Renewable Energy 37 (2012) 259–265

Contents lists available at ScienceDirect

Renewable Energy

journal homepage: www.elsevier.com/locate/renene

Fermentation medium and oxygen transfer conditions that maximize the xylose conversion to ethanol by *Pichia stipitis*

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ARTICLE INFO

Article history:
Received 8 November 2010
Accepted 20 June 2011
Available online 23 July 2011

Keywords:
Xylose
Ethanol
Pichia stipitis
Nutrient
Volumetric oxygen transfer coefficient

ABSTRACT

The xylose conversion to ethanol by *Pichia stipitis* was studied. In a first step, the necessity of supplementing the fermentation medium with urea, $MgSO_4 \times 7H_2O$, and/or yeast extract was evaluated through a 2^3 full factorial design. The simultaneous addition of these three nutritional sources to the fermentation medium, in concentrations of 2.3, 1.0, and 3.0 g/l, respectively, showed to be important to improve the ethanol production in detriment of the substrate conversion to cell. In a second stage, fermentation assays performed in a bioreactor under different K_La (volumetric oxygen transfer coefficient) conditions made possible understanding the influence of the oxygen transfer on yeast performance, as well as to define the most suitable range of values for an efficient ethanol production. The most promising region to perform this bioconversion process was found to be between 2.3 and 4.9 h^{-1}, since it promoted the highest ethanol production results with practically exhaustion of the xylose from the medium. These findings contribute for the development of an economical and efficient technology for large scale production of second generation ethanol.

1. Introduction

The beginning of this century is marked by the large incentive given to biofuel use in replacement of gasoline, which has been motivated by several reasons, including the rising oil prices and recognizing that the global oil reserves are exhausting fast; the concern about fuel emissions; the requirements of the Kyoto Protocol and the Bali Action Plan on carbon emissions, and the provision of alternative outlets for agricultural producers [1]. Currently ethanol is the main bio-fuel used in the world and the worldwide prospects are the expansion of the production and consumption of ethanol.

Polysaccharides present in lignocellulosic materials, including cellulose and hemicellulose, are of great interest as feedstocks for second generation ethanol production due to their large availability, richness in sugars, and mainly because they do not affect the food provision. However, while technologies to produce ethanol from sugar or starch are well established, the technologies to produce bioethanol from lignocellulosic biomass are still under development all over the world. Hemicellulose is one of the three major components of lignocellulosic biomass together with cellulose and lignin, and can be easily hydrolyzed to monomeric sugars under mild conditions [2]. Xylose is the main sugar obtained by hydrolysis of this fraction, and its bioconversion is an important step in the use of lignocellulosic materials for ethanol production. *Pichia stipitis* is a promising yeast strain for industrial application on ethanol production due to its ability to rapidly convert xylose to ethanol, with high yield [3–5]. Moreover, this yeast is also able to ferment several other sugars besides xylose, including glucose, mannose, galactose and cellobiose along with mannan and xylan oligomers [6], which is an important characteristic considering that hemicellulosic hydrolyzates usually contain these other sugars in mixture with xylose.

As is well know, the efficiency of a fermentation process is affected by the cultivation medium composition and operational conditions used. Different yeast strains require different sources and amounts of nutrients to efficiently convert the sugars in the product of interest. Specific nutrients such as nitrogen, trace elements or vitamins, may be required to obtain rapid fermentation and high ethanol levels, desirable to minimize capital costs and distillation energy [7]. Among the operational conditions, the oxygen availability is considered the most important factor affecting the sugars fermentation by yeasts, since it determines the partitioning of the carbon flux between growth and product formation [8]. The establishment of an adequate oxygen level is thus of great importance to obtain an efficient process with high

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doi:10.1016/j.renene.2011.06.032

values of conversion and productivity. In this sense, the volumetric oxygen transfer coefficient (K_La) is an important parameter to be observed in fermentations performed in bioreactors. The K_La value depends on several factors such as the agitation speed and aeration flow rate, the geometry of the reactor, the rheological properties of the medium (density and viscosity), and environmental variables (temperature and pressure) [9]. Through the K_La value, the oxygen transfer for different processes can be compared, and the scaling up operations for different geometry of bioreactors are facilitated [10].

Although several studies have been carried out on ethanol production from xylose, much work remains to be done for achieving high ethanol yields and productivities. Aiming to contribute for the development of an efficient technology for large scale production of second generation ethanol, the present study evaluated the influence of medium composition and volumetric oxygen transfer coefficient on ethanol production from xylose by *P. stipitis*. Initially, assays in Erlenmeyer flasks revealed the necessity of supplementing the medium with urea, $MgSO_4 \times 7H_2O$, and/or yeast extract. In a second step, assays in bioreactor under different K_La conditions made possible understanding the influence of the oxygen transfer on yeast performance, as well as to define the most suitable range of values for an efficient ethanol production by this yeast strain.

