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Enhancing Clostridial Acetone-Butanol-Ethanol (ABE) Production and Improving Fuel Properties of ABE-enriched Biodiesel by Extractive Fermentation with Biodiesel

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Abstract The extractive acetone–butanol–ethanol (ABE) fermentations of *Clostridium acetobutylicum* were evaluated using biodiesel as the in situ extractant. The biodiesel preferentially extracted butanol, minimized product inhibition, and increased production of butanol (from 11.6 to 16.5 gL^{-1}) and total solvents (from 20.0 to 29.9 gL^{-1}) by 42% and 50%, respectively. The fuel properties of the ABE-enriched biodiesel obtained from the extractive fermentations were analyzed. The key quality indicators of diesel fuel, such as the cetane number (increased from 48 to 54) and the cold filter plugging point (decreased from 5.8 to 0.2 °C), were significantly improved for the ABE-enriched biodiesel. Thus, the application of biodiesel as the extractant for ABE fermentation would increase ABE production, bypass the energy intensive butanol recovery process, and result in an ABE-enriched biodiesel with improved fuel properties.

Keywords Biobutanol · Biodiesel · Butanol inhibition · *Clostridium* · Extractant · Low cold filter plugging point (CFPP) · Solvent extractive fermentation

Introduction

The decline of reserve, the rising price, and the concern about the environmental impact of petroleum-based fuel have initiated interest in renewable biofuel [1, 2]. Vegetable oil and/or

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animal fat-based biodiesel is one of the most promising biofuels that has the potential to partially replace petroleum-based diesel fuel [3, 4]. However, biodiesel has not been widely used in the fuel market, mainly because of its high production cost and poor flow property at low temperature. The flow property of biodiesel is usually characterized by the cold filter plugging point (CFPP) [5]. A high CFPP biodiesel will limit its use in winter in large parts of China. Blending butanol with biodiesel has shown an improvement in flow property of the butanol-enriched-biodiesel [6–8]. To this end, microbial produced biobutanol will be the best choice to enrich and improve the fuel property of biodiesel.

In China, clostridial acetone–butanol–ethanol (ABE) fermentation has been in operation since the 1950s [9]. In recent years, over a dozen ABE plants have been built or resumed operation to expand ABE production [10]. However, the majority of these ABE products are used as solvents in various applications. In spite of its superior fuel property, biobutanol derived from ABE fermentation is currently not an economically competitive fuel. Costs of substrate and butanol recovery from the fermentation broth are the major burden.

In clostridial ABE fermentation, butanol inhibition limits product titer, which in turn contributes to the high cost of product recovery [11, 12]. Therefore, in situ solvent extractive fermentation has been proposed as one of the approaches to minimize butanol inhibition and increase product titer [13–18]. However, the market value of the extractant and the subsequent cost of extractant recycling have prevented them from being applied on large scales. An ideal in situ extractant should be one that has a direct end-use as a fuel, which will bypass the expensive butanol recovery and extractant recycling procedure [19, 20].

In this communication, we report the application of biodiesel as an in situ extractant for clostridial ABE fermentation, which increased butanol and total solvent production by 42% and 50%, respectively. The resulting ABE-enriched biodiesel has significantly improved fuel properties such as a lower CFPP (0.2 °C) and a higher cetane number (54), compared to those (5.8 °C and 48) of the original biodiesel. This improved ABE-enriched biodiesel can be used as a substitute to petroleum-based diesel fuel.

Materials and Methods

Microorganism, Media, and Fermentation Conditions

The bacterial strain *Clostridium acetobutylicum* ATCC 824 was used in this study. The medium composition used for strain maintenance and ABE fermentation are as follows (per liter): 100 g glucose, 2 g yeast extract, 6 g tryptone, 3 g CH_3COONH_4, 0.3 g $MgSO_4{\cdot}7H_2O$, 0.5 g KH_2PO_4, and 10 mg $FeSO_4{\cdot}7H_2O$. The initial medium pH was adjusted to 6.5 before sterilization.

The biodiesel was obtained from Hubei Tianji Bioenergy Technology Development Co. Ltd (Wuhan, China). The fatty acid methyl ester composition of the biodiesel is listed in Table 1. The biodiesel was washed multiple times with hot water until it reached a neutral pH, and then treated with charcoal to remove residual water and color.

