Extrusion as a pretreatment for lignocellulosic biomass: Fundamentals and applications

Aleta Duque, Paloma Manzanares, Mercedes Ballesteros

Biofuels Unit, Energy Department-CIEMAT, Avda. Complutense, 40, 28040-Madrid, Spain

*Corresponding author: Aleta Duque. Phone: 00 34 91 3466054. E-mail: aleta.duque@ciemat.es

Abstract

Pretreatment of lignocellulosic biomass is an essential step to obtain sugars from such biomasses, aimed at breaking the recalcitrant structure of lignocellulose and facilitate the access of enzymatic hydrolytic agents to carbohydrates. Among the variety of pretreatment technologies that have been investigated in the past years, extrusion is a promising thermo-mechanical pretreatment. It is a continuous process, highly versatile, having good mixing and heat transfer capabilities and being able to operate at high solids loadings. However, its energetic and economic feasibility needs still to be evaluated before it can be an actual alternative to conventional biomass pretreatments.

With the aim to provide a further insight into extrusion pretreatment, this work reviews its fundamentals and the influence of the most relevant operation variables (screw profile and speed, residence time, temperature, liquid to solid ratio and downstream operations) on extrusion performance, analyzed according to the existing bibliography. To complete this overview, the effects of extrusion on lignocellulosic biomass structure, composition and enzymatic digestibility are studied, with a focus on the performance of catalyzed extrusion.

Keywords: lignocellulosic biomass, pretreatment, extrusion, operation variables, particle size, enzymatic digestibility

Abbreviations: EH – enzymatic hydrolysis; L/D – length to diameter ratio; L/S – liquid to solid ratio inside the extruder; LCB – lignocellulosic biorefineries; LB – lignocellulosic biomass; SEM – Scanning electron microscopy; SSA – Specific surface area

Introduction

Lignocellulosic biorefineries (LCB) success will be based on maximizing the value of complex biomass materials by extracting and valorizing all components while minimizing the processing needs.[1] LCB relies on an efficient separation or fractionation process (pretreatment) that results in the separation of main components (cellulose, hemicellulose and lignin), to be processed into biofuels and biochemicals. Carbohydrate fraction is mostly used for the production of fermentable carbohydrates by enzymatic conversion, resulting in a stream of fermentable sugars that can be sent directly to the production of biofuels, bioproducts and biochemicals. Lignin represents a valuable source of aromatic chemicals or can be used as a fuel in the lignocellulosic biorefinery.

Extensive literature on pretreatment technologies to fractionate lignocellulosic biomass (LB) into its main constituents has been published in the last years.[2-4] Commercial methods are based on the use of high pressure and temperature, with or without chemical agents. Extrusion, however, stands out as a technology that can effectively fractionate lignocellulosic biomass at mild temperature and lower chemicals, preventing the formation of inhibitory by-products occurring at more severe conditions.[5] Extrusion is a relatively novel pretreatment, in which the material is submitted to mixing, heating and shearing, resulting in physical and chemical modifications.[6] It is a continuous process characterized for its ability to provide high shear, rapid heat transfer, and efficient and fast mixing.[7] Furthermore, extrusion is able to work at high solid concentrations and it is a highly versatile pretreatment that can be used alone, or combined with both chemicals (reactive extrusion) and other pretreatment methods. It can be run at moderate temperature and pH conditions, which reduce sugars degradation and avoids the generation of toxics.[5]

As a consequence of these favorable features, an increasing number of research studies have been published, dealing with the effectiveness of extrusion to produce fermentable sugars from a wide range of lignocellulosic biomasses. The results vary depending on the particular conditions of the pretreatment and the biomass used, but in all cases there is an improvement of the sugar production yield from extruded materials (hereinafter, extrudates) compared to the raw material.

A look at the existing bibliography about LB extrusion shows that agricultural wastes and herbaceous biomasses such as corn stover,[8-11] are feedstocks mostly used for this pretreatment. These kind of herbaceous materials is usually preferred because they are softer and easier to extrude; however, extrusion of woody biomasses such as poplar,[12] hardwood,[13] Douglas fir and eucalyptus,[14,15] or pine wood chips,[16] have also resulted, under proper conditions, in substrates with enhanced enzymatic digestibility.

Although some research has been carried out about extrusion as sole pretreatment for lignocellulosic biomass,[8-11,17-20] there is a clear trend towards using extrusion in combination with other kinds of pretreatments. In fact, the efficiency of extrusion pretreatment and, therefore, its effect on the enzymatic digestibility, seems to be limited when no chemical agents are used.[21] This topic will be developed later on this paper under paragraph 4.

On the other hand, the important prospective shown by extrusion as LB pretreatment, has led to a number of processes having been patented for the treatment of lignocellulosic substrates, as can be seen in Table 1. First patents were issued in the late 80´ with the use of alkaline extrusion,[22] or extrusion in combination with other pretreatments such as steam-explosion[23] or acid hydrolysis.[24] From that time on, other process configurations involving extrusion have been patented, more intensively in the last 10 years.[25-32]

-----------------------------(Insert Table 1)---------------------------

All patents shown in the Table 1 claim advantages of the disclosed processes (all of them comprehending extrusion) compared to the reference pretreatments; reduced costs,[24-26] time[22,27,28] and heat consumption,[22,29] and production of substrates with increased hydrolysis efficiency[25,27,30,31] and less degradation of hemicelluloses.[24,33]

Coinciding with the increasing attention drawn by extrusion pretreatment, some reviews have recently appeared[33-35] and the present work aims at contributing to a more comprehensive study of extrusion pretreatment by covering all aspects of the technology. Thus, this paper aims at providing an insight on the fundamentals and operation of extrusion, analyzing the particular effect of each operation variable on the extrusion performance. In addition, the effects of this pretreatment on the biomass are analyzed based on results from abundant research effort carried out during the last years on numerous lignocellulosic substrates at different extrusion conditions.

1. Extrusion process

Extrusion is a technology that was first used for metal conformation, but soon expanded to many other applications in several fields such as ceramics, rubber, food processing, chemical, polymer, composites and energy industries. Extrusion is a thermo-mechanical process that is based on the action of one or two screws that spin into a tight barrel, which is equipped with temperature control (Figure 1). This action produces high shearing forces between the raw material, the screw and the barrel that lead to locally temperature and pressure rise along the extruder.

------------------(Insert Figure 1, 1.5 column)-----------------------------

The main distinction among extrusion machines is made between single-screw and twin-screw extruders. Single-screw extruders are made of one single solid piece, while twin-screw are comprised of small pieces called screw elements that are assembled in the shafts. In twin-screw extruders there are three main types of screw elements that can be used: kneading blocks, reverse screws and conveying screws (Figure 2). Additionally, twin-screw extruders can be classified as counter-rotating and co-rotating. The first development of a single screw extrusion machine dates back to the second half of the 19th century. Co-rotating twin-screw extruders started to be used in the 1940's and 50's,[36] leading to the licensing of the technology in 1957.[37] From this point, extruder design kept on evolving, incorporating new features such as the modular technology or the modelling of processes, that allowed to broaden the functionalities of the equipment to, for instance, evaporation, or reaction (from 1970's).[36]

-----------------(Insert Figure 2, single column)--------------------

-----------------(Insert Figure 3, single column)--------------------

As it can be seen in Figures 2 and 3, these screw elements have different forms, which produce different effects on the biomass; transport, mixing, shearing and combinations of the three that are responsible for the disintegration of the LB, the good flowability of the material and the complete mixing that characterize extrusion pretreatment. The arrangement of the screw elements is called the screw profile (or screw configuration) and it defines the position of each element, its characteristics in terms of pitch, stagger angle, and length, and how the different elements are spaced.[38] The screw profile is a very important factor in extrusion processing (its influence will be analyzed later in section 3.1), and affects significantly the transport of the material in twin-screw extruders.

The different types of extrusion machines differ in the way of mixing and in the flow patterns developed inside the barrels. Single-screw extruders produce mainly a distributive mixing in which the blending agent spreads through the lignocellulose matrix, obtaining a good spatial distribution. Moreover, in single-screw extruders, the forces that lead the conveyance of the material are exclusively frictional or viscous and the screw is made up of one piece. In contrast, full intermeshing twin-screw extruders produce both distributive and dispersive mixing, which involves the reduction in size of solid particles, meaning that there are changes on the physical properties of the material.[39] In co-rotating twin-screw extruders, the shearing and plasticizing effect is axial (the maximum velocity being achieved at the intermeshing zone), while in counter-rotating equipment, the effect is radial (highest velocity achieved at the screw tips).[40,41] There are references in the literature about studies that use single-screw extruders[7,17,42,43] and also counter-rotating twin-screw extruders for biomass pretreatment.[44] However, the counter-rotating twin-screw extruders are traditionally used for extrusion of polymers due to the higher pressure developed, since are considered more suitable for mixing and chemical reaction operations,[41] having also the advantage of being self-cleaning and leaving no dead zones between the screws.[45] The most common equipment for biomass pretreatment is the full-intermeshing co-rotating twin-screw extruder. The use of co-rotating twin-screw extruders has several advantages such as better heat and mass transfer capabilities, good control of residence time distribution and mixing.[21] Moreover, full-intermeshing co-rotating twin-screw extruders are able to operate at smoother conditions, while producing high shearing forces along the process. They are able to form dynamic plugs inside the barrels, which increase pressure, due to the combination of the different types of screw elements. The formation of such dynamic plugs is influenced by the flux properties of the particular biomass and the potential use of catalysts or viscosity modifying agents.

1.1. Energy considerations

The main energy source for extrusion is electricity. Concerning the energy consumption of extruders, they need two types of energy: thermal energy to reach and keep the operation temperature, and mechanical energy of the motor to rotate the screws.

Thermal heating is a big consumer of energy in LB extrusion processing and thus, it is crucial to make extrusion competitive with thermochemical processes by optimizing process temperature. In recent years, some authors have explored the application of LB extrusion pretreatments at low temperatures (<100ºC) with promising results,[21,46-49] which means less thermal energy consumed.

In relation to mechanical energy, a key parameter to understand extrusion is the torque. It can be defined as the energy required by the motor to rotate the screws, and it is closely related to process variables such as the screw speed, fill and viscosity of the material inside the extruder.[42] The torque is inversely proportional to the screw speed, meaning that the mechanical energy is lower at higher screw speeds. As every continuous process, a certain time is required in extrusion before a continuous regime is established. Torque values tend to peak quickly and fluctuate in an erratic way during the first moments of operation.[50] This can be attributed to the intermittent formation of discrete fiber plugs in the zones with functional screw elements (kneading blocks and reverse flux elements), until the steady-state is achieved.

