**Direct Conversion of Cellulose and Hemicellulose to Fermentable Sugars by a
Microbially-Driven Fenton Reaction**

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Abstract

The aim of this work was to develop a microbially-driven Fenton reaction that fragments cellulose and hemicellulose, degrades cellodextrins and xylodextrins, and produces short-chain oligosaccharides and monomeric sugars in a single bioreactor. The lignocellulose degradation system operates at neutral pH and does not require addition of conventional lignocellulose-degrading enzymes, thus avoiding problems associated with enzyme accessibility and specificity. The ability to produce useful bioproducts was demonstrated by production of the bioplastic polyhydroxybutyrate with the xylan degradation products as starting substrate.

Key words:

Fenton reaction, *Shewanella oneidensis*, Fermentable sugars

1. Introduction

Lignocellulose represents the most abundant renewable resource for the biofuel and biorefinery industries (Gontikaki et al., 2015). The main components of lignocellulose include complex carbohydrate (cellulose and hemicellulose) and aromatic (lignin) polymers (Lange, 2007). The carbohydrate polymers are composed of sugar monomers (mainly glucose and xylose) tightly bound to lignin via ester and ether linkages. Lignocellulose is recalcitrant to enzymatic degradation due to the crystalline structure of the cellulose polymers and strong bonding to lignin (Lee et al., 2014). More than 50% of the terrestrially-derived lignocellulose flux from the continents to the ocean, however, is remineralized in coastal marine environments by cellulolytic bacteria (Gontikaki et al., 2015). Microbial degradation of lignocellulose is initiated by enzymes produced by lignocellulolytic fungi or bacteria, including carbohydrate hydrolases (e.g., cellulases and hemicellulases) and oxidative lignin-degrading enzymes (e.g., laccase and peroxidases) (Gontikaki et al., 2015; Lynd et al., 2002; Sindhu et al., 2016).

Conventional bioprocesses for production of biofuel or biorefinery products from lignocellulose consist of pretreatment (including fragmentation), enzymatic degradation, fermentation, and downstream bioproduct recovery (Sun et al., 2016). Fragmentation initiates lignocellulose breakdown and increases lignocellulose susceptibility to cellulose- and hemicellulose-degrading enzymes (Sun et al., 2016). Enzymatic degradation of fragmented lignocellulose produces sugars such as glucose and xylose that are subsequently metabolized to useful bioproducts. Simultaneous saccharification and co-fermentation (SSCF) and consolidated bioprocessing (i.e., combined hydrolase production, lignocellulose hydrolysis, and sugar fermentation) have been developed to reduce the costs of converting lignocellulose to useful bioproducts (Lynd et al., 2005; Lynd et al., 2002). Current physical and chemical lignocellulose

pretreatment technologies include milling, steam explosion, ozonolysis, acid or alkaline hydrolysis, and ionic liquid fragmentation (Rabemanolontsoa & Saka, 2016). The pretreatment technologies are hindered, however, by production of toxic pollutants, safety concerns, high energy demand, and requirements for detoxification of phenolic inhibitory byproducts after fragmentation (Jonsson et al., 2013; Jonsson & Martin, 2016; Peng et al., 2015; Rabemanolontsoa & Saka, 2016). Complete enzymatic degradation of fragmented cellulose and hemicellulose to fermentable sugars also requires a multitude of lignocellulose-degrading enzymes (Lynd et al., 2002; Zhang, 2008).

Enzymatic oxidation is currently an attractive technology for lignocellulose fragmentation and degradation (Kim et al., 2014; Sindhu et al., 2016). Enzymatic oxidation of lignocellulose is driven by fungal lignolytic enzymes (multi-copper containing laccases, lytic polysaccharide monooxygenases (LPMOs), manganese-dependent peroxidases) that degrade cellulose and partially degrade lignin via Fenton reaction-based chemistry (Hammel et al., 2002; Kerem et al., 1998; Kerem et al., 1999; Kim et al., 2014; Ruiz-Duenas & Martinez, 2009). The reactive oxygen species produced in the enzyme active sites include η^1 superoxo intermediate of LPMOs, and phenoxy and hydroxyl ($\text{HO}^\bullet$) radicals of laccases and peroxidases (Kim et al., 2014; Ruiz-Duenas & Martinez, 2009).

