Production of Galacturonic Acid from the Enzymatic Hydrolysis of Citrus Processing Waste Biomass

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Introduction

Galacturonic acid (GA) is one of the main compounds produced after the enzymatic hydrolysis of pectin-rich biomass generated as waste from the processing of citrus fruits, apples, and sugar beet. Data for the last five years indicate that several million (MM) tons of these wastes annually accumulate in USA at an approximate range of 3.0 to 3.5 MM tons for citrus processing waste (CPW), 2.0 to 2.5 MM tons (dry weight) for sugar beet processing waste (SBPW), and 0.20-0.25 MM of dry apple processing waste (APW) (NASS, 2011 and 2013). GA has the potential to be considered as a renewable platform for the synthesis of relevant green chemicals or it may be used in direct applications as a green chemical. Some of the potential applications are its fermentation to bioethanol, reduction to sugars and polyols, oxidation to galactaric, glucaric and succinic acids, synthesis of pharmaceutics, cosmetics, and nutraceutics products or applied as a biodegradable water softener in detergent formulations.

A new approach for the enzymatic hydrolysis (depolymerization) and simultaneous adsorption of specific monomers is being developed to convert pectin-rich biomass to enhance the reaction and adsorption rates, and increase the yield of products. An experimental study based on the self-buffered enzymatic hydrolysis of pectin and simultaneous adsorption of galacturonic acid (GA) is being performed to determine the reaction and adsorption kinetics. Pectinases, cellulases, and β-glucosidase are used for the depolymerization reaction, and weakly basic anion exchange resins (WBAER) are utilized for the adsorption process. The broader goal is the development of a process for the production and separation of GA during the enzymatic hydrolysis of the pectin-rich citrus biomass contained in grapefruit processing waste (GPW), The resulting kinetic parameters will be used to design a multifunctional reactor for the conversion of GPW into fermentable sugars, galacturonic acid, and other value-added products (such as Flavonoids) including the simultaneous separation of GA to be utilized as a green chemical. The developed process will be applied for the conversion of other pectin-rich biomasses such as CPW, SBPW, and APW.

Materials and Methods

Aqueous solutions of pectin (0.5 wt %) are hydrolyzed using a commercial pectinases complex (Pectinex Ultra SP-L from Novozymes). Thus, 100 mL of pectin solution are neutralized with Sodium Hydroxide 0.5 N in an Erlenmeyer flask at room temperature to reach pH 4.8 measured with a pH-meter. Once the solutions are neutralized these are poured into a 125 mL bottom-baffled Erlenmeyer flask, these are introduced into a water bath shaker at 45 °C and stirred at 175 rpm. Weakly basic anion exchange resin (WBAER), such as Amberlite® IRA-67 and IRA-96, is added into the solutions (between 0.2 to 1 grams) for the adsorption of the galacturonic acid produced by hydrolysis. Temperature probe and pH electrodes are inserted in the cap of Erlenmeyer flasks for the monitoring of the reaction. Once time that all initial reaction conditions are registered, 100 µL of Pectinex Ultra SP-L are added into the reaction system and the pH is monitored each 2.5 min during the first 20 min, then each 5 min. during next 30 min, and finally each 10 min for the following 3 hours of the enzymatic hydrolysis/adsorption process. Similar procedure is applied when aqueous slurry (at 4wt% of insoluble solids) of finely milled grapefruit processing waste (GPW) is used as source of pectin.

Analytical Methods

A GC-MS Analytical method was developed to analyze the Oxime-Trimethylsilyl (OTMS) derivatives of sugars, and Trimethylsilyl (TMS) derivatives of carboxylic acids and polyols and other organic oxygenated compounds. A DB-5 capillary column is used for the separation of the compounds which are identified and quantified using the MS detector. Details of the derivatization method, sample preparation and analytical conditions are outlined in a separate publication.

Preliminary Results and Discussion

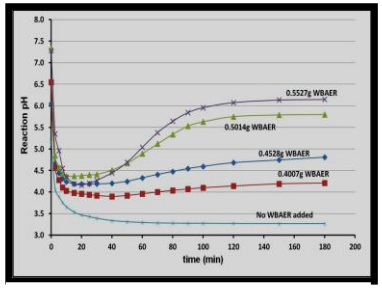

Figure1. Behavior of the pH during the enzymatic hydrolysis of Pectin and simultaneous adsorption of galacturonic

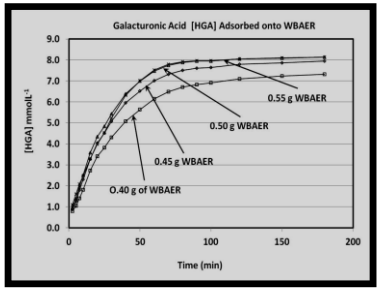

Figure 2. Concentration of GA adsorbed on WBAER during the enzymatic hydrolysis of Pectin.

Based on the experimental and modeling results, the application of multifunctional reactor concept for the self-buffered enzymatic hydrolysis reaction and simultaneous adsorption operation, enhances the synergistic effect of the self-buffered system (conformed initially by the reaction with NaOH of carboxylic groups in pectin structure and after by the [HGA]/[GA^-]-system formed during the enzymatic reaction), improving the reaction rate, the yield of sugars and GA at low concentration of enzyme complexes. Furthermore, helps to avoid the enzymatic inhibition due to the reduction of pH when GA is produced, part of the GA is adsorbed on the WBAER driving the pH of the self-buffered system at optimum ranges (4.5 to 5.5). GA can easily be recovered from WBAER using high purity water.

References

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