Chapter 2
Physical–Chemical Properties of Solid Substrates

Abstract Physical–chemical properties of solid substrates are important parameters of high-solid and multi-phase bioprocess. The chemical properties refer to the biomass recalcitrance and heterogeneity which caused by its chemical composition. The physical properties include porous properties, rheology properties, and water state. In this chapter, the composition and recalcitrance of the solid substrates are analyzed, and the change laws of physical–chemical properties such as porous properties, rheology properties, and water states are revealed, which is significant for bioconversion of biomass in high-solid and multi-phase bioprocess. In addition, solid effects caused by physical–chemical properties of solid substrates were also systematically discussed and investigated with the expectation of guiding bioconversion process of biomass.

Keywords High solid · Chemical composition · Physical property
Recalcitrance · Solid effects

High-solid and multi-phase bioprocess has merits of high concentration of substrate and production. In high-solid and multi-phase bioprocess, "solid effect" is caused by the increase of solid substrates, which is critical to the bioconversion efficiency of high-solid and multi-phase bioprocess. Researches indicated that the "solid effect" includes solid substrates composition effects, adsorption and desorption effects of cellulolytic enzymes, water-binding effects, and inhibitor effects [1, 2]. These can be the challenge of the large-scale utilization of renewable solid resources, changing transfer and reaction characteristics of the system, increasing process energy consumption, and reducing efficiency of the reaction.

Traditional researches on renewable resources aimed to improve the process efficiency with little cognition of the solid substrates' properties. Therefore, it could not solve the bottleneck problems in bioconversion of lignocellulose, resulting in the economic infeasibility of the large-scale utilization of renewable resources. The factors, influencing the efficiency of renewable solid resources conversion, include porous structure (e.g., pore size and volume, curvature, grain size, and specific surface area), chemical component (e.g., lignin, hemicellulose, acetyl, and

pectin), and chemical structure (crystallinity and the degree of polymerization of cellulose) [3]. It is closely related to the multicomponent and porous characteristics of renewable solid resources. The complex multicomponent characteristics of the renewable solid resources determine that using single technology and component is not benefit to the efficiency of the conversion process. Biomass is the typical porous medium material in which mass and heat transfer are different from the traditional liquid system; this phenomenon is more obvious in the situation of high-solid content. "Solid effect" is related to the intrinsic characteristics of renewable solid resources. Therefore, analyzing the relationship between the intrinsic characteristics of renewable solid resources and "solid effect" is essential to break down the "solid effect" and realize the efficient conversion of renewable resources.

2.1 Composition and Preparation of Solid Substrates

2.1.1 Solid Substrates Composition and Recalcitrance

Lignocellulose is a typical biomass with complex chemical and physical properties. Chemical components mainly include cellulose (30–50% of the total), hemicellulose (10–40% of the total), lignin (10–30% of the total), and other components such as wax, pectin, protein, pigment, and ash. The multicomponents of lignocellulose are macromolecules with specific functions, which are consisted of different monomers. There are differences among these multicomponents of different types of lignocellulosic biomass, while hardly differences exist in their basic elements (including carbon, hydrogen, oxygen, and nitrogen) [4–6].

Lignocellulosic materials include softwoods, hardwoods, and herbal plant biomass. Softwood contains 43% cellulose, 29% hemicellulose, and 28% lignin averagely. Hardwood contains about 45% cellulose, 34% hemicellulose, and 21% lignin, and cellulose content is similar to the softwoods. Hardwood has higher content of hemicellulose than softwood. In hardwood hemicellulose, there are 20–25% poly4-O-Methylglucuronic acid and 1–3% polydextrose mannose, while hemicellulose of softwoods has 15–20% poly-galactosidase-glucose mannose–acetate, 10% poly4-O-methyl-glucuronide arab xylose, and 1–3% arabinogalactan [5]. Therefore, there is more polyxylose in the softwoods but a higher content of polymannose, polygalactose, and araban in the hardwoods. Lignin content of softwoods is higher than that of hardwoods. The main components of lignin in softwoods are guaiacol propane and some phydroxyphenyl propane. The main components of lignin in hardwood are guaiacol propane, lilac propane, and small phydroxyphenyl propane [5]. The components of herbal plants are similar to that in hardwood. Cellulose content of most herbal plants is close to wood, except for straw (corn stalks and sorghum stalks) [5]. The chemical compositions of different types of biomass fiber raw materials are summarized in Table 2.1.

Table 2.1 Chemical composition of different biomasses

	Softwoods fibrous materials	Hardwoods fiber materials	Gramineae fiber materials
Carbohydrate	65–80%	65–80%	50–80%
Cellulose	40–45% (averagely 43%) length of the molecular chain is about 5000 nm, average polymerization degree is 10000	40–45% (averagely 45%) Similar to softwoods	30–45% Low polymerization degree of cellulose averagely
Hemicellulose	25–30% (averagely 28%), A high content of Poly-galactosidase-glucose mannose–acetate (15–20%), 10% content of glucuronic acid as well as galacturonic acid and araban-4-O-methylglucuronic acid xylose. Less content of arabinogalactan is about 1–3%	30–35% (averagely 34%), large amount of araban-4-O-methylglucuronic acid xylose (20–25%), less polydextrose mannose (1–3%)	20–35%, mainly component is polyxylose, small molecular weight, low polymerization degree
Lignin	25–30%(averagely 29%), mainly contains guaiacyl propane and a little phydroxyphenyl propane	20–25% (averagely 21%), mainly contains guaiacyl propane, syringyl, and a little phydroxyphenyl propane	10–25%, mostly at a low content level which are closely to that of hardwoods, except for the bamboo that is similar to softwoods
Extractives	Low content of hot water extractives and 1% NaOH extractives. High content of organic extractives, which main component are abietic acid, terpenoid, fatty acid and unsaponifiable matter, etc., which are existing in resin duct and xylem ray parenchymal cell.	Low content of hot water extractives and 1% NaOH extractives. Less than 1% content of organic extractives than softwoods which mainly contains free or esterifiable aliphatic acids and neutral components, without or few terpenoid compounds, mainly existing in xylem ray cell and parenchymal cell.	High content of hot water extractives and 1% NaOH extractives, and less than 1% ether extractives which mainly contains wax and few higher aliphatic acid and higher alcohols et al. And with quite high content of benzene–ethanol extractives (3–6%).
Protein	<0.5%		5–10%
Inorganic substance	0.1–1.0%		0.5–10%

Cellulose is the main component of the cell wall, which binds to hemicellulose, lignin, and pectin. The binding mode determines the property of lignocellulosic biomass. Cellulose is linear polymer which composed of D-glucose by 1, 4-β-glycosidic bond, and its chemical formula is $(C_6H_{10}O_5)_n$, where n is the degree of polymerization. Cellulose is composed of 44.4% carbon, 6.2% hydrogen, and 49.4% oxygen. Structure of cellulose is shown in Fig. 2.1 [5, 6].

Cellulose and lignin are linked together closely by hemicellulose. Hemicellulose almost exists in all plant cell wall. It is one of the main components of cell wall, and the content is lower than cellulose. Content, component, and structure of hemicellulose in various materials are different. The structure units of hemicellulose mainly contain D-xylose, D-mannose, D-glucose, D-galactose, L-arabinose, L-rhamnose, and L-fucose.

Taking herb plant cell wall as an example (Fig. 2.2), xylan is the main component of hemicellulose. The degree of polymerization of hemicellulose is only 150–200, much smaller than that of cellulose.

Lignin is a natural polymer with three-dimensional structure which is consisted of phenylpropane structure unit linked by ether bond and carbon–carbon bond. Phenylpropane structure unit forms the basic skeleton of nature hemicellulose, but there is a little difference in aromatic part. Based on the number of $-OCH_3$, structure units are divided into three kinds including guaiacyl propane (G), syringyl (S), and phydroxyphenyl propane (H) (Fig. 2.3). As a natural adhesive, lignin exists in microfiber and intercellular layer of plant cell walls, sticking adjacent cells together.
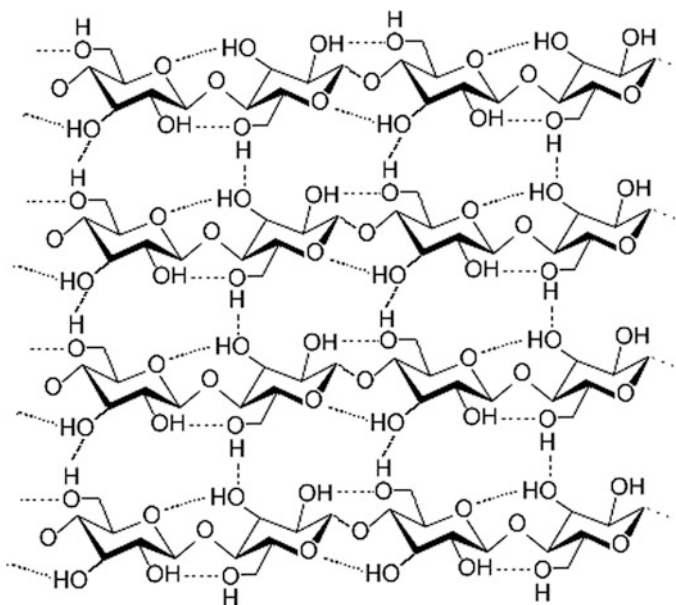

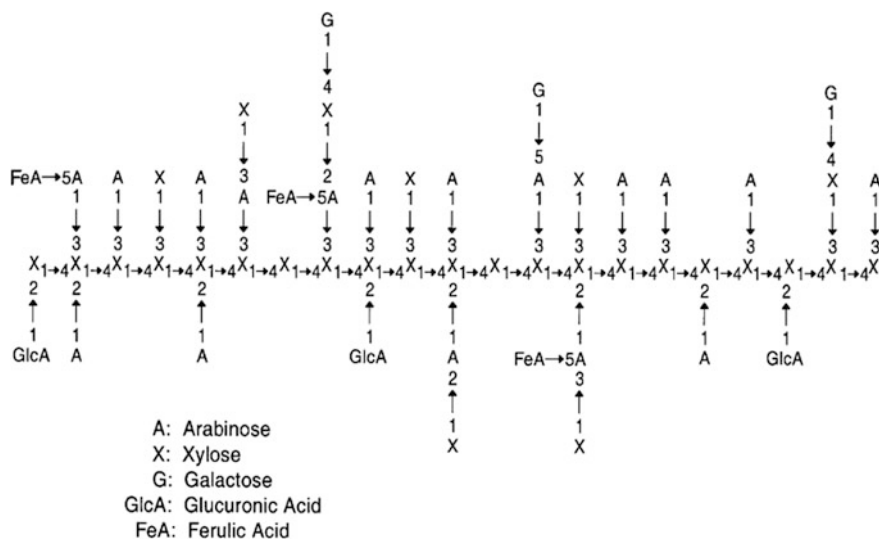Fig. 2.1 Chemical structure of cellulose

Fig. 2.2 Schematic structure of hemicellulose in maize fiber. Reprinted from Ref. [7]. Copyright 2003, with permission from Springer

Lignin plays a supporting role in maintaining the mechanical properties of the plant cell wall and reduces the transverse water permeability of cell wall. Besides, lignin can help to prevent microbial attack to glycan. The distribution of lignin in plant cell wall is inhomogeneous. The content and component of lignin of plant differ from each other, which depend on the plant species, growth time, and grown zone. Above all, lignin is the third largest nature polymer, whose content is only lower than cellulose and chitin in nature [4–6, 8].

According to the aromatic nuclear structure, lignin can be divided into guaiacyl propane, syringyl, and phydroxyphenyl propane type. And lignin has various functional groups (including aromatic ring and side chain), such as methoxy group ($-OCH_3$), phenolic hydroxyl group ($-OH$), and carbonyl group ($-C=O$), which is
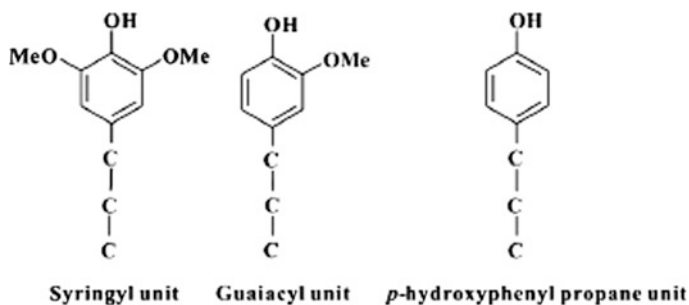

Fig. 2.3 Typical lignin aromatic polymer: syringyl (S), guaiacyl (G), and hydroxyphenyl (H)

shown in Fig. 2.4. Lignin is the biggest obstacle to the utilization of chitosan. To achieve efficient utilization of chitosan, it must change the structure of lignin or remove lignin.

