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# Innovative combined dry fractionation technologies for rice straw valorization to biofuels

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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).

## Introduction

Biofuels production from agricultural residues has been recently investigated as a renewable energy alternative to current fossil fuels. Lignocellulosic 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)[1, 2, 3]. Polysaccharides account for over 70% in lignocellulosic 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[4], RS can potentially produce yearly over 200 billion liters of bioethanol[3]. RS composition typically comprises cellulose (35-40%), hemicelluloses (25-30%), lignin (10-15%) and ash (8-15%)[1, 5]. The interest of RS for biofuel production relates to its high carbohydrate and low lignin content as compared to others lignocellulosic feedstocks and residues. Ethanol production from lignocellulosic 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 are known to be the rate-limiting steps in biofuels fermentation of most lignocelluloses substrates[6, 7]. The structural heterogeneity and complexity of the lignocellulosic matrix is in fact one of the obstacles to enhance hydrolysis efficiency and subsequent bioconversion. In a perspective of lignocellulosic biorefinery, the separation/fractionation of lignocellulose 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 lignocellulosic biomass aiming to maximise ethanol production[1, 2, 6, 7]. These include mechanical, chemical, physical-chemical and biological methods or a combination of them[1]. 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 lignocellulosic 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 valorisation, 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[1, 8].

As example, Papatheofanous *et al*.[9] 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[9]. 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[10]. Interestingly, samples with different particles sizes obtained via dry fractionation protocols were reported to have remarkably different chemical compositions[11]. 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 lignocellulosics[11]. Wheat bran has also been fractionated using a combination of ultrafine milling and electrostatic separation in an electrical field (positively+ and negatively- charged electrodes)[12, 13]. 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[12, 13]. 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[14]. 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[14].

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 valorisation. 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.

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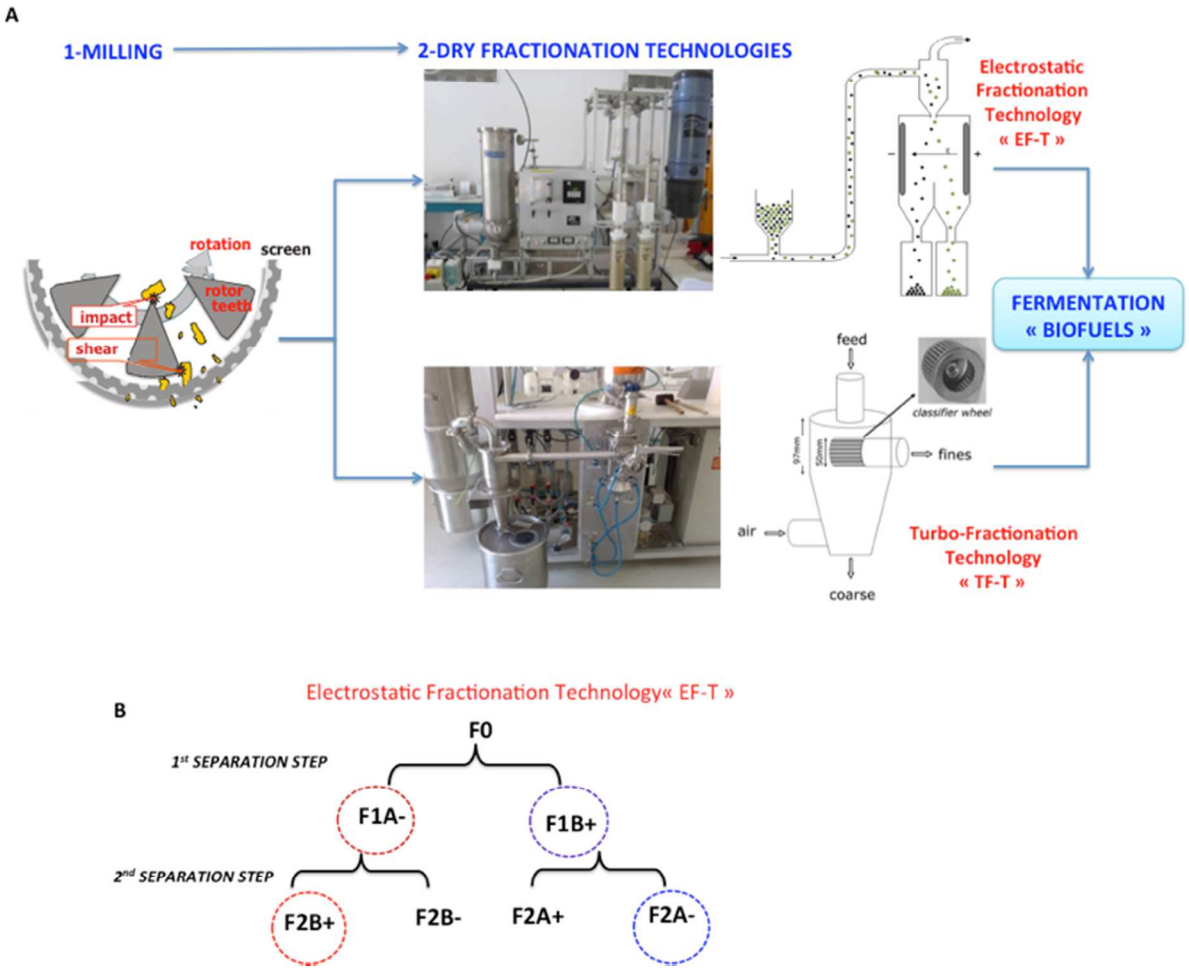