Lignocellulose fragmentation via $\text{HO}^\bullet$ radicals produced by the abiotic (purely chemical) Fenton reaction improves subsequent enzymatic degradation of lignocellulose and increases overall sugar yields (Baures et al., 2008; Kato et al., 2014). In the purely chemical Fenton reaction, hydrogen peroxide (H_2O_2) reacts with ferrous iron (Fe(II)) to produce ferric iron (Fe(III)), hydroxyl ion (OH^-), and lignocellulose-degrading $\text{HO}^\bullet$ radical.

Conventional Fenton reaction-driven lignocellulose degradation is limited, however, by the high concentrations of Fenton reagents Fe(II) and H₂O₂ that must be continuously supplied to produce HO• radicals (Arantes & Milagres, 2006; Kato et al., 2014). UV irradiation is often employed to induce Fe(III) re-reduction and photolytic radical production in photo-Fenton systems. The UV irradiation systems, however, are limited by UV light penetration, and H₂O₂ must still be continuously supplied to drive the conventional Fenton reaction (Silva et al., 2013). Although degradation of cellulose and hemicellulose by the Fenton reaction produces reducing sugars, the identity of the oligosaccharides produced from Fenton reaction-based lignocellulosic degradation remains unknown (Arantes & Milagres, 2006; Baures et al., 2008; Kato et al., 2014).

Microbially-driven Fenton reactions that alternately produce the Fenton reagents H₂O₂ and Fe(II) extracellularly at neutral pH alleviate the need for continual addition of H₂O₂ and Fe(II) under acidic conditions (Sekar & DiChristina, 2014). The microbially-driven Fenton reaction was recently employed to oxidatively degrade the environmental contaminants pentachlorophenol (PCP) and 1,4-dioxane (McKinzi & DiChristina, 1999; Sekar & DiChristina, 2014). In the microbially-driven Fenton reaction, batch liquid cultures of the Fe(III)-reducing facultative anaerobe *Shewanella oneidensis* were amended with Fe(III) and contaminants and subsequently exposed to alternating aerobic and anaerobic conditions. During the aerobic period, *S. oneidensis* produced H₂O₂ (via microbial O₂ respiration), while during the anaerobic period *S. oneidensis* produced Fe(II) (via microbial Fe(III) reduction). During the transition from aerobic-to-anaerobic conditions, H₂O₂ and Fe(II) interacted chemically via the Fenton reaction to produce extracellular HO• radicals that degraded PCP (McKinzi & DiChristina, 1999) and 1,4-dioxane (Sekar & DiChristina, 2014).

In the present study, the microbially-driven Fenton reaction was employed to generate extracellular HO[•] radicals that i) fragmented cellulose and hemicellulose, ii) degraded cellodextrins (G1-6) and xylodextrins (X1-6), and iii) produced short-chain oligosaccharides and monomeric sugars in a single bioreactor system that operated at neutral pH conditions and did not require addition of conventional lignocellulose-degrading enzymes. The ability to produce useful bioproducts was confirmed by production of the bioplastic polyhydroxybutyrate using the xylan degradation products as starting substrate.