Complex physical structure and chemical features of lignocellulose show different protection mechanisms against microbial attack and polysaccharide degradation, which is called biomass recalcitrance [9]. Recalcitrance determines the efficiency of refining and economical efficiency of conversion process. Himmel et al. have put forward various key factors which can measure lignocellulosic biomass recalcitrance to chemical reagents, microorganism, and enzyme [9], including (1) epidermal tissue of plant, especially cuticle and cuticular wax; (2) the arrangement and location of vascular bundle; (3) the relative content of parenchymatous tissue; (4) degree of lignification; (5) structural heterogeneity and complexity of cell wall components; (6) challenge of enzyme action in insoluble substrate; (7) inhibitory effect of the fermentation process from the inhibitor which exits naturally in cell wall or being generated in transformation process; and (8) crystalline cellulose of cytoderm
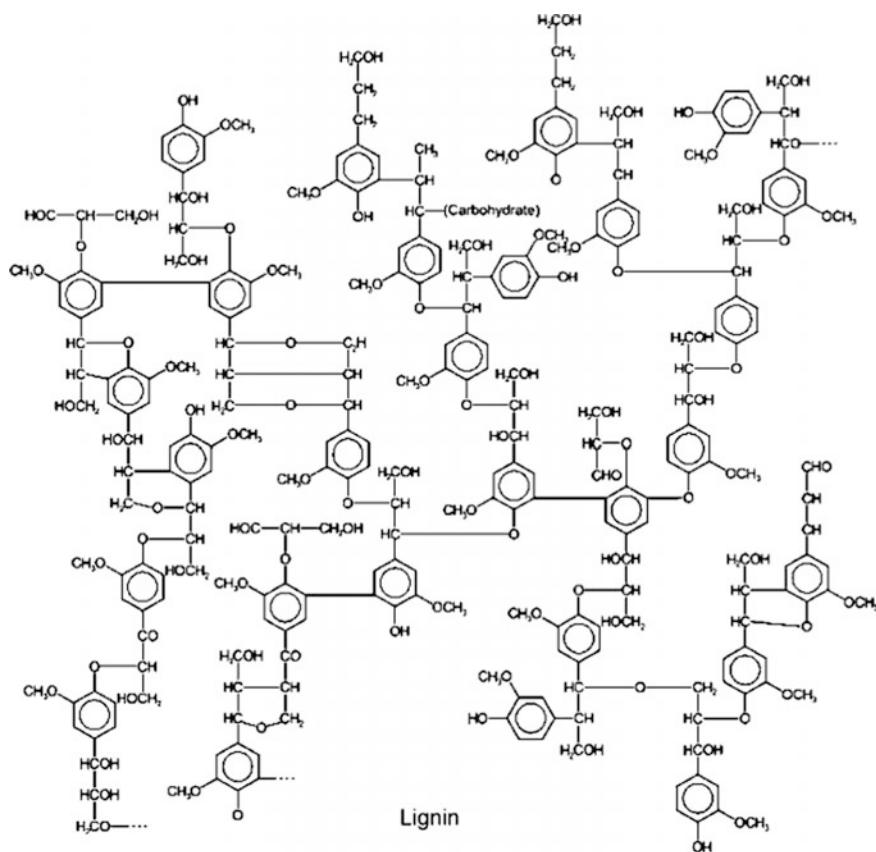

Fig. 2.4 Structure characterizations of lignin fraction in plant cell wall

microfiber hinders the chemical and biological degradation in molecular level. In conclusion, biomass recalcitrance, in the macroscopic and microscopic level, hinders the transmission of chemical reagent and enzyme, which reduces the transformation efficiency. Thus, in order to realize the efficient utilization of lignocellulosic biomass, it is necessary to develop the most efficient technology to overcome recalcitrance of lignocellulose.

Chemical composition and physical structure of lignocellulosic biomass are different in various organs, tissues, and cells, and even the same organ tissue cells. Additionally, linkage mode between monomers changes with monomer and chemical components of functional macromolecule. These qualitative differences are defined as heterogeneity of lignocellulosic biomass which influences the refining efficiency of lignocellulose biomass.

Complex structure of organs, tissues, cells, and cell wall of the plant was formed in the process of evolution. Take herb plants as example; the straw consists of several joints with a relative poor intensity. Internode and node are joined with growth band, which consists of parenchyma cells. Second, the midsection of internode will shrink in most herb plants. Vascular bundle in stick distributes scattered without epidermis and stele boundary, which mainly contains epidermis, parenchyma, and vascular bundle. Epidermis is the outer layer of the herb plants stick, which is arranged alternately by one long cell and two short cells. Vascular bundle is a vascular tissue system consisted of xylem and phloem, where intra-xylem tightly packages the outside phloem. Herb plants cell can be divided into several kinds: (1) fibrocyte, which is taper off both sides; (2) parenchymal cell, with varieties of shapes rhabditiform, rectangle, square, and spheroidal; (3) duct, channel organization in plant with ringlike structure, heliciform, trapezoid, and reticulate, whose diameter is larger than that of fibrocyte; (4) epidermal cells; (5) sieve tube and companion cell; and (6) sclereid, it is sclerenchyma cell contrary to fibriform with spheroidal, oval shape, and quite much lignification, phellemmication, or cornification. The size is quite small.

Plant cell walls mainly contain middle lamella, primary wall, secondary outer layer, central layer, and inlayer. (1) The adhesion layer between cells is middle lamella where lignin and pectin are the main chemical components, and the corner of cell is called corner aero of middle lamella. (2) Primary wall is a sac-like matter formed at the primary growth of cell with amount of hemicellulose and lignin, which links with middle lamella closely. (3) Secondary wall is the wall layer formed in primary wall. It is the metabolic sedimentation of protoplast which is the major structure of cell wall. Secondary wall can be divided into three layers. The outer layer consists of cellulose and hemicellulose. Cellulose microfibers interlacedly wrapped around the fiber cell walls in order forming into inter-helix. The cellulose microfibers of central layer of secondary are helical arrangement parallel structure, which is almost parallel to the fiber axis, and the angle between microfibers and the fiber axis presents a gradient trend from inner to outer. The inlayer takes a small part compared to the whole cell wall. However, the wrapping pattern of microfibers is similar to that of the outer layer. Cellulose macromolecule chains regularly arrange in the protofibril which arranged in microfiber. Cellulose is formed by several microfibers arranged in

different ways. Previous researches proved that one protofibril with a 3 nm diameter consisted of about forty cellulose macromolecules. One sub-microfiber with a 12 nm diameter consists of 16 protofibrils, and one protofibril with 25 nm diameter consisted of four sub-microfibers. Hemicellulose monomolecular layer exists inside of protofibril which exists inside of sub-microfiber. Inter-microfiber is filled with hemicellulose and lignin, which are linked by chemical bond to form lignin–glycan complex polymer.

In order to recognize heterogeneity, corn stover is split into five morphological fractions based on functional and structural property at the plant organ level (Fig. 2.5). Table 2.2 shows that the chemical compositions of corn stover were significantly different at the plant organ level ($P < 0.05$). Among whole corn stover and five morphological fractions, stem rind had the highest glucan content and total sugar content (61.8%), while leaf sheath had the lowest glucan content and total sugar content (49.5%). Xylan and araban contents increase as follows: stem node < stem pith < stem rind < whole corn stover < leaf < leaf sheath. Acetyl content in different fractions showed a similar trend to xylan and araban. High acetyl content could facilitate the dissolution of hemicellulose, and hence the auto-hydrolysis effect in steam explosion [10]. Stem node had the highest lignin content, whereas stem pith had the lowest ($P < 0.05$). Higher lignification may hinder chemicals or enzymes permeating into plant cell wall and decrease the conversion efficiency [11]. Water extractives content increased in the order of leaf
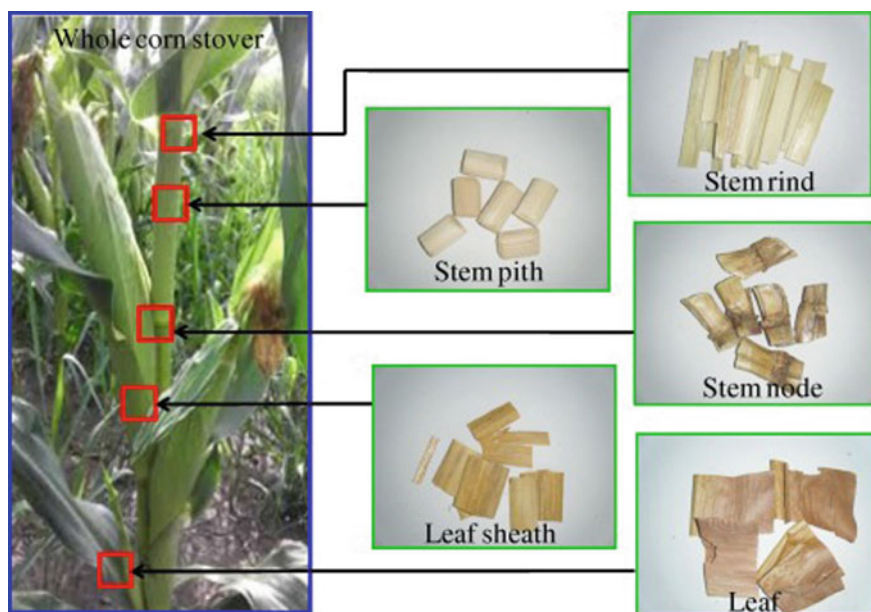

Fig. 2.5 Selective structure fractionation of corn stover biomass into five morphological fractions on the plant organ level

Table 2.2 Chemical compositions of whole corn stover and different morphological fractions before steam explosion

Compositions (% of total)	Different morphological fractions					
	Stem rind	Stem pith	Stem node	Leaf sheath	Leaf	Whole corn stover
Glucan	41.4 (0.6)	36.5 (0.9)	33.6 (0.5)	25.9 (1.2)	27.1 (0.8)	33.6 (1.1)
Xylan	17.7 (0.5)	16.6 (0.4)	15.9 (0.3)	20.2 (0.5)	19.8 (0.4)	19.1 (0.7)
Araban	2.7 (0.2)	3.0 (0.1)	2.8 (0.2)	3.4 (0.3)	3.1 (0.2)	3.1 (0.1)
Acetyl	2.7 (0.1)	2.4 (0.1)	2.4 (0.2)	3.5 (0.3)	3.2 (0.1)	2.9 (0.3)
Acid-insoluble lignin	17.9 (0.7)	13.8 (0.5)	18.0 (0.2)	14.4 (0.3)	15.3 (0.6)	16.1 (0.5)
Acid-soluble lignin	1.2 (0.1)	0.8 (0.1)	1.2 (0.2)	0.9 (0.1)	1.3 (0.1)	1.1 (0.1)
Water extractives	17.8 (0.3)	22.5 (0.5)	20.7 (0.4)	7.1 (0.6)	10.9 (0.5)	17.9 (0.7)
Glucose	8.1 (0.1)	9.5 (0.4)	8.2 (0.2)	2.4 (0.2)	1.7 (0.1)	7.7 (0.4)
Xylose	2.5 (0.3)	2.9 (0.2)	2.8 (0.3)	1.7 (0.1)	1.6 (0.2)	2.3 (0.1)
Ash	3.7 (0.1)	3.1 (0.2)	3.4 (0.2)	6.9 (0.3)	7.3 (0.2)	4.6 (0.1)
Total	105.1 (–)	98.6 (–)	97.9 (–)	82.3 (–)	88.0 (–)	98.4 (–)

Note Water-extractable glucose and xylose contents were calculated based on the total dry matter weight of feedstock; standard deviations are shown in parentheses

Table 2.3 Chemical compositions of whole corn stover and different morphological fractions after steam explosion

