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Bacteriocins of lactic acid bacteria as a hindering factor for biohydrogen production from cassava flour wastewater in a continuous multiple tube reactor

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ARTICLE INFO

Article history:
Received 1 August 2015
Received in revised form
9 October 2015
Accepted 11 November 2015
Available online 25 March 2016

Keywords:
Continuous multiple tube reactor
Cassava wastewater
Biohydrogen
Lactic acid bacteria
Bacteriocins
LC-MS/MS

ABSTRACT

Studies on fermentative systems applied to cassava processing wastewaters usually indicate favorable scenarios for biohydrogen ($BioH_2$) production, considering the appreciable levels of carbohydrates found on such wastewaters. To assess the suitability of cassava flour wastewater (CFWW), a high-strength effluent from cassava flour industries, for $BioH_2$ production, a continuous multiple tube reactor (CMTR) was applied in bench-scale assays. The CMTR is an innovative bioreactor configuration that promotes continuous biomass discharge and prevents the accumulation of solids in the long-term. Continuous experiments were conducted using raw and heat-treated CFWW, with and without nutrient supplementation. Although the carbohydrate conversion exceeded 90%, little to no hydrogen production was observed regardless of the feeding conditions. The poor performance of the CMTR could be associated with the presence of organic acids but is likely attributed primarily to bacteriocins Nisin A and Nisin Z in the CFWW, as an evidence of the presence of lactic acid bacteria. The type of cassava wastewater may severely affect hydrogen production; therefore, prior characterization of the CFWW influent is essential to determine its suitability for acidogenic systems.

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http://dx.doi.org/10.1016/j.ijhydene.2015.11.186

Introduction

Hydrogen production from the anaerobic bioconversion of organic residues (i.e. biohydrogen or $BioH_2$) comprises a promising approach for the generation of energy, based on important environmental benefits compared to conventional thermo- and electrochemical processes, with emphasis on lower energy requirements [1]. Considering the implementation of $BioH_2$ fermentative systems in full-scale treatment plants, the use of continuous systems is imperative to handle the continuous generation of wastewaters [2]. Although most studies on continuous $BioH_2$ production by dark fermentation are based on the use of continuously stirred tank reactors (CSTRs), the occurrence of biomass washout in such systems may limit the activity of hydrogen producing bacteria (HPB) [3]. Therefore, reactor configurations that maintain high cell density within the systems, such as packed-bed (APBR), fluidized-bed (AFBR), sludge-bed (UASB), sequencing batch (ASBR) and membrane (AMBR) reactors, are considered attractive approaches to enhance hydrogen production in fermentative systems [4–6].

Focusing on fixed-bed systems, despite the suitability previously pointed, studies on $BioH_2$ production often associate limitations to the application of APBRs in fermentative systems, considering unstable and decreasing production rates even for short-term operations [5,7–9]. The literature usually associates such poor performances with the accumulation of biomass within the reactors [10–12], which leads to inadequate conditions for the food-to-microorganism ratio (F/M) or specific organic loading rate (SOLR). At excessive biomass concentrations, substrate shortage conditions may be established, which stimulates the activity of homoacetogenic bacteria, characterized by autotrophically growing on carbon dioxide and using molecular hydrogen as electron donor in the Wood-Ljungdahl pathway [8,12,13]. Decreasing $BioH_2$ production in acidogenic systems submitted to excessive biomass concentrations may also result from both substrate competition between HPB and non-hydrogen producing bacteria and inhibitory effects from the accumulation of extracellular polymeric substances, as observed by Lee et al. [14] from the operation of an AMBR.

In this context, the continuous multiple tube reactor (CMTR), which is an innovative reactor configuration suitable for biological systems [15], may represent an alternative to overcome the limitations regarding the control of the SOLR. CMTRs are designed to provide a larger surface area to the attachment of solids compared to conventional tube reactors without support material [15], as the reaction region is formed by a group of parallel small diameter tubes. Simultaneously, the high superficial velocity applied to the tubes is expected to control the formation of the biofilm by continuously discharging solids accumulated in excess, which potentially maintains an adequate biomass concentration for $BioH_2$ production. The concept of the CMTR is analogous to multitubular heat exchangers in chemical engineering, in which the larger surface area enhances both heat dissipation in highly exothermic processes and heat absorption in highly endothermic processes [16].

The applicability of the CMTR to fermentative hydrogen production was initially tested by Gomes et al. [15], who achieved continuous $BioH_2$ production for periods of approximately 20–25 days in experiments that used sucrose as the carbon source. However, the literature still lacks studies on the application of the CMTR in fermentative anaerobic systems that use real wastewaters as substrate. Organic matter-rich wastewaters, such as vinasses and effluents from biodiesel production, cassava processing and cheese whey, constitute important substrates for $BioH_2$ production, as residual compounds from agroindustrial processes may be suitable substrates for HPB. Lin et al. [17] reviewed aspects regarding hydrogen production from different wastewaters in fermentative systems, assessing the feasibility of bioenergy recovery through $BioH_2$ in integrated acidogenic-methanogenic anaerobic processes. The aforementioned authors compiled a list of operating conditions in which $BioH_2$ production was attained, including substrate concentration, pH, temperature and hydraulic retention time (HRT) ranging respectively from 0.25 to 160 g COD L^{-1}, 4–8, 23–60 °C and 0.5–72 h, with various types of reactor configuration. Among the available wastewater streams potentially suitable to $BioH_2$ production, this study highlights the use of the cassava flour wastewater (CFWW), which is a type of cassava processing wastewater characterized by high levels of carbohydrates (approximately 37 g L^{-1} as fructose and glucose) and a high chemical oxygen demand (COD, approximately 60 g L^{-1}) [18]. Theoretically, the high organic matter content of cassava wastewaters, especially in terms of readily available sugars, makes these residues suitable substrates for hydrogen production, as HPB use carbohydrates as their main carbon source.

The literature includes several studies on $BioH_2$ production from cassava wastewaters using different approaches, such as the application of continuous and batch processes [19–22]; different continuous reactor configurations, such as CSTR, ASBR and AFBR [22–25]; mesophilic and thermophilic conditions [22,24]; and co-digestion with other substrates [25,26]. Although these studies typically report successful scenarios for $BioH_2$ production from cassava wastewaters, eventual performance losses inherent in acidogenic systems may be observed due to limitations in the availability of nutrients [27] and the presence of undesirable microorganisms and inhibitory compounds in the wastewaters. Lucas et al. [22] observed higher $BioH_2$ production from synthetic sucrose-based effluent compared to cassava processing wastewater, which may indicate the occurrence of inhibitory processes over hydrogen production from cassava-related byproducts. In fact, depending on the type of processing applied to cassava, specific metabolic compounds that result from the presence of lactic acid bacteria (LAB) may be found in the wastewater, such as organic acids, especially lactic acid [28,29] and bacteriocins [30,31]. Bacteriocins are biologically active peptides, which present antimicrobial properties against other bacterial species, increasing the permeability of cell membranes and enhancing the efflux of essential compounds in such bacteria [32]. Therefore, in addition to the competition between LAB and HPB by substrate [33], the excretion of bacteriocins by LAB may inhibit $BioH_2$ production by directly damaging the cellular membrane of