Available online at www.sciencedirect.com

ScienceDirect

http://www.elsevier.com/locate/biombioe

Fermentative metabolism of an anaerobic, thermophilic consortium on plant polymers and commercial paper samples

Sarah M. Carver [a,b,1], Michael C. Nelson [c,2], Zhongtang Yu [c], Olli H. Tuovinen [a,b,*]

[a] Department of Microbiology, Ohio State University, 484 West 12th Avenue, Columbus, OH 43210, USA
[b] Department of Chemistry and Bioengineering, Tampere University of Technology, P.O. Box 541, FI-33101 Tampere, Finland
[c] Department of Animal Science, Ohio State University, 2029 Fyffe Road, Columbus, OH 43210, USA

ARTICLE INFO

Article history:
Received 10 May 2014
Received in revised form
21 January 2015
Accepted 6 February 2015
Available online 27 February 2015

Keywords:
Anaerobic biodegradation
Biohydrogen
Cellulose biodegradation
Fermentation
Plant polymers

ABSTRACT

The purpose of the study was to examine the feasibility and capacity of a thermophilic microbial consortium to produce fermentative metabolites from plant polymers. The consortium comprised of cellulolytic anaerobes that were originally enriched from a compost pile using cellulose as the substrate. Fermentative metabolism was examined with monosaccharides, disaccharides, hemicellulose, starch, pectin, chitin, and eight commercial paper samples without further enrichment of the culture to each specific substrate. In general, H_2, CH_4, CO_2, and organic acids were the main metabolites on all substrates but the metabolite profiles varied with the substrate. Similar H_2 yields of 2–3 mol mol^{-1} substrate at 48 h were obtained with all monosaccharides and disaccharides. The CO_2 yields were higher with disaccharides than with monosaccharides, 4.5 vs 2 mol mol^{-1} substrate. Metabolite yields were relatively low with glyceraldehyde, glycerol, and arabinose. Paper samples containing high amounts of chemical pulp produced the highest metabolite yields, and biodegradation accounted for ≤74% of total dry weight loss. The fermentative metabolism of the paper samples varied with the pulp composition and the amount of inorganic material. Bacterial community analysis using pyrosequencing analysis of 16S rRNA gene showed a predominance of members of the order Clostridiales, including members of genera *Clostridium* and *Lutispora*, which contain known cellulolytic organisms. Most differences among the samples were attributed to small taxonomic groups represented by ≤10% of total sequences.

* *Corresponding author*. Department of Microbiology, Ohio State University, 484 West 12th Avenue, Columbus, OH 43210, USA. Tel.: +1 614 292 3379; fax: +1 614 292 8120.
E-mail addresses: carver.84@gmail.com (S.M. Carver), michael.c.nelson@uconn.edu (M.C. Nelson), yu.222@osu.edu (Z. Yu), tuovinen.1@osu.edu (O.H. Tuovinen).
[1] Present address: Central Research Service, Kraft Foods Group, Inc., Northfield, IL 60093, USA.
[2] Present address: Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06269, USA.
http://dx.doi.org/10.1016/j.biombioe.2015.02.005
0961-9534/

1. Introduction

Recent research into sustainable energy has focused on cellulose and cellulosic biomass as renewable resources. Agricultural and wood residues and other forms of plant biomass are complex mixtures of polymers that include cellulose, hemicelluloses, lignin, starch, and pectin. Cellulose microfibrils are buried within a multifaceted matrix that is covalently bound with other polymers [1–3]. The most readily biodegradable plant polysaccharide is starch, a polymer composed of glucose linked with α1-4 and α1-6 glycosidic bonds [4]. Hemicellulose is a heterogeneous substrate with a backbone of β1-4 linked xylose with branches of pentoses and hexoses, including arabinose, mannose, and glucuronic acid. Some of the saccharides within the hemicellulose structure are modified through reactions such as esterification [5,6]. Pectin, commonly present in fruit biomass, consists of an α1-4 galacturonic acid backbone with side chains of fucose, xylose, and/or rhamnose with a high degree of methylation and acetylation [5,7].

Residues containing plant polysaccharides have been shown to be good substrates for value-added products such as ethanol, methanol, methane, and biohydrogen [8–10]. Pulp and paper mills generate both liquid and solid residue streams that contain many biodegradable plant polysaccharides. Within US landfills, the paper and cardboard fraction has decreased from 38 to 28% of the total solid waste between 1999 and 2009 [11,12]. Although recycling rates have increased, paper and other cellulose products still constitute a major fraction of municipal solid waste and could provide a large source of renewable feedstock for bioenergy purposes.