Steam explosion conditions	Steam-exploded solids	Glucan	Xylan	Araban	Acetyl	Lignin	Ash	Total
1.5 MPa, 6 min	Stem rind	53.4 (1.0)	5.9 (0.2)	1.1 (0.2)	0.2 (0.1)	31.2 (1.0)	2.3 (0.3)	94.1 (–)
	Stem pith	52.8 (0.9)	2.6 (0.3)	0.8 (0.1)	–	33.8 (1.1)	1.7 (0.1)	91.7 (–)
	Stem node	45.0 (0.3)	6.3 (0.5)	1.2 (0.2)	0.8 (0.1)	30.8 (1.3)	1.9 (0.2)	86.0 (–)
	Leaf sheath	39.5 (1.4)	7.6 (0.4)	1.4 (0.2)	–	26.5 (1.5)	3.1 (0.2)	78.1 (–)
	Leaf	41.5 (1.1)	7.0 (0.3)	1.3 (0.1)	–	27.4 (1.1)	3.2 (0.1)	80.4 (–)
	Whole corn stover	46.4 (1.0)	5.6 (0.2)	1.3 (0.2)	0.3 (0.1)	29.8 (1.0)	2.8 (0.1)	86.2 (–)
1.2 MPa, 6 min	Stem rind	48.4 (0.6)	7.3 (0.3)	2.0 (0.1)	1.1 (0.1)	27.8 (1.2)	2.4 (0.2)	89.0 (–)
	Stem pith	46.1 (1.0)	3.7 (0.5)	1.9 (0.1)	0.5 (0.1)	29.3 (0.9)	1.8 (0.1)	83.3 (–)
	Stem node	39.9 (0.7)	7.6 (0.4)	2.0 (0.2)	1.2 (0.2)	25.1 (1.3)	2.1 (0.2)	77.9 (–)
	Leaf sheath	35.3 (0.9)	9.1 (0.2)	2.7 (0.1)	1.0 (0.1)	22.7 (1.4)	3.5 (0.3)	74.3 (–)
	Leaf	36.7 (1.2)	9.5 (0.1)	2.6 (0.2)	0.8 (0.1)	23.6 (1.1)	3.6 (0.2)	76.8 (–)
	Whole corn stover	42.1 (1.1)	7.5 (0.3)	2.1 (0.2)	1.0 (0.1)	26.1 (1.2)	3.3 (0.2)	82.1 (–)

Note Lignin is acid-insoluble lignin; Standard deviations are shown in parentheses

sheath, leaf, stem rind, whole corn stover, stem node, and stem pith. Free sugars (glucose and xylose) showed a similar trend to water extractives. Interestingly, glucose content in stem pith, stem rind, and stem node is more than 8.0%, which is 3–4 times higher than that in leaf sheath and leaf. Free glucose content is easily degradable at high temperature, which should be adverse to the efficiency of steam explosion. The efficiency of auto-hydrolysis effect in steam explosion is closely related to the amount of ash content [8, 12]. Leaf sheath and leaf have higher ash content compared with other morphological fractions. These results suggested that different morphological fractions have different compositions and their unique effect on the efficiency of pretreatment and hydrolysis.

Chemical compositions of whole corn stover and different morphological fractions after steam explosion are given in Table 2.3. Results suggested that steam explosion increased glucan and lignin content while selectively dissolved xylan, araban, and ash. Glucan content in steam-exploded corn stover increased at 1.5 MPa and 1.2 MPa which were 11.5–16.3 and by 6.4–9.6%, respectively. Lignin content increased by 11.2–20.0% (1.5 Mpa) and 7.1–15.5% (1.2 MPa). However, xylan content in steam-exploded corn stover decreased by 9.6–13.9% (1.5 MPa) and 8.3–12.8% (1.2 Mpa) compared with that in untreated cornstalk. After steam explosion treatment, stem pith had the highest increased glucan and lignin content and the highest decreased xylan content at both 1.5 and 1.2 MPa, whereas stem node had the lowest. Acetyl content in stem pith, leaf sheath, and leaf was not detected at 1.5 MPa (Table 2.3), suggesting that all acetyl groups were removed after steam explosion. Ash content was also decreased after steam explosion especially in leaf sheath and leaf.

2.1.2 Solid-Medium Preparation Principles

High-solid and multi-phase is a bio-reaction process including solid matrix degradation and microbial growth. In general, solid matrix has complex structure caused by compact intertwine among the macro-molecular components. This natural structural feature exposed physical recalcitrance for degradation of the solid matrix and mycelium expand. In traditional solid-sate fermentation process, chemical nutrients have been focussed in culture medium preparation. However, physical properties of solid matrix are featured by porosity which has significant effect on matrix degradation and microbial growth. Specifically, accessibility governed by surface area and pore affects matrix degradation, while porosity affects mass and heat transfer in high-solid and multi-phase system. Therefore, apart from chemical nutrients in high-solid and multi-phase system, physical properties should also be paid attention. To be specific, large surface should be provided for enzyme binding or mycelium expanding, while pores are provided for enhancing heat and mass transfer.

2.2 Porous Properties of Solid Substrates

2.2.1 Cognition on Porous Properties of Solid Substrates

Porous medium is the common space occupied by the multi-phase materials and also a combination of coexisted multi-phase material. The space without solid skeleton is called pore, which is occupied by liquid phase or gas phase. Porous media has the characteristics such as porosity and heterogeneity. Porosity is the ratio of pore volume to total volume of porous media. The effective pore refers to part pore interconnected porous medium and not occupied by water, which is the ratio of effective pore volume to total volume of porous media. The dead-end pore is that one side of the porous media connects to other pores and another side of the porous media is sealed. Heterogeneity refers to the three phases, solid, liquid, and gas phases that can coexist. In porous medium, solid phase is the skeletons and the gas phase distributes in the unsaturated zone.

2.2.2 Correlation Between Substrate Porous Properties and Bioprocess

The lignocellulosic material is the typical multiscale porous material, which has the porous and frame construction. From crystalline region, amorphous region of cellulose, cell pit, cell lumen, cell wall, and vascular bundle to pellet, each scale of lignocellulosic biomass reflects the concepts of porous material such as porosity, pore size, and specific surface area. Lignocellulose aperture distribution with different dimensions is shown in Table 2.4.

There are obvious differences among coniferous wood, hardwood, and herbaceous plants in the organization structure.

In general, there are few types of cell in coniferous wood. Resin canal exists in coniferous wood, while catheter does not. Tracheid, the main component, has

Table 2.4 Lignocellulose aperture distribution with different dimensions

Level	Source of the pore size	Width (μm)	Level	Source of the pore size	Width (μm)
Tissue cell	Epidermis cell	20–35		Pit	0.5–50.0
	Pore	2–10		Plasmodesmata	0.03–0.06
	Vessel cell	30–130	Cell wall	Porosity between macrofibrils	0.001–0.100
	Sieve tube	5–50			
	Cribrate cell	5–50		Porosity between microfibrils	0.001–0.030
	Fiber cell	13			
	Intercellular layer	<1		Porosity between macromolecule	0.001–0.030
	Cube corner	≈1			

function of conducting and mechanical support, which accounted for over 90% of the timber volume; a typical cell is 3–5 mm long and 0.03–0.05 mm wide [5]. Hardwood consists of many types cell with high evolution degree. There is not resin canal in hardwood. However, catheter exists in hardwood which has function of conducting moisture, mechanical support to wood fiber. Broadleaf wood also contain three kinds of fiber cells including toughness wood fiber, fiber tracheid, and tracheid, which is known as wood fiber. Wood fiber is one of the important anatomical molecules of broadleaf timber with average length of 1 mm and the width of less than 20 μ. It accounts for 60–80% of timber volume [5]. Herbaceous plants have many cell types, including fiber cells, catheters, parenchyma cells, skin cells, screen, and companion cells. Catheter cell content in herbaceous plants is high and its diameter is much bigger than fiber cells. Stems, for example, their common characteristic is scattered identify vascular bundle throughout a large number of basic organization, not the boundaries between the cortex and the stele. Fiber cells distribute in exterior subcortical and vascular bundle, the average fiber length is 1.0–1.5 mm, and the average width is 10–20 μm, general accounting for volume content of 40–70% [5]. Basic organization takes a significant share in the stem and consists of parenchyma cells. Herbaceous plants in Gramineae stem center of basic parenchyma tend to fracture in the process of development and form hollow medullary cavity. Table 2.5 summarizes the different biomass fiber raw materials. Fiber closely packed in coniferous wood due to relatively few types of cells and is also known as the "non-pored wood". The fiber is long and the content is high in coniferous wood. It is the structural support of the plant material organization. Fiber cells usually have secondary wall due to the high lignification thickness and compact structure. Water vapor is difficult to penetrate the material inside the pore, causing a large thermal mass transfer resistance in steam explosion process. Hardwood has catheter tissue which plays the role of conveying water. Catheter cells are large and long pipe, affected by fiber arrangement regularity than coniferous wood, known as "porous wood". Parenchyma cell content is two times much more than that of softwood. Parenchyma cell is large with thin cell wall and short cavity. It plays the role of storing nutrition in plant growth. Those features in parenchyma cell are conducive to heat and mass transfer and water vapor in the flash physical tear. In herbaceous plants, the secondary xylem is embedded basic tissue (parenchyma). Large number of parenchyma is good for the heat transfer via water. At present, in the research of relationship of biomass physical structure and steam explosion, judgement of difficulty in material explosion is still based on qualitative analytic. Related structural parameters or specific theoretical model has not been formed to reflect the effect of multiscale physical structure of biomass on the steam explosion process transfer, the reaction, and blasting effect. Therefore, further cognitive about the structure foundation of steam-exploded biomass still has a long way to go.

Table 2.5 Structural characteristics of different kinds of biomass

	Fibrous materials of coniferous wood	Fibrous materials of broad-leaved wood	Fibrous materials of poaceae
Cell types	Simple composition,tracheid occupied about 90–95%, xylem ray occupied about 1.5–7%, axial parenchyma cells occupied about 7–8%, and epithelial cell occupied about 0–1.5%	Complex composition, wood fiber occupied about 60–80%, vessel element occupied about 20%, xylem ray occupied about 17%, and parenchymal cell occupied about 13%	Complex composition, fibrocyte occupied about 40–70%, with high content of parenchymal cell, and vessel, sieve tube, companion cells, and chrotoplast
Characteristics of arrangement	Lined up along the radial on the transverse section	As affected by the catheter, regularity of fiber is not good than coniferous wood, and big difference of different material regularities	Misaligned and scattered of vascular bundle
Parenchyma cells	Relative low content of xylem parenchyma, whose volume account for 7–8%	Relative high content of xylem parenchyma within 20%, more than twice as coniferous wood	High content of parenchymal cell, up to 46% in straw. Parenchymatous tissue is easily to be fractured and hollow medullary space is formed of Gramineae stem center in the development process
Fiber cells	Fiber is thick and long, playing a support and conducting role, with average length 3–5 mm and width 0.03–0.05 mm about 1/100 of its length	Fiber is short and thin。 wood fiber account for 60–80% of broad-leaved wood, with about average length of 1 mm and width within 20 μm	Average fiber length of 1–1.5 mm and average width of 10–20 μm. General fiber cells account for 40–70% of the total cells. And low content of corn stalk is about 30%
Duct	Nothing	There are ring, thread, scalariform, checker, and pitted vessel inner the duct. Duct cells arrange end, perforation and pit shape and size of the catheter itself pattern vary with tree species, are important basis for hardwood species identification	Relative high content of vessel cells, its diameter is much bigger than fiber cells, with shapes of circular, spiral, trapezoidal, and checker

<div align="right">(continued)</div>

Table 2.5 (continued)

	Fibrous materials of coniferous wood	Fibrous materials of broad-leaved wood	Fibrous materials of poaceae
Pit	Obvious fiber pit, the tracheid wall pit are bordered pit, wood ray parenchyma cells in the pit to pit, in the cross field are connected to the wood rays to pit tracheid form half of bordered pit. Different tree species with different shapes and sizes is the main basis to identify needles	Most of the fiber pit is not obvious	Fiber cell walls have a single pit, and some without pit
Resin duct	Part of coniferous possess with volume composition less than 1%	None	None

2.2.3   Enhancement of Seepage Transfer of Porous Solid Substrates for Bioprocess

Porous solid substrates of high-solid and multi-phase bioprocess may exist in some fluid; these may be condensed water which flows on the surface of porous solid substrates or penetrate into the pores. The movement of the fluid abides by certain rules which have a great impact on the heat and mass transfer in high-solid and multi-phase bioprocess. The rule of seepage fluid in porous solid substrates is called Darcy's law. The microorganisms and nutrients needed are present in the water phase as the form of solute, which transport with water as the carrier. Periodical dynamic change of air is a method to strengthen mass transfer and heat transfer for high-solid and multi-phase bioprocess, which is based on hydrostatics, and its power source is the normal force. High-solid and multi-phase bioprocess could be a biogeocenose in which quality, heat, and energy exchange can be in progress. The force of periodical dynamic changes of air with air pressure pulsation on the closed pressure vessel could make the molecular diffusion into forced convection diffusion, which could enhance heat transfer and mass transfer performance [13].

2.3   Water Properties of Solid Substrates

2.3.1   Cognition on Water States in Solid Substrates

Water is critical factor in high-solid and multi-phase bioprocess, but often being overlooked. Functions of water in pretreatment are concluded as follows:

(1) reactant, forming mild acidic conditions due to a decrease in the pKw of water at high temperature [14, 15]; (2) solvent or mass transfer medium of intermediates, end products, and inserted catalytic substances [16]; (3) heat transfer medium, determining the heat pattern and efficiency throughout the cellular structure [17, 18]; (4) plasticizer, maintaining a moist and soft texture of fibers which influence cell size and fiber strength [19]; and (5) explosion medium for analogous explosion pretreatments, tearing materials into small pieces and disrupting microstructures when immediate discharge of high pressure occurs [20]. As water is directly related to the interaction between substrate and pretreatment medium, most pretreated materials are dry substrates with water content (WC) $\leq 15\%$. Rehydration operation before pretreatment is necessary for enhancing pretreatment efficiency.

