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# Improvement of acetone–butanol–ethanol (ABE) production from switchgrass pretreated with a radio frequency-assisted heating process

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## HIGHLIGHTS

- Switchgrass hydrolysate through RF was conducted first time for ABE production.
- Hydrolysates through RF performed consistently better for ABE fermentation than WB.
- RF heating generated harsher condition, produced more nutrients for fermentation.

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## ABSTRACT

Radio frequency (RF) heating assisted alkaline pretreatment was evaluated as an emerging technology to generate hydrolysates for acetone–butanol–ethanol (ABE) fermentation. Traditional water bath (WB) heating was utilized as a control. ABE fermentation was conducted with *Clostridium beijerinckii* 8052 using the hydrolysates as carbon sources with or without yeast extract (YE, as supplemented complex nitrogen sources). Results indicated that the hydrolysates generated through RF pretreatment (RFH) performed consistently better for ABE fermentation than the hydrolysates generated through WB (WBH), with or without yeast extract (YE) supplementation. Without YE addition, fermentation with RFH generated 50% higher ABE than that with WBH. YE supplementation (up to 5 g/L) enhanced fermentation under all conditions and diminished the difference among performances of fermentations. ABE production with 1 and 2 g/L YE supplementation increased by 15% and 107% than those without YE for WBH, while for RFH the increase were 1% and 55%, respectively. Supplementation of 5 g/L YE brought the final solvent production to be very close to each other with either WBH or RFH. Therefore, we concluded that RFH contained more nutrients than WBH which made a big difference for the ABE fermentation. RF could be explored as an efficient method for biomass pretreatment. This study provided valuable references for developing a sustainable system to convert lignocellulosic biomass into bioenergy in an economically efficient manner.


## 1. Introduction

In recent years, the finite nature of fossil fuel resources as well as associated negative environmental effects including emission of greenhouse gases and global warming have raised many public concerns [1,2]. Bio-based fuels and materials produced from renewable sources have been considered as a solution for this problem [3]. Lignocellulosic biomass such as agricultural residues (corn cob, rice straw, wheat straw, etc.), forestry residues (branches, sawdust, etc.), dedicated crops and trees (switchgrass, eucalyptus, etc.) and municipal waste is abundant and low-value renewable resources [4,5] that can be used for sustainable production of biofuels and biochemicals. Biosolvents such as acetone, butanol and ethanol (ABE) produced by microbial fermentation from renewable resources have attracted a lot of attentions because of their considerable value as industrial chemicals and fuels. Among them, biobutanol (n-butanol) has been of particular interest due to its various advantages as biofuel source and excellent value for industrial chemical [6]. As a fuel source, butanol has high volumetric energy content, which is comparable to gasoline and 30% higher than that of ethanol; butanol is less corrosive, and thus it can be delivered through existing infrastructure; butanol has low vapor pressure, making it easier to handle; butanol can be used as a replacement for gasoline at any percentage and no modification is required for the existing engine [7]. While as an industrial chemical, butanol can be used in many aspects, including (but not limited to) latex surface coating, enamels and lacquers industries, antibiotic, vitamin and pharmaceuticals manufactures,

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and food and flavor industries, etc. Besides, acetone is also an important solvent with many industrial applications [8]. The biosolvents, namely ABE, are usually produced through fermentation using solventogenic clostridia through a process called ABE fermentation [9]. For ABE fermentation, the cost of substrate is an important part out of the overall cost [10]. Exploration of inexpensive feedstocks is a desirable mean for improving the economics of the ABE fermentation process. As discussed above, lignocellulosic biomass is abundant and cheap renewable resources that can be explored for ABE production [11].

In order to convert lignocellulosic biomass into biobased fuel and chemicals through a downstream fermentation process, a pretreatment process (along with necessary enzymatic hydrolysis of the generated cellulose and hemicellulose to fermentable sugars) is usually required. In the past decades, various pretreatment technologies have been developed [12]. These various methods have their own advantages. However, most of these approaches involve high temperature heating and/or high pressure treatment conditions, which induce high energy consumption [13,14]. Meanwhile, most of these pretreatment methods generate significant levels of inhibitors which would suppress the performance on microbial fermentation steps [12]. Compared to the convection traditional heating technologies, dielectric heating has various advantages, especially for applications such as biomass pretreatment. Convection heating is based on superficial heat transfer, while dielectric heating transforms electromagnetic energy into heat in a volumetric and fast way. At the same time, the electromagnetic field could generate non-thermal effects, which can also accelerate destruction of lignocellulosic biomass crystallinity structure [15,16]. Depending on the wavelength, dielectric heating can be divided into two categories, namely microwave and radio frequency (RF) heating. Microware heating has already been investigated by many researchers for biomass pretreatment purposes [17–20], while RF heating has higher electricity to electromagnetic power conversion efficiency, and a much deeper penetration of RF energy into a wide array of materials [21,22]. With such properties, RF based heating approaches are believed to be easier to scale up.

Therefore, the objective of this study was to investigate RF heating as an efficient biomass pretreatment approach, and further evaluate the generated hydrolysates as inexpensive substrate for ABE production. In order to fulfill this purpose, switchgrass was pretreated by RF-assisted heating (traditional heating using water bath (WB) as control) and further hydrolyzed to generate the hydrolysates. ABE fermentation was carried out with *Clostridium beijerinckii* NCIMB 8052 using the hydrolysates as carbon sources. Moreover, the effects of yeast extract (YE, as supplemented complex nitrogen sources, up to 5 g/L) was investigated on the fermentation performance.