**Fig 1**: Electrostatic (EF-T) and Turbo (TF-T) Fractionation technologies route developed for rice straw (RS) in this study (A) Fractions prepared by electrostatic fractionation (B).

## Results and discussion

### Fractionation of RS combining ultrafine milling with electrostatic separation technology (EF-T)

The eelectrostatic fractionation technology (EF-T) concept is depicted in Figure 1. EF-T is based on a conveying of lignocellulosic 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 0.1mm particle size) as starting material (see Figure 1B). Samples were collected after each separation step yielding two sets of three fractions (F1A-, F2A-, F2A+, F1B+, F2B+ and F2B+). Table 1 summarizes recovery yields and particle size of different fractions prepared via EF-T. Results clearly pointed out that EF-T could have a significant influence on particle size diameter ($D_{50}$). Positively charged "F+" fractions possessed reduced sizes with respect to negatively charged fractions "F-" and untreated starting materials "F0" (ca. 10 um smaller, Table 1). Positively and negatively charged fractions were also characterized in terms of particle surface, biochemical composition, crystallinity and enzymatic accessibility.

**Table 1:** Physicochemical properties of RS fractions prepared by electrostatic-fractionation technology

| Fraction | Recovery (% w/w) | $D_{50}$ (µm) | *CrI* (%) | *SA* ($m^2/g$) |
|---|---|---|---|---|
| **F0** | - | 64.8 | 63.7 | 57 |
| **F1A-** | 38.5 | 68.1 | 67.8 | 54 |
| **F2A-** | 33.3 | 72.7 | 68.3 | 52 |
| **F2B-** | 49.3 | 69.6 | 64.6 | 54 |
| **F1B+** | 61.5 | 61.3 | 57.2 | 60 |
| **F2B+** | 66.7 | 56.1 | 56.7 | 62 |
| **F2A+** | 50.7 | 54.6 | 59.8 | 67 |

*SA*= (*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 ($g/m^3$)

The general characteristics and physicochemical properties of studied lignocellulosic 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 1). These results were in agreement with previous literature reports[15, 16]. *SA* of treated wheat straw was recently reported to be highly sensitive to particle size and pretreatment (i.e. increasing with ball milling and chemical treatment)[16]. EF-T also influenced the biochemical composition of different RS fractions depending on charged particles (Table 2). Positively charged fractions "F+" were richer in cellulose as compared to "F0" and negatively charged fractions "F-". Comparably, 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 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. Tables 1 and 2 essentially point out that positively charged particles generated upon EF-T fractionation possessed improved physicochemical properties 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).