The fermentation was carried out in 500-ml flask containing 250 ml medium (10% inoculum, 30 °C, strict anaerobic condition) for 72 h. The biodiesel was added to the fermentation broth at a ratio of 1:2 (v/v) at different time (0 to 64 h with 8-h intervals). A

Table 1 The fatty acid methyl ester composition of the biodiesel.

Fatty acid methyl ester[a]	Percentage (%)
Myristic acid methyl ester (C14:0)	0.27
Palmitic acid methyl ester (C16:0)	17.5
Stearic acid methyl ester (C18:0)	15.1
Oleic acid methyl ester (C18:1)	37.5
Linoleic acid methyl ester (C18:2)	9.87
Linolenic acid methyl ester (C18:3)	4.74
Arachidic acid methyl ester (C20:0)	0.34
Saturated fatty acid methyl ester	43.21
Unsaturated fatty acid methyl ester	52.11

[a] The fatty acid methyl ester was determined by GC/MS

sample was taken from the biodiesel and the fermentation broth to determine the concentrations of acetone, butanol, and ethanol.

Determination of the Partition Coefficients

The partition coefficient of the expected ABE fermentation products was determined as follows: a stock solution was prepared by dissolving 2.0 g butanol, 1.0 g acetone, 0.5 g ethanol, 0.2 g acetic acid, and 0.5 g butyric acid in 100-ml fermentation medium broth. Fifty milligrams of biodiesel was mixed with the stock solution in a conical flask, which was then sealed with a rubber stopper and incubated at 30 °C for 24 h in a constant temperature and humidity chamber. The concentrations of butanol, acetone, ethanol, acetic acid, and butyric acid in the biodiesel and medium broth were then determined. The partition coefficient was calculated by the following formula: $K=C_E/C_B$. Where C_E is the concentration of the analyst in the biodiesel and C_B is the concentration of the analyst in the medium broth [16].

Analysis

The fatty acid ester composition of the biodiesel was determined by a GC/MS (Thermo-Finnigan, USA). The concentrations of acetone, butanol, ethanol, acetic acid, and butyric acid were determined by a GC equipped with a FID detector (Thermo-Finnigan, USA) and a polyethylene glycol phase capillary column (Agilent, USA). Isobutanol was used as the internal standard. The fuel properties of the biodiesel were analyzed based on the methods published by the American Society for Testing and Materials (ASTM) [20].

Results and Discussion

Preferential Extraction of Butanol and Butyric Acid

It is well documented that butanol is the major inhibiting compound in clostridial ABE fermentation, resulting in low titers of butanol (10–12 gL^{-1}) and total solvent (18–20 gL^{-1}) [12]. Minimizing butanol inhibition is the primary goal for in situ extractive fermentation,

which requires butanol being extracted preferentially by the extractant. To evaluate whether biodiesel can serve such a preferential extraction role, the partition coefficient of the major ABE fermentation products were initially determined in a biodiesel/medium broth mixture. As shown in Table 2, with a partition coefficient of 1.23 and 1.62, respectively, butanol and butyric acid were preferentially extracted by the biodiesel. The other products have a much lower partition coefficient (0.16–0.22). These results indicated that biodiesel would preferentially extract butanol (and butyric acid), and minimize butanol inhibition during biodiesel extractive clostridial ABE fermentation.

In Situ Extractive ABE Fermentation

Preferential extraction of butyric acid may not favor butanol production because clostridial ABE fermentation poses sequential acidogenic and solventogenic biphasic fermentation. In the acidogenic phase, which usually takes place in the exponential growth phase, substrate is converted into acetic acid, butyric acid, H_2, and CO_2. Next, during the solventogenic phase, which usually occurs in the stationary phase, acetic acid, and butyric acid are re-assimilated and converted into acetone, butanol, and ethanol [12, 21, 22]. The preferential extraction of butyric acid means butyric acid will not be available for butanol production.

To understand the extent of the potential negative impact of preferential extraction of butyric acid, we determined the amount of butyric acid produced in a preliminary fermentation. The results showed that butyric acid reached its maximum titer, 5 gL^{-1}, at 32 h, and then declined to about 1 gL^{-1} at 48 h. Based on this result, the biodiesel extractant should be added later than 48 h in order to avoid the negative impact of preferential extraction of butyric acid. However, to minimize butanol inhibition, early addition of biodiesel is preferred.

