Chapter 20

Carbohydrates from Biomass: Sources and Transformation by Microbial Enzymes

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/51576

1. Introduction

In the last decades the destination of biomass, especially agro-industrial residues, is an important world problem that has been target of many researches. Accumulation of agro-industrial residues in the environment can cause serious ecological problems. On the other hand, these kinds of rich carbohydrate materials can aggregate economical value to different biotechnological process as for example in the microbial fermentative processes. According to this, the proposal of this chapter is to describe and discuss the utilization of biomass from agro-industrial residues and products and its transformation by microbial enzymes to obtain products (saccharides) with industrial interest. This is a subject that has attracted the attention of many researches and industrial sectors. To organize the information concerning this subject in a chapter is very interesting to qualify the state of the art on the utilization and importance of carbohydrates from the agro-industrial residues and products. The importance of microorganism for the transformation of biomass is another important aspect that will be highlighted.

Microorganisms, as bacteria and fungi, are able to use a great variety of inorganic and organic compounds as nutrients, reflecting an interesting metabolic diversity. Among these nutrients, nitrogen and carbon sources are indispensible for a primary metabolism. Others nutrients are required at low concentration, as vitamins. According to the growing, microorganisms are able to produce many enzymes that can show interesting biochemical properties for biotechnological application (Guimarães et al., 2006). Among these enzymes, some are constitutive while others are inducible. The induction of enzyme production by microorganisms can be obtained by use of properly biomass as carbon sources. Microorganisms are able to produce a diversity of enzymes as, for instance, the carbohydrate-active enzymes (figure 1). The glycoside hydrolases are enzymes able to acts on disaccharides, oligosaccharides and polysaccharides where can be found important enzymes as cellulases, amylases, inulinases and invertases (Table 1). Carbohydrate esterase

is involved in the removal of O-(ester) and N-Acetyl moieties from carbohydrates. The polysaccharide lyase catalyzes the β-elimination reaction on uronic acid glucosides while the glycosyltransferase acts forming glycosidic bonds using activated sugar donors.

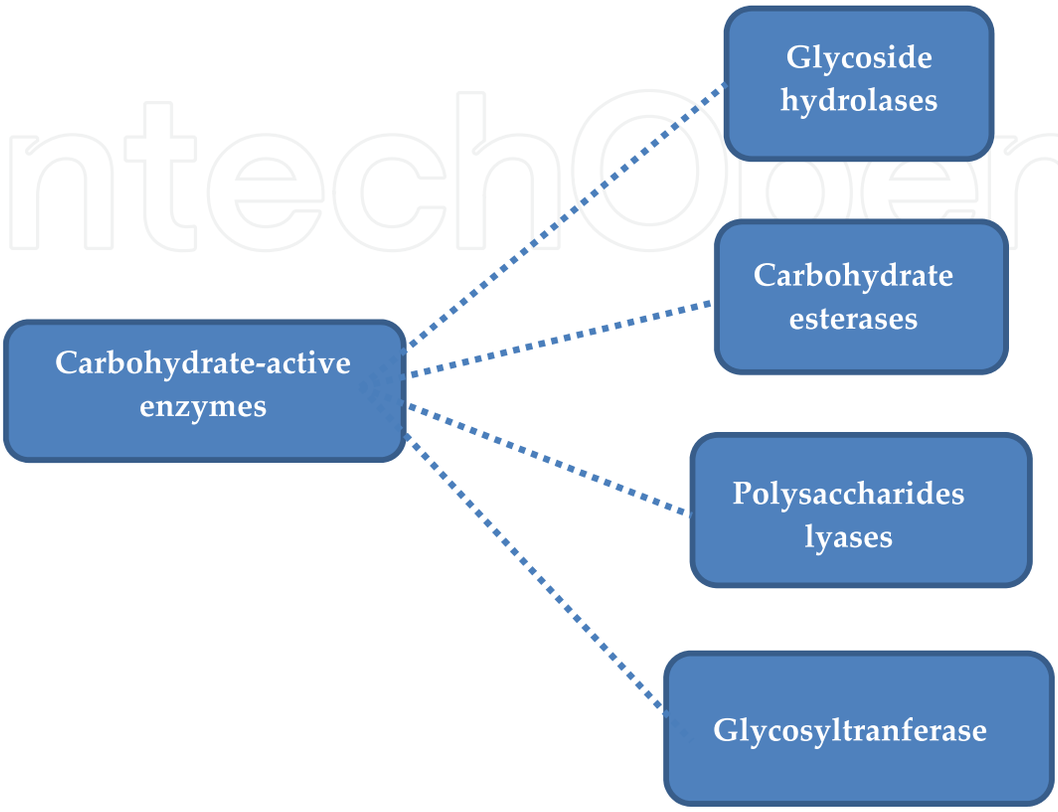

Figure 1. Classes of carbohydrate-active enzymes.

Microorganisms	Enzymes				
	Cellulase*	Xylanase	Invertase	Inulinase	Amylase
Bacteria					
Acremonium cellulolyticus	◆	◆			
Arthrobacter sp.				◆	
Bacillus amyloliquefaciens	◆				◆
Bacillus cellulyticus	◆				
Bacillus circulans		◆			◆
Bacillus licheniformis		◆			◆
Bacillus subtilis	◆	◆			◆
Bifidobacterium sp.			◆		
Cellulomonas sp.	◆	◆			
Clostridium cellulolyticum	◆				
Clostridium thermocellum	◆	◆			
Lactobacillus sp.			◆		◆
Pseudoalteromonas sp.	◆				
Streptomyces sp.		◆			

Microorganisms	Enzymes				
	Cellulase*	Xylanase	Invertase	Inulinase	Amylase
Fungi					
Aspergillus aculeatus	◆	◆			
Aspergillus caespitosus		◆	◆		
Aspergillus japonicus		◆	◆		
Aspergillus niger	◆	◆	◆	◆	◆
Aspergillus ochraceus		◆	◆		
Aspergillus oryzae	◆	◆	◆		◆
Aspergillus phoenicis		◆	◆		
Aspergillus terreus	◆	◆			
Chaetomium thermophilum		◆			◆
Emericella nidulans	◆	◆	◆		
Fusarium oxyporum		◆			
Humicola grisea	◆				◆
Humicola insolens	◆				◆
Neurospora crassa	◆	◆	◆		
Penicillium sp.	◆	◆		◆	◆
Trichoderma viride	◆	◆			

*Including endo-1,4- β-glucanases, exo-1,4- β-glucanases or cellobiohydrolases and 1,4- β-glucosidases. Data obtained from BRENDA (The Comprehensive Enzyme Information System).

