



# Comparison of various milling modes combined to the enzymatic hydrolysis of lignocellulosic biomass for bioenergy production: Glucose yield and energy efficiency



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## ABSTRACT

Bagasse is an abundant by-product from sugarcane production that can be used for conversion into biofuels. Nonetheless, the recalcitrant structures of lignocellulosic fibers required a pretreatment prior conversion into biofuels. In this study, four mechanical deconstruction methods were compared in terms of energy demand and energy efficiency at lab scale: BM (ball mill), VBM (vibratory ball mill), CM (centrifugal mill) and JM (jet mill). Results indicate that VBM was more effective compared to BM, JM and CM in enzymatic accessibility and sugars solubilization: VBM-3h > BM-72 h > JM-5000 rpm > CM-0.12 mm. However, preliminary energetic assessment showed that at lab scale, the CM (centrifugal mill) as mechanical fractionation process appears to be the most efficient in terms of energy-efficiency (kg glucose/kWh) compared to BM, VBM and JM. A comparison with literature pretreatments data highlighted that fine and/or ultrafine milling process (BM, VBM, CM) are simpler saccharification technologies, which not required any chemical or water inputs, thus minimizing waste generation and treatment.

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## 1. Introduction

In the future, petroleum refineries will be substituted by bio-refineries, where wastes will be cracked into oil, synthons, bio-energy or biomaterials [1]. Many crops, agro-resources, or agro-industrial wastes are used for this reason. Among them, sugar cane is an ideal plant to serve as a basis for biorefining [2,3]. Indeed, sugarcane produces sugars and several molecules of high added value [4], but also BG (bagasse), which is a lignocellulosic biomass, with an amount of 279 MMT (million metric tons) generated annually on a global level [5]. BG is a fibrous residue, which consists mainly of cellulose, hemicellulose and lignin. Enzymatic hydrolysis of the main components of the sugarcane bagasse is one of the promising methods to further upgrading it into biofuels [6]. The enzymatic hydrolysis can be influenced by various physicochemical parameters like accessible surface area, cellulose crystallinity,

lignin content and distribution, hemicelluloses structure and lignin–hemicelluloses–cellulose association [6–9]. A pretreatment process is therefore recommended in order to: i) modify the structure and architecture of the lignocellulose, ii) reduce particles size and cellulose crystallinity, and iii) increase the accessible surface area and porosity [10–12]. A number of pretreatment methods have been developed to improve the conversion of sugars polymers (i.e. cellulose, hemicellulose) into fermentable sugars aiming to maximize biofuels production [9,11,13]. Pretreatment technologies include mechanical, chemical, physicochemical and biological methods or a combination of these techniques [10].

Most of lignocellulosic biomass pretreatments are currently based on the use of expensive chemical processes multistep, which, in some cases, consume large amount of water and chemical quantities and generate significant wastes (i.e. effluents). Low or no water consumption during lignocellulosic pretreatment can decrease the effluents, and also reduce the energy input for the biomass pretreatment [9,10,14,15]. For this purpose, mechanical fractionation of lignocellulosic biomass could be one promising alternative for a sustainable future dry biorefinery with low water and chemical consumption and thus low waste production.

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### Abbreviations

BG	bagasse
BM	ball milling
VBM	vibro-ball milling
JM	jet milling
CM	centrifugal milling
CrI	crystallinity index
SA	surface area
$E_{TER}$	total energy requirement
DM	Dry matter

In recent years, the development of environmentally friendly pretreatment such as milling, ultrasonic, plasma and wet explosion has been studied with woods, bagasse, rice and wheat straw [14,16–21]. Mechanical fractionation of biomass by hammer mills, knife mills, disk mills, ball mills, vibratory ball mills, colloid mills, centrifugal mills, tumbling ball mills, planetary ball mills, jet mills produces fine particles which increase surface area, reduce cellulose crystallinity, resulting in an improved conversion of sugars polymers during enzymatic hydrolysis. For example, studies have shown that particles sizes must be reduced to 0.5–2 mm in order to reach a well-accepted level of digestibility [22]. Currently, milling processes are not cost-effective because of high investment costs but also high-energy requirements. Total energy requirement ( $E_{TER}$ ) of milling processes will depend on the physicochemical properties of biomass and also on the ratio of particle size distribution of materials before and after milling, which also depend strongly on the equipment or machine used.  $E_{TER}$ , glucose yields and energy efficiency are generally used to compare performance and efficiency of different pretreatment processes [10,14,15,21]. However, literature concerning the comparison of energy consumption, and energy efficiency of lignocellulosic biomass remain scarce.