In recent years, in terms of relationship between water state and steam explosion, many researches have been conducted on the mechanism of auto-hydrolysis and optimization of water content in target materials [17, 21–23]. Cullis et al. evaluated the effect of water content on the bioconversion efficiency of softwood. Increased water content reduced the relative severity of steam explosion pretreatment, generating improved recovery efficiency of solids and hemicellulose-derived carbohydrate due to a "buffering effect" of the water [21]. Ewanick and Bura confirmed that SO_2 steam pretreated biomass increases ethanol yield by 18–28% after simultaneous saccharification and fermentation, indicating the positive effect of increasing water content on SO_2 permeability into biomass [22]. Ferreira et al. found that straw with high water content increased methane yield by 4–10%. However, high water content also increased heating energy, leading to high cost of the process [23]. The author has previously reported water transfer mechanism during multistage steam explosion process of corn stover and its contribution to processing bottlenecks because it gives important insights for designing industrial processes to impregnate cellulosic feedstocks with pure water, preservatives, or other materials [18].

Though scholars have recognized critical importance of water in biomass pretreatment technologies, their strides made from the point of water content are far from enough in elucidating water's acting essence in pretreatments, due to the complex interactions between water and plant biomass.

When water is adsorbed to lignocellulosic matrix, it is subjected to interactions caused by the chemical and physical compositions and the structure and composition of the plant produce different states and locations of water in turn [15]. Within the matrix, these associated water molecules have properties highly different from properties of bulk water. They become localized, more structured, and comparatively limited in available degrees of freedom, kinetic motion, and ability to exchange with other water molecules compared to water in the bulk [24]. These different water states certainly influence the feedstock properties and closely relate to pretreatment process, which should be the key issue that affects the extent of pretreatment efficiency. Thus, a deep understanding of water functions in pretreatment requires an adequate consideration of water states in the architecture of biomass, which provides insights into fundamental mechanism of water function contributing to the development of pretreatments.

2.3.2   Correlation Between Substrate Water States and Bioprocess

One of the main factors that influence high-solid enzymatic hydrolysis efficiency is the mass transfer [20]. Fully contact between substrate and enzyme is the premise of effective enzymatic hydrolysis [25]. However, free water decreases with increased solid content. When solid–liquid ratio is above 15%, water bound by lignocellulose turns into bound water and capillary water, resulting in hardly free water exists in the system, which is called "water-binding effect" of high-solid enzymatic hydrolysis [25–27]. In high-solid and multi-phase system, there are five main forms of water, including primary bound water, secondary bound water, capillary water, gravity water, and free water. Water-binding effect is closely related to lignocel-lulosic materials species, pretreatment method and condition, chemical composi-tion, and physical structure of the solid. Reduced free water significantly affects the mass transfer efficiency and rheological properties of enzymatic hydrolysis process. On one hand, water is the diffusion medium of enzymes and product, and takes part in the reaction in the enzymatic hydrolysis process as substrate. Reduced free water can slow down dissolution rate and transmission efficiency of product and enzymes, affecting the enzymatic hydrolysis efficiency. On the other hand, low free water content increases the viscosity of the system, increasing the requirement for mixing yield stress and energy consumption of mixing [26–28].

Roberts et al. studied the effect of combination of solid water and cellulose on mass transfer and enzymatic hydrolysis efficiency. High solid–liquid ratio increases the physical restriction of water in the system, and addition of glucose and mannose increases the water bound effect [25, 28]. Inhibition of enzymatic hydrolysis rate and increased water bound caused by monosaccharide showed the same tendency. Cellobiose was not found in the system, indicating that monosaccharide reduces the efficiency of saccharification by increasing water bound effect. There was a positive correlation between water bound and diffusion of monosaccharide and enzymes. Due to interaction between water and cellulose, water and soluble content, the system viscosity increased, leading to the increase of diffusion resistance and decrease of enzymatic and hydrolysis efficiency. Effective diffusion coefficient of BSA decreased by two-thirds with the solid–liquid ratio increased from 5 to 20%, and the intrinsic diffusion coefficient reduces by nearly 1/2 [28, 29]. Selig et al. investigated the relationship between water bound caused by macromolecule polymers and its inhibition on enzymatic hydrolysis. Relaxation time curve (T2) of added polymer system measured by low-field nuclear magnetic resonance close to zero within a short time and conversion rate of cellulose was decreased by adding polymer. The trend is consistent with water bound and reduced conversion rate, proving that water bound reduces the efficiency of high-solid enzymatic hydrolysis [30]. Selig et al. studied the influence of water availability and soluble substances on high-solid lignocellulosic saccharification. Effect of solute produced by enzymatic hydrolysis on water availability plays a determinative role in conversion rate during high-solid enzymatic hydrolysis process [31]. In addition, Hodge et al. studied

enzymolysis of corn stalk pretreated by dilute acid, finding that mass transfer become one of the main factors limiting conversion rate when solid–liquid ratio is higher than 20%. This can be explained as that mass transfer mainly depends on diffusion due to low degree liquefaction of the system, while convection efficiency is low. In addition, decreased water content limits the diffusion of the sugar from enzyme activity sites, inhibiting saccharification is suppressed, and enzyme is unreachable to new catalytic site [11]. Different types of lignocellulose, pretreatment, physics structure, and chemical composition also affect the water state and distribution of high-solid enzymatic hydrolysis process, and then affect the quality of mass transfer [31–34]. Mass transfer limit in high-solid enzymatic hydrolysis process significantly inhibits hydrolysis efficiency. Therefore, new strategies need to be developed to tackle this problem.

In order to investigate biomass–water interactions, the water pool (T2 relaxation time) distributions of untreated corn stover and steam-exploded corn stover under different moisture contents were determined, and the results are shown in Figs. 2.6 and 2.7, and Supplementary material 2 [35]. Results showed that the peak height and peak area of water pools in untreated corn stover and steam-exploded corn stover increased with the increase of moisture content. Figure 2.6 also shows that the T2 relaxation time of each water pool in steam-exploded corn stover and untreated corn stover increased with the increase of moisture content. The observations of extended relaxation times with the increase of moisture content may be due to the interactions between water molecules, which would be more frequent at higher moisture content. It is interesting to note that untreated corn stover had two peaks below fiber saturation point while steam-exploded corn stover had one peak (Fig. 2.6A, B). The reason may be that the order and rigid untreated corn stover makes the interactions of cellulose and water difficult. A part of water entered into the inner of cellulose fiber and the other part of water adsorbed to the surface of cellulose fiber. As for steam-exploded corn stover, the order and rigid structure were disrupted and cellulose fiber was obviously exposed by steam explosion, generating more accessible area for water. When moisture content was above 23%, the peak height of water pool in steam-exploded corn stover was higher than that in untreated corn stover (Fig. 2.6), while the peak width at half-height (Wh/2) was lower. Additionally, the T2 relaxation time of water pool in steam-exploded corn stover was obviously shorter than that in untreated corn stover under low moisture content (10–50%) (Supplementary material 2). These results indicated that the interactions of steam-exploded corn stover and water were stronger than those of untreated corn stover and water under low moisture content (10–50%).

Figures 2.6 and 2.7 show that water pools 1, 2, and 3 have appeared sequentially with the increase of moisture content. The result should be due to the fact that as water continues to be added and moisture content of biomass increases further, the biomass–water interactions in inner and/or surface of cellulose eventually reach an equilibrium state where water begins to accumulate in micropore and macropore thereafter. It is interesting to note that the peak height of main water pool in steam-exploded corn stover (pool 2 in Fig. 2.7B1, pool 2 in Fig. 2.7B2, and pool 3 in Fig. 2.7B3) was lower than that in untreated corn stover (pool 2 in Fig. 2.7A1, pool 3
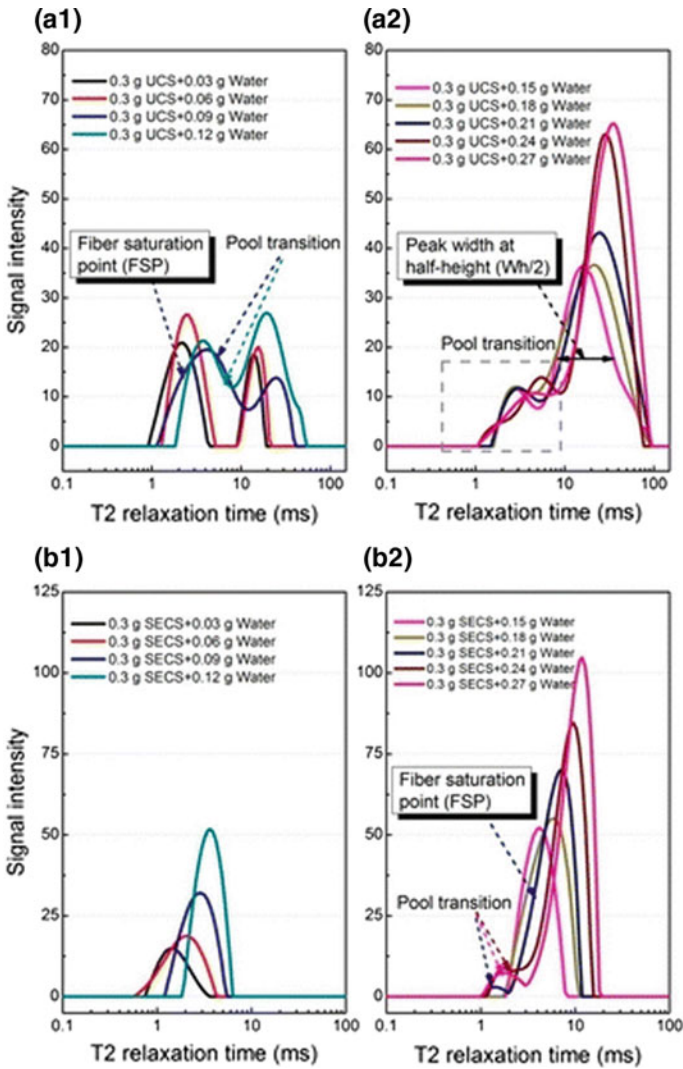
Fig. 2.6 Water pool distribution (T_2 relaxation time) and fiber saturation point of untreated (A1 and A2) and steam-exploded (B1 and B2) corn stover below 50% moisture content. UCS is untreated corn stover and SECS is steam-exploded corn stover. "0.3 g UCS + 0.03 g Water" stands for the mixture of 0.3 g of UCS and 0.03 g of water. Reprinted from Ref. [36]. Copyright 2013, with permission from Elsevier

