Analytical Biochemistry 485 (2015) 86–96

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Hydrophilic compounds in liquids of enzymatic hydrolyzed spruce and pine biomass

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ARTICLE INFO

Article history:
Received 15 December 2014
Received in revised form 2 June 2015
Accepted 3 June 2015
Available online 10 June 2015

Keywords:
Wood
Fermentation
Bioreactor
Hydrophilic compounds
Capillary electrophoresis
Liquid chromatography

ABSTRACT

This study was focused on organic acids and metals in biofluids of wood. Without seasoning, fresh woods from spruce and bark, phloem, and heartwood from pine were used as materials, which were degraded with either microbes of oyster mushroom, baker's yeast, or lactic acid bacteria. Due to neutral pH of the fluids, ambient temperature, atmospheric pressure, and short reaction time, native wood microbe populations were supposed to be present. The water content of the fresh woods was 4 to 20%. The study showed that process methodology and experimental conditions affected the generation of lactic, citric, succinic, and adipic acids, which are considered as source chemicals in the biopolymer industry. In addition to the organic acids and metals, the process produced monosaccharides, polysaccharides, and phenolic acids such as benzoic, salicylic, cinnamic, vanillic, tannic, and conifer (ferulic) acids. Concentrations of total acids and acetic and succinic acids in pine fluids from bark, phloem, and heartwood were 58.4 g/kg and 3.5 to 6.9 g/kg, respectively. In spruce, the most dominant acids were L-lactic and L-malic acids. As for metals, Ag and Cr were detected at 0.01-g/kg quantities in pine bark. Alkali metals K, Mg, Sr, and Ca were detected at 10, 8, 1.3, and 4 g/kg, respectively.

Biomass has significant importance among different resources because it is renewable and can reduce CO_2 emissions to the environment [1]. Biomass is often described as plant-based materials. However, it includes a broad range of other materials such as wood, agricultural residues, food waste, industrial waste, and energy crops. The most abundant biomass on earth is wood. Examples of different wood materials include birch, spruce, pine, poplar, aspen, eucalyptus, and willow, which can be processed to biofuels or biochemicals with online coupled material pretreatment, hydrolysis, and fermentation technologies [2].

In Finland, the wood used in biomass production is pine, spruce, birch, aspen, and alder [3–5]. The main chemical constituents of all wood types are cellulose, hemicelluloses, and lignin. Furthermore, there are other polymeric compositions such as pectin, starch, and proteins that are not at high quantities. Beside those macromolecular components, nonstructural and low-molecular substances (extractives, water-soluble organics, and inorganics) are found in both hardwood and softwood, which have almost the same content of cellulose (40–45% of the dry solid). Softwood and hardwood also contain hemicelluloses and lignin, which are 25 to 30% and 30 to 35% of the dry wood solids, respectively [4–7]. For these reasons, wood biomass is an excellent matrix for innovative and novelty-value production of biochemicals. The processed products can be used as precursors to many industrially important compounds for the food, chemical, and pharmaceutical industries. As an example of the volumes in production, the global succinic acid market was projected to reach 144.7 thousand tons by the year 2015, driven by the anticipated rise in the use of bio-based succinic acid as an eco-friendly and low-cost replacement for the conventional petroleum-based succinic acid [8].

Traditionally, disassembling of trees for lignin degrading occurs with peroxidases and laccases enzymes [9]. However, biological pretreatment of organic material is done with microorganisms, which inter alia can be yeast or brown-, white-, and soft-rot fungi. They not only are selective to degrade lignin but also are important catalysts that enable solubilization and degradation of hemicelluloses. The most effective white-rot fungi microbes studied [10–13] are *Phanerochaete chrysosporium*, *Ceriporia lacerate*,

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http://dx.doi.org/10.1016/j.ab.2015.06.004

Cyathus stercolerus, Ceriporiopsis subvermispora, Pycnoporus cinnarbarinus, and *Pleurotus ostreatus* [2]. Although biological pretreatment of lignocelluloses is made with various fungi, none of them is superior because they all had equally good delignification efficiency. In addition to fungi, new types of bacteria have also been shown to improve selective fermentation. Some novelty-valued bacteria are very specific for succinic acid production. One of them is *Mannheimia succiniciproducens* MBEL55E, which has been isolated from bovine rumen. It is known to use xylose and glucose as the carbon source [10,14,15]. Unfortunately, those kinds of bacteria are very expensive in routine processes because their isolation and use in process demand specific instrumentation. They are seldom useful when the whole process system is online coupled with the whole process in industry.

In biological processes, the starting wood material may also be treated by hydrolyzation with a strong mineral acid such as sulfuric acid. In that case, cellulose degrades to hexoses. Simultaneously, the wood hemicelluloses degrade to acetic acid and pentose, which are further changed to furans with an extension reaction. Thereafter, the reaction goes to furfural and other wood-based compounds. Furthermore, wood lignin goes to phenols and thereafter to phenolic compounds of low molecular weights [10]. Lignocelluloses can be processed to biochemicals and fuels with pretreatment, hydrolysis, fermentation, and product separation [2,14–16]. Currently, lignocelluloses are converted via saccharification followed by biochemical or catalytic process to sugars. The process is time-consuming because it still needs either acid or enzyme hydrolysis. After the bioprocesses, the valuable chemicals are in solutions, which is why usually the biomass liquids need screening and identification and not the solid parts [14].

