Biochemical Engineering Journal 92 (2014) 41–46

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Regular Article

Aeration-enhanced bioethanol production

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ARTICLE INFO

Article history:
Received 3 March 2014
Received in revised form 2 May 2014
Accepted 7 May 2014
Available online 21 May 2014

Keywords:
Aeration
Ethanol
E. coli KO11
Bioreactors
Oxygen transfer
Batch processing

ABSTRACT

In recent years, growing attention has been devoted to maximize the product yield for the conversion of biomass into ethanol. In this study, microaerated conditions were established to enhance the ethanol yield by *Eschericia coli* KO11. According to the results, limited aeration was found to be an important factor to increase the ethanol yield by improving the consumption of sugars and the production of biomass. The best result was obtained using oxygen transfer rate (OTR) of 5 mmol/L/h, reaching 19.66 g/L of ethanol at 48 h using quince pomace as substrate. The assays showed that less than 5% of the initial sugar remained at the end of the fermentation, achieving a biomass concentration of 7.3 g/L. In conclusion, we successfully carried out lab-scale production of bioethanol from quince pomace using the ethanologenic *E. coli* KO11. In particular, microaerobic ethanol fermentation at OTR = 5 mmol/L/h is suggested for the efficient utilization of sugars in quince pomace. Considering the abundance of raw material and the ease of large-scale production, this improvement will have a considerable impact on the total cost of bioethanol.

1. Introduction

Ethanol is considered as one of the best alternative energy resources because it reduces the CO_2 emission in to the air [1]. In this regard, bioconversion of biomass to ethanol has been receiving a great amount of interest worldwide. In order to promote bioethanol utilization, it is necessary to reduce the production cost using agroindustrial wastes. Conventionally, bioethanol has been produced by physiochemical treatment of the biomass containing multi glucose units, using microbial catalysis and metabolic engineering under unaerated conditions [2]. As a promising biocatalyst for ethanol production from agroindustrial wastes, various ethanologenic recombinant *Escherichia coli* strains have been studied intensively by many researchers. In particular, *E. coli* KO11 has been employed as a promising biocatalyst to convert hemicellulosic waste into ethanol, because *E. coli* is capable of assimilating fructose and sucrose as well as glucose, which are the predominant sugars accounting for of 99% of dried quince pomace [3]. The strain has a higher product yield of 0.4 g/L under unaerated conditions [4]. However it was shown that under microaerated conditions the ethanol yield reached to 0.47 g/L with a 25% higher sugar uptake ratio in comparison to unaerated conditions [5].

The gas–liquid mass transfer and oxygen uptake rate are strongly influenced by the hydrodynamic conditions in the bioreactors. These conditions are known to be a function of energy dissipation that depends on the operational conditions, the physicochemical properties of the culture, the geometrical parameters of the bioreactor and also on the presence of oxygen consuming cells. Oxygen mass transfer controls the performance of bioreactors. Successful design depends on the comprehension and elucidiation of the complex hydrodynamic interactions within the reactor [6].

Aeration conditions influence mass transfer by affecting the bubble size, air hold up and turbulance within the vessel as well as biomass production. A wide range of environmental conditions are required to obtain maximum productivity in a bioreactor depending on the specific process. Attempts of optimization with respect to productivity and process economics should take into account design variables such as aeration capacity, sparger type and etc.

From the viewpoint of the industrial-scale production of bioethanol, it is a very simple and practical operation to control fermentation performance through the oxygen transfer rate (OTR), because the OTR can theoretically be controlled by adjusting both aeration rates and durations. In this study, the effect of aeration and OTR on the fermentation performance was investigated as an operational parameter for the control of pilot scale bioethanol production. Cemeroglu and Karadeniz [3] reported that the quince pomace was directly used as a substrate without any chemical pretreatment (such as acid or base hydrolysis) due to the availability of the sugars (mostly glucose and fructose) in the pomace for microorganisms. There are several studies on the utilization of

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http://dx.doi.org/10.1016/j.bej.2014.05.011
1369-703X/

Abbreviations

D	impeller diameter, m
k	constant with magnitude dependent on the geometry of the impeller, dimensionless
N	impeller rotation speed, rpm
N_P	power number for stirred tank, dimensionless
P	unaerated power consumption for stirred tank, W
P_g	aerated power consumption for stirred tank, W
Re	impeller Reynolds number, dimensionless
V_L	working volume of the stirred tank, m^3
X	biomass concentration, g/L
t_m	mixing time, s
υ_{tip}	impeller tip speed, m/s
k_La	oxygen mass transfer coefficient, 1/s
q_{O_2}	Specific oxygen uptake rate, mmol/g/h
C^*	dissolved oxygen concentration at saturation in the bulk, g/m^3
C_L	dissolved oxygen concentration at any time, g/m^3
OTR	oxygen transfer rate in the reactor, $mmol/m^3/h$
$Y_{P/S}$	product yield, g/g
Q_P	volumetric productivity, g/L/h
v_s	superficial gas velocity, m/s
Q	air flow rate, m^3/s