Table 1. Some microbial (bacteria and fungi) sources of the enzymes involved in the utilization of carbohydrates found in the plant biomass.

2. Microbial cultivation using biomass

Different kinds of biomass have been used as carbon sources in the microbial cultivations under submerged and solid-state fermentations. Agro-industrial residues and products as, for example, rice straw, fruit peels, sugar cane bagasse and oat meal are important alternatives of carbon sources for both kinds of fermentation. The solid-sate fermentation is characterized as a system constituted by solid material in absence of free water where microorganisms are able to grown. This condition is more similar than that found by microorganisms in the environment if compared to the submerged condition. In addition, some other advantages for use of solid-state fermentation has been mentioned as: i) higher yields of products; ii) similar or higher yield if compared to submerged fermentation; iii) uniform dispersion of spore suspension; iv) higher levels of aeration and v) reduction of problems with contamination by bacteria and yeast. It is also important to consider that the medium for solid-state fermentation is simple and low cost substrates as agro-industrial residues can be used.

According to the substrate nature, two processes can be used for solid-state fermentation. The solid substrate, in the first case, is used as both support and nutrient source. These substrates are obtained from agriculture activity or from by-products from food industry.

Generally, they are heterogeneous and water insoluble. When substrates with amylaceous or lignocellulosic nature will be used, a pre-treatment is required to convert raw substrate into a suitable substrate. After, the liquid medium containing nutrients necessaries to the microbial growth can be used to moisten the inert support. The microbial growth and the product synthesis in solid state fermentation is influenced by environmental factors, such as water activity, moisture content of the substrate, mass transfer processes, temperature and pH. The control of these factors is not easy, configuring a negative aspect from solid-state fermentation. However, under economic view, solid-sate fermentation can be applied in different sectors for biotransformation of crop residues, food additives, biofuels, bioactive products, production of organic acids, detoxification of agro-industrial wastes, bioremediation, biodegradation and enzyme production (Pérez-Guerra et al., 2003).

The use of agro-industrial residues and/or products as substrates/ carbon source for SSF should be considered under some aspects. According to the biomass characteristics a pre-treatment step is necessary as cited above. To transform the raw material to the available form for microbial utilization it is necessary, many times, to reduce the size of the material using for example grinding among others. Other possibility is to promote damages on the superficial substrate layers using cracking, grinding or pearling. The utilization of chemical or enzymatic pre-treatments, cooking or vapor treatment and elimination of contaminants can be also utilized. According to the nutritional exigency of the microorganism, supplementation with phosphorus and nitrogen sources and salts can improve the microbial growth and the product yield. On the other hand, the influence of environmental factors on the SSF system also deserves consideration. The microbial growth as well as the obtainment of the products in SSF is directly affected by the moisture content. Excessive or reduced moisture content is prejudicial to the microorganism and, consequently, to the product recovery. According to this, the moisture content should be adjusted for each microorganism used in process considering the nature of the matrix used as substrate, and it has been used water content of substrate from 30% to 70% (Pérez-Guerra et al., 2003).

In SSF the gases and nutrients diffusion are severally affected by the matrix structure and also by the liquid phase in the system. The aeration permits an effective supplement of oxygen that can be used for aerobic metabolism and, at the same time, it promotes the removing of CO_2 and water vapour as well as the heat and volatiles compounds produced by the microbial metabolism. The temperature in the SSF system is a consequence of the microbial metabolism if the heat is not removed. The acquirement of nutrients depends on both hydrolysis of the polymeric structure to obtain monomers and after diffusion through the cell membrane from outside to inside the cell. The pH is another factor that affects the SSF system but its control is difficult (for review, see Pérez-Guerra et al., 2007).

Advantages for enzyme and secondary metabolites productions have been reported for both fermentations. In addition, agro-industrial residues and products are excellent alternatives as substrates for solid-state fermentation. These substrates, and consequently their carbohydrate content, can be transformed by action of a set of enzymes instead the chemical conversion. Enzymatic technology is a clear and secure process minimizing the environment problems while chemical process can generate pollulents.

Sugar cane bagasse is one of the most important agro-industrial residues accumulating biomass in the environment (figure 2) that can be used for microbial transformation. In Brazil, around 80 million of ton of sugar cane bagasse is produced per year. This raw material is constituted by 26-47% cellulose, 19-33% hemicelluloses, 14-23% lignin and 1-5% ashes. Part of this biomass as used for electric energy generation, but the most part is accumulated without