Lately, a great deal of attention has been given to biotechnological products, especially when they are produced by cell cultivation technology [16–18]. In pilot scale, bioreactor processing and biocompound separation are the two essential technical platforms that are needed to provide evidence of production methods of a new technology with profitability. It is also an axiom that only a combination of monitoring and identification of bioprocesses has potential both to improve the understanding of the processes and to develop the ways to influence and control them. When optimizing bioprocesses, the lack of accurate real-time data and the different effects of physical, chemical, and biological hydrolysis may represent a significant bottleneck phenomenon. With that in mind, we have recently used an online capillary electrophoresis (CE)[1] system to control and monitor carboxylic acid production in the presence of *Kluyveromyces lactis* and *Saccharomyces cerevisiae* during two different bioreactor processes [17–20]. The successful tests demonstrated that this kind of a system was really needed to maximize the biotechnological processes in an economical way and to monitor the reliability of the process using natural materials.

In enzymatic hydrolysis, usually the enzymes are naturally occurring proteins that have catalyzing effects for specific reactions in enzymatic and direct microbial conversion [20]. The important advantage of biochemical hydrolysis is that degradation of carbohydrates can be prevented with the use of enzymes favoring 100% selective conversion of cellulose to glucose. Nevertheless, such processes are slow due to structural characteristics of lignocelluloses such as lignin and hemicelluloses content, surface area, and cellulose crystallinity [14]. The advantage of degradation of wood in biological methods is that, due to pH 4.8 and low temperature (45–50 °C), the process itself does not cause unknown variables in the system such as corrosion of the reactor materials by complicating the reaction kinetics. Nowadays, using newly developed enzymes, high yields of the products (75–85%) may be obtained. Enzymatic hydrolysis is also an environmentally friendly alternative to mineral acidification.

In traditional biological treatment of wood, carbohydrate degrading enzymes, such as celluloses and hemicelluloses that are used to hydrolyze lignocelluloses to fermentable sugars [18], activate the reactions. They are further changed to organic acids and interesting medical products [16–20,14,21]. Although wood hydrolytes are good sources for glucose and xylose, the fluids contain toxic or inhibitory compounds for cells, resulting in low efficiency of bioconversion. Lately, the toxic compounds have been identified to be furfurals and compounds with similar structure [22]. There are not many microbes that can resist the toxicity of furfurals. However, oyster mushrooms, which live in hardwoods, excrete enzymes that stand for the toxicity and can break organic bonds in wood materials in the presence of furfurals.

In this study, we show a new way to process biomass using lignocellulosic spruce and pine bark, phloem, and heartwood as carbon sources and using yeast and oyster mushroom fungi as microbial sources. Fermentation of the chips was done with microbes to prevent the high production of furfurals, which are processed by mineral acid catalysis.

Oyster mushrooms live in hardwoods. They excrete enzymes that break organic bonding in wood materials. Therefore, because oyster mushroom microbes are also efficient in toxic chemical solutions, they were preferred in the study. The current study was conducted to identify the main organic acids in spruce and pine bioliquids after they were processed with microbes. The wood materials were carefully selected and were degraded with microbes that were not earlier used for the wood processing. The intention was to study the use of the new methodology to process valuable organic acids that are possibly used as monomers for polymerization of new biopolymers. Furthermore, metals in nitric acid extracts of pine species were investigated to estimate their concentrations in the wood. Metals were assumed to originate from the soil and environment.

Materials and methods

Materials

The organic acid chemicals were at analytical purity grade. Acetic acid, adipic acid, ascorbic acid, butyric acid, citric acid, ferulic acid, formic acid, fumaric acid, D-lactic acid, L-lactic acid, maleic acid, D-malic acid, L-malic acid, malonic acid, oxalic acid, pyruvic acid, salicylic acid, succinic acid, and tartaric acid were Sigma–Aldrich, Merck, and J.T. Baker products. Sodium hydroxide pellets (purity > 99%), sodium hydroxide (1 M) solution, and hydrochloride acid (37%) were purchased from Merck (Darmstadt, Germany). Acetonitrile (purity $\geqslant$ 99.8%) was obtained from J.T. Baker, benzoic acid was obtained from Fluka, and water-free methanol (purity $\geqslant$ 99.9%), *trans*-cinnamic acid, vanillic acid, *N*-cyclohexyl-3-aminopropanesulfonic acid (CAPS), and 2,3-pyrazine-dicarboxylic acid (97%) were obtained from Sigma–Aldrich (Germany). Myristyltrimethyl ammonium hydroxide (MTAH) was obtained from Waters. The inorganic salts used for preparation of electrolyte solution for organic acids were $CaCl_2{\cdot}2H_2O$ and $MgCl_2{\cdot}2H_2O$ (99%, Riedel-de Haen, Germany).

The inductively coupled plasma atomic emission spectroscopy (ICP–AES) standards (Ag, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, P, Pb, S, Sn, Ti, V, and Zn) were commercial products. Calcium and silver solutions were obtained from ROMIL (Waterbeach, Cambridge, UK). Titanium and some other elements

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[1] *Abbreviations used:* CE, capillary electrophoresis; CAPS, *N*-cyclohexyl-3-aminopro panesulfonic acid; MTAH, myristyltrimethyl ammonium hydroxide; ICP–AES, inductively coupled plasma atomic emission spectroscopy; LC, liquid chromatography; UV, ultraviolet; HPLC, high-performance liquid chromatography; DAD, diode array detector; MAE, microwave-assisted extraction; RSD, relative standard deviation.