The relationship between temperature (thermal energy) and torque is negative; higher temperatures lead to lower torque requirements, due to the thermal softening of biomass and decrease of viscosity.[42] The moisture level of the feeding (or liquid to solid ratio in the extruder) has also a great influence on torque. It is nearly a universal trend that the torque increases as the moisture decreases.[51]

Although the torque is a parameter adequate to measure the mechanical energy of an extruder, torque values vary greatly depending on the size of the equipment, the type of biomass and the extrusion conditions. For this reason, to facilitate the comparison among the different pretreatments, a new parameter that considers the throughput of the process is used: the specific mechanical energy factor (SME). The SME measures the mechanical energy consumption in the extrusion treatment by estimating the mechanical energy required to produce a unit weight of pretreated biomass in a particular extrusion machine. It can be calculated through the following expression:[52]

$$\mathrm{SME\ (Wh/kg)} = 3{,}6 \times \frac{\left(\frac{\tau(\%) - \tau_0(\%)}{100}\right) \times \frac{\mathrm{S_S(Hz)}}{\mathrm{S_{rated}(Hz)}} \times \mathrm{P_{rated}(W)}}{\mathrm{Q\ (kg/h)}}$$

Where τ is the operating torque, τ_0 is the no load torque, S_S is the operating screw speed, S_{rated} the maximum screw speed, P_{rated} is the rated motor power, and Q is the total mass flow.

From the expression above, it can be inferred that achieving a high throughput of pretreated material is crucial to keep the energy consumption of the extruder at low values.

To summarize, extrusion pretreatment offers the possibility of reducing its energy consumption by working at low temperatures, which means less thermal energy consumed, and by setting process conditions that lead to low values of torque and SME, to minimize the mechanical energy spent.

1.2. Rheological considerations

Extrusion pretreatment is capable to operate at high-solids loadings. Since extruders are equipped with specialized mixing sections that permit a good material and energy transfer, it is possible to obtain the desired solids concentration at the output, which will determine the liquid and solid flows inside the extruder and the screw profile and energy consumption. This potentially offers many advantages including increased sugar concentrations and decreased production and capital cost, since extrusion allows overcoming the typical mixing and transferring phenomena problems at high solid concentrations.

As a continuous process, it is essential to achieve a proper flow of the material inside the extruder. Rheological behavior of lignocellulosic biomass is highly dependent on the type of feedstock and composition, due to the nature of the materials treated and the conveying mechanism inside the extruder. If the system is not correctly designed and operated, blockages, lack of homogeneity in the product and flow interruptions can occur.

According to Senturk-Ozer et al.,[13] extrusion pretreatment of high aqueous biomass suspensions suffer from a lack of flowability that leads to low pretreatment rates, low biomass/liquid flow ratios and, in some cases, high temperature peaks. These authors proposed to add a gelation agent to improve the elasticity of the biomass suspension; however, this could also reduce the effective shear stress transmitted to the material. In reactive extrusion, the catalyst itself may act as a flux modifier, as is the case of alkaline extrusion.

In summary, it is important to highlight the key ability of extrusion to operate at high-solids loadings (≥15%), unlike many other pretreatments that show important limitations in this sense. However, to achieve a good flow inside the extruder, it is necessary to count on the presence of a fluid (water, chemical catalysts, or a specific gelation agent) that aids to the processing of LB. The fluid type will depend on the characteristics of the process.

2. Variables affecting the extrusion

Many variables affect the extrusion performance such as the screw profile, the screw speed and the temperature of extrusion.[34] Other important parameters are the torque,[42,50] the liquid to solid ratio inside the extruder,[17,53,54] the global pretreatment configuration[7] and the possible use of a catalyst. Most of these variables are inter-related, and usually more than one factor is involved, so the actual contribution of each variable to the total effect is not easy to distinguish.

An analysis of the main variables influencing the performance of extrusion is shown below, based on the references in the scientific literature.

2.1. Screw profile, screw speed and residence time

Screw profile is set by the combination of the different screw elements described in point 2 (Figure 2) and it is one of the most important factors during extrusion processing. The screw profile not only influences the residence time distribution and the mechanical forces developed inside the extruder, but it is also a factor to take into account for setting other operation variables such as the L/S ratio and the feedstock particle size. However, as there is a very high potential number of elements combinations, usually optimization of operation variables is performed using a fixed screw profile. In this regard, Shah and Gupta[41] point out that three-dimensional flow simulation software is a valuable tool to design twin-screw extruder's profile. It is known that the basic single screw or the forward conveying elements in twin-screw extruders are not enough to achieve a good mixing effect, and other types of elements need to be included in the screw profile.[45] Senturk-Ozer et al.[13] extruded a mixture of hardwoods in a configuration with three mixing zones that combined right- and left-handed fully-flighted screw elements and kneading disks. The second mixing zone, containing kneading disks staggered in the forward, neutral and reverse directions, was the toughest. They found that the effects on the biomass were greater within the second mixing zone, precisely at the reverse kneading block, due to the higher pressure and stresses developed by this kind of elements. Thus, the presence of reverse elements is very important to the fibrillation of lignocellulosic biomass.

Supporting this idea, Choi et al.[55] compared different screw profiles in a dilute sulfuric acid extrusion pretreatment of rape straw. It was shown that the configuration that alternates mixing and reverse elements along the extruder was the more effective in concentrating the cellulose and also in increasing enzymatic digestibility of the pretreated rape straw.

Furthermore, Zheng et al.[46] studied extrusion followed by NaOH treatment of sweet corn corncobs and the influence of replacing a conveying element by a kneading or reverse element on various features of the pretreated substrates. The authors concluded that extrusion treatment with functional screw elements affected lignin, so it was easier to remove it with a subsequent chemical treatment.

Recently, Kuster Moro et al.[56] found that the insertion of a reverse element into the screw configuration increased the residence time in the extruder up to 2.5 times and it greatly enhanced the effects of the pretreatment in sugarcane bagasse and straw in terms of enzymatic digestibility and crystallinity reduction. The position and space of reverse elements were also demonstrated to have a great influence on the filtration performed inside an extrusion machine and the sugar recovery in the liquid fraction.[57]

Thus, it can be concluded that the use of functional screw elements in a screw profile is essential to achieve a good fiber destructuration and to produce chemical changes in the pretreated biomass. In Table 2 a compilation of conditions, analyzed parameters, and main results of the above mentioned research work is shown to summarize the main findings in relation to the effect of screw profile on extrusion of different biomasses.

In which concerns the screw speed, a direct consequence of varying it is the change of the residence time of the substrate inside the extruder. As a general rule, at fixed input flows, the mean residence time decreases with increasing screw speeds. Most of the studies about extrusion pretreatment use relative low screw speed for the experimentation (≤ 2.5 Hz), seeking to increase the short residence time in this kind of equipment, as it can be seen in Table 2. In extruders used at research level, the residence time varied within a wide range, from 1 min to 10 min.[12,17,47,54]

Authors such as Karunanithy and Muthukumarappan[7], Zhang et al.[9] or Yoo et al. [51] have reported that the increase of the screw speed has a positive effect on the enzymatic digestibility of extrudates up to a certain point that, when overcome, results in lower enzymatic hydrolysis (EH) yields. Optimum screw speed of 1.33 Hz for corn stover[9] and 5.8 Hz for soybean hulls[51] extruded in a twin-screw extruder, and 1.66 Hz for switchgrass pretreated in a single-screw extruder have been reported.[7] In fact, the effect of screw speed on fiber deconstruction is determined by two combined effects. One is the variation of the residence time (shorter as the screw speed increases) and the other is the influence on the mechanical energy exerted on the material (the torque is higher at lower speeds).[34] It is not clear in the literature the reason of the decrease in enzymatic hydrolysis at high screw speeds. Zhang et al.[9] attributed the decrease of the yields to extremely reduced residence time at screw speeds over 1.33 Hz, while Karunanithy and Muthukumarappan[7] claimed that it is due to the lignin behavior. Nevertheless, it has been also reported in the literature that the influence of screw speed on the efficiency of the pretreatment, measured as the sugar yield after EH, depends on other variables such as the specific feedstock,[7] the barrel temperature,[52] and/or the use or not of a catalyst.[9,58] Another factor that conditions both the screw speed and the residence time is motor power and extruder size and the geometry of the machine, i.e. length/diameter ratio (L/D) and. L/D ratio is an indication of the treatment capacity of the extruder, and, at the same screw speed, a higher L/D value will result in a longer residence time. Because of all these interactions, it is difficult to distinguish the singular behavior of each variable on the performance of the pretreatment.

---------------------(Insert Table 2)-----------------------------------

2.2. *Temperature*

Temperature is an essential variable of extrusion pretreatment and values can typically fluctuate between 40 and 200ºC. It has usually a positive impact on the enzymatic digestibility of the extrudates,[16] and sometimes can even overcome the magnitude of the effect of screw speed as found by Karunanithy and Muthukumarappan in switchgrass.[17] However, for some biomasses and operation conditions, a temperature limit exists, from which the barrel temperature negatively affects the sugar yield.[7,47,59] This limitation could also be related to the recondensation of lignin at high temperatures. Under certain conditions (140ºC temperature, 11.1 and 16.6 % solids load, 0.25 Hz screw speed, use of an ionic liquid as a catalyst and two extrusion passes), it was observed that high temperature can even cause partial charring of the biomass.[59]

In general, barrel temperature promotes the plasticizing of the substrates[54] and decreases the viscosity inside the extruder, which affects the flowability of the suspension and reduces the residence time inside the extruder. However, Yoo et al.[51] observed that some lignocellulosic materials do not melt even at high temperatures, which causes poor flow inside the extruder. Thus, these differences prove the significance that the type of LB has on the effect produced by temperature on extrusion.