2. Materials and Methods

2.1. Culture Medium and Chemical Reagents.

Bacterial strains and plasmids used in this study are listed in Table S16. *S. oneidensis* was routinely cultured aerobically in LB medium (10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl) at 30°C (Sambrook et al., 1989). carboxymethyl cellulose (CMC), xylan, cellodextrin and xylodextrin degradation experiments were conducted in minimal salt solution (LS; pH 7.0, Supplementary Table S3) amended with 50 mM lactate as carbon and energy source and 10 mM Fe(III)-citrate as anaerobic electron acceptor. Fe(III) citrate was prepared by previously described methods (DiChristina & Delong, 1994). *Ralstonia eutropha* was grown aerobically in nutrient broth media at 30°C. When required for selection, chloramphenicol (25 µg mL⁻¹) was amended to basal growth media. All chemicals unless otherwise specified were obtained from Sigma-Aldrich. Cellotriose, cellotetraose, cellopentaose, cellohexaose, xylobiose, xylotriose, xylotetraose, xylopentaose, and xylohexaose were obtained from Megazyme Inc. The sugar compounds were designated as follows: Glucose = G1, Cellobiose = G2, Cellotriose = G3, Cellotetraose = G4, Cellopentaose = G5, Cellohexaose = G6, Xylose = X1, Xylobiose = X2,

Xylotriose = X3, Xylotetraose = X4, Xylopentaose = X5, Xylohexaose = X6. In the microbially-driven Fenton degradation experiments, sugar compounds were added to the batch reactor at the following concentrations: CMC - 1% w/v, xylan - 1% w/v, G1-6 - 100 μ M, and X1-6 -100 μ M.

2.2. Microbially Driven Fenton Degradation of Carboxymethyl Cellulose and Xylan on Solid Agar Medium.

The ability of the *S. oneidensis*-driven Fenton reaction to fragment and degrade CMC and xylan was tested during colony growth on solid agar growth medium. *S. oneidensis* cells were serially-diluted on LS agar medium supplemented with CMC or xylan (1% w/v), Congo red dye (0.04% w/v), 10 mM Fe(III), 50 mM lactate and incubated at 30°C for 48 h under aerobic condition. Congo red dye specifically binds to beta-glucans to form red-colored dye-polysaccharide complexes (Shin et al., 2010). A series of three control incubations were carried out on solid agar growth medium with the following changes: CMC or xylan omitted, Fe(III) omitted, and incubations in the presence of HO $\cdot$ scavenging compound thiourea (40 mM). The resulting colonies were visually scored for the presence of clear halos around the aerobically grown colonies that indicated depolymerization of CMC and xylan (Jung et al., 1998; Shin et al., 2010).

2.3. Design of a Microbially Driven Fenton Reaction for Degradation of Carboxymethyl Cellulose, Xylan, Cellodextrin, and Xylodextrin Compounds.

The experimental condition for the microbially-driven Fenton degradation of CMC, xylan, cellodextrins and xylodextrins (sugar oligosaccharides) was similar to the batch reactor system previously employed (Sekar & DiChristina, 2014). *S. oneidensis* was grown aerobically in LB, harvested and resuspended in LS medium (amended with 10 mM Fe(III) and 50 mM lactate) to a

final cell density of 10^9 cells mL^{-1} in 60-mL glass serum bottles. Anaerobic stock solutions of sugar oligosaccharides were added and the cell cultures were incubated under anaerobic conditions by injecting (hydrated) compressed nitrogen until 10 mM Fe(III) was reduced to 8.6-9.7 mM Fe(II) (6 h of anaerobic incubation; Fe(II) generating phase) (Fig. 1A). The compressed nitrogen line was replaced by a (hydrated) compressed air line to initiate aerobic conditions and 8.6-9.7 mM Fe(II) was oxidized to 0.7-1.6 mM (6 h of aerobic incubation; $\text{HO}^\bullet$ production phase) (Fig. 1A). During the aerobic phase, the Fenton reagents Fe(II) (produced by microbial Fe(III) reduction) and H_2O_2 (produced by microbial O_2 reduction) reacted to generate $\text{HO}^\bullet$ radicals. G1-6 and X1-6 concentrations were monitored throughout the experiments via high pressure liquid chromatography (HPLC). Following the first aerobic phase, the anaerobic and aerobic phases were alternated for 12 h and 6 h respectively for 156 h (Fig. 1A) and the reactor temperature (25°C) and pH (7.0) were held constant throughout the experiment.

