The Effect of Dry Matter Content on the Simultaneous Saccharification and Fermentation of Citrus Processing Waste

WEIYANG ZHOU1*, WILBUR WIDMER1, AND KAREL GROHMANN2

1USDA/ARS Citrus and Subtropical Products Laboratory, Winter Haven, FL 33881
2Renewable Spirits, LLC, Delray Beach, FL 33444

ADDITIONAL INDEX WORDS. Citrus sinensis, citrus processing waste, ethanol, dry matter content, SSF, enzyme loading, biomass

Citrus processing waste (CPW) is an attractive feedstock for making fuel ethanol. In order for ethanol production from citrus waste to be economically viable, it is desirable to obtain a high ethanol yield and concentration with a small amount of enzymes. In this study, we investigated the effects of dry matter content and pectinase loading on the simultaneous saccharification and fermentation of pretreated CPW. Two levels of substrates (10% and 20% dry matter) were compared at three different pectinase loadings. It was found that the ethanol yield (as percentage of the theoretical) obtained from 20% dry-matter substrate was similar to that obtained from 10% dry-matter substrate. These results could lead to significant savings in enzyme and distillation costs for ethanol production from CPW.

Materials and Methods

CPW (Citrus sinensis var. Valencia) was obtained from a local orange processing plant and frozen (−20 °C) for later use. The thawed CPW was first cut with a knife into appropriately 2 × 2 cm pieces, and then placed in a preheated reactor. The reactor was capped, evacuated to remove air from the tissues, and subsequently pressurized with dry steam. Once the temperature in the reactor reached 160 °C (heating time was 45 s), reaction timing began. The pressure relief valve was partially opened to allow steam flow through the reactor at 7.2 kg/h while maintaining pressure at 75 psi (steam stripping). After 6 min, both the steam supply and pressure relief valves were closed, and a butterfly valve on the top of the reactor was suddenly opened to rapidly expel the contents into a large, vented vessel (steam explosion). After a sample was taken for the analyses of total dry matter, dissolved dry matter, carbohydrate composition and limonene content, the remaining pretreated material was immediately frozen at −10 °C, and stored for SSF experiments.

SSF experiments were performed at high (18% to 20%) and low (9% to 10%) solids content with low solids experiments done after dilution of the high solids CPW with an equal amount of deionized water. SSSFs were done by adding 100 g of either high or low solids CPW into a 250-mL flask, followed by the addition of 0.75 g calcium carbonate or 0.38 g in the case of low solids CPW, and enzyme mixtures adjusted for dry weight contents. The remaining pretreated material was immediately frozen at −10 °C,

Acknowledgment. The authors would like to acknowledge Sandra Matlack, Jerica Scales, and Tommy Long for their technical support with all the experiments and analyses. This research was supported in part by CRADA agreement 503K9581250.

Mention of a trademark or proprietary product is for identification only and does not imply a guarantee or warranty of the product by the U.S. Department of Agriculture. The U.S. Department of Agriculture prohibits discrimination in all its programs and activities on the basis of race, color, national origin, gender, religion, age, disability, political beliefs, sexual orientation, and marital or family status.

*Corresponding author; email: weiyang.zhou@ars.usda.gov; phone: (863) 293-4133, ext. 118
A/S (Bagsvaerd, Denmark) and the pectinase preparation was a kind gift from DSM Food Specialties (Delft, Netherlands). For all experiments, while the cellulase and β-glucosidase loadings were kept constant at 16.4 FPU/100 g dry and 136.4 IU/100 g dry, respectively, pectinase loading was varied from 103 to 25.8 IU pectinase/100 g dry matter. The SSF of pretreated CPW was conducted for 48 h in a rotating bottle at 37 °C. Calcium carbonate was added to neutralize galacturonic acid released from the demethylation and hydrolysis of pectin. Initial pH of the substrate was between 5.0 and 5.6, and it was maintained between 4.0 and 4.5 throughout the SSF experiments.

Samples were taken from the fermented CPW after 24 h and 48 h, and analyzed for dry dissolved matter (DDM), ethanol, and galacturonic acid content. DDM was determined by the removal of residual insoluble solids by the filtration of fermented CPW through a pre-weighed 1.2-μm glass fiber filter (grade GF/C, Whatman International, Maidstone, UK) followed by rinsing with deionized water. Filters were then dried to constant weight for 24 h at 70 °C followed by drying in vacuum oven at 75 °C for 1 h and recording the difference. Galacturonic acid and ethanol were determined by HPIMP chromatography using an Aminex HPX-87 H column (Bio Rad Corp, Hercules, CA) heated to 65 °C, and 0.01 N aqueous sulfuric acid at 0.7 mL/min flow rate as an eluant. All compounds were determined using a Model LC-30 refractive index detector (Perkin Elmer Corp., Boston, MA) and isopropanol as an internal standard. The yields of ethanol and galacturonic acid were calculated based on the total fermentable sugars and galacturonic acid obtained after hydrolysis of raw CPW using an excess of enzymes. Each experiment was conducted 2–3 times, and the results averaged.

**Results and Discussion**

Figures 1 and 2 compare the yields of DDM, ethanol, and galacturonic acid obtained from the substrates containing 20% dry matter with those obtained from the substrate containing 10% dry matter at three different pectinase loadings after 24 h and 48 h SSF, respectively. It was noticed that the results seldom
changed after the first 24-h SSF, indicating that SSF was close to completion in 24 h. The amount of dry matter in the substrate did not affect the yields of DDM, ethanol, or galacturonic acid. This suggested that high SSF of high solids CPW can be done without sacrificing ethanol yields.

Figures 1 and 2 also show that the reduction in pectinase loading significantly decreased the yields of galacturonic acid, but had a little effect on the ethanol and DDM yields. This was unexpected because galacturonic acid is the major constituent of pectin and accounts for 15% to 20% of CPW dry matter. One possible explanation is that a large portion of pectin was solubilized by pretreatment, and the soluble pectin was only partially hydrolyzed into pectin fragments when the pectinase loading was low.

Conclusions

This study shows that high ethanol yield and concentration can be achieved by the SSF of high-solid-content pretreated CPW using a relatively low pectinase loading. Pectinase significantly affected galacturonic acid yields, but its effects on ethanol yield and dissolved dry matter were insignificant.

Literature Cited


