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Liquefied petroleum gas as solvent medium for the treatment of immobilized pectinases

Iloir Gaio[a], Carolina Elisa Demaman Oro[b], Adriana Márcia Graboski[b], Cindy Elena Bustamante-Vargas[b], Marcus Vinícius Tres[c], Alexander Junges[b], Rogério Marcos Dallago[b], Eunice Valduga[b,*], Agenor Furigo Jr.[a]

[a] Department of Chemical Engineering, Federal University of Santa Catarina (UFSC), R. Eng. Agronômico Andrei Cristian Ferreira, s/n – Trindade, Florianópolis, SC 88040-900, Brazil
[b] Department of Food Engineering, URI Erechim, Av. Sete de Setembro 1621, Erechim, RS 99700-000, Brazil
[c] Federal University of Santa Maria (UFSM), Cachoeira do Sul, RS 96506-302, Brazil

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ABSTRACT

This work is aimed at assessing the influence of pressurized fluids treatment on the enzymatic activity of pectin lyase (Pectinex® Ultra SP-L and Pectinex® Mash) immobilized in hybrid polymer-inorganic of alginate/gelatin/calcium oxalate (AGOCa). The effects of system pressure (30–190 bar), exposure time (1–6 h) and depressurization rate (20–100 bar/min), using liquefied petroleum gas (LPG) on the enzymatic activity of pectin lyase and pectin methyl esterase have been evaluated through Central Composite Rotational Design (CCRD) 2^3. The pectin lyase Pectinex® Ultra SP-L presented a 14% increase in residual activity using LPG at 30 bar during 1 h exposure with a 100 bar/min depressurization rate and the commercial Pectinex® Mash had a 38% increment observed at 190 bar for 1 h treatment at 20 bar/min depressurization rate. Enzyme extracts immobilized on alginate-gelatin remained at approximately 60% of pectin lyase (Pectinex® Mash) activity on the sixth recycle after treatment LPG. However, approximately 53% of the pectin lyase (Pectinex® Ultra SP-L) remained until the third recycle. The treatment with pressurized LPG increases the catalytic power of pectinases, hence constituting an interesting solvent to improve the enzyme activity before its use in the process or to carry out reactions using LPG as reaction medium in a enzymatic gas–solid system.

1. Introduction

Enzymes are biocatalysts with properties that make them highly attractive as they increase the chemical reaction rate without undergoing transformation. Apart from being active and versatile, they rapidly catalyze a number of selectively transformations under mild reaction conditions, which differs from conventional catalysts. Another advantage is the ease in regulating enzyme activity, since it is sufficient to change the reaction medium's nature, by changing the pH or adding supplements. There is generally a high specificity as a result of its three-dimensional conformation (Hernalsteens and Maugeri, 2010; Pizarro and Park, 2003; Patel, 2002).

Pectinases are a group of enzymes that degrade pectin by different modes of action, being degrading esters (pectin esterase), the de-polymerizing (hydrolases and lyases) and the proto pectinases. Their classification can also be based on preference for the substrate, such as pectate or pectic acid, which is the polygalacturonic acid that do not have any methoxylation and the pectinic acid or pectin, that are the polygalacturonic acids containing variable amounts of methoxyl groups (Alkorta et al., 1998). Pectin can be degraded by pectinolytic enzymes in different combinations produced by plants and micro-organisms such as fungi, yeast and bacteria. These enzymes are widely used by fruit juice industries to reduce viscosity and to increase filtration and clarification efficiencies. They are also used by wine making industries on preliminary treatment for grapes, maceration, liquefaction and extraction of plant tissues, tea, coffee and cocoa fermentation and to improve vegetable oils and tomato pulp extraction, as well as in the treatment and degumming of natural fibers for textiles and paper (Uenojo and Pastore, 2007; Kashyap et al., 2001).