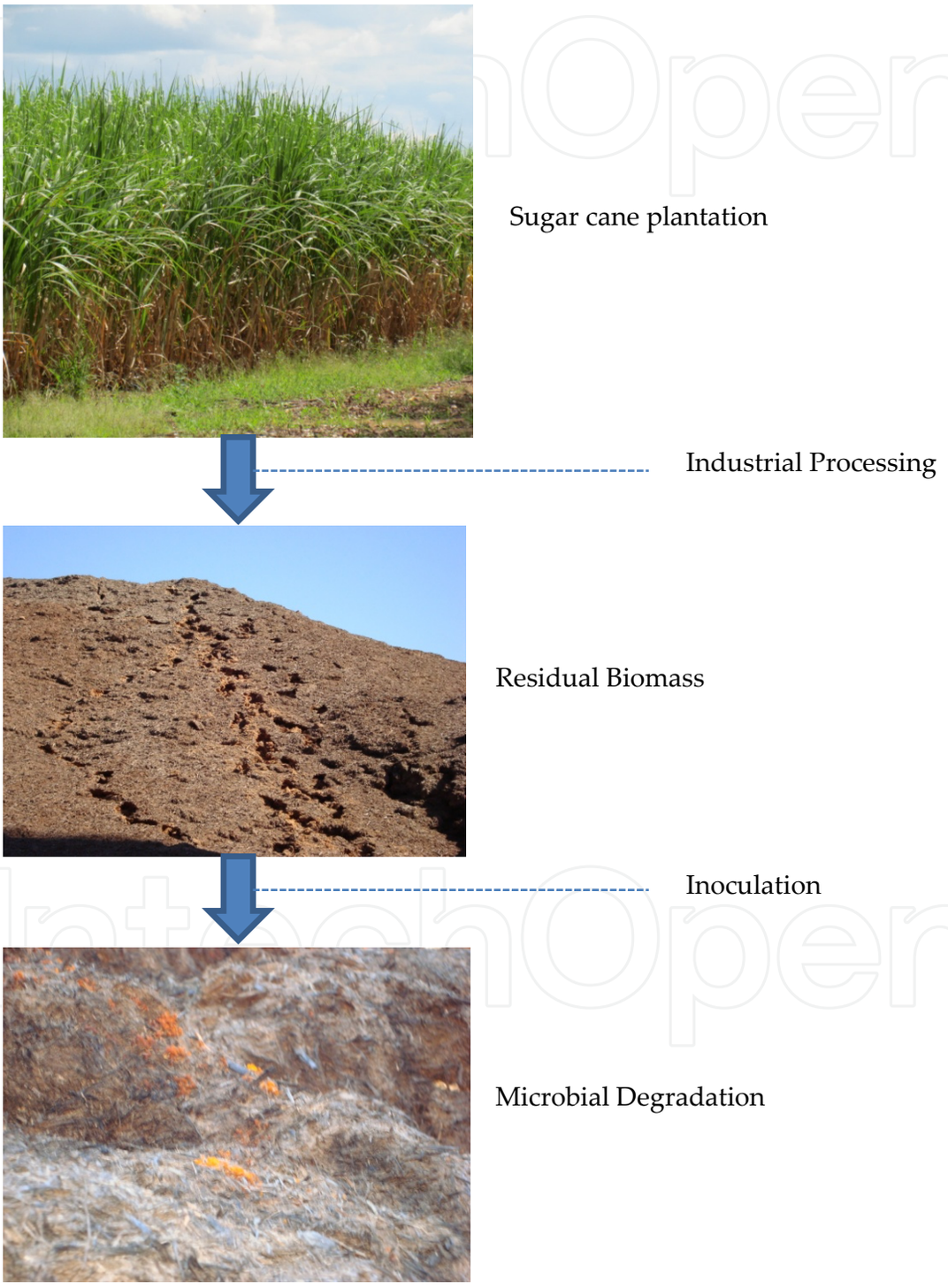

Figure 2. Utilization of sugar-cane bagasse as biomass source for microbial activity (photos by Guimarães L.H.S).

destination. So, sugar cane bagasse can be used for many other purposes as paper production, fertilizer, feeding for ruminant animals and ethanol production, among others. It is an interesting carbon source/substrate for microbial cultivation and enzyme production as verified for many filamentous fungi. High levels of β-fructofuranosidase were obtained using sugar cane bagasse as carbon source in submerged cultivation from *Aspergillus niveus* (Guimarães et al., 2009) and *Aspergillus ochraceus* (Guimarães et al., 2007).

Many others agro-industrial residues can be used for microbial cultivation such as wheat bran, which was used for invertase production by *Aspergillus caespitosus* under submerged and solid-state fermentation (Alegre et al., 2009).

Although the submerged fermentation and solid state fermentation as good option for microbial cultivation, a new kind of fermentation has been proposed. The biofilm fermentation (BF) is characterized by the fungal growing on the inert support as can be observed in the figure 3.

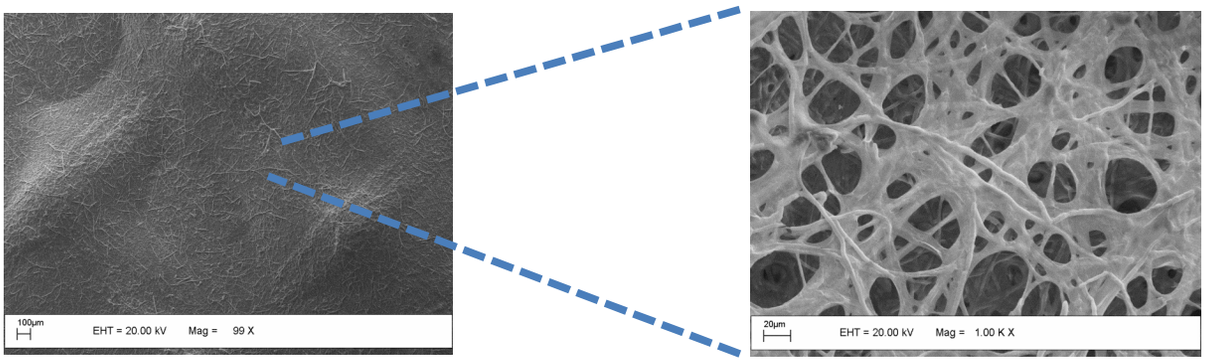

Figure 3. The *Aspergillus phoenicis* biofilm on the polyethylene as inert support.

The adhesion on surface is a natural process observed for the filamentous fungi in the environment. This complex process involves the production of adhesive compounds that fix the spore to the substrate, germ tube formation, hyphae elongation and, finally, surface colonization. These events can be observed in the *Aspergillus niger* biofilm formation on polyester cloth as reported by Villena and Gutiérrez-Correa (2007). These authors observed that the morphological pattern for *A. niger* growth attached to the surfaces is similar to that found in microbial biofilms with micro colonies development, extracellular matrix production and formation of pores and channels. The fungal morphology is an important factor to the enzyme production and, compared to the submerged fermentation, biofilms are more productive and more efficient if considered the metabolism associated with the specific enzymes which act on biomass, as for instance lignocellulosic enzymes. Recently, the use of *Aspergillus phoenicis* biofilms was reported for fructooligosaccharides production by one simple step (Aziani et al., 2012).

3. Biomass conversion by microbial enzymes

Residues and products of plant origin are recognized by their carbohydrate composition. According to this composition, the action of different enzymes on polysaccharides permits

the obtainment of a variety of mono- and oligosaccharides which can be used by different sectors including biofuel, food, beverage and pharmaceutical among others.

Nowadays, the future of our energy sources and consequently the life in the planet is target of discussion around the world with participation of different sectors of the society as researches, politicians, undertakers and third sector. It has been noted that there is an increasing interest on biomass utilization as renewable energy source since that there is a conscience that the fossil fuels are restricted. In addition, this kind of fuel is a determinant factor of pollution to the atmosphere, where the CO_2 concentration has been increased. Hence, the utilization of biomass from plant residues for biofuel production is pointed-out as an important alternative for reduction of the energetic and environment problems. Brazil and USA are the main producer countries of ethanol to be used as fuel, the former using sugar cane and the later using the corn. For example, ton of sugar cane bagasse is generated as residue from the ethanol production in Brazil, which could be used for obtainment of fermentable sugars by enzymatic hydrolysis. In the next step, these sugars can be used for fermentation process to obtain ethanol.

