Kinetics of the Self-Buffering Enzymatic Hydrolysis of Pectin in a Batch Reactor

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Introduction

During 2008 to 2011, almost 10.5 MM tons of waste were generated in the USA by processing of grapefruits and oranges.[1] In a conventional process, the citrus processing waste (CPW) is dried and used as a low-cost ruminant feed for livestock.[1] The drying operation requires a significant amount of energy, such as natural gas, to reduce the moisture content of the raw wet waste before it can be transformed into livestock feed pellets. The costs associated with the drying operations, when combined with the low nutritional value of the livestock feed, are often not economical.[1] If the CPW is not converted to a livestock feed, it must be sent to a landfill, which creates an environmental hazards and results in significant processing costs. Grapefruit processing is the primary source of CPW in Texas and can be used as a renewable source of pectin-rich biomass and value added products[1]. Grapefruit waste typically has a pectin content of 7 to 9 wt%, which is large enough to justify developing an efficient means of converting it to more useful organic products, such as galacturonic acid (GA).[1]

The primary objective of this work was to study the kinetics of self-buffering enzymatic hydrolysis of pectin. This is later extended to study the enzymatic hydrolysis of GPW biomass, which can be used for manufacture of value-added chemicals that can be used in specialty chemical, pharmaceutical, food and nutraceutical industries.[1]

Materials and Methods

All the experiments were performed with commercially available pectin from citrus peel (Sigma-Aldrich, P9135) and raw GPW obtained from the grapefruit processing facility located in the Rio Grande Valley in Mission, TX. The enzymes used were commercially available Pectinase from aspergillus aculeatus (Pectinex® Ultra SPL, Sigma) and Accellerase® XY (Dupont-Genecor). Tetracycline hydrochloride (Sigma-Aldrich), chloramphenicol 98% (Sigma-Aldrich), acitidione (Fluka) and Thimerosal (Sigma-Aldrich) were added as growth inhibitors. The neutralizing agent used was 1N NaOH. Sodium acetate (NaOAc, 75 mM) was used as a buffer solution. The reactions were carried out in Erlenmeyer flasks (100 mL working volume) with bottom baffles (Corning, Inc.). The samples were collected in 20 ml plastic centrifuge tubes and the extraction solvent used was 95% EtOH (Sigma-Aldrich). The extracted samples were collected in 2 ml vials. Nitrogen gas was used for drying the samples and pyridine (Sigma-Aldrich), hydroxylamine hydrochloride (J.T. Baker) and salicin (Sigma-Aldrich) and BSTFA/TMCS® (Sigma-Aldrich) were used for derivatization[3]. A solution pH of 4.8 was used for hydrolysis of pectin while a pH of 5.5 was used for hydrolysis of GPW. The scale-up experiments for hydrolysis of GPW were performed in a bioreactor (BioFlo 3000) having two different vessels whose working volumes are 5L and 15L, respectively.

Analytical Methods

GC/MS was used to analyze the reaction products. The samples were collected in the 2 ml GC vials were dried under an inert N_2 stream and then derivatized in a two-step process: (1) Oxime reaction and (2) TMS-Oxime reaction, in which the –COOH and –OH functional groups are converted to their corresponding trimethylsilyl-oxime (TMSO) equivalents. This procedure allows the sample matrix to be resolved using a DB-5 capillary column on an Agilent 6890 and identified with a 5973 MSD. Details of the derivatization method are outlined in a separate publication.[2]

Results and Discussion

Initial experiments were performed using pectin as the substrate versus GPW to determine the feasibility of the proposed hydrolysis protocol. These experiments showed that the final concentrations of GA produced in the self-buffered system at various initial pectin concentrations were similar to the final concentration generated using the buffered system (see Figure 1).

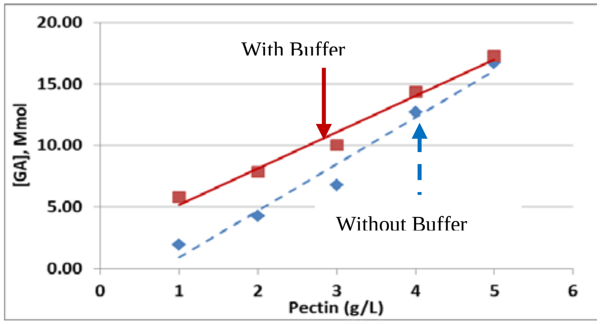

Figure 1. Final Concentrations of GA produced, buffered and self-buffered system

In addition, kinetic experiments showed that the self-buffered system could be described by the Michaelis-Menten rate equation. Based on this analysis, subsequent experiments were performed without a buffering solution.

Scaling-up the self-buffering hydrolysis from the 100mL shaker flasks to the well-stirred Bioflo reactor in two different solution volumes (5L and 15L, respectively) shows that transport-kinetic interactions may be present, depending upon the solid loading and other factors. The importance of developing an understanding of scale-up issues will be discussed.

References

1 Rivas, R., Jones, K., Mills, P., (2013), In: "A citrus waste based biorefinery as a source of renewable energy: technical advances and analysis of engineering challenges". Waste management and research.

2 Raul, R., (2013), Citrus waste biorefinery: Process development and modeling for pretreatment and enzymatic hydrolysis of Grapefruit Processing Waste biomass (doctoral dissertation, to be published)