The temperature of the extrusion is also influenced by the use or not of a catalyst. Generally, in non-catalyzed extrusion the temperatures are higher,[8,9,11,16] compared to catalyzed extrusion. Acid extrusion[12,53,54] is usually carried out at higher temperatures than alkaline extrusion.[18,47,48]

Table 3 summarizes the feedstocks, conditions, analyzed parameters and results of some of the case studies referenced in this paragraph. As it can be seen, the temperature has a general positive effect on the release of sugars from LB, but above a certain point (that depends on the type of LB and the catalyst employed), the temperature causes a negative impact on the enzymatic digestibility of the extrudates. On the other hand, it can be stated that because the temperature factor is closely related to the energy demand of the extruder, the use of lower temperatures would be favorable from the energy point of view. Another reason to limit the temperature of extrusion pretreatment is to avoid the excessive degradation of lignin and hemicellulose-derived sugars, especially at low pH conditions.

----------------------------(Insert Table 3)---------------------------------

2.3. Liquid to solid ratio (L/S)

The liquid to solid ratio inside the extruder (L/S) is calculated as the total amount of liquid added to the process, considering also the contribution of the moisture of the raw material, divided by the biomass flow in dry weight basis. In the cases where no additional liquid is introduced in the extrusion trial, this concept refers to moisture. The L/S parameter is essential to achieve a proper flow inside the extruder and always a minimum amount of liquid (or moisture) must be introduced to avoid excessive wearing of the equipment. Moreover, it promotes the softening of cellulose and allows the shearing forces to break the microfibrils bound. For every process configuration, there is a maximum L/S allowable in the extruder, which depends on screw profile, temperature, or flow properties of the suspension. Overcoming this limit would result in bad performance of the equipment or excessive dilution of the final products.[60]

In non-catalyzed extrusion, the feedstock moisture influences the thermal softening of the material and the rate of shear development.[7] In such processes, low moisture means higher friction forces and thus, greater effect on the biomass, as confirmed by Senturk-Ozer et al. on hardwood.[13] The inverse relationship between moisture and torque was also proved by Yoo et al.[51] in soybean hulls at moisture contents between 40 and 50%. On the other hand, an excess of moisture may form thin slurry, which reduces the effectiveness of the pretreatment, as proved by Lin et al.[61] in experiments with wet ball milling of corn stover.

In reactive extrusion processes, it is more difficult to differentiate the actual influence of the L/S ratio, since it is linked to the amount of catalyst added. In these cases, higher L/S ratios usually lead to a higher effectiveness of the combined extrusion plus catalyst pretreatment.[5,62,59] Thus, L/S values tend to be higher in reactive extrusion pretreatments compared to only extrusion processes, as it can be seen in Table 4, which summarizes the experimental conditions and findings of the research works above referenced.

In conclusion, L/S ratio in extrusion is delimited by a minimum and maximum value that ensures the proper flow and treatment of the LB. These limits vary for each process depending on the characteristics of the feedstock, the extruder device and the other operation conditions. Within the range of allowable L/S ratios, lower values will be preferred, since they lead to higher shearing stresses, taking into account also the influence of the amount of catalyst, if that is the case.

-------------------------(Insert Table 4)-----------------------------------

2.4. *Downstream operations*

In reactive extrusion, the catalysts and chemicals (NaOH, acids, ionic liquids, organic solvents) used can affect the pH, and can also affect downstream operations. In fact, a common concern in reactive extrusion is the cost of the catalyst and the amount of water used to remove it. In this regard, Cha et al.[63] reported the use of an screw-type separator placed after a twin-screw extruder to wash and separate the black liquor from the solids, and so allow for the recycling of the catalyst. They achieved almost 70% saving in NaOH after recycling twice the black liquor although, on the other hand, it resulted in lower pretreatment efficiencies.

Usually, the residual catalyst that is left in the substrate after the pretreatment must be somehow removed, and/or the pH must be adjusted before submitting the extrudate to EH. A common practice among the different studies found in the scientific literature is to wash the extrudate with distillate water after the extrusion. This step is fully integrated into the experimental procedures and, although washing step is relevant for the enzymatic hydrolysis step, only few researchers have investigated its impact on the enzymatic digestibility.

For instance, Lamsal et al.,[52] extruded wheat bran and soybean hulls in different ways: only with water; previously impregnating the biomass with a mixture of sodium hydroxide, urea and thiourea; or applying a post-treatment with $CaCl_2$. They washed the pretreated substrates and compared their enzymatic digestibility to that of the unwashed extudates. The washed materials showed higher reducing sugars yield in all cases, regardless if they have been submitted to additional chemical treatment or not. The authors hypothesized that washing would remove the possible chemical residues and enzyme inhibitors produced by extrusion.

Furthermore, Zhang et al.[58] compared the enzymatic digestibility of extruded corn stover previously soaked in different alkali concentrations solutions, with and without washing after extrusion. They found that washing was somehow negative for low alkali concentrations (1 and 3%), but it improved greatly the glucose and xylose yields at 5 and 14% NaOH. Another result was that the sugars yield of unwashed samples did not vary significantly with the increasing alkali concentrations, while washing after extrusion allowed to attain higher yields (from 5%NaOH) and to see clear differences among the alkali concentrations proved.

On the other hand, if the catalyst concentration is low enough to make the pH of the resulting extrudate compatible with enzymes requirements, Karunanithy and Muthukumarappan[18] defend that it is unnecessary to wash the extrudates, considering also that during the washing step a certain solid and sugar loss can take place (6-7% in their case), which is an unwanted consequence. In spite of this, the advantages of washing on the enzymatic digestibility of extrudates have been sufficiently proved to incorporate this operation to the process.

------------------------(Insert Figure 4, 1.5 column)--------------------

In an step ahead in the integration of downstream operations in extrusion, Duque et al.[5] have successfully proven the possibility of performing the pretreatment and every conditioning operation (neutralizing, washing) in the same equipment (Figure 4), producing therefore a substrate suitable for its direct incubation with enzymes or microorganisms for sugar production. This high level of integration, together with the wide range of different process configurations that can be designed based on extrusion, adds an evident value to this technology as a LB pretreatment method.

In summary, concerning the downstream operations, washing is necessary in many extrusion processes. Some solutions have been proposed concerning the integration of this operation in the same machine and the possible re-use of the catalyst.

3. Effects of extrusion pretreatment on biomass

In general, since extrusion is essentially a mechanical pretreatment, its action on the biomass can be assimilated to that kind of treatments. Thus, studies performing extrusion report on a reduction of the particle size distribution, increase of the specific surface area (SSA), changes in the crystallinity, and visible changes in the biomass structure, which can also be confirmed by Scanning Electron Microscopy (SEM) images. When extrusion is combined with other pretreatments or chemicals, similar effects may be observed, although it is sometimes difficult to distinguish the actual contribution of extrusion to the global effect.

Chen et al.[64] identified devillication of cellulose fibers of rice straw as the unique feature of extrusion that distinguishes this process from chemical, biological, and even other mechanical treatments such as milling, thermal and hydrothermal pretreatments. They claim that the extrusion mechanism is not due to changes in the chemical compositions and structures of the LB, but to significant physical modifications (including particle size distribution, water holding capacity, specific porosity and SSA) and devillication of fibers.

To characterize the extension of the changes on the biomass caused by extrusion, it can be useful to consider the classification of the mechanical effects on biomass as Class I and Class II biomass refining reported by Leu and Zhu.[65] Class I refining is more superficial, as it increases the fiber external surface by cutting, breaking, separating the fiber bundles and mildly fibrillating the surface, while Class II refining causes total cell wall disruption, reducing the size of fibers to micro or nanoscale, breaking the cross-linked micro-fibrils up, and thus, inducing the internal fibrillation of the material. In extrusion pretreatment, the extent of such physical effects depend on the type of biomass, if the biomass is previously pretreated or not, and the equipment used.[10]

An extended analysis of the principal effects reported for pretreatments involving extrusion of lignocellulosic biomass, based on the existing bibliography is given below.

3.1. Particle size reduction and increase of the surface area

The particle size reduction is one of the main effects of extrusion pretreatment, and it is due, in a major part, to the shearing forces exerted in the biomass fibers by the rotating screws. Process parameters such as the barrel temperature and the liquid to solid ratio inside the extruder (L/S) influence the extent of size reduction, as proved by Um et al.[66], working on rape straw pretreated with hot water inside a twin-screw extruder. At higher temperature and lower L/S ratio, greater particle size reduction and finer skewness of the particle size distribution are found. This relationship between low moisture and size reduction was also demonstrated by Chen et al.[64] on rice straw at moistures ranging from 10 to 80%. Furthermore, screw configuration used in the extrusion process has been reported to be related to the extent of the particle size reduction, especially when using kneading disks arranged in reverse or forward direction resulting in high stress on the fibers (Table 2).

Also in relation to particle size reduction in extrusion, it is important to consider the influence of the use of catalysts (reactive extrusion). For instance, Chen et al.[53] used rice straw with a particle size ≤ 1 mm and found particles size between 0.4 and 0.5 mm only with extrusion pretreatment. When they used a combination of extrusion and aqueous extraction, the authors achieved a smaller mean particle size, between 0.09 and 0.3 mm. This fact illustrates the positive effect of combining extrusion with other pretreatments on the particle size of the pretreated substrate. The observation was corroborated by Um et al.,[66] who obtained a further reduction of the particle size when they added an acid or alkaline catalyst to the extrusion, instead of hot water.

Likewise, Duque et al.[67] found a different particle size reduction when they submitted barley straw (5 mm) to NaOH-extrusion and, afterwards, to extrusion with enzymes. As it is shown in Figure 5, after the first extrusion with alkali, the proportion of particles between 3.14 and 0.52 mm increased from 28 to 54%, and the mean particle size was also slightly reduced (from 2.5 to 2.2 mm). The effect of second extrusion pass with enzymes was greater. The particles above 3.14 mm almost disappeared, and the proportion of particles under 0.52 mm increased up to 37%. A new fraction of particles under 0.063 mm was found and the mean particle size dramatically decreased to 0.6 mm.

----------------------(Insert Figure 5, one column)----------------------

In general, the available surface area is closely related to the particle size mentioned above and to the volume of the pores generated on the substrates by the effect of the pretreatment. The increase of the surface area can be due to physical changes in the fiber as a consequence of the mechanical effects (reduction of particle size, fibrillation, increase of pore size), or by chemical action on the lignocellulose components. In the last case, either the hemicelluloses or the lignin that wrap the cellulose fibers are affected or removed to clear the path for the enzymes to the cellulose.

