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Scale-up and kinetic modeling for bioethanol production

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HIGHLIGHTS

- Methodologies of scale up procedure for bioethanol production.
- Kinetic modeling for the behaviors of *Escherichia coli* KO11.
- Importance of the rheological behavior of fermentation broth.
- Hydrodynamic parameters of bioreactors were evaluated.

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ABSTRACT

Bioethanol was produced from acidic hydrolysate of rice hulls using recombinant *Escherichia coli* KO11. Two different issues (scale-up and kinetic modeling) were evaluated simultaneously and concomitantly for bioethanol production. During the step-wise scale-up process from 100 mL shaken flask to 10 L stirred-tank bioreactor, the constant Reynolds number and the constant impeller tip speed were evaluated as scale-up methodologies under laboratory conditions. It was determined that the volumetric bioethanol productivity was 88% higher in 10 L bioreactor in comparison to the value of 0.21 g L^{-1} h^{-1} in shaken flask. The modified *Monod* and *Luedeking-Piret* models provided an accurate approach for the modeling of the experimental data. Ethanol concentration reached the maximum level of 29.03 g/L, which was 5% higher than the value of model prediction in 10 L bioreactor. The findings of this research could contribute to the industrial scale productions especially from lignocellulosic raw materials.

1. Introduction

Bioethanol is considered as one of the most promising next-generation automotive fuels, as it is carbon neutral and can be produced from renewable resources, like lignocellulosic materials (Tamburini et al., 2011). The demand for bioethanol in the EU is expected to rise to 28.5 billion liters by 2020 (Wang et al., 2012). Statistics indicate that the world ethanol production is 60% from sugar crops, 33% from other crops and 7% from chemical synthesis (Dexter and Fu, 2009) and only two countries U.S. (corn-derived) and Brazil (sugar cane-derived) monopolize more than 90% of the world's bioethanol production (Serrano-Ruiz et al., 2012). Even though bioethanol is the predominant biofuel today, it has important compatibility, energy–density and water-absorption issues that limit its further implementation in the current fuel infrastructure (Serrano-Ruiz and Dumesic, 2011; Serrano-Ruiz et al., 2012).

The biorefinery industry is looking for cheaper, abundant and more accessible feedstock for producing bioethanol (Wang et al., 2012). In rice industry, biomass residues that are commonly utilized for energy generation are rice straw and rice hull, which are crop residue and agro-industrial residue, respectively. Rice hull is the outer layer of a rice seed, and removed from the rice seed as a by-product during the milling process. It consists of 28.6% cellulose, 28.6% hemicelluloses, 24.4% lignin and 18.4% extractive matter. Its annual production is approximately 137.05 million tons which is distributed in Africa (4.9 million tons), America (7.62 million tons), Asia (123.65 million tons), Europe (0.82 million tons), and Oceania (0.06 million tons) (Lim et al., 2012). The rice hulls in these quantities could be converted to 38.4 billion liters bioethanol per year, approximately.

With increasing interest in the industrial application of batch alcoholic fermentation, various kinetic models have been examined for microbial growth, product formation and substrate consumption. Kinetic modeling may be regarded as an important step in developing a fermentation process, since models help in process control, reducing process costs and increasing product quality (Dodic et al., 2012). Microbial processes are inherently complex, and it is of critical importance in practical applications to develop models that provide an accurate description of the process without unnecessary complexity (Luong et al., 1988). Unstructured kinetic models are frequently used because of their simplicity and adequacy for technical purposes (Garcia-Ochoa et al., 1995; Richard and Margaritis, 2004). In general, these models

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Nomenclature

A_c	cross-sectional area or area available for oxygen exchange, m^2
EtOH	ethanol
D_i	impeller diameter, m
D_{int}	internal diameter of vessel, m
d_f	largest inner diameter of shaken flask, m
d_o	shaking diameter, m
DT	doubling time, h
Fr	Froude number, dimensionless
g	gravitational acceleration, 9.81 m s^{-2}
HAc	acetic acid
HFor	formic acid
HLac	lactic acid
HSuc	succinic acid
i	the number of experimental data points, dimensionless
j	degree of product inhibition, dimensionless
k	constant with magnitude dependent on the geometry of the impeller, dimensionless,
K_s	saturation constant, g/L
K_I	inhibition constant, g/L
N	impeller rotation speed, rpm
N_p	power number for stirred tank, dimensionless
N'_p	modified power number for unbaffled shaken flask, dimensionless
n	shaking frequency, rpm
P	product concentration, g/L
P_m	maximum product concentration, g/L
Ph	phase number, dimensionless
P_o	unaerated power consumption for stirred tank, W
P_f	power consumption for shaken flask, W
P_o/V_L	power consumption per unit volume of liquid, W m^{-3}
q_A	specific reaction rate of A, h^{-1}
Re_i	impeller Reynolds number, dimensionless
Re_f	Reynolds number for shaken flask, dimensionless
S	substrate concentration, g/L
V_L	working volume, m^3
υ_{tip}	impeller tip speed, m s^{-1}
X	biomass concentration, g/L
Y_{AB}	yield of A from B, g g^{-1}
Z	minimizing objective function of the discrepancy between results, dimensionless

