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Inhibition of fermentative H_2 production by hydrolysis byproducts of lignocellulosic substrates

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ABSTRACT

Lignocellulosic materials are potential renewable substrates for fermentative H_2 production; however, most of the methods available to hydrolyze these materials produce fermentation inhibitors. This study assessed the effect of three different groups of inhibitors on fermentative H_2 production by a mixed culture: (1) acetic acid; (2) furan derivatives, such as furfural and 5-hydroxymethylfurfural (HMF); and (3) phenolic monomers, such as vanillin, syringaldehyde, and 4-hydroxybenzoic acid (HBA). Conduction of batch assays in the presence of glucose and different concentrations of inhibitors helped to assess how the inhibitors affected the kinetic parameters of the modified Gompertz model (R_m, H_{max}, and λ). The concentrations of inhibitors that reduced 50% of the maximum H_2 production rate (IC50) were estimated. In terms of IC50, HBA provided the largest inhibition, 0.38 g L^{-1}, which is a novel result in the literature. HBA was followed by HMF and furfural, 0.48 and 0.62 g L^{-1}, respectively. Vanillin, syringaldehyde, and acetic acid at 0.71; 1.05; and 5.14 g L^{-1} provided the same inhibition level, respectively. Knowledge about the degree of inhibition of these compounds shall contribute to sustainable H_2 production from lignocellulosic substrates.

1. Introduction

Hydrogen (H_2) is a clean energy source—its combustion produces water only. However, the methods that are currently available to obtain H_2, such as oil or coal processing, consume fossil fuels and a large amount of energy, which makes them non-sustainable [1,2].

In recent years, researchers have considered biological processes for H_2 production, because most of these processes occur at ambient temperature and pressure [3]. Among the existing biological methods, fermentative H_2 production stands out: it can generate H_2 from renewable materials such as carbohydrate-rich wastes [4,5].

In this context, scientists have focused on the use of lignocellulosic materials as substrate for sustainable fermentative H_2 production [6,7]. Unfortunately, these carbohydrate-rich materials have a complex chemical structure and often require pretreatment and/or hydrolysis with dilute acid or alkali at elevated temperatures before they can serve as substrate in the fermentation process [8]. Although pretreatment and hydrolysis make these substrates more bioavailable, they generate decomposition byproducts like organic acids, furanes, and phenolic monomers, which may negatively interfere in fermentation [9–11]. Three main classes of compounds have emerged as potential fermentation inhibitors during hydrolysis of lignocellulosic materials: (1) organic acids such as acetic acid arising from the hydrolysis of acetyl groups from hemicellulose; (2) furan derivatives like furfural or 5-hydroxymethylfurfural (HMF), which originate from pentoses and hexoses dehydration, respectively; and (3) phenolic monomers, such as vanillin, syringaldehyde, and 4-hydroxybenzoic acid (HBA), products of lignin decomposition [12–15].

For ethanolic fermentation, the inhibitory effects of these compounds on yeast have been thoroughly investigated [11–15], but effects of these compounds on fermentative H_2 production by mixed cultures remain to be properly studied. Indeed, papers along these lines are limited in number or in terms of the concentrations of the assessed inhibitors.

This study investigated how compounds representing the three different classes of byproducts from the hydrolysis of lignocellulosic materials, namely organic acids, furan derivatives, and phenolic compounds, affect biological H_2 production by a mixed

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culture of microorganisms. This work also estimated the inhibitor concentration that elicited 50% inhibition of H_2 production rate by a mixed culture (IC50). The present findings shall pave the way to apply lignocellulosic materials as substrates in fermentative H_2 production.

2. Materials and methods

2.1. Inoculum

The mixed culture (sludge) used as inoculum was collected from an upflow anaerobic sludge blanket (UASB) reactor used to treat the effluent from a sugar and ethanol (vinasse) mill situated in the Region of Ribeirão Preto-SP, Brazil. The sludge was maintained in the laboratory by feeding with glucose (as carbon source) and nutrient solution consisting of NH_4Cl (0.11 g L^{-1}), $MgSO_4.7H_2O$. (0.1 g L^{-1}), KH_2PO_4 (0.136 g L^{-1}), and Na_2HPO_4 (0.148 g L^{-1}) as macronutrients; this nutrient solution also contained 1 mL L^{-1} of the trace elements $FeCl_2.4H_2O$ (2.0 mg L^{-1}), H_3BO_3 (50.0 mg L^{-1}), $ZnCl_2$ (50.0 mg L^{-1}), $CuCl_2.2H_2O$ (38.0 mg L^{-1}), $MnCl_2.4H_2O$ (500.0 mg L^{-1}), $(NH_4)_6Mo_7O_24.4H_2O$ (50.0 mg L^{-1}), $AlCl_3.6H_2O$ (90.0 mg L^{-1}), $CoCl_2.6H_2O$ (2.0 mg L^{-1}), $NiCl_2.6H_2O$ (142.0 mg L^{-1}), $Na_2SeO.5H_2O$ (164.0 mg L^{-1}), EDTA (1.0 mg L^{-1}), and HCl 36% (1.0 mg L^{-1}). All the chemicals were analytical grade.

Before use, the inoculum (sludge) was dried at 105 °C for 12 h, which enrich it with H_2-producing bacteria. After heat treatment, the sludge was macerated, sieved in #35-mesh sieves (500 μM), and stored at ambient temperature (25 °C), in a dark flask. The volatile solids (VS) content of the dry sludge was analyzed according to APHA et al. (1995) [16].

