior SA as compared to negatively charged particles or F0 (Table IV- 1).

Table IV- 1 *Physicochemical properties and biochemical Composition of RS fractions prepared by electrostatic-fractionation technology*

Fraction	Recovery (% w/w)	D_{50}(µm)	*CrI (%)*	*SA (m^2/g)*	*LiG*	*Cell*	*Hem*	*Ash*	*Ash/Cell*	*LiG/Cell*
F0	-	64.8	63.7	57	13.8±0.6	49.8±1.2	22.5±0.7	13.8±1.1	0.28	0.28
F1A-	38.5	68.1	67.8	54	17.1±0.8	42.4±1.4	26.3±1.0	14.1±0.8	0.33	0.40
F2A-	33.3	72.7	68.3	52	17.4±1.4	40.3±0.9	26.2±0.5	16.1±0.4	0.40	0.43
F2B-	49.3	69.6	64.6	54	15.6±0.9	41.9±1.1	27.0±0.8	15.5±1.6	0.37	0.37
F1B+	61.5	61.3	57.2	60	11.1±0.8	52.6±1.6	24.3±0.2	12.0±0.3	0.23	0.21
F2B+	66.7	56.1	56.7	62	9.0±0.2	59.4±1.5	22.7±1.1	8.9±0.4	0.15	0.15
F2A+	50.7	54.6	59.8	67	13.4±0.9	47.6±1.2	24.9±0.7	14.1±1.2	0.30	0.28
$SA = (Sp/Vp)/\rho$ Sp = surface of particle (m^2)= $4\pi((Zp/2)^2)$; Zp : Particle size (m); Vp=volume of particle (m^3) =$4/3\pi((Zp/2)3)$; ρ: density of particle (g/m3)					**RS: rice straw; Cell: cellulose; Hem: Hemicellulose; LiG: lignin; SA: surface area; CrI: Crystallinity index**					

These results were in agreement with previous literature reports. SA of treated wheat straw was recently reported to be highly sensitive to particle size and pretreatment (i.e. increasing with ballmilling and chemical treatment). EF-T also influenced the Biochemical composition of different RS fractions depending on charged particles (Table IV- 1). Positively charged fractions "F+" were richer in cellulose as compared to "F0" and negatively charged fractions "F−". Similarly, negatively charged fractions "F−" were rich in lignin and ash with respect to positively charged fractions "F+". No significant differences could be observed in hemicelluloses and ash content. "F2B+" exhibited a very singular composition; 22.7% hemicelluloses, 9.0% lignin, 8.9% ash as well as remarkable 59.4% cellulose content (16% increase in cellulose content and ash/lignin decrease of 54% with respect to F0, Table IV- 1). In comparison, "F2A−" contains about 26.2% of hemicelluloses, richer in lignin and ash with approximately 17.4% and 16.1%, respectively, and less rich in cellulose with 40.3% compared to "F0" and "F2B+". Cellulose crystallinity was also influenced by EF-T, with

positive fractions again exhibiting a lower CrI as compared to F0 and "F−" fractions. Highly crystalline cellulose is less accessible to cellulase attack (or chemical hydrolysis) with respect to low crystalline amorphisized cellulose. An increase in cellulose content (with lower crystallinity) while decreasing hemicellulose, ash and lignin content can in principle facilitate the process of enzymatic hydrolysis and biofuels production. Table IV- 1essentially points out that positively charged particles generated upon EF-T fractionation possessed improved physicochemical properties compared to those of untreated RS. These included a significant increase in cellulose content (with less lignin) accounting for a 53% (after the first separation step) and 60% (after the second step).

These results confirmed the microstructure analysis using the fluorescence microscopy analyses (Figure IV- 2), which confirmed the separation of different tissues with different physicochemical properties. Figure IV- 2 showed that the positively charged fractions "F2B+" were more "blue" as compared to negatively charged fractions "F2A−" (more "brownish"), whereas the starting material "F0" is a mixture of two fractions. Figure IV- 2 shows that the morphology of the positively charged fractions differed from negatively charged fractions and untreated RS "F0". The positively charged fractions are characterized by crumbly and more homogeneous small particles. In contrast, the negatively charged fractions contained more heterogeneous and fibrous long particles. These findings were in good agreement with previous results for wheat bran after successive electrostatic separations. The observed difference in color and morphology could be due to differences in composition depending on the origin of the tissues.

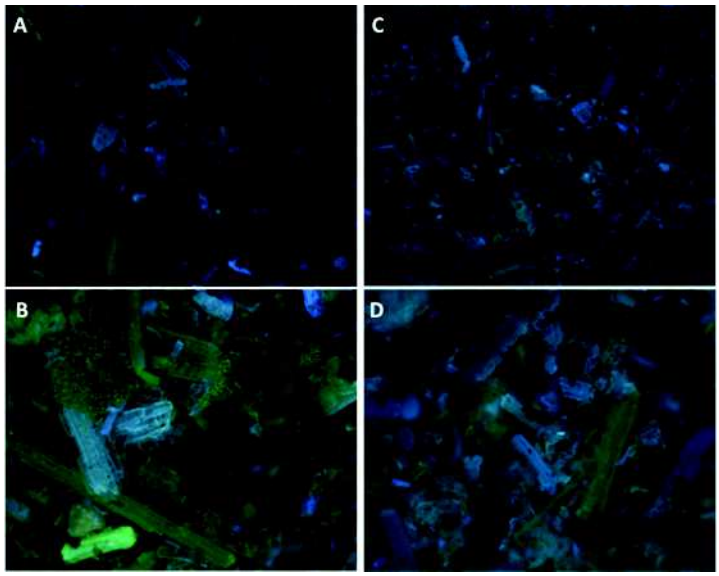

Figure IV- 2 *Micrographs showing the morphology of positively charged fractions F2A−: (A) ×5 and (B) ×25, and negatively charged fractions F2B+: (C) ×5 and (D) ×25. Samples were imaged without any staining using the multizoom AZ100M microscope (Nikon) in epifluorescence mode using the UV2A filter cube (Nikon) (excitation filter: 325–375 nm, dichroic mirror: LP 400 nm,*

emission filters: LP 420 nm). The observation was made with a Plan Fluor 5× (NA = 0.5) objective and the optical zoom was set at 1 or 5, leading to a global magnification of 5× or 25×. At a 25× magnification, a Z-series of images were acquired and the "extended depth of focus" image was calculated using NIS-Element software (Nis Element v4.13, Nikon, Japan).

Electrostatic fractionation could therefore provide an efficient separation of LC fractions displaying improved structures and more appropriate biochemical compositions for subsequent fermentation steps to biofuels, minimizing waste generation (*e.g.* toxic effluents) in the absence of any added chemical and/or solvents.

IV.1.3 Enzymatic hydrolysis and ethanol fermentation of EF-T rice straw fraction

All RS fractions prepared via EF-T (Figure IV- 1B) were subsequently hydrolyzed with a commercial enzyme cocktail at biomass loadings of 10% in buffer and enzyme loadings of 20 FPU/g for 72 h.16 The effects of each pretreatment step were evaluated via determination of released sugar yields (glucose and xylose; mg/gRS). Figure IV- 3 shows that maximum glucose yields after 72 h could be obtained from "F+" fractions (ca. 220–250 mg/gRS). The observed increase in glucose yields represents a remarkable increase in the 65–83% range as compared to untreated RS. Interestingly, there were very significant differences obtained for positively and negatively charged fractions in terms of xylose and particularly glucose yields (Figure IV- 3). Both were clearly superior in "F+" fractions, pointing to a higher accessibility by enzymes. EF-T can then be considered a particularly useful and effective fractionation technique to facilitate separation and isolation of poor enzymatically accessible fractions "F−" from improved accessible fractions "F+". Interestingly, averaging numbers of reducing sugars and ethanol yield from "F−" and "F+" fractions pretty much leads to numbers obtained for F0 (untreated RS).

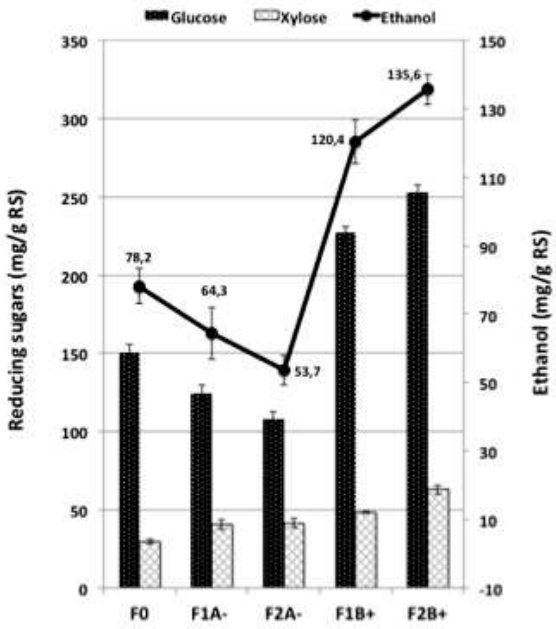

Figure IV- 3 *Reducing sugars and ethanol yields (mg/gRS) of rice straw fractions obtained from EF-T. The maximum ethanol yield obtained was 136.6mg/gRS*

Results were in good agreement with literature data in which cellulose crystallinity, particle size and reactive surface area, lignin and ash content were reported as main factors influencing the rate of accessibility of LC biomass by enzymes and microorganisms for bioconversion to biofuels. "F−" fractions are in fact richer in lignin and ash (therefore less accessible) and produce low glucose as compared to untreated RS "F0". Comparatively, "F+" was richer in cellulose and has low lignin and ash content, leading to larger quantities of reducing sugars. RS fractions obtained by EF-T after each separation step was eventually fermented for bioethanol production using the SSF method (simultaneous saccharification and fermentation). Data presented in Figure IV- 3 also show that the maximum ethanol yield after 72 h of SSF (120–140 mg g^{-1} RS) was obtained with positively charged fractions "F+". These values represented an increase between 64 and 74% as compared to those obtained with untreated RS (78 mg g^{-1}, Figure IV- 3). "F+" fractions again proved to be more accessible to microorganisms and thus able to produce more ethanol with respect to "F−" and untreated RS ("F0"). The combination of simultaneous milling and electrostatic fractionation has been proved to be an advanced LC valorization strategy to improve the rate of saccharification and ethanol production avoiding the utilization of any chemical, water and additional heat inputs, minimizing waste generation (*e.g.* toxic effluents) as compared to conventional LC pretreatments.

IV.1.4 Fractionation of RS combining ultrafine milling with turboseparation technology (TS-T)

Superfine grinding combined with dry separation (air classification or turbo-separation) or jet-milling technologies have attracted significant interest in recent years due to their use in the formation of superfine powders (with decreased particle sizes and improved reactive surface areas). Such dry environmental technologies have been considered similar to traditional mechanical grinding and conventional separation technologies in terms of energy consumption. Ultrafine milling combined with air classification (turbo-fractionation technology, TF-T) is also reported in this work as an alternative fractionation process to EF-T in order to obtain different fractions with varying particle structure, size and composition for enzymatic hydrolysis to sugars and ethanol production. TF-T entails the use of an air or turbo-classifier at different rotational speeds (from 3000 to 12 000 rpm) combined with a previous dry milling step (Figure IV- 1A). Untreated RS ("F0", D50: 64.8 μm) was introduced as feed (1 kg/h) to produce two different fractions, denoted as fine fractions "Ff" and coarsed fractions "Cf". These different fractions were characterized in terms of particle size, bulk density and reactive surface area, biochemical composition, enzymatic hydrolysis and ethanol production.

Table IV- 2 *Physicochemical properties and biochemical Composition of RS fractions prepared by turbo-fractionation technology*

Speed (rpm)		Recovery (% w/w)	D_{50} (µm)	*CrI* (%)	*SA* (m^2/g)	*LiG*	*Cell*	*Hem*	*Ash*	*Ash/ Cell*	*LiG/ Cell*
---	**F0**	-	64.8	63.7	57	13.8±1.6	49.8±1.3	22.5±0.8	13.8±1.6	0.28	0.28
3000	**Cf**	4.3	133.9	64.3	27	14.1±0.8	51.4±1.8	25.8±1.4	8.6±0.6	0.17	0.28
	Ff	94.9	70.7	61.8	52	13.4±1.1	49.0±1.6	24.3±0.9	13.3±1.1	0.27	0.27
5000	**Cf**	50.2	110.3	63.5	33	13.9±0.8	50.4±1.8	24.9±0.2	10.8±0.5	0.21	0.27
	Ff	48.0	47.9	61.4	74	12.8±0.8	48.5±1.3	24.4±0.8	14.3±1.1	0.30	0.27
7000	**Cf**	76.1	75.9	62.7	45	13.1±0.9	49.5±1.2	25.6±1.0	11.7±0.8	0.14	0.27
	Ff	17.5	26.6	61.3	138	12.6±0.8	49.6±1.2	23.4±0.6	14.3±0,.0	0.28	0.25
10000	**Cf**	44.9	71.6	64.4	51	12.9±0.8	52.4±1.6	26.3±0.5	10.0±0.7	0.19	0.25
	Ff	54.1	10.2	60.4	329	11.8±0.2	54.0±0.8	20.5±1.3	13.7±1.3	0.25	0.22
12000	**CF**	81.3	71.6	62.4	51	13.5	52.8	24.6	9.7	0.18	0.26
	Ff	16.1	8.5	60.5	433	13.1	52.6	22.1	14.1	0.25	0.25

CrI : crystallinity index ; D_{50} : Diameter median

SA (surface area) **= (*Sp*/*Vp*)/ ρ**

Sp **= surface of particle (m^2)= $4\pi((Zp/2)^2)$; *Zp* : Particle size (m)**

Vp=volume of particle (m^3) = $4/3\pi((Zp/2)3)$

ρ: density of particle (g/m^3)

RS: rice straw; Cell: cellulose; Hem: Hemicelluloses; LiG: Lignin

Table IV- 2 summarizes recovery yields, physicochemical properties and biochemical composition of different TF-T fractions. Characterization parameters of TF-T fractions clearly indicate the significant influence of rotational speeds of the classifier on obtained differences between Cf and Ff fractions in terms of recovery yields, particle size and chemical composition (Table IV- 2). The observed values are remarkably dissimilar as compared to results under EF-T. Recovery yields of "Ff" fractions ranged from 16 to 95% (5 to 80% for "Cf" fractions) when the rotational speed was varied from 3000 to 12 000 rpm (Table IV- 2). An increase in rotational speed of the classifier led to a decrease of particle size. In principle, a rotational speed of 12000 rpm generated the finest fractions "Ff" (9 µm). However, a 16% recovery yield could only be achieved under these conditions, with a 74% recovery of "Cf" (72 µm particle size). Such a low "Ff" yield makes these conditions (TF-T, 12 000 rpm) economically unfeasible. Interestingly, a rotational speed of 10 000 rpm also produced a very fine particle size fraction (10 µm) with high yields (55% and 45% recovery of "Cf" with a particle size of 72 µm). These are certainly optimum conditions as compared to results obtained under reduced rotational speeds (3000, 5000, 7000 rpm). Results significantly predate previous literature reports on the use of jet mill (FJM-200) classifiers, which generated larger particles from RS pretreatment (60 µm) at 4544 rpm at low yields (typically 28%).

TF-T can therefore improve the economic feasibility of jet milling in terms of lower energy consumption and generation of superfine particles with different physicochemical structures in a

short time, easily converted to biofuels. “Ff” and “Cf” collected fractions upon ultrafine milling-TF-T were subsequently studied in terms of biochemical (cellulose, hemicelluloses, lignin and ash, Table IV- 2) composition and physicochemical properties (crystallinity, density and surface area, Table IV- 2). The reactive surface area (SA) was observed to significantly vary according to the rotational speed of the classifier and particle size of “Ff” and “Cf” fractions (Table IV- 2).

In general, the finer fractions exhibited larger surface areas as compared to coarse fractions (Table IV-2), in some cases reaching very large numbers (433 m^2 g^{-1}, Ff-12000). These values increased with increasing rotational speeds and were remarkably different from typical values obtained for Cf and untreated RS (ca. 50 m2 g^{-1}). As opposed to EF-T, TF-T did not appreciably influence cellulose crystallinity in samples or their biochemical composition (as expected in an air-classification approach, Table IV- 1 vs. Table IV- 2). TF-T fractionated samples also exhibited no significant differences in cellulose, hemicelluloses and lignin content for both finer and coarse fractions obtained at different rotational speeds (Table IV- 1 vs. Table IV- 2). These corresponded to a lignin/cellulose ratio of 0.28 (untreated RS) as compared to 0.25–0.26 for most Ff and Cf-fractions (Table IV- 2). Only an increase in cellulose content (10%) with a 14% decrease in lignin was observed for Ff-10 000 rpm, corresponding to a lignin/cellulose ratio of 0.22. Coarse fractions were found to be richer in ash content compared to finer fractions, which varied with rotational speeds of the classifier. In some cases, these values were significant (i.e. a decrease of 60 and 42% in ash for Cf-3000 and Cf-12 000 rpm respectively compared to untreated RS, Table IV- 2).

IV.1.5 Enzymatic hydrolysis and ethanol fermentation of RS TF-T fractions

“Cf” and “Ff” RS fractions obtained at different speeds after fractionation were subsequently hydrolyzed with a commercial enzyme cocktail at biomass loadings of 10% and enzyme loadings of 20 FPU g^{-1} biomass for 72 h at 37 °C. The effect of the classifiers’ rotational speed on glucose and xylose yields (degree of hydrolysis of glucan and xylan) was subsequently evaluated. Data presented in Figure IV- 4 illustrates significant differences observed in reducing sugar yields for “Cf” and “Ff” fractions as a function of the TF-T rotational speeds. Optimum yields of glucose (280 mg glucose g^{-1} RS, a 102% increase as compared to untreated RS) could be obtained after 72 h for Ff-10 000 and Ff-12000.

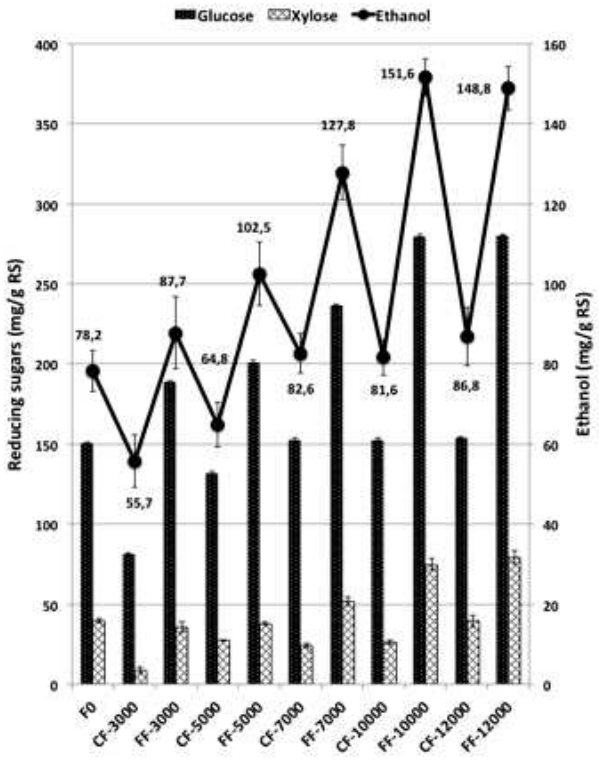

Figure IV- 4 *Reducing sugars and ethanol yields (mg/gRS) of rice straw fractions obtained from TF-T. The maximum ethanol yield obtained was 148.8 mg/gRS*

Figure IV- 4 also summarizes bioethanol yields from different TF-T fractions. The maximum ethanol yield (SSF, 72 h) was achieved with fractions Ff-10000 and Ff-12000, accounting for ca. 150 mg g^{-1} RS. These values represent an increase of about >90% in bioethanol production as compared to untreated RS (ca. 78 mg g^{-1}). In this case, superfine fractions "Ff" readily hydrolyzes, releasing large quantities of glucose without any remarkable changes in cellulose content or crystallinity as compared to RS subjected to EF-T (Tables IV-1 vs. IV-2). Fermentation to bioethanol also followed a similar trend, with very similar yields obtained for EF-T "F+" and TF-T "Ff" samples (Figure IV- 3 vs Figure IV- 4; 136 vs. 150 mg g^{-1}), pointing to a positive effect of particle size and surface area. The generation of significantly higher SA may relate to the differences observed between EF-T and TF-T values, particularly for bioethanol production.

These results were in good agreement with most literature data to date, in which a positive impact of reduced particle sizes on enzymatic and chemical hydrolysis of cellulose and LC materials was reported (Taherzadeh and Karimi 2008; Maache-Rezzoug, Pierre et al. 2011). Maache-Rezzoug et al., 2011 recently reported glucan conversion studies on wheat straw in which glucose yields increased at decreased particle sizes (102 mg g^{-1} and 150 mg g^{-1} of glucose for particle sizes of 800–1000 μm and 50–600 μm, respectively (Maache-Rezzoug, Pierre et al. 2011). Experimental findings for the combination of ultrafine grinding-TF-T fractionation illustrate the potential of the

technology, in a similar way to that highlighted for EF-T, in the valorization of RS to a better digestible and easily hydrolysable/fermentable feedstock for bioethanol production. The importance of maximizing SA and reducing particle size using TF-T is also another additional advantage of the proposed combined technology.