Extrusion has been reported to cause an increase in the surface area and fibrillation of the biomass.[9,14,15,17,53] In biomass feedstocks such as giant reed and wheat straw, Yan et al.[49] described an increase of the surface area, measured by dye absorption, 16 times higher than untreated material for giant reed, and 3.3 times higher for wheat straw, using a screw press to pretreat them. The authors attribute the larger surface are to the shear and pressure forces exerted during extrusion that cause cellulose structure disruption and thus increased accessible surface area. Concerning the fibrillation effect, evidence is provided by Lee et al.,[15] who observed that most of the fibers had been fibrillated to a sub-micron scale (< 5 µm) and some of them had diameters less than 100 nm, when woody biomass previously pretreated by hot-compressed water was submitted to extrusion.

Images taken by SEM of reed straw submitted to screw press pretreatment showed higher disintegration of particles, and fiber bundles compressed, twisted, and partly separated from the initial connected structure, compared to the same biomass treated with a cutting mill.[49] Liu et al.[68] observed similar mechanical effects (defibration, delamination, and increase of the porosity) on alkali-extruded corn stover, effects that were more pronounced as the NaOH loading increased. From their observations, these authors classified the structural changes as mainly Class I size reduction, with a small proportion of Class II.

As in other effects above discussed, the addition of a catalyst during extrusion may influence the variations found in surface area and pore size. It has been reported that in acid or hot-water catalyzed extrusion, where the main effect is the partial hydrolysis of the hemicellulose chains, an increase in the number and size of pores occurs, making it easier for the enzymes to attack them.[12,15,54,69] Likewise, an extrusion catalyzed by alkali, based on the modification and removal of lignin, results in a similar effect as reported by several authors.[9,48,52,68,70,71]

From the discussion above, it can be stated that extrusion is a pretreatment that results in an increase of the surface area and effective fibrillation of lignocellulosic biomass, thanks to the mechanical forces developed inside the machine and the use of catalysts, in the case of reactive extrusion.

3.2. Biomass composition

In absence of a filtration step embedded into the process configuration, extrusion is essentially a dry to dry process, which means that there are no material losses and composition of the biomass does not vary.[21,56,72,73] This fact could be an advantage, since the pretreated substrate would contain all the carbohydrates initially present in the raw biomass, and so the virtual amount available for sugar production will be the maximum. On the other hand, in reactive extrusion, the catalyst is usually able to solubilise part of the biomass, resulting in composition changes in the extrudates. The values vary according to the severity of the pretreatment and the catalyst used. Alkaline pretreatments lead to solubilisation of hemicelluloses and partial delignification, depending on the concentration of the catalyst.[47] On the other hand, acids solubilise mainly the hemicelluloses, and affect the cellulose in harsh conditions.[12] Accordingly, several authors have reported delignification and hemicellulose losses,[21,48,68,70,74] and increased concentration of cellulose in extrudates, compared to raw material.[46,47,54,75]

Besides changes in biomass components ratio, some studies also indicate that extrusion could substantially affect the lignin fraction of biomass, especially when high temperatures and/or shearing forces are exerted on the biomass.[9,16] According to Zheng et al.,[46] lignin can be highly affected by the heat, compression and shear in the extruder, causing lignin condensation or pseudolignin complex formation, so influencing the enzymatic digestibility. Although the temperature used in this work to extrudate corncobs is only 75ºC, the authors hypothesize that the presence of a reverse screw in the profile generates high shear forces that lead to local high temperatures resulting in lignin relocalization.

The changes in the physicochemical characteristics of main components as those in lignin above reported, as well as variations in composition due to components removal during extrusion significantly influence the accessibility of the enzymes to the carbohydrates, which is discussed in more detail in the next paragraph.

3.3. Enzymatic digestibility

From the results of extrusion process applied to different lignocellulosic biomasses, it can be stated that, in general, extrusion leads to an increase of the enzymatic digestibility of the extrudate in comparison to raw material. Data from literature regarding enzymatic digestibility after extrusion are difficult to compare since the expressions by which yields are calculated can be different, depending on the basis it is calculated (raw material, extrudate, weight of one of the components). Thus, only values comparing extruded and raw biomass digestibility are given in this review.

Extrusion has demonstrated to favorably compare to conventional acid and alkaline pretreatments in terms of hydrolysis efficiency. An example can be found in the work by Yoo et al.,[51] who compared the saccharification yield of raw extruded soybean hulls with the material extruded and pretreated with acid and alkali. According to their results, extrusion was as effective as alkaline pretreatment and superior to acid pretreatment, leading to 132% improvement in the saccharification yield over the raw material. In a later study by the same authors, Yoo et al.[20] optimized the moisture of the feedstock and the screw speed of the extrusion process and obtained up to 155% increase in glucose yield of extruded soybean hulls as compared to the control. The enhancement of the enzymatic digestibility by extrusion is achieved by a combination of all the effects previously described; particle size reduction, increase of the superficial area, the number of pores, and changes in biomass composition Supporting this view, Karunanithy and Muthukumarappan[8] found glucose, xylose and combined sugar yields 2.0, 1.7 and 2.0 times higher in extruded corn stover in comparison to untreated biomass and attributed the increase to the disturbance of the cell wall produced by the friction inside the extruder. Zhang et al.[9] declared sugars yields 2.2, 6.6 and 2.6 times higher for glucose, xylose and combined sugars, respectively, from extruded corn stover, and associated these improvements to a greater surface area.

Moreover, when a catalyst is added in the extrusion, combined effects are introduced and so, trying to distinguish the actual contribution of each effect to the enzymatic digestibility can become challenging. Um et al.[66] analyzed the weight of each of element (physical and chemical) by studying the enzymatic digestibility of rape straw, raw and pretreated by extrusion, with hot water, NaOH or H_2SO_4. They measured the increase in the cellulose conversion and quantified the individual contributions of the separate factors. Particle size reduction had the main impact on enzymatic digestibility, which was approximately double the value from untreated rape straw. Next, the chemical effect was especially important in the process with sulfuric acid. Finally, the reduction of the crystallinity had only a small impact. Overall, the enzymatic digestibility was improved between 2 and 3 times over the untreated biomass. Duque et al.[47] have reported significant effect of alkali addition on barley straw enzymatic digestibility. The authors observed that the amount of catalyst added significantly affected the enzymatic digestibility of extrudates, obtaining glucan and xylan saccharification efficiencies 5 and 9 times over that of the raw biomass, respectively. For wheat straw the enzymatic digestibility of glucan increased 13 times and 11 times that of xylan, working in similar conditions.[76] During alkaline extrusion, the extrusion action would be enhanced by the chemical agent, which is able to partially solubilise hemicellulose and lignin, as well as produce a swelling of the biomass fibers, so promoting substrate digestibility.

An interesting view is to consider the existence of a synergism in the mechanical and chemical actions occurring during reactive extrusion. This idea was already contemplated in 1987 by Hemling et al.,[77] who claimed a synergistic effect between chemical and mechanical forces by using an extruder to treat wheat straw previously impregnated with alkaline hydrogen peroxide. Much later Razumovski et al.[78] studied the joint action of catalysts as NaOH, succinic acid or $CoSO_4$ and extrusion on birch wood and compared with the material submitted to pretreatments separately. The authors demonstrated that the combined action of additives and mechanical forces lead to synergic effects shown by an increase of water-soluble fractions and appearance of low-molecular weight oligosaccharides that could not be observed by pretreating birch wood separately by extrusion or by chemical catalysts. Advantages such as reduced working temperature (relative to thermohydrolysis) and prevention of side products are claimed in relation to synergistic effect in reactive extrusion.

On the other hand, the combination of different pretreatments with extrusion performed in a consecutive way has been shown to result in an improvement of the enzymatic digestibility, compared to each pretreatment alone. For example, Lee et al.[15] found that extrusion increased the glucose yield by enzymatic saccharification after a hot-compressed water (autohydrolysis) pretreatment of softwood and hardwood. This effect was especially significant in the case of Douglas fir, in which only mechanical kneading with water had been previously found to produce a limited improvement on the enzymatic digestibility of the substrate.[44] It was proven that the partial removing of hemicellulose and lignin due to the autohydrolysis treatment allowed a finer fibrillation of the biomass during extrusion and enhanced its enzymatic digestibility. Furthermore, mechanical refining through twin-screw extrusion (among other methods) has proved its potential as post-treatment for improving the enzymatic digestibility of corn stover pretreated by acid steam explosion at low severity conditions, getting closer or even reaching the values of enzymatic digestibility obtained at more severe pretreatment conditions.[10] The enhancement is believed to be due to Class II refining of the biomass. When extrusion was followed by a secondary pretreatment such as ozonation, Karunanithy et al.[79] found that it allowed to greatly decrease the ozone consumption and ozonation time compared to ozone pretreatment alone, since the previous extrusion had already opened up the LB structure. The enzymatic digestibility was increased around 1.6-fold by the combination of pretreatments compared to extrusion alone. In a paper by the same authors[80], the combination of extrusion plus microwave pretreatment was not as effective, but they achieved anyway an increased enzymatic digestibility of about 1.2-fold over the extruded only LB.

Extrusion has been proven, thus, to be an effective pretreatment to enhance the enzymatic digestibility of LB, resulting in higher enzymatic hydrolysis yields than those of the raw materials, effect that increases when talking about reactive extrusion. Moreover, extrusion has been demonstrated to perform well in combination with other pretreatments, allowing achieving highly digestible substrates even when the severity of the secondary pretreatment is decreased. Although the mechanisms that cause this positive effect of extrusion in enzymatic digestibility are not completely clear, from the discussion above it can be concluded that physical changes such as disruption of fibers (fibrillation), particle size reduction, and increase of SSA are behind the increased enzymes accessibility. In the case of reactive extrusion, catalyst addition can provoke partial removing of hemicellulose and lignin, so facilitating the action of enzymes. Particularly for alkaline extrusion, the authors[47] have already reported that alkaline agent enhances the action of extrusion by degradation of ester bonds and cleavage of glycosidic linkages in the cell wall matrix, as well as it causes the swelling of the fibers.