2. Materials and methods

2.1. Microorganism and inoculum

P. stipitis NRRL Y-7124 was the microorganism used in the experiments. Cultures of this yeast were maintained on malt extract agar slants at 4 °C. For inoculum preparation, cells of the yeast in the maintenance medium were transferred to 500-ml Erlenmeyer flasks containing 100 ml of the medium composed by (g/l): xylose (30.0), glucose (5.0); arabinose (5.0); urea (2.3), $MgSO_4 \times 7H_2O$ (1.0), and yeast extract (3.0). The inoculated flasks were incubated at 30 °C, 200 rpm, during 24 h. After this time, the cells were recovered by centrifugation (2000 × *g*, 20 min) and resuspended in the fermentation medium to obtain an initial concentration of 1 g/l.

2.2. Fermentation medium and conditions

To evaluate the influence of medium composition on ethanol production, assays were performed in 125-ml Erlenmeyer flasks containing 50 ml of a 90 g/l xylose solution supplemented or not with nutrients (urea, $MgSO_4 \times 7H_2O$, or yeast extract) according to the experimental design given in Table 1. The flasks were inoculated with an initial cell concentration of 1 g/l and maintained in a rotary shaker at 30 °C, 150 rpm, during 48 h. Fermentation runs were monitored through periodic sampling to determine the cell growth, xylose consumption, and ethanol production.

To evaluate the influence of the volumetric oxygen transfer coefficient (K_La) on ethanol production, assays were performed in a 1.6 L stirred tank bioreactor (Autoclavable Benchtop Fermenter Type R'ALF, Bioengineering AG, Wald, Switzerland), containing 1.2 L of the following fermentation medium (g/l): xylose (90.0), glucose (15.0), arabinose (15.0); urea (2.3), $MgSO_4 \times 7H_2O$ (1.0), and yeast extract (3.0). After inoculated with 1 g/l of cells, the fermentation runs were maintained at 30 °C during 96 h. The K_La values used in each assay are shown in Table 2. During the experiments, samples were taken each 12 h for sugars, ethanol, and cell growth determinations.

Table 1
Experimental conditions for evaluation of the fermentation medium supplementation with nutrients, on ethanol production and xylose consumption by *Pichia stipitis*.

Assay	Variables			Responses	
	Urea g/l	$MgSO_4 \times 7H_2O$ g/l	Yeast extract g/l	Total consumed xylose (%)	Maximum ethanol production (g/l)
1	0.0	0.0	0.0	4.0	0.00
2	2.3	0.0	0.0	5.8	0.00
3	0.0	1.0	0.0	4.8	0.00
4	2.3	1.0	0.0	8.1	0.00
5	0.0	0.0	3.0	47.3	15.70
6	2.3	0.0	3.0	60.3	19.83
7	0.0	1.0	3.0	45.5	15.17
8	2.3	1.0	3.0	74.0	24.17
9	1.15	0.5	1.5	41.0	12.25
10	1.15	0.5	1.5	40.5	12.21
11	1.15	0.5	1.5	42.2	12.20

2.3. K_La determination

For the K_La determination, initially the polarographic oxygen probe was calibrated at atmospheric pressure by setting zero and 100% saturation under nitrogen and air sparging, respectively. Then, the bioreactor was filled with the culture medium not inoculated with cells, and oxygen was stripped from the medium by sparging with nitrogen. In the sequence, the oxygen saturation time course was monitored as the air flow and stirring conditions chosen for use during the fermentation were resumed. The K_La value was calculated by the integrated form of the equation proposed by Stanbury et al. [11], where the value of $-K_La$ is equal to the slope of the resulting straight line representing the ln (C^*-C_L) versus time.

2.4. Analytical methods and determination of fermentation parameters

Cell growth was estimated by measuring the fermentation broth absorbance at 600 nm, which was correlated to a calibration curve (dry weight × optical density). Glucose, xylose, arabinose, and ethanol concentrations were determined by high performance liquid chromatography (HPLC) in a Jasco chromatograph equipped with a refractive index detector and a Bio-Rad Aminex HPX-87H column (300 × 7.8 mm) at 60 °C, using 0.005 M sulfuric acid as eluent in a flow rate of 0.7 ml/min.

The ethanol yield factor ($Y_{P/S}$, g/g) was calculated by the ratio between ethanol concentration (g/l) and substrate (glucose + xylose) consumed (g/l), while the cell yield factor ($Y_{x/S}$, g/g) was determined by the ratio between cell concentration (g/l) and substrate consumed

Table 2
Fermentation parameters obtained during the ethanol production from xylose by *Pichia stipitis*, under different K_La values.

K_La (h^{-1})	$Y_{P/S}$(g/g)	$Y_{x/S}$(g/g)	Q_P(g/l h)
0.7	0.33	0.15	0.24
2.3	0.26	0.13	0.27
4.9	0.32	0.15	0.32
11.7	0.30	0.19	0.33
12.1	0.31	0.18	0.34
18.7	0.23	0.21	0.38
58.0	0.13	0.51	0.17
65.8	0.00	0.54	0.00

$Y_{P/S}$: ethanol yield factor; $Y_{x/S}$: cell yield factor; Q_P: ethanol volumetric productivity.