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Biomass pretreatment with reactive extrusion using enzymes: A review

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ABSTRACT

Introducing enzymes during the extrusion process has been mainly used as new pretreatment techniques in the starch degradation process and, more recently, in the second generation bioethanol production. The technique, called the bioextrusion, is a special case of reactive extrusion. Starch and lignocellulose bioextrusion examples underline the good mixing capacities as a way to initiate the enzymatic reaction in high solid content conditions. Starch bioextrusion results show a low dextrinization yield but a real effect on the polymer size decrease which allows higher and faster subsequent saccharification. It also considerably reduced the recrystallization phenomenon that limits the saccharification efficiency. Bioextrusion of lignocellulose resulted in a better sugar production. Very short residence times limit the use of bioextrusion to a pretreatment technique. However, unique flexibility of the extrusion technique allows to subsequently pretreat, in the same extruder, with physical and/or chemical constraining conditions, followed by a milder bioextrusion.

1. Introduction

Bioextrusion is defined as the use of one or several types of enzymes as biocatalysts during the extrusion process. The extruders' adaptability allows the introduction of liquid enzyme solutions at different steps of the process. Even if all the known examples of this combination concern the treatment of biomasses, theoretically, all kinds of substrates can be processed with bioextrusion. Enzyme deactivation by extrusion has also been studied on different food products like cereal grains (Fretzdorff and Seiler, 1987) and fish muscles (Choudhury and Gogoi, 1996). These studies intended to deactivate enzymes involved in the product degradation in order to improve product shelf-life (Linko et al., 1981). Proteins involved in the product degradation like proteases, lipases, lipoxidases, ureases and peroxidases, can be denatured thanks to shear fields and high temperatures. In these cases enzymes are not used as biocatalysts and do not correspond to the bioextrusion definition. This is why it won't be detailed in this review. This review summarizes the advantages and limitations of the combination between the extrusion process and the enzyme biotechnology. These characteristics will be studied through two main examples of its application, starch liquefaction and lignocellulose deconstruction.

1.1. A specific case of reactive extrusion

The extrusion is a continuous process that can easily be brought to

an industrial level. This characteristic has led the early developments of this technique. It is a determining factor regarding volumes and quantities of biomasses that are processed for the food, feed or chemicals production. It is a flexible and versatile technique. Screw extruders are characterized by a highly versatile screw configuration which allows various constraint profiles. It can handle different substrates with different viscosities, rheological behaviors, phases. Processing conditions can easily be adapted and extrusion modules can be changed to add or remove functionalities (physical separation, degassing) (Bouvier and Campanella, 2014).

As enzymes can be seen as biocatalysts, bioextrusion can be regarded as a specific case of reactive extrusion. Historically chemical and biochemical reactions have been carried out in diluted conditions. But these conditions imply the use of solvents or diluents from 5 to 20 times the weight of the desired product, requiring costly facilities to recycle it. As shown by Vergnes and Berzin (2004), contrary to batch reactors, extruders can handle very viscous materials. Higher physical and chemical modification rates place it as an intensification process. In addition, low processing volumes and residence times permit to make economies in energy, materials and consumables. With increasing concerns about energy and environmental issues, efficient and continuous reactors like extruders are seen as sustainable processing technologies (Janssen, 2004). While solving the problem regarding the solvents use, it raises issues linked to the mixing of high viscous reactants or products and difficulties to achieve homogeneous mixtures.

Abbreviations: BAB, blue agave bagasse; BS, barley straw; DE, dextrose equivalent; GPC, gel permeation chromatography; HTST, high temperature and short time extrusion; OPEFB, oil palm empty fruit bunch; SC, sweet corn co-products; SEM, scanning electron microscopy; SME, specific mechanical energy; WSI, water solubility index

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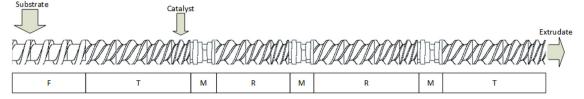


Fig. 1. reactive extrusion standard screw configuration. F corresponds to a feeding zone, T and R are respectively transport and reaction areas with conveying elements and M are mixing zones with kneading discs.

Extruders are continuous reactors and in-line addition of reactants associated with restrictive screw elements (reverse or kneading blocks) permit to isolate specific barrel modules in order to carry out discrete chemical processes (Bouvier and Campanella, 2014). An example of this screw configuration is presented in Fig. 1. Four main classes of chemical reactions are realized in screw extruders, bulk reactions, control of molecular weight of polymers, chemical modifications and reactive liquid/solid extraction-pressing. These reactions can be applied to synthetic materials as well as biomaterials such as biomass. Reactive extrusion has especially been studied in continuous monomers polymerization in bulk reactions, avoiding the use of solvents while enhancing process productivity and flexibility (Brown, 1991). It is still highly complicated to build a model of these reactions due to the lack of relevant kinetic data that were, for most of it, obtained in diluted conditions. Most of these reactions are carried out in intermeshing corotating twin-screw extruders.

