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Combination of dry dark fermentation and mechanical pretreatment for lignocellulosic deconstruction: An innovative strategy for biofuels and volatile fatty acids recovery

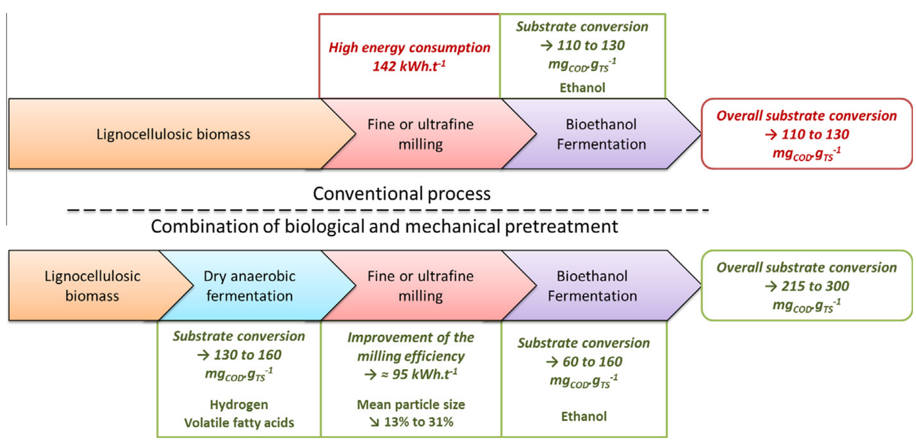
Jean-Charles Motte [a,b], Cecilia Sambusiti [a], Claire Dumas [b], Abdellatif Barakat [a,*]

[a] INRA, UMR 1208 Ingénierie des Agropolymères et Technologies Emergentes 2, Place Pierre Viala, F-34060 CEDEX Montpellier, France
[b] INRA, UR0050, Laboratoire de Biotechnologie de l'Environnement, Avenue des étangs, F-11100 Narbonne, France

HIGHLIGHTS

- A novel combination of solid-state fermentation and fine milling was developed.
- Biological pretreatment produces valuable bioproducts (VFA and biohydrogen).
- Solid-state dark fermentation improves considerably the milling efficiency.
- Bioethanol yield was higher after a strong particle size reduction.
- Substrate conversion was two times higher than conventional processes.

GRAPHICAL ABSTRACT



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ABSTRACT

In the present study, the feasibility of combining dry dark fermentation and mechanical pretreatment of wheat straw was studied in order to improve substrate valorization, save energy input, decrease the environmental impact and diversify biofuels and volatile fatty acids production. To this end, dark fermentation of wheat straw was performed at 55 °C and 35 °C under solid-state conditions (23% of total solid content) and it was considered as a biological pretreatment. Both biologically treated and raw straws were reduced at four particles size to cover the range of fine ($50 < X < 500$ µm) and ultrafine milling (<50 µm). Biological pretreatment led to a substrate conversion of 16% and 14%, mainly into volatile fatty acids and biohydrogen. Biological pretreatment improved the substrate grindability with a reduction of mean particle size up to 31% and a reduction of the milling specific energy consumption up to 35% compared to untreated straw. Finally, related to untreated straw, this combination of biological and mechanical treatments improved the bioethanol yield up to 83%, which leads to an enhancement of the overall substrate conversion up to 131%. Based on these high yields, this combination of dry biological–mechanical pretreatments appears more attractive and efficient in terms of bioproducts production, energy efficiency and environmental impact, compared to conventional pretreatments.

Abbreviations: WS, wheat straw; BMT, mesophilic biological pretreatments; BTT, thermophilic biological pretreatments; BM, ball milling; CM, centrifugal milling; TS, total solid; VFA, volatile fatty acids; SSF, Simultaneous Saccharification and Fermentation.

* Corresponding author. Tel.: +33 (0) 4 99 61 25 81; fax: +33 (0) 4 99 61 30 76.
E-mail address: barakat@supagro.inra.fr (A. Barakat).

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1. Introduction

Pretreatments of lignocellulosic biomass appeared to be critical stages for biorefinery development to improve transformation yields and rates. These processes aim to solubilize carbohydrates in order to enhance their conversion into biofuels (*i.e.* bioethanol and biogas). To date, different pretreatments have been investigated in literature, such as mechanical pretreatment (milling), thermal pretreatment (liquid hot water, steam explosion, CO_2 explosion...), chemical pretreatment (acid, alkaline, oxidative...) or biological pretreatment (fungal, enzymes, fermentation) [1,2]. The choice to use a single or a combination of pretreatments is often due to a compromise between carbohydrates solubilization efficiency, energy consumption and effluent management [3,4]. Pretreatments that operated under dry conditions are particularly interesting, since they save energy and generate small quantity of effluent [5,6]. Among them, strong particle size reduction is often considered as a process of interest for its ability to homogenize substrate, to reduce the cellulose crystallinity and to improve the carbohydrates solubilization [6,7]. The application of fine ($50 < X < 500$ μm) and ultrafine (<50 μm) milling is particularly efficient to improve the enzymatic hydrolysis [8,9] and consequently the bioethanol production from lignocellulosic substrates [10,11]. However, the high energy consumption required to break the lignocellulosic structure [5,7,12] is generally considered as the main limiting factor for the application of mechanical pretreatments. Recently, combination of chemical and mechanical pretreatments have been proved to reduce the energy consumption of milling step and to maximize the efficiency of glucose release [9].