IV.1.6 Efficiency of ultrafine grinding EF-T and TF-T fractionation technologies compared to other pretreatments of RS

Hydrolysis is directly affected by porosity (available surface area) of LC biomass as well as by ash and lignin content. A large number of pretreatment methodologies have been developed in recent years (Mosier, Wyman et al. 2005; Galbe and Zacchi 2007; Taherzadeh and Karimi 2008; Yang and Wyman 2008; Zhu, O'Dwyer et al. 2008; Kumar, Barrett et al. 2009; Kumar and Wyman 2009). Some chemical, physicochemical, physical and mechanical pretreatments and/or fractionation technologies have been proved to be effective, but have several disadvantages in terms of energy consumption/ energy inputs, corrosion, inhibition effects in bioconversion, a large number of separation and purification steps, etc (Barakat, de Vries et al. 2013; Barakat, Chuetor et al. 2014) Many chemical pretreatments also involve the utilization of large water quantities, solvents and chemical reagents, which increase pretreatment costs and reduce at the same time their green credentials (e.g. waste generation, toxic effluents, number of steps, etc.). The amounts of glucose recovered after enzymatic hydrolysis (mg glucose g^{-1} RS), bioethanol yields (mg g^{-1} RS), water inputs (L kg^{-1} biomass) and quantities of chemical products (g kg^{-1}) are generally utilized to compare both performance, efficiency and environmental impacts of different RS pretreatment processes (Table IV- 3). A comparison of proposed technologies with existing processes indicates that glucose and bioethanol yields are comparable (in some cases remarkably superior) to conventional processes including mechanical treatment without fractionation "T0". The energy consumption of proposed fractionation technologies developed in this work has been worked out from the equation: ET = EM + EEF. The specific energy requirements (EM) to reduce the particle size of RS using a 0.1 mm knife mill (Figure IV- 1A) were 135.4 Wh kg^{-1}. EF-T and TF-T fractionation technologies also consumed only 12.5 and 22.4 Wh kg^{-1} RS, respectively (calculated specific energy consumption measurements). This indicates clearly that EF-T and TF-T technologies consume low or unimportant energy compared to milling equipment used to reduce particle size such as knife and ball milling and to thermal pretreatments such as steam and hot water (Zhu and Pan 2010; Kratky and Jirout 2011; Barakat, de Vries et al. 2013). The total energy (E_T) required for RS pretreatment was 147.9 and 157.8 Wh kg^{-1} for EF-T and TF-T, respectively.

Total energy requirements (E_T) were used to calculate the energy efficiency of the process (η), defined as kg glucose produced per kWh of energy consumed. The more effective pretreatment/fractionation process will have the highest η. The energy efficiency of acid and alkaline pretreatments of oilseed rap (OSR) straw has been previously evaluated (Mathew, Chaney et al. 2011). The measured η was 0.94 g glucose Wh^{-1} from a pretreatment time of 60 min. However, the highest η was obtained with alkaline pretreatment (1.42 g glucose Wh^{-1}) by pretreating biomass for 30 min at 130 °C using a NaOH concentration of 0.63 and 0.75 mol dm^{-3}. A higher glucose concentration could be extracted from OSR straw per Wh of energy consumed when alkaline pretreatment was used in contrast to acid pretreatment. Da Silva et al., 2010 (da Silva, Inoue et al. 2010) also studied the efficiency of wet disk milling "WDM" on bagasse and sugarcane straw for bioethanol production. Maximum sugar yields were obtained after 20 WDM cycles for both bagasse and straw, which yielded 213 and 245 g glucose kg^{-1} biomass, respectively. However, the highest η obtained was 0.046 and 0.027 g glucose Wh^{-1}, for bagasse and straw biomass, respectively, after only 10 WDM cycles, while 20 cycles consumed the highest amount of energy, corresponding to the lowest η. Hideno et al., 2009 (Hideno, Inoue et al. 2009) compared also the efficiency energy of BM, WDM and hot compressed water treatment (HCWT). They suggested that the optimal milling time was 60 min with the highest yield of glucose (331 mg glucose g^{-1} RS). However, BM treatment at 60 min resulted in lower η compared to WDM-5 min and -10 min for the pretreatment of RS. The highest η obtained was 0.078 g glucose Wh^{-1} for RS after BM at 5 min. These results indicate clearly that energy efficiency is an important parameter that can be used in the comparison of the efficiency of different LC pretreatments.

Table IV- 3 *Comparison of various RS pretreatment with the fractionation technologies developed in this study*

Pretreatment conditions	Enzymatic Hydrolysis and fermentation conditions	Glucose (Kg Kg^{-1})	Ethanol (g Kg^{-1})	Water or effluent* (L Kg^{-1})	Chemical reagent* (Kg Kg^{-1})	*E*-factor	Ref
Alkaline: 0.25mm RS, 12% Na_2CO_3.Na_2SO_3, 140°C for 60h. The residue was washed extensively with distilled water	Cellulase 20FPU/g biomass shaking incubator 180rpm for 48h at 50°C and pH 4.8	0.28	n.d.	6	0.12	6.84	(Yang, Cao et al. 2013)
Alkaline: 2% NaOH at 85°C for 60 min. The residue was washed extensively with distilled water	*T.reesei* ZM4-F3, shaking bed (180rpm) at 35°C for 120h and pH 4.5	0.26	n.d.	4	0.02	4.76	(Zhang and Cai 2008)

Alkaline + Ultrasonic (300w): NaOH 2.96% at 82°C for 60 min. The residue was washed extensively with distilled water	Trichoderma reesei (60FPU/g) and β-glucosidase from Aspergillus niger (30CBU/g) at 50°C for 72h, pH 4.8	0.26	n.d.	4	0.10	4.85	(Kim and Han 2012)
Torrefaction: RS particles<0.065mm, 220°C for 40min without oxygen	1g RS + 400µl Cellulclast1.5L+ 200µl Novozyme+ 200µl Viscostar, pH 4.5 50°C, 72h, 150 rpm	0.20	150	0	0	0.79	(Sheikh, Kim et al. 2013)
Popping pretreatment: 200°C, 1.96 MPa. The residue was washed extensively with distilled water	Enzyme cocktail Cellulase 23FPU/g and Xylanase 62IU/g biomass, pH 4.8 37°C, 48h	0.39	172	3.5	0	4.11	(Wi, Choi et al. 2013)
Acid: RS 5% w/w, 1% H_2SO_4, 60°C, 24h at 200rpm + 15min at 121°C, 15 lb pressure. The residue was washed extensively with distilled water	*Clostridium acetobutylicum*, 72h, 37°C at 120rpm	n.d.	60	20	0.01	–	(Ranjan and Moholkar 2013)
Acid + Enzyme : RS 5% w/w, 1% H_2SO_4, 60°C, 24h at 200rpm + 15min at 121°C, 15 lb pressure +enzyme. The residue was washed extensively with distilled water		n.d.	93	20	0.02	–	
Milling+Turbo-Fractionation: RS particle 0.062mm +turbo-separation (TF-T) without using chemicals, water and heat	Enzyme cocktail (*Trichoderma Longibrachiatum* C9748) (20 FPU g^{-1} biomass) 10 % (w/v) (pH 5.0 37°C, 72h)	**0.28**	**152**	0	0	0.71	This study
Milling + Electrostatic Fractionation: RS particle 0.062mm +electrostatic fractionation (EF-T) without using chemicals, water and heat		**0.25**	**136**	0	0	0.75	This study
n.d.: not determined; * used only in the pretreatment							

As compared to 150 mg glucose g^{-1} RS obtained under ultrafine milling "T0" (this work), ca. 250–280 mg glucose g^{-1} RS were obtained for EF-T and TF-T processes. These values are at least comparable (and superior in most cases) to previously reported work on biomass saccharification pretreatments.

Most importantly, the calculated energy efficiency factors (η) obtained for the combination milling/TF-T and milling/ EF-T were 1.77 and 1.72 g glucose Wh^{-1} respectively, as com- pared to only 1.10 g glucose Wh^{-1} obtained for milled RS. These findings represent a significant advantage of 0.65 g glucose Wh^{-1} and 0.78 g glucose Wh^{-1}, for RS after 5 min BM pretreatment as reported by Hideno et al (Hideno, Inoue et al. 2009).

Table IV- 3 summarizes a comparative study of various pretreatment technologies developed for RS saccharification for bioethanol production. Energy efficiency η (g glucose extracted Wh^{-1}) is normally utilized to compare pretreatment/fractionation performance (Zhu and Pan 2010; Barakat, Chuetor et al. 2014). However, the literature concerning energy consumption and energy efficiency of chemical, physicochemical and mechanical treatment of RS is scarce (Table IV- 3). However, various inputs and outputs have been utilized to compare these different pretreatment processes in terms of *E-factor* (Table IV- 3).

The calculated *E-factor* for the different pretreatments varies between 0.7 and 7. A higher E-factor implies more waste and, consequently, a worse environmental impact.

The performance of sodium carbonate–sodium sulfite (Na_2CO_3–Na_2SO_3) pretreatment in improving the enzymatic hydrolysis of rice straw was also investigated. The results indicated that both Na_2CO_3 and Na_2SO_3 pretreatments can effectively improve the enzymatic digestibility of RS(Yang, Cao et al. 2013). The highest glucose recovery of pretreated RS, ca. 282 mg g^{-1} RS, was obtained at cellulase loading of 20 FPU g^{-1} cellulose after pretreatment at 140 °C for 60 h, a 12% chemical input and a 0: 1 Na_2CO_3–Na_2SO_3 ratio (Table IV- 3). The corresponding *E-factor* was 6.84.

To minimize the cost of cellulase production, both RS pre- treatment and on-site enzyme production were carried out. RS was first alkali pretreated using 2% NaOH at 85 °C for 60 h, which could increase cellulose by 54.8% and decrease hemicellulose (61.1%) and lignin (36.2%), respectively (Zhang and Cai 2008). After hydrolysis for 120 h, the production of glucose could achieve 258 mg g^{-1} RS, but with an *E-factor* of 4.76. A maximum literature reported glucose yield of 254.5 mg g^{-1} RS could only be obtained after alkaline pretreatment under optimized conditions (2.96% NaOH, 81.79 °C and 56.66 min) (Kim and Han 2012).

The addition of an ultrasound step to the alkali pretreatment (Table IV- 3) showed a slightly higher digestibility yield but the difference was not significant (Kim and Han 2012). However, this study used large quantities of water and chemicals resulting in an *E-factor* of 4.85 (Table IV- 3). Sheikh et al. (Sheikh, Kim et al. 2013) reported a highest yield of 201 mg glucose g^{-1} RS obtained after torrefaction treatment at 220 °C for 40 min, representing a 60% increase with respect to untreated materials. Based on ethanol studies conducted on RS, this estimated quantity of sugars could

produce 150 mg g^{-1} ethanol, a 50% increase compared with untreated feed- stocks. This study was generally fine from the green chemistry standpoint, with a low *E-factor* of 0.79 (Table IV- 3).

Using optimized enzyme conditions and popping pretreatment of RS (15% substrate loading, w/v), a large glucose yield of 394 mg g^{-1} (total reducing sugars: 567 mg g^{-1}) was obtained after 48 h, which was significantly higher than that from untreated RS (total reducing sugars: 270 mg g^{-1} biomass) (Wi, Choi et al. 2013). Nevertheless, the popping pretreatment consumed large quantities of water and generated significant quantities of waste, which correspond to a high *E-factor* of 4.11 (Table IV- 3). Fermentation of the hydrolysates by Saccharomyces cerevisiae resulted in 172 mg ethanol g^{-1} RS after 24 h, equivalent to 80.9% of the maximum theoretical yield (based on the amount of glucose in the raw material) (Wi, Choi et al. 2013). Ranjan et al., 2013 (Ranjan and Moholkar 2013) compared also various rice straw pretreatment methods (steam exploded, acid treated, and enzyme assisted acid treated), conducted at high temperatures and pressures with a large water consumption (Table IV- 3). Enzyme-assisted acid treatment released the highest amount of glucose (nearly 38%) and produced a high ethanol yield of about 93 mg g^{-1} RS from hydrolysates(Ranjan and Moholkar 2013).

A close comparison of discussed protocols and methodologies with herein reported ultrafine milling/EF-T or TF-T clearly demonstrates that EF-T and TF-T are comparably less energy consuming and simpler technologies which can effectively improve the rate of saccharification and bioethanol production without the need for any chemical or water inputs with an *E-factor* of approximately 0.7–0.75, thus minimizing waste generation while maximizing the value of the lignocellulosic feedstock (Table IV- 3).

The proposed combination is envisaged to pave the way for the utilization of a wide range of feedstocks for a more efficient biofuels production, which will be reported in due course.

IV.1.7 Conclusion

The combination of ultrafine milling with turbo- and electro- static separation allowed the production of interesting fractions in a short processing time. Fractions exhibited distinctive particle structures, size and composition depending on the utilized methodology. The maximum glucose yield obtained after EF-T or TF-T was approximately in the range of 253–280 mg glucose g^{-1} RS, equivalent to an 83–103% increase as compared to untreated RS. The maximum ethanol produced upon fermentation (72 h) of EF-T and TF-F was found to be in the 136 and 152 mg g^{-1} RS range, representing an increase between 75 and 95% with respect to untreated RS. The combination of milling with electrostatic and turbo-fractionation of LC particles appears to be an interesting continuous process and a new technology for the development of environmentally sound LC

biorefineries for bio- fuels which avoid the utilization of chemicals and water as well as waste minimization, being at the same time comparable, in terms of energy consumption, to other available technologies.

IV.2 Grinding of rice straw analyzed by a hydro-textural approach

Une partie de cette section a fait l'objet d'une communication au 8ème Colloque Science et Technologie des Poudres qui s'est tenu à Nancy du 8 au 10 Avril 2015 (STPMF 2015). Repris sous une version étendue, ces résultats ont été soumis pour publication dans la revue Powder Technology sous le titre suivant : Grinding of rice straw analyzed by a hydro-textural approach, par CHUETOR Santi, BARAKAT Abdellatif, ROUAU Xavier, RUIZ Thierry. Nous proposons une version intégrale de l'article rédigé en anglais, ainsi que des résultats complémentaires portant sur le tassement et disposés en fin de section. Un résumé en français du contenu de ce travail est proposé ci-dessous :

La matière lignocellulosique présente dans les pailles de végétaux tels que les céréales, représente une ressource en biomolécules d'intérêt multiple mais dont l'extraction est sujette à une succession de traitements très consommatrice en énergie. Ces matériaux sont réfractaires dans le sens où ils se prêtent mal à une séparation de leurs constituants, de par leur constitutions fortement hétérogène et leur structuration à différents niveaux d'échelles (de l'organisation moléculaire, au niveau cellulaire, à la structure en fibres jusqu'au tissus végétal). Les pailles doivent être préalablement broyées afin que les éléments d'intérêts puissent être séparés par des procédés mécaniques, électrostatiques ou par voie chimique. On constate que l'opération de broyage nécessite elle-même la mise en œuvre de prétraitement afin améliorer la qualité et l'efficacité du fractionnement. Ces prétraitements, essentiellement (thermo) chimiques, ont pour objectif de déstructurer les liaisons intermoléculaires afin de faciliter le broyage. Cependant, comment identifier précisément leur rôle dans l'ensemble des processus impliqués lors du broyage ? Et plus généralement comment contribuer à identifier puis modéliser les processus mis en jeu lors du broyage avec ou sans prétraitement ?

L'objectif de ce travail est de compléter la caractérisation du procédé de broyage par une approche hydro-texturale appliquées aux milieux granulaires afin d'aider à la reconnaissance des processus mis en jeu, puis de quantifier l'impact d'un prétraitement chimique. Le travail expérimental a été réalisé à partir de pailles de riz, destinées à une valorisation énergétique par bioconversion des polysaccharides. Plusieurs moutures de tailles granulométriques décroissantes ont été réalisées (de ~1000µm à ~25 µm) à partir de trois différentes technologies de broyage. Les poudres ont été caractérisées selon certaines de leurs propriétés physiques (densité, cohésion, coefficient de friction, coulabilité) et ont fait l'objet d'une description sur un diagramme hydro-

textural : compacité (ϕ), teneur en eau (*w*). Leur sensibilité volumétrique à la variation de teneur en eau a été estimée par la quantification du retrait induit lors d'un séchage en étuve.

Les résultats font apparaitre que le mode de rupture correspond à une « perte de la porosité » et ce, quelle que soit la taille les poudres obtenues. La fragmentation semble localisée dans les « zones de plus fortes porosités ». Le broyage « libère » les structures les plus denses qui contiennent l'eau résiduelle dont la diminution au fur et à mesure du broyage n'est que très faible. En supposant que l'eau soit localisée au sein des cellules (eau intracellulaire), on introduit, à partir de la loi puissance qui relie la compacité et le diamètre médian (d_{50}), une « taille limite » caractéristique de la limite physique du broyage et dont la valeur calculée à partir du modèle est voisine de l'épaisseur des parois des cellules : $\lim_{\phi \to 1} d_{50} = e_{cell} \sim 2\mu m$. On constate par ailleurs que le coefficient apparent de friction augmente quand la taille des poudres diminue mais que la cohésion apparente diminue. Ce fait est lié à la forme des particules qui influe fortement sur les propriétés frictionnelles apparentes. Enfin, l'étude de la déformabilité par retrait au séchage, met en évidence l'impact « chimique » du prétraitement en confortant le fait que la déstructuration à bien lieu au niveau cellulaire.

IV.2.1 Introduction

Rice straw (RS) is considered as a renewable source for biofuel production such a bioethanol, biohydrogen as well as biogas (Wyman 1994). Bio-based fuels and products may contribute to energy supplies and economic development. Likely, rice straw contains biomolecules as cellulose, hemicellulose and lignin that could be converted into multiple products (Sun and Cheng 2002). In order to valorize RS, a biorefinery process must be developed to recover biomolecules of interest such as fermentable sugars that could be converted into biofuel (Wang, Wang et al. 2011). Biorefinery is a global process aiming to valorize all constituents of biomass to bioproducts as well as bioenergy. Nevertheless, the structural complexity of RS hinders the enzymatic accessibility to release fermentable sugars. Several factors affect the enzymatic digestibility like effects of chemical composition and physical features (Barakat, Chuetor et al. 2014). In order to facilitate the recovery of their interesting constituents, a pretreatment of starting material is an inevitable process in biorefinery (Taherzaeh, J. et al. 2004; Barakat, de Vries et al. 2013). Various pretreatments lead to fractionate and, or alter the RS structure. These processes have been generally divided into different categories: physical, chemical, physic-chemical and biological as well as their combinations. Physical pretreatment, like mechanical comminution, is considered as a crucial process in biorefinery that is associated with grinding, chopping, milling. Objective is to reduce the particle

size, which leads to the rupture of plant cell wall and the dissociation of the tissues. So, it contributes to increase the specific surface area increasing by this way the matter transfer rate. The physical characteristics of RS affect also the enzymatic accessibility. The objective of mechanical pretreatment by grinding is to reduce the particle size, cellulose crystallinity eventually to change the shape, to increase the bulk density and the specific surface area and to improve flow properties. High specific surface area leads to increase the kinetics of enzyme reactions (Schell and Harwood 1994; Bitra, Womac et al. 2009). The particle size reduction by grinding permits also a decrease in cellulose crystallinity as well as polysaccharide degree of polymerization, consequently increasing enzymatic hydrolysis yields (Barakat, de Vries et al. 2013). The size reduction modifies also the mechanical behavior of the resulting powdered product, which had consequences on the flow properties. The straw is considered as an elasto-plastic material (Annoussamy, Richard et al. 2000; Muller 2003) with an irregular form. How the chemical composition is distributed and how the cell wall structure of RS is ruptured during grinding remains largely unknown. The physical, mechanical as well as rheological characterizations of the RS powder are considered as relevant parameters to describe the deconstruction of this material. Moreover, how follow and model the dissociation phenomena mechanism during grinding process in a conceptual approach, which integrates this multidisciplinary problem? This current study aims to characterize the raw material state without chemical pretreatment during a grinding process by a hydro-textural approach defined for granular materials (Ruiz et al., 2005 and 2011). The studies of hydro-textural parameters allow depicting the state of the powders after each steps of grinding.

IV.2.2 Particle size distributions and powder morphologies

Particle size is the parameter directly impacted by the grinding and milling process provoking fragmentation. Table IV- 4 presents the characteristics of particles obtained with each different process of grinding and milling. Each distribution is mono-disperse, so we proposed to characterize the particle size distribution by the median diameter (d_{50}) and the span calculated by: $Span = \frac{(d_{90}-d_{10})}{d_{50}}$. We observed that d_{50} decreased with increasing grinding/milling process energy. Moreover, the more small the particle size, the more wide the size distribution. Figure IV- 5a 1a illustrates the logarithmic dependence of these factors ($Span\ \alpha\ -Ln\ (d_{50})$. This result indicates that the size reduction comes along with an increase of polydispersity. It is assumed that this gain of polydispersity does not improve the flowing properties of powders (Shaebani, Madadi et al. 2013; Nguyen, Azema et al. 2014).

Table IV- 4 *Values of physical properties of powders obtained after grinding process.*

Grinding techniques	Sample	d_{50} (µm)	SPAN	Aspect ratio (l_{min}/l_{max})	σ
Knife milling (KM)	KM_6	908	1.404	0.424	0.065
	KM_2	356	1.559	0.448	0.032
Impact milling (IM)	$IM_{0.5}$	238	2.603	0.435	0.079
	$IM_{0.1}$	108	2.704	0,471	0,088
Vibro-ball milling (VBM)	VBM_{20}	46	3.699	0.556	0.119
	VBM_{40}	26	4.151	0.623	0.153

In order to investigate the shape of grinded particles, SEM pictures have been used to analyze the distribution of aspect ratios. Examples of observed powder morphologies are presented in Figure IV- 6. Fibrous forms were more important for the big particles than the smaller. It shows that, initially rice straw is mostly in fibrous form when it was chopped with different screens of knife milling (Figure IV- 6a and Figure IV- 6b). The different screens allow only reducing the particle size. On the other hand, the impact milling using screen 0.5 and 0.1mm respectively changed the form of particles as can be seen in Figure IV- 6c and Figure IV- 6d. Figure IV- 6e and Figure IV- 6f shows the particles resulting from milling by VBM_{20} and VBM_{40}. It is clear that the particles have been relatively more destroyed compared to two previous milling technologies.