Even though this technique provides many advantages, two main drawbacks are to be highlighted. Firstly the temperature regulation difficulties, because of the limited cooling capacities. Secondly, and most importantly, the limited residence time. In general it is comprised between 1–5 min, allowing only fast reactions to occur. In these fast conditions, species distribution depend on both bulk flow and molecular diffusion (Bouvier and Campanella, 2014).

1.2. Enzymatic hydrolysis of biomass

Processing biomass brings issues that have already been solved by some living organisms. Enzymes are the key tools selected by the evolution to efficiently degrade and assimilate biomass, from storage organs (seeds, tubers, rhizomes) to structural organs with complex lignocellulosic composition. Historically enzymes have served as efficient biocatalysts for biomass. Processes involving fermentation such as brewing, baking and the production of alcohol have been known and used as controlled natural transformations since prehistoric times. From its discovery in 1887 by Kühne to its wide application today, enzyme catalysis has progressively been developed in the biomass processing, from food to non-food sectors.

In the 1960s the first enzyme preparation (amyloglucosidase) was produced, allowing the complete breakdown of the starch polymer into glucose. Greater yields, higher degree of purity and facilitated crystallization led to the rapid conversion of the glucose production from acid hydrolysis to enzymatic hydrolysis. Later, in the 1990s, enzyme technology was applied for the degradation of complex polysaccharides present in plant cell walls. This was done in order to improve the extraction of intracellular components in fruits (production of fruit juices) and seeds, involving enzymes such as pectinases, hemicellulases, and cellulases. Firsts applications in the non-food industry have been developed at the same time within the textile, paper and more recently biofuels industries (Rehm and Reed, 1998). Enzyme catalysis is as an alternative to conventional physical, physiochemical or chemical treatments. Because of the rapid development of biotechnologies it has been more accessible for large scale applications (Guzmán-Maldonado et al., 1995; Norman, 1981). It offers different advantages compared

with conventional treatments that can be underlined concerning the biomass hydrolysis. The better specificity limits the production of byproducts that can later be inhibitory for the process, while increasing the reaction yield. These reactions can be done under mild conditions, using non-toxic solvents which is particularly important in the food industry where products have to be edible and respect norms of food safety (Rosenthal et al., 1996). However, some limitations concerning biomass processing have to be cited. The main limitation is the reaction time that can extend to several days. Moreover, as highlighted in the 1.1 section, an optimal catalysis reaction requires diluted reaction conditions. Therefore the solid to liquid ratio is an important criterion since the enzyme, in its free form, needs a vector to move from a substrate to another. Increasing the solid concentration decreases the enzyme's mobility. It is observed in solid/liquid mixtures of insoluble substrates like lignocellulose, as well as with soluble substrate in high concentration that give highly viscous solution with diffusion limitation (Baks et al., 2008). In the case of insoluble lignocellulosic substrate, hydrolysis is limited by several factors. Particles size, available surface area, crystallinity of the cellulose, moisture content, lignin content and polymerization degree control the biomass accessibility (Hendriks and Zeeman, 2009). The use of one or several pretreatment methods is often required to improve the enzyme accessibility and therefore catalysis.

1.3. Twin-screw extrusion: thermomechanical pretreatment of biomass

Extrusion of biomass historically appeared in the food industry of the mid-1930s with the production of pasta and the cereal-processing industry. Kneading, cooking, forming and texturizing functions of the extruder are used in the cereals transformation. Extrusion also widely developed in the oilseed-processing industry because of the fractionation capacities of the machine (Harper, 1981). More recently, in the 1980s, extrusion process has been applied to lignocellulosic biomass degradation, for applications such as paper industry (De Choudens et al., 1989), components extraction (N'Diaye et al., 1996) or pretreatment for bioethanol production, as summarized by (Zheng and Rehmann, 2014). As detailed earlier, extrusion pretreatment presents unique advantages and flexibility. Biomass processing commonly uses the high shear, high pressure and temperature capabilities of the extruder, to physically deconstruct feedstock. With chemical resistant materials, it is also used for acid or alkali reactive treatments. These hard conditions that are enabled during the extrusion process aren't compatible with enzyme mild conditions. This is why it won't be detailed in this review, especially since Karunanithy Muthukumarappan (2013) already summarized the thermomechanical pretreatment of a large range of feedstocks. Among other advantages, it points out the good mixing capacities that removes the softened parts while exposing the interior surface to thermal and chemical action, thus improving the material deconstruction. And finally, it has to be underlined that the flexible and continuous aspects of the extruder allow to sequentially pretreat biomass while permitting, in a subsequent part of the machine, good conditions for enzyme catalysis, which is one of the main advantage of this technology (Vandenbossche et al., 2016).

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