2.4. Inhibition of the Microbially-Driven Fenton Reaction in Control Incubations.

A series of four control incubations were carried out to confirm that sugar oligosaccharides were degraded by $\text{HO}^\bullet$ radicals generated by the *S. oneidensis*-driven Fenton reaction. The four control incubations were carried out in the batch reactor system described above with the following changes: In the first set of control incubations, sugar oligosaccharide concentrations were monitored under alternating anaerobic and aerobic conditions with 15 mM nitrate replacing 10 mM Fe(III) as electron acceptor. In the second set of control incubations, the sugar oligosaccharide degradation process was carried out in the presence of the $\text{HO}^\bullet$ radical scavenging compound thiourea (20 mM) added to the batch reactor system. In the third set of control incubations, sugar oligosaccharide degradation was monitored in the absence of either

Fe(III) or *S. oneidensis* cells (abiotic control). In the fourth set of control incubations, sugar oligosaccharide concentrations were monitored in abiotic sealed serum bottles maintained under strict anaerobic conditions without compressed nitrogen sparging for the entire incubation period. G1-6 and X1-6 concentrations were monitored throughout the experiments.

2.5. Cloning and Expression of *R. eutropha* *phaCAB* in *S. oneidensis*.

The primers and plasmids used for cloning *phaCAB* are listed in Tables S16. The PHB biosynthetic gene cluster *phaCAB* from *R. eutropha* was cloned into a pBBR1MCS expression vector (Kovach et al., 1994) and expression controlled by a pBAD promoter induced by arabinose. The plasmid was transformed into the xylose metabolizing *S. oneidensis* XM1 strain (Sekar et al., 2016). The resulting recombinant *S. oneidensis* strain was cultured in LB media with 25 $\mu\text{g mL}^{-1}$ chloramphenicol at 30°C for 24 hours for *phaCAB* expression. Arabinose concentration of 0.5 mM was used to induce *phaCAB* expression at $\text{OD}_{600} = 0.2$.

2.6. Polyhydroxybutyrate Production from Xylose as Sole Carbon and Energy Source by Xylose-Adapted *S. oneidensis* Strain XM1.

The *S. oneidensis* XM1 strain harboring the PHB biosynthetic gene cluster *phaCAB* of *R. eutropha* was cultured aerobically in LB medium with 0.5 mM arabinose. Cells were harvested, washed, and resuspended in minimal media with varying xylose concentrations (0, 0.1, 0.5, 5, 50, and 100 mM) and incubated at 30°C for 96 h under aerobic condition. Samples were periodically drawn to determine the concentration of xylose and PHB.

2.7. Polyhydroxybutyrate Production Derived From Fenton Degradation of Xylan by Xylose-Adapted *S. oneidensis* strain XM1.

The *S. oneidensis* XM1 strain harboring the PHB biosynthetic gene cluster *phaCAB* of *R. eutropha* was cultured aerobically in LB medium with 0.5 mM arabinose. Cells were harvested, washed, and resuspended in spent culture supernatants derived from bioreactors which had previously degraded xylan by the microbially-driven Fenton reaction and incubated at 30°C for 96 h under aerobic condition. The spent supernatant contained only xylodextrins as the sole carbon source since lactate was completely consumed to below detection limit after 132 h of Fenton reaction (Fig. S1 and Table S1). Samples were periodically drawn to determine the concentration of xylose and PHB.

2.8. Analytical Techniques.

Samples were withdrawn and centrifuged at 6000 x g for 10 min. HCl-extracted Fe(II) concentrations were determined with a previously described Ferrozine-based detection technique (DiChristina, 1992). H₂O₂ concentrations were determined using a previously described spectrophotometric assay (Sekar & DiChristina, 2014): a solution consisting of 400 mM KI, 50 mM NaOH, and 170 μM of ammonium molybdate tetrahydrate was mixed with equal volume of 100 mM potassium hydrogen phthalate. Samples were added and absorbance of the resulting solution was measured at 350 nm to determine H₂O₂ concentration. H₂O₂ spectrophotometric measurements were not possible in Fe(III)-containing LS medium due to Fe(III) interference. Calibration curves were generated from standards to determine the H₂O₂ concentrations. Cellodextrins and xylodextrin concentrations were measured via HPLC according to a previously described protocol (Kratschmar et al., 1999). 300 μL of an aqueous solution of sample was