## 2. Materials and methods

### 2.1. Raw materials

Switchgrass was acquired from the Bioenergy and Bioproducts Center at Auburn University. The materials were air-dried and stored at 4 °C prior to usage (less than one week). Before carrying out the pretreatment, the switchgrass was milled to an average particle size of $1.0 \times 2.0 \times 0.3$ cm$^3$ ($L \times W \times H$) with a Wiley mill (Thomas Scientific, Philadelphia, PA) and stored in sealed plastic bags at room temperature.

### 2.2. Biomass pretreatment

Switchgrass was soaked in 2 M NaOH solution at a solid to liquid ratio of 1:10 (w/v) at room temperature overnight. Then the sample was subjected to heating on a RF heater (SO6B; Strayfield, Berkshire, England) at a frequency of 27.12 MHz and maximum power output of 6 kW [23]. The distance between the two electrodes was fixed at 8.5 cm. During the heating process, four fiber-optic sensors (UMI, FISO Technologies, Quebec, Canada) were employed to record the temperature. When the temperature reached 90 °C, samples were kept at approximately 90 °C by turning RF heater on and off for 60 min. This pause-heating manner was repeated until the predetermined total heating time was reached. For the conventional heating (as the control for RF heating) pretreatment with WB, WB was preheated to 90 °C. Then the sample was put into the WB and heated (at 90 °C) for 60 min.

For both pretreatment approaches, the samples were cooled down to room temperature after the heating process. The pretreated material was collected by filtration through a Whatman No. 4 filter paper in a Buchner funnel, and washed with warm deionized water for at least three times to neutralize the pH value to 7.0. The wet pretreated material was used directly (without drying again) for the chemical compositional analysis followed by enzymatic hydrolysis [24].

### 2.3. Enzymatic hydrolysis

Enzymatic hydrolysis was performed in 125 mL of 50 mM sodium citrate buffer (pH 4.8) at 2% glucan (w/v) with Novozym 22C (Novozymes, Franklinton, NC). The filter paper activity and β-glucosidase activity of Novozym 22C was 100 FPU/mL and 343 IU/mL, respectively. The enzyme loading used was 10 FPU/g glucan. The hydrolysis was carried out in a shaker with agitation of 150 rpm at 50 °C for 72 h. Samples were periodically taken for sugar analysis using an Agilent 1260 Infinity Quaternary LC VL HPLC (Agilent Technologies, Santa Clara, CA) with refractive index detector (RID) following the National Renewable Energy Laboratory (NREL) standard protocol [25]. Aminex HPX-87P column and a 30 mm × 4.6 mm i.d. guard column of the same material (Bio-Rad, Hercules, CA) was used to separate and quantify individual sugars.

### 2.4. Elemental analysis

Elemental analysis was performed on a Perkin–Elmer CHNS/O analyzer (model 2400, Series II) to quantify the carbon, hydrogen, nitrogen and sulfur contents in the raw and pretreated materials. Moreover, the supernatant of RF and WB pretreated switchgrass was analyzed using an Agilent 6890N GC connected with an Agilent 5973 mass-selective detector (MSD) equipped with a DB-1701 column (60 m × 0.25 mm, 0.25 μm film thickness).

### 2.5. Fermentation

A laboratory stock of *C. beijerinckii* 8052 was routinely stored as spore suspension in sterile double distilled water at 4 °C. Spores were heat-shocked at 80 °C for 10 min, followed by cooling on ice for 5 min. The heat-shocked spores were inoculated at a 1% inoculum level into 20 mL tryptone–glucose–yeast extract (TGY) medium. The TGY medium contains 30 g/L of tryptone, 20 g/L of dextrose/glucose, 10 g/L of yeast extract, and 1 g/L of cysteine-HCl monohydrate. The culture was then incubated at 35 ± 1 °C in an anaerobic chamber under $N_2$:$CO_2$:$H_2$ (volume ratio of 85:10:5) atmosphere. When the TGY culture grew to an optical density ($OD_{600}$) of 0.8–1.0 (took ~12–14 h), it was inoculated at a 5% ratio into 30 mL model solution in 100 mL Pyrex bottles for fermentation in the same anaerobic chamber. The fermentation solution consisted of the substrate (either biomass hydrolysates or synthetic substrate as control) supplemented with 1% of P2 stock solutions. The P2 medium contained the following compounds (in g/L):

$KH_2PO_4$, 0.5; $K_2HPO_4$, 0.5; $CH_3COONH_4$, 2.2; $MgSO_4{\cdot}7H_2O$, 0.2; $MnSO_4{\cdot}H_2O$, 0.01; $FeSO_4{\cdot}7H_2O$, 0.01; NaCl, 0.01; p-aminobenzoic acid, 0.001; thiamine-HCl, 0.001; and biotin, 0.00001 [26]. Prior to inoculation, the pH value of the fermentation broth was adjusted to 6.8–7.0 with filter-sterilized KOH solution.

ABE fermentation was carried out in parallel using various carbon sources (supplemented with P2 medium), including switchgrass hydrolysates generated from RF pretreatment (RFH), switchgrass hydrolysates generated from WB pretreatment (WBH), and a control (CON) with mixed sugars and acetic acid at the same levels as in RFH (henceforth in this paper, we also used RFH, WBH and CON to represent the fermentations carried out using RFH, WBH and CON, respectively, as carbon sources). Moreover, the effects of yeast extract (providing nitrogenous compounds, carbon, sulfur, trace nutrients, vitamin B complex and other important growth factors for cell growth) were investigated on the fermentation performance. Cell density and the concentration of fermentation metabolites were monitored through the course of fermentation.