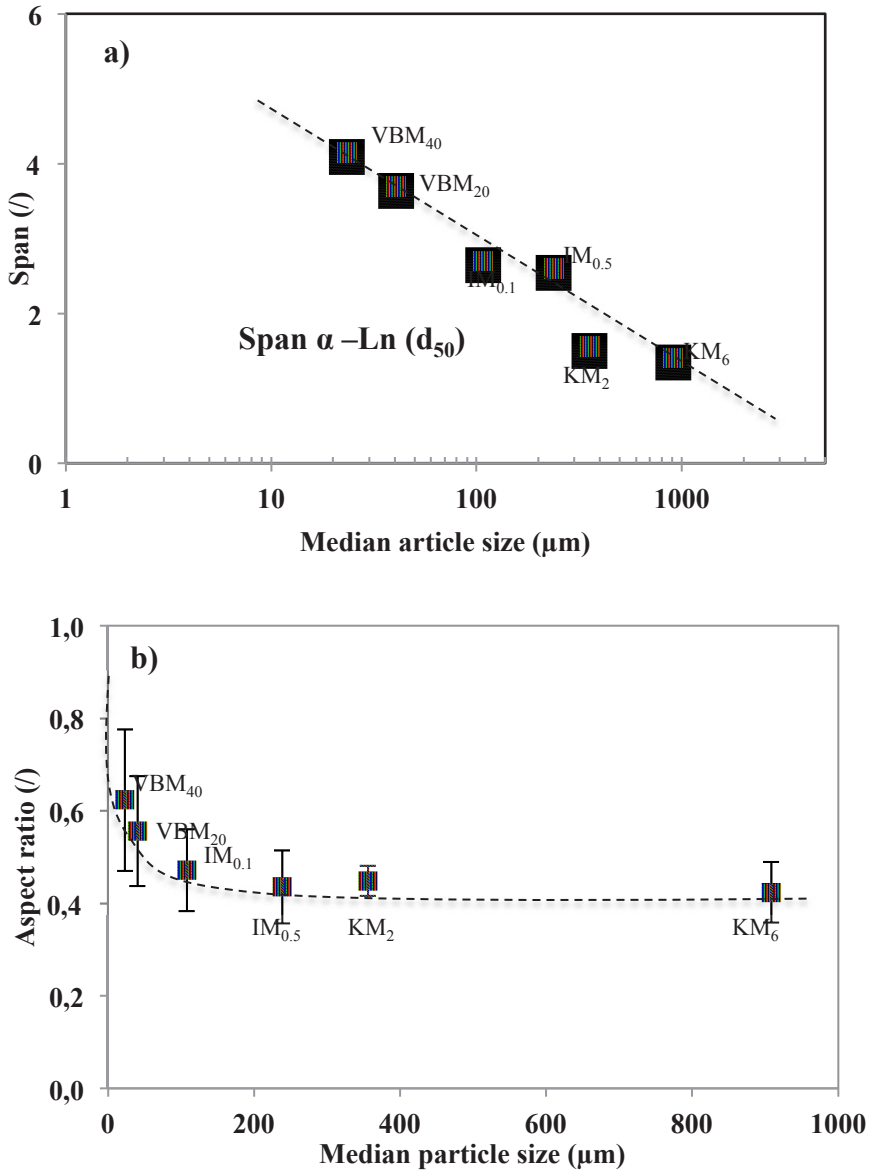

Figure IV- 5 *Variations of powder properties with the size for each grinding process.*

-a) Span of the distribution size. -b) Average aspect ratio of particles.

In order to compare the forms of particles resulting from mechanical processes, the aspect ratios were calculated and reported in Figure IV- 5b. For powders KM_6, KM_2, $IM_{0.5}$ and $IM_{0.1}$, the aspect ratio tends being stable to a fiber shape. It varied between 0.4 and 0.45. Below 100µm, the aspect ratio increased and reached a maximum at 0.63 for VBM_{40} process. Particle shape tended then to a more spherical form. From the sizes 50-100 µm, particles attempted the size of plant cell wall. Grinding/milling broke down these structures in fragments, the shape of which became less and less fibrous. This result indicates that these fibrous cells were statistically cut perpendicularly to the larger size.

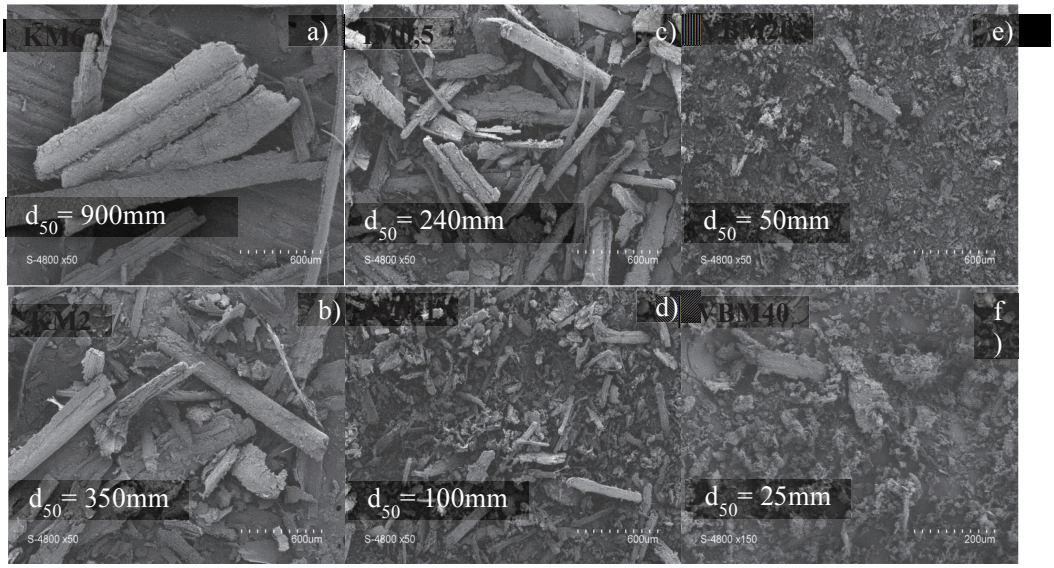

Figure IV- 6 *Illustration of each powder fractions by SEM pictures. – a) powder KM_6 – b) powder KM_2 – c) powder $IM_{0.5}$ –d) powder $IM_{0.1}$ –e) powder VBM_{20} –f) powder VBM_{40}.*

IV.2.3 Hydro-textural analysis

This section concerns the parameters, which are linked to the hydro-textural description (ϕ, w, S). All rice straw characteristics have been presented in the Table IV- 5. We observe that moisture content decrease when the particle size decreases (Figure IV- 7a). However, this loss of water, which might be due to the heating during grinding operation, is not significant. From 900µm to 26µm corresponds to~97% of a size reduction, ~11% of removal water. It would mean that a milling/grinding operation could not only break plant cell down but it also decreases the amount of water contained in the material.

Table IV- 5 *Values of hydro-textural and density parameters obtained after grinding process.*

Grinding/milling techniques	Samples	w (/)	ϕ (/)	s (/)	ρ_s^* (g.cm^{-3})
Knife milling (KM)	KM_6	0.096	0.679	0.253	1.246
	KM_2	0.095	0.703	0.361	1.610
Impact milling (IM)	$IM_{0.5}$	0.093	0.743	0.450	1.665
	$IM_{0.1}$	0.090	0.758	0.480	1.700
Vibro-ball milling (VBM)	VBM_{20}	0.089	0.830	0.698	1.618
	VBM_{40}	0.086	0.853	0.809	1.629

This decrease in moisture content affects directly the degree of saturation (Figure IV- 7a), which increases when the particle size decreases. This variation is explained by the effect of size reduction that involves also an increase in powder density between ~1.2 and ~1.6-1.7. It means that (i) rice straw powders are in an unsaturated state which is the consequence of their drying and (ii) the more particles are grinded, the more they are densified as confirmed by the increasing compactness (Table IV- 5). This unsaturated state tends to saturation with size reduction: $\lim_{d_{50}\to 0} S = 1$ (Figure IV- 7a). Hence, it indicates that when the particle size was reduced, there are less void zones in the porous rice straw and the quantity of water content was maintained nearly constant, which consequently increases the degree of saturation. Most of water is still contained in the cells or their fragments as a compact and saturated medium. It means that a grinding operation eliminates a void part of material. Beside, an increase in compactness indicates that the local mechanism is effective to break down the straw at the level of void localization. Moreover, observing that the compactness of rice straw powders is correlated with median diameters according to a power law:$\phi = (\frac{d_{50}}{d_{50}^{\circ}})^{s}$, with s an exponent related to the geometry of the straw, equals to *s* = - 0,064, and d_{50}° the median size of a smallest structure which could be ground by this process. It corresponds to: $d_{50}^{\circ} = \lim_{\phi\to 1} d_{50}$ and is equal to ~2 µm. This power law fits experimental data with a good correlation (R^2 = 0.97) above two decades (Figure IV- 7b). The order of magnitude of $d_{50}^{\circ}$is comparable with the thickness of the rice straw cells shaped in a fiber form (Moghaddam and Wilman 1998). This evident remark is consistent with the fact that the cells should be preferentially cut in the transversal rather than in the longitudinal orientation.

The relevance of this power law allows to interpret the straw grinding as a fractal deconstruction mechanism (Dang and Nguyen, 2007) whose fractal dimension would be given by: $s = D_f - 3$ with D_f the fractal dimension of the considered object equals 2.93 in this case (3 is the space dimension including the object). This result could be linked with the theoretical fractal geometry of straw, provided that the morphogenesis of rice straws follows such a growth process (Mandelbrot, 1982, Gardiner et al., 2012). The structures involved that the histology of straw could therefore be agented with hierarchized void levels (from cells to fibers to epidermal system…).

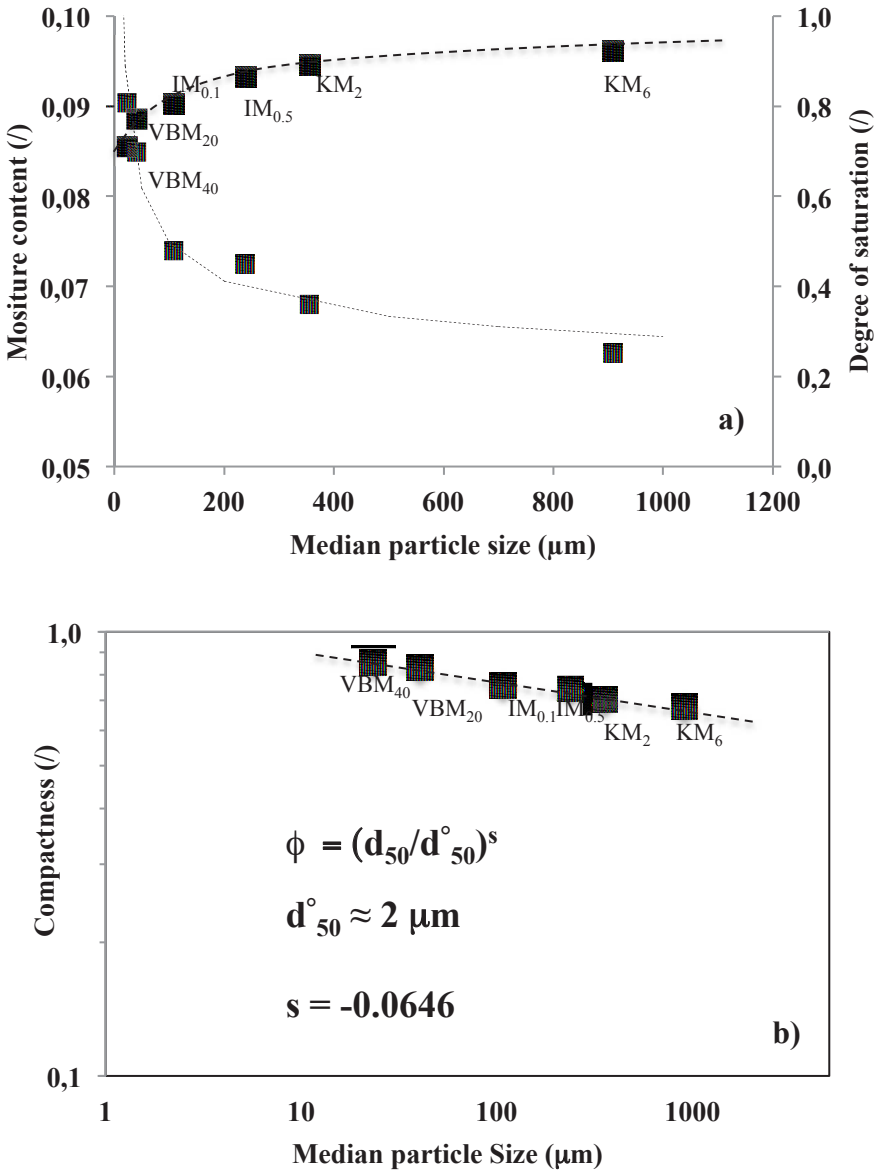

Figure IV- 7 *Variations of hydrotextural properties of particles with the size for each grinding process.*

-a) □ Moisture content and ● degree of saturation. -b) Compactness

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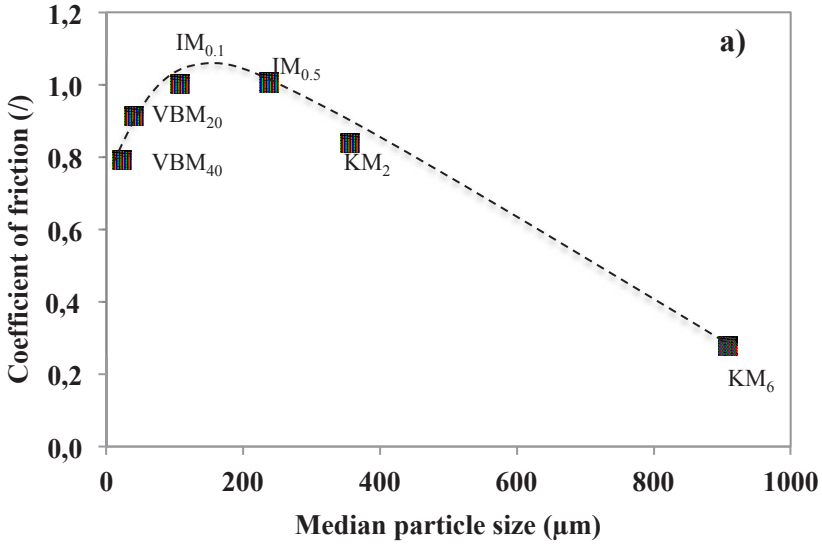
IV.2.4 Mechanical properties

Mechanical characterization of the powder obtained by grinding the rice straw was performed in shear cell (FT4 powder rheometer). From Mohr circles, the Coulomb strait line allows to define the apparent friction coefficient and the apparent cohesion. The apparent cohesion and friction coefficient are measured at the pallet scale not at the particle scale. Generally, in literature the LC powder materials have been poorly studied.

Concerning the evolution of the coefficient of friction (Figure IV- 8a) and cohesion (Figure IV- 8b) with the size of powder particles, we observe a surprising dependency. For the straw cut by milling, the coefficient of friction has the smallest value ($\mu \approx 0.25$), which is smaller compared to other powders and the cohesion has the higher value ($C \approx 2$ kPa), which is not significantly different from the other powders. This powder could be considered as a low cohesive powder. The particle size reduction increase the coefficient of friction till reaching a maximum and it then starts decreasing for the smallest particle size powders. The cohesion decreases until a minimum, which corresponds to the size where the coefficient of friction reached its maximum and then, for fine powders ($d_{50} \leq 100\mu m$), increased. The grinding operations are normally able to increase the specific surface areas that make consequently the cohesion increases. According to the results, we can give an explanation of this counterintuitive behavior. For powders ground up to~100 µm, the particles are small fibers that make physicochemical and steric interactions not significant. The aspect ratio corroborates the fiber shape of the particles (Figure IV- 5b). The physicochemical interactions are not significant in considering the steric entanglement of fibers and at the same time particles can move one to the others with a low flowing threshold. The particle is attached by mechanical force due to geometrical complexity. Below~100 µm, the particles become more and more spherical (Figure IV- 5b) what makes a decrease in steric interactions. The cohesion increases then by a gain of specific surface area, which promotes the attractive interactions.



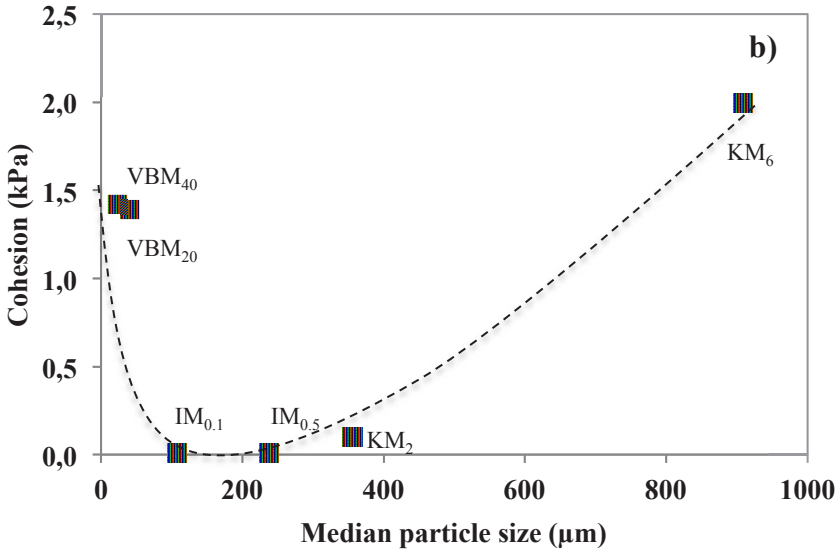


Figure IV- 8 *Variations of mechanical properties according to Coulomb criterion with the size for each grinding process. -a) Coefficient of friction. -b) Cohesion.*

IV.2.5 Kinetics of rice straw powder densification

Le suivi de l'évolution du volume apparent des lits de poudre (V_L) disposés une éprouvette et soumis à des chocs répétitifs et de régulière intensité, permet de quantifier leur aptitude au tassement. Connaissant la valeur de la masse sèche de poudre introduite, il est possible de déduire de ces mesures l'évolution de la compacité apparente du lit : $\phi_L = M_s/(\rho_s^* V_L)$. Pour les six poudres de riz, la Figure IV- 9 présente, sur une échelle semi-logarithmique, les évolutions de la compacité du lit en fonction du nombre de coups. On constate que les distributions granulométriques des poudres impactent directement ces « cinétiques » de tassement, tant leur allure que les niveaux de compacité atteints. D'un point de vue général, les niveaux initiaux de compacité du lit sont faibles : de ~ 0,036 pour KM_6 à ~ 0,208 pour VBM_{40} (Table IV- 6). Ces faibles compacités « vrac » sont la signature de milieux très aérés, plutôt fibreux désordonnés. On constate que pour les échantillons de KM_6, KM_2 et $IM_{0.5}$ l'évolution de la compacité n'augmente que très légèrement, ce qui traduit leur difficile réarrangement sous une telle sollicitation. Pour la poudre $IM_{0.1}$, et surtout pour BM_{40} et BM_{20} l'évolution de la compacité est beaucoup plus significative et présente, sous se mode de représentation, trois phases de densification : une latence initiale à laquelle succède très rapidement une phase densification rapide et « forte » puis, enfin, une phase de relaxation lente qui conduit progressivement le milieu vers son état de compacité maximal.

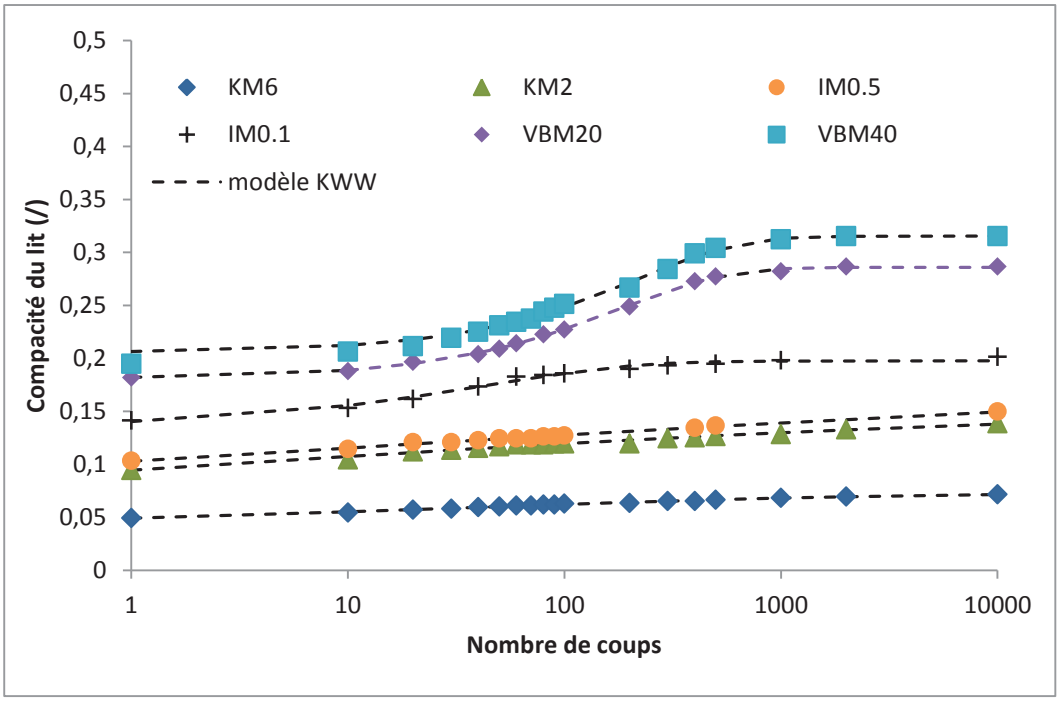


Figure IV- 9 *Essais de tassement sur les différentes poudres de paille de riz – Confrontation avec le modèle KWW.*

Il s'est avéré, conformément au constat issu de la bibliographie, que le modèle KWW est celui qui interpole le mieux au sens des moindres carrés, ces courbes expérimentales (Figure IV- 9; Table IV- 7). Le paramètre τ^{-1} peut être interprété comme un nombre de coups caractéristique à partir duquel le lit se densifie moins fortement et sa compacité rejoint asymptotiquement sa valeur maximale. La variation du paramètre τ avec la taille médiane des particules est présentée Figure IV- 10. Cette dépendance a la même allure que la dépendance de la cohésion des poudres, même si pour la poudre KM_6, l'interpolation a très probablement conduit à une surestimation de τ. Il est très probablement possible d'attribuer ce gain de capacité de réarrangement des lits de poudres les plus fines, par l'action conjointe de l'augmentation du SPAN et de l'augmentation du rapport d'aspect.