**Table 2**: Biochemical Composition of RS fractions prepared by electrostatic-fractionation technology

| Fractions | *LiG* | *Cell* | *Hem* | *Ash* | *Ash/ Cell* | *LiG/ Cell* |
|---|---|---|---|---|---|---|
| **F0** | 13.8 ±0.6 | 49.8 ±1.2 | 22.5 ±0.7 | 13.8 ±1.1 | 13.8 | 0.28 |
| **F1A-** | 17.1 ±0.8 | 42.4 ±1.4 | 26.3 ±1.0 | 14.1 ±0.8 | 14.1 | 0.40 |
| **F2A-** | 17.4 ±1.4 | 40.3 ±0.9 | 26.2 ±0.5 | 16.1 ±0.4 | 16.1 | 0.43 |
| **F2B-** | 15.6 ±0.9 | 41.9 ±1.1 | 27.0 ±0.8 | 15.5 ±1.6 | 15.5 | 0.37 |
| **F1B+** | 11.1 ±0.8 | 52.6 ±1.6 | 24.3 ±0.2 | 12.0 ±0.3 | 12.0 | 0.21 |
| **F2B+** | 9.0 ±0.2 | 59.4 ±1.5 | 22.7 ±1.1 | 8.9 ±0.4 | 8.9 | 0.15 |
| **F2A+** | 13.4 ±0.9 | 47.6 ±1.2 | 24.9 ±0.7 | 14.1 ±1.2 | 14.1 | 0.28 |

RS: rice straw; Cell: cellulose; Hem: Hemicellulose; LiG: lignin; SA: surface area; CrI: Crystallinity index

These results confirmed the microstructure analysis using the fluorescence microscopy analyses (Fig 2), which confirmed the separation of different tissues with different physicochemical properties. Fig 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. Fig.2 shows that the morphology of the positively charged fractions differed from negatively charged fractions and untreated WS "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.[12,13] The observed difference in color and morphology could be due to differences in composition depending on the origin of the tissues.

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

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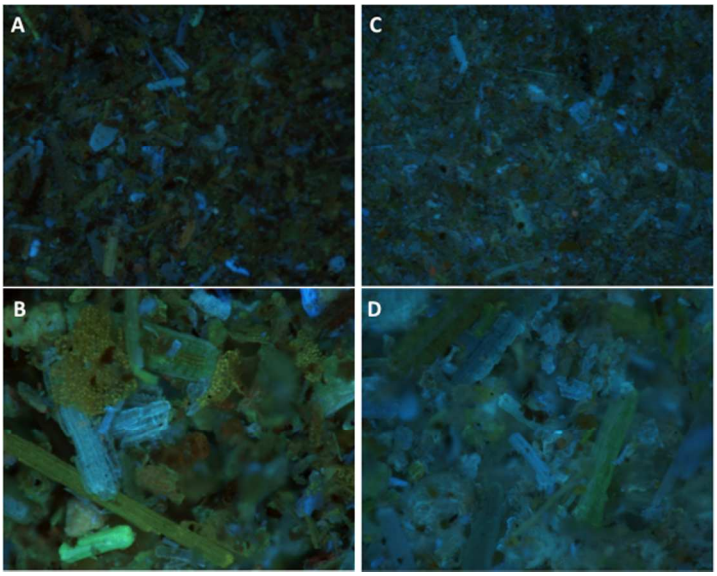


**Fig.2**: Micrographs showing the morphology of Positively charged fractions F2A-: A) x5 and B) x 25, and negatively charged fractions F2B+ C) x 5 and D) x 25. Samples were imaged without any staining using the multizoom AZ100M microscope (Nikon) in epifluorescence mode using the UV2A filter cube (Nikon) (excitation filter: 325-375nm, dichroïc mirror: LP 400nm, emission filter LP 420nm). The observation was made with a Plan Fluor 5x (NA=0.5) objective and the optical zoom was set at 1 or 5, leading to a global magnification of 5x or 25x. At a 25x 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).

**Enzymatic hydrolysis and ethanol fermentation of EF-T rice straw fractions**

All RS fractions prepared via EF-T (Figure 1B) were subsequently hydrolyzed with a commercial enzyme cocktail at biomass loadings of 10% in buffer and enzyme loadings of 20 FPU $g^{-1}$ for 72 $h^{16}$. The effects of each pretreatment step were evaluated *via* determination of released sugar yields (glucose and xylose; mg $g^{-1}$ RS). Fig 3 shows that maximum glucose yields after 72 h could be obtained from "F+" fractions (ca. 220-250 mg $g^{-1}$ RS). 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 3). Both were clearly superior in "F+" fractions, pointing to a higher accessibility by enzymes. EF-T can be then 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).