This work is a first attempt to compare different milling processes (i.e. BM, VBM, CM, JM) in term of sugars yields after enzymatic hydrolysis, energy requirement and energy efficiency. The physicochemical properties changes, enzymatic digestibility, energy consumption and energy efficiency were evaluated and compared to chemical and physicochemical pretreatment developed in literature for BG biomass.

## 2. Experimental section

The fractionation of raw BG was carried out using various mechanical processes governed by mechanical stresses such as impact, compression, friction, and shear (Fig. 1); all may coexist in one commercial equipment [10,23,24]. For example, in a JM (jet milling), the particles are projected against each other in an air stream; major mechanical stresses generated are impact and friction between particles. CM (centrifugal milling) consists of a rotor driving different tools. The rotor speed is generally adjustable. A sieve or a screen allows control of the particle size of the final product. These mills generate more impact and shear. Finally, in VBM (vibratory ball mill); the raw materials suffer impact and compression stresses when collisions between balls and walls occur. VBM is similar to BM, except that the mill is vibrated instead of rotated. The efficiency of different milling modes as a pretreatment of Moroccan BG for biofuels production was evaluated by: i) evaluating the total yield of sugars such as glucose, xylose and arabinose recovered from enzymatic hydrolysis of different bagasse fractions; ii) looking for the physicochemical properties of fractionated bagasse; iii)

calculating the total energy consumption and further assess the energy efficiency.

### 2.1. Feedstock and mechanical fractionation

Sugarcane Bagasse was generously provided by COSUMAR (Company located in Morocco). BG was dried to moisture content of 8% and coarsely cut to less than 2 mm by knife milling (Retsch SM 100, Germany). Then, different milling equipment with different mechanical stresses such as impact, compression, friction, and shear were applied on BG samples (Fig. 1). Mechanical pretreatments investigated were i) CM (centrifugal milling) (Retsch ZM 200, Germany), operated at ambient temperature and speed of 12,000 rpm, with 0.5 and 0.12 mm screen size (the material was milled until it passed through the grid), ii) BM (ball milling) (Marne n°55, FAURE, France) operated at ambient temperature at speed of 50 rpm for 24, 48 and 72 h, using a jar of 1 L with 1/3 of balls (diameter of 0.5 cm) and 1/3 of biomass, iii) VBM (vibratory ball milling) (Retsch MM 400, Germany) operated at ambient temperature at a frequency of 15 s<sup>-1</sup> for 1 h and 3 h, using a ball of 1.5 cm of diameter, iv) JM (jet milling) (100 AFG, Hosokawa alpine, Japan) operated at ambient temperature at speeds of 4000 rpm and 5000 rpm for 15 min (Fig. 1).

### 2.2. Enzymatic hydrolysis

Enzymatic hydrolysis of BG was performed using an enzyme cocktail (*Trichoderma longibrachiatum* C9748) obtained from Sigma Aldrich (20 FPU g<sup>-1</sup> cellulose in biomass). Enzymatic hydrolysis was carried out at a solid concentration of 5% (w/v) in a 50 mM sodium acetate buffer (pH 5.0) at 37 °C for 72 h with agitation (100 rpm). The experiment was performed in triplicate. The enzymatic digestibility was assessed by the obtained soluble sugars (i.e. glucose, xylose, arabinose in mg g<sup>-1</sup> BG) determined by HPLC (high performance liquid chromatography) analysis using BioRad HPX-87H column [16].

### 2.3. Biochemical and physical analysis

The carbohydrate and lignin composition of lignocellulose samples was measured after concentrated acid hydrolysis. The sugars analysis was done with a combined HPLC Water system, using a BioRad HPX-87H column at 40 °C and 0.3 mL/min [16]. All the determinations reported here were duplicate results. The particles size was analyzed by laser granulometry (Mastersizer2000, Malvern Instrument). The crystallinity of different BG fractions was determined by X-ray diffraction. Powder X-ray diffraction patterns were recorded on a Bruker diffractometer D8 Advance. The measurements were conducted on powder compacted on small mats. XRD (X-ray diffraction) data were collected from 2θ = 5° to 50° with a step interval of 0.02°. The degree of crystallinity can be expressed as the percentage crystallinity index [16]. The surface area was determined using BET (Brunauer–Emmett–Teller) method. The gas adsorption data were collected using a Micrometrics (France) 3Flex Surface characterization analyzer using N<sub>2</sub>. Prior to N<sub>2</sub> sorption, all samples were degassed at 50 °C overnight. The specific surface areas were determined from the nitrogen adsorption/desorption isotherms (at –196 °C).

### 2.4. Measurement of energy requirement “ $E_{TER}$ ”

The total energy requirement “ $E_{TER}$ ” during the various mechanical fractionation processes was measured according to Eqs. (1) and (2). The power active, active electric energy (Wh), frequency hertz and time were logged into a computer card at 1-s intervals.

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