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Ultrasound assisted enzymatic depolymerization of aqueous guar gum solution

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ABSTRACT

The present work investigates the effectiveness of application of low intensity ultrasonic irradiation for the intensification of enzymatic depolymerization of aqueous guar gum solution. The extent of depolymerization of guar gum has been analyzed in terms of intrinsic viscosity reduction. The effect of ultrasonic irradiation on the kinetic and thermodynamic parameters related to the enzyme activity as well as the intrinsic viscosity reduction of guar gum using enzymatic approach has been evaluated. The kinetic rate constant has been found to increase with an increase in the temperature and cellulase loading. It has been observed that application of ultrasound not only enhances the extent of depolymerization but also reduces the time of depolymerization as compared to conventional enzymatic degradation technique. In the presence of cellulase enzyme, the maximum extent of depolymerization of guar gum has been observed at 60 W of ultrasonic rated power and ultrasonic treatment time of 30 min. The effect of ultrasound on the kinetic and thermodynamic parameters as well as the molecular structure of cellulase enzyme was evaluated with the help of the chemical reaction kinetics model and fluorescence spectroscopy. Application of ultrasound resulted in a reduction in the thermodynamic parameters of activation energy (E_a), enthalpy (ΔH), entropy (ΔS) and free energy (ΔG) by 47%, 50%, 65% and 1.97%, respectively. The changes in the chemical structure of guar gum treated using ultrasound assisted enzymatic approach in comparison to the native guar gum were also characterized by FTIR. The results revealed that enzymatic depolymerization of guar gum resulted in a polysaccharide with low degree of polymerization, viscosity and consistency index without any change in the core chemical structure which could make it useful for incorporation in food products.

1. Introduction

Guar gum (GG) is a seed endosperm consisting of a linear backbone of mannose units with galactose units as side chains and typically obtained from guar plant known as *Cyamopsis tetragonolobus*. Globally, guar gum and its derivatives have been extensively used as stabilizing and thickening agent in various industries such as food, pharmaceuticals, cosmetics, textile, oil recovery and biomedical field [1,2]. For many of these applications, guar gum needs to be processed in order to improve or customize the physical properties. Depolymerization is intended to impart desired properties to the gaur galactomannan like increased solubility in water, solution clarity, increased shelf life and ionic character according to the needs of the specific applications. Depolymerization of guar gum is specifically very important for the various applications in the food processing due to dependency of nutritional values on the intrinsic viscosity or the molecular weight [1]. Low molecular weight guar galactomannan, i.e., partially hydrolyzed guar gum (PHGG) is considered as a rich source of dietary fiber and hence has significant use in nutraceutical products and functional foods showing physiological benefits like increase in defecating frequency and reduction in serum cholesterol, free fatty acid and glucose concentration [3]. Preparation of partially hydrolyzed guar gum by various methods such as ultrasonication, free radical reactions, acid hydrolysis, gamma radiation and microwave techniques has been widely studied [1,2,4–6]. Various studies have also been attempted for the depolymerization of guar gum using enzymes such as endo-β-mannanase [7], cellulase [1] and pectinase [8]. The endo- and exo-β-1,4 linkages on the D-mannose backbone and the α-1,6 linkage between the mannose unit and the galactose side-chain bonds in guar gum are more susceptible to enzymatic hydrolysis [7]. However, conventional enzymatic method requires significantly higher time for depolymerization of polymers due to slower reaction rate and also gives less degree of depolymerization for some polymers. These disadvantages of conventional enzymatic method

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can be overcome using the ultrasound-assisted enzymatic hydrolysis and hence has been investigated in the present work.

Over the last decades, ultrasound has been widely used for enzyme inactivation but in recent times it has been reported that ultrasound does not inactivate all enzymes mainly under mild operating conditions [9]. Ultrasound has also shown significant application in food and biotechnology processes [10,11]. Although high ultrasonic intensity or higher ultrasonic irradiation time can denature the enzymes, ultrasonic treatment at suitable frequencies and intensity levels can give significant enhancement in the enzyme activity [9,12]. Ultrasonic irradiation can also result in favorable conformational changes without changing the structural integrity of the enzymes [13]. The ability of ultrasound to enhance the enzyme activity and improve the mass transfer rate during the enzymatic reaction is mainly due to better interaction between enzyme and substrate molecules [14]. In the present study, effect of ultrasound has been investigated on the activity of cellulase (cellulase from *Aspergillus niger*) enzyme and also on the enzymatic depolymerization. Cellulase is involved in breakage of bonds between mannose units and results in a decrease in chain length of the polymer or breaks guar gum into smaller polysaccharides or completely into glucose units. Cellulase (endo-1,4-β-D-glucanase) is a group of enzymes produced mainly by fungi, bacteria, and protozoans and can catalyze the process of hydrolysis of guar gum. Cellulase enzymes are widely used in various fields such as pulp and paper industry, textile industry and laundry detergents, bioethanol industry, wine and brewery industry, food processing industry, animal feed industry and agricultural industries, etc. [15]. Mudgil et al. [1] studied the effect of cellulase enzyme on the depolymerization of guar gum using agitation at a speed of 100 rpm at 50 °C till 4 h of treatment time. It was reported that enzymatic hydrolysis of native guar gum resulted in the reduction of intrinsic viscosity and molecular weight reduced from 9 dL/g to 0.28 dL/g and 889,742–7936 respectively. The effect of pectinase enzyme on the 1% aqueous solution of guar galactomannan was studied by Shobha et al. [8]. It was reported that at optimized conditions of temperature of 50 °C and pH of 5, the viscosity and molecular weight (M_w) reduced from 5100 cps (centipoise per second) to 400 cps and 240 kDa (kilo dalton) to 70 kDa respectively at the end of 60 min as the hydrolysis treatment time.