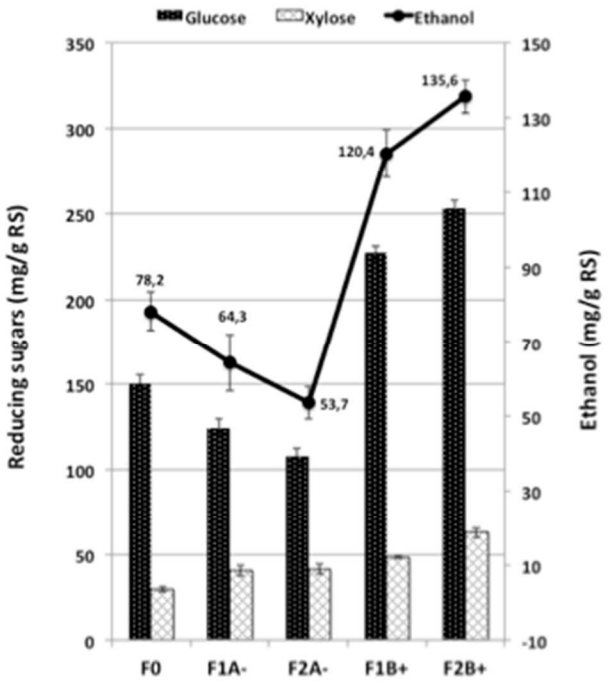


**Fig 3**. Reducing sugars and ethanol yield (mg $g^{-1}$ RS) of rice straw fractions obtained from EF-T. Maximum ethanol yield obtained 135.6 mg/ g RS.

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 lignocellulosic biomass by enzymes and microorganisms for bioconversion to biofuels[1, 17, 18]. "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+" are richer in cellulose and have 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 Fig 3 also shows that the maximum ethanol yield after 72hrs of SSF (120-140 mg $g^{-1}$ RS) was obtained with positively charged fractions "F+". These values represented an increase between 64-74% as compared to those obtained with untreated RS (78 mg $g^{-1}$, Fig 3). "F+" fractions were 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 was proved to be an advanced lignocellulosic valorisation strategy to improve the rate of saccharification and ethanol production avoiding the utilisation of any chemical, water and additional heat inputs, minimising waste generation (e.g. toxic effluents) as compared to conventional lignocellulosic pretreatments.

**Table 3: Physicochemical properties of RS fractions prepared by turbo-fractionation technology**

| Speed (rpm) | | Recovery (% w/w) | $D_{50}$ (µm) | *CrI (%)* | *SA* ($m^2/g$) |
|---|---|---|---|---|---|
| --- | **F0** | - | 64.8 | 63.7 | 57 |
| **3000** | **Cf** | 4.3 | 133.9 | 64.3 | 27 |
| | **Ff** | 94.9 | 70.7 | 61.8 | 52 |
| **5000** | **Cf** | 50.2 | 110.3 | 63.5 | 33 |
| | **Ff** | 48.0 | 47.9 | 61.4 | 74 |
| **7000** | **Cf** | 76.1 | 75.9 | 62.7 | 45 |
| | **Ff** | 17.5 | 26.6 | 61.3 | 138 |
| **10000** | **Cf** | 44.9 | 71.6 | 64.4 | 51 |
| | **Ff** | 54.1 | 10.2 | 60.4 | 329 |
| **12000** | **CF** | 81.3 | 71.6 | 62.4 | 51 |
| | **Ff** | 16.1 | 8.5 | 60.5 | 433 |

***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 ($g/m^3$)**

## Fractionation of RS combining ultrafine milling with Turbo-separation technology (TS-T)

Superfine grinding combined with dry separation (air classification or turbo-separation) or jet-milling technologies have attracted a significant interest in recent years due to their possibilities 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 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 12000rpm) combined with a previous dry milling step (Fig 1A).