The increasing emphasis on the use of biocatalysts due to their favorable properties, such as mild and environmentally compatible reaction conditions and their high specificity, have resulted in an increased use of immobilized enzymes, since the interaction between enzyme and support can favorably alter its physical and chemical

* Corresponding author.
E-mail address: veunice@uricer.edu.br (E. Valduga).

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properties (Fernandez-Arrojo et al., 2013; Abdelmajeed et al., 2012; Buga et al., 2010).

Many studies have been seen in the literature recently regarding the use of alternative solvents for biocatalysis (Zhao et al., 2006; Knez and Habulin, 2002). Considerable efforts have been reported in the literature towards green chemical reactions, with emphasis on enzymatic reactions performed in ionic liquids (Erbeldinger et al., 2006; Lou et al., 2006; Lau et al., 2000; Kim et al., 2001) and in sub and super-critical fluids (Silva et al., 2013a, 2013b; Kuhn et al., 2011a, 2011b; Kumar et al., 2004; Oliveira and Oliveira, 2000; Jessop and Leitner, 1999). The use of pressurized fluids as solvents for chemical and biochemical reactions could be a promising route in eliminating solvent traces from the reaction products. Furthermore, industrial processes near the solvent conditions critical points can be advantageous in terms of energy consumption, ease of product recovery and minimizing by-products formation. The super-critical carbon dioxide has, for instance, special characteristics such as low toxicity and working temperature, it may also increase reaction rates (Knez and Habulin, 2002).

Stability and enzymatic activity may depend on many factors, such as; the enzyme, the solvent characteristics, the water content, the relationship between the enzyme/support/reaction medium and the variables process involved, implying that different effects can be obtained depending on the system's characteristics that are under investigation (Franken et al., 2010; Fadiloglu and Erkmen, 2002). Studies reveal that some enzymes increase their activity and others diminish and/or lose their activity after exposure to pressurized fluids. The use of these solvents can be an interesting alternative not only to conduct enzymatic reactions, but also to either increase enzyme systems activity or to inactivate or even, reduce their activity (Silva et al., 2014, 2013a, 2013b; Kuhn et al., 2011a, 2011b; Oliveira et al., 2006a, 2006b; Fricks et al., 2006; Tedjo et al., 2000; Giebauf and Gamse, 2000).

Pectinases have been immobilized by encapsulating in different organic and inorganic polymeric supports, as agar-agar (Rehman et al., 2014), polyvinyl alcohol foam (Esawy et al., 2013) and sodium alginate (Bogra et al., 2013). From these, calcium alginate noteworthy for its low cost and biocompatibility however has as drawback its low mechanical and chemical resistance. In our prior work, a polymer-inorganic hybrid matrix for the immobilization of crude pectinase from *Aspergillus niger* ATCC 9642, constituted by spheres of calcium alginate recovered by a biomimetic layer of gelatin and calcium oxalate, has been successfully synthesized for biomimetic mineralization technique (Bustamante-Vargas et al., 2015).

The aim of this study was to evaluate the usage effects of pressurized fluid (liquefied petroleum gas - LPG), on pectin lyase (PMGL) activity of immobilized enzymes (Pectinex® Ultra SP-L e Pectinex® Mash) on biomimetic matrix of alginate/gelatin/calcium oxalate (AGOCa).

2. Material and methods

2.1. Chemicals and enzyme

Two commercial pectinases were used in free form to conduct the study, kindly provided by LNF Latino Americana Ltda. Their trade names are Pectinex® Mash and Pectinex® Ultra SP-L and both enzymes are produced by submerged fermentation of *Aspergillus aculeatus* and *Aspergillus niger* microorganisms. The liquefied petroleum gas was supplied by Petrobras Inc. (Oil Company), consisting of a mixture of propane (50.3%), n-butane (28.4%), isobutane (13.7%), ethane (4.8%) and other minor components (methane, pentane, isopentane, among others).