### 2.6. Fermentation products analysis

ABE, acetic acid, and butyric acid were quantified using HPLC (Agilent Technologies 1260 series) equipped with an automatic sampler/injector and a RID using an HPX-87H column (Bio-Rad, Hercules, CA, USA). Fermentation samples were centrifuged at 13,200 rpm for 10 min, and the supernatant were diluted fivefold with distilled water before the HPLC analysis. The 5 mM $H_2SO_4$ solution was used as the mobile phase at a flow rate of 0.6 mL/min at 45 °C.

During the experiment, cell concentration was quantified as optical density ($OD_{600}$). ABE (or butanol) yield (g/g) was defined as total ABE (or butanol) produced (g/L) divided by the total sugar consumed (g/L) during the fermentation process.

### 2.7. Statistical analysis

All experiments were performed in triplicate. Multiple one-way analyses of variance (ANOVA) were conducted to investigate the effect of different pretreatment approaches on ABE production.

## 3. Results and discussion

### 3.1. Biomass pretreatment and enzyme hydrolysis

The composition of the switchgrass before and after heating treatment is illustrated in Table 1. Untreated switchgrass contained 21.4% lignin and 67.3% carbohydrates. The major portion of the carbohydrates was glucan. Both WB and RF pretreatment led to significant decrease in lignin content and increase of glucan fraction compared to the raw feedstock. Although the RF pretreatment generated a little bit higher glucan while lower xylan contents than WB, generally speaking, the pretreatment efficiency (based on the analyzed chemical composition) of both heating approaches were pretty much similar. NaOH pretreated switchgrass with RF heating generated a glucan yield of 72.6%, which was about 20% higher than that from the conventional pretreatment with WB heating as control (51.8%). 14.9 g/L of glucose was produced from the hydrolysates generated from WB heating pretreatment, while 15.5 g/L glucose was generated from the hydrolysates by RF heating pretreatment. Low levels of xylose and arabinose were also detected (at similar levels for both hydrolysates) for WB heating pretreated switchgrass. Hydrolysates generated from RF heating produced much higher (by ~40%) acetic acid than that from WB heating. Acetic acid is usually generated during the pretreatment process due to the acetylation of hemicellulose and to some extent lignin [27,32]. The higher level of acetic acid generated in the RF heating process demonstrated that RF heating might have brought about a harsher condition to switchgrass than WB heating during the pretreatment leading to severer acetylation.

### 3.2. Fermentation with switchgrass hydrolysates

The switchgrass hydrolysates generated in this study was very dilute and the total sugar concentration was only around 15 g/L. We attempted to investigate whether the dilute carbon sources can be directly (without further step such as concentration) converted to value-added bioproducts (such as biosolvents) through fermentation. ABE fermentation was carried out in parallel using various carbon sources (supplemented with P2 medium), including RFH, WBH, and CON.

Generally, cells grew rapidly in all the growth media with very short lag time, despite that RFH and WBH experienced a little bit longer lag phase compared to CON (Fig. 1A). Interestingly, RFH reached the highest maximum optical density of around 4.2 (approximately 24 h after the start of fermentation), which is even higher than that of CON. WBH reached a maximum $OD_{600}$ of around 3.3, which is 22% lower than that of RFH. As illustrated in Fig. 1B–D, ABE production was detected less than 10 h fermentation process, and reached the maximum levels at about 24 h. Corresponding to the cell growth profiles, RFH generated the highest ABE levels (2.96 g/L, of which butanol accounted for 1.95 g/L), which is 50% higher than that of WBH (2.00 g/L, of which butanol accounted for 1.38 g/L). CON only produced 1.56 g/L ABE, which was only about half of that generated from RFH. There were remarkable levels of acids (acetic and butyric acids) observed at the end of all the fermentations (Table 2). For the sugars presented in the hydrolysates, as shown in Fig. 1E, the consumption of both glucose and xylose started from the very beginning of the fermentation, however, the consumption rate was much higher for glucose than that for xylose, until concentrations for both sugars became below 2 g/L. Since arabinose only presented at trace amount, the consumption was not apparent over the fermentation process. Although the difference among individual sugar concentrations might have an impact on their consumption kinetics, the results indicated that hexoses are more preferable for ABE fermen-

**Table 1**
Chemical composition (% dry basis) of switchgrass before and after alkaline pretreatment assisted with RF or WB heating and sugar content (g/L) of the hydrolysates generated from RF (RFH) and WB (WBH) based pretreatment processes.

| Biomass | Klason lignin | Acid-soluble lignin | Glucan | Xylan | Galactan | Arabinan | Mannan | Extractives |
|---|---|---|---|---|---|---|---|---|
| Untreated | 21.39 ± 0.79 | 2.37 ± 0.19 | 43.66 ± 0.23 | 23.57 ± 0.24 | 1.06 ± 0.08 | 3.03 ± 0.11 | NA | 0.99 ± 0.07 |
| WB | 11.18 ± 0.62 | 1.96 ± 0.42 | 58.38 ± 0.65 | 18.55 ± 0.35 | 2.18 ± 0.67 | 4.79 ± 0.09 | 0.52 ± 0.06 | 0.71 ± 0.05 |
| RF | 10.39 ± 0.14 | 1.94 ± 0.27 | 60.70 ± 0.55 | 16.56 ± 0.19 | 2.47 ± 0.08 | 5.54 ± 0.71 | 0.51 ± 0.15 | 0.67 ± 0.04 |
| | Glucose | Xylose | Arabinose | Acetic acid | | | | |
| RFH | 15.54 ± 0.11 | 5.60 ± 0.20 | 0.22 ± 0.01 | 2.19 ± 0.33 | | | | |
| WBH | 14.87 ± 0.17 | 5.20 ± 0.18 | 0.21 ± 0.04 | 1.58 ± 0.18 | | | | |