**Table 4**: Physicochemical properties of RS fractions prepared by turbo-fractionation technology

| Speed (rpm) | | *LiG* | *Cell* | *Hem* | *Ash* | *Ash/ Cell* | *LiG/ Cell* |
|---|---|---|---|---|---|---|---|
| --- | **F0** | 13.8 ±1.6 | 49.8 ±1.3 | 22.5 ±0.8 | 13.8 ±1.6 | 0.28 | 0.28 |
| **3000** | **Cf** | 14.1 ±0.8 | 51.4 ±1.8 | 25.8 ±1.4 | 8.6 ±0.6 | 0.17 | 0.28 |
| | **Ff** | 13.4 ±1.1 | 49.0 ±1.6 | 24.3 ±0.9 | 13.3 ±1.1 | 0.27 | 0.27 |
| **5000** | **Cf** | 13.9 ±0.8 | 50.4 ±1.8 | 24.9 ±0.2 | 10.8 ±0.5 | 0.21 | 0.27 |
| | **Ff** | 12.8 ±0.8 | 48.5 ±1.3 | 24.4 ±0.8 | 14.3 ±1.1 | 0.30 | 0.27 |
| **7000** | **Cf** | 13.1 ±0.9 | 49.5 ±1.2 | 25.6 ±1.0 | 11.7 ±0.8 | 0.14 | 0.27 |
| | **Ff** | 12.6 ±0.8 | 49.6 ±1.2 | 23.4 ±0.6 | 14.3 ±0.0 | 0.28 | 0.25 |
| **10000** | **Cf** | 12.9 ±0.8 | 52.4 ±1.6 | 26.3 ±0.5 | 10.0 ±0.7 | 0.19 | 0.25 |
| | **Ff** | 11.8 ±0.2 | 54.0 ±0.8 | 20.5 ±1.3 | 13.7 ±1.3 | 0.25 | 0.22 |
| **12000** | **CF** | 13.5 | 52.8 | 24.6 | 9.7 | 0.18 | 0.26 |
| | **Ff** | 13.1 | 52.6 | 22.1 | 14.1 | 0.25 | 0.25 |

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

Untreated RS ("F0", $D_{50}$: 64.8 µm) was introduced as feed (1kg/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. Tables 3 and 4 summarize 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 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 12000 rpm (Table 2). An increase on 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" (72µm particle size). Such low "Ff" yield makes these conditions (TF-T, 12000rpm) economically unfeasible. Interestingly, a rotational speed of 10000rpm also produced a very fine particle size fraction (10µm) with high yields (55% and 45% recovery of "Cf" with a

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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 of 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 4) composition and physic-chemical properties (crystallinity, density and surface area, Table 3). 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 3).

In general, the finer fractions exhibited larger surface areas as compared to coarse fractions (Table 3), in some cases reaching very large numbers (433 $m^2$/g, Ff-12000). These values increased at increasing rotational speeds and were remarkably different to typical values obtained for Cf and untreated RS (ca. 50 $m^2$/g). 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, Tables 1 vs 3). 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 (Tables 1 vs 4). 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 4). Only an increase in cellulose content (10%) with a 14% decrease in lignin was observed for Ff-10000rpm, corresponding to a *lignin/cellulose* ratio of 0.22. Coarsed 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-12000rpm respectively compared to untreated RS, Table 4).

**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 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 3 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-10000 and Ff-12000.

Fig 4 also summarizes bioethanol yields from different TF-T fractions. The maximum ethanol yield (SSF, 72h) 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 hydrolyse, releasing large quantities of glucose without any remarkable changes in cellulose content or crystallinity as compared to RS subjected to EF-T (Tables 2 vs 4). Fermentation to bioethanol also followed a similar trend, with very similar yields obtained for EF-T "F+" and TF-T "Ff" samples (Fig 3 vs 4; 136 vs 150 mg $g^{-1}$), pointing to a positive effect of particle size and surface area. The generation 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 in enzymatic and chemical hydrolysis of cellulose and lignocellulosic materials was reported.[19, 20] Maache-Rezzoug *et al.*[19] 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[19]). 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 valorisation of RS to a better digestible and easily hydrolysable/fermentable feedstock for bioethanol production. The importance of maximising *SA* and reducing particle size using TF-T is also another additional advantage of the proposed combined technology.