Table IV- 6 *Valeurs des caractéristiques granulométriques des poudres, des compacités initiale et finale et des paramètres du modèle KWW.*

Sample	$D_{50}(\mu m)$	SPAN	Aspect ratio	ϕ_0	$\phi\infty$	$\tau(/)$	β
KM_6	908	1,404	0,424	0,036	0,072	36,578	0,229
KM_2	356	1,559	0,449	0,001	0,149	0,995	0,104
$IM_{0.5}$	238	2,603	0,435	0,000	0,176	4,678	0,083
$IM_{0.1}$	108	2,704	0,471	0,135	0,198	43,778	0,622
VBM_{20}	46	3,669	0,556	0,181	0,286	183,240	0,881
VBM_{40}	26	4,151	0,623	0,206	0,315	218,006	0,904

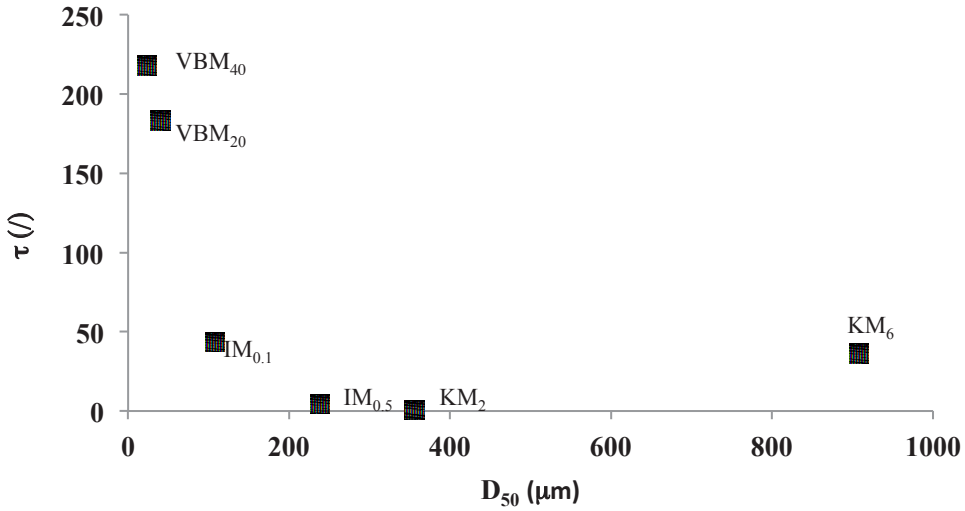


Figure IV- 10 *Variation du paramètre τ du modèle KWW avec la taille médiane des particules du lit de poudres*

Les particules de poudres ayant des compacités différentes (Figure IV- 7b), il est possible de quantifier la part strictement due au réarrangement interparticulaire en calculant la compacité qui lui est associée : $\phi_{inter.} = \phi_L \phi_p^{-1}$ où ϕ_p est la compacité des particules dite intraparticulaire. La Figure IV- 11 illustre la fait que la contribution de la différence de compacité des particules suivant leur taille est négligeable sur le différentiel entre compacité du lit et compacité interparticulaire. L'étude de la compacité du lit permet donc d'analyser directement les réarrangements entre grains.

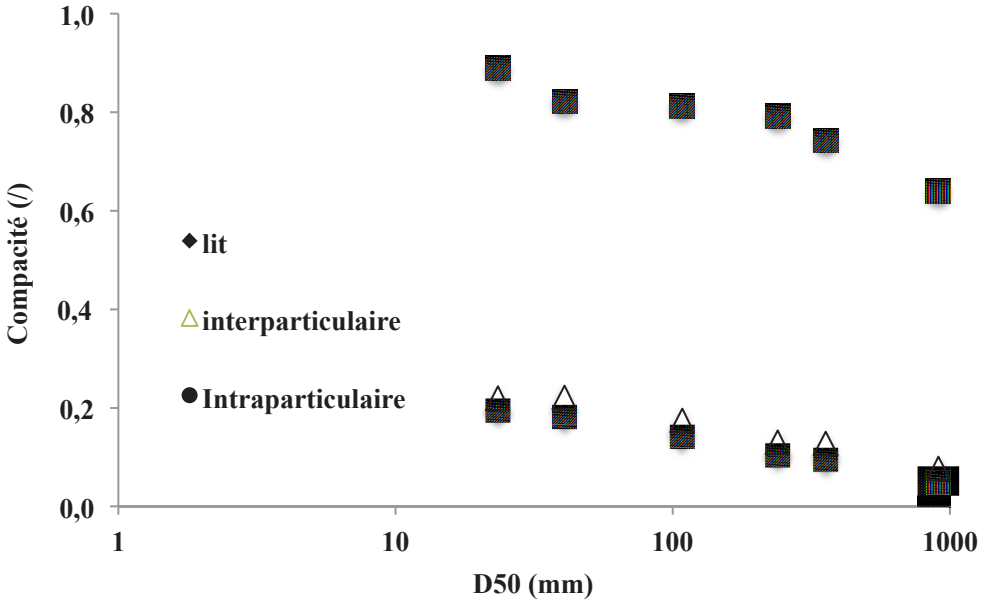


Figure IV- 11 *Comparaison des valeurs des compacités initiale du lit (ϕ_L), interparticulaire ($\phi_{inter.}$) et intraparticulaire (ϕ_p) en fonction du diamètre médian des poudres.*

On constate que plus les particules sont petites, plus la variation de compacité du lit $\Delta\phi_L = \phi_{L\infty} - \phi_{L0}$ augmente. Cette dépendance est plutôt exponentielle avec le diamètre médian et plutôt linéaire avec le SPAN de la distribution (Figure IV- 12).

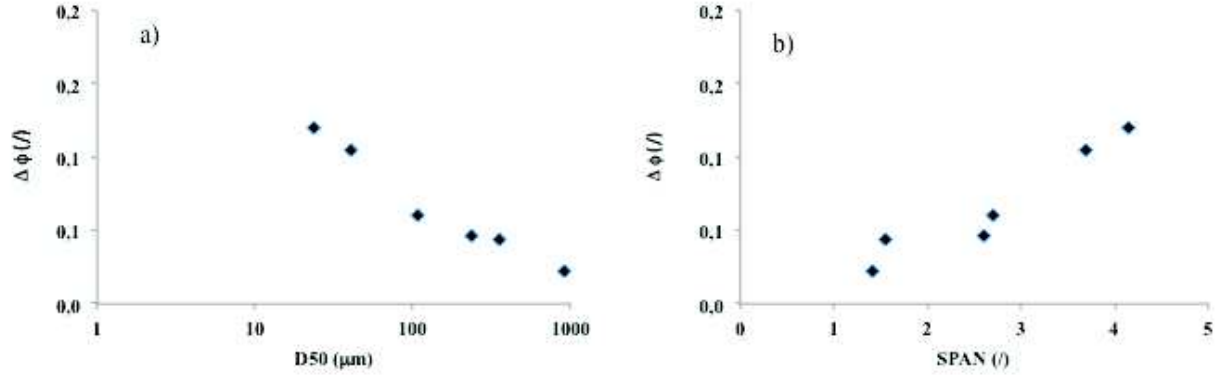


Figure IV- 12 *Comparaison des valeurs des compacités* $\boldsymbol{\Delta\phi_L}$ *en fonction a) du diamètre médian des particules, b) du SPAN de la distribution granulométrique des particules.*

Les lits de poudres issues de broyages de plus en plus poussés, sont donc de plus en plus compressibles. Cette caractéristique a des répercussions sur leur rhéologie dont la valeur les indicateurs : indice de Carr et rapport d'Hausner (Shah, Tawakkul et al. 2008), permet de classer technologiquement la coulabilité (Table IV- 7). La Figure IV- 13 montre que l'indice de Carr et le rapport d'Hausner augmentent lorsque la taille des particules diminue. Nous pouvons situer les particules de poudre de riz sur le diagramme de Geldart (Boerefijn, Ghadiri et al. 2007; van Ommen, Valverde et al. 2012; Valverde Millán 2013) où se matérialise la trajectoire technologique due au broyage (Figure IV- 14). Si les prétraitements mis en jeu permettent d'obtenir des poudres de plus en plus fine, avec un gain énergétique intéressant, en revanche, la qualité d'écoulement des poudres se déprécie. Ce facteur pourrait aussi devenir une propriété d'usage explicitement recherchée, car les poudres sont amenées à être convoyées d'un réacteur à l'autre au sein de la filière de valorisation énergétique des pailles de riz.

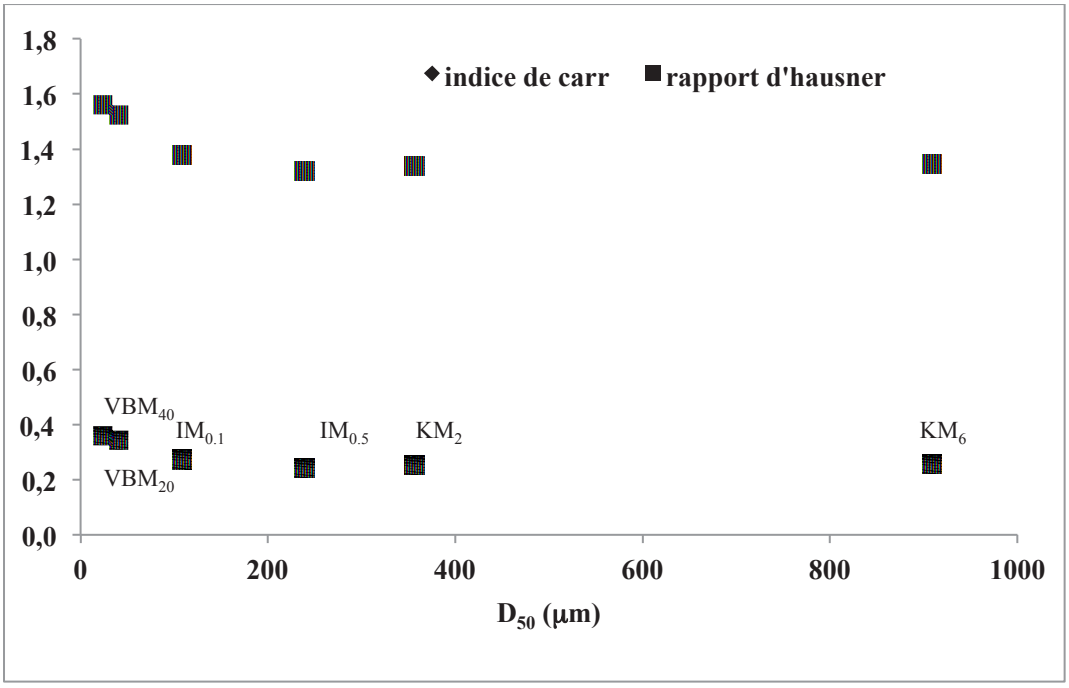


Figure IV- 13 *Variations du rapport d'Hausner et de l'indice de Carr en fonction du diamètre médian des particules*

Table IV- 7 *Classification des poudres de pailles de riz*

échantillon (mm)	D_{50} (µm)	ρ_s^* (g.cm^{-3})	Indice de Carr	Rapport d'Hausner	classification Geldart	coulabilité
KM_6	908	1.246	0,258	1,348	A fusante	Mauvaise
KM_2	356	1.610	0,253	1,339	A fusante	Mauvaise
$IM_{0.5}$	238	1.665	0,244	1,323	A fusante	Mauvaise
$IM_{0.1}$	108	1.700	0,274	1,377	A fusante	Mauvaise
BM_{20}	46	1.618	0,344	1,525	C cohésive	Mauvaise
BM_{40}	26	1.629	0,36	1,561	C cohésive	Très mauvais

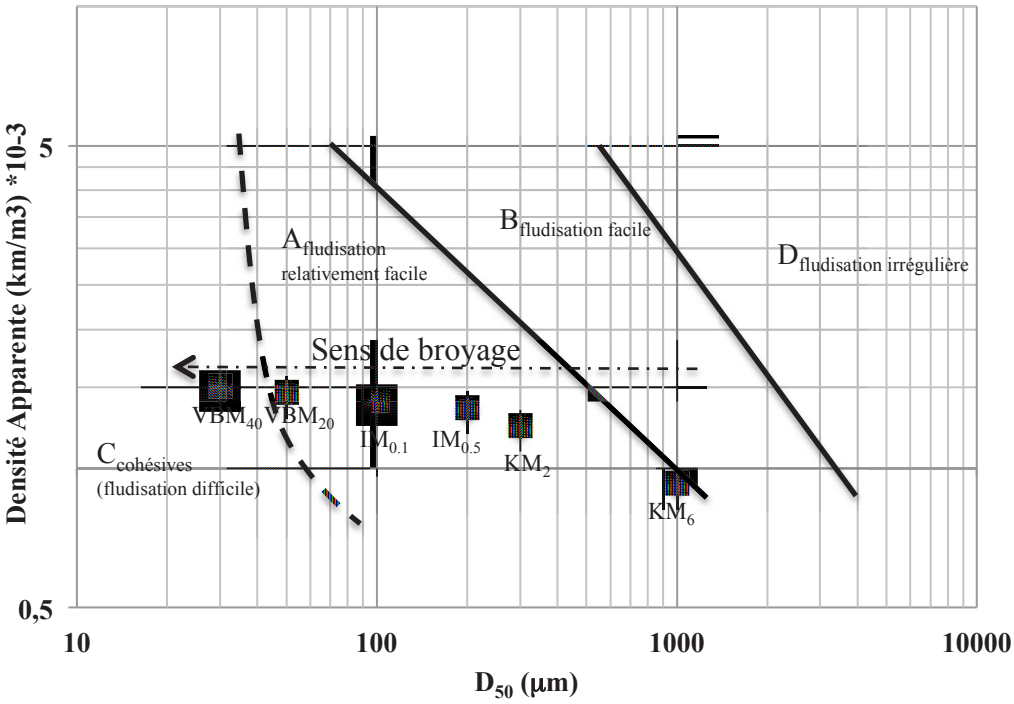


Figure IV- 14 *Situation des poudres de pailles de riz sur le diagramme de Geldart (Valverde Millán 2013)*

IV.2.6 Conclusion

The current study presented the new characterization methodologies of LC powder using different technologies allowing understanding more the structural organization of the LC matrix and their fractionation. A hydro-textural approach has been used to describe and follow the wet granular state of rice straw upon different grinding operations. Along with the grinding process, particles become smaller, more spherical, and denser and more compact, as well as more saturated, which would improve rice straw sensitivity to further enzymatic hydrolysis and also fermentation. A power law had been proposed to describe the relation between size reduction and increase in compactness. This model highlights a theoretical value of a grinding limit size that seems to correspond to the thickness of straw cell wall. This value is in an order of magnitude consistent with the process limits generally observed for this kind of material. The mechanical studies had shown changes in cohesion and friction properties during the grinding process linked to aspect ratio evolutions. The key parameters identified in this study would be further affected in case of combination of grinding with other chemical or physical-chemical pretreatments of the starting material, allowing improved technical and economic efficiency of biorefinery processes and lower environmental impacts.

IV.3 Comportement des poudres de pailles sous sollicitations hydrique - Essais d'imbibition

IV.3.1 Introduction

Les pailles de riz qui servent de support à cette étude, ont été préalablement séchées et leur texture n'est plus directement en lien avec l'état physiologique de la plante sur pied. Cette déshydratation génère notamment un retrait mécanique, se traduisant par la constriction des tissus et une modification de l'agencement textural. Ces modifications mécaniques peuvent être accompagnées de modulations des propriétés physico-chimiques vis-à-vis des propriétés cinétiques de sorption d'eau, mais aussi des propriétés thermodynamiques comme la relation activité/teneur en eau. Les procédés humide et semi-humide de prétraitement considérés dans cette étude, mettent en jeu une réhydratation des pailles (i) plutôt « volumique » pour le procédé humide et (ii) plutôt « surfacique » pour le semi-humide. Le mouillage des fibres et l'imbibition d'eau dans les pailles représentent donc des phénomènes important dans le sens où ils sont préliminaires à toutes interactions chimiques entre la paille et la soude ou tout autre composé en solution dont l'action chimique est déstructurante. La capacité de reprise d'eau et la localisation de la pénétration d'eau peuvent en conséquence représenter des phénomènes limitants l'efficacité chimique des prétraitements. Nous avons considéré dans ce travail, que l'évaluation du « degré de réversibilité » de la relation texture/état hydrique des pailles est un point de départ à l'analyse des phénomènes mis en jeu dans les prétraitements.

IV.3.2 Cinétique de transfert d'eau

La méthodologie d'analyse repose sur une étude hydro-texturale comme développée au chapitre précédent, mais adaptée ici pour suivre l'imbibition des pailles (Ruiz, Rondet et al. 2011) étude expérimentale est tout d'abord conduite sur des morceaux de paille native de quatre centimètres de long. Les pailles ont été fendues selon une génératrice, déroulées puis mise à plat, enfin, des « lanières » de deux millimètres de large sont découpées. L'hypothèse expérimentale revient à considérer que ces fragments macroscopiques sont représentatifs du « matériau paille » dans la mesure où ils intègrent toutes les échelles de la microstructure. L'expérience d'imbibition vise à mesurer les évolutions de la teneur en eau, de la déformation, du degré de saturation et de la compacité à l'échelle du matériau au cours d'une imbibition conduite par immersion des pailles directement dans l'eau de la cuve de la balance hydrostatique (Figure III- 11). La pression hydrostatique sous laquelle l'eau pénètre dans les pailles ($P_{hydro.} = \rho_w gh$), correspond à une colonne d'eau de 5 cm soit : 0,490 kPa. En absence d'agitation mécanique, les conditions cinétiques

d'imbibition sont entièrement pilotées par la diffusion d'eau. A différents instants, les poids apparents « dans l'air » (noté : $M_h g$) et « dans l'eau » (poids corrigé de la poussée d'Archimède, noté : $M_{app.} g$) sont mesurés (Figure IV- 15). Le poids apparent « dans l'eau » est ici négatif, ce qui indique que la densité apparente de la paille est inférieure à celle de l'eau. Les pailles flottent, c'est pourquoi un petit système d'immersion constitué d'une « cage métallique » a été mis en place.

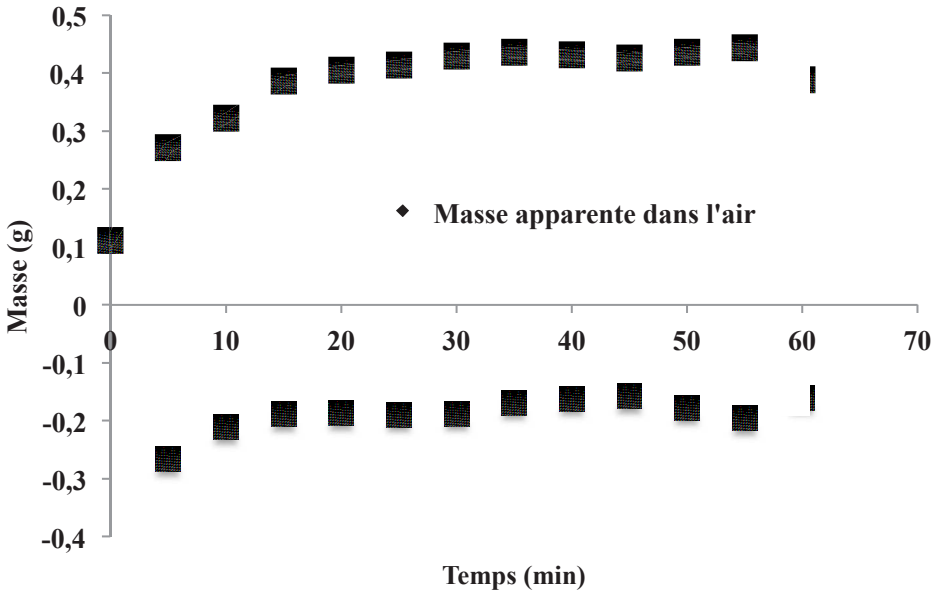


Figure IV- 15 *Evolution de la masse apparente de la paille durant l'imbibition, mesurée dans l'air et dans l'eau*

A partir de la cinétique de masse apparente dans l'air (M_h) et en mesurant la masse sèche des pailles en fin d'essai après étuvage (notée : M_s), on calcule l'évolution de la teneur en eau : $w = (M_h - M_s)/M_s$. La Figure IV- 16 représente une cinétique de teneur en eau relative à l'imbibition des pailles natives. L'imbibition s'initie par une première phase « rapide » à laquelle succède un ralentissement puis une stabilisation lente vers la teneur en eau de saturation. Sur la seule durée de cet essai, la cinétique se modélise bien par un modèle de type cinétique chimique d'ordre 1 : $dw/dt = \tau^{-1} w$ avec $w(t = 0) = 0$ et $\lim_{t\to\infty} w = w_{sat}$, et où τ^{-1} est le temps caractéristique de ce transfert (i.e. τ est la constante de vitesse). La solution, de la forme : $w/w_{sat} = 1 - \exp(-t/\tau)$ est représentée Figure IV-15, avec $\tau^{-1} \approx$ 8 min et $w_{sat} \approx$ 380%. Le milieu n'étant pas saturé, cette description cinétique qui correspond à la stabilisation de la teneur en eau, n'est pas conforme à la réalité car la paille continue à absorber de l'eau. Ce modèle décrit exclusivement la première période de transfert d'eau, qui est piloté par les conditions d'imbibition imposées. On montrera par le raisonnement qui va suivre que la cinétique de prise d'eau qui se prolonge au-delà des 60 min

d'essai, est aussi contrôlée par le gonflement des pailles, phénomène qui échappe à la description de ce modèle d'ordre 1.

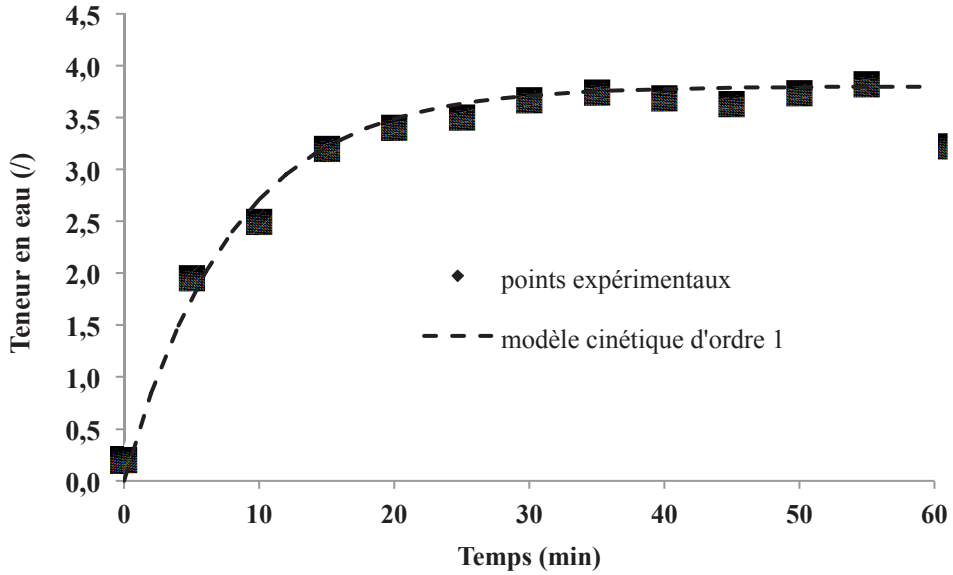


Figure IV- 16 *Cinétique de teneur en eau au cours d'un essai d'imbibition de la paille. Modélisation de cette période initiale d'imbibition par une cinétique d'ordre 1*

IV.3.3 Couplage entre transfert d'eau et le gonflement de la paille

A partir de la mesure de la masse apparente dans l'eau (notée $M_{app.}$) et du théorème d'Archimède qui donne l'expression du poids apparent de la paille immergée dans l'eau : $P_{app.} = M_{app.}g = M_h g - \rho_e V_p g$, connaissant les masses volumiques de la paille (ρ_s^*) et de l'eau (ρ_e), il est possible de calculer à chaque instant le volume de la paille : V_p (***Figure IV- 17***). On démontre facilement que la variation de masse apparente est directement liée aux variations de teneur en eau et de compacité ($M_{app.}/M_s = 1 + w - d_s^{*-1}\phi^{-1}$) : $\delta(M_{app.}/M_{s)} = \delta w - d_s^{*-1}\delta\phi^{-1}$, avec d_s^* la densité de la paille.