Greek symbols

μ_{max}	maximum specific growth rate, 1/h
λ	Kolmogorov's eddy size, m
γ	shear rate, 1/s
τ	shear stress, N/m^2
ρ	fluid density, kg/m^3
η	dynamic viscosity of fluid, kg/m/s
υ	kinematic viscosity of fluid, m^2/s

the fruit pomaces such as apple pomace without pretreatment process for the bioconversion of value-added bioproducts [7–9]. In this study quince pomace as an agro-industrial biomass was used for bioethanol production under microaerated conditions, eliminating the pretreatment step. To our knowledge, this is the first report on the successful utilization of quince pomace for bioethanol production on the lab scale fermentation under microaerated conditions. Also, the existing micoaeration studies using *E. coli* KO11 are conducted using full aeration during fermentation which requires more air feeding and power consumption.

2. Materials and methods

2.1. Growth conditions

Recombinant *E.coli* KO11 (pLOI 1910) strain was provided by courtesy of Professor L.O. Ingram from University of Florida. Stock cultures were stored in 20% glycerol at −86 °C. Working cultures of KO11 were maintained on modified Luria-Bertani (LB) agar containing (per liter) 5 g of NaCl, 5 g of yeast extract, 10 g of tryptone, 20 g of glucose, 15 g of agar, and 600 mg of chloramphenicol at 4 °C.

For inoculation, cells from 3 fresh colonies were transferred into 500 mL flasks containing 150 mL LB medium supplemented with 50 g/L glucose. Seed cultures were incubated under static conditions for 16 h at 30 °C. Cells were harvested by centrifugation (5000 × *g*, 5 min, 5 °C) and washed with the fermentation medium. The initial cell density was adjusted to the given concentration range of 0.33 g-dry cell weight/L. No chloramphenicol was included in seed cultures or fermentations.

Table 1
Elementel analysis and sugar composition of dried quince pomace.

The elemental composition	(%)	Sugar[a]	(%)
C	34.50	Glucose	28.76
N	0.23	Fructose	55.71
C/N		Sucrose	10.12

[a] Ref. [3].

2.2. Substrate

Quince pomace was used as a substrate for ethanol production instead of glucose. Quinces were pressed and dried to constant weight at 70 °C in a pasteur oven (Memmert, Germany) to remove bound-water. Dried pomace was grinded to 0.1 mm in size. No further pretreatments were carried out.

The total carbon (C) and nitrogen (N) contents of dried quince pomace were determined by the methods described elsewhere [10,11] and are shown in Table 1. Quince pomace was added to the reactor as carbon source to give a C:N ratio of 14.33 g/g, which was determined based on the elemental composition of the LB medium supplemented with glucose.

2.3. Reactor conditions

Batch fermentations were carried out in 5 L (Sartorius A plus stat.) bioreactors with a working volume of 2 L, containing quince pomace and LB broth without glucose. Quince pomace and the supported LB ingredients were autoclaved separately and mixed aseptically before fermentation. The fermentation was carried out at constant pH 5.5 at 35 °C. 2 M KOH solution was automatically added to prevent acidification. No antifoam or antibiotic were used in reactor experiments. All experiments were carried out in duplicate.

2.3.1. Aeration and k_La determination

Different aeration rates and different aeration durations were studied with quince pomace supported LB medium in order to determine the influence of oxygen addition on sugar consumption and ethanol production by *E. coli* KO11. Thus, a one factor at a time design was used. For the first set of experiments, different aeration rates of 0, 0.02, 0.035, 0.047 and 0.062 vvm were fed to the reactor for the first 8 h of the fermentation, corresponding to OTR values of 0, 2, 5, 9, 16 mmol/L/h, respectively. The results were compared in terms of product yield for the fermentation period of 64 h. For the second set, 0.035 vvm air was fed to the reactor for the first 6, 8, 10 and 12 h to determine the most appropriate aeration duration for the ethanol production period of 64 h.

The volumetric oxygen transfer coefficient, k_La, was determined using a polarographic oxygen sensor calibrated with nitrogen and air sparging to set zero and 100%, respectively. The determination of the k_La values was done following the unsteady state method of gassing out using Eq. (1) [12];

$$\mathrm{OTR} = \frac{dO_2}{dt} = k_La(C^* - C_L) - q_{O_2}X \quad (1)$$

where k_La is the oxygen mass transfer coefficient; C^* is the dissolved oxygen concentration at saturation in the bulk; C_L is the dissolved oxygen concentration at any time; q_{O_2} is the specific oxygen uptake rate and X is the biomass concentration.

2.3.2. Mathematical equations

Mixing time was experimentally determined using the pH-response technique [13]. Mathematical equations in order to determine rheological behavior of the reactor for bioethanol production are presented in Table 2. For stirred-tank bioreactors, the