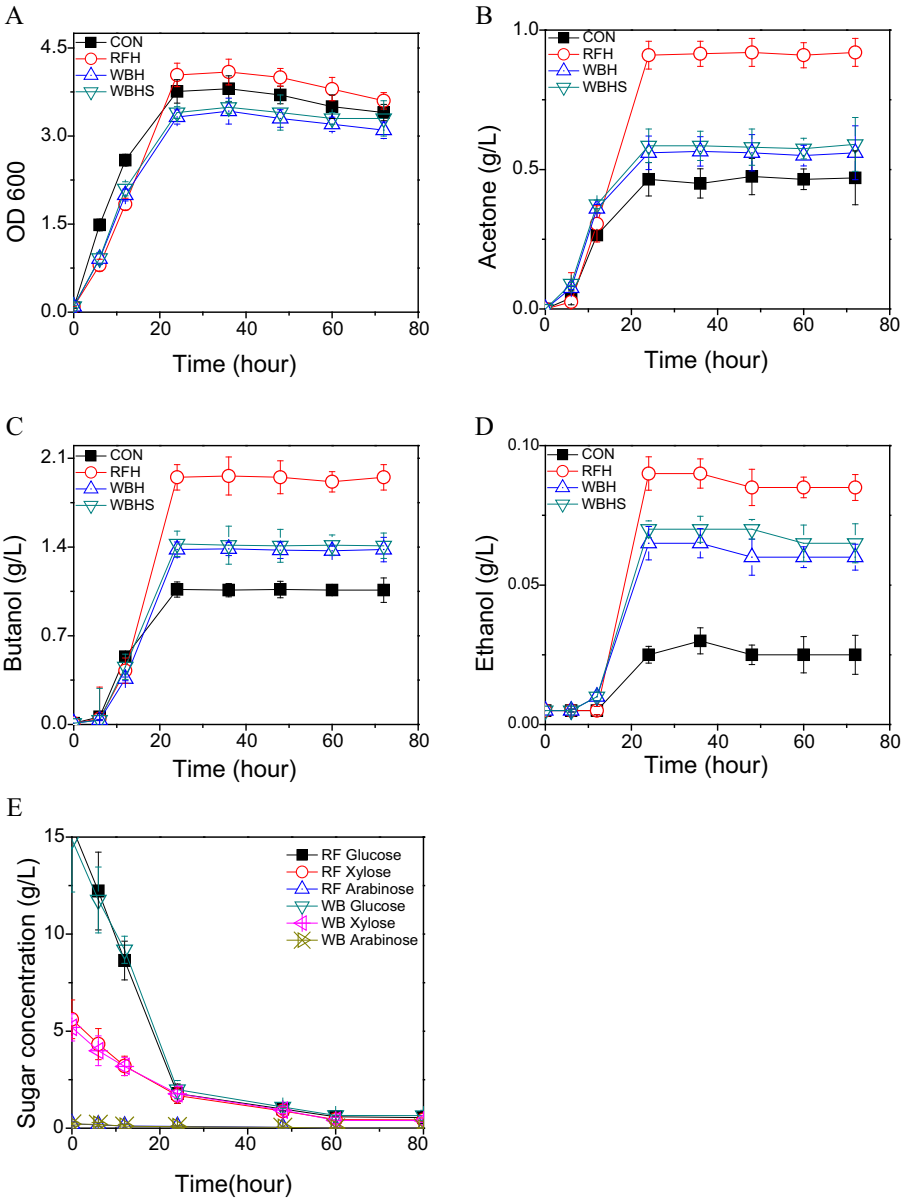


**Fig. 1.** ABE fermentation profiles with different carbon sources (supplemented with P2 medium). (A) Cell growth; (B) acetone production; (C) butanol production; (D) ethanol production; (E) sugar consumption kinetics in RFH and WBH. CON: synthetic medium with the same levels of carbon sources as presented in RFH. RFH: hydrolysates generated from radio frequency (RF) pretreatment and enzymatic hydrolysis; WBH: hydrolysates generated from water bath (WB) pretreatment and enzymatic hydrolysis; WBHS: WBH supplemented with various carbon sources to make the levels of carbon sources the same as in RFH.

**Table 2**
Summary of fermentation results with CON, RFH, WBH and WBHS as carbon sources.

| | Acetone (g/L) | Butanol (g/L) | Ethanol (g/L) | ABE (g/L) | Acetic acid (g/L) | Butyric acid (g/L) | Butanol yield (g/g) | ABE yield (g/g) |
|---|---|---|---|---|---|---|---|---|
| CON | 0.47 ± 0.09 | 1.06 ± 0.09 | 0.03 ± 0.01 | 1.56 ± 0.11 | 0.9 ± 0.19 | 0.39 ± 0.10 | 0.07 ± 0.01 | 0.10 ± 0.01 |
| RFH | 0.92 ± 0.05 | 1.95 ± 0.10 | 0.09 ± 0.00 | 2.96 ± 0.18 | 1.4 ± 0.10 | 0.56 ± 0.18 | 0.13 ± 0.04 | 0.20 ± 0.03 |
| WBH | 0.56 ± 0.10 | 1.38 ± 0.09 | 0.06 ± 0.00 | 2.00 ± 0.14 | 1.7 ± 0.19 | 0.79 ± 0.19 | 0.09 ± 0.01 | 0.13 ± 0.01 |
| WBHS | 0.59 ± 0.10 | 1.41 ± 0.10 | 0.07 ± 0.01 | 2.07 ± 0.20 | 1.8 ± 0.17 | 0.85 ± 0.20 | 0.09 ± 0.02 | 0.14 ± 0.02 |