On constate immédiatement que la variation de volume d'eau qui pénètre dans le matériau est plus importante que la variation de volume du matériau (***Figure IV- 17***).

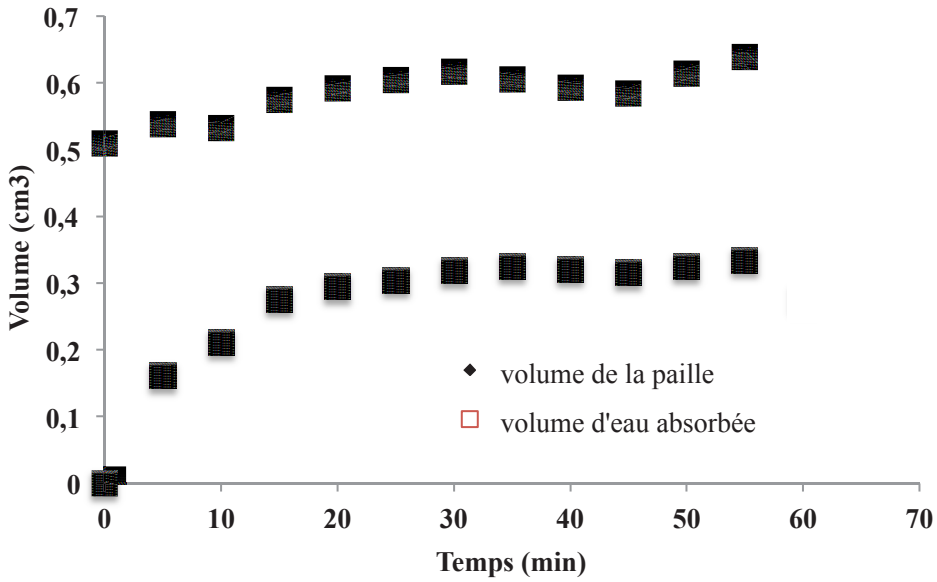


Figure IV- 17 *Evolution du volume de la paille et du volume d'eau absorbée durant l'imbibition.*

L'analyse de la déformation volumique définie par : $\varepsilon = \Delta V / V_0$ avec V_0 permet de compléter l'interprétation du transfert d'eau (***Figure IV- 17***). On constate que les pailles commencent par se remplir d'eau sans gonfler dans un même rapport. Si le gonflement était idéal, la variation de volume de la paille serait exactement égale à la variation de volume d'eau ayant pénétrée le matériau. On démontre très facilement que la condition : $\Delta V = \Delta V_e$, se traduit par une dépendance linéaire entre la déformation volumique et la teneur en eau (***Figure IV- 17***) : $\varepsilon = d_s^* \phi_0 (w - w_0)$ où ϕ_0 est la compacité initiale de la paille. L'infiltration initiale de l'eau correspond donc à un remplissage rapide d'une grande quantité d'eau (~300%) (Figure IV- 16) qui n'induit qu'une faible déformation (~5%). Puis, le ralentissement de la cinétique de transfert d'eau est concomitant avec un fort gonflement du matériau dont l'évolution de la déformation volumique est au moins comparable à un gonflement idéal. Ces cinétiques de transfert/gonflement, illustrent le déphasage du couplage hydro-mécanique qu'il y a entre ces phénomènes.

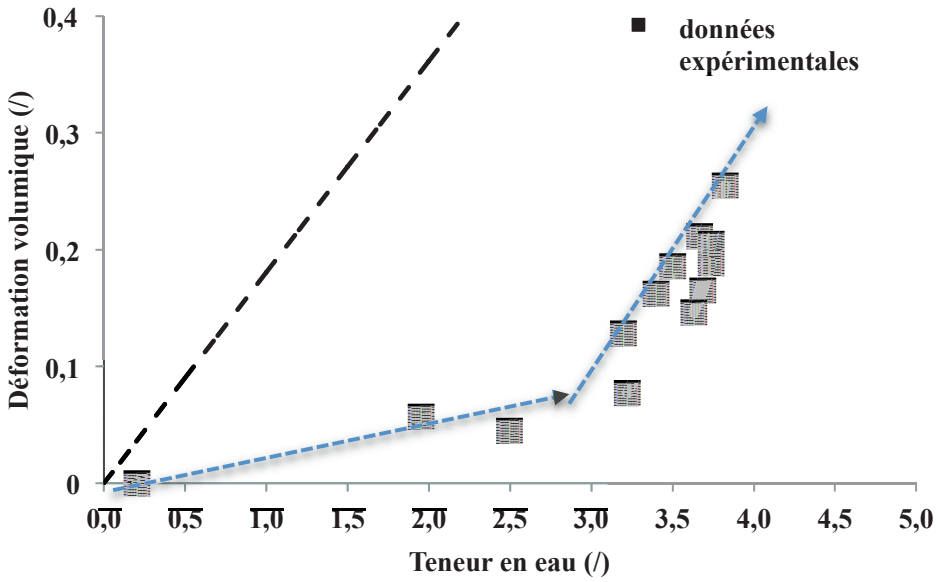


Figure IV- 18 *Evolution de la déformation volumique de la paille en fonction de la teneur en eau durant l'imbibition.*

La mesure de la masse sèche en fin d'essai, permet aussi de calculer la compacité de la paille : $\phi = M_s/(\rho_s^* V_p)$. Le degré de saturation peut alors être calculé par : $S = d_s^* w/(\phi^{-1} - 1)$. Les cinétiques de transfert d'eau et de déformation conduisent à la saturation progressive de la paille (Figure IV- 18). Le matériau suit une loi de saturation de type loi puissance décrite par Rondet et al., 2010 (Rondet, Delalonde et al. 2010) et représentative d'un phénomène de percolation en milieu poreux : $S = \left(\frac{w}{w_{sat}}\right)^n$, avec w_{sat} la teneur en eau atteinte à saturation (~580%) et n le coefficient de couplage hydro-mécanique (~0,93). Ce modèle de description est représenté Figure IV- 18. La forte teneur en eau à saturation indique que le matériau peut reprendre encore beaucoup d'eau, cependant, la forte valeur du coefficient n indique qu'il n'est pas très fortement déformable sous cette action hydrique. Notons que les pailles sont ici réhumidifiées, leur séchage ayant très certainement généré des collapses irréversibles limitant le gonflement.

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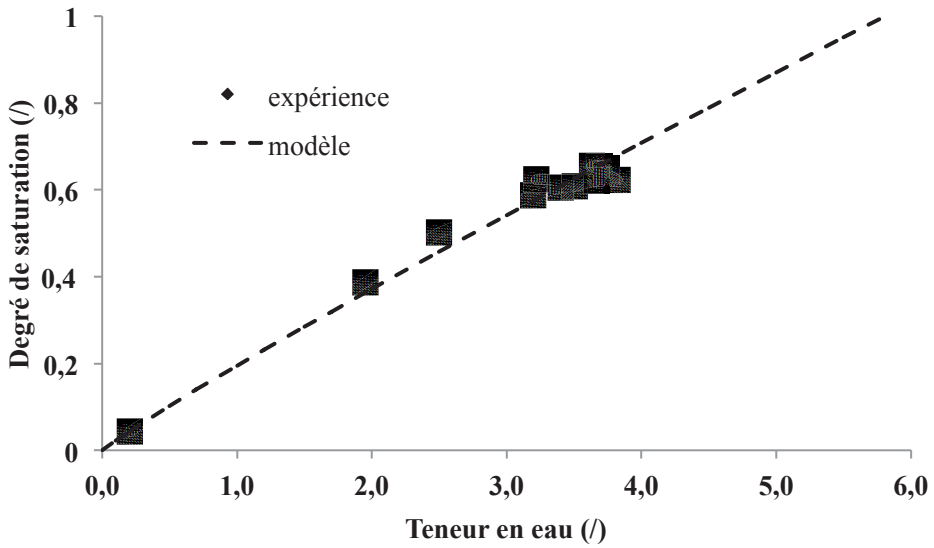


Figure IV- 19 *Evolution du degré de saturation avec la teneur en eau durant l'imbibition.*

Cette analyse des phénomènes peut être représentée sur le diagramme hydro-textural des pailles au travers du « chemin d'imbibition » (Figure IV- 19). Bien en lien avec la cinétique de prise d'eau, la compacité des pailles suit une variation modélisable par : $\phi^{-1} = 1 + d_s^* w_{sat}^n w^{1-n}$, représentée Figure IV- 19. Le modèle hydro-textural, qui nécessite la connaissance de la densité de la paille, de la teneur en eau à saturation w_{sat} et du coefficient n, permet de quantifier le lien entre déformation et teneur en eau.

Cette information est importante pour qualifier le couplage entre transferts d'eau et gonflement au cours des prétraitements humide et semi-humide. Ce travail nous permet de conclure que tant que la teneur en eau reste inférieure à 300% on peut considérer que le matériau est quasiment indéformable. Cette hypothèse permet une simplification lourde de conséquence expérimentale dans la mesure où il n'est plus nécessaire de mesurer l'évolution du volume apparent dans l'expérience de prise d'eau. Avec $\phi \approx$ cte, la mesure seule directe de la variation masse apparente directement dans l'eau, permet d'accéder à la cinétique de teneur en eau : $\delta(M_{app.}/M_{s)} = \delta w$. Par ailleurs, la gamme de compacité des six poudres de pailles de riz broyées, est située entre 0,679 pour KM_6 et 0,853 pour VBM_{40} (Table IV- 6). Sur la Figure IV- 20, est reportée en encart, la situation des points KM_6 et VBM_{40} dont la comparaison avec l'état hydro-textural des pailles natives montre bien que les gammes de compacités et de teneurs en eau sont très différentes. Les poudres de pailles pourront dont être considérées comme indéformables au cours de leur réhydratation que les prétraitements humide et semi-humide imposent.

Cependant, les essais d'imbibition à l'eau des pailles broyées n'ont pas été concluants car la dissolution des molécules solubles a rendu la mesure de la matière sèche impossible en cours d'expérience. Il serait nécessaire de mesurer la quantité de matière dissoute au cours d'un essai pour suivre l'évolution des paramètres hydro-texturaux. Une corrélation entre densité optique (mesure spectrophotométrique) et masse sèche n'ayant pas pu être mise en place dans les temps, cette analyse complète ne sera pas aboutie dans le cadre de cette thèse.

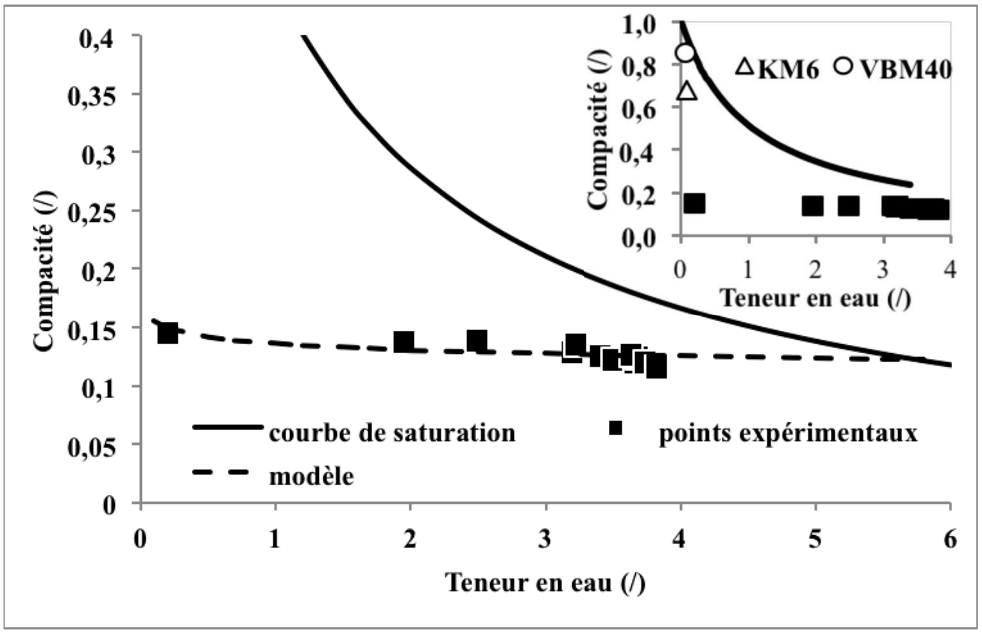


Figure IV- 20 *« chemin d'imbibition » sur le diagramme hydro-textural de la paille.*

IV.4 Méthodologie de quantification des processus de déconstruction des pailles de riz

IV.4.1. Introduction

Les chapitres précédents ont permis de montrer que l'analyse hydro-texturale pouvait représenter un cadre scientifique pertinent pour étudier les mécanismes de broyage, de séchage et d'imbibition des pailles de riz. Il s'agit à présent d'appliquer cette approche à l'étude de l'impact des prétraitements chimiques humide et semi-humide. Cette partie reprend donc les principaux éléments de la démarche, et l'étude des transferts d'eau à l'échelle des particules par une approche hydro-texturale, permettra de quantifier leurs impacts dans la déstructuration chimique des pailles. Cette analyse servira de base différentielle d'étude du rôle spécifique des réactifs chimiques (soude, acide,…) dans la déconstruction. Il ne s'agit donc pas d'une étude des cinétiques classiques des transferts de matière au cours des opérations de séchage et d'imbibition. Elle est volontairement axée sur le recours à une analyse purement hydro-texturale qui permet d'analyser les processus locaux de transformation de l'état multiphasique interne aux particules. En soit, cette restriction nous prive des apports de la démarche « Génie des Procédés », notamment de l'analyse dimensionnelle qui permettrait d'établir les bases d'un scale-up. Mais, ce travail préliminaire est avant tout orienté vers la consolidation d'une méthodologie de travail générique complémentaire qui permette de construire un raisonnement fiable d'analyse de l'impact des procédés sur les transformations des pailles à l'échelle de leur microstructure. Une fois cet objectif atteint, l'intégration d'une approche produit/procédé plus classique pourra largement être envisagée, la démarche hydro-texturale ayant fait les preuves de son applicabilité au traitement des boues de station d'épuration(Ruiz and Wisniewski 2008), à l'extrusion/sphéronisation d'excipients pharmaceutiques (Galland, Ruiz et al. 2009) ou encore l'élaboration du couscous (Saad, Barkouti et al. 2011).

IV.4.2. Tailles médianes et masses volumiques des poudres

Comme présenté dans la section précédente, nous retrouvons ici la paille de riz qui a été broyée par les différents modes de broyage et conduit à des poudres de tailles différentes. Les tailles médianes des poudres de paille broyée varient entre 23 et 900 micromètres. Comme précédemment, nous proposons de caractériser les états hydro-texturaux des particules qui constituent ces poudres. Nous complétons cette description des états résultant de l'opération de broyage, par l'analyse de

l'influence des transferts d'eau dans ces particules par séchage et par imbibition. Le Table IV- 8 recense les tailles, teneurs en eau hygroscopique ainsi que les masses volumiques des poudres obtenues avec les différentes technologies de broyage. L'exposant « ° » se réfère à la masse volumique apparente des particules de la poudre, mesurée par la balance hydrostatique. L'autre masse volumique est mesurée par pycnométrie à l'azote. L'indice « s » se réfère à un état anhydre tandis que l'indice « h » caractérise un état humide et plus précisément l'état d'équilibre hygroscopique à la température et l'humidité relative du laboratoire.

Table IV- 8 *Valeurs des principales grandeurs physiques caractéristiques de la paille de riz broyée.*

Technique de broyage	Abréviation	D_{50} (µm)	w (/)	ρh^*	ρs^*	$\rho°h^*$	$\rho°s^*$
Broyeur à couteau	KM_6	908	0,096	1,449	1,246	0,927	0,935
	KM_2	356	0,095	1,669	1,610	1,239	1,261
Broyeur UPZ	$IM_{0.5}$	238	0,093	1,710	1,665	1,353	1,338
	$IM_{0.1}$	108	0,090	1,729	1,700	1,404	1,410
Broyeur à billes	VBM_{20}	46	0,089	1,778	1,618	1,462	1,428
	VBM_{40}	26	0,086	1,696	1,629	1,508	1,496

Le Table IV- 8 fait apparaître le fait que toutes ces masses volumiques augmentent avec la diminution de la taille des particules. Ce point illustre le fait que plus les particules sont petites, plus elles sont denses. On constate que les masses volumiques semblent converger vers une même valeur avec la diminution de la taille des particules.

Pour chacune des poudres, il n'y a pas de différences notables entre les masses volumiques des états anhydre et hygroscopique (Figure IV- 21). L'eau présente dans les particules est dans un état thermodynamique dit « lié », et qui plus est, constitutive des cellules. Sa contribution « volumique » à la texture des particules n'est pas significative. Par contre, l'évaluation du volume des particules par immersion dans l'azote (volume sans pores internes hors porosité occluse) ou l'huile de paraffine (volume apparent des particules) met en évidence l'existence d'une porosité interne dont la valeur est donnée par : $1 - \rho_h^*/\rho_s^* \approx 1 - \rho_h^{°*}/\rho_s^{°*}$. Cette porosité décroit avec la diminution de la taille des particules (convergence des masses volumiques) ce qui indique que les poudres anhydre et hygroscopique ont des comportements comparables, dictés par la nature de la microstructure de la paille.

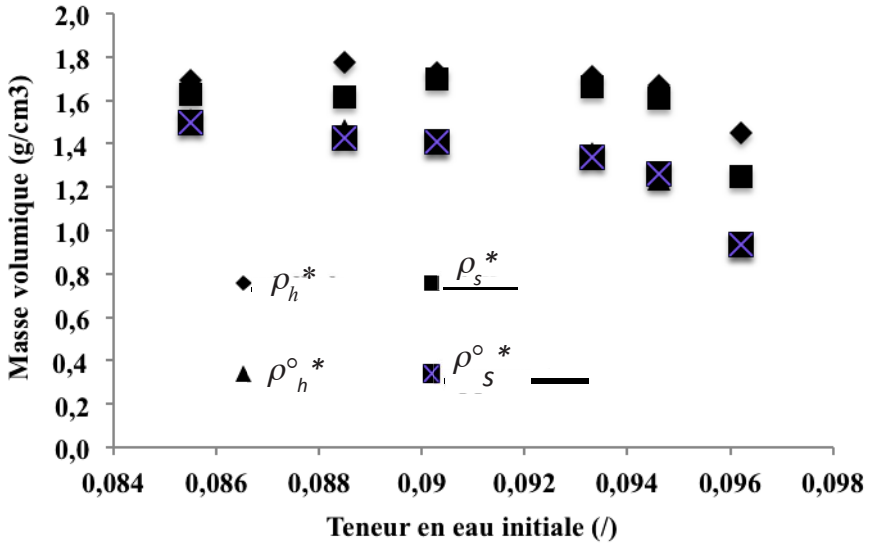


Figure IV- 21 *Variation des masses volumiques apparente et réelle des différentes poudres dans les états anhydre et hygroscopique, avec leurs teneurs en eau initiales.*

IV.4.3. Construction du diagramme hydro-textural des particules d'une poudre

Le diagramme hydro-textural est un diagramme de phase qui représente la relation entre la compacité et la teneur en eau des particules (Ruiz, Delalonde et al. 2005; Ruiz and Wisniewski 2008). Cette représentation permet de situer l'état de l'agencement entre les phases solide, liquide et gazeuse qui constituent le matériau, lui-même assimilé à un milieu granulaire triphasique. Ce graphe est construit par l'ensemble des états « physiques » que le matériau peut occuper. Dans le cas du broyage des pailles riz, nous serons conduits à représenter l'état initial de la poudre, puis les états intermédiaires de broyage, les états secs (après étuvage) et enfin les états fortement hydratés (après imbibition).

La Figure IV- 22 constitue le cadre du diagramme hydro-textural des particules de paille de riz. Les courbes de saturation des poudres obtenues par les six niveaux de broyage différents sont tracées dans le repère (w,ϕ). Une courbe de saturation représente l'état de compacité qu'aurait la poudre si l'eau qu'elle contient saturait totalement la porosité intra-particulaire. Cette relation indique que le volume de la particule saturée en eau est la somme des volumes du solide et de l'eau. On montre alors simplement que cette courbe a l'équation d'une hyperbole: $\phi_{sat} = 1/(1 + d_s^* w)$, où $d_s^* = \rho_s^*/\rho_e^*$ est la densité sèche de la poudre avec ρ_e^* la masse volumique de l'eau. Plus la densité des particules est importante et plus la courbure de l'hyperbole est forte (Figure IV- 22). Ainsi pour une teneur en eau donnée, plus les particules sont de petites tailles, plus elles sont denses (Table IV-

8) et plus la compacité qui correspond à l'état de saturation est basse. Autrement dit, pour une particule de compacité donnée, il y aura besoin d'atteindre une teneur en eau plus élevée pour saturer une grosse particule qu'une petite.