In paper and cellulose manufacturing, pulp is generated through mechanical, chemical, or combined processes. Mechanical pulping utilizes physical forces in the presence of water to strip fibers from wood and suspend the free fibers in water. This process recovers 90–95% of the wood fiber but the pulp is of a low quality with hemicelluloses and recalcitrant lignin present [13,14]. Chemical pulping utilizes either acidic, H_2SO_3 and HSO_3^-, or alkaline, NaOH and NaS_2, chemicals along with heat and/or pressure to separate the fibers. While the pulp yield is 40–50%, much lower than that of mechanical pulping, the pulp is of higher quality as lignin and some hemicelluloses are dissolved during the chemical process [15]. After pulping, the slurry is diluted and mixed with the biologically inert mineral fillers necessary for mechanical strength and integrity before it is pressed into paper. Bleaching of the pulp prior to pressing is optional and likely affects the polymer characteristics. The paper can be surface coated, usually with conventional pigments (e.g., kaolin, $CaCO_3$, TiO_2) or silica pigment and polyvinyl alcohol, to achieve the desired final paper quality, gloss, and void fraction of ink volume for printing. Different pulping and paper making processes can result in residue streams that vary in types and amounts of individual substrates, which can affect bacterial degradation and fermentation.

Paper products have been tested as a feedstock for simultaneous saccharification and fermentation to ethanol [16–18] or lactic acid [19–21]. Ntaikou and co-workers [22] tested paper biodegradation with *Ruminococcus albus*, a cellulolytic anaerobe, and reported a positive relationship between total carbohydrate content in the paper and high H_2 yields. To date, however, the composition of the pulp has not been considered in paper biodegradation studies.

In our previous study, we investigated cellulose biodegradation by a thermophilic, anaerobic consortium TC52 [23]. A subculture of TC52, which was maintained on cellulose at 60 °C, was previously found to contain mostly *Clostridium*, *Acetivibrio*, and *Thermoanaerobacter* spp. [23]. Because of the phylogenetic diversity of the TC52 consortium, it was hypothesized that the culture can use a broad spectrum of plant-derived complex substrates. In view of the capacity of TC52 to produce value-added metabolites from cellulose, the present study was undertaken to examine the fermentative metabolism of TC52 with multiple carbon sources including monosaccharides, disaccharides, plant polysaccharides, and eight types of commercial paper. Pyrosequencing of 16S rRNA genes of representative samples was used to characterize changes in the makeup of the consortium during the degradation of the polysaccharides.

2. Materials and methods

2.1. Substrates

Simple sugar substrates were ≥98% pure forms of glyceraldehyde, glycerol, ribose, arabinose, xylose, glucose, galactose, fructose, mannose, lactose, sucrose, cellobiose, and maltose. Polysaccharides analyzed were chitin from crab shells, amylose, and pectin from apples (Sigma–Aldrich, St. Louis, MO). A heterogeneous hemicellulose of unknown composition was obtained from a paper mill company (UPM Corp., Helsinki, Finland). The hemicellulose sample was homogenized with a mortar and pestle before use. Commercial paper samples were obtained from two paper mill companies (UPM and M-Real Corp., Helsinki, Finland). Information provided by the companies on the standardized paper composition is listed in Table 1. Stock paper samples were manually cut to approximately 0.5 × 0.5 cm for use as a substrate and designated as paper types A–H.

2.2. Culture conditions

The microbial consortium, designated TC52, originated from the interior of a compost heap through enrichment on cellulose as the sole substrate at 55 °C. It has been maintained on cellulose for years and used in two previous studies [24,25]. The TC52 consortium was grown anaerobically in medium containing (per dm^3) 2 g trypticase, 1 g yeast extract, 4 g Na_2CO_3, 0.23 g K_2HPO_4, 0.18 g KH_2PO_4, 0.36 g NH_4Cl, 0.04 g NaCl, 0.09 g $MgSO_4 \cdot 7H_2O$, 0.06 g $CaCl_2 \cdot 2H_2O$, 5.66 g acetic acid, 1.62 g propionic acid, 0.68 g *n*-butyric acid, 0.23 g isobutyric acid, 0.20 g isovaleric acid, 0.20 g *n*-valeric acid, 0.20 g 2-methylbutyric acid, 0.001 g resazurin, 0.25 g cysteine-HCl, 0.25 g $Na_2S \cdot 9H_2O$, and 4 g microcrystalline cellulose (Sigmacell Type 20, Sigma–Aldrich).

Monosaccharides and disaccharides were prepared anaerobically as sterile 50 mmol dm^{-3} stock solutions and added as single substrates to anaerobic media following autoclaving to