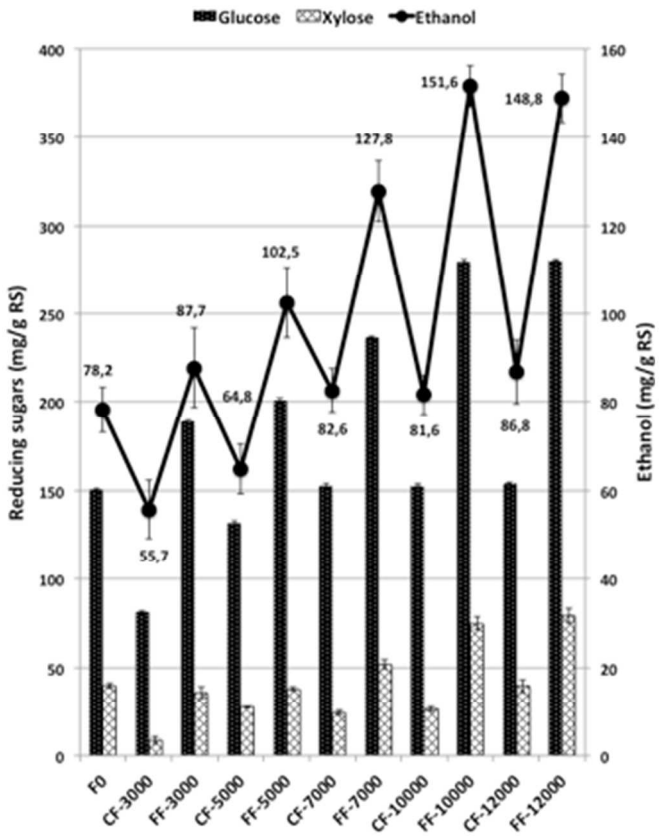


**Fig 4**. Reducing sugars and ethanol yield (mg $g^{-1}$ RS) of rice straw fractions prepared by TF-T. Maximum ethanol yield obtained 148.8 mg/g RS.

## 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 lignocellulosic biomass as well as by ash and lignin content. A large number of pretreatment methodologies have been developed in recent years[6, 20, 21, 22 , 23, 24 , 25]. Some chemical, physicochemical, physical and mechanical pretreatments and/or fractionation technologies have 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[1,16]. Many chemical pre-treatments also involve the utilisation 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 amount 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 utilised to compare both performance, efficiency and environmental impacts of different RS pretreatment processes (Table 5). A comparison of proposed technologies with existing processes indicates that glucose and bio-ethanol 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: ($\boldsymbol{E_T = E_M + E_{EF}}$). The specific energy requirements ($E_M$) to reduce particle size of RS using a 0.1 mm knife mill (Fig 1A) were 135.4 $WhKg^{-1}$. EF-T and TF-T fractionation technologies also consumed only 12.5 and 22.4 $Whkg^{-1}$ RS, respectively (calculated specific energy consumption measurements). This indicates clearly that EF-T and TF-T technologies consumes 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[1, 26, 27]. Total energy ($E_T$) required for RS pretreatment was 147.9 and 157.8 $Whkg^{-1}$ 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 pre-treatments of oilseed rap (OSR) straw have been previously evaluated[28]. 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*.[29] 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 cycles WDM, while 20 cycles consumed a highest amount of energy, corresponding to the lowest η. Hideno *et al.*[30] 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 lignocellulosic pretreatments.

As compared to 150 mg glucose $g^{-1}$ RS obtained under

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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 compared 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.*[30].

Table 5 summarises a comparative study of various pretreatment technologies developed for RS saccharification for bioethanol production. Energy efficiency η (g glucose extracted $Wh^{-1}$) is normally utilised to compare pretreatment/fractionation performance[16, 31]. However, literature concerning nergy consumption and energy efficiency of chemical, physicochemical and mechanical treatment of RS is scarce (Table 5). However, various inputs and outputs have been utilised to compare these different pretreatments processes in terms of *E*-factor (Table 5).

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.

**Table 5:** Comparison of various RS pretreatments 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 | 32 |
| **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 | 33 |
| **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 | 34 |
| **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 | 35 |
| **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 | 36 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **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 | – | 37 |
| **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 +electrostatic fractionation (EF-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 +turbo-fractionation (TF-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

The performance of sodium carbonate–sodium sulfite ($Na_2CO_3$–$Na_2SO_3$) pretreatment on 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[32]. 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 5). The corresponding *E*-factor was 6.84.

To minimize the cost of cellulase production, both RS pretreatment 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[33]. 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 optimised conditions (2.96% NaOH, 81.79°C and 56.66 min)[34].