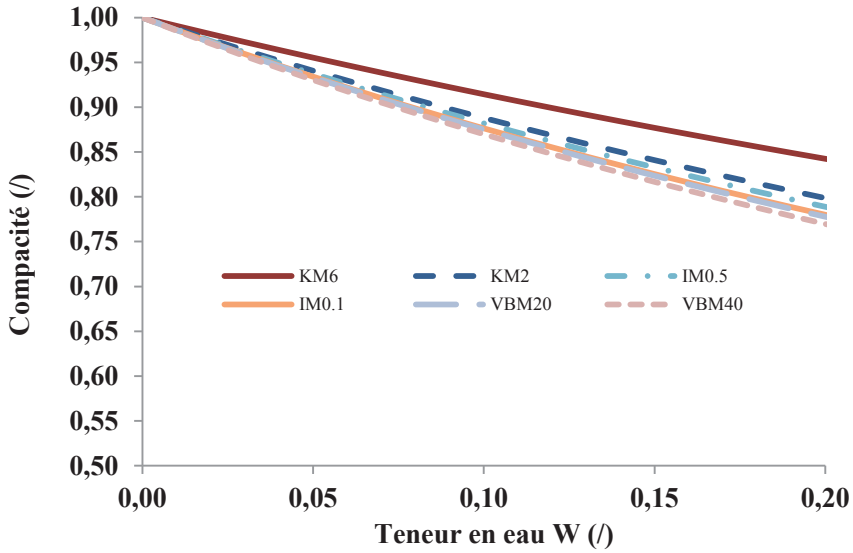


Figure IV- 22 *Représentation des courbes de saturation des particules issues des six différents broyages.*

L'état initial des particules qui correspondent au broyage KM_6 (Figure III- 1) ainsi que la courbe de saturation est placés sur le diagramme (Figure IV- 23). Les particules constituant la poudre résultante de ce broyage ont une teneur en eau moyenne : w = 9,6% et une compacité moyenne : ϕ = 68%, ce qui correspond à un état non saturé (S = 25%).

Au cours des sollicitations thermo-hydro-mécaniques que cette poudre sera amenée à subir, si le matériau est indéformable (i.e. si les particules sont intrinsèquement déformables, étant entendu que le lit de particules, la poudre, est globalement déformable), dans le sens où la quantité d'eau qu'il contient n'influence pas son volume apparent (gonflement ou retrait intrinsèque des particules), la compacité resterait alors constante et l'évolution de l'état de la poudre avec la teneur en eau serait une droite parallèle à l'axe des abscisses. A l'opposé, l'évolution de l'état d'un matériau dont le volume changerait sans que sa teneur en eau ne varie, serait représentée par une droite verticale. Si les particules sont déformables et que la teneur en eau varie, l'état hydro-textural peut alors se situer dans tous les états sous la courbe de saturation, accessibles par le procédé de transformation. L'analyse que nous proposons, consiste à identifier expérimentalement les états résultants des

opérations de broyage, de séchage et d'imbibition (traitée au chapitre suivant), afin de caractériser la façon dont les processus mis en jeu par ces procédés ont fait évoluer les poudres. Cette démarche constitue la base de l'analyse hydro-texturale proposée par Ruiz et al. (2005, 2008) (Ruiz, Delalonde et al. 2005; Ruiz and Wisniewski 2008).

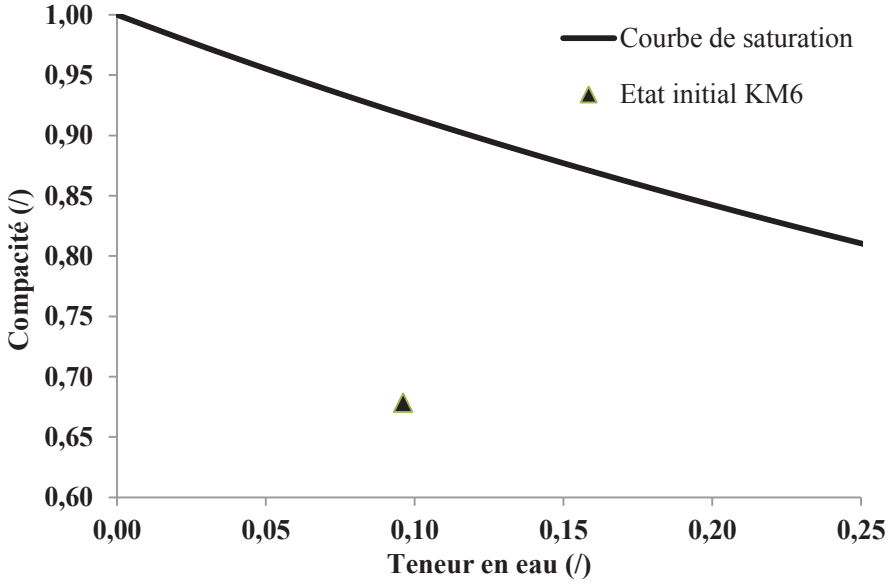


Figure IV- 23 *Représentation de l'état initial de la paille de riz broyée (KM_6) sur le diagramme hydro-textural avec sa courbe de saturation.*

IV.4.4. Analyse du broyage à partir du diagramme hydro-textural des particules de paille non traitée

A partir des données du Table IV- 9, la compacité (ϕ_h donnée Table IV- 9) et le degré de saturation sont calculés pour chaque échantillon. Les compacités et teneurs en eau constituent alors les points du diagramme dit « hydro-textural de la poudre de paille de riz » (Figure IV- 24). Pour des facilités de lecture, nous n'avons reporté sur ce diagramme hydro-textural du broyage que les courbes de saturation qui correspond aux cas « extrêmes » de KM_6 et VBM40 (Figure IV- 24).

Plus les particules sont petites et plus elles sont compactes. De plus, le broyage module en l'atténuant l'hygroscopicité des poudres dans le sens où malgré le fait que la diminution de taille puisse être associée à l'augmentation de la surface spécifique, la teneur en eau hygroscopique est

plus basse. Ces deux résultats, établis dans la partie **IV.2.3** où ils sont illustrés Figure IV- 7, sont reliés indépendamment de la taille des particules grâce à cette représentation dans le repère (w,ϕ).

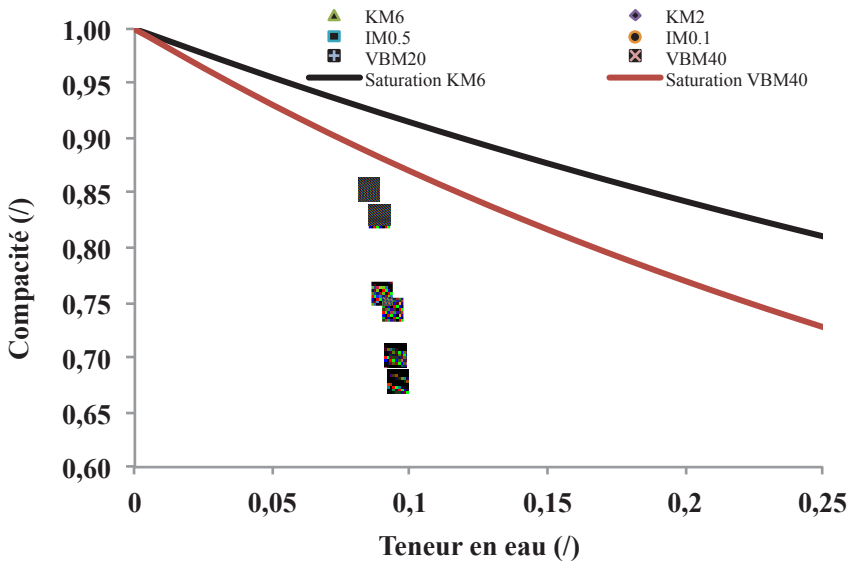


Figure IV- 24 *Diagramme hydro-textural des particules issues des différents broyages de la paille de riz.*

L'analyse hydro-texturale des particules issues des différentes conditions de broyage corrobore les résultats que nous avions présentés section **IV.2.3**. La relation entre la taille médiane des particules et leur compacité moyenne est bien représentée par une loi puissance (Figure IV- 7). L'ensemble de ces analyses permet de représenter la microstructure à l'échelle des particules. La variation de l'état hydro-textural entre chaque broyage est due à l'évolution particulière des microstructures cellulaires des poudres issues des pailles de riz. C'est encore la microstructure qui sera mise à contribution lors de sollicitations thermo-hydro-mécaniques d'autres natures. Le séchage est l'une de ces opérations, et afin de révéler le rôle de la microstructure des particules, nous allons analyser leur réponse « hydro-texturale » à un séchage contrôlé. Ce type de sollicitation représente aussi une opération unitaire que l'on trouve dans la filière de valorisation des pailles de riz, au niveau des prétraitements humide et semi-humide (Barakat, Chuetor et al. 2014). Le retrait mécanique induit par les contraintes de séchage liées au départ d'eau, est une déformation qui peut conduire à la déstructuration des poudres par collapse et fissuration. S'il va de soi que le séchage seul des poudres de pailles contribue à cette déstructuration recherchée, il est important de pouvoir en quantifier l'effet afin de mesurer, par une analyse différentielle, le rôle effectivement joué par les agents chimiques amenés à contribuer fortement à cet effet. Nous proposons tout d'abord de

poursuivre l'analyse hydro-texturale des mêmes fractions en les soumettant à un séchage par étuvage. Enfin, le prétraitement humide à la soude d'une seule fraction de pailles sera analysé avec la même procédure.

Une fois séchées, les poudres anhydres (w = 0 et S = 0) sont soumises aux protocoles de classification de la distribution de taille et de détermination du volume de leurs particules. Pour chacune des fractions, la valeur des tailles médianes et des compacités des particules avant et après séchage est reportée Table IV- 9. Pour chaque échantillon, ces points expérimentaux sont tracés sur le diagramme hydro-textural où l'on peut visualiser le chemin de transformation par séchage (Figure IV- 25).

Table IV- 9 *Valeurs des tailles médianes et des compacités des différentes poudres à l'état hygroscopique (noté h) et l'état sec (noté s).*

Technique de broyage	Echantillons	W (/)	ϕ_h(/)	D_{50h} (µm)	ϕ_s(/)	D_{50S} (µm)
Broyeur à couteau	KM_6	0,096	0,672	908	0,750	N/A
	KM_2	0,095	0,697	356	0,783	348
Broyeur UPZ	$IM_{0.5}$	0,093	0,745	238	0,803	265
	$IM_{0.1}$	0,090	0,763	108	0,830	107
Broyeur à billes	VBM_{20}	0,089	0,826	46	0,883	49
	VBM_{40}	0,086	0,827	26	0,918	25

On constate que les particules sont des matériaux déformables qui se sont rétractés au cours du séchage. L'augmentation de la compacité, rend compte du phénomène de retrait mécanique qui impacte la microstructure des particules. Ce collapse est observé pour toutes les particules testées, et quelle que soit leur taille, il semblerait que son intensité soit la même (Figure IV- 25). En effet, la variation de compacité associée au départ d'eau ne semble pas être dépendante de la taille des particules. La déformation volumique : $\varepsilon = \Delta V/V_0 = 1 - \phi_h/\phi_s$, est en moyenne de 9%. Ce résultat peut être interprété comme le fait que le broyage n'a pas modifié drastiquement la microstructure des particules natives. Les poudres de plus en plus fines sont des fragments dont la microstructure est comparable à celle de la paille « mère ». Le seul effet d'échelle notable est celui de l'augmentation de la compacité qui, comme nous l'avions indiqué dans la section **IV.2.2**, est due au fait que le processus majeur de réduction de taille correspond à une brisure dans les zones de plus forte porosité. Cette « perte » progressive de porosité au cours de la réduction de taille ne serait donc pas accompagnée d'une modification notable de la microstructure des fragments générés. Le

broyage seul, est ici un processus qui « déplace » la distribution granulométrique, mais maintient « homothétiquement » la microstructure initiale, c'est dans ce sens que l'on peut le qualifier de faiblement déstructurant.

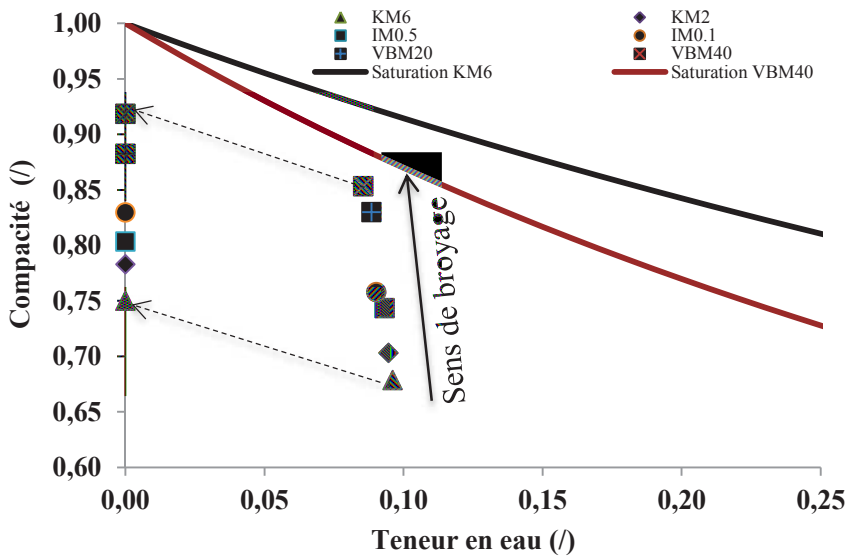


Figure IV- 25 *Diagramme hydro-textural des poudres de paille de riz - représentation du chemin de séchage entre les états hygroscopique et sec.*

Pour corroborer cette analyse, nous avons cherché à modéliser la relation entre la taille des particules sèches et leur compacité par une loi puissance comme proposé section IV.2.3 : $\phi = (\frac{d_{50}}{d_{50}^{\circ}})^{s}$***. Le***

Table IV- 10 reporte les valeurs des paramètres d_{50}°, taille « limite » pour laquelle la compacité est égale à 1 et $s = D_f - 3$ avec D_f la dimension fractale des particules. Cette analyse indique que la structure des objets secs peut elle aussi, être considérée comme fractale. Là encore la loi puissance est calée sur plusieurs décades de tailles (Table IV- 9**),** ce qui renforce sa pertinence. Les dimensions fractales des particules hygroscopiques et des particules sèches sont très voisines. Cet argument est en faveur de la caractéristique d'indépendance d'échelle que possède la microstructure. En revanche la taille des « éléments » compacts (de compacité égale à 1) est le double pour les particules sèches. On peut interpréter cette différence par le collapse local de quelques parois cellulaires, agrégées en amas par compactage au cours du séchage.

Table IV- 10 *Variation des paramètres hydro-texturaux de la paille de riz.*

	s (/)	D_f (/)	d_{50}° (µm)
Etat hygroscopique	- 0,064	2,93	2
Etat sec	- 0,056	2,94	5

Les propriétés mécaniques des poudres à l'état sec sont évaluées par cisaillement annulaire sous contrainte verticale (rhéomètre à poudres FT4) dans les mêmes conditions que les expériences présentées au chapitre précédent. Les coefficients de friction ainsi que les cohésions apparentes de chaque échantillon sont représentées en fonction de la taille des particules ***Figure IV- 26***.

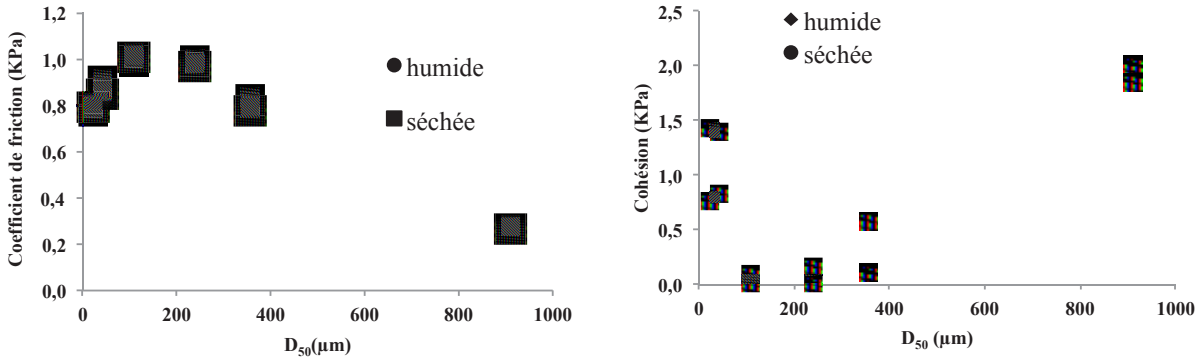


Figure IV- 26 *A) coefficient de friction et B) cohésion en fonction de la taille des particules.*

Quelle que soit la fraction considérée, le séchage ne semble pas affecter les propriétés de friction et de cohésion des particules. Les hypothèses précédemment proposées pour rendre compte des variations de ces paramètres avec la taille des particules sont inchangées. En revanche, ce résultat renforce l'idée que l'eau présente dans les particules ne joue pas de rôle sur ces propriétés. Cette eau, dont la teneur hygroscopique moyenne est voisine de 10% pour toutes les poudres, doit effectivement être essentiellement contenue dans les cellules et n'est pas présente à la surface des particules.

IV.4.5. Analyse hydro-texturale des effets des prétraitements chimiques

Les fractions de paille de riz obtenues avec le broyeur à couteaux et correspondant à la dénomination KM_2 ont été choisies pour servir de support expérimental à une analyse hydro-texturale des impacts des prétraitements à la soude en conditions humide (noté KM_2 + NaOH) et semi-humide (noté RS_{sh}) (Figure IV- 27**).** Les poudres présentées dans cette partie sont issues des protocoles décrits au chapitre III.4 (Figure III- 12). Concernant le prétraitement « semi-humide », les poudres retenues pour qualifier le procédé correspond à celles ayant été traitées avec un temps de réaction de 1h. Les temps d'exposition plus long n'ont pas amélioré ces résultats. Les pailles natives, prétraitées en conditions humide et semi-humide ont été ensuite broyées par un « centrifugal milling » (Retsch, ZM 200). L'énergie mise en jeu dans cette opération est mesurée afin de servir de base à l'évaluation de la contribution du prétraitement à l'atténuation de la dépense énergétique. Si les résultats présentés ne sont relatifs qu'à ces strictes conditions opératoires (restrictives), et ne peuvent être directement extrapolés à d'autres configurations, la méthodologie d'analyse en revanche, présente un caractère générique volontairement mis en avant.

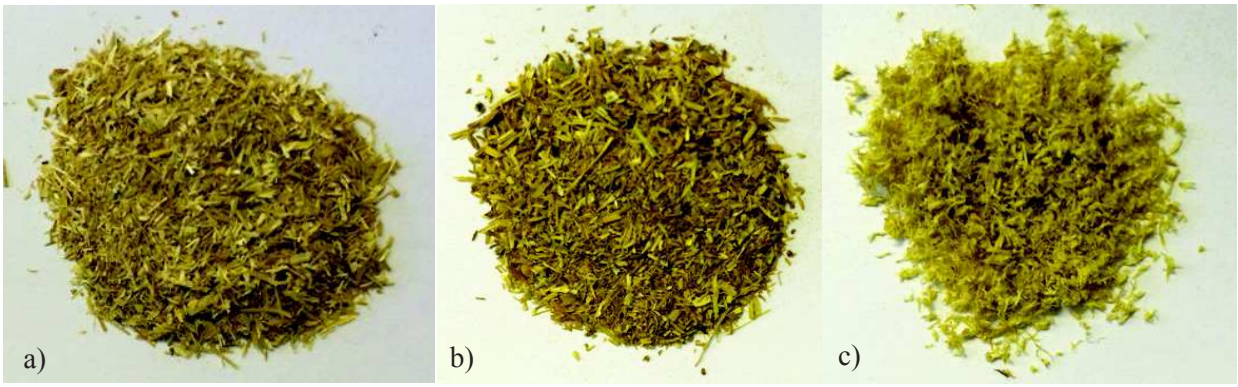


Figure IV- 27 *Aspects des poudres de paille de riz a) non traitées (KM_2), b) prétraitées par le procédé semi-humide (RS_{sh}), c) prétraitées par le procédé humide (KM_2 + NaOH).*

Pour les conditions opératoires présentées, on constate que l'absence de prétraitement génère des poudres dont la taille médiane est la plus élevée, tandis que le traitement humide génère les poudres les plus fines et enfin la granulométrie des poudres issues du traitement semi-humide leur est intermédiaire (Table IV- 11).

Table IV- 11 *Valeurs des teneurs en eau, compacités, tailles médianes et teneur en cellulose des particules issues des trois différents traitements. Energie de broyage des trois pailles***.**

Echantillons	w (/)	ϕ_h (/)	ϕ_s (/)	d_{50h} (µm)	d_{50s} (µm)	Teneur en cellulose (mg/gMS)	Energie de broyage (KJ/Kg)
KM_2	0,0946	0,697	0,783	352	348	34,57	4319

RS_{sh}	0,0966	0,683	0,739	347	340	35,06	3924
KM_2 + NaOH	0,0878	0,654	0,851	298	285	66,83	3832

Le prétraitement chimique par la soude permet de solubiliser les hémicelluloses et la lignine. A l'échelle de l'ultrastructure les propriétés de résistance mécanique sont en partie imputables à la lignine et certains composés moléculaires comme les acides féruliques qui pontent les chaînes d'arabinooxylane (hémicellulose) (Sun, Xu et al. 2005; Kristensen, Börjesson et al. 2007). En conséquence, la teneur en cellulose, qui n'est pas attaquée par la soude, est plus élevée dans les poudres traitées que non traitées. On constate que le traitement humide conduit à une plus forte dissolution des lignines et hémicelluloses que son homologue semi-humide qui ne semble pas avoir activé de dissolution notable de lignine (Table IV- 11). En effet, sa teneur en cellulose est la même que celle des pailles non traitées. Le prétraitement humide devrait donc fortement accroitre la déformabilité des particules de poudre de paille de riz et cette propriété devrait être contrastée entre les deux poudres issues des traitements humide et semi-humide.

Les couples compacité/teneurs en eau (Table IV- 11) sont disposés sur le diagramme hydro-textural (Figure IV- 28). Pour les deux traitements chimiques, la soude joue un rôle sur l'organisation en feuillet de la cellulose (Moharram and Mahmoud 2007; Liu and Sun 2010) en augmentant les écarts entre les feuillets. Cet effet se traduit à l'échelle des particules par une diminution de la compacité par rapport aux pailles non traitées. Le procédé humide conduit à des particules plus poreuses que le procédé semi-humide, effet d'une déstructuration très probablement plus « à cœur ». On constate de plus que ce traitement conduit aussi à modifier sensiblement les propriétés hygroscopiques des poudres via la modification de leur composition. Là encore, c'est le traitement humide qui parvient à module plus sensiblement cette propriété. L'hypothèse d'une action plus à cœur peut être encore avancée. Le chemin de séchage des particules prétraitées par le procédé semi-humide est similaire à celui des particules non traitées (Figure IV- 28), la déformabilité étant du même ordre de grandeur (Figure IV- 28). En revanche, le procédé humide génère des particules beaucoup plus déformables dont le retrait mécanique dû au séchage est de l'ordre de 24% (Figure IV- 29).