in Fig. 2.7A2, and pool 3 in Fig. 2.7A3) at the same moisture content, respectively, while the peak width at half-height (Wh/2) of main water pool showed an opposite trend. Additionally, the T2 relaxation time of main water pool in steam-exploded corn stover was shorter than that in untreated cornstalk. It should be noticed that the peak height of water pool 3 in untreated corn stover was about 6–7 times higher than that of
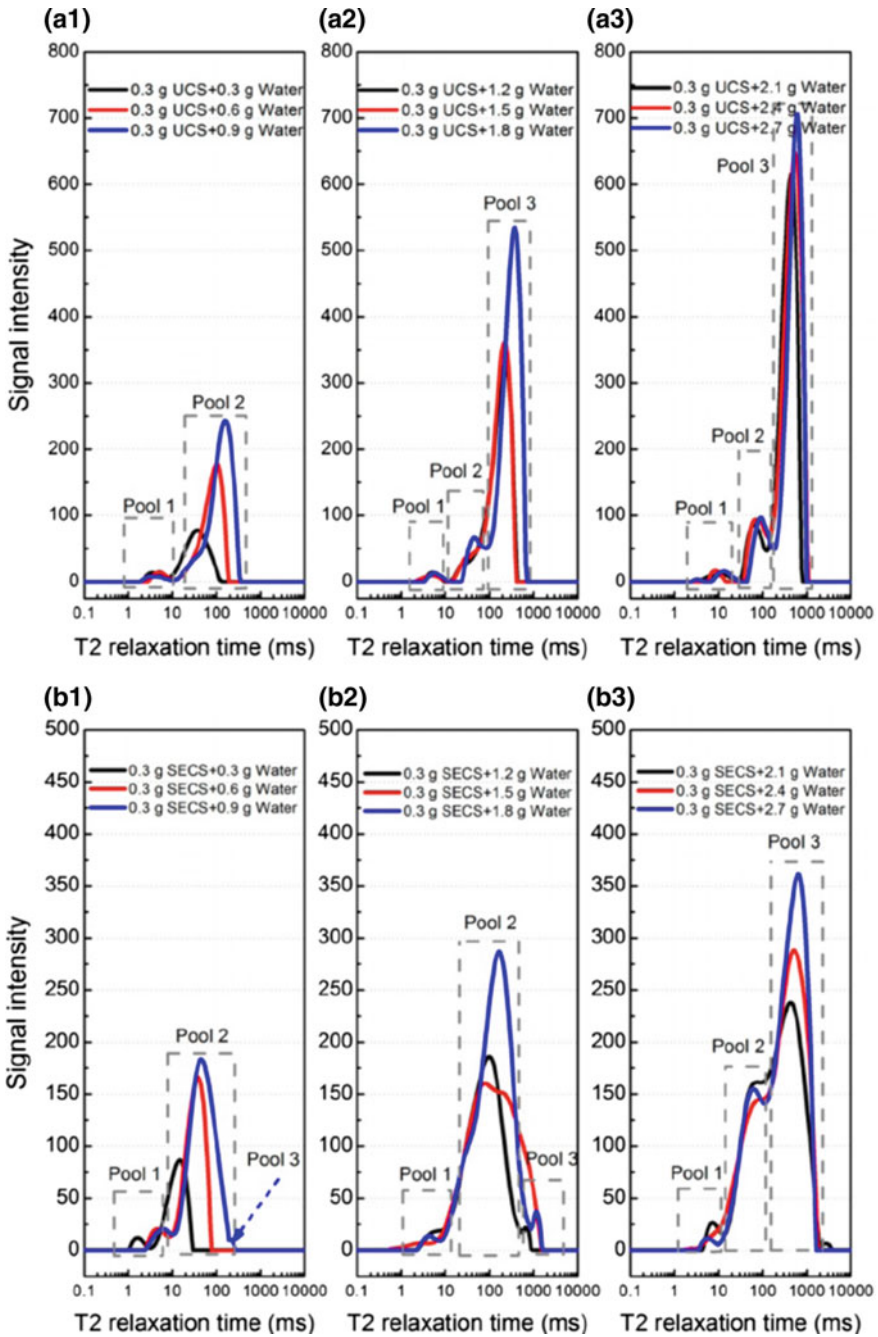
Fig. 2.7 Water pool distribution (T_2 relaxation time) of untreated (A1, A2, and A3) and steam-exploded (B1, B2, and B3) corn stover above 50% moisture content. UCS is untreated corn stover, and SECS is steam-exploded corn stover. Reprinted from Ref. [36]. Copyright 2013, with permission from Elsevier

water pool 2 beyond 87.5% of moisture content (Fig. 2.7A3), while the peak height of water pool 3 in steam-exploded corn stover was only 1.5–2.3 times higher than that of water pool 2 (Fig. 2.7B3). The states and locations of water in steam-exploded corn stover were obviously changed by steam explosion compared with these untreated cornstalks. Therefore, these results also indicated that the interactions of steam-exploded corn stover and water were stronger than those of untreated corn stover and water under high moisture content (50–90%).

The total peak area of water pools is closely related to moisture content, and the relations between total peak area and moisture content should reflect the interactions of biomass and water. The total peak area of water pools in both untreated corn stover and steam-exploded corn stover has positive correlation with the increase of moisture content (Fig. 2.8A), and the corresponding relations should conform to the exponential model as follows:

$$M(t) = A \times \exp(-t/T_0) + C_0 \tag{1}$$

where M is the total peak area; t is the moisture content, %; and A, T_0, and C_0 are the constants. The fitting results for the total peak area of untreated corn stover and steam-exploded corn stover with the increase of moisture content are given in Table 2.6, and the fitting exponential model was shown as follows (Fig. 2.8A):

$$M_{ucs} = 122.5 \times \exp(t/19.7) + 192.6 \tag{2}$$

$$M_{secs} = 105.3 \times \exp(t/18.9) + 127.3 \tag{3}$$

The correlation coefficient R^2 was 0.9990 for untreated corn stover and 0.9999 for steam-exploded cornstalk, respectively, which suggested that the exponential model can express untreated cornstalk–water interactions and steam-exploded cornstalk–water interactions well. Furthermore, it gave a conclusion that the interactions of untreated cornstalk/steam-exploded corn stover and water under lower moisture content were stronger than those under higher moisture content.

Compared with untreated cornstalk, the decreased total peak area of water pools in steam-exploded corn stover was also fitted with moisture content by exponential model (Fig. 2.8B). The correlation coefficient R^2 was 0.9338, and the fitting exponential model was given as follows:

$$\text{MDTPA} = 87.5 \times \exp(t/22.6) - 1.3 \tag{4}$$

Results suggested that the steam-exploded cornstalk–water interactions were stronger than the untreated corn stover–water interactions below 87.5% of moisture content especially below 50% of moisture content. The reason may be that steam explosion modified the structure and altered the composition of steam-exploded corn stover, and hence enhanced the interaction of steam-exploded corn stover and water under low moisture content. With the increase of moisture content, the
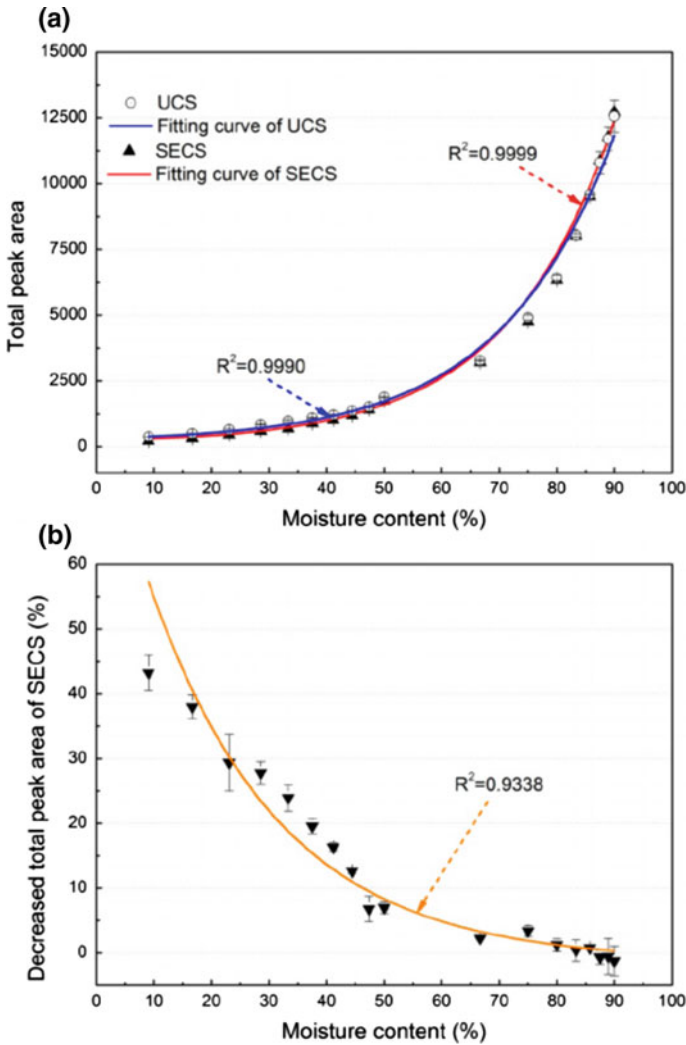
Fig. 2.8 Fitting model for the total peak area of water pools in untreated or steam-exploded corn stover with an increase in moisture content (A) and for the decreased total peak area of water pools in steam-exploded corn stover compared with untreated corn stover (B). UCS is untreated cornstalk, and SECS is steam-exploded corn stover [35]

interactions between water molecules became significant, resulting in the approximate total peak area of water pools for untreated corn stover and steam-exploded corn stover. Therefore, the exponential model can express the interactions of untreated cornstalk/steam-exploded corn stover and water well and also suggested that the interactions were stronger under lower moisture content.

Table 2.6 Exponential model fitting results for relations between total peak area of water pools in untreated and steam-exploded corn stover and moisture content. Reprinted from Ref. [35]. Copyright 2017, with permission from Elsevier

Samples	A	Standard error 1	T_0	Standard error 2	C_0	Standard error 3	R^2
M_{UCS}	122.5	9.8	−19.7	0.36	192.6	20.7	0.9990
M_{SECS}	105.3	13.6	−18.9	0.49	127.3	81.6	0.9999
M_{DTPA}	87.5	12.4	−22.6	2.2	−1.3	0.5	0.9338

2.3.3   Water-Binding Effects of Solid Substrates for Bioprocess

Water plays a key role in the enzymatic hydrolysis process. It is the medium for enzyme into the reaction site and the medium for product diffusing away from the reaction site. Additionally, water takes part in reaction and is the substrate of glycosidic bond hydrolysis during the enzymatic hydrolysis process, which determines the efficiency of enzymolysis reaction. Water existing in the lignocellulosic biomass enzymolysis system has five different states: primary bound water, secondary bound water, capillary water, limited gravity water, and free water, which is shown in Fig. 2.9. The water bonding with cellulose or other polysaccharides by hydrogen bonds is called primary bound water. Water adsorbed on the cell wall or surface glycan is called secondary bound water or film water. Water restricted in pores or voids by capillary force is called capillary water. The water restricted by macropore is called limited gravity water. Water which is not affected by solid or other solutes is called free water. The phenomenon that water reacts with other substances and causes fluidity reduced is called bound water effect, and the water is called bound water. With the increase of solid loading, the water is bound in the matrix and the free water reduces or even disappears, affecting the enzymatic hydrolysis efficiency.
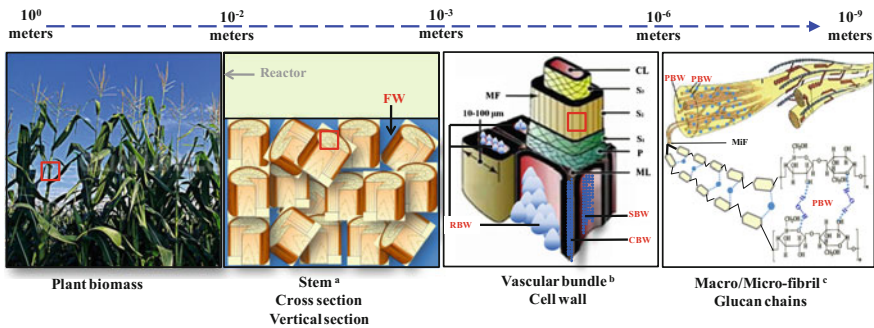
FW, Free water; RBW, Restricted bulk water; CBW, Capillary bound water; SBW, Secondary bound water; PBW, Primary bound water; MF, Macro-fibril; MiF, Micro-fibril; CL, Cell lumen; S, Secondary cell wall (S1, S2, S3); P, Primary cell wall; ML, Middle lamella.

Fig. 2.9 Schematic displaying water pools of lignocellulosic biomass in multi-length scale of its conversion process

2.4 Solid Rheology Properties in High-solid and Multi-phase Bioprocess

2.4.1 Solid Rheology Properties Variation in High-solid and Multi-phase Bioprocess

The rheology property of high-solid and multi-phase system is one of the key factors influencing enzymatic hydrolysis efficiency of lignocellulose conversion. It is also an important index of reactor design [29, 36]. Rheological property of high-solid and multi-phase system is influenced by physical and chemical properties of matrix, such as particle size and distribution, flexibility of fiber, elasticity of matrix skeleton, and material composition [36]. The water holdup affects rheological properties of mixture significantly when solid–liquid ratio is over 15%. The disappearance of solid particles lubrication results in a solid state of the enzymatic hydrolysis system. Rheological models were used to describe the enzymatic hydrolysis process of solid. Um and Hanley have studied rheological properties of enzymolysis, while the solid–liquid ratio is 15–20% and found that it conformed to the pseudoplastic fluid model [37]. Viamajala et al. found that particle size could significantly affect the rheological properties of mixture and further affect the energy consumption of agitation. Small particle size resulted in little viscosity, improving transformation efficiency. Knutsen and Liberatore found that adding surfactant can effectively reduce the yield stress of enzymatic hydrolysis, and elevated temperature can reduce viscosity to some extent [38, 39]. Increased solid–liquid ratio affects rheological properties significantly, affecting local material concentration, product removal, and heat transfer. In addition, rheological transformation demands higher stirring power and energy consumption, even agitation method is subversive, which brings new challenges to the process design and operations. Therefore, new strategy must be developed to alter the rheological properties so as to improve the efficiency of high-solid enzymatic hydrolysis.

Lignocellulosic mechanical properties (such as winding, intensification, tensile, compression, shear, and torsion) are the important parameters affecting process intensification. Although a few study of lignocellulosic mechanical properties has been reported (such as the bending force, moment of inertia, modulus of elasticity, flexural rigidity, shear strength, bending strength, the relationship between the straw stalk lodging resistance and compression characteristics, and the relationship between straw mechanical properties and harvesting and processing equipment with low energy consumption and high efficiency), the researches of intrinsic characteristics of lignocellulose mechanics are still less and dispersed. Particularly, the scholars did not recognize the intrinsic characteristics of lignocellulose mechanics for its important role on conversion process. There is no report about the inner relationship of lignocellulosic mechanical characteristics, rheology of system, and process intensification.