The addition of an ultrasound step to the alkali pretreatment (Table 5) showed a slightly higher digestibility yield but the difference was not significant[34]. However, this study used large quantities of water and chemicals resulting in an *E*-factor of 4.85 (Table 5). Sheik *et al*.[35] 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 feedstocks. This study was generally fine from the green chemistry standpoint, with a low *E*-factor of 0.79 (Table 5).

Using optimized enzyme condition 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 48h, which was significantly higher to that from untreated RS (total reducing sugars: 270 mg $g^{-1}$ biomass)[36]. Nevertheless, the popping pretreatment consumed large quantities of water and generates significant quantities of waste, which corresponding to a high *E*-factor of 4.11 (Table 5). Fermentation of the hydrolyzates by *Saccharomyces cerevisiae* resulted in 172 mg ethanol $g^{-1}$ RS after 24h, equivalent to 80.9% of the maximum theoretical yield (based on the amount of glucose in the raw material)[36]. Ranjan *et al*.[37] 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 5). 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[37].

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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 of any chemical or water inputs with an *E*- factor of approximately 0.7-0.75, thus minimizing waste generation while maximizing value of the lignocellulosic feedstock (Table 5).

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

## Experimental

### Raw material

Rice straw (RS) was obtained from a local farm (Languedoc-Roussillon region, France). RS was coarsely cut to less than 2 mm by knife milling (Retsch SM 100, Germany). The milled rice straw was then sieved using UPZ milling (0.1 mm) for electrostatic technology (EF-T) and turbo-fractionation or air classification technology (TF-T).

### Ultrafine milling

Two-millimeter at moisture content of 8-10% was ground using an impact mill (Hosokawa-alpine, type UPZ, Augsburg, Germany), operated at ambient temperature at a speed of 18000 rpm, with 0.1 mm screen size (the material was milled until it passed through the grid). After UPZ milling, the size was analyzed by laser granulometry (Mastersizer2000, Malvern Instrument). Density of particles was determined using a pycnometer (Ultra Pycnometer 1000, Quantachrome Instrument).

### 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 1. 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+".

### 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 1). 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").

### Measurement of specific energy consumption

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 Equation 2:

$$E_{EF-T} = \frac{\int_0^t (P_t - P_0)dt}{m} = \frac{\int_0^t \Delta P_t dt}{m}$$

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

**Carbohydrate analysis**

The carbohydrate and lignin composition of lignocellulose 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.

**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). 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 azide 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[16].

**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.

**Conclusion**

The combination of ultrafine milling with turbo- and electrostatic 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, an equivalent to a 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- 95% with respect to untreated RS. The combination of milling with electrostatic and turbo-fractionation of lignocellulosic particles appears to be an interesting continuous process and new technology for the development of environmentally sound lignocellulosic biorefineries for biofuels 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.

**Notes and references**

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**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

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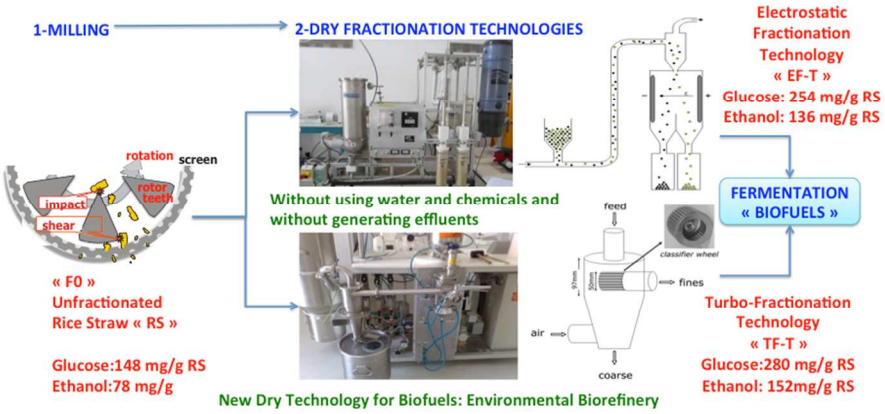


Development of an innovative lignocellulosic biorefinery: Milling combined with electrostatic (EF-T) and turbo (TF-T) fractionation technologies of lignocellulose biomass. EF-T and TF-T appear to be interesting technologies for biofuels production from waste feedstocks (e.g. rice straw) without any chemical or water inputs and minimizing waste generation