Solid-state biological processes that operate at a total solid (TS) content higher than 20%, have been recently applied for dark fermentation of lignocellulosic substrates [13–15]. Dark fermentation corresponds to the first step of the anaerobic digestion after inhibition of the methanogenic activity, and it conducts simultaneously to the production of high amount of hydrogen (H_2) and valuable volatile fatty acids (VFA) [15,16]. These organic acids, commonly acetic, propionic, butyric and lactic acids, have been investigated as precursors of various biotechnological applications: (1) for biodiesel production through the synthesis of single cell oils by oleaginous yeast [17]; (2) as substrate for microbial fuel cell [18]; (3) as external carbon source for the biological denitrification of wastewaters rich in nitrogen [19]; (4) as potential substrate for the production of hydrogen via photofermentation by mix microbial cultures [20]; (5) as the building blocks of various organic compounds including alcohols, aldehydes, ketones, esters and olefins [21] and (6) recently VFAs have been considered valuable substrates to produce bioplastics (*i.e.* poly-β-hydroxybutyrate, poly(hydroxy alcanoate)s) [22]. Dark fermentation can be considered as a substrate pretreatment due to a combination of both biological degradation and acidic conditions [13,23]. To perform dark fermentation process, an anaerobic digestate is generally used as inoculum after inhibition of its methanogenic activity, induced for example by a thermal or an acid shock [16]. Nevertheless, acidification can be also easily induced under solid-state condition, reducing the amount of inoculum (*i.e.* with a substrate/inoculum ratio higher than 20) [24]. These fermentation conditions provide a solid fraction poor in water and sludge content, which do not require much treatment (cleaning) before further use of the lignocellulosic biomass.

The aim of this study was the development of a novel scheme of lignocellulosic biomass pretreatment to improve and diversify biofuels and volatile fatty acids production in a general concept of solid-state biorefinery. To this end, a combination of solid-state fermentation and fine milling was developed (Fig. 1). The different scenarios were tested and evaluated in term of biofuels (bio-hydrogen, methane and ethanol) yield, volatile fatty acids, and specific energy consumption.

2. Material and methods

2.1. Substrate characterization

Wheat straw (*Triticum aestivum*) was harvested during summer 2010 from a farm located in the south of France (Languedoc Roussillon Region, Herault department). A cutting mill (Retsch® SM100) equipped with a 10 mm grid, was used to grind straw coarsely. Total solids content (TS) was measured after drying sample at 105 °C during 48 h, while volatile solids (VS) was measured

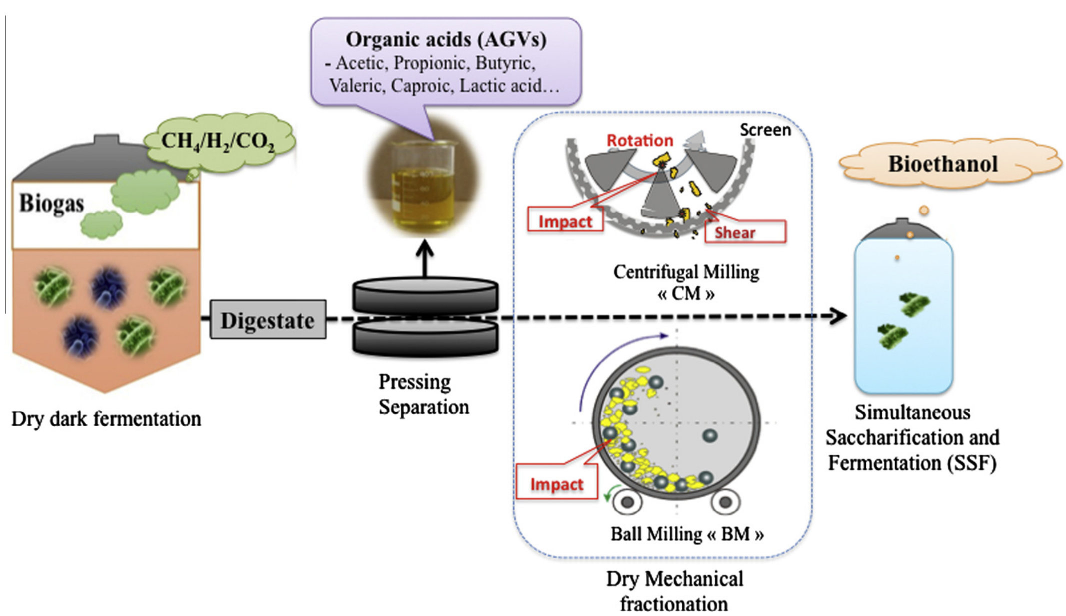

Fig. 1. Combination of dry dark fermentation and mechanical pretreatment proposed in this study.