3.4. Production of sugar degradation and other toxic compounds

One of the relevant advantages of extrusion pretreatment over other technologies is that it can be carried out at moderate operation conditions of temperature and pressure, which prevents the formation of potential fermentation inhibitors.[70] These toxic compounds include degradation and oxidation products of lignin and oligomeric sugars, due to severe pH and temperature conditions in other conventional hydrothermal conditions.[81]

Negro et al.[82] could detect neither furfural nor HMF in filtrates from alkaline extrusion of olive tree pruning at 70, 90 and 110ºC. The same applies to the extrusion of switchgrass and prairie cord grass,[83] pine wood chips[16] and alkali-impregnated big bluestern,[18] at temperatures between 50 and 180ºC. Furfural and HMF were found at hardly detectable levels (under 0.05 g/l of furfural and under 0.023 g/l of HMF) after extrusion of soybean hulls at 80ºC.[20] On the other hand, slightly higher concentrations of these degradation compounds were detected in experiments carried out with acid and extrusion, as reported by Chen et al.,[53] who found less than 1 g/l furfural after extruding rice straw with dilute sulfuric acid.

In contrast, the concentrations of acetic acid in extruded materials, produced by the hydrolysis of acetyl groups of hemicelluloses, tend to be greater, especially in alkaline extrusion. In different studies with extruded biomasses, Karunanithy and Muthukumarappan found acetic acid concentrations between 0.02 and 0.132 g/l.[8,83] Working with dilute sulfuric acid as catalyst, Chen et al.[53] detected acetic acid in a concentration about 3.7-5.9 g/l after acid extrusion of rice straw. Furthermore, in experiments with NaOH-extrusion and barley straw, the concentration of acetic acid in filtrate was around 4 g/l, meaning a deacetylation about 95% based on the content of acetyl groups of raw barley straw.[5]

The combination of extrusion with other pretreatments has been shown to have a positive effect on inhibitors production. For instance, steam explosion combined with extrusion[84] could be carried out at lower temperature and acid concentration than conventional steam explosion, and the amount of fermentation inhibitors were therefore lower. The amount of formic acid, acetic acid, furfural and HMF was greatly reduced when a combination of pretreatments was applied, compared to just acid-catalyzed steam explosion.

This low production of inhibitory compounds is one of the key advantages of extrusion pretreatment, since it avoids the necessity of costly detoxification post-treatments and facilitates the conversion of the pretreated substrates by biological agents.

4. Process configurations

Although in the discussion carried out throughout this work, several examples of combination of pretreatments with extrusion have been described, this section aims to summarize the different configurations of extrusion with other technologies and give some particular examples. Such combination of extrusion and other treatment methods can be made in several ways:

i. Sequence of treatments, by applying a pretreatment to raw biomass before extrusion, or submitting the extrudate to a post-treatment. The other treatment can be a hydrothermal, physical or chemical one.
ii. Reactive extrusion, by introducing a chemical or biological catalyst inside the extruder.
iii. Combination of technologies in one reactor by specially modified extrusion machines to combine various pretreatments.

Examples of cases of study, chosen from the recent literature to illustrate the above mentioned process configurations involving extrusion pretreatment, are presented in Table 5.

-------------------------(Insert Table 5)-------------------------------

The process configuration can refer to a multiple pretreatment step, in which extrusion is only one step of the existing operations. In this research line, Cha et al.[21] integrated an extrusion equipment in a bigger pretreatment system for *Miscanthus sacchariflorus*, in which extrusion is used as first step followed by chemical soaking in NaOH in a continuous stirred tank and further incubation in a single screw reactor. The advantages of this system are shorter incubation time, higher biomass loading, higher sugar yield and relatively low temperature reaction. The initial extrusion step is believed to prepare the substrate for a more efficiently pretreatment afterwards. Extrusion has been also successfully coupled to steam-explosion by Chen et al.[84] and this combined process has been even object of patent.[32] In these kinds of processes, the main advantages that the extrusion reactor provides are the continuous operation, and the high pressures and shearing forces developed inside the machine.

The examples presented in Table 5 show the large variety of process configuration that can be applied to extrusion and the main proven effects on biomass composition, physical characteristics and enzymatic digestibility of pretreated LB. Our aim here is to give a glimpse on what can be the future of the technology applied to the pretreatment of LB.

5. Conclusions

In this paper, a comprehensive view about the extrusion technology applied to the pretreatment of lignocellulosic biomass is presented. Co-rotating twin-screw extruders seem to be the more adequate equipment type to pretreat lignocellulosic biomass and they have good perspectives to continue developing new biomass deconstruction pretreatments, or to be integrated within a multiple pretreatment process. Functional screw elements (kneading blocks and reverse elements) need to be included in the screw profile to take fully advantage of the mixing and fiber destructuration capabilities of this type of extruders.

The extrusion operation depends on several variables, many of which are inter-related, so it is not easy to determine the individual influence of each one. Due to this complexity, usually some of the variables are fixed, according to values obtained from previous experimentation or by setting operative limits, while the others vary to optimize the operation of the equipment. As a general rule, low energy extrusion processes can be achieved by keeping a low working temperature and using a relatively low screw speed.

In addition, to achieve a proper flow of the material, it is essential to attain a certain L/S ratio inside the extruder by adding water, a chemical catalyst or any agent that aids to the fluidification of the biomass suspension. This L/S ratio has to be optimized for each process, but it must be kept as low as possible in order to favor the development of high shearing forces inside the equipment.

The need for washing the extrudates after the pretreatment and the way in which it is performed has to be evaluated depending on each particular case. Anyway, minimizing the amount of washing water and catalyst used is a priority that will result in a more cost-effective and environmentally sustainable pretreatment.

On the one hand, as a mechanical treatment, extrusion causes physical changes such as particle size reduction, fibrillation and increase of the surface area in the LB substrates through the application of high shearing forces. Also, when reactive extrusion is used, chemical effects on LB components occur, like hemicellulose and/or lignin solubilisation. As a result of the combined mechanical and chemical effects, LB structure and components are altered and the susceptibility of the extrudate to the enzymatic attack is significantly enhanced in comparison to raw material. Furthermore, a particular feature of extrusion is the low production of inhibitory compounds, which is a key advantage in the ethanol production process by enzymatic hydrolysis and fermentation.

The overview presented in this paper is considered only a first approach to the extrusion operation that reflects some of the possibilities of this technology for the pretreatment of lignocellulosic biomass. The effective mixing that extruders are able to provide, even at high solids content, and the possibility to work at relatively low temperatures are two important advantages of this pretreatment. Moreover, the results reported in the scientific literature in terms of structural changes on the LB and improvement of the enzymatic digestibility are encouraging enough to support the interest arisen by extrusion pretreatment. The high versatility of extrusion is one of the strengths of this technology whose future could be either its application alone as a complete pretreatment or as an intermediate step in a larger process. However, there are still a number of issues that need to be addressed before determining its actual potential, including the energy consumption and economic impact of extrusion.

Acknowledgements

The authors wish to thank the European Commission (7th FP, Project BABETHANOL reference 227498) and Community of Madrid (Consejería de Educación, Project RESTOENE-2, reference 2013/MAE-2882) for financial support.

References

1. Ghosh D, Dasgupta D, Agrawal D, Kaul S, Adhikari DK, Kurmi AK, Arya PK, Bangwal D and Negi MS, Fuels and chemicals from lignocellulosic biomass: an integrated biorefinery approach. *Energy Fuels* **29**: 3149-3157 (2015).

2. Alvira P, Tomás-Pejó E, Ballesteros M and Negro MJ, Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresour Technol* **101**: 4851-4861 (2010).

3. Galbe M and Zacchi G, Pretreatment: the key to efficient utilization of lignocellulosic materials. *Biomass Bioenergy* **46**: 70-78 (2012).

4. Ravindram R and Jaiswal AK, A comprehensive review on pre-treatment strategy for lignocellulosic food industry waste: Challenges and opportunities. *Bioresour Technol* **199**: 92-102 (2016). DOI: 10.1016/j.biortech.2015.07.106

5. Duque A, Manzanares P, Ballesteros I, Negro MJ, Oliva JM, Sáez F and Ballesteros M, Study of process configuration and catalyst concentration in integrated alkaline extrusion of barley straw for bioethanol production. *Fuel* **134**: 448-454 (2014).

6. Zhan X, Wang D, Bean SR, Mo X, Sun XS and Boyle D, Ethanol production from supercritical fluid extrusion cooked sorghum. *Ind Crops Prod* **23**: 304-310 (2006).

7. Karunanithy C and Muthukumarappan K, Effect of extruder parameters and moisture content of switchgrass, prairie cord grass on sugar recovery from enzymatic hydrolysis. *Appl Biochem Biotechnol* **162**: 1785-1803 (2010).

8. Karunanithy C and Muthukumarappan K, Influence of extruder temperature and screw speed on pretreatment of corn stover while varying enzymes and their ratios. *Appl Biochem Biotechnol* **162**: 264-279 (2010).

9. Zhang S, Xu Y and Hanna MA, Pretreatment of corn stover with twin-screw extrusion followed by enzymatic saccharification. *Appl Biochem Biotechnol* **166**: 458-469 (2012).

10. Chen X, Kuhn E, Wang W, Park S, Flanegan K, Trass O, Tenlep L, Tao L and Tucker M, Comparison of different mechanical refining technologies on the enzymatic digestibility of low severity acid pretreated corn stover. *Bioresour Technol* **147**: 401-408 (2013).

11. Lin M and Ryu GH, Effects of thermomechanical extrusion and particle size reduction on bioconversion rate of corn fiber for ethanol production. *Cereal Chem* **91**: 366-373 (2014).

12. Kim TH, Choi CH and Oh KK, Bioconversion of sawdust into ethanol using dilute sulfuric acid-assisted continuous twin screw-driven reactor pretreatment and fed-batch simultaneous saccharification and fermentation. *Bioresour Technol* **130**: 306-313 (2013).

13. Senturk-Ozer S, Gevgilili H and Kalyon DM, Biomass pretreatment strategies via control of rheological behavior of biomass suspensions and reactive twin screw extrusion processing. *Bioresour Technol* **102**: 9068-9075 (2011).

14. Lee SH, Teramoto Y and Endo T, Enzymatic saccharification of woody biomass micro/nanofibrillated by continuous extrusion process I - Effect of additives with cellulose affinity. *Bioresour Technol* **100**: 275-279 (2009).

15. Lee SH, Inoue S, Teramoto Y and Endo T, Enzymatic saccharification of woody biomass micro/nanofibrillated by continuous extrusion process II: Effect of hot-compressed water treatment. *Bioresour Technol* **101**: 9645-9649 (2010).

16. Karunanithy C, Muthukumarappan K and Gibbons WR, Extrusion pretreatment of pine wood chips. *Appl Biochem Biotechnol* **167**: 81-99 (2012).

