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Enhancement of biobutanol production by electromicrobial glucose conversion in a dual chamber fermentation cell using *C. pasteurianum*

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ABSTRACT

A set of experiments have been performed to investigate the production of biobutanol as a novel applicable biofuel in a bioelectrolysis cell (BEC). The objective of this work was to understand the mechanism and production rate of the biobutanol by bioelectrosynthesis (BES) using glucose as a substrate. Four main factors, such as electrode materials, substrate concentration, operating temperature, and poised applied voltage were investigated in batch mode to achieve optimum condition for producing maximum butanol by *C. pasteurianum* in BEC. Standard modified P2 medium (MP2) and standard minimal medium (SMM) were used as fermentation media in batch operation mode. Numerical optimization using central composite design (CCD) method has been used to maximize the butanol production within the experimental range. The maximum butanol production 13.31 g/L was obtained by applying 1.32 V indicating the suitability of this procedure. The results showed that by applying optimum conditions in SMM, the butanol could be enhanced remarkably by electroactive microorganisms in cathode chamber.

1. Introduction

The rapidly growing demand for biofuel is gaining attention nowadays, especially for biobutanol beyond bioethanol. Biobased butanol fuel is known as a second generation alcoholic biofuel which includes higher energy density and lower volatility compared to ethanol. A number of companies are looking for developing biobutanol on industrial scale. This biofuel is able to compete with $80 bbl oil. The advantages of biobutanol are its variety of commercial usages in a current market worth over $5 billion dollars [1]. Biobutanol that has such physicochemical properties can be properly used in compression ignition (CI) engine. Using *n*-butanol as pure or blends with diesel has demonstrated promising potential for enabling operation in diesel engines. Among different fuel properties, the long ignition delay, and high oxygen content of *n*-butanol are key factors to amend the fuel-air mixing and lower the NO_x and ash emission [2]. The impact of biobutanol and biobutanol–diesel blends on the combustion and emission features in an ignition engine showed that the indicated specific fuel consumption (ISFC) of the mixed fuels was higher than that of diesel. Nevertheless, the exhaust temperature was less than that of diesel. Moreover, nitrogen oxide (NO_x), carbon monoxide (CO) and soot from Bu20 (20% biobutanol and 80% diesel) were lower than those values from diesel fuel; however, hydrocarbons (HC) were higher than those from diesel [3]. Biobutanol is also an important chemical and solvent which has been recently addressed as one of the evolving second generation liquid biofuels. As a liquid transportation fuel, butanol is preferred to the first-generation biofuel, bioethanol, because of its higher mixing rate with gasoline without engine modification, octane enhancing number, and suitable delivery using current pipeline infrastructure. Furthermore, its energy content is higher, since butanol contains 96% of the energy of a gasoline volume unit, while ethanol only produces 73% of gasoline energy per unit volume [4]. Apart from biofuel application of butanol, it is a global chemical with over 4 million tons of annual demand, and *n*-butanol is an applicable chemical utilized in grave high value applications in a broad range of commercial markets. It is also an intermediate used to produce resins and specialty solvents used in final product formation, such as paints and coatings to cosmetics and perfumes, adhesives and inks, and even food flavors and extracts. It can be used in plastics and polymers, brake fluids, lubricants, synthetic rubber, fire retardants as well [5].

Conventional biobutanol fermentation suffers several limiting constraints. One of the main challenges of traditional biobutanol production is low productivity which can be enhanced by one of

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Nomenclature

a	exponent of inhibitory product
ANOVA	analysis of variance
ATP	Adenosine triphosphate, is the energy currency of life. Which is found in every cell
B	butanol concentration (g/L)
BEC	bio-electrolysis cell
BES	bio-electrosynthesis
Bu20	20% biobutanol and 80% diesel
CCD	central composite design
CI	compression ignition
CO	carbon monoxide
DNS	dinitrosalicylic acid
EEB	electron equivalent balance
EET	extracellular electron transfer
EMS	electro-microbial synthesis
MES	microbial-electrosynthesis
ISFC	indicated specific fuel consumption
K_S	Monod or substrate saturation constant (g/L)
NADH	Nicotinamide adenine dinucleotide (reduced form), a coenzyme found in all living cells
NAD^+	Nicotinamide adenine dinucleotide (oxidized form)
NR	neutral red
NO_x	nitrogen oxides
MECS	microbial electrochemical synthesis
MP2	modified P2 medium
m, n	power constants
OF	objective function
P	concentration of inhibitory product (g/L)
P^*	critical concentration of inhibitory product above which cells do not grow (g/L)
S	initial substrate (glucose) concentration (g/L)
S^*	critical glucose concentration above which cells do not grow (g/L)
SMM	standard minimal medium
T	temperature (°C)
t	time (h)
V	voltage (V)
X	biomass (g)
x_i	coded variable