3.1. Cellulases and lignocelullosic biomass

Considering the plant biomass, the main component from plant cell wall is the cellulose, the more abundant carbohydrate found in the planet. Structurally, this saccharide is constituted by glucopyranose monomers linked by β-1,4 glycosidic bonds with two distinct regions, the crystalline and amorphous regions. For the complete cellulose hydrolysis, an enzymatic complex (known as cellulases) constituted by endo-1,4- β-glucanases (EC 3.2.1.4), exo-1,4- β-glucanases or cellobiohydrolases (EC 3.2.1.91), and 1,4- β-glucosidases (EC 3.2.1.21), is necessary. Cellulases are modular enzymes included in the GH family (glycoside hydrolases). These enzymes have a complex structure with different modules as one or more catalytic domain and/or CBD module in the same protein. The CBD module is able to modify the catalytic domain and, consequently the cellulase properties, facilitating the interaction catalytic domain/crystalline cellulose. Cellulases can act using two main catalytic mechanisms, inversion or retention of the anomeric carbon. Two catalytic carboxylate residues are involved in both mechanisms and they are responsible for the acid-base catalysis in the reaction. Endoglucanases (EG; carboxymethiylcellulases, CMCase) catalyze random cleavage of cellulose internal bonds at amorphous region. Exoglucanases, also known as cellobiohydrolases (CBH) act at the chains ends (CBHI at the reducing end and CBHII at non-reducing end), releasing cellobiose that can act as competitive inhibitor, while β-glucosidases (BGL) convert short cellooligosaccharides and cellobiose to glucose monomers. It is important to detach that the BGL activity is competitively inhibited by glucose. The GH1 family includes BGL obtained from bacteria, plant and mammalian and the GH3 family includes BGL from bacteria, fungi and plants. However, the full hydrolysis of cellulose depends on the previous hydrolysis of the other cell wall compounds, *i.e.* hemicelluloses and lignin (Dashtban et al., 2009; Bayer et al., 1998).

Hemicelluloses are polymeric molecules constituted by pentoses (as xylose and arabinose), hexoses (as mannose, glucose, galactose) and sugar acids. Because their heterogeneity, the hemicelluloses hydrolysis is only obtained by the action of different enzymes called hemicellulases. The most important hemicellulase is the enzyme that catalyzes the breakdown of β-1,4 linkages in the xylan, a polymer constituted by monomers of xylose. The oligomers obtained from this reaction are now substrates to the reaction catalyzed by β-xylosidase to obtain xylose (Dashtban et al., 2009).

Lignin is a heterogeneous aromatic polymer constituted by non-phenolic and phenolic structures that is able to link both cellulose and hemicelluloses making difficult the access of the enzymatic preparations to the cellulose and hemicelluloses. The enzyme able to catalyze the lignin hydrolysis are generically named as ligninases, which can be divided in two main families, phenol oxidase (laccase) and peroxidases that includes manganese peroxidase (MnP) and lignin peroxidase (LiP) (Dashtban et al., 2009).

The conversion of the lignocelluloses biomass from different sources to ethanol as can be observed in the figure 4 should take in account different steps as pre-treatment of the material, hydrolysis of the cellulose and hemicelluloses, fermentation and, finally distillation and evaporation. The pre-treatment will facilitate the asses of the hydrolases to the polyssacharides (they will be cleaved by cellulases and hemicellulases) through the lignin breakdown using physical-chemical or enzymatic process. The separated lignin can be used as matrix for energy production, as electricity. The hydrolysis and fermentation steps can be conducted separately or through of simultaneous saccharification and fermentation as shown in SSF square. Some considerations that will be done in next lines on the microorganism selection can be used for the others enzymes discussed in the next pages.

All enzymes from the cellulolitic complex, hemicellulases and lignin hydrolase can be obtained from microorganism as filamentous fungi. In the nature, filamentous fungi are able to produce and secrete different enzymes to the extracellular medium to hydrolyze polymeric compounds to obtain monomers that can be used as nutritional source.

3.2. Amylases, starch sources and structure

Another interesting carbohydrate found in the plant biomass is the starch, the main form carbohydrate reserve in these organisms. The starch is the result of interaction of two structure, one linear structure formed by glucose monomers linked by α-1,4 glycosidic bonds (amylose) with molar mass of 10^1 e 10^2 Kg/mol and another branched structure with α-1,6 bonds (amylopectin) with molar mass of 10^4 and 10^6 Kg/mol. The units of amylopectin can be classified in tree groups, A, B and C. The type A is simple and characterized by the α-1,6 linkage to the amylopectin structure. The type B is subdivided in B_1, B_2, B_3 e B_4 according to the size and the group formation. The type C is a mixture of A and B types. The starch granule is formed by 25% and 75% of amylase and amylopectin, respectively. The last one is responsible for the granule crystalinity. According to the crystallographic structure the

native starch can be classified as cereal starch (type A), tuber starch (Type B) and leguminosea starch (type C), that corresponds to the amylopectin groups. The more stable structure for the α-1,4 chains, as observed for starch, is the helix with high degree of spiralization with intra chain hydrogen bridge. The helicoidal structure has six residues per loop where each glucose residue forming an angle of 60° with the next residue (Yoshimoto et al., 2000; Ritte et al., 2006).