17. Karunanithy C and Muthukumarappan K, Optimization of switchgrass and extruder parameters for enzymatic hydrolysis using response surface methodology. *Ind Crops Prod* **33**: 188-199 (2011).

18. Karunanithy C and Muthukumarappan K, Optimization of alkali, big bluestem particle size, and extruder parameters for maximum enzymatic sugar recovery using response surface methodology. *BioResour* **6**: 762-790 (2011).

19. Karunanithy C and Muthukumarappan K, Optimization of alkali soaking and extrusion pretreatment of prairie cord grass for maximum sugar recovery by enzymatic hydrolysis. *Biochem Eng J* **54**: 71-82 (2011).

20. Yoo J, Alavi S, Vadlani P and Behnke KC, Soybean hulls pretreated using thermo-mechanical extrusion - Hydrolysis efficiency, fermentation inhibitors, and ethanol yield. *Appl Biochem Biotechnol* **166**: 576-589 (2012).

21. Cha YL, Yang J, Park Y, An GH, Ahn JW, Moon YH, Yoon YM, Yu GD and Choi IH, Continuous alkaline pretreatment of *Miscanthus sacchariflorus* using a bench-scale single screw reactor. *Bioresour Technol* **181**: 338-344 (2015).

22. Inoi T, Akabane T, Kurokawa Y and Matsouka , Process for pretreating biomasses in advance of their enzymatic treatment. US Patent US4642287 (1987).

23. Delong EA, Method of rendering lignin separable from cellulose and hemicellulose in lignocellulosic material and the product so produced. Canada Patent CA1217765 (1987).

24. Huber GR, Wenger LG, Hauck BW, Rokey GJ, Schmelzle LE, Hartter TR, inventors; Wenger Manufacturing Inc., assignee. Extrusion method and apparatus for acid treatment of cellulosic materials. US Patent US4728367. 1 March 1988.

25. Litzen D, Dixon D, Gilcrease P and Winter R, Pretreatment of biomass for ethanol production. US Patent US2006141584 (2006).

26. Mingzai L, Natural fabric hydrolysis reactor. Canada Patent CN101418353 (2009).

27. Steiner M, Steiner W, Dreier U and Beckers R, An improved process for providing ethanol from plant material. Euopean Patent EP2226387 (2010).

28. Vilarem G, Rigal L, Vandenbossche V, Brault J, Hernández-Luna M, Manzanares P, Ballesteros M and Duque A, Method of enzymatic treatment of a solid lignocellulosic material. US Patent US 2015299751 (2015).

29. Stirl A, Gerath H and Knitter C, Method for hybrid decomposition of biomass containing lignocelluloses. European Patent EP1978086 (2008).

30. Barbier J, Bataille F, Briand A and Soum S, Method for producing intermediate material intended for ethanol production, and resulting intermediate material. Worldwide Patent WO2010063980 (2010).

31. Dale BE and Weaver JK, Process and apparatus for treating cellulosic materials. Worldwide Patent WO 9956555 (1999).

32. Dottori FA, Cooper RA and Benech RO, Two-stage continuous pre-treatment of lignocellulosic biomass. Canada Patent CA2842781 (2013).

33. Lin Z, Liu L, Li R and Shi J, Screw extrusion pretreatments to enhance the hydrolysis of lignocellulosic biomass. *J Microb Biochem Technol* (2012). DOI: 10.4172/1948-5948.S12-002.

34. Karunanithy C and Muthukumarappan K, Thermo-mechanical pretreatment of feedstocks, in *Green biomass pretreatment for biofuels production*, ed by Tingyue G. Springer-Verlag, Berlin Heidelberg, pp 31-65 (2013). DOI: 10.1007/978-94-007-6052-3_2

35. Zheng J and Rehmann L, Extrusion pretreatment of lignocellulosic biomass: A review. *Int J Mol Sci* **15**: 18976-18984 (2014).

36. Kholgrüber K and Wiedeman W, Co-rotating twin-screw extruders: fundamentals, technology and applications, Carl Hanser Verlag, Munich, pp 59-89 (2008).

37. Erdmenger R, Mixing and kneading machine. US Patent 2814472 (A) (1957).

38. Vandenbossche V, Brault J, Vilarem G, Hernández-Meléndez O, Vivaldo-Lima E, Hernández-Luna M, Barzana E, Duque A, Manzanares P, Ballesteros M, Mata J, Castellón E and Rigal L, A new lignocellulosic biomass deconstruction process combining thermo-mechano chemical action and bio-catalytic enzymatic hydrolysis in a twin-screw extruder. *Ind Crops Prod* **55**: 258-266 (2014).

39. Kalyon DM and Malik M, An integral approach for numerical analysis of coupled flow and heat transfer in co-rotating twin screw extruders. *Int Polym Process* **22**: 293-302 (2007).

40. Raquez JM, Narayan R, Dubois P, Recent advances in reactive extrusion processing of biodegradable polymer-based compositions. *Macromol Mater Eng* **293**: 447–470 (2008).

41. Shah A and Gupta M, Comparison of the flow in co-rotating and counter-rotating twin-screw extruders. *Society of Plastics Engineers Annual Technical (ANTEC) Papers* **50**: 443-447 (2004).

42. Karunanithy C and Muthukumarappan K, A comparative study on torque requirement during extrusion pretreatment of different feedstocks. *BioEnergy Res* **5**: 263-276 (2012).

43. Brudecki G, Cybulska I and Rosentrater K, Integration of extrusion and clean fractionation processes as a pre-treatment technology for prairie cordgrass. *Bioresour Technol* **135**: 672-682 (2013).

44. Lee SH, Teramoto Y and Endo T, Enhancement of enzymatic accessibility by fibrillation of woody biomass using batch-type kneader with twin-screw elements. *Bioresour Technol* **101**: 769-774 (2010).

45. Lawal A and Kalyon DM, Mechanism of mixing in single and twin-screw extruders. *Polym Eng Sci* **35**: 1325-1338 (1995).

46. Zheng J, Choo K and Rehmann L. The effects of screw elements on enzymatic digestibility of corncobs after pretreatment in a twin-screw extruder. *Biomass Bioenergy* **74**: 224-232 (2015).

47. Duque A, Manzanares P, Ballesteros I, Negro MJ, Oliva JM, Sáez. F and Ballesteros M, Optimization of integrated alkaline-extrusion pretreatment of barley straw for sugar production by enzymatic hydrolysis. *Process Biochem* **48**: 775-781 (2013).

48. Han M, Kang KE, Kim Y and Choi GW, High efficiency bioethanol production from barley straw using a continuous pretreatment reactor. *Process Biochem* **48**: 488-495 (2013).

49. Yan Q, Miazek K, Grande PM, Domínguez De María P, Leitner W and Modigell M, Mechanical pretreatment in a screw press affecting chemical pulping of lignocellulosic biomass. *Energy Fuels* **28**: 6981-6987 (2014).

50. Scott CT, Samaniuk JR and Klingerberg DJ, Rheology and extrusion of high-solids biomass. *Tappi J* **10**: 47-53 (2011).

51. Yoo J, Alavi S, Vadlani P and Amanor-Boadu V, Thermo-mechanical extrusion pretreatment for conversion of soybean hulls to fermentable sugars. *Bioresour Technol* **102**: 7583-7590 (2011).

52. Lamsal B, Yoo J, Brijwani K and Alavi S, Extrusion as a thermo-mechanical pre-treatment for lignocellulosic ethanol. *Biomass Bioenergy* **34**: 1703-1710 (2010).

53. Chen WH, Xu YY, Hwang WS and Wang JB, Pretreatment of rice straw using an extrusion/extraction process at bench-scale for producing cellulosic ethanol. *Bioresour Technol* **102**: 10451-10458 (2011).

54. Choi CH, Um BH, Kim YS and Oh KK, Improved enzyme efficiency of rapeseed straw through the two-stage fractionation process using sodium hydroxide and sulfuric acid. *Appl Energy* **102**: 640-646 (2013).

55. Choi CH and Oh KK, Application of a continuous twin screw-driven process for dilute acid pretreatment of rape straw. *Bioresour Technol* **110**: 349-354 (2012).

56. Kuster Moro M, Sposina Sobral Teixeira R, Sant'Ana da Silva A, Duarte Fujimoto M, Albuquerque Melo P, Resende Secchia A, Pinto da Silva Bon E, Continuous pretreatment of sugarcane biomass using a twin-screwextruder. *Ind Crop Products* **97**: 509-517 (2017).

57. Zheng J, Choo K and Rehmann L, Xylose removal from lignocellulosic biomass via a twin-screw extruder: The effects of screw configurations and operating conditions. *Biomass Bioenergy* **88**: 10-16 (2016).

58. Zhang S, Keshwani DR, Xu Y and Hanna MA. Alkali combined extrusion pretreatment of corn stover to enhance enzyme saccharification. *Ind Crops Prod* **37**: 352-357 (2012).

59. Da Silva AS, Teixeira RSS, Endo T, Bon EPS and Lee SH, Continuous pretreatment of sugarcane bagasse at high loading in an ionic liquid using a twin-screw extruder. *Green Chem* **15**: 1991-2001 (2013).

60. Kadam KL, Chin CY and Brown LW, Continuous biomass fractionation process for producing ethanol and low-molecular-weight lignin. *Environ Prog Sustainable Energy* **28**: 89-99 (2009).

61. Lin Z, Huang H, Zhang H, Yan L and Chen J, Ball milling pretreatment of corn stover for enhancing the efficiency of enzymatic hydrolysis. *Appl Biochem Biotechnol* **162**: 1872-1880 (2010).

62. Choi CH, Uma BH and Oh KK, The influence of screw configuration on the pretreatment performance of a continuous twin screw-driven reactor (CTSR). *Bioresour Technol* **132**: 49-56 (2013).

63. Cha Y, Yang J, Seo S, An G, Moon Y, You G, Lee J, Ahn J and Lee K, Alkaline twin-screw extrusion pretreatment of *Miscanthus* with recycled black liquor at the pilot scale. Fuel 164: 322-328 (2016). DOI: 10.1016/j.fuel.2015.10.006

64. Chen X, Zhang Y, Gu Y, Liu Z, Shen Z, Chu H and Zhou X, Enhancing methane production from rice straw by extrusion pretreatment. *Appl Energy* **122**: 34-41 (2014).