tation despite both hexoses and pentoses can be consumed simultaneously by *C. beijerinckii* [28]. We demonstrated here that the dilute carbon sources in the switchgrass hydrolysates could be successfully fermented for ABE production without concentrating hydrolysates or detoxification. The inhibitors in the hydrolysates only resulted in slightly delay for the cell growth, while no obvious hysteresis observed in terms of solvent production for RFH or WBH compared to CON. Surprisingly, both RFH and WBH generated much higher levels of ABE (RFH even demonstrated better cell growth) than CON which contained the same level of carbon sources (as RFH) but no inhibitors. This might be because, in the hydrolysates, besides the carbon sources, there were also other nutrients (such as nitrogen sources) generated during the pretreatment through the breakdown of the biomass. These nutrients likely made a difference for the cell growth and solvent production.

The fermentation performance of RFH was much better than that of WBH. The total sugar concentration in RFH was slightly higher than that in WBH (Table 1), which could have led to the better performance. In order to testify this hypothesis, we carried out another fermentation with WBH supplemented with various sugars (glucose, xylose, and arabinose) and acetic acid (this fermentation was termed as WBHS) to make the carbon sources in WBH to be at the same levels as in RFH. As shown in Fig. 1 and Table 2, WBHS demonstrated very similar fermentation profiles and produced around the same level of solvents as WBH. So, we concluded that the better fermentation performance of RFH than WBH was not merely due to the slight higher levels of carbon sources in RFH, but probably also because of other nutrient sources in the medium.

### 3.3. Effects of yeast extract on ABE fermentation

Since the difference in carbon source concentrations was not the main factor leading to better fermentation performance for RFH than WBH, in the next step, we attempted to inspect the effects of other nutrient sources on this matter. The effects of yeast extract (YE) as a complex nutrient supplement on the fermentation performance was investigated. First, fermentations were carried out with various carbon sources (RFH, WBHS and CON) supplemented with 1 g/L YE, which were termed respectively as RFH1YE, WBHS1YE and CON1YE. Compared to the fermentation without YE supplementation, the addition of 1 g/L YE has significantly improved the cell growth (Fig. 2); the $OD_{600}$ of RFH1YE reached the maximum of 5.5, while that of WBHS1YE also reached the maximum of around 5.0. The fermentations took about the same time to complete (<24 h) as those without YE supplementation. The YE addition led to notable re-assimilation of acetic and butyric acids in all three fermentations; the final acids concentration decreased by 40–50% compared to the corresponding fermentation without YE. Both the butanol and ABE production in CON1YE increased by ~27% than those in CON. The butanol and ABE production in WBHS1YE increased by 8.5% and 15.0% respectively than those in WBHS. However, interestingly, the solvent production in RFH1YE did not increase significantly than that in RFH. Therefore, the supplementation of 1 g/L YE reduced the difference of performances between the fermentations based on RF and WB generated hydrolysates.

Furthermore, fermentations with supplementation of 2 g/L YE based on various carbon sources (RFH, WBHS and CON; termed the new fermentations as RFH2YE, WBHS2YE and CON2YE, respectively) were carried out in order to further elucidate the effects of YE on the fermentation performance. As shown in Table 3 and Fig. 3, the cell growth was further enhanced for all fermentations. The maximum $OD_{600}$ of RFH2YE reached 8.0, which was slightly higher than that of WBHS2YE (~7.7). While the cell growth of CON2YE followed very similar growth profile as RFH2YE and reached about the same maximum of OD. The solvent production in all fermentations increased significantly compared to the conditions when there was no YE added. ABE concentrations in RFH2YE and WBHS2YE reached 4.57 and 4.28 g/L, which were 55% and 107% increases respectively compared to those of RFH and WBH. The ABE concentration in CON2YE attained 3.69 g/L, which was a 136% increase compared to that of CON. Generally, the addition of 2 g/L YE further enhanced the solvent production for all the fermentations and abated the difference of the ABE production levels among three fermentations. However, although the solvent production in WBHS2YE was very close to that in RFH2YE, the CON2YE still produced ~20% less ABE than RFH2YE or WBHS2YE.

Supplementation of YE was then increased to 5 g/L based on various carbon sources (RFH, WBH and CON; termed the new fermentations as RFH5YE, WBHS5YE and CON5YE, respectively). The