2.2. Fermentative H_2 production assays

Batch tests for fermentative H_2 production used glucose (Vetec, Brazil) concentrations ranging from 5 to 60 g L^{-1}. These tests helped to identify the concentration of glucose that afforded the maximum H_2 production rate.

Batch assays that helped to test H_2 production with addition of inhibitors involved the use of 120 mL of glucose solution (40 g L^{-1}), 1.0 mL of the nutrient solution described above, and 1.5 g of sludge containing 43% of VS. This condition provided the maximum H_2 production rate. Addition of different concentrations of inhibitors helped to evaluate how the byproducts from the hydrolysis of lignocellulosic materials affected fermentative H_2 production. The inhibitors represented the three classes of hydrolysis products, as follows: (1) organic acid—acetic acid (98%) between 0.50 and 10.0 g L^{-1}; (2) furan derivatives—furfural (99%) between 0.25 and 2.00 g L^{-1} and HMF (99%) between 0.10 and 1.00 g L^{-1}; and (3) phenolic monomers—vanillin (99%) between 0.25 and 2.00 g L^{-1}, syringaldehyde (99%) between 0.25 and 2.00 g L^{-1}, and hydroxybenzoic acid 4 (99%) between 0.15 and 1.00 g L^{-1}. These compounds were purchased from SIGMA—ALDRICH (USA).

The pH in the bioreactors was adjusted to 6.0 at the beginning of the tests. Argon gas was bubbled through the reactor, to maintain anaerobiosis. All the tests were performed in duplicate. Bioreactors were placed in a temperature-controlled bath shaker, at 37 °C, and stirred at 80 rpm. The pipes for gas collection were coupled to the bioreactors and to a gas measurement system consisting of an inverted flask containing a NaOH 5% (w v^{-1}) solution and a flask, which helped to determine the volume displaced by the produced gas. The volume of displaced NaOH solution corresponded to the total volume of generated gas, except for the CO_2 retained in NaOH. The gas composition was analyzed by gas chromatography (CG), and the H_2 volume was obtained by multiplying the total volume of the gas by the percentage of H_2 in the gas. Gas samples were collected from the bioreactors headspace every 2 h for analysis of the gas composition at the beginning of the batch fermentative assays, to determine the time elapsed before the onset of H_2 production and the maximum H_2 production rate. After that, the gas was analyzed at longer intervals, until maximum H_2 production was reached and until neither the volume nor the gas composition was altered.

2.3. Analytical determinations

The gas composition of the bioreactor headspace was analyzed by gas chromatography (GC). For injection into the GC system, a 50-μL aliquot of the safety flask was collected with a gas tight syringe on a regular basis. The analysis was conducted on a GC 2014 Shimadzu (Japan) chromatograph equipped with a thermal conductivity detector (TCD). The column consisted of 5-A molecular sieves and measured 2 m × 4.7 mm. Argon at a flow rate of 30 mL min^{-1} was the carrier gas. The temperatures of the injector, column, and detector were 80, 50, and 100 °C respectively.

The concentrations of the substrate (glucose) and of the fermentation metabolites, such as acetic acid, lactic acid, butyric acid, and ethanol, were analyzed in samples kept in bioreactors before the start and after the end of the kinetic assays by high performance liquid chromatography (HPLC) conducted on an equipment from Shimadzu (Japan). The methodology described by Datar et al. (2007) was employed [4].

2.4. Kinetic model

The H_2 volume accumulated along the assay was introduced in the *Statistica* 7 program and modeled according to the modified Gompertz model (Equation (1)), to obtain the kinetic parameters R_m, H_{max}, and λ.

$$H = H_{max} \cdot \exp\left\{ -\exp\left[\frac{R_m \cdot e}{H_{max}}(\lambda - t) + 1\right]\right\} \quad (1)$$

H = cumulative H_2 volume in tests, H_{max} = maximum potential of H_2 production (mL), R_m = Maximum H_2 production rate (mL h^{-1}),

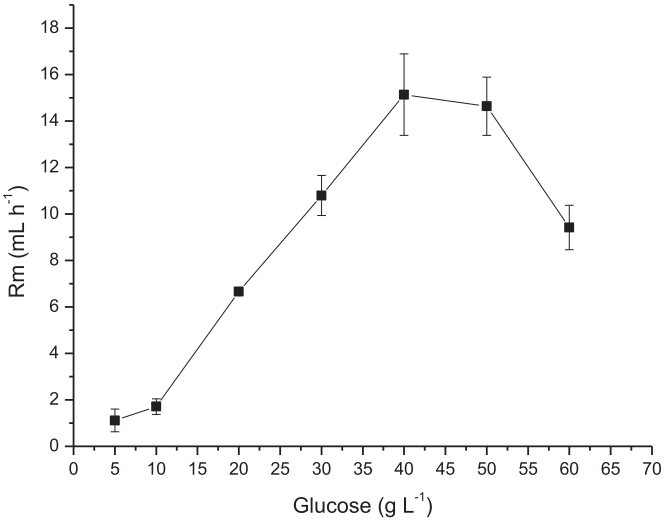

Fig. 1. Maximum H_2 production rate (R_m) obtained by the modified Gompertz model, in different glucose concentrations. R_m is represented as the mean of the replicates and the average deviation.