Subscripts

Exp	experimental
Cal	calculated

Greek

μ	specific growth rate (h^{-1})
μ_{max}	maximum specific growth rate (h^{-1})
α_0	average value of the responses of the assays
α_i	principal effect of each factor i on the response
α_{ij}	interaction effect between factor i and factor j on the response
β	the number of NADH requirements per mole of metabolism
ΔP	formation of final products in mol or mmol

the novel techniques, such as electromicrobial synthesis (EMS) or bioelectrosynthesis (BES). In such a process, bacteria catalyze reduction reactions (by utilizing hydrogen atom and electron) of organic molecules (on the cathode) to produce high value-added product. On the other hand, on the anode surface, the hydrogen ion is produced and passes through exchange membrane. In fact, on the anode, under abiotic conditions, water is oxidized to protons and oxygen in the anode half reaction and the protons pass through the permeable membrane. At the cathode, half reaction occurs by conversion of carbon sources to carbon-bearing compounds by biological film provided on cathode. The mechanism of extracellular electron transfer is divided into the following steps: (1) direct electron transfer: nanowire, or direct contact; and (2) mediators-shuttled: [6].

Solventogenic *Clostridia* species (*C. acetobutylicum*, *C. beijerinckii*, *C. saccharobutylicum*, and *C. saccharoperbutylacetonicum*) are widely used for solvent production (acetone, butanol, and ethanol) especially butanol [7]. On the other hand, some kind of *Clostridia* such as *C. tyrobutyricum* can produce high organic acid (butyrate) using glucose [8]. Special heterotroph microorganisms like *C. pasteurianum* are able to produce biochemicals utilizing organic fermentable matters. A lot of studies have been performed to produce solvents by *C. pasteurianum* by using glycerol utilization, while using glucose as substrate has not been attended due to low solvent production rather than organic acid production. In this study we tried to discover that by the aid of the electricity, the more solvents especially butanol can be produced by this strain. By bioelectrosynthesis, microorganisms are able to drive electrons from electrode for organic matter reduction. The electricity energy can be supplied by external energy resources.

Biological redox cofactor (NAD^+/NADH) plays an important role in bacterial metabolism. The ability of microorganisms to exchange electrons directly or indirectly with cathode, and therefore drive novel reductive reactions at electrodes led to the improvement of microbial electrochemical synthesis (MECS) over the last decade [9]. In microbial metabolism, NADH is generated (NAD^+ to NADH) by oxidation reactions (e.g., 2 NADH generated from 1 glucose by glycolysis) which have to be consumed in vise versa reaction by oxidant for an appropriate redox balance [8]. The goal of this work was to maximize the butanol production during batch fermentation process in MEC. The common glucose fermentation pathway by *C. pasteurianum* and its conversion into butanol, ethanol, butyrate along with release of H_2 and other components are presented in Fig. 1. The formation of each end-product from glucose with formation of butanol released H_2O. Therefore, to maximize the production of specific product (butanol) in fermentation broth, electricity supplement can be a suitable method. In fact, in this report, understanding the mechanism of bioelectrosynthesis (BES) and optimizing the operating conditions for butanol production are the main targets using glucose as a substrate and *C. pasteurianum* as a microorganism in BEC.

2. Materials and methods

2.1. Microorganism and medium

Choi et al. [10] showed that, among *C. pasteurianum*, *C. acetobutylicum* and *C. tyrobutyricum*, just *C. pasteurianum* was the most suitable reduction peak for BES. *C. pasteurianum* ATCC 6013™ was purchased from CEDARLANE (American Type Culture Collection). Freeze-dried microorganism was revived and grown (after heat shock at 80 °C) in peptone-yeast extract-glucose (PYG) media under anaerobic condition for 36 h at 37 ± 0.01 °C and 150 rpm in 125 mL serum vials (working volume 50 mL) [4]. The medium