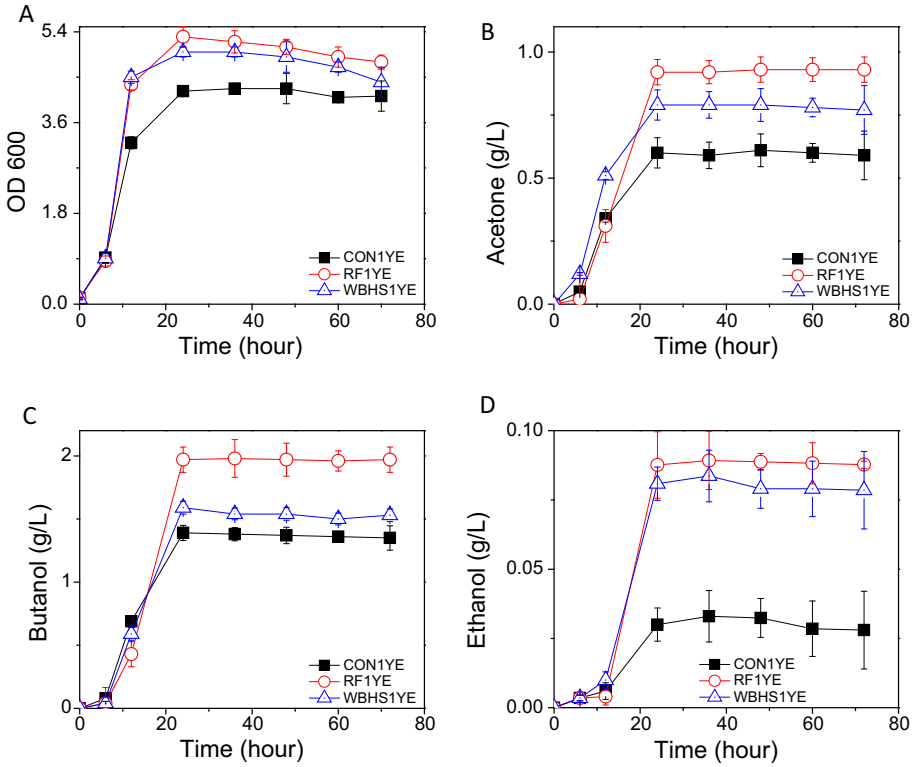


**Fig. 2.** ABE fermentation profiles with different carbon sources (supplemented with P2 medium and 1 g/L yeast extract): CON1YE, RFH1YE, and WBHS1YE. (A) Cell growth; (B) acetone production; (C) butanol production; (D) ethanol production. CON1YE: synthetic medium with the same levels of carbon sources as presented in RFH supplemented with 1 g/L yeast extract. RFH1YE: hydrolysates generated from radio frequency (RF) pretreatment and enzymatic hydrolysis supplemented with 1 g/L yeast extract; WBHS1YE: WBH supplemented with various carbon sources to make the levels of carbon sources the same as in RFH and with addition of 1 g/L yeast extract.

**Table 3**
Summary of fermentation results in various media with yeast extract supplementation.

| | Acetone (g/L) | Butanol (g/L) | Butanol yield (g/g) | Ethanol (g/L) | ABE (g/L) | ABE yield (g/g) | Acetic acid (g/L) | Butyric acid (g/L) |
|---|---|---|---|---|---|---|---|---|
| CON1YE | 0.59 ± 0.09 | 1.35 ± 0.10 | 0.09 ± 0.01 | 0.03 ± 0.01 | 1.97 ± 0.14 | 0.13 ± 0.01 | 0.51 ± 0.03 | 0.2 ± 0.03 |
| RFH1YE | 0.93 ± 0.05 | 1.97 ± 0.09 | 0.13 ± 0.01 | 0.09 ± 0.00 | 2.99 ± 0.71 | 0.20 ± 0.01 | 0.78 ± 0.03 | 0.4 ± 0.03 |
| WBHS1YE | 0.77 ± 0.11 | 1.53 ± 0.05 | 0.10 ± 0.02 | 0.08 ± 0.01 | 2.38 ± 0.38 | 0.16 ± 0.02 | 1.01 ± 0.10 | 0.43 ± 0.10 |
| CON2YE | 1.10 ± 0.09 | 2.50 ± 0.19 | 0.17 ± 0.04 | 0.09 ± 0.05 | 3.69 ± 0.39 | 0.25 ± 0.01 | 0.61 ± 0.05 | 0.35 ± 0.19 |
| RFH2YE | 1.33 ± 0.12 | 3.13 ± 0.20 | 0.21 ± 0.03 | 0.12 ± 0.00 | 4.57 ± 0.51 | 0.30 ± 0.05 | 0.59 ± 0.09 | 0.32 ± 0.10 |
| WBHS2YE | 1.30 ± 0.19 | 2.88 ± 0.10 | 0.19 ± 0.04 | 0.11 ± 0.01 | 4.28 ± 0.66 | 0.29 ± 0.01 | 0.77 ± 0.05 | 0.34 ± 0.10 |
| CON5YE | 1.45 ± 0.19 | 3.25 ± 0.18 | 0.22 ± 0.05 | 0.14 ± 0.01 | 4.84 ± 0.51 | 0.32 ± 0.01 | 0.40 ± 0.05 | 0.25 ± 0.01 |
| RFH5YE | 1.45 ± 0.10 | 3.45 ± 0.09 | 0.23 ± 0.07 | 0.15 ± 0.01 | 5.05 ± 0.69 | 0.34 ± 0.05 | 0.39 ± 0.10 | 0.25 ± 0.19 |
| WBHS5YE | 1.40 ± 0.08 | 3.25 ± 0.18 | 0.22 ± 0.06 | 0.14 ± 0.01 | 4.79 ± 0.78 | 0.32 ± 0.03 | 0.45 ± 0.04 | 0.27 ± 0.10 |

