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Research paper

Production of polyhydroxybuyrate (PHB) from switchgrass pretreated with a radio frequency-assisted heating process

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ABSTRACT

Effects of radio frequency (RF) heating as a biomass pretreatment process to generate hydrolysates for polyhydroxybuyrate (PHB) were evaluated in all production steps from pretreatment to enzymatic hydrolysis to fermentation. Switchgrass was pretreated under alkaline conditions with RF-assisted heating (traditional water bath (WB) heating as control) and subsequently enzymatically hydrolyzed. Fermentation was conducted with recombinant *Escherichia coli* strain for PHB production using the hydrolysates as carbon sources with or without yeast extract (YE) supplemented. Results indicated that the hydrolysates generated through RF pretreatment performed consistently better for PHB production than WB (50% higher PHB levels without YE). YE supplementation (up to 5 g/L) enhanced fermentation under all conditions and diminished the difference among performances of fermentations. When adding 2 g/L YE, PHB production increased by 200% and 80% for WB and RF hydrolysates, respectively. Supplementation of 5 g/L YE brought the final PHB concentration to be very close to each other for all three fermentation conditions. Compared to traditional heating process, the unique heating mechanism of RF generates harsher conditions (under regular pressure) to disrupt the biomass structure more completely and generate more nutrients for bacterial fermentation. RF was therefore proved to be an efficient process for biomass pretreatment.

1. Introduction

Nowadays, plastics are used in almost every corner of our life, but most of them are not biodegradable. Moreover, petroleum is currently the primary raw material to produce plastics. More and more concerns have been raised because of the deteriorating consequence of petrochemical based routes on the natural environment [1–5]. Therefore, it becomes urgent and inevitable to replace the non-biodegradable plastics by the biodegradable ones and petroleum with bio-based materials [1,6,7]. Among the various biomaterials that have been evaluated for producing biodegradable plastics, polyhydroxybutyrate (PHB) is attracting more and more attention from the publics because of its unique characteristics. The pure PHB have similar physical and chemical properties to those of commonly used plastics derived through petrochemical routes, e.g. polypropylene. In terms of mechanical property, the melting temperature, Young's modulus and tensile strength of PHB are all comparable to those of polypropylene and polystyrene and other bulk plastics. Moreover, PHB is resistant to water and moisture and 100% biodegradable [8,9]. For PHB production, the cost of feedstocks is an important part for the overall cost. Recently, corn starch was widely used by many companies such as Cargill Dow Polymers, LLC for biopolymer production [10,11]. However, on one hand, the price of corn starch is high, and on the other, the utilization of corn starch for biochemical/biomaterial production cannot avoid the competition with global food/feed supplies. Exploring cheap and suitable feedstocks for PHB production is of great importance from the cost effectiveness standpoint. Therefore, using inexpensive renewable energy crop as feedstocks could provide tremendous advantage to the economics of PHB production [12]. Switchgrass was identified as a representative species of herbaceous energy crop by US Department of Energy [13,14]. It shows great potentials for bioeconomy industries because of the high productivity, suitability for marginal land utilization, low water and nutritional requirements, environmental benefits, and flexibility for multipurpose uses [15]. In addition, switchgrass contains nutrients such as N, P, K, and Na that are suitable for microbial cultivation [14]. In order to convert switchgrass into bio-based chemicals, a

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pretreatment process (along with necessary enzymatic hydrolysis of the generated cellulose and hemicellulose to fermentable sugars) is generally required. In the past decades, various pretreatment technologies have been developed, most of which involved high temperature heating (and/or high pressure) treatment conditions, which induced high energy consumption and generate remarkable fermentation inhibitors [16–18]. Compared to the convection traditional heating technologies, Radio frequency (RF) is a promising dielectric heating technology that transforms electromagnetic energy into heat which is volumetric and fast. At the same time, the electromagnetic field could generate non-thermal effects, which can also accelerate the destruction of biomass crystallinity structure. In addition, RF based heating approaches are believed to be easier for scaling up [19–21]. Generally, RF heating has a much higher energy conversion efficiency from electricity than traditional convection/conduction-based heating process. Therefore, the cost of the RF heating would be much lower than the traditional heating.

In order to produce biodegradable plastics with bio-based materials, utilization of switchgrass hydrolysate as a potential feedstock for PHB production employing the recombinant *Escherichia coli* strain was investigated in the current study. RF heating was evaluated as an efficient approach for switchgrass pretreatment. The generated hydrolysates (followed by further enzymatic hydrolysis) were used for PHB production without concentration or detoxification steps. Moreover, the effects of yeast extract (YE, as supplemented complex nitrogen sources, from 1 g/L to 5 g/L) on switchgrass hydrolysates fermentation performance was investigated.