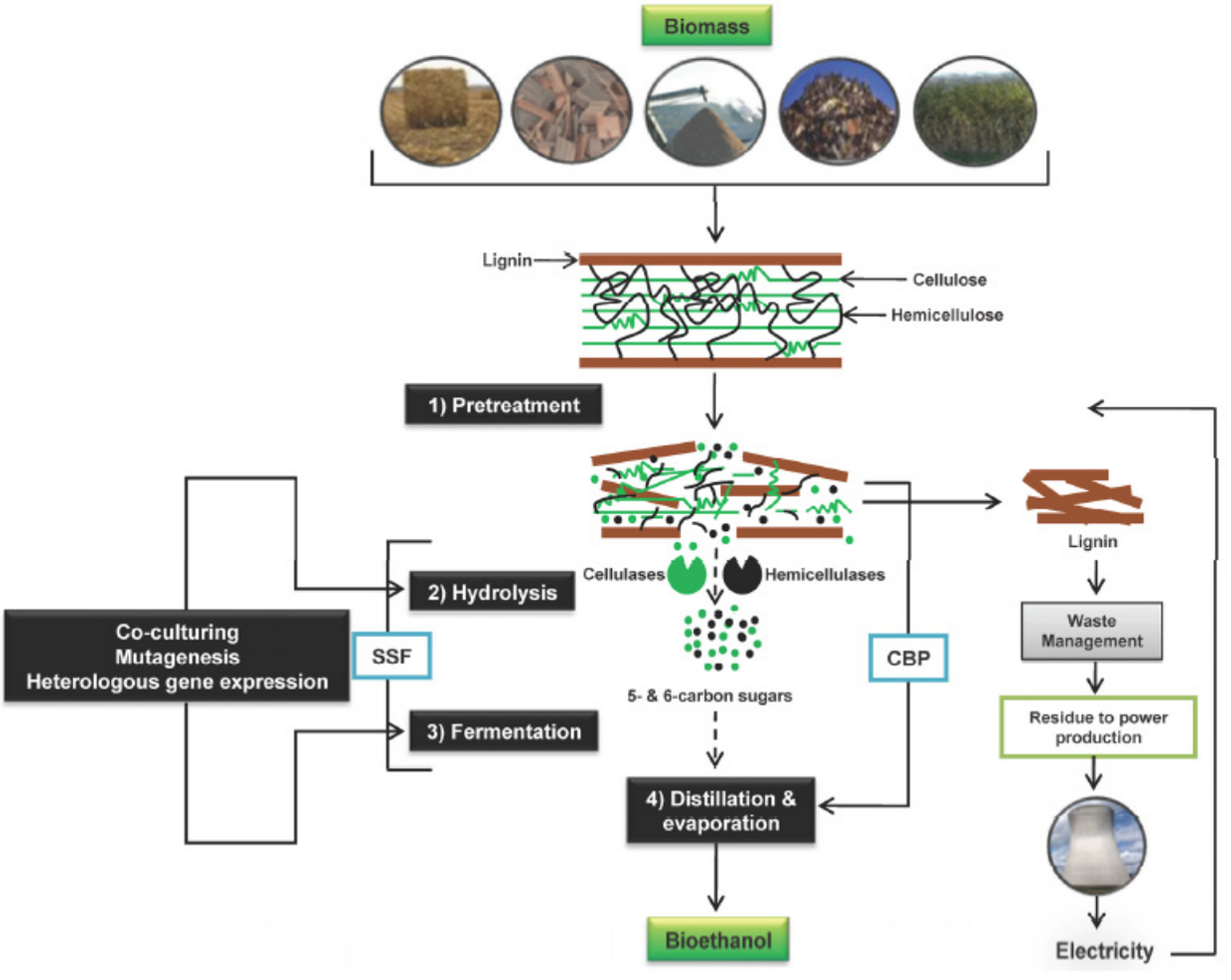

Figure 4. Schematic picture for the conversion of lignocellulosic biomass to ethanol, including the major steps (Original figure from Dashtban M., Schraft H., Qin W. Fungal Bioconversion of Lignocellulosic Residues; Opportunities & Perspectives. Int. J. Biol. Sci. 2009; 5(6):578-595. Available from http://www.biolsci.org/v05p0578.htm)

The starch synthesis is realized in the plastids and it is characterized, at the first step, by the conversion of glucose-1-phosphate and ATP to ADP-glucose and Pi by the ADP-glucose phosphorylase. After, the ADP-glucose can be used as a donor of glucose to different starch synthases that are able to elongate the glucan chain in the α-1,4 positions for both amylase and amylopectin. It is important to highlight the participation of the branching enzymes that

are responsible to add α-1,6 linkages by re-organization of linear pre-existent chains. Some introduced branching can be removed by other enzymes to finalize the starch structure. The starch is highly hydrated because there are many hydroxyl groups permitting the interaction with the water. The starch is accumulated in the leaves during the day and it is used at night to maintain the respiration, the sucrose export and the grown. This transitory starch can be use through two ways, i) hydrolytic way to obtain maltose and ii) phosphorolytic way to provide carbon to the reactions in the chloroplast during the light phase.

Similar to the cellulose, the starch cleavage occurs under the action of an enzymatic complex (figure 5). The enzymes found in this complex are organized in four main groups: debranching enzymes; endoamylases; exoamylases; and transferases. The debraching enzymes are able to act exclusively on the α-1,6 glycosidic bonds and they are separated in isoamylase (EC 3.2.1.68) and pullulanase (EC 3.2.1.41). The former hydrolyze this kind of bond exclusively in amylopectin while the later hydrolyzes the α-1,6 bond from amylopectin and pullulan. Endoamylases acts on α-1,4 glycosidic bonds inside the amylose and amylopectin structure. In this group are located the α-amylases that release oligosaccharides with different length from their substrates. They can be found in many microorganisms as bacteria and fungi. Exoamylases are enzymes that hydrolyze the external bonds of amylase and amylopectin to release only glucose or maltose and β-limiting dextrin. Three main hydrolytic characteristics can be recognized for the exomylases: the specific breakdown of α-1,4 glycosidic bonds catalyzed by β-amylases (EC 3.2.1.2) and the breakdown of both α-1,4 and α-1,6 bonds catalyzed by amyloglucosidase (glucoamylase; EC 3.2.1.3) and α-glucosidase (EC 3.2.1.20). β-amylases as well as glucoamylase are able also to convert the anomeric configuration from α to β in the released maltose. In addition, glucoamylases act better on long-chain polysaccharide while α-glucosidases have preference on maltooligosaccharides. The last group of starch-converting enzymes, *i.e.* transferases, acts on α-1,4 glycosidic bonds of a donor molecule transferring part of this molecule to a glycosidic acceptor producing a new glycosidic bond. In this group are found the enzyme known as amylomaltase (EC 2.4.1.25), cyclodextrin glycosyltranferase (EC 2.4.1.19) and branching enzymes (EC 2.4.1.18). Amylomaltase and cyclodextrin glycosyltranferase have similar mechanism of reaction. Although the reduced hydrolytic activity from these enzymes, they are able to catalyze the transglycosylation reaction to obtain cyclodextrins by breakdown of α-1,4 glycosidic bonds and linkage of the reducing to the non-reducing end. However the product obtained by amylomaltase activity is linear while the cyclic product is obtained by cyclodextrin glycosyltranferase action (van der Maarel et al., 2002).