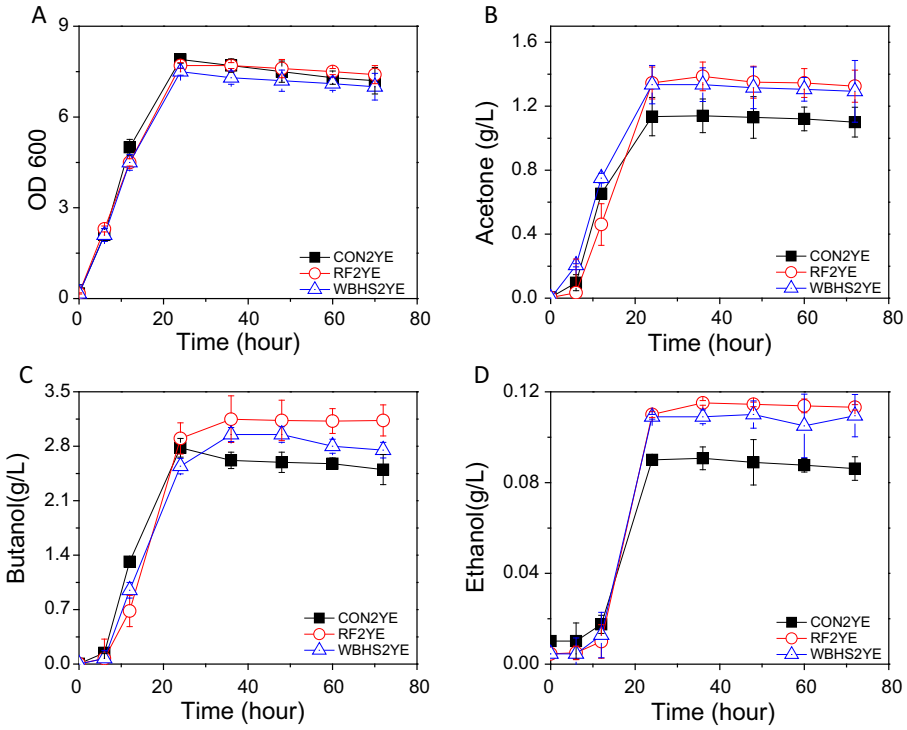


**Fig. 3.** ABE fermentation profiles with different carbon sources (supplemented with P2 medium and 2 g/L yeast extract): CON2YE, RFH2YE, and WBHS2YE. (A) Cell growth; (B) acetone production; (C) butanol production; (D) ethanol production. CON2YE: synthetic medium with the same levels of carbon sources as presented in RFH supplemented with 2 g/L yeast extract. RFH2YE: hydrolysates generated from radio frequency (RF) pretreatment and enzymatic hydrolysis supplemented with 2 g/L yeast extract; WBHS2YE: WBH supplemented with various carbon sources to make the levels of carbon sources the same as in RFH and with addition of 2 g/L yeast extract.

cell growth was further enhanced and followed very similar kinetics for all fermentations. The maximum $OD_{600}$ increased to ~8.7 for all three conditions (Fig. 4). Similarly, the supplementation of 5 g/L YE further increased the solvent production, and brought the final solvent production to be very close to each other for all three fermentations, with final solvent production in RFH5YE was slightly (by ~5%) higher than WBHS5YE and CON5YE (Table 3 and Fig. 4).

Without YE supplementation, RFH demonstrated much better fermentation performance than WBH, WBHS and CON. Although fermentation inhibitors might have presented in biomass hydrolysates, they (RFH, WBH and WBHS) worked much better than CON for the ABE production. Generally, YE supplementation enhanced the cell growth, acid re-assimilation and solvent production for all the conditions with various carbon sources. Meanwhile, YE addition decreased differences of fermentation performances among various carbon sources, and when the YE increased up to 5 g/L, all fermentations performed very similarly and generated similar levels of ABE. Most of biomass pretreatment methods involve high temperature heating processes, which are usually achieved through convection- or conduction-based heating [29]. Dielectric heating transforming electromagnetic energy into heat is a promising and alternative method for conventional heating. RF, along with microwave, is a type of dielectric heating; compared to microwave, RF, with longer wavelength and thus higher energy, can penetrate dielectric materials more deeply [21,30]. At the same time, the electromagnetic field of RF could generate non-thermal effects, which can also accelerate the destruction of crystallinity structure of the biomass [16,31]. Therefore, in this study, we speculate that the RF heating based pretreatment had broken down the biomass more completely and thus generated more nutrients than the WB heating based pretreatment, such as nitrogen sources and others. For both hydrolysates carbon sources (RF or WB heating generated), they provided extra nutrient sources generated from the biomass (even various fermentation inhibitors might also present), and thus enabled better fermentation performance than CON. However, although hydrolysates generated through RF treatment (RFH) provided more nutrients for the fermentation, along with WBH and CON, the nutrients in the fermentation media was not sufficient for a good ABE fermentation performance. YE, as an organic nitrogen source, can provide various amino acids, vitamins, minerals and growth factors that can promote the growth

