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Acetone-butanol-ethanol (ABE) fermentation using the root hydrolysate after extraction of forskolin from *Coleus forskohlii*

Shirish M. Harde [a, b], Swati B. Jadhav [a, b], Sandip B. Bankar [b], Heikki Ojamo [b], Tom Granström [b], Rekha S. Singhal [a], Shrikant A. Survase [b, *]

[a] *Food Engineering and Technology Department, Institute of Chemical Technology, Matunga, Mumbai 400019, India*
[b] *Department of Biotechnology and Chemical Technology, Aalto University School of Chemical Technology, P.O. Box 16100, 00076 Aalto, Finland*

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ABSTRACT

The biomass obtained after the extraction of forskolin from the roots of *Coleus forskohlii* was evaluated as a substrate for the production of acetone-butanol-ethanol (ABE). The spent biomass constituting more than 90% of the raw material showed 50–70% carbohydrates with starch and cellulose being the major constituents. This study was undertaken to optimize enzymatic hydrolysis of *C. forskohlii* roots for maximum release of fermentable sugars and subsequent fermentation to ABE. The root biomass was hydrolyzed using the Stargen® 002 and Accellerase® 1500. Cocktail of both enzymes (16U Stargen® 002 and 60 FPU Accellerase® 1500) could produce 41.2 g/l of total reducing sugars (glucose equivalent to 32.33 g/l). The production of ABE was optimized in a batch fermentation using *Clostridium acetobutylicum* NCIM 2877. The maximum ABE production using the root hydrolysates was 0.55 g/l. Pretreatment with lime and Amberlite XAD-4 increased the production of total solvent to 5.33 g/l.

1. Introduction

Coleus forskohlii Briq, a herbal plant belonging to the *Lamiaceae* family, is native to India and is reported in Ayurvedic *Materia Medica* under the Sanskrit name Makandi and Mayini [1]. Presently, about 40,000 acres of land is under cultivation of *C. forskohlii* in India, Africa and South East Asia for its tuberous roots [2]. The cultivation may provide an average yield of 800–1000 kg/ha of dry tubers which can be improved to 2000–2200 kg/ha of dry tubers by applying proper practices. The cultivation of *C. forskohlii* has been on an increase due to its commercial utilization [3]. The roots of the *C. forskohlii* plant are a unique source of forskolin (FSK), a labdane diterpene compound. FSK has been shown to be useful in the treatment of asthma, glaucoma, cardiovascular diseases and certain types of cancer [4].

The spent biomass obtained after extraction of forskolin from the root has no commercial value. It constitutes more than 90% of carbohydrate-rich raw material which could be used as a substrate for the production of value added chemicals and fuels such as ABE solvents. Currently, tonnes of *C. forskohlii* root biomass is either dumped or burnt which are environmentally hazardous. There is an increased interest among the researchers to develop various strategies to utilize waste biomass for useful and value-added purpose.

Increase in petroleum prices and depletion of fossil fuels are the key reasons for ongoing search of energy alternatives worldwide [5]. Bioconversion of waste biomass to alcoholic fuels such as bioethanol, biobutanol, and biodiesel is rapidly emerging as an area of interest among researchers [6]. Currently, countries like USA and Brazil contribute 20–30% of biobutanol production in fuel market, while in Asia the bioethanol production is at a very early stage of development [7,8]. Biobutanol has attracted the attention of researchers and investors due to its various advantages over other biofuels such as like high heating value, low freezing point, high hydrophobicity, and low heat of vaporization that are closer to gasoline [9–11]. The current production of n-butanol is about 5–6 million tons per year with a worldwide market sale of US$7–8.4 billion [12]. The market demand is anticipated to increase dramatically, if n-butanol can be produced cost-effectively [13].

Butanol can be obtained from renewable biomass by ABE fermentation [14]. *Clostridium acetobutylicum* utilizes a range of carbon sources to produce butyric acid and acetic acid in the first phase (acidogenesis), and acetone, butanol and ethanol in the second phase (solventogenesis). The process of biobutanol production

* Corresponding author. Tel.: +358 400368375; fax: +358 9 462 373.
E-mail address: shrikantraje1@gmail.com (S.A. Survase).

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