Plant biomass is a biological composite with multiscale structure and special molecular mechanics phenomenon [40]. In macro-scale, it is formed by intercellular

layer cells cohered into highly anisotropic natural porous materials. In microscopic scale, the plant cell wall can be regarded as natural fiber-reinforced composites composed of hemicellulose, lignin, and microfibril. In nanoscale, the plant biomass is high polymer materials composed of cellulose, hemicellulose, and lignin. The multistage structure shows that mechanism of macro- and micro-mechanical behavior is closely related to the steam explosion process. The macro- and micro-mechanical performance of biomass material directly determines the macro- and microphysical tear effect of steam explosion process. The effects include microlevel of material, the cracking damage degree, and the broken degree of cell wall. Biomass is featured by heterogeneous, anisotropic, and natural existence of micro- and macropore, defect, or damage. The irregular evolution behavior of initial pore, defect, or damage when biomass is loaded decides the macroscopic mechanical behavior [41]. Although mechanical properties of biomass have important influence on steam explosion effect, little related research has been reported. It is unclear that the resistance is based on biomass to steam explosion from the perspective of mechanics and physical damage mechanism of steam explosion.

At present, physical and mechanical properties of plant have been thoroughly and deeply studied, such as pulping, the drying process in wood processing areas. It could provide the reference and ideas for the studying of relationship between material mechanical properties and steam explosion process. The mechanical characteristics of raw material are greatly affected by the structure and composition change.

Wood is a multiple cell-layered biological composite material. From the view of mechanics, wood cells can be divided into two main kinds: one is wood fiber composed of vascular bundle, thick-walled cells, which is the main component in determining mechanical properties of wood. The other is the parenchyma cell which plays the role of uploading and buffer between the vascular bundle. Therefore, if the wood fiber is considered as reinforced body and the other parts are substrate material, timber can be seen as strong structural features of biological composite material. Its performance and damage rule depend not only on the mechanical properties of component materials, but also on its structural characteristics including the volume fraction, distribution, and shape of enhancement, as well as the nature of the interface.

Therefore, wood microstructure consists of many cells with different functions. In cell walls, orderly arranged cellulose chains form microfibril bundles, which is the main source of cell wall strength. Lignin fills in cellulose and enhances the mechanical strength of the cell wall. Besides cellulose, hemicellulose is another carbohydrate in the cell walls. It penetrates in the skeleton of material in an amorphous state and plays the role of cementation, enhancing the overall strength of fiber [40]. Factors influencing the mechanical properties of wood mainly include density, microfibril angle, chemical composition, and moisture content. Distribution, combination method, and the quality of wood cell wall components have important influence on its macroscopic mechanical properties. Water is one of important factors that affect the mechanical properties of the tracheid. Cell walls affect the intensity and elastic modulus. It affects mechanical properties of the tracheid through affecting structure and chemical composition performance. Microfibril angle is a micro-cellular structure characterization, which is closely related to the mechanical properties of the cell wall [40].

The efficiency of steam explosion is determined by the physicochemical property of plant biomass. Steam explosion included auto-hydrolysis stage and explosion stage after penetration of high-pressure steam into plant cell wall [42]. During auto-hydrolysis at high temperature and pressure, hemicellulose was solubilized and degraded. Acetic acid generated from acetyl groups further catalyzed the hydrolysis of hemicellulose. Lignin was melt, solubilized, and recondensed partly. Water soluble compositions were dissolved [10, 43]. The reaction rate (k) of these compositions was related with activation energy (Ea) according to Arrhenius formula:

$$\ln k = -Ea/RT + \ln A \tag{5}$$

Each chemical composition has its unique chemical potential energy and activation energy of reaction, resulting in the difference of reaction rate (k). Chemical compositions of whole corn stover and morphological fractions were varied, leading to a different reaction rate (k) during auto-hydrolysis at high temperature and pressure. Additionally, chemical bond connection mode of polymers in whole corn stover and morphological fractions is also different. Chemical bond mainly determined the reaction rate (k) of compositions. These results suggested that the auto-hydrolysis efficiency of steam explosion was determined by morphological fractions and the utilization of whole corn stover may be unsatisfactory. Selective fractionation can split plant biomass into different morphological fractions, which had their respective optimal steam explosion conditions for obtaining the high auto-hydrolysis efficiency.

During explosion stage in steam explosion, plant biomass particles are exploded into small pieces, the ordered structure is disrupted, and the cellulose fibers are separated by mechanical effect [10, 43]. The mechanical property of morphological fractions was measured by the compression method (Tables 2.7 and 2.8). Hardness of first cycle, reflecting the highest force used to fracture material, increased in the order of leaf < stem pith < leaf sheath < stem rind < stem node under 30% moisture content (Table 2.7). Stem node and stem rind had the maximum hardness, suggesting higher pretreatment severity was needed to achieve the same pretreatment performance as other morphological fractions. Hardness deformation percentage, reflecting the deformation of objects from force bearing point to fracture point, showed an opposite trend to hardness. Compression work of first cycle, which is the work to overcome the internal structure force of material, also increased in the order of leaf < stem pith < leaf sheath < stem rind < stem node. Results suggested that in order to obtain the same pretreatment efficiency, more work was needed in steam explosion to overcome the internal structure force of stem node and stem rind compared with other morphological fractions. Interestingly, adhesive force and adhesiveness showed a similar trend to hardness.

The mechanical property of stem pith with different moisture contents was determined by the compression method (Table 2.8). Hardness of first cycle increased with moisture content increasing from 5–90%. However, hardness deformation percentage and compression work of first cycle increased with moisture content

Table 2.7 Mechanical property of whole corn stover and different morphological fractions before steam explosion by compression method mode test. Reprinted from Ref. [42]. Copyright 2015, with permission from American Chemical Society

Parameters	Different morphological fractions (30% moisture content)					
	Steam rind	Steam pith	Stem node	Leaf sheath	Leaf	Whole cornstalk
Hardness of first cycle (g)	4085	244	6648.5	1261	190	–
Hardness deformation percentage (%)	19.4	30.7	21.2	30.7	31.3	–
Compression work done of first cycle (mJ)	49	5.1	67.5	21.9	3.4	–
Total work of 1st cycle (mJ)	49.1	5.1	67.6	22.9	3.8	–
Adhesive force (g)	68.5	12	220.1	32	2.5	–
Adhesiveness (mJ)	1.49	0.12	2.2	0.22	0.03	–
Elastic work done (mJ)	1	0.02	1.23	0.18	0.01	–
First fracture work done (mJ)	9.9	2.4	12.6	8.8	0.8	–
First fracture deformation percentage (%)	7.4	24.1	4.3	25.7	7.5	–

Table 2.8 Mechanical property of stem pith under different moisture contents before steam explosion by compression method mode test. Reprinted from Ref. [42]. Copyright 2015, with permission from American Chemical Society

Parameters	Moisture content of stem pith			
	5%	30%	60%	90%
Hardness of first cycle (g)	84	244	311	358
Hardness deformation percentage (%)	14.3	30.7	39.1	10.2
Compression work done of first cycle (mJ)	1.7	5.1	5.4	2.4
Total work of first cycle (mJ)	1.7	5.1	6.2	2.4
Adhesive force (g)	15.5	12	3	10
Adhesiveness (mJ)	0.29	0.12	0.11	0.2
Elastic work done (mJ)	0.14	0.02	0.06	0.17
First fracture work done (mJ)	0.4	2.4	2.3	0.9
First fracture deformation percentage (%)	8.2	24.1	24.2	9

increasing from 5 to 60%, and then decreased with the further increase of moisture content. Elastic work showed a similar trend to adhesive force and adhesiveness. Results suggested that moisture content significantly affected the mechanical property of stem pith because moisture can enter into plant cell wall and interact with the macromolecules by hydrogen bond and van der Waals force [15, 44], which changed the internal structure force of plant biomass. Furthermore, the swelling effect of cellulose should contribute to altering the mechanical property of plant biomass.

Mechanical property of plant biomass is key factor affecting the design and process optimization [45, 46]. It affects the energy consumption of stirring and hence the efficiency of mass transfer in enzymatic hydrolysis. The results of mechanical property measured by TPA method showed that hardness of first cycle of untreated solids increased in the order of stem pith < leaf < leaf sheath < stem rind < whole corn stover < stem node (Table 2.9). Apparent modulus, reflecting the difficulty of compression, showed the similar trend to hardness and total work of first cycle. Interestingly, apparent modulus of steam-exploded solids was 20–90% lower than that of untreated ones. Untreated stem node and stem rind obtained higher adhesive force and adhesiveness, while steam-exploded stem pith and whole corn stover showed higher adhesive force and adhesiveness. Elastic work reached the maximum value for whole corn stover before and after steam explosion.

Elasticity index is used to indicate the recovery of deformation. Before steam explosion treatment, stem pith had the highest springiness index, while whole corn stover had the lowest one. After steam explosion treatment, stem pith had the lowest springiness index, while stem rind had the highest one. Elasticity index increased by 14–65% after steam explosion treatment except for stem pith, whose elasticity index decreased by 50%. The reason was that SE disrupted the physical structure and changed the chemical composition of different morphological fractions and hence changed the springiness index.

Mechanical properties of whole corn stover at different solid loadings were determined using TPA method (Table 2.10). Hardness of first cycle of untreated whole corn stover decreased from 5800.0 to 434.0 g with decrease of solid loading from 95 to 40%. It increased to 702.0 g at 20% solid loading and then decreased to 32.0 g at 10% solid loading. Hardness of first cycle of steam-exploded whole corn stover decreased from 336.0 to 222.0 g with decrease of solid loading from 95 to 70%, and increased to 592.0 g at 20% solid loading and then decreased to 266.0 g at 10% solid loading. The possible reason was that whole corn stover mixture was swelled and the bulk density decreased with solid loading decreasing from 95 to 40%, resulting in the decrease of hardness. Along with decrease of solid loading from 40 to 20%, the bulk density increased, resulting in the increase of hardness. When solid loading was below 10%, whole corn stover mixture shows a slurry state, resulting in the decrease of hardness.

2.4.2 Correlation Between Solid Rheology Properties and Bioprocess

Rheology refers to the physical mechanics of material deformation or flow from the aspects of stress and strain. Stress–strain behaviors of steam-exploded whole corn stover and different morphological fractions were evaluated in enzymolysis. Hardness and total work of first cycle at 0 h in incubator shaker enzymolysis and periodic peristalsis enzymolysis with 10% solid loading increased in the order

Table 2.9 Mechanical property of whole corn stover and different morphological fractions before and after steam explosion with 5% (w/w) moisture content by texture profile analysis (TPA) method. Reprinted from Ref. [41]. Copyright 2015, with permission from American Chemical Society

Parameters	Untreated morphological fractions						Steam-exploded morphological fractions					
	Stem rind	Steam pith	Stem node	Leaf sheath	Leaf	Whole cornstalk	Stem rind	Steam pith	Stem node	Leaf sheath	Leaf	Whole cornstalk
Hardness of first cycle (g)	2666	352	8374	2598	2162	5800	358	564	1446	574	654	336
Compression work done of first cycle (mJ)	48	9.5	146.9	49.4	42	86	11.1	14.1	27.2	14.6	15.5	10.1
Recoverable work done of first cycle (mJ)	7.4	1.4	16.3	7.1	7.9	9	2.6	1.9	4.6	3	3.7	2.1
Total work of first cycle (mJ)	55.3	10.9	163.2	56.5	49.9	95	13.6	16	31.8	17.5	19.1	12.2
Apparent modulus (kPa)	118	18	391	111	89	317	29	15	61	16	20	24
Adhesive force (g)	20	4	10	6	6	6	8	14	6	8	6	8
Adhesiveness (mJ)	2.1	0.8	2.7	1.1	1.1	1.8	1.1	2.2	1.2	1.2	0.3	2.5
Elastic work done (mJ)	1.2	0.7	1.5	1	1.1	1.7	1	2.1	1.1	1	0.1	2.4
Hardness of second cycle (g)	1586	248	5718	1504	1320	3262	272	384	1032	404	494	252
Compression work done of second cycle (mJ)	10.4	3.9	46.4	12.2	11.6	16.1	6.5	3.7	8.8	5.2	7	1.9
Cohesiveness	0.22	0.41	0.32	0.25	0.28	0.19	0.59	0.27	0.32	0.35	0.45	0.18
Recoverable work done of second cycle (mJ)	3	0.8	8.6	3.7	4.5	4.4	1.8	1.6	2.7	1.9	2.3	1.3
Total work of second cycle (mJ)	13.4	4.7	55	15.9	16	20.5	8.4	5.3	11.5	7.1	9.3	3.2
Springiness index	0.33	5.02	0.39	0.38	0.41	0.25	0.48	0.29	0.33	0.46	0.47	0.41
Mean peak (g)	2126	300	7046	2051	1741	4531	315	474	1239	489	574	294