mixed with 100 μ L of a 1.4 M sodium cyanoborohydride solution in water, 700 μ L of a methanolic solution of 0.6 M aminobenzoic butyl ester (ABBE) and 100 μ L of 10% acetic acid. The solution was mixed and heated at 80°C for 60 min. Subsequently, the aqueous phase was extracted three times with 0.5 mL chloroform and directly injected into the HPLC system. The sugar compounds were detected using a SUPELCOSIL LC-18-DB column with mobile phase comprised of 0.1 M ammonium acetate, pH 4.5 (solvent A), and acetonitrile (solvent B). Separation was carried out with a mixture of mobile phase A-acetonitrile (75:25, v/v) at a flow rate of 0.5 mL/min. Chromatograms were generated at 260 nm for the sugar compounds with retention times as shown in Table S2. Calibration curves were generated from standards to determine the concentrations of each compound. Lactate was analyzed via HPLC using a SUPELCOGEL C-610H column with 0.1% H₃PO₄ as the mobile phase and a constant flow rate of 0.3 ml/min (Fig. S1). Chromatograms were generated at 210 nm for lactate with retention times of 29.4 min. Calibration curves were generated from standards to determine the concentrations.

To determine the total number of reducing ends of the residual fraction of CMC and xylan, 50 mL of spent culture was washed with 3X volume of cold acetone and ethanol. The mixture was centrifuged at 15,000 x g for 5 min and the sugar pellet was weighed and resolubilized in 2 mL of 3 M NaOH solution. The mixture was centrifuged at 15,000 x g for 5 min. The total number of reducing ends was determined by the dinitrosalicylic acid (DNS) assay. 100 μ L of the supernatant was added to 900 μ L of DNS solution. DNS solution was prepared as follows: 0.75% 3,5-dinitrosalicylic acid, 1.4% sodium hydroxide, 21.6% potassium sodium tartrate, 0.55% phenol and 0.55% sodium metabisulfate dissolved in water. The mixture was then boiled for 5 min, centrifuged at 15,000 x g for 5 min and the optical density of the supernatant was measured at 545

nm. Reducing sugar concentrations were calculated using glucose and xylose as standards for CMC and xylan respectively (Miller, 1959).

PHB was analyzed as described previously (Kocharin et al., 2012; Li et al., 2013). Cell pellets were boiled in 0.5 mL of concentrated sulfuric acid for 60 min and then diluted with 1 mL of 14 mM H₂SO₄. Samples were centrifuged for 15 min at 16,000g cell debris and the supernatant was analyzed via liquid chromatography (LC) using a ZORBAX SB-C18 column with 5% aqueous acetonitrile at pH 2.8 as the mobile phase and a constant flow rate of 0.3 mL/min. Chromatograms were generated at 210 nm at a retention time of 7 min.

3. Results and Discussion

3.1. Microbially Driven Fenton Degradation of Carboxymethyl Cellulose and Xylan on Solid Agar Medium.

The ability of the microbially-driven Fenton reaction to fragment and degrade cellulose (carboxymethyl cellulose; CMC) and hemicellulose (xylan) was initially tested on solid agar medium amended with CMC or xylan, lactate as carbon and energy source, Fe(III) as anaerobic electron acceptor, and the CMC- and xylan-staining compound Congo red. After 48 h of aerobic incubation, *S. oneidensis* colonies displayed distinct halos (4 and 5.5 mm diameter for CMC and xylan respectively), which indicated that CMC and xylan were depolymerized in the colony periphery. HO• radicals were implicated in the CMC and xylan depolymerization process by addition of the HO• radical-scavenging compound thiourea, which inhibited CMC and xylan depolymerization activities and halo formation.