Greek symbols

μ	Specific growth rate, h^{-1}
μ_{max}	Maximum specific growth rate, h^{-1}
α	Inhibition constant for nonspecific inhibitors in the hydrolysate, dimensionless
ε	The rate of energy dissipation per unit mass of fluid, W kg^{-1}
λ	Kolmogorov's eddy size, μm
γ	Shear rate, s^{-1}
τ	Shear stress, N m^{-2}
ρ	Fluid density, kg m^{-3}
η	Dynamic viscosity of fluid, kg m^{-1} s^{-1}
υ	Kinematic viscosity of fluid, $m^2 s^{-1}$

use a form of the logistical equation to describe biomass growth and a form of the *Luedeking-Piret* model to describe biopolymer formation as a function of biomass. In order to fit their experimental data, several researchers have also used *Monod* type equations to better describe biomass growth and product formation in terms of the limiting substrate (Moraine and Rogovin, 1966; Garcia-Ochoa et al., 1995) and others have proposed substrate dependent equations which are not of the *Monod* type (Quinlan, 1986; Richard and Margaritis, 2004).

The two main objectives of the present study were: (i) to evaluate the use of both the constant Reynolds number and the constant impeller tip speed as scale-up methodologies under laboratory conditions for the step-wise scale-up process from 100 mL shaken flask to 10 L stirred-tank bioreactor, considering whether an increase in the bioethanol yield can be achieved, and (ii) to validate the kinetic models applied for better describing the behaviors of *Escherichia coli* KO11 during bioethanol fermentation from rice hulls hydrolysate.

2. Methods

2.1. The preparation and dilute acid pre-treatment of rice hulls

The rice hulls were obtained from Kahya rice factory, Izmir, Turkey. They were milled in a hammer mill (Brook Crompton Series 2000, UK) to pass through a 1 mm screen. The milled rice hulls were dried in an oven (Memmert GmbH, Germany) at 70 °C for one night.

The milled rice hulls at a solid loading of 30% (w/w) were mixed with 0.40 M H_2SO_4 and pretreated in an autoclave at 121 °C under the pressure of 0.10 MPa for 60 min. The detoxification process was carried out using 340 mM $Ca(OH)_2$ at 60 °C for 30 min at 400 rpm. The hydrolysate was adjusted to initial pH 6 with 6 M KOH and then separated (10,000 rpm, 30 min) using centrifuge separator (Westfalia Seperator Mineral oil Systems GmbH D-59302, Germany) to remove any precipitate formed before using it as substrate. The dry LB ingredients were added to the hydrolysate and were not sterilized for fermentation (Moniruzzaman and Ingram, 1998).

2.2. Strain maintenance and preparation of Inocula

E. coli KO11 was provided by courtesy of Professor L.O. Ingram (University of Florida, USA). The recombinant *E. coli* KO11 is the derivative of *E. coli* B and contains the chloramphenicol acyl transferase gene (*cat*) and the *Z. mobilis* genes encoding alcohol dehydrogenase (*adhB*) and pyruvate decarboxylase (*pdc*) for ethanol production. Stock cultures were stored in 40% glycerol at −80 °C.

The cells from a single well-isolated colony were inoculated into 250 ml cotton-plugged-conical flasks containing 50 ml of modified Luria–Bertani (LB) medium (Moniruzamman et al., 1997) with 50 g/L glucose. The cultures were incubated under static condition for 16 h at 30 °C in the absence of antibiotic. Then, the cells were harvested by centrifugation (6000*g*, 5 min and 5 °C) and used as inocula for the experiments at the initial dry weight of 0.72 g cell/L.

2.3. Bioethanol production conditions

For the small-scale production, the cells were incubated on an orbital shaker (IKA® KS 4000ic Thermoshake, Werke GmbH & Co. KG, Germany) with a 20 mm shaking diameter at a shaking frequency of 228 rpm in 100 mL flask (62 mm diameter) containing 80 mL of rice hulls hydrolysate with dry LB ingredients at the temperature of 30 °C under semi-anaerobic condition during 72 h of