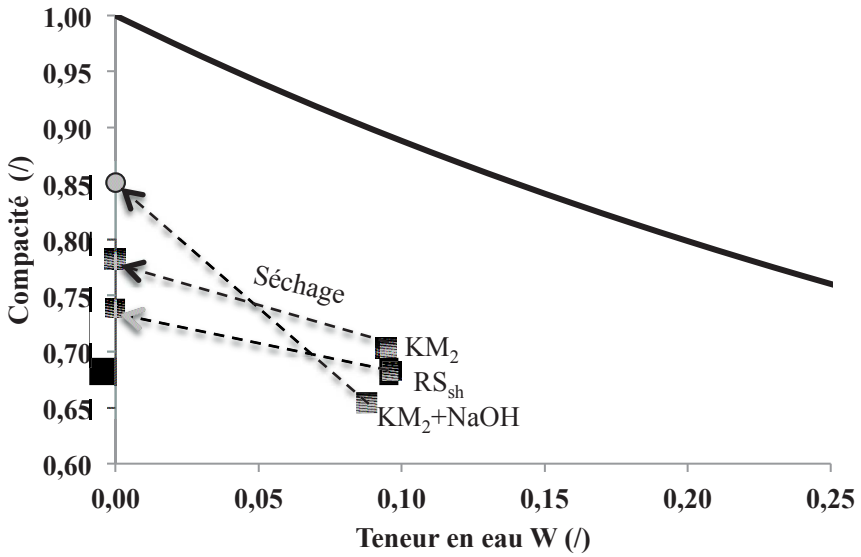


Figure IV- 28 *Etats hydro-texturaux hygroscopique et sec des particules prétraitées par le procédé humide (KM_2 + NaOH) et par le procédé semi-humide (RS_{sh}). Comparaison avec la paille non traitée (KM_2).*

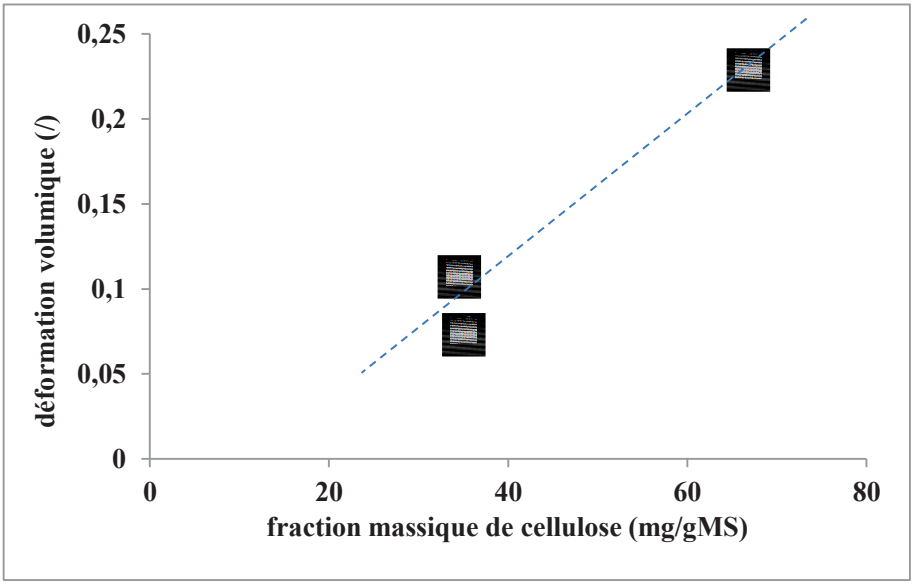


Figure IV- 29 *Variation de déformation volumique des particules après séchage, avec la fraction massique de cellulose pour les trois essais.*

Cet accroissement du collapse provient d'une déconstruction plus forte à l'échelle de l'ultrastructure. Dans le procédé semi-humide, la solution alcaline de soude pulvérisée à la surface des particules ne doit pas parvenir à pénétrer à l'intérieur et déconstruit essentiellement la périphérie des particules (Figure IV- 30). Il s'agirait d'une action plus en surface au regard de l'action plus à cœur que le procédé humide autorise.

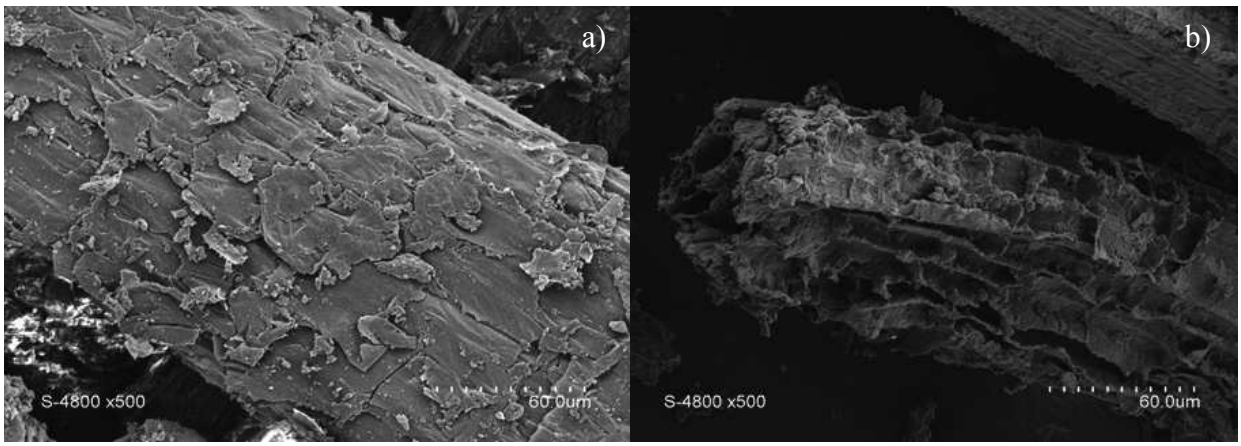


Figure IV- 30 *Photographies MEB des poudres de paille de riz a) non traitées (KM_2), b) prétraitées par le procédé semi-humide (RS_{sh}).*

Les effets contrastés que les deux modes de prétraitement génèrent sur les particules, permettent-ils de considérer le procédé humide comme plus efficace que le semi-humide ? Lorsque l'on évalue la consommation énergétique nécessaire au broyage **(**Table IV- 11**),** on confirme que le prétraitement humide qui génère des particules de teneur en cellulose élevée, assure une énergie de broyage plus basse qu'en l'absence de prétraitement chimique (Figure IV- 30). On remarque Cependant que cette tendance n'est pas respectée par les particules issues du prétraitement semi-humide. L'énergie de broyage est nettement inférieure à celle nécessaire à broyer les poudres élaborées sans prétraitement chimique, alors que les fractions de cellulose sont identiques **(**Figure IV- 31**).** Un élément d'explication peut être avancé en considérant la variation de l'énergie consommée avec la compacité des particules (Figure IV- 32). Plus la compacité des particules, est élevée et plus l'énergie mise en œuvre pour les broyer est élevée. Pour les conditions opératoires considérées dans cette étude, on constate que le prétraitement semi-humide associé au broyage n'est pas aussi performant en terme de consommation énergétique que le tandem prétraitement humide/broyage. En revanche il apporte un gain très significatif par rapport à l'absence de prétraitement chimique tout en minimisant les transferts de liquide réactif.

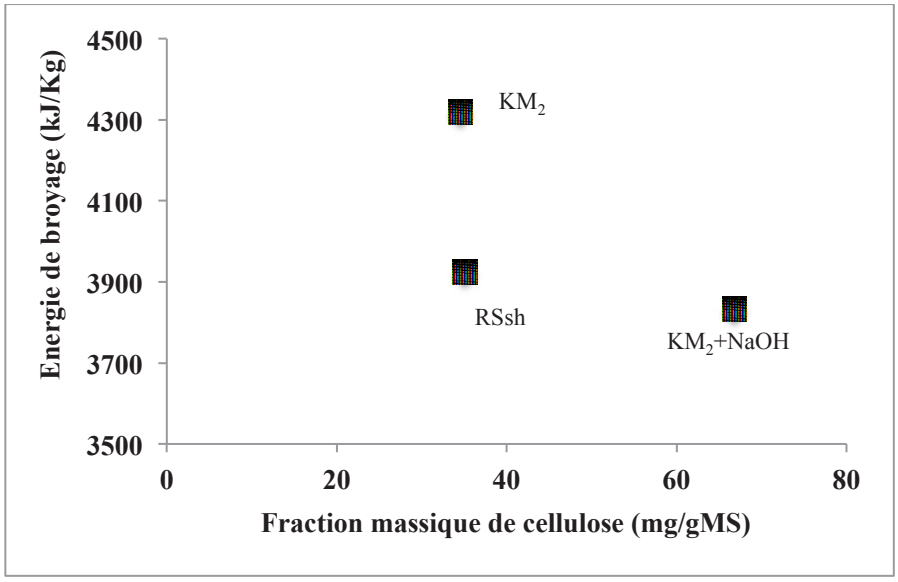


Figure IV- 31 *Variation de l'énergie de broyage avec la fraction massique de cellulose pour les trois essais.*

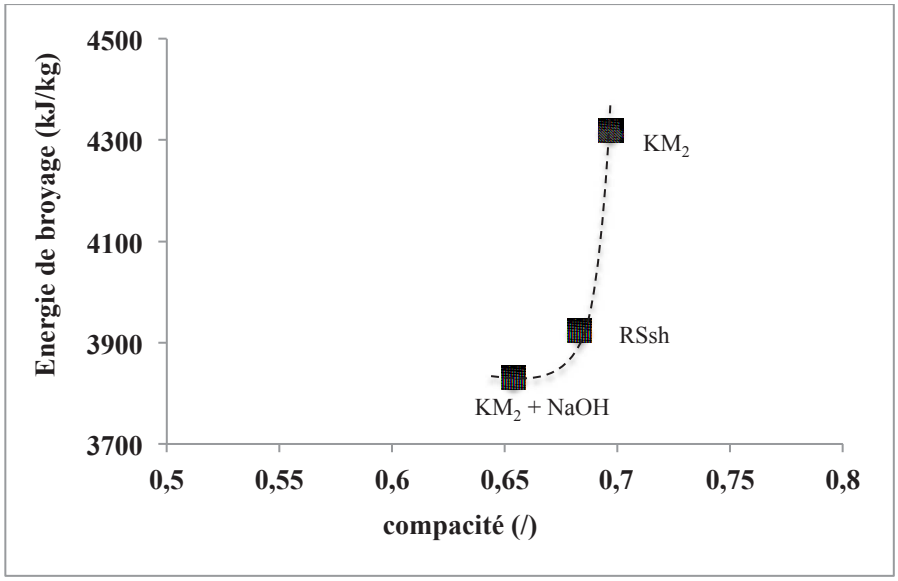
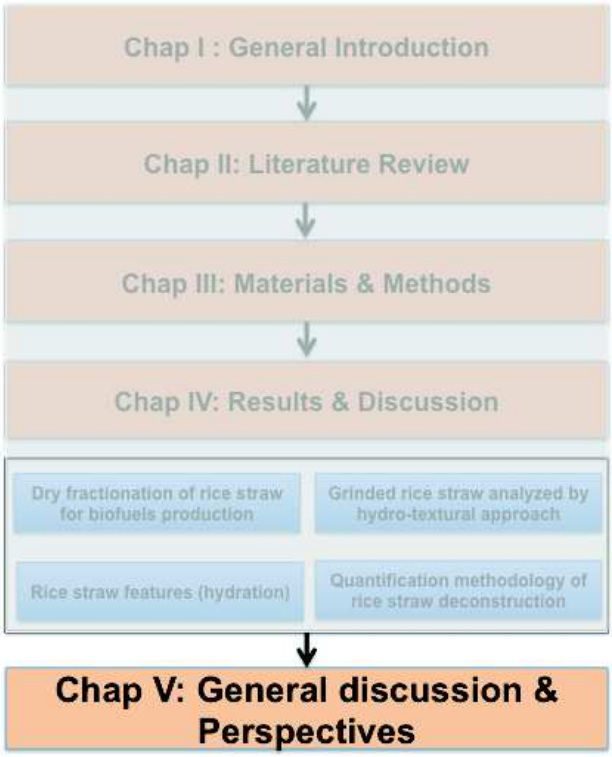


Figure IV- 32 *Variation de l'énergie de broyage avec la compacité des particules pour les trois essais.*

Chapter V:

General discussion & Perspectives



Les travaux de cette thèse s'insèrent dans l'objectif global d'établir une filière durable de valorisation de la matière lignocellulosique. La paille de riz a été choisie comme modèle d'une source de lignocellulose en raison de sa composition biochimique, de son comportement physique ainsi que mécanique. Le procédé combiné chimique et mécanique a été choisi afin de mieux répondre aux contraintes techniques, économiques ainsi que éco-environnementales. Dans cette thèse nous avons développé une approche qui a permis de décrire les mécanismes de déconstruction mécanique de la paille de riz et de tester un procédé innovant de fractionnement.

Dans ce chapitre, nous présentons une discussion générale qui conclue le travail de thèse. Les perspectives de ces travaux sont ensuite présentées.

Contextes et objectifs

Dans les schémas de bioraffinerie des lignocelluloses, les prétraitements sont des étapes indispensables afin de rendre la matière lignocellulosique plus accessible notamment la cellulose, par les microorganismes et les enzymes pour permettre des bioconversions efficaces. L'objectif de prétraitements est de diminuer la taille macroscopique des substrats et par conséquent d'augmenter la surface spécifique des particules, d'altérer la structure ligneuse et de diminuer la cristallinité de la cellulose. Ces différents paramètres (cristallinité de la cellulose, structure de la lignine, surface spécifique des particules) sont considérés comme des facteurs limitant l'accessibilité et la biodégradabilité de la cellulose et la LC. Certains travaux ont montré une forte corrélation entre la cristallinité de la cellulose, la quantité de la lignine et l'hydrolyse enzymatique de la cellulose native par des cellulases (Sun and Cheng 2002; Taniguchi, Suzuki et al. 2005). Cependant, dans le cas des LC cette corrélation n'est pas trop claire en raison de la complexité et l'hétérogénéité de la matrice LC avec la contribution des liaisons covalentes et non-covalentes inter polymères LC mais également due à l'organisation moléculaire, supramoléculaire et tissulaire très complexe de la matrice LC. D'autre part, la plupart de prétraitements proposés actuellement induisent des coûts énergétiques forts et génère des effluents toxique nécessitent des investissements supplémentaires. , Le fractionnement par voie sèche *via* la fragmentation mécanique (broyage) constitue un bon compromis technique afin de répondre aux exigences technico-économiques. En effet, une étape importante dans la bioraffinerie est la réduction de la taille de la matière de la macrostructure (mm) jusqu'à la microstructure (µm). Le fractionnement par voie sèche peut constituer un prétraitement efficace de la LC pour des bioconversions (augmentation de la surface de réaction et diminution de la cristallinité), sans consommation d'eau ni production d'effluents.

La fragmentation mécanique par broyage permet également de produire des substrats (poudres) pour l'extraction de molécules plateformes qui sont ensuite valorisées vers diverses applications (biocarburants, biomolécules et biomatériaux). Le couplage broyage/prétraitements modérés, devrait permettre de réduire très significativement l'énergie de broyage tout en préservant la cellulose.

Pour aller plus loin dans la fragmentation mécanique, un procédé combiné « chemo-mécanique » en voie semi-humide a été développé, avec pour objectif d'augmenter à la fois la réactivité des produits et aussi de diminuer les intrants et les effluents. Le prétraitement chimique permet ici de fragiliser la microstructure (rupture des liaisons inter polymères) afin d'augmenter la broyabilité de la matrice LC.

Ce travail de thèse a donc pour but i) d'accéder à la compréhension des mécanismes de déconstruction de la paille de riz au cours de fractionnement mécanique ou mécanique-chimique en couplage, ii) de suivre la transformation de la paille de riz par une approche hydro-texturale et iii) d'analyser les effets de prétraitements chimiques couplés à la fragmentation mécanique de la paille de riz. L'état granulaire de la matière ainsi transformée, introduit des caractéristiques spécifiques aux poudres : distribution des tailles et des formes, rhéologie, réactivité de surface, … L'un des challenges de ce travail a été de considérer la nature particulaire des substrats

Fractionnement par voie sèche (combinaison du broyage ultrafine et de la séparation des particules)

Dans cette étude, nous avons tout d'abord testé l'effet du broyage ultrafin (<100μm) couplé à la séparation. La paille de riz a été broyée pour obtenir des fractions fines (<100μm) qui ont été ensuite séparées avec deux techniques de séparation : le tri-électrostatique et la turbo-séparation ont été comparés en terme d'efficacité pour obtenir des fractions ou des tissues de propriétés et compositions différentes. L'objectif appliqué de ce couplage a été de produire des fractions ou tissues de différentes réactivités pour la production de bioéthanol. Le tri-électrostatique consiste ici à séparer des particules chargées par frottements (tribo-charge) dans un champ électrique selon leurs charges électriques nettes acquises (positives ou négatives en fonction de leur composition de surface). Cette technique a donc permis d'obtenir des fractions enrichies soit en cellulose qui permettent de produire l'éthanol, soit en lignine qui pourraient être valorisées en biomatériaux ou en source de molécules pour la chimie.. En revanche, la turbo-séparation est basée sur les propriétés aérodynamiques des particules, reliées principalement à leur densité. Cette technique a permis d'obtenir deux fractions différant par leur taille moyenne de particules (grossière et fine). Les résultats obtenus ont montré que les fractions fines sont significativement plus accessibles aux enzymes que les grossières grâce à une plus grande surface de réaction des particules.

La combinaison des opérations de broyage de la paille de riz et de tri électrostatique et aérodynamique en milieu sec strict, a permis d'isoler des fractions concentrées en cellulose, en hémicelluloses-lignine et en minéraux sans modifications chimiques contrairement aux procédés de fractionnement chimiques développés jusqu'à présent. La technologie de tri-électrostatique sur les végétaux encours de développement à IATE à donner des résultats très intéressants sur d'autres matières premières comme les tourteaux. La combinaison des opérations de broyage des tourteaux d'oléagineux (Colza, Tournesol…) et de tri électrostatique a permis d'isoler des fractions riches en

protéines jusqu'à 60% et 45% en lignine comparés seulement à 30% et 20% w/w, respectivement dans la matière première (Barakat, Jérôme et al. 2015). Les résultats obtenus sur la LC, montre clairement la complexité et la difficulté à séparer très efficacement les polysaccharides par exemple de la lignine ou la cellulose des hémicelluloses, ceci due à l'organisation très complexe de la matrice LC. Ces résultats montre également que la partie dissociation à une échelle donné (moléculaire, tissulaire…) par le broyage un paramètre très important à étudier.

Caractérisation des poudres de paille de riz

Trois modes de fragmentation mécanique ont été utilisés pour produire les six échantillons de poudres ayant différentes tailles de particules (KM_6, KM_2, $IM_{0.5}$, $IM_{0.1}$, VBM_{20} et VBM_{40}). Ces trois modes sollicitations mécaniques sont i) un broyeur à couteaux avec des grilles de 2 et 6mm ; ii) un broyeur par impact (UPZ palette) avec des grilles de 0,1 et 0,5mm et iii) un broyeur à billes avec des temps de 20 et 40min. Ces trois techniques de broyage ont permis de produire des poudres avec différentes tailles dont la taille médiane varie entre 900 à 20 µm.

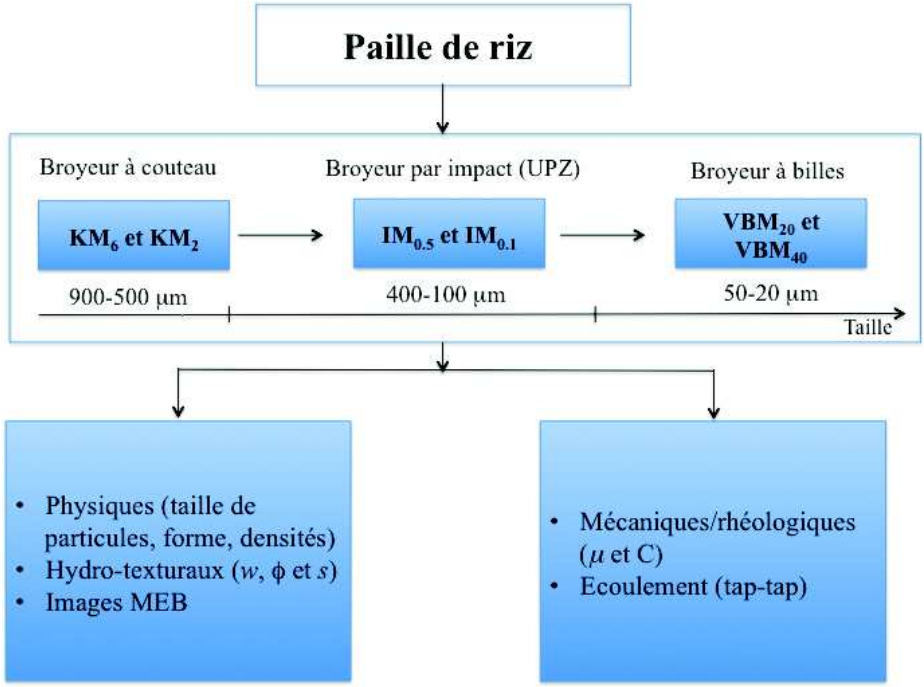


Figure V- 1 *Schéma de déconstruction de la paille de riz et méthodes de caractérisation.*

La déconstruction structurale du réseau lignocellulosique de l'échelle macroscopique à l'échelle microscopique peut être abordée par une approche couplée produit-processus-procédé. Concernant l'aspect produit, le critère important est la fonctionnalité des produits obtenus après la mise en œuvre. En revanche, l'aspect procédé concerne son optimisation (consommation énergétique,

impacts environnementaux). Les processus mis en jeu dans les transformations de la matière lignocellulosique se doivent d'être considérés dans le cadre spécifique de la matière granulaire. Dans ce sens, il existe plusieurs méthodes caractérisations aux différentes échelles mises en jeu. A l'échelle de la macrostructure (mm) nous parlons du procédé mise en œuvre. Dans ce cas, l'énergie de broyage peut être mesurée. A l'échelle de la microstructure, la caractérisation biochimique est pertinente. Cependant, à une échelle mésoscopique, intermédiaire entre la macro- et la microstructure, il y a une difficulté de caractérisation pour comprendre ce qu'il se passe lors de la déconstruction. Dans cette partie des méthodes de caractérisation spécifique pour suivre l'évolution de transformation de la matière à l'échelle de la « meso »-structure, ont été développées.