Many of these enzymes are involved in the complete hydrolysis of the starch. First, disbranching enzymes should act on the α-1,6 bonds to expose the linear structure that can be hydrolyzed by α-amylase and β-amylase (figure 5). Many authors have demonstrated the importance of obtainment of maltooligosaccharides from complex carbohydrate using amylolytic enzymes. Maltooligosaccharides can be used in different industrial sectors as food. Fungi are able to produce amylases. In addition, other important application of the

starch is related with its use as source of renewable energy in the production of bioethanol as an alternative to the fossil fuels.

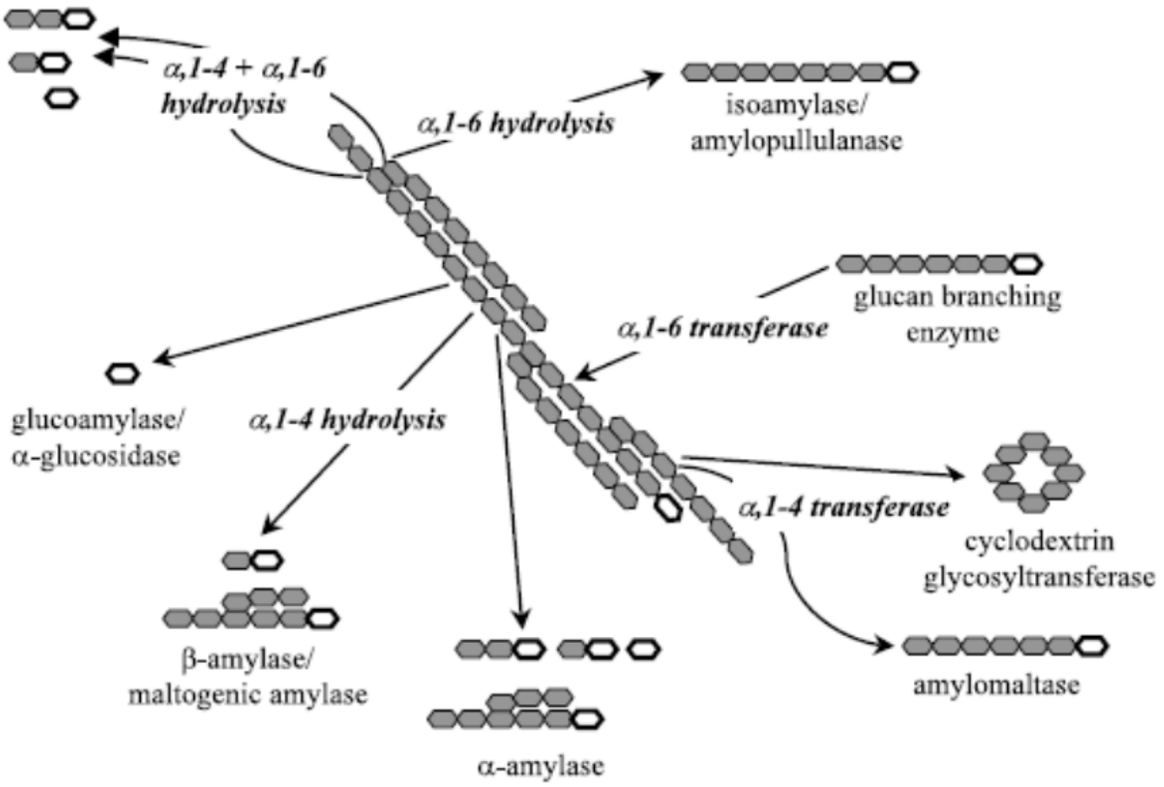

Figure 5. Different enzymes involve in the degradation of the starch. The open ring structure represents the reducing ends of a polyglucose molecule (Original figure from van der Maarel MJEC, van der Veen B, Uitdehaag JCM, Leemhuis H, Dijkhuisen L. Properties and applications of starch-converting enzymes of the α-amylase family. J. Biotechnol. 2002; 94: 137-155).

3.3. Fructosidases and other carbohydrates from biomass

Other saccharides can be also obtained from plant sources as inulin and sucrose, which are substrates for fructofuranosidase action, as β-fructofuranosidases and inulinases, which can be produced by microorganism as for example yeast and filamentous fungi. These enzymes have an important role in the microbial nutrition since monomers can be obtained and used in the metabolism. On the other hand, the enzymes involved in this process can be used with biotechnological goal.

3.3.1. The inulin and its utilization

Inulin, a polymer constituted by linear chain of β-2,1 fructufuranose residues terminated by a glucose residue, can be obtained from plant sources, especially from tubers and roots, as for example chicory, dahlia, yacon and Jerusalen artichoke. The plant sources of inulin have been considered as renewable raw material for many applications such as ethanol, obtainment of fructose syrup and fructooligosaccharides (FOS) production. This

carbohydrate can be hydrolyzed by action of inulinases (2,1 β-D-fructan fructanohydrolase; EC 3.2.1.7), which can be classified into endoinulinase that hydrolyze the internal linkages from inulin to obtain inulotriose, inulotetraose and inulopentaose as end products, and exoinulinase that acts removing the terminal fructose from the non-reducing end from the inulin until the last linkage to release glucose (Ricca et al., 2007). It is important to observe that the type of enzymatic action depends on the microbial source. The most of fungal inulinases acts using the exo-mechanism. However, it was demonstrated that *Aspergillus ficuum* was able to produce endo- and exo-inulinases with different properties. The mixture of both enzymes can be considered as a good strategy to increase the conversion of the inulin to fructose (for review, see Ricca et al., 2007). Despite the action and affinity similarities for sucrose as substrate, with fructosidases as invertase, inulinases has been separated since invertase has reduced activity on high molecular mass substrates as inulin. The relation S/I has been used to separate inulinase from invertase. The S/I values depends on the inulin sources and also on the methodology used to determine the enzyme activity. However, kinetic studies are good methodologies that can be help the differentiation of these enzymes as for example considering the substrate affinity and catalytic efficiency. On the other hand, the enzymes recognized as true invertases have no activity on inulin.