3.2. Microbially Driven Fenton Reaction for Degradation of Carboxymethyl cellulose and Xylan in liquid batch cultures.

The ability of the microbially-driven Fenton reaction to degrade CMC and xylan was subsequently confirmed in Fe(III)-amended liquid batch cultures exposed to nine alternating aerobic and anaerobic phases (Sekar & DiChristina, 2014). During the nine anaerobic phases, *S. oneidensis* reduced Fe(III) to approximately 8-9 mM Fe(II), while in the subsequent nine aerobic phases, O₂ oxidized the produced Fe(II) to 1-2 mM levels (Fig. 1A). In control experiments with nine alternating aerobic and anaerobic phases, *S. oneidensis* produced H₂O₂ to approximately 25 µM during the initial 6 h aerobic phase, and H₂O₂ remained at 20-25 µM levels throughout the remaining 150 h time period (Fig. 1B). Thus, during the anaerobic period *S. oneidensis* produced Fe(II) (via microbial Fe(III) reduction) while during the aerobic period, *S. oneidensis* produced H₂O₂ (via microbial O₂ respiration). During the transition from aerobic-to-anaerobic conditions, H₂O₂ and Fe(II) interacted chemically via the Fenton reaction to produce extracellular HO• radicals. During the first four alternating aerobic and anaerobic phases (initial 78 h time period), cellodextrins and xylodextrins were produced at low levels (Fig. 1C and 1D). However, during the initial 78 h time period, the total number of CMC- and xylan-reducing ends increased sharply, which indicated that CMC and xylan were fragmented and reducing ends were exposed during the first four alternating aerobic and anaerobic phases (Fig. 1C, 1D). At the 78-h mark, the concentrations of the cellodextrin and xylodextrin increased significantly (Figs. 1C, 1D and tables S12-S15). These results demonstrate that shorter oligosaccharides were produced via degradation of the partially fragmented CMC and xylan polymers.

During the subsequent five alternating aerobic and anaerobic phases (78-156 h time period), the total number of CMC- and xylan-reducing ends remained constant (Fig. 1C and 1D),

while the concentrations of cellodextrins and xylodextrins increased significantly (Fig. 1C, 1D, and Table S12-13). These results suggest that CMC and xylan were fragmented during the first four alternating aerobic and anaerobic phases (designated the fragmentation phase) and the fragmented CMC and xylan intermediates were subsequently degraded to shorter oligosaccharides and monomeric sugars during the subsequent five alternating aerobic and anaerobic phases (designated the degradation phase). The total number of xylan-reducing ends exposed during xylan fragmentation was 2-fold greater than the number of CMC-reducing ends exposed during CMC fragmentation (Figs. 1C, 1D). In addition, the total concentration of xylodextrins produced during xylan degradation was 8-fold greater than total concentration of cellodextrins during CMC degradation (Fig. 1 and Table S13), which most likely reflects the less ordered and more amorphous structure of xylan compared to CMC (Dimarogona et al., 2012). The inability of the *S. oneidensis* wild-type strain to metabolize sugar oligosaccharides resulted in accumulation of oligosaccharides including glucose and xylose in spent culture supernatant during the microbially-driven Fenton reaction (Rodionov et al., 2010) (Figs. 1C and 1D). The newly developed system did not require cellulose- and xylan-degrading enzymes to produce glucose and xylose (Lynd et al., 2002; Shin et al., 2010).

The total number of CMC- and xylan-reducing ends remained constant at low levels throughout the nine alternating aerobic and anaerobic phases in parallel control incubations lacking either Fe(III) or *S. oneidensis* cells (abiotic control). These results demonstrate that the microbially-driven Fenton reaction is required for CMC and xylan fragmentation. Identical patterns of *S. oneidensis*-catalyzed Fe(III) reduction and O₂-catalyzed Fe(II) oxidation were observed in parallel control incubations with CMC or xylan omitted. Thus, the rates of microbially-catalyzed Fe(III) reduction and O₂-catalyzed Fe(II) oxidation were not affected by

the presence of CMC or xylan.