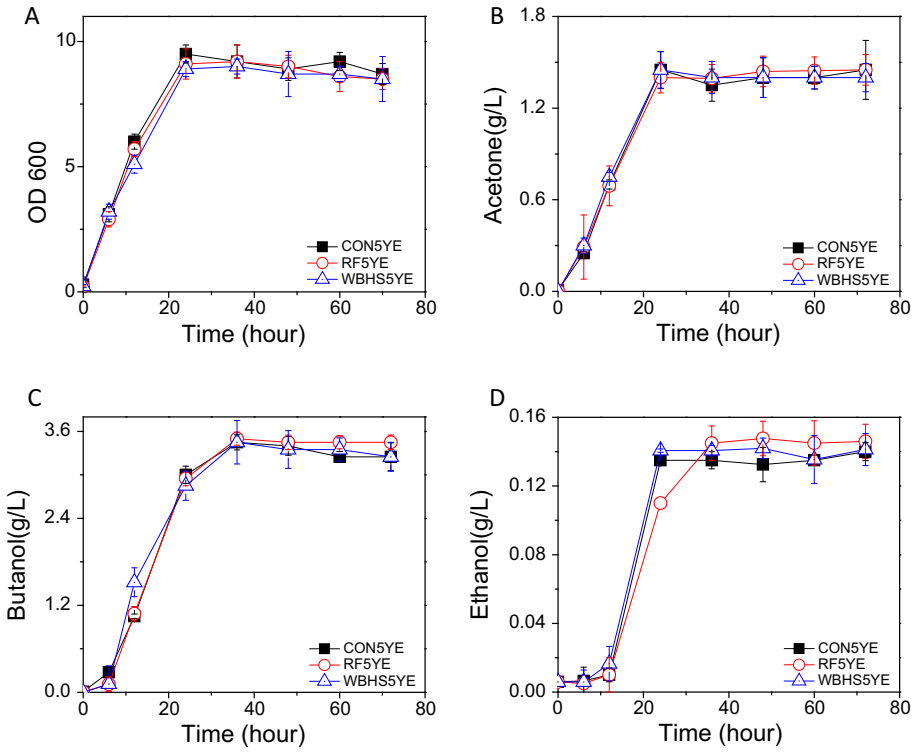


**Fig. 4.** ABE fermentation profiles with different carbon sources (supplemented with P2 medium and 5 g/L yeast extract): CON5YE, RFH5YE, and WBHS5YE. (A) Cell growth; (B) acetone production; (C) butanol production; (D) ethanol production. CON5YE: synthetic medium with the same levels of carbon sources as presented in RFH supplemented with 5 g/L yeast extract. RFH5YE: hydrolysates generated from radio frequency (RF) pretreatment and enzymatic hydrolysis supplemented with 5 g/L yeast extract; WBHS5YE: WBH supplemented with various carbon sources to make the levels of carbon sources the same as in RFH and with addition of 5 g/L yeast extract.

of microorganisms [31]. The supplementation of YE in this study significantly enhanced the fermentation performance for all conditions, and meanwhile diminished the difference of fermentation performance among different conditions based on various carbon sources. Li et al. [33] reported that by adding YE into cassava meal medium, the phase shift was triggered and fermentation performances were consequently improved. Total butanol concentrations/butanol productivities increased 15% compared to those with cassava substrate alone. Madihah et al. [34] reported that the use of a mixture of organic and inorganic nitrogen source (YE & $NH_4NO_3$) enhanced the growth of *Clostridium acetobutylicum* and solvent production compared to the use of YE alone. In our case, the biomass hydrolysates might contain both organic and inorganic nitrogen sources (along with other nutrients) which were generated from the biomass and provided benefits for the fermentation.

### 3.4. Elemental analysis

To confirm our hypothesis that RF pretreatment led to higher nutrient content in the biomass hydrolysates, we performed a CHNS analysis on the pretreated switchgrass, as well as switchgrass hydrolysates generated from RF or WB heating based pretreatment approaches (shown in Table 4).

The nitrogen content in the RF pretreated biomass was 27% higher than that in the WB pretreated biomass (0.57% vs 0.46%; $p$-value = 0.02), while the nitrogen content in RFH was 29% higher than that in WBH (1.19% vs 0.92%; $p$-value = 0.01). The results demonstrated that, compared to WB pretreatment, RF pretreatment generated more nutrients (especially nitrogen sources) and led to better cell growth and solvent production. This was in concert with the fermentation results as we discussed above.

Moreover, our GC–MS analysis on the biomass hydrolysates revealed that some specific compounds including hydroxylacetaldehyde, propanoic acid, 2-methoxy-4-vinyl phenol, 2-methoxy phenol and 1,2-benzenediol (catechols) were observed in hydrolysates obtained through RF pretreatment, but not in the hydrolysates obtained through WB pretreatment (data not shown). Interestingly, these compounds were also reported to be observed in the bio-oils generated through fast pyrolysis of biomass which experienced super high temperature [35–37]. This confirmed that RF pretreatment, with a different heating mechanism, provided a much harsher heating condition than WB pretreatment (although the same temperature was applied for both approaches) and led to very different composition of nutrients (along with slightly higher levels of carbon sources) resulting in a better ABE fermentation performance.

**Table 4**
Summary of elemental analysis results (% dry basis).

| Sample[a] | Carbon | Hydrogen | Nitrogen | Sulfur |
|---|---|---|---|---|
| Raw | 47.38 ± 3.19 | 6.61 ± 0.98 | 0.62 ± 0.09 | 1.43 ± 0.39 |
| RF pretreated | 53.01 ± 2.93 | 6.01 ± 0.77 | 0.57 ± 0.17 | 1.21 ± 0.38 |
| WB pretreated | 49.13 ± 4.07 | 6.62 ± 0.68 | 0.46 ± 0.15 | 1.54 ± 0.24 |
| RFH | 2.28 ± 0.78 | 11.51 ± 1.86 | 1.19 ± 0.09 | 0.64 ± 0.01 |
| WBH | 1.51 ± 0.39 | 11.33 ± 1.98 | 0.92 ± 0.07 | 0.65 ± 0.02 |

RF pretreated: solid fraction of switchgrass biomass after RF-heating pretreatment.
WB pretreated: solid fraction of switchgrass biomass after WB-heating pretreatment.
RFH: hydrolysates generated from RF pretreatment and enzymatic hydrolysis.
WBH: hydrolysates generated from WB pretreatment and enzymatic hydrolysis.
[a] Raw: raw switchgrass biomass prior to pretreatment.

## 4. Conclusion

Results demonstrated that switchgrass hydrolysate pretreated by RF showed consistently better performance during ABE fermentation than that was generated through WB pretreatment or the

synthetic medium (as fermentation control). YE supplementation enhanced cell growth, acids re-assimilation and solvent production, and diminished the difference among fermentations with various carbon sources. Thus, RF pretreatment produced much harsher condition to biomass under normal pressure, broke down biomass more completely and generated more nutrients for the fermentation. RF-based dielectric heating approach could be a promising means to pretreat biomass for biofuel and biochemicals from lignocellulosic biomass.

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