The inulinases obtained from yeast are enzymes that can be linked to the cell membrane and partially secrete to the extracellular environment. In addition the synthesis of these enzymes is subject to the catabolic repression. In addition, inulinases are recognized as inducible enzymes and they are encoded by *INU* genes. The enzymes obtained from filamentous fungi has demonstrated optimum of pH activity from 4.5 to 6.0 differing than that observed for some bacterial inulinases with higher pH of activity. The optimum of temperature for activity for the most inulinases is from 30°C to 55°C but higher temperatures can be also found.

3.3.2. The sucrose and its utilization

Sucrose, a disaccharide constituted by D-glucose and D-fructose linked by α-1,2 glycosidic bond, is the main carbohydrate produced by plants using photosynthesis to generate ATP and NADPH, which will be used in the Calvin Cycle to fix CO_2 in the dark step. Two main enzymes are involved in the sucrose synthesis, the sucrose-phosphate syntase (EC 2.4.1.14) and the sucrose-phosphate phosphatase (EC 3.1.3.24) (Winter and Huber, 2000). After the synthesis, the sucrose produced in the photosynthetic leaves is distributed to the other plant organs and tissues. The ethanol production in Brazil is performed using rich-sucrose sugar cane juice. However, after the sucrose extraction, the residual sugar cane bagasse also has residual sucrose that can be used to obtain monosacharides by microbial action. These monomers can be used together with other monosaccharides obtained from the hydrolysis of polysaccharides present in the sugar cane bagasse, as cellulose, to fermentation process. The sucrose hydrolysis (figure 6) is catalyzed by the β-fructofuranosidases (invertases; EC 3.2.1.26), which are found in many microorganisms. The product obtained is an equimolar (1:1) mixture of the monosaccharides and residual sucrose known as invert sugar with wide application in the food and beverage industries. The fructose is much more attractive for

application since it can has liquid and non-crystalizable constitution. The β-fructofuranosidases are located in the GH32 family of the glycosil hydrolases and grouped in different isoforms according to their pH of actuation as acid, alkaline and neutral enzymes (Vargas et al., 2003).

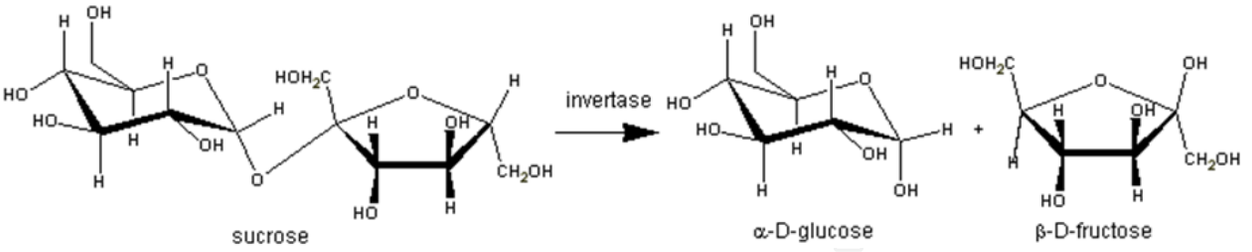

Figure 6. Hydrolysis of sucrose by invertase.

The production of β-fructofuranosidases (FFases) by microorganisms has been characterized, especially for the yeast *Saccharomyces cerevisiae*. In this microorganism it was observed the synthesis of two isoforms of FFases where one is gylcosylated and another non-glycosylated. Both enzymes are result from the two mRNA (1.8 and 1.9 kb) encoded by the same gene *SUC2*. The glycosylated enzyme is found in the periplasmic space while the non-glycosylated is found in the citosol (Belcarz *et al*., 2002). In *S. cerevisiae* the sucrose metabolism occurs throughout two main ways: i) the sucrose is hydrolyzed in the extracellular environment by extracellular invertases to liberate glucose and fructose, which can be transported to inside of the cell by hexose transporters and ii) the sucrose is actively transported to inside of the cell by proton-symport mechanism and after hydrolyzed by intracellular invertase. The expression of the *SUC2* gene that encodes both enzymes is severally regulated by glucose (Basso et al., 2011).

The production of FFases by other microorganism, especially filamentous fungi as *Aspergillus* genera, among others, has been studied. In this situation, different fermentation processes are used, which many times the residual biomass are used as carbon sources (submerged fermentation) and/or substrates (solid-sate fermentation). Some microorganisms are able to produce multiple β-fructofuranosidases as observed for *Aureobasidium pullulans* (Yoshikawa et al., 2006). In this situation the authors observed the presence of five FFases (I, II, III, IV and V) with high FFase I activity at the initial times of culture and reduced FFase II-V activities. After initial times the FFase II-V activities are increased. In addition, the multiple FFases produced by *A. pullulans* have distinct properties as suggested by authors. FFase IV has high hydrolytic activity acting as FOS-degrading enzyme at the FOS-degrading period while the participation of FFases II, III and V is uncertain, since they have significant transfructosylating activity and they are present in the FOS-degrading periods (Yoshikawa et al., 2006).

The most of β-fructofuranosidases are dimmers but monomers also can be found. The optimum of temperature and pH of reaction considering all microbial sources are variable. This carbohydrate has a negative influence on the invertase synthesis by *A. niger* as well as fructose. Only β-fructofuranoside saccharides were able to induce the invertase synthesis

(Rubio & Navarro, 2006). It was observed that the *A. niger* is able to produce two β-fructosidases known as SUC1 and SUC2. Both enzymes catalyzed the sucrose hydrolysis but only SUC 2 was able to act on inulin. Other fungal strains have been used for invertase production as *Aspergillus ochraceus* (Guimarães et al., 2007), *Aspergillus niveus* (Guimarães et al., 2009), *Aspergillus caespitosus* (Alegre et al., 2009), *Aspergillus phoenicis* (Rustiguel et al., 2011) and *Paecylomyces variotii* (Giraldo et al., 2012) using both submerged and solid-state fermentation with agro-industrial residues as carbon source/substrate. Thermostable FFases has been obtained by cultivation of *A. ochraceus* and *A. niveus* using sugar cane bagasse as carbon source.