To confirm that CMC and xylan were fragmented and degraded by HO[•] radicals generated by the microbially-driven Fenton reaction, a series of control incubations were carried out in the presence of the HO[•] radical-scavenging compound thiourea. During the first four alternating aerobic and anaerobic phases in the presence of thiourea (0-78 h time period), the total number of CMC- and xylan-reducing ends remained constant at low levels. These results indicated that HO[•] radicals derived from the microbially-driven Fenton reaction were involved in CMC and xylan fragmentation. CMC and xylan were not fragmented in control experiments in which nitrate replaced Fe(III) as anaerobic electron acceptor. Microbial Fe(III) reduction was thus required to drive the CMC and xylan fragmentation process. The absence of CMC and xylan fragmentation in strictly anaerobic Fe(III)-reducing control incubations indicated that CMC and xylan were not enzymatically degraded by *S. oneidensis*.

3.3. Microbially Driven Fenton Reaction for Degradation of Cellodextrin, and Xylodextrin Compounds in Liquid Batch Cultures.

Observed decreases in concentrations of cellodextrins and xylodextrins during the microbially-driven Fenton reaction suggested that the fragmented oligosaccharides may also be degraded to shorter forms by HO[•] radicals (Figs. 1C and 1D). Cellodextrin and xylodextrin were continuously degraded to shorter oligosaccharides during the alternating aerobic and anaerobic phases of the microbially-driven Fenton reaction (Figs. 2, S2, and Tables S4-7). The degradation rates of glucose and xylose were the lowest of the cellodextrins and xylodextrins, which correlated with the highest accumulation rates of glucose and xylose during CMC and xylan degradation (Tables S8-11, S14-15). G5 was degraded at the fastest rate among the cellodextrins

(approximately 3-fold higher than G1-4 and G6), which most likely resulted in G5 degradation to below detection limits during CMC degradation by the microbially-driven Fenton reaction (Fig. S2E and Tables S1, S8-9). The degradation rate of even-numbered xylohextrins (X2, X4 and X6; 262 nM h⁻¹) was approximately 3-fold higher than odd-numbered xylohextrins (X1, X3 and X5), most likely due to the inhibitory effects of the alternating side chains (arabinose, glucuronic acid, and acetyl) on HO[•] radical-catalyzed xylohextrin degradation (Tables S10-11) (Shekiri et al., 2012; Shin et al., 2010). Cellodextrins and xylohextrins were not degraded in control experiments with *S. oneidensis* cells omitted (abiotic controls), with Fe(III) replaced by nitrate as anaerobic electron acceptor, in the presence of the HO[•] radical scavenging compound thiourea, or under strict anaerobic Fe(III)-reducing conditions. These results indicated that the cellodextrins and xylohextrins were degraded by HO[•] radicals produced by the microbially-driven Fenton reaction.

A total carbon balance was subsequently carried out to account for differences in carbon input and output during degradation of the cellodextrins and xylohextrins by the microbially-driven Fenton reaction. The total carbon accumulated during the degradation of the cellodextrins and xylohextrins was approximately 47% and 56% lower than the corresponding carbon input (Tables S5 and S7). This discrepancy may be caused by production of aldonic acid degradation products (e.g., gluconic acid and cellobionic acid) that were not analyzed in the present study (Bey et al., 2013; Li et al., 2015).

3.4. Polyhydroxybutyrate Production Derived from Fenton Degradation of Xylan by Xylose-Adapted *S. oneidensis* strain XM1.

Wild-type *S. oneidensis* is unable to metabolize xylose as sole carbon and energy source (Rodionov et al., 2010). Thus, to determine if the xylan degradation products resulting from the microbially-driven Fenton reaction may be fermented to useful bioproducts, spent culture supernatants of xylan degradation products were fed to genetically engineered *S. oneidensis* strain XM1 (previously isolated for the acquired ability to uptake and grow aerobically on xylose as carbon and energy source) (Sekar et al., 2016). Strain XM1 was genetically engineered to harbor the polyhydroxybutyrate (PHB) biosynthetic gene cassette *phaCAB* (Steinbuchel, 2001). In control experiments with pure xylose as sole carbon and energy source, *phaCAB*-containing Strain XM1 produced PHB at rates proportional to initial xylose concentrations and xylose consumption rates (Fig. S3 and Table S17). *phaCAB*-containing Strain XM1 was subsequently incubated aerobically with spent supernatant derived from bioreactors which had previously degraded xylan by the microbially-driven Fenton reaction. *phaCAB*-containing Strain XM1 consumed the xylan-derived xylose and concomitantly produced PHB at near-Strain XM1 rates with pure xylose as starting substrate (Fig. 1D, 3 and Tables S1, S12-13, S17). Xylan degradation products resulting from the microbially-driven Fenton reaction may thus be fermented to the useful bioproduct PHB.