65. Leu SY and Zhu JY, Substrate-related factors affecting enzymatic saccharification of lignocelluloses: Our recent understanding. *Bioenergy Res* **6**: 405-415 (2013).

66. Um BH, Choi CH and Oh KK, Chemicals effect on the enzymatic digestibility of rape straw over the thermo-mechanical pretreatment using a continuous twin screw-driven reactor (CTSR). *Bioresour Technol* **130**: 38-44 (2013).

67. Duque A, Manzanares P, Ballesteros I, Negro MJ, Oliva JM, González A and Ballesteros M, Sugar production from barley straw biomass pretreated by combined alkali and enzymatic extrusion. *Bioresour Technol* **158**: 262-268 (2014).

68. Liu C, Van Der Heide E, Wang H, Li B, Yu G and Mu X, Alkaline twin-screw extrusion pretreatment for fermentable sugar production. *Biotechnol Biofuels* **6**: 97-108 (2013).

69. Choi CH, Kim JS and Oh KK, Evaluation the efficacy of extrusion pretreatment via enzymatic digestibility and simultaneous saccharification & fermentation with rapeseed straw. *Biomass Bioenergy* **54**: 211-218 (2013).

70. De Vrije T, De Haas G, Tan GB, Keijsers ERP and Claassen PAM. Pretreatment of *Miscanthus* for hydrogen production by *Thermotoga elfii*. *Int J Hydrog Energy* **27**: 1381-1390 (2002).

71. Kang KE, Han M, Moon SK, Kang HW, Kim Y, Cha YL and Choi GW, Optimization of alkali-extrusion pretreatment with twin-screw for bioethanol production from *Miscanthus*. *Fuel* **109**: 520-526 (2013).

72. Hjorth M, Gränitz K, Adamsen APS and Møller HB, Extrusion as a pretreatment to increase biogas production. *Bioresour Technol* **102**: 4989-4994 (2011).

73. Karunanithy C and Muthukumarappan K, Effect of extruder parameters and moisture content of switchgrass, prairie cord grass on sugar recovery from enzymatic hydrolysis. *Appl Biochem Biotechnol* **162**: 1785-1803 (2010).

74. Carr ME and Doane WM, Modification of wheat straw in a high-shear mixer. *Biotechnol Bioeng* **26**: 1252-1257 (1984).

75. Choi WI, Oh KK, Park JY and Lee JS, Continuous sodium hydroxide-catalyzed pretreatment of empty fruit bunches (EFB) by continuous twin-screw-driven reactor (CTSR). *J Chem Technol Biotechnol* **89**: 260-296 (2014). DOI: 10.1002/jctb.4119

76. Coimbra M, Duque A, Saéz F, Manzanares P, Garcia-Cruz C and Ballesteros M, Sugar production from wheat straw biomass by alkaline extrusion and enzymatic hydrolysis. *Renew Energy* **86**: 1060-1068 (2016).

77. Helmling O, Arnold G, Rzehak H, Fahey GC, Berger LL and Merchen RR, Improving the nutritive value of lignocellulosics: the synergistic effects between alkaline hydrogen peroxide and extrusion treatments. *Biotechnol Bioeng* **33**: 237-241 (1989).

78. Razumovskii SD, Podmaster'ev VV and Zelenetskii AN, Mechanochemical methods of activating processes of biomass pretreatment. *Catalysis in Industry* **3**: 23-27 (2011).

79. Karunanithy C, Muthukumarappan K and Gibbons WR, Sequential extrusion-ozone pretreatment of switchgrass and big bluestem. *Appl Biochem Biotechnol* **172**: 3656-3669 (2014).

80. Karunanithy C, Muthukumarappan K and Gibbons WR, Sequential extrusion-microwave pretreatment of switchgrass and big bluestem. *Bioresour Technol* **153**: 393-398 (2014).

81. Oliva JM, Sáez F, Ballesteros I, Negro MJ, Manzanares P and Ballesteros M, Effect of lignocellulosic degradation compounds from steam explosion pretreatment on ethanol fermentation by thermotolerant yeast *Kluyveromyces marxianus*. *Appl Biochem Biotechnol* **105-108**: 141-153 (2003).

82. Negro MJ, Duque A, Manzanares P, Sáez F, Oliva JM, Ballesteros I and Ballesteros M, Alkaline twin-screw extrusion fractionation of olive-tree pruning biomass. *Ind Crops Prod* **74**: 336–341 (2015).

83. Karunanithy C and Muthukumarappan K, Effect of extruder parameters and moisture content of switchgrass, prairie cord grass on sugar recovery from enzymatic hydrolysis. *Appl Biochem Biotechnol* **162**: 1785-1803 (2010).

84. Chen J, Zhang W, Zhang H, Zhang Q and Huang H, Screw extrude steam explosion: A promising pretreatment of corn stover to enhance enzymatic hydrolysis. *Bioresour Technol* **161**: 230-235 (2014).

Table 1. Summary of patented pretreatment strategies comprising extrusion process, description of the process and main findings

Type of process	Description of the invention	Findings of the patent	Patent number	Publication date	Ref.
Extrusion	Twin screw extrusion of lignocellulosic biomass (preferred wood chips or sawdust) by prior to enzymatic hydrolysis to produce ethanol.	Significant increase in sugar recovery from such biomass.	US2006141584	2006-06-29	[25]
	Natural fiber hydrolytic reactor to treat plant cellulose and hemicellulose mixed with starch or raw materials to prepare a sugar solution.	No further additive needed. Environmental-friendly process, simple control and reduced manufacturing cost.	CN101418353	2009-04-29	[26]
Reactive extrusion	Extrusion of lignocellulosic material at 60-180ºC with 200-450% water plus 0.5-4% strong acid or base for ethanol production.	Fibrillation of the material. 8 fold increase of the enzymatic hydrolysis yield.	WO2010063980	2010-06-10	[30]
Alkaline extrusion	Twin-screw extrusion of lignocellulosic biomass in a with an alkaline solution (NaOH and/or Na_2S) 3 to 30% weight at 60- 230ºC, prior to enzymatic hydrolysis.	Good contact biomass/alkaline solution, continuous pretreatment, short time, low heat required.	US4642287	1987-02-10	[22]
Acid hydrolysis and extrusion	Extruder device composed of two chambers separated by a pressure sensitive die. In the extrusion zone, materials are pretreated at high pressure and temperature, and then pass into a reaction zone where they are mixed with an acidic solution at lower pressure and temperature.	Improved low cost energy efficient extrusion device, unique die construction that avoids surging and blowing of the fibrous materials, minimum acid degradation of the extruder components.	US4728367	1988-03-01	[24]

Steam explosion and extrusion	Pretreatment process of lignocellulosic biomass with multiple applications (ruminant feed, conversion to sugars and alcohols, lignin extraction) consisting in a treatment with steam at high pressure to raise temperature (185-240ºC) in a short time, followed by the release of the pressure by extrusion through a die.	Severe mechanical destructuration of lignocellulosic materials, minimization of hemicellulose degradation, enhanced enzymatic digestibility.	CA1217765	1987-02-10	[23]
	Pretreatment of lignocellulosic biomass in two steps with intermediate filtration. In which step two is carried out in a similar steam explosion-extrusion reactor as in the example above.	Improvement of the process referred above. Sequential hydrolysis of the material that allows removing hemicelluloses and possible inhibitors after the first pretreatment stage.	CA2842781	2013-01-31	[32]
Ammonia fiber explosion and extrusion	Extrusion process of cellulosic material with ammonia to expand the fiber to use it for animal feed or nutrient source for fermentation.	Increased enzymatic and ruminant digestibility, reduction in apparent lignin, high rate of digestion.	WO9956555	1999-11-11	[31]
Extrusion and microwave treatment	Extrusion process on wet lignocellulosic biomass for producing biogas or bioethanol, consisting in a single or twin-screw machine coupled to a microwave process at high temperature (150-250ºC) and pressure where thermal and possibly chemical hydrolysis (if catalyst is added) takes place, followed by anaerobic fermentation.	For biogas production, the dry fermentation process time is reduced to 10-20 days and biomass utilization increases up to 65-80% dry weight basis. For bioethanol, sugar yields increase to 75-85% of polysaccharides in the dry biomass, inhibitory products furfural and HMF remain in low concentrations and final ethanol yields increase.	EP1978086	2008-10-08	[29]
Vacuum extrusion	Pretreatment to produce bioethanol or other	Increase in at least 19% ethanol	EP2226387	2010-09-08	[27]

	valuable products from biomass, consisting in a vacuum extrusion (-3 kPa or more).	production compared to material not subjected to vacuum extrusion.			
Bioextrusion	Mechano-chemical treatment for the production of sugars from lignocellulosic biomass (to ethanol production) comprising consecutive steps of introduction of an alkaline extrusion, neutralization, filtration, and addition of enzyme solution into the extruder.	Reduced treatment time, low or no production of inhibitors for the enzymatic hydrolysis and fermentation, method easy to implement in pre-existing industrial installations.	US2015299751	2015-10-22	[28]

Table 2. Summary of feedstocks, extrusion conditions, analyzed parameters and main results from selected cases of study that investigate the screw profile, screw speed and residence time.