At high sucrose concentration, some β-fructofuranosidases are able to catalyze transfructosylation reaction to obtain fructooligosaccharides (FOS) as 1-kestose (GF2), 1-nystose (GF3) and fructofuranosyl nystose (GF4). The molecular structure of these FOS can be observed in the figure 7. The GF2 is constituted by two molecules of fructose binding to the D-glycosyl unit at the non reducing end while the GF3 and GF4 by three and four fructose residues, respectively. These oligosaccharides have functional properties that have attracted the attention of different sectors. FOS are no caloric sugars that can be used by diabetic peoples with security since they are not metabolized by the organism. In addition, FOS can also stimulate the bifidobacteria development in the intestine and minimize the colon tumor. It has been demonstrated that some components of plant sources (biomass) used in pet foods exhibit FOS concentration of GF2, GF3 and GF4, as for example wheat bran, peanut hulls and barley, among others. The hydrolysis of FOS by microbial sources as bacteria using enzymes that act on these saccharides was demonstrated in some reports. Hence, enzymes that are able to act on FOS can be used to obtain saccharides as glucose from the biomass containing GF2, GF3 and/or GF4. On the other hand, different approaches have been used to obtain FOS as the utilization of immobilized enzymes on lignocellulosic materials and by substrate and enzyme engineering. Recently, the one-step FOS production was obtained using *Aspergillus phoenicis* biofilms in rich-sucrose medium as demonstrated in our laboratory (Aziani et al., 2012).

In the same way, the fuctosyltransferases as levansucrases (sucrose:2,6-β-fructans: 6-β-D-fructosyltransferase; EC 2.4.1.10), inulosucrases (sucrose:2,1-β-D-fructan: 1-β-D-fructosyltransferase; EC 2.4.1.9) and fructosyltransferase (sucrose:2,6-β-fructan:6-β-D-fructosyltranferase; EC 2.4.1.10) should be considered. The former is responsible by the synthesis of microbial levans using glucose or levan as acceptor to the β-D-fructosyl residues while the inulosucrases are able to catalyze the transference of the β-D-fructosyl residues to the sucrose or inulin as acceptors. The later are involved in the levan synthesis but it is not able to catalyze hydrolysis or exchange reactions as observed for the levansucrase (Velázques-Hernandez et al., 2009). These enzymes that catalyze the fructans synthesis are inserted in the GH68 family of glycoside hydrolases. In general, five main domains are recognized in the fructosyltransferases of microorganisms: a signal peptide; an N-terminal domain with variable length; a catalytic domain with around of 500 amino acids; a cell wall binding domain; and a C-terminal domain with variable length (Van Hijum et al., 2006).

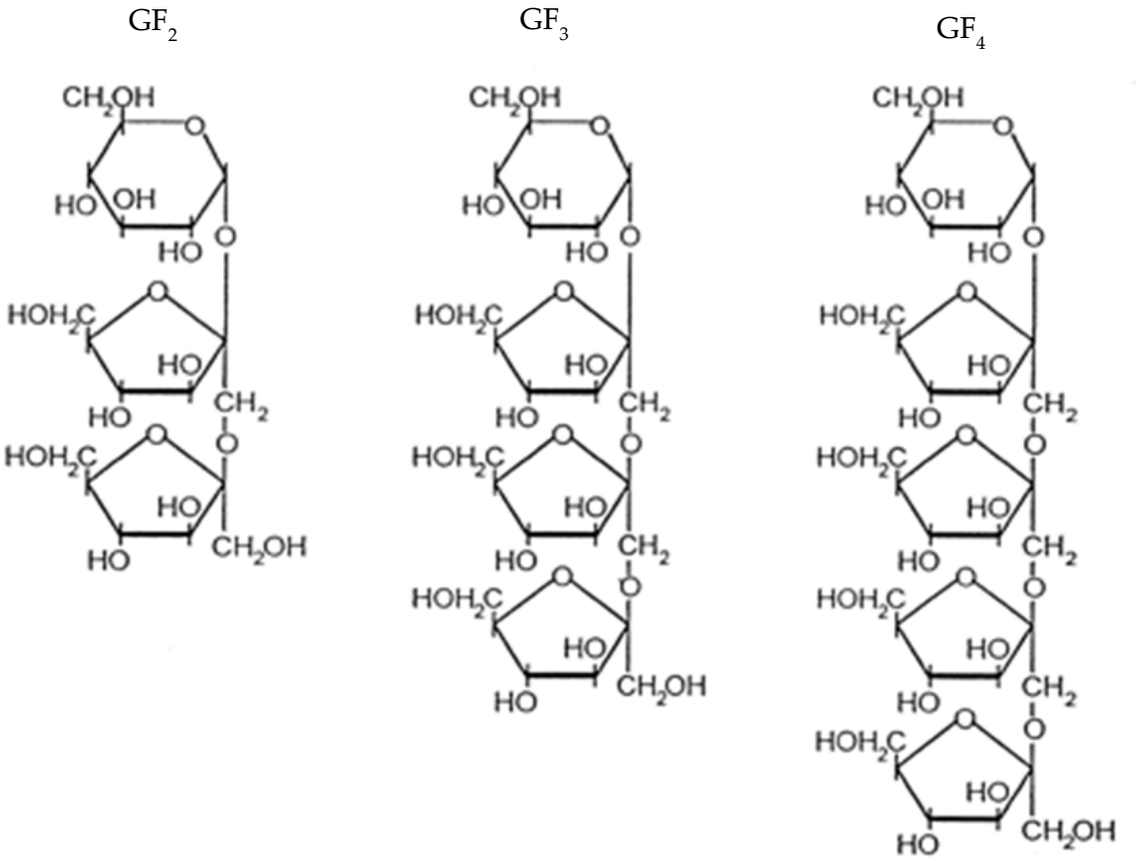

Figure 7. Molecular structure from the fructooligosaccharides nystose (GF2), 1-kestose (GF3) and fructosyl nystose (GF4).

4. Conclusion

In conclusion, the biomass that has been accumulated around the world as residue can be widely used for different applications considering its carbohydrate composition which can be accessed by microbial activity according to the enzymatic potential of each one. Microorganisms show metabolic versatility permitting the carbohydrate utilization and transformation from biomass since they are important sources of enzymes with biotechnological potential. According to this, different products can be obtained from biomass and applied in different industrial sectors. The view of the biomass as an important renewable energy source is very important to the future of the life in our planet, especially if considered the agro-industrial residues. In addition, the environment problems of bioaccumulation of residues can be reduced. Future studies to improve the biomass utilization are important as well as on the carbohydrate-active enzymes produced by microorganisms to optimize this process.

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