To achieve a balance between minimized butanol inhibition and negative impact of preferential extraction of butyric acid, an optimized time point should be determined for biodiesel extraction. A series of in situ extractive fermentations were then carried out by adding biodiesel at different time points (0 to 64 h with 8 h intervals). The results are shown in Fig. 1. Surprisingly, butanol and total solvent production were increased by 25–42% and 22.5–50%, respectively, for all in situ extractive fermentations regardless of the biodiesel addition time. These results indicate that minimizing butanol inhibition has a greater impact on ABE fermentation than minimizing extraction of butyric acid. Nevertheless, the highest titers of butanol (16.5 gL^{-1}) and total solvent (29.9 gL^{-1}) were achieved when biodiesel

Table 2 Partition coefficient of the ABE products in the biodiesel.

Products	Partition coefficient[a]
Acetone	0.19
Ethanol	0.16
Butanol	1.23
Acetic acid	0.22
Butyric acid	1.62

[a] The partition coefficient was calculated by dividing the concentration of the product in the biodiesel by the concentration of the product in the medium broth in a biodiesel/medium broth mixture. This mixture was setup by mixing 50 ml biodiesel with 100 ml medium broth containing 2 g butanol, 1 g acetone, 0.5 g ethanol, 0.2 g acetic acid, and 0.5 g butyric acid, and incubated at 30 °C for 24 h

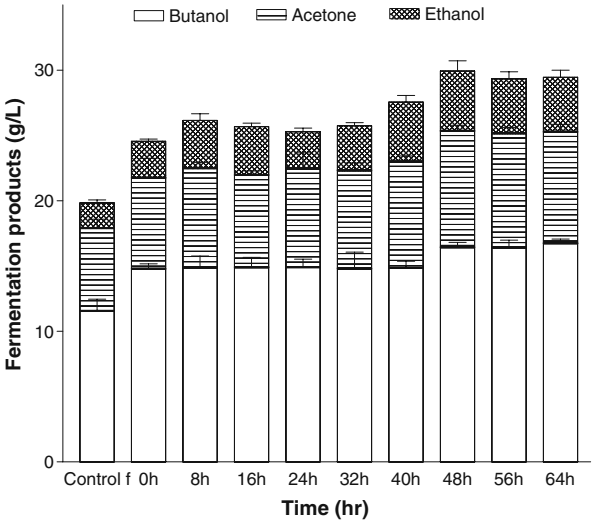

Fig. 1 Butanol and total solvent production in the biodiesel extractive ABE fermentation. The time intervals (0 to 64 h) of the *x-axis* indicate the time when biodiesel was added to the medium (at a 1:2 ratio) during fermentation. Control has no biodiesel added. The *y-axis* represents total solvent production

was added at 48 h; this is an optimal time to minimize both butanol inhibition and negative impact of butyric acid extraction.

Fuel Properties of ABE-enriched Biodiesel

The fuel properties of ABE-enriched biodiesel from extractive fermentation were analyzed. As shown in Table 3, compared to those of the original biodiesel, most fuel parameters (except for flash point) were improved for ABE-enriched biodiesel. The major quality indicators of diesel fuel, the cetane number and CFPP, were significantly improved. This ABE-enriched biodiesel can be used as a substitute to petroleum-based diesel fuel.

Table 3 Comparison of the fuel properties of the biodiesel and the ABE-enriched biodiesel.

Fuel property	Biodiesel	ABE-enriched biodiesel
Water and sediment	0.03	0.05
CFPP[a]	5.8	0.2
Kinematic viscosity, 40°C	4.2	3.1
Flash point	140	95
Sulfated ash	0.019	0.012
Cetane number	48	54
Carbon residue	0.055	0.043
Acid number	0.75	0.37
Phosphorus content	0.003	0.003

Fuel properties were tested based on the procedures of the American Society of Testing and Materials (ASTM)

[a] *CFPP* cold filter plugging point

Conclusion

Biodiesel can be used as an in situ extractant for clostridial ABE fermentation. Optimal fermentation improvement can be achieved when biodiesel extraction starts at 48 h during fermentation. The advantage of biodiesel extractive ABE fermentation is bypassing the expensive processes of product recovery and extractant recycling. The resulting ABE-enriched biodiesel has improved fuel properties such as a higher cetane number and a lower CFPP, allowing for being used as a substitute to petroleum-based diesel fuel.

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