La nature triphasique des poudres puis du lit de poudre, est considérée via trois grandeurs d'état physiques reliées à la déconstruction de la paille de riz triphasée (solide, liquide, gaz). Ces grandeurs qui sont définies pour exprimer l'état physique lié à la texture, sont qualifiées de grandeurs hydro-texturales : i) la teneur en eau, ii) le degré de saturation et iii) la compacité. Leur mesure permet de suivre le processus de fragmentation.

Les résultats ont montré que lors du broyage, la quantité d'eau contenue (teneur en eau) dans la paille de riz a légèrement diminué ce qui a affecté directement le degré de saturation. La quantité d'eau perdue est égale à l'augmentation du volume de solide et qui par conséquent, conduit à augmenter le degré de saturation des particules. De plus, l'augmentation de la compacité des particules signifie que lors du broyage, la zone la plus poreuse de la paille de riz a été détruite créant des populations de particules plus petites et plus compactes (Figure IV- 16).

En parallèle, les propriétés rhéologiques ont été analysées afin de comprendre à la fois l'organisation de matériau et également l'évolution de propriétés d'écoulement. Conformément à la loi du Coulomb, le coefficient de friction et la cohésion apparente ont été déterminés pour chacune des poudres. Les résultats obtenus ont montré que le coefficient de friction et la cohésion apparente sont corrélés à la taille des particules des poudres mais aussi à la forme des particules. Les particules grossières sont moins cohésives mais génèrent plus de frictions que dans le cas de KM_2 $IM_{0.5}$ et $IM_{0.1}$. Par contre, dans le cas de KM_6, le coefficient de friction est petit mais la cohésion est élevée ; ce phénomène peut être dû à l'enchevêtrement des grosses particules dont la morphologie se rapproche de celle de fibres. Concernant les poudres fines (VBM_{20} et VBM_{40}), le coefficient de friction est petit mais la cohésion augmente ce qui pourrait être attribué à l'intensification des interactions de surface à l'échelle moléculaire (électrostatique etc.) via l'augmentation de la surface spécifique des particules.

D'autre part, nous avons qualifié les propriétés d'écoulement des poudres par un essai de tassement. Cette étude a permis d'indiquer l'évolution des propriétés d'écoulement des poudres avec la réduction de taille. Ces informations enrichissent la connaissance des propriétés fonctionnelles des poudres obtenues qui auront à s'écouler par exemple lors du transport dans un silo ou encore lors de la mise en œuvre dans les bioréacteurs. Dans les bioraffineries, il y a beaucoup d'opérations unitaires qui nécessitent de « bonnes » caractéristiques d'écoulement des poudres. Les connaitre est important pour designer et fabriquer des dispositifs et équipements adaptés. Dans cette partie, nous avons utilisé une méthode de densification par tap-tap qui représente un essai classique dans le domaine de la pharmacie industrielle. Les résultats obtenus ont montré que les cinétiques de densification en fonction du nombre de coups peuvent se comportent en deux phases. Dans les cas de KM_6, KM_2 et $IM_{0.5}$, les courbes de densification augmentent progressivement jusqu'à atteindre leur stabilisation. Pour les $IM_{0.1}$, VBM_{20} et VBM_{40}, les courbes de densification augmentent fortement jusqu'à atteindre un plateau. A partir de ces résultats, nous avons classé les six poudres sur le diagramme de Geldart et calculé le rapport d'Hausner et l'indice de Carr. Dans ce diagramme, nous avons constaté que les poudres se disposent dans deux zones : zone cohésive (VBM_{20} et VBM_{40}) avec une très mauvaise coulabilité et zone fusante (KM_6, KM_2, $IM_{0.5}$ et $IM_{0.1}$) avec simplement une mauvaise coulabilité. Dans cette partie, nous avons appliqué des modèles décrivant les cinétiques de relaxation de milieux granulaires humides, soumis à des vibrations verticales. Parmi les différents modèles testés, le modèle KWW est celui qui est le plus proche des résultats expérimentaux. Ce modèle heuristique donne une très bonne approximation du comportement au tassement des poudres.

Aptitude aux transferts de liquide et quantification des processus de déconstruction des pailles de riz

Un diagramme de phase « hydro-textural » est employé pour représenter l'état d'agencement des phases solide et fluides (liquide et gaz) à l'échelle des grains et à l'échelle de la poudre, du lit de grains. Cette analyse permet de représenter la relation entre trois paramètres nécessaires et suffisants pour caractériser l'état hydro-textural de la microstructure : la teneur en eau (w), la compacité (ϕ) et le degré de saturation (s). On constate que l'état initial des poudres des pailles broyées est non saturé (i.e. coexistence d'une phase liquide, eau interstitielle, et d'une phase gazeuse). Dans cette partie, nous avons utilisé la description hydro-texturale pour étudier l'influence des transferts d'eau durant des processus d'imbibition et de séchage. Les prétraitements envisagés

dans les procédés humide et semi-humide, mettent en jeu systématiquement une étape de « mouillage » des pailles par la solution chimique puis une étape de séchage.

L'imbibition des pailles est plus ou moins modulée suivant le processus considéré (humide ou semi-humide). L'analyse mise en place permet de suivre l'évolution de l'entrée d'eau dans les pailles et de quantifier leur gonflement. On constate que le gonflement de la paille est un phénomène qui se déroule sur une échelle de temps importante. Les pailles se « remplissent » tout d'abord, sans variation notable de leur volume. Leur gonflement ne devient signification qu'une fois qu'elles sont suffisamment imbibées, Bien que nous ayons quantifié le phénomène de gonflement d'un point de vue cinétique et hydro-textural, l'étude montre que le temps caractéristique de ce phénomène est grand par rapport au temps de l'expérience de prétraitement chimique. Aussi, le gonflement peut être négligé durant cette opération. La mise au contact des pailles avec le liquide de mouillage peut donc être considérée comme une étape au cours de laquelle l'interaction avec la microstructure est réduite à la solubilisation des composés. La « matrice » lignocellulosique demeure un milieu poreux très peu déformable, siège de réactions chimiques à l'interface solide/liquide. L'avancée du front d'imbibition liquide/solide, constitue le facteur limitant de cette opération.

Concernant le séchage, les résultats obtenus ont montré que les pailles sèches sont plus compactes que dans un état humide. Cet effet est l'impact du retrait mécanique qui est couplé au départ d'eau, sur la microstructure des pailles. Le retrait mécanique provoque un collapse qui densifie les particules. On remarque que quelle que soit la taille des particules humides soumises à un même protocole de séchage, la variation de compacité entre les états humide et sec est la même. Ce point est lié au fait que la déformation volumique des particules est indépendante de leur taille (sur la gamme de diamètres médians testés) ce qui montre que le rôle majeur de la nature de la microstructure qui ne semble pas avoir été fortement remaniée par le broyage. L'essai de transfert d'eau par séchage est donc conduit ici comme s'il représentait un essai mécanique sous sollicitation isotrope. Nous montrons qu'il est possible d'en extraire des informations relatives à l'état de la microstructure des poudres et, notamment, à l'impact du broyage sur la microstructure. L'étape suivante a consisté à utiliser cet essai pour caractériser l'impact d'un prétraitement chimique de déconstruction, sur l'intégrité de la microstructure.

Les essais rhéologiques plus classiques, montrent en revanche, que le retrait de l'eau n'a pas d'influence notable sur les propriétés rhéologiques (μ et C) coefficient de friction et cohésion apparente. Ce résultat signifie que le retrait de l'eau contenue dans la paille n'influence pas des propriétés rhéologiques des poudres, entendues comme le lit de particules.

Nous avons choisi un échantillon KM_2 caractérisé par une analyse hydro-texturale pour quantifier les effets d'un prétraitement chimique par la soude (NaOH) soit en processus semi-humide (RS_{sh}) soit en processus humide (KM_2 + NaOH). Les résultats ont montré que les tailles médianes de particules se classent ainsi : (KM_2 + NaOH) > (RS_{sh}) > KM_2 (Table IV- 11). Nous avons également comparé la consommation énergétique du broyage par le broyeur centrifuge. La mesure a montré que le broyage de la paille native (KM_2) consomme plus d'énergie que celles traitées respectivement par voie semi-humide (RS_{sh}) et voie humide (KM_2 + NaOH). Le prétraitement chimique joue le rôle attendu et fragilise la microstructure de la paille (ce résultat qui parait évident ne l'est pas forcément, les prétraitements à la soude auraient pu avoir une action essentiellement surfacique sans toucher à la microstructure). De plus, le prétraitement chimique par la soude permet de solubiliser une partie de la lignine ainsi que des hémicelluloses qui sont associés avec la cellulose dans la matrice lignocellulosique. Par conséquent, les fractions massiques de la cellulose sont plus importantes dans le cas du prétraitement humide (KM_2 + NaOH).

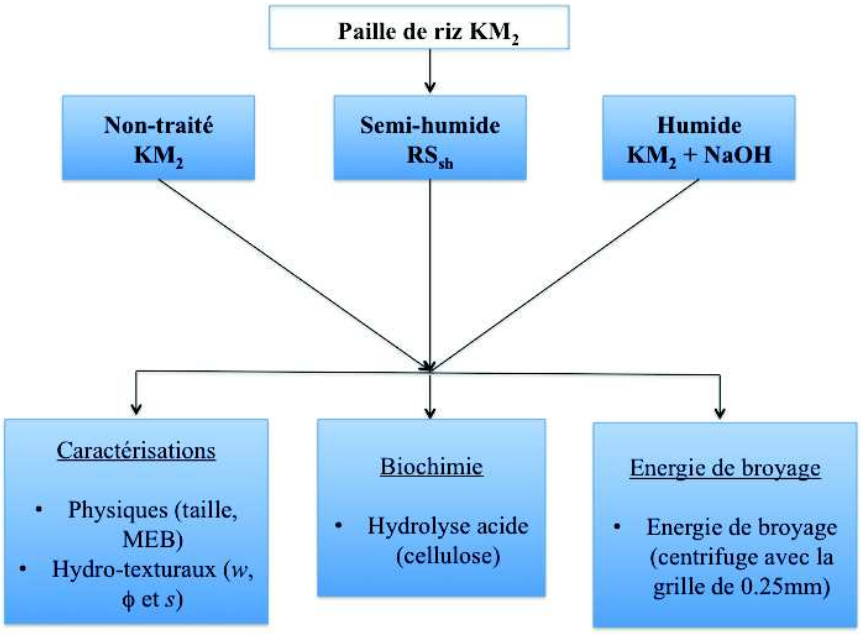


Figure V- 2 *Schéma de 3 procédés prétraitements de la paille de riz*

Ce phénomène destructif par le prétraitement chimique spécifiquement par voie humide (KM_2 + NaOH), a affecté fortement la déformabilité ainsi que les propriétés texturales de la paille de riz. Nous avons constaté que les prétraitements semi-humide (RS_{sh}) et humide (KM_2 + NaOH) peuvent significativement améliorer la réduction énergétique au broyage. Cette diminution d'énergie est voisine de 9 et 12% respectivement par rapport au témoin de KM_2 (Table IV- 11). Notons que dans le cas du prétraitement semi-humide, la fraction massique de cellulose n'est pas augmentée par rapport au témoin KM_2 ce qui peut expliquer par la photo à la MEB (Figure IV- 22). Nous avons vu

que le prétraitement semi-humide permet de déstructurer principalement la surface des pailles, est n'a pas d'action suffisamment notable au niveau de la microstructure interne.

Perspectives

Les travaux initiés dans cette thèse montrent la faisabilité du prétraitement semi-humide en tant que préalable au broyage des pailles de riz. Au-delà de l'étude spécifique de ce procédé, nous avons proposé une approche plus générique qui permette de suivre la transformation des pailles en poudres selon trois différentes sollicitations hydro-mécaniques de types mouillage/séchage/broyage (i) humide sans réactif, (ii) humide avec réactif et (iii) semi-humide avec réactif. Cette ressource ligno-cellulosique représente un gisement de biomolécules très important pour la Thailande, ce qui renforce l'intérêt de se projeter plus en avant dans l'amélioration et l'exploitation plus opérationnelle des résultats de ce travail.

L'approche scientifique ainsi que la méthodologie qui ont été appliquées et développées, sont de nature pluridiciplinaires. La perspective générale que nous souhaiterions indiquer porte sur le renforcement de cette approche par des éléments théoriques issus de la physique des milieux granulaires, du génie des procédés et de la biotechnologie. L'intégration des interactions liquide/solide dans la représentation hydrotexturale, qui puisse permettre de quantifier la mouillabilité des pailles ainsi que leur imprégnation, préalable à la création d'une interface réactionnelle, permettrait de renforcer l'analyse locale des phénomènes au niveau de la microstructure.

Du point de vu des procédés étudiés, l'écriture de bilan de matière (bilan de population) et de bilan énergétique à l'échelle des réacteurs, renforcerait le pilotage des procédés. La mise en œuvre d'outil de transposition d'échelle (analyse dimensionnelle), via quelques critères adimensionnels (flux spray adimensionné pour caractériser la pulvérisation de gouttes sur les pailles, nombre de puissance dans le réacteur agité,…) ouvrirait l'étude de régimes de transformation des poudres en fonction des conditions opératoires. Dans ce contexte, un plan d'expérience par procédés (prétraitement, séchage et broyage) permettrait d'identifier rationnellement les points de fonctionnement.

Du point de vue des produits obtenus, la meilleure caractérisation des fonctionnalités attendues pour la biotransformation ou l'extraction de molécules d'intérêt, parait indispensable. Une définition rationnalisée de la séparabilité des différentes fractions, du degré de déconstruction et de la capacité à générer des fractions d'intérêt, devrait émerger de l'emploi de techniques de caractérisation plus fines. Citons en particulier les propriétés d'écoulement des poudres en vue de leur manutention et

l'étude de leur ré-imbibition pour une application bioconversion. Ecoulement et instantanéisation des poudres (en vue d'une bioconversion), peuvent mettre en jeu des processus limitants et énergivores qui contrarient l'efficience du procédé de broyage avec prétraitement. L'extension de ces expériences à d'autres substrats lignocellulosiques transformables en poudres d'intérêt, confèrerait un caractère plus générique à ce procédé.

Ces quelques perspectives, constituent des axes de recherche à part entière, qui nous apparaissent cependant comme un préalable nécessaire à l'efficience du développement technologique de cette opération.

Chapter VI:

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Communications issues de cette thèse

Articles scientifiques

Barakat, A., S. Chuetor, et al. (2014). "Eco-friendly dry chemo-mechanical pretreatments of lignocellulosic biomass: Impact on energy and yield of the enzymatic hydrolysis." *Applied Energy* **113**(0): 97-105.

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CHUETOR Santi*, BARAKAT Abdellatif, ROUAU Xavier, RUIZ Thierry "Grinding of rice straw analyzed by a hydro-textural approach" in *Récent Progrès en Génie des Procédés, Numéro 107-2015 ISSN: 1775-335X; ISBN: 978-2-910239-81-7, Ed.SFGP, Paris, France* (*submission*)

Chuetor S, Barakat A, Rouau X and Ruiz T., "Comportement des poudres de pailles sous sollicitations hydrique- Essais d'imbibition" *En préparation*

Chuetor S, Barakat A, Rouau X and Ruiz T., "Méthodologie de quantification des processus de déconstruction des pailles de riz" *En préparation*

National & International communications:

- TSAC 2014 Toulouse, France (Oral presentation) " *Development of lignocellulosic biomass pretreatment*",
- Journée de l'école doctorat Sciences des Procédés- Sciences des Aliments (SPSA) Montpellier supagro (Poster session) "*Optimization of chemo-mechanical pretreatments in semi-humid medium of lignocellulose biomass for biofuels production*"
- TSB 2014 (TSB International Forum 2014 "Green Bioprocess Engineering") Bangkok, Thailand (Oral presentation), "*Dry fractionation of rice straw for biofuels production: Physical and biochemical characterization*"
- STPMF 2015 (Colloques Science et Technologie des Poudres et Poudres et Matériaux Frittés) Nancy France (Poster presentation), « *Grinding of rice straw analyzed by a hydro-textural approach* »
- TSAC 2015 Uppsala, Sweden (Oral presentation), "*Dry fractionation of rice straw prior to bioethanol production*"

- BFFM 2015 (Biorefinery for Food & Fuel and Material Symposium) Montpellier, France (Oral presentation), "*Chemo-mechanical pretreatments of rice straw for biofuels and bioproducts: physical and biochemical characterization*"

- 250th American Chemical Society National Meeting & Exposition (Innovation from discovery to application) Boston, USA (Oral presentation), "*Dry fractionation of straw prior to biofuels production*"

Abstract

Combination of chemo-mechanical pretreatments of rice straw in semi-humid pathway: effect on physicochemical, rheological properties and reactivity.

Lignocellulosic biomass (LC) is considered as a promising alternative in order to produce not only biofuels, but also green chemicals and materials and bio-based molecules for the synthesis of polymers, which could substitute those from petrochemicals. The LC biomass mainly consists of cellulose, hemicelluloses and lignin. Its composite nature and its heterogeneous matrix microstructure make difficult its enzymatic degradation and its bioconversion. The pretreatment of LC biomass is a required step that allows deconstructing the LC matrix and improving the accessibility to the parietal polymers, in particular for the production of bio-based molecules.

Dry fractionation of LC biomass can take place in biorefinery with favorable arguments with regards to the durability (no water consummation, no drying, and no effluent generation). The improvements of resolution in LC fractionation, the reduction of energy consumption as well as the enhancement of reactivity/features of products constitute three priority objectives of research in the dry fractionation field. One inconvenience of grinding operation is high-energy consumption. So, the development of mild pretreatments, which can facilitate the grinding process of the LC matrix and the accessibility to substrates, might contribute (i) to improve the resolution of dry fractionation, (ii) to reduce significantly the energy consumption by grinding and (iii) to improve the product reactivity.

The objective of the thesis concerns the characterization of the combination of chemical and mechanical pretreatments of rice straw. This study focuses in particular on an innovative process of chemical pretreatment by semi-humid pathway, which allows weakening the LC matrix in order to facilitate its mechanical deconstruction. The semi-humid chemo-mechanical coupled processes allowed at the same time increasing the product reactivity, decreasing the energy consumption as well as eliminating some steps, while generating no effluents. The results of dry fractionation showed that the combination of an ultrafine grinding and separation steps is a useful alternative technique in biorefinery to obtain interesting fractions with contrasted properties. These results permit to propose a pretreatment technique that can be adapted to many kind of biomass.

A hydro-textural approach at the particle size scale is then proposed in order to better understand the mechanisms of fractionation and evaluate the effects of chemical pretreatments. The role of microstructure in response to the chemo-mechanical pretreatments is analyzed, in particular by studying the mass transfers (water) in powders (imbibition and drying). A physical characterization of powders completes the description of the properties conferred to the ground rice straws. Beyond the obtained results, this study allowed to propose an original way to describe and understand the effects of fractionation tools that can be applied further to other kind of biomasses and other processes.

Keywords: biorefinery, rice straw, pretreatment, grinding, hydro-textural approach, rheological properties, energy consumption, bioconversion

Résumé

Couplage de procédés de prétraitements chimio-mécaniques de la paille de riz en voie semi-humide : effets sur les propriétés physicochimiques, rhéologiques et réactivité.

La biomasse lignocellulosique (LC) est considérée comme une alternative prometteuse pour produire des biocarburants, mais aussi extraire des biomolécules et synthons pour la synthèse de polymères et des matériaux afin de les substituer à ceux issues de la pétrochimie. La biomasse LC est principalement composée de cellulose, d'hémicellulose et de lignine. Sa nature composite et sa microstructure matricielle hétérogène rendent difficiles sa digestibilité et sa bioconversion. Le prétraitement de la biomasse LC est une étape indispensable permettant de dissocier la matrice LC et d'améliorer l'accessibilité des polymères pariétaux, étape-clé notamment pour la production de synthons.

Le fractionnement par voie sèche des LC s'insère dans les schémas de bioraffinerie de la biomasse avec des arguments favorable à la durabilité (pas d'eau consommée, pas de séchage, pas d'effluents). L'amélioration de la résolution du fractionnement, la réduction de la dépense énergétique et l'amplification de la réactivité/fonctionnalité des produits constituent des objectifs de recherche prioritaires dans le champ du fractionnement sec. Un des inconvénients de l'opération de broyage de la LC native est son importante consommation énergétique. La mise en œuvre de prétraitements modérés qui favorisent la broyabilité de la matrice LC et l'accessibilité aux molécules d'intérêt, peut permettre (i) d'améliorer la résolution du fractionnement, (ii) réduire très significativement l'énergie de broyage et (iii) amplifier la réactivité des produits.

L'objectif de la thèse porte sur l'analyse de la mise en œuvre de prétraitements chimiques couplés au fractionnement mécanique de paille de riz, qui a été choisie comme substrat valorisable de référence. Cette étude s'appuie notamment sur un procédé innovant de prétraitement chimique par voie semi-humide, qui permet de fragiliser et déstructurer la matrice LC afin de faciliter une déconstruction mécanique. Le couplage de procédés chimio-mécaniques semi-humide ont permis à la fois d'augmenter la réactivé des produits et de diminuer la consommation énergétique ainsi que supprimer certaines étapes et ne pas générer des effluents. Les résultats du fractionnement par voie sèche ont montré que la combinaison d'un broyage ultrafin et d'une séparation est une alternative de bioraffinerie technique pour obtenir des fractions intéressantes pour différentes propriétés. Ces résultats permettent d'améliorer les méthodes de prétraitements adaptées aux plusieurs types de biomasse dans la bioraffinerie des LC.

Une approche hydro-texturale à l'échelle des particules est ensuite proposée pour identifier les mécanismes de fractionnement et évaluer l'impact des prétraitements chimiques. Le rôle de la microstructure dans les prétraitements chemo-mécaniques est notamment analysé par le biais de l'étude des transferts d'eau dans les poudres (imbibition et séchage). Une caractérisation physique des poudres complète la description des propriétés conférées aux pailles de riz broyées. Au-delà des résultats spécifiques aux pailles de riz, cette étude a été conduite de façon a présenter un degré de généricité suffisant pour extrapoler la démarche et les connaissances acquises au traitement d'autres biomasses annuelles ou pérennes.

Mots-clés : bioraffinerie, paille de riz, prétraitements, broyage, approche hydro-texturale, propriétés rhéologiques des poudres, consommation énergétique, bioconversion