4. Conclusions

In the present study, cellulose and xylan were fragmented and degraded by a microbially-driven Fenton reaction in a single bioreactor. The single-step reaction produced a mixture of short oligosaccharides that were dominated by glucose and xylose as the major byproducts, thus

providing a consolidated bioprocess for lignocellulose conversion to fermentable sugars. The microbially-driven Fenton reaction thus provides the foundation for development of consolidated bioprocesses for lignocellulose degradation that may be linked to downstream production of a myriad of useful bioproducts, including ethanol, butanol, biodiesel, bioplastic, and lactic acid (Sawatdeenarunat et al., 2016).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version.

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FIGURE CAPTIONS

Fig. 1. Microbially-driven Fenton reaction for degradation of CMC and xylan in batch liquid cultures of *S. oneidensis*. **(A)** Fe(II) concentration profiles: blue (◆), CMC; red (■), xylan. **(B)** H₂O₂ concentration profiles: blue (◆), CMC; red (■), xylan. **(C)** Cellodextrin concentration profiles during microbially-driven Fenton reaction for degradation of CMC in batch liquid cultures of *S. oneidensis* amended with 10 mM Fe(III) and 1% w/v CMC and exposed to nine alternate aerobic and anaerobic cycling periods: blue (◆), G1; red (■), G2; green (▲), G3; violet (X), G4; black (*), G5; orange (●), G6; black (◆), total number of CMC-reducing ends. **(D)** Xylodextrin concentration profiles during microbially-driven Fenton reaction for degradation of xylan in batch liquid cultures of *S. oneidensis* amended with 10 mM Fe(III) and 1% w/v xylan and exposed to nine alternate aerobic and anaerobic cycling periods; blue (◆), X1; red (■), X2; green (▲), X3; violet (X), X4; black (*), X5; orange (●), X6; black (◆), total number of xylan-reducing ends. Grey shaded areas correspond to anaerobic phases, and unshaded areas correspond to aerobic phases. Values represent means of triplicate samples; error bars represent one standard deviation.

Fig. 2. Concentration profiles during microbial Fenton degradation of xylodextrins in batch liquid cultures of *S. oneidensis* amended with 10 mM Fe(III) and 100 µM xylodextrins with an aerobic and anaerobic cycling period of 6 and 12 h respectively: **(A)** X2 degradation; **(B)** X3 degradation; **(C)** X4 degradation; **(D)** X5 degradation; **(E)** X6 degradation; solid blue (◆), X1; solid red (■), X2; solid green (▲), X3; solid violet (x), X4; solid black (*), X5; solid orange (●), X6. Grey shaded areas correspond to anaerobic phases, and unshaded areas correspond to aerobic phases. Values represent means of triplicate samples; error bars represent one standard deviation.

Fig. 3. Xylose and PHB concentration profiles during PHB production by *phaCAB*-containing *S. oneidensis* Strain XM1. *phaCAB* gene expression was induced by the addition of 0.5 mM arabinose for 24 h. Following *phaCAB* gene expression, cells were resuspended in spent culture supernatant derived from bioreactors in which xylan had previously been degraded by the microbially-driven Fenton reaction. DCW – dry cell weight. Xylose; blue (◆), PHB; red (■). Values represent means of triplicate samples; error bars represent one standard deviation.

549 **Fig. 1**

C

D

562 **Fig. 2**

818 **Fig. 3**

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