Variable	Feedstock	Extrusion conditions	Analyzed parameters	Results	Ref.
Screw profile	Hardwood	0.83 Hz; 70-120ºC; L/S = 1.1-2.7 Catalyst: White/black liquor **Screw profile composed of three mixing zones with different severity**	• Collecting of samples from the different mixing zones along the screw and analysis by SEM	• Visible destructuration and separation of the cellulosic fiber bundles after the most severe mixing zone (including reverse elements)	[13]
	Sweet corn cobs	1.67 Hz; 75ºC; Moisture 40% **3 screw profiles changing one element (conveying, by kneading or reverse)** Subsequent NaOH treatment	• Carbohydrate analysis • Particle size measurements • Crystallinity • SEM • Sugars released by EH	• Increased lignin removal (after NaOH treatment) with functional elements profiles • Pores blockage with reverse element (due to lignin re-distribution) • Enhanced enzymatic digestibility, increases after NaOH treatment in profiles with kneading and reverse elements	[46]
	Rape straw	0.5-1 Hz, 160-175ºC L/S =13.4 Catalyst: H_2SO_4 0.013-0.067 g/g biomass **3 screw profiles**	• Carbohydrate analysis • Sugars released by EH	• No differences in biomass composition among profiles • Increased sugars release with increasing severity of the profiles	[55]
	Sugarcane bagasse (B) and straw (S)	2.5 Hz (B)-1.4 Hz (B); 150ºC (B)-85ºC (S) Catalyst: 0.75 (B)-0.53 (S) weight glycerol/biomass 3 extrusion passes **2 screw profiles (with/without**	• Residence time • Crystallinity • Glucose released by EH	• Increased residence time from 1-4 min to 5-10 min with reverse element • No changes in crystallinity for (B)/Reduction of CrI for (S) with reverse	[56]

		reverse)		• Increased glucose EH yield 1.4-fold with reverse compared without	
	Steam-exploded corn cobs	1.67 Hz; 100ºC; L/S =0.73 (H_2O/biomass) 6 min residence time **8 screw profiles**	• Pressure profile • Liquid/solid separation • Xylose recovery in filtrate	• Increasing pressure, flow of filtrate and xylose recovery as the number of reverse elements increases	[57]
Screw speed and residence time	Switchgrass Prairie cord grass	**0.83-2.5 Hz;** 50-150ºC; Moisture 15-45%	• Sugars released by EH	• Switchgrass: increasing glucose released with increasing screw speed up to 1.66 Hz, then decrease; other sugars release increase as screw speed increase • Prairie cord grass: increasing sugars released as screw speed decreases	[7]
	Corn stover	**0.67-2.33 Hz**; 50-140ºC; Moisture 22.5-27.5%	• Glucose released by EH	• Increasing glucose released with increasing screw speed up to 1.33 Hz, then decrease	[9]
	Soybean hulls	**4.7-7 Hz**; 80ºC; Moisture 40%	• Glucose released by EH	• Increasing SME with increasing screw speed • Increasing glucose yields as screw speed increases up to 350 rpm, then decrease	[51]
	Wheat bran	**3.7-7 Hz**; 110-150ºC	• Reducing sugars after EH	• Effect of screw speed depending on temperature. Same yield by low screw speed (high residence time) and mild temperature, or high screw speed (low residence time) and high temperature.	[52]
	Corn stover	**0.66-1.67 Hz**; 50-140ºC Previous soaking in NaOH (0.004-0.04	• Glucose released by EH	• Compared to the prominent effects of alkali concentration,	[58]

		g/g biomass)		screw speed was non-significant • Behavior different from extrusion only with water in ref. 9 above	

Table 3. Summary of feedstocks, extrusion conditions, analyzed parameters and main results from selected cases of study that investigate temperature.

Variable	Feedstock	Extrusion conditions	Analyzed parameters	Results	Ref.
Temperature	Switchgrass Prairie cord grass	0.83-2.5 Hz; **50-150ºC**; Moisture 15-45%	• Sugars released by EH	• Switchgrass: sugars released increased up to 100ºC, then decrease • Prairie cord grass: sugars release decrease from 100ºC	[7]
	Pine wood chips	1.67-3.3 Hz, **100-180ºC** Moisture 25-45%	• Sugars released by EH	• Increasing sugars released as temperature increases	[16]
	Switchgrass	0.33-2 Hz, **45-225ºC** Particle size 2-10 mm Moisture 10-50%	• Sugars released by EH	• Increasing sugars released as temperature increases • Temperature was the most prominent effect on xylose release, over screw speed	[17]
	Barley straw	2.5 Hz, **50-100ºC** Catalyst: NaOH 0.025-0.75 g/g dry biomass	• Sugars released by EH	• Increasing glucose released with increasing temperatures up to 75ºC, then slight decrease • Increasing xylose released as temperature increases	[47]
	Sugarcane bagasse	0.2 Hz, **140-180ºC** L/S = 2-8 1 to 3 passes Catalyst: 1-ethyl-3-methylimidazolium acetate	• Sugars released by EH • SEM	• Glucose released did not increase from 140 to 160ºC and decreased above that point • Xylose released increased with temperature up to 160ºC, then decreased • Greater structure disruption and fibrillation (<100 nm fiber diameter) up to 160ºC • At 180ºC, collapsed structure	[59]

				and partial charring	

Table 4. Summary of feedstocks, extrusion conditions, analyzed parameters and main results from selected cases of study that investigate L/S.

Variable	Feedstock	Extrusion conditions	Analyzed parameters	Results	Ref.
L/S	Switchgrass Prairie cord grass	0.83-2.5 Hz; 50-150ºC; **Moisture 15-45%**	• Sugars released by EH	• Sugars release decrease as moisture increases for both LB	[7]
	Hardwood	0.83 Hz, 70-120ºC **Moisture 44-60%** Catalyst: NaOH, CMC	• SEM	• Increasing torque and pressure as moisture decreases • Reduction of the fibers diameter as moisture decreases	[13]
	Barley straw	2.5 Hz, 50-100ºC **L/S= 0.25-0.75** Catalyst: NaOH 0.025-0.75 g/g dry biomass	• Sugars released by EH	• Higher L/S means higher catalyst addition (concentration of the catalyst solution constant) • Sugars released increase with increasing L/S	[47]
	Soybean hulls	7 Hz, 80ºC **Moisture 45-50%** 0-5% starch addition	• Glucose released by EH	• Torque increases with low moisture • Glucose yield increases at low moisture	[51]
	Rape straw	0.33 Hz, 150-170ºC **L/S= 9-13** Catalyst: H_2SO_4 (31-48% w/w biomass)	• Carbohydrates composition	• More liquid and more acidity result in higher cellulose content and lower hemicellulose content in the solid fraction of extrudates	[62]
	Sugarcane	0.2 Hz, 140-180ºC	• Sugars released by EH	• Sugars released decrease as	[59]

	bagasse	**L/S = 2-8** 1 to 3 passes Catalyst: 1-ethyl-3-methylimidazolium acetate		biomass loading increases	

Table 5. Examples of different case pretreatment configurations using extrusion process found in recent literature and its effects on the LB.

Configuration	Pretreatment	Biomass	Operation conditions	Proven positive effect on*	Ref.
Only extrusion	Single-screw extruder	Pine wood chips	2.5 Hz, 180ºC, 25% moisture	CH, NTCR	[16]
	Twin-screw extruder	Corn fiber	5 Hz, 140ºC, 30% moisture Die 3 mm	CH, PSR	[11]
Sequence of treatments	Autohydrolysis + extrusion	Douglas fir Eucalyptus	0.83-2 Hz, room temperature Previous autohydrolysis at 140-180ºC, 30’	CH, FB	[15]
	Impregnation with glycerol + extrusion	Sugarcane bagasse and straw	0.5 Hz, 50ºC 0.53-0.75 weight glycerol/biomass	CH	[56]
	Impregnation with ionic liquids + extrusion	Sugarcane bagasse	0.25 Hz, 140ºC 25%wt ionic liquid (1-ethyl-3-methylimidazolium acetate)	CH, SSA	[59]
	Extrusion + ozonation	Switchgrass Big bluestern	Extrusion: 2.6 Hz, 176ºC/180ºC, 20% moisture 8 mm particle size Ozonation : 25%moisture, 2.5 min 37/365 mg ozone/h	CH, NTCR	[79]
	Extrusion + microwaves	Switchgrass Big bluestern	Extrusion: 2.6 Hz, 176ºC/180ºC, 20% moisture 8 mm particle size Microwave: 25% moisture, 2.5 min 450W	CH, NTCR	[80]
	Extrusion + organic solvent	Prairie cord grass	Extrusion: 1.1 Hz, 90ºC, 20% moisture 8 mm particle size Organic solvent: 39 min, 129ºC	CH, XS, LR	[43]

			0.7% catalyst (H_2SO_4) 28% methyl isobutyl ketone		
Reactive extrusion	NaOH	Barley straw	2.5 Hz, 68ºC 0.06 g NaOH/g dry biomass Neutralization with H_3PO_4 and filtration inside the extruder	CH, NTCR	[47]
	H_2SO_4	Rapeseed straw	1 Hz, 165ºC 0.27 g H_2SO_4/d dry biomass	CH	[55]
	Hydrolytic enzymes	Barley straw	2.5 Hz, 68- 50ºC 0.06 g NaOH/g dry biomass Neutralization with H_3PO_4, filtration and addition of enzymes inside the extruder 10 mg protein/g dry extrudate	CH, PSR, NTCR	[67]
Combination of technologies in one reactor	Steam explosion & extrusion	Corn stover	150ºC, 2 min	CH, XS, LR	[84]
	Alkaline extended pretreatment	*Miscanthus*	140ºC, 22% moisture 3 mm particle size 0.16 g NaOH/g dry biomass	CH, LR	[21]
	Hemicelluloses extraction	Steam exploded corn cobs	1.25-1.67 Hz, 65-100ºC 0-0.7 g water/g biomass	XS	[57]

* CH – carbohydrate hydrolysis; PSR – particle size reduction; FB – fibrillation; SSA – specific surface area; NTCR – no toxic compounds release; XS – xylose solubilisation; LR – lignin removal

Figure 1. Simplified diagram of a twin-screw extruder. A) Motor; B) Hopper; C) Thermal-regulated barrels; D) Screws; E) Pumps for the addition of catalysts.

Figure 2. Illustration of the three different types of screw elements in a twin-screw extruder. A) Conveying screw; B) Kneading block; C) Reverse screw.

Figure 3. Example of screw profile assembled in a co-rotating twin-screw extruder Evolum EV25 (Clextral®).

Figure 4. Filtered configuration (F) for the extrusion of barley straw in the twin-screw extruder. Inputs, outputs, screw profile and reaction and neutralization zones are shown. T: transport effect, M: mixing effect; S: shearing effect.

"Reprinted from Fuel, 134, Duque A, Manzanares P, Ballesteros I, Negro MJ, Oliva JM, Sáez F and Ballesteros M, Study of process configuration and catalyst concentration in integrated alkaline extrusion of barley straw for bioethanol production, 448-454, Copyright (2014), with permission from Elsevier."

Figure 5. Particle size distribution (% relative amount) of raw barley straw, extrudate and bioextrudate materials.

"Reprinted from Bioresource Technology, 158, Duque A, Manzanares P, Ballesteros I, Negro MJ, Oliva JM, González A and Ballesteros M, Sugar production from barley straw biomass pretreated by combined alkali and enzymatic extrusion, 262-268., Copyright (2014), with permission from Elsevier."