Table 2.10 Mechanical property of whole corn stover before and after steam explosion at different solid loadings by texture profile analysis (TPA) method

| Parameters	Solid loading (w/w) of whole cornstalk before steam explosion					Solid loading (w/w) of whole cornstalk after steam explosion				
	95%	70%	40%	20%	10%	95%	70%	40%	20%	10%
Hardness of first cycle (g)	5800	610	434	702	32	336	222	260	592	266
Compression work done of first cycle (mJ)	86	15.8	12.2	18.5	0.6	10.1	6.8	7.6	13.5	8.8
Recoverable work done of first cycle (mJ)	9	1.2	0.9	0.6	0.5	2.1	0.5	0.9	1.7	0.2
Total work of first cycle (mJ)	95	17	13.1	19	1.2	12.2	7.4	8.5	15.2	9.1
Apparent modulus (kPa)	317	19	12	22	1	24	5.8	7	19	6
Adhesive force (g)	6	8	12	22	12	8	22	12	10	12
Adhesiveness (mJ)	1.8	0.6	1.7	2.3	1	2.5	2.6	4	0.3	2.3
Elastic work done (mJ)	1.7	0.4	1.6	2.1	0.7	2.4	2.5	3.3	0.1	0.4
Hardness of second cycle (g)	3262	550	402	514	20	252	194	244	530	246
Compression work done of second cycle (mJ)	16.1	4.7	2.7	5.2	0.1	1.9	2.9	3.3	5.8	2.9
Cohesiveness	0.19	0.3	0.22	0.28	0.17	0.18	0.42	0.43	0.43	0.32
Recoverable work done of second cycle (mJ)	4.4	1	0.9	0.4	0.5	1.3	0.5	0.6	1.3	0.3
Total work of second cycle (mJ)	20.5	5.6	3.5	5.5	0.6	3.2	3.3	3.9	7	3.1
Springiness index	0.25	0.35	0.35	0.49	0.91	0.41	0.29	0.26	0.37	0.54
Mean peak (g)	4531	580	418	608	26	294	208	252	561	256
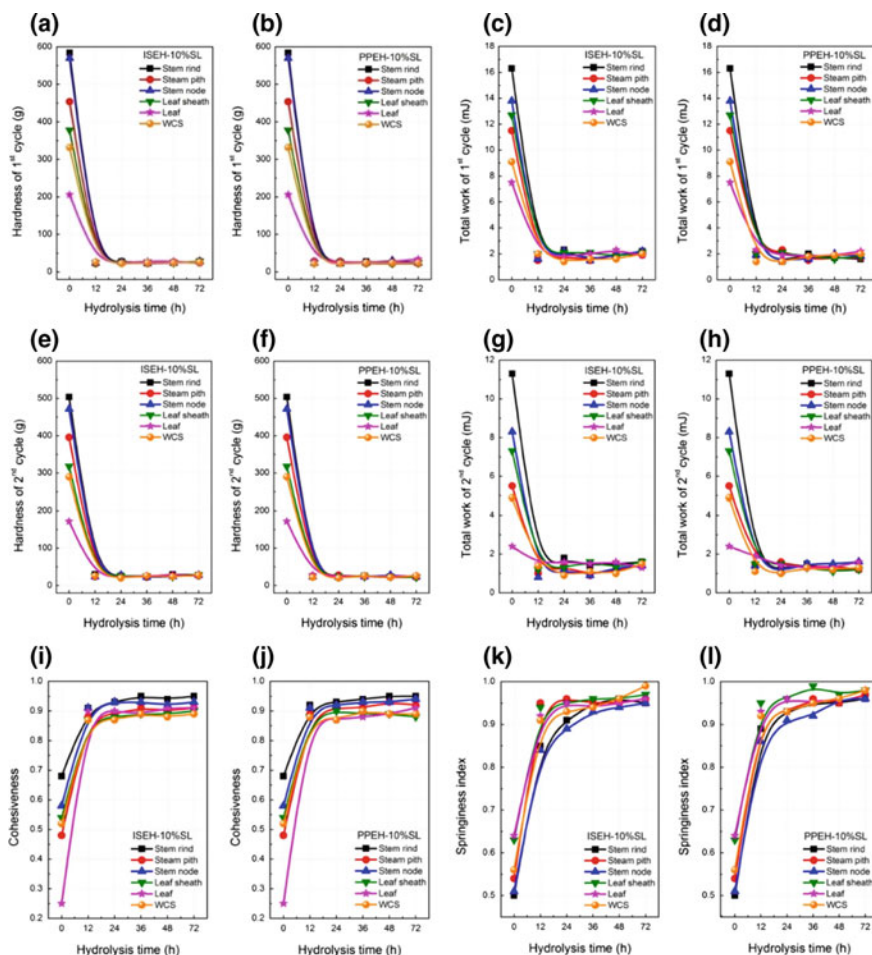
Fig. 2.10 Mechanical properties of steam-exploded whole corn stover (WCS) and morphological fractions in enzymolysis at 10% solid loading. ISEH, incubator shaker enzymolysis; PPEH, periodic peristalsis enzymolysis; SL, solid loading. Reprinted from Ref. [43]. Copyright 2015, with permission from American Chemical Society

leaf < whole corn stover < leaf sheath < stem pith < stem node < stem rind (Fig. 2.10A–D), and decreased to similar value after 12 h. Results suggested that treatment of stem mode and stem rind should be more difficult than that of whole corn stover and other morphological fractions. Hardness and total work of second cycle was lower than those of first cycle, showing same trends to those of first cycle (Fig. 2.10E–H). Interestingly, the maximum decrease of hardness and total work of hydrolysis mixture at 12 h was consisted with the maximum increase of glucan conversion. Results indicated that the mechanical property of corn stover was closely related to the enzymolysis performance. Viscidity increased in the order of
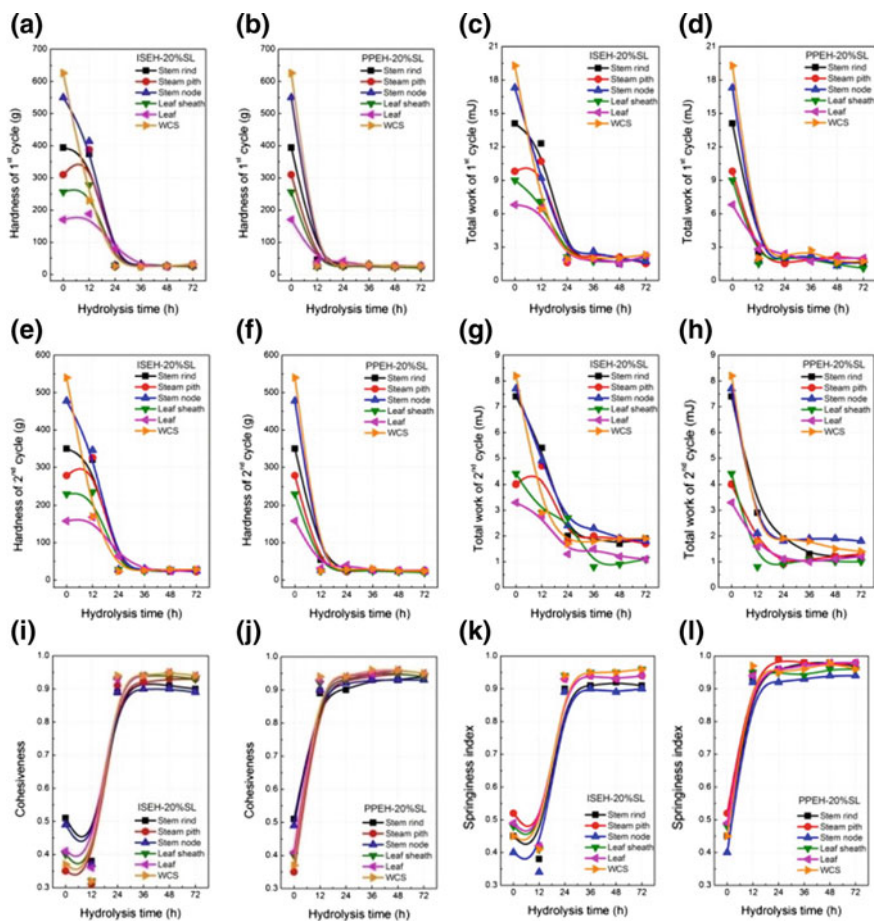
Fig. 2.11 Mechanical properties of steam-exploded whole corn stover (WCS) and morphological fractions in high-solid enzymolysis at 20% solid loading. ISEH, incubator shaker enzymolysis; PPEH, periodic peristalsis enzymolysis; SL, solid loading. Reprinted from Ref. [43]. Copyright 2015, with permission from American Chemical Society

leaf < stem pith < whole corn stover < leaf sheath < stem node < stem rind at 0 h in incubator shaker enzymolysis and periodic peristalsis enzymolysis (Fig. 2.10I, J). But it increased to 0.85–1.0 with hydrolysis progression. The higher cohesiveness indicated the stronger inner structure force of solid matrixes. The reason was that the hydrolysis mixture showed slurry state after 12 h and the hydrolyzed solid was effectively mixed with water. Elasticity index increased in the order of stem rind < stem node < stem pith < whole corn stover < leaf sheath < leaf at 0 h in incubator shaker enzymolysis and periodic peristalsis enzymatic hydrolysis (Fig. 2.10K, L), showing an opposite trend to hardness and total work. Results suggested that the mixing efficiency of steam-exploded stem mode and stem rind
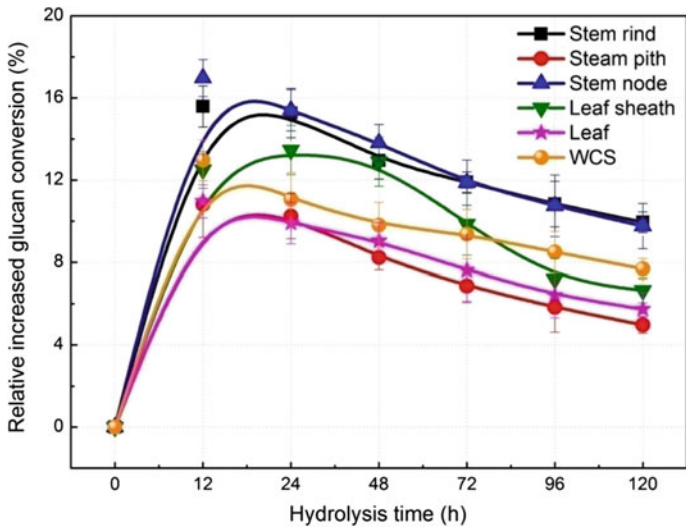
Fig. 2.12 Relative increased glucan conversions in periodic peristalsis enzymolysis compared with incubator shaker enzymolysis at 20% solid loading with an enzyme loading of 15 FPU/g glucan. ISEH, incubator shaker enzymolysis; PPEH, periodic peristalsis enzymolysis; WCS, whole corn stover. Reprinted from Ref. [43]. Copyright 2015, with permission from American Chemical Society

may lower than that of steam-exploded whole corn stover and other morphological fractions. Elasticity index increased to 0.9–1.0 after 12 h in incubator shaker enzymolysis and periodic peristalsis enzymatic hydrolysis.