To the best of our knowledge, the depolymerization of guar gum has not been reported using ultrasound assisted enzymatic method. Enzymes are typically used at their optimal conditions, where they exhibit highest activity, thus achieving maximum reaction rate. Thus it is necessary to understand the impact of the ultrasound on the effectiveness of the enzyme and establish the optimum conditions. In the present work, depolymerization of aqueous guar gum solution was investigated using cellulase enzyme under the influence of ultrasonic irradiation. Various process parameters such as enzyme loading, temperature, ultrasonic rated power were optimized and thermodynamic properties were investigated with the help of the Eyring transition state theory and Arrhenius equation. Comparison of ultrasound assisted approach for depolymerization has also been performed with conventional stirring based approach for enzymatic reactions.

2. Materials and methods

2.1. Materials

Guar gum was obtained from Premcem Gums Pvt. Ltd., Mumbai, and had a particle size of 200 mesh and viscosity of 3500 cps. Cellulase enzyme was obtained from Advanced Biotechnologies, Mumbai, India as a gift sample with enzyme activity of 205,000 unit per gram (U/g). 3,5-Dinitrosalicylic acid (DNSA), NaOH and potassium sodium tartrate were procured from S.D. Fine Chemicals. All the chemicals were of analytical grade and used as received from the supplier, without any pretreatment. Distilled water has been used as a solvent to prepare the required solutions.

2.2. Cellulase-catalyzed depolymerization of guar gum

Ultrasound assisted enzymatic depolymerization of guar gum using cellulase as a catalyst was investigated using ultrasonic horn (20 kHz frequency and maximum rated power of 240 W with probe tip diameter of 2.1 cm) obtained from M/s Dakshin, Mumbai, India. The actual power dissipated in the system was observed to be 10.7 W for a supplied power of 60 W giving an energy efficiency of 17.8% as per the calorimetric measurements [16].

The schematic of the experimental setup for ultrasound assisted operation has been shown in Fig. 1. All experiments involving ultrasound were performed using ultrasonic horn operated with 5 s ON and 5 s OFF pulse at rated power dissipation of 60 W and fixed reaction volume of 100 ml with fixed concentration as 0.5% (w/v). The experiments were carried out in a glass beaker with a maximum capacity of 200 ml. Agitation was provided with the help of magnetic stirrer and water bath was used to maintain the temperature of reaction mixture constant at the desired set value. The effect of ultrasonic treatment time was investigated over the range of 5–90 min, whereas the effect of ultrasonic rated power was evaluated over the range 40–120 W. The effect of enzyme loading has been investigated with experiments involving various concentrations of cellulase enzyme (0.03–0.09 g), whereas effect of temperature has also been investigated by performing experiments over different temperature range of 30 °C to 60 °C, under the optimized ultrasonic treatment time of 40 min and 60 W as rated power dissipation.

2.3. Analysis

2.3.1. Assay of cellulase activity

The enzyme activity of cellulase was determined as per details provided in our earlier work by Subhedar and Gogate [9].

2.3.2. Intrinsic viscosity

Samples were collected at regular time intervals to monitor the progress of depolymerization. The withdrawn samples were cooled to 25 °C ± 2 °C in ice cold water bath to stop the reaction and analyzed using glass capillary Ubbelohde viscometer for measuring the intrinsic viscosity. The measurements in terms of the intrinsic viscosity reduction gives a simple way of establishing the degree of

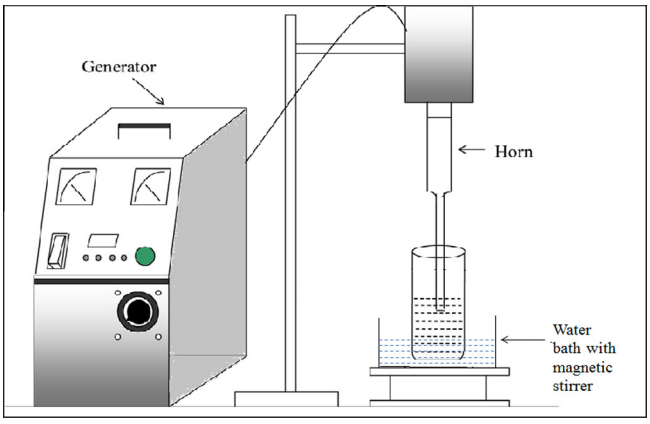

Fig. 1. Schematic of experimental setup of ultrasonic assisted enzymatic depolymerization.