2.2. Pectinases immobilization

The immobilization methodology used in this work was adapted from Bustamante-Vargas et al. (2015). Initially, a 2% (p/v) sodium alginate solution dissolved in sodium oxalate buffer (100 mM, pH 5.5) was prepared. After the solubilization, the enzymatic extract was added at different proportions: 3:10 and 5:10 (v/v), considering the relation enzyme extract to sodium oxalate buffer. The gel obtained was dripped in a $CaCl_2$ (75 mM) solution and gelatin 1% (p/v), under constant agitation. The beads formed were kept 10 min in the solution of $CaCl_2$ and gelatin. After, the beads were washed with 100 mL of distilled water and 100 mL sodium acetate buffer (100 mM, pH 5.5). After the washing process, the beads were filtered with vacum pump (Tecnal TE-085) during 20 min and stored in desiccator at 4 °C.

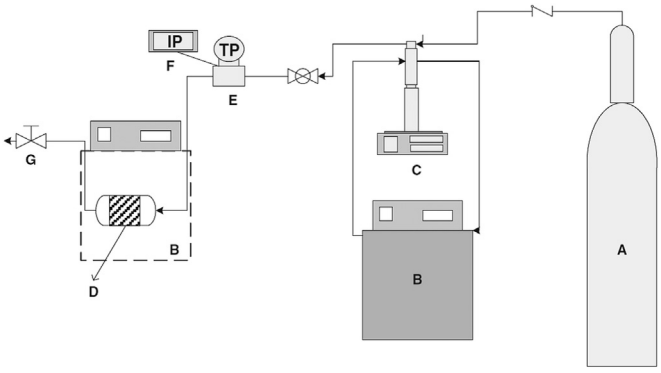

Fig. 1. Schematic diagram of the apparatus used in the enzymatic treatment of the complex with the pressurized fluid. A – Solvent reservoir; B1 – Thermostatic bath of the pump; B2 – Thermostatic bath of the reactor; C – Syringe pump; D – Reactor/steel cell; E – Pressure indicator; F – Pressure transducer; G – Micrometric valve.

2.3. High-pressure enzymes treatment

The commercial enzyme treatment was performed with pressurized fluid (LPG) using the operational conditions based on the enzyme experimental design. The experimental apparatus consisted basically of a solvent reservoir, two thermostatic baths, a syringe pump (ISCO 260D), a stainless steel reactor with 3 mL of internal volume, an absolute pressure transducer (SMAR, LD301) equipped with a portable programmer (SMAR, HT201) with a ± 0.4 bar accuracy. The apparatus' schematic diagram is shown in Fig. 1. The apparatus was built to conduct experiments of up to 220 bar and 80 °C (Franken et al., 2010; Fricks et al., 2006). All lines of experimental apparatus employed stainless steel pipes with a 1/16" (HIP) outside diameter. A check valve (15 41AF1 HIP-T316SS) was placed between the pump and the solvent reservoir to prevent pressurized solvent back flowing. Two other micrometric valves (HIP 15 11AF2 316SS) supplemented the experimental apparatus, one located after the syringe pump, in the high pressure cell inlet, allowing solvent loading and the other one located after the cell, for solvent discharging. The high pressure cell was submerged in a water bath and supported by a basic device, while the micrometric valves were located outside the bath. The unit's schematic diagram and the reactor used in the experiments can be seen in Fig. 1.

The assays were performed after the thermostatic baths' temperatures adjustment. The bath pump was adjusted to 5 °C in order to liquefy the gas and the reactor bath set at 37 °C, the same applied to all experiments. Once the temperature stabilized, the immobilized enzyme (average 36 g pre-weighted) was packed in the cell and coupled to the system. After this procedure, the system was fed with pressurized LPG to the pressure set in the experimental design for a given time. After the stipulated time expired, the system was depressurized at a well-established rate by the experimental design.

2.4. Experimental conditions

A central composite design 2^3 was performed with the objective of evaluating the effects of process variables (pressure, depressurization rate (pressure, depressurization rate (R) and exposure time) on the