As for 20% solid loading, hardness and total work of first cycle at 0 h in incubator shaker enzymolysis and periodic peristalsis enzymolysis increased in the order leaf < leaf sheath < stem pith < stem rind < stem node < whole corn stover (Fig. 2.11A–D). However, it should be noticed that they decreased with hydrolysis progress from 0 to 24 h in incubator shaker and 0–12 h in periodic peristalsis enzymatic hydrolysis. Hardness and total work of second cycle was lower than that of first cycle, showing same trends to those of first cycle (Fig. 2.11E–H). Results showed that periodic peristalsis enzymolysis reduced hardness and total work at 12 h compared with incubator shaker enzymolysis. The above results showed that periodic peristalsis increased the glucan conversion compared with incubator shaker enzymolysis at 12 h (Fig. 2.12). The reduction of hardness and total work by periodic peristalsis enzymolysis compared with incubator shaker enzymolysis was consistent with the increased glucan conversion. Interestingly, hardness and total work at 0 h showed the same trends to glucan conversion at the final stage of enzymatic hydrolysis except for whole cornstalk. Higher glucan conversion of whole corn stover can be explained as that the decrease of hardness and total work of whole corn stover were more rapid than that of five morphological fractions during hydrolysis process. Viscosity increased in the order of stem pith < whole corn stover < leaf sheath < leaf < stem node < stem rind at 0 h in incubator shaker
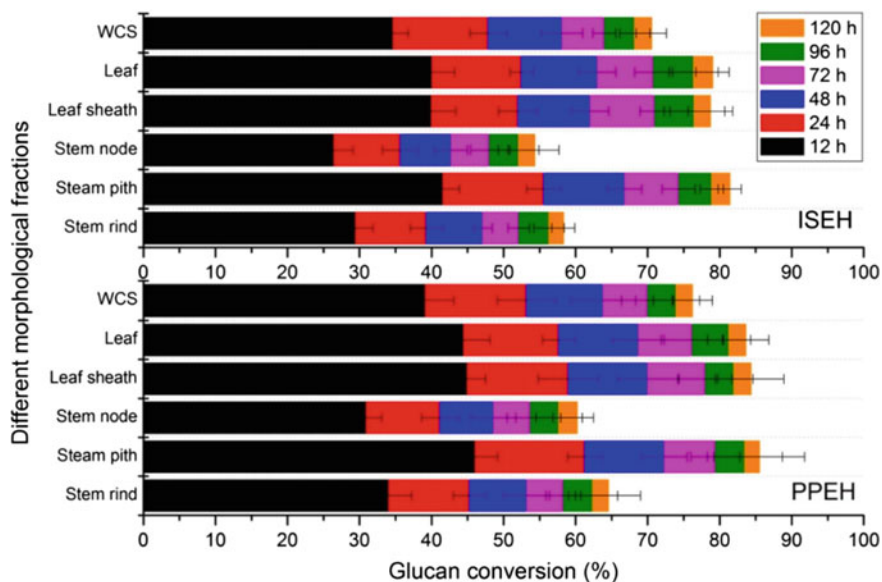
Fig. 2.13 Enzymatic hydrolysis kinetics of steam-exploded whole corn stover (WCS) and different morphological fractions at 20% solid loading. Steam explosion conditions: 1.5 MPa and 6 min; enzymolysis conditions: 15 FPU/g glucan, 120 h, and 30 rpm for periodic peristalsis enzymolysis and 200 rpm for ISEH. ISEH, incubator shaker enzymolysis; PPEH, periodic peristalsis enzymolysis. Reprinted from Ref. [43]. Copyright 2015, with permission from American Chemical Society

and periodic peristalsis enzymatic hydrolysis (Fig. 2.11I, J). The viscosity in incubator shaker decreased with hydrolysis time increasing from 0 to 12 h, and then increased to 0.9–1.0 with hydrolysis progression. But it increased from 0 to 12 h in periodic peristalsis enzymolysis. It can be explained that incubator shaker led to the agglomeration of steam-exploded solid before 12 h while periodic peristalsis rapidly converted the solid into slurry. Elasticity index increased in the order of stem node < stem rind < whole cornstalk < leaf sheath < leaf < stem pith at 0 h in incubator shaker and periodic enzymatic hydrolysis (Fig. 2.11K, L), showing an opposite trend to hardness and total work. Results suggested that the mixing efficiency of steam-exploded stem mode and stem rind may be lower than that of steam-exploded whole corn stover and other morphological fractions.

2.4.3 From Shearing-Force Stirring of Low Solids to Normal-Force Peristalsis of High Solids

The traditional mechanical agitation enhances molecular relative motion and admixture on the interfaces of gas–liquid and liquid–solid, and strengthens the mass
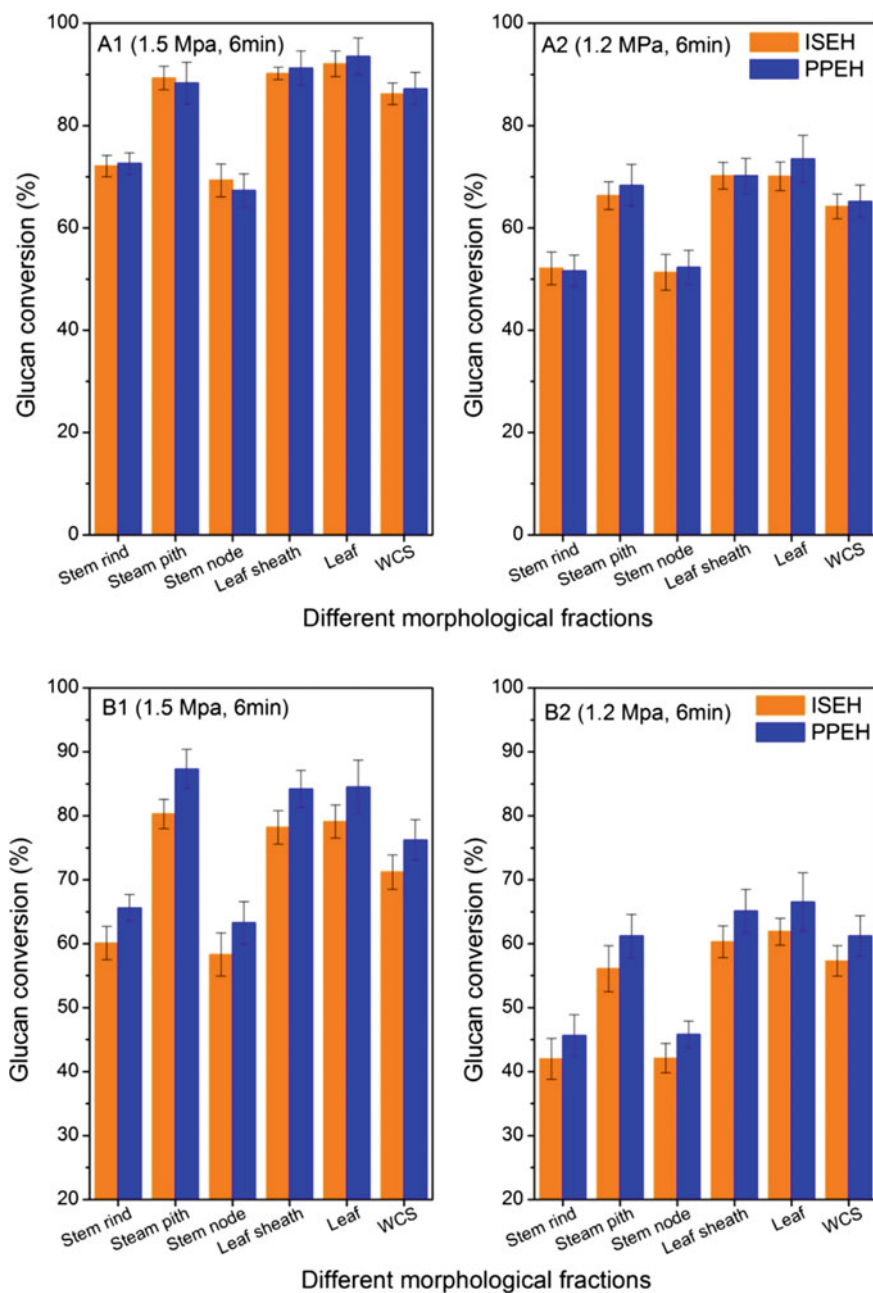
Fig. 2.14 Enzymatic hydrolysis of steam-exploded whole corn stover (WCS) and different morphological fractions at 1% (A) and 20% (B) solid loading. Steam explosion conditions: 1.5 or 1.2 MPa and 6 min. ISEH, incubator shaker enzymolysis; PPEH, periodic peristalsis enzymolysis. Reprinted from Ref. [43]. Copyright 2015, with permission from American Chemical Society
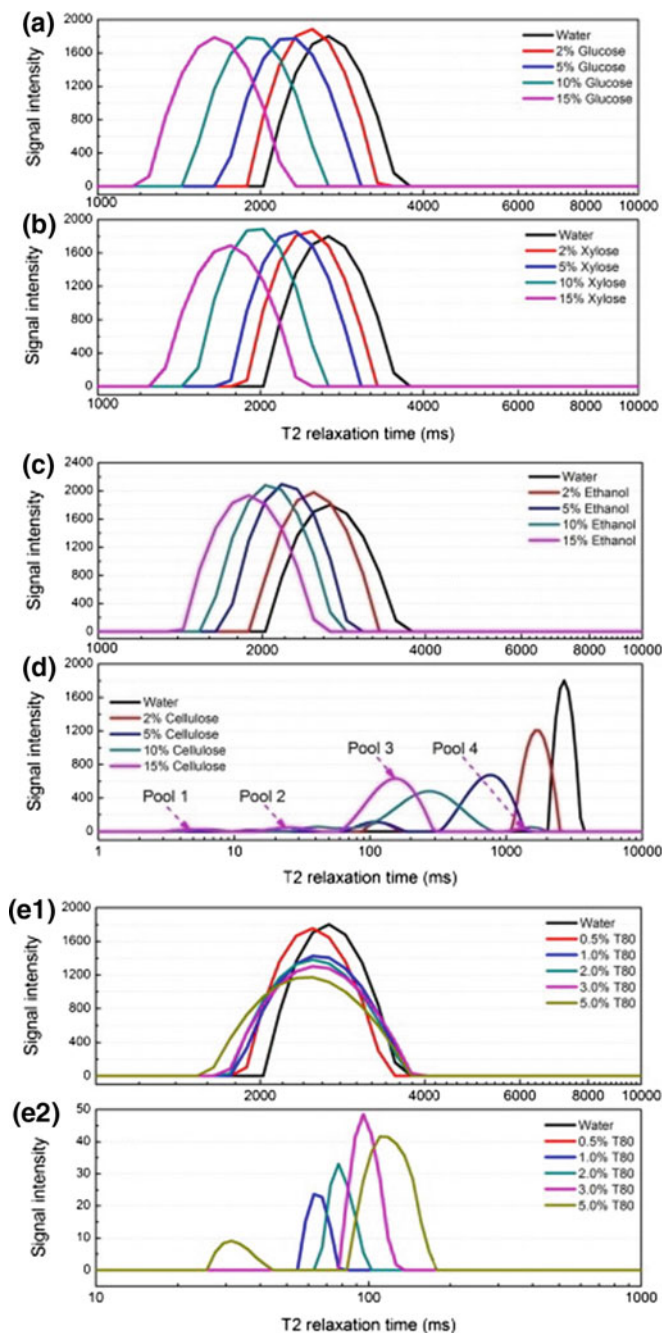
Fig. 2.15 Effects of chemicals (A for glucose, B for xylose, C for ethanol, D for cellulose, and E for Tween 80) on T_2 relaxation time of water pools in different solute concentrations (w/w). 2% glucose means that glucose concentration is 2% (w/w). T 80 is Tween 80. Reprinted from Ref. [48]. Copyright 2017, with permission from Elsevier

transfer and the heat transfer by tangential friction of momentum transfer. When mechanical stirring is applied to biological reactor, the organisms suffer the larger damage from stirring shear stress, leading to worse performance secondary metabolites synthesis. In high-solid and multi-phase bioprocess, in order to eliminate the destructive effect of shear stress caused by mechanical stirring on microbial, and enhance heat and mass transfer in reactor, periodic peristalsis is proposed. Periodic peristaltic avoids the effect of shear stress on microorganisms, which strengthens heat and mass transfer by periodic normal-force peristalsis [47].

Enzymolysis kinetics at 20% solid loading showed that glucan conversion of steam-exploded whole corn stover and five morphological fractions increased with the hydrolysis time in periodic peristalsis enzymolysis and incubator shaker enzymolysis (Fig. 2.13). Glucan conversion at the initial (at 12 h) and final (at 120 h) stage of enzymatic hydrolysis increased in the following order: stem node < stem rind < whole corn stover < stem pith < leaf sheath < leaf, which showed the same trend to the above results (Fig. 2.14).

Relative increased glucan conversion in periodic peristalsis enzymolysis compared with incubator shaker enzymolysis was calculated (Fig. 2.15). The relative increased glucan conversion among steam-exploded whole corn stover and five morphological fractions in periodic peristalsis enzymolysis compared with incubator shaker enzymolysis reached 10–17% before 24 h and then decreased to 5–10% with hydrolysis progression. Results indicated that periodic peristalsis significantly improved the glucan conversion before 24 h compared with incubator shaker. The relative increased glucan conversion at a specific hydrolysis time increased according to the order of stem pith < leaf < whole corn stover < leaf sheath < stem rind < stem node, which showed an opposite trend to glucan conversion. The possible reason was that stem node and stem rind had higher hardness compared with other morphological fractions, which reduced the mixing efficiency in incubator shaker enzymolysis at high-solid loading. Periodic peristalsis enhanced the mixing efficiency compared with incubator shaker, and hence increased the relative glucan conversion. Results suggested that the increase of glucan conversion by periodic peristalsis varied depending on morphological fractions.

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