2. Materials and methods

2.1. Raw materials

Switchgrass was acquired from the Bioenergy and Bioproducts Center at Auburn University. Commercial cellulase, Novozym 22C, was kindly donated by Novozymes (Franklinton, NC). PHB, chloroform, ethanol, sodium hypochlorite (5% active chlorite), and the 99.8% atom-D chloroform containing 0.03% TMS, were all purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA).

2.2. Biomass pretreatment

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

Commercial blender (Dynamics Corporation of America, New Hartford, CT).

Before pretreatment, switchgrass was soaked in NaOH solution (0.2 g NaOH/g Biomass) at a solid to liquid ratio of 1:10 in a 500-mL plastic container at room temperature for overnight. Then the sample was subjected to RF heating (SO6B; Strayfield, Berkshire, England) at a frequency of 27.12 MHz and maximum power output of 6 kW. During the heating process, four fiber-optic sensors (Neoptix, Inc., Québec City, Québec, Canada) were employed to measure temperature of switchgrass mixture. When the temperature of sample reached 90 °C (less than 7 min), the RF heater was paused for 0.5 min followed by another round of heating (~1 min) in order to keep the sample at approximately 90 °C. This pause-heating pattern was repeated until the predetermined total heating time (60 min) was completed. Water bath (WB) was used as a conventional heating method (as control for RF heating) in pretreatment. The 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 substrate 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 to 7.0. The wet pretreated substrate was used directly (without drying) for the chemical compositional analysis and followed by enzymatic hydrolysis [21]. Chemical compositional analysis was determined using the NREL protocol [22].

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis of the pretreated biomass was performed in 125 mL of 50 mM sodium citrate buffer (pH 4.8) at 2% glucan (w/v) with commercial enzyme (Novozym 22C). 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 reaction was conducted at 50 °C and 150 rpm for 72 h in a benchtop incubator shaker (Excella E24R, Eppendorf Company, Edison, NJ). Samples were periodically taken for sugar analysis using an Agilent 1260 Infinity Quaternary High Performance Liquid Chromatography (HPLC) (Agilent Technologies, Santa Clara, CA) with refractive index detector (RID) following the NREL protocol [23]. 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

The compounds of supernatant of RF and WB pretreated switchgrass was analyzed with Gas Chromatography-Mass Spectrometry (GC-MS). An Agilent 6890N GC coupled with an Agilent 5973 mass-selective detector (MSD) equipped with a DB-1701 column (60m × 0.25 mm, 0.25 μm film thickness) was employed for this purpose. Moreover, the carbon, hydrogen, nitrogen and sulfur contents in the raw and pretreated materials were quantified with a Perkin-Elmer CHNS/O analyzer (model 2400, Series II).

2.5. Fermentation

The *E. coli* XL1-blue strain hosting pBHR68 plasmid for PHB production was kindly provided by Dr. Charles Miller at Utah State University. The pBHR68 plasmid contains the three genes (phaA, phaB, and phaC) needed for PHB synthesis and confers ampicillin resistance [24,25]. During all the strain cultivation steps, 100 μg/mL ampicillin was supplemented unless otherwise specified. The strain was first grown in Luria-Bertani (LB) medium. Then the overnight culture was inoculated into standard M9 medium in an orbital shaker operating at 220 rpm and 37 °C [26,27]. M9 minimal medium contains 0.6% Na_2HPO_4, 0.3% KH_2PO_4, 0.05% NaCl, 0.1% NH_4Cl, 0.02% $MgSO_4$, and 0.001% $CaCl_2$. Overnight culture was then used to seed the media for PHB production at the same agitation rate and temperature. The initial optical density (OD_{600}) after inoculation was set around 0.05 for each fermentation. At the beginning of fermentation, 0.1 mm Isopropyl β-D-1-thiogalactopyranoside (IPTG) (Gold Biotechnology, Inc. St. Louis, MO) was added to induce the gene expression for PHB production. Cell density was monitored through the course of fermentation. For PHB quantification, cell mass was harvested from 50 mL fermentation broth with centrifugation at 4000 rpm and 4 °C for 20 min and was then freeze-dried. Samples were kept in a desiccator and weighed to determine dry cell weight (DCW) for measurement of cell mass concentration and PHB content. Lyophilized cell biomass was used

to determine DCW and construct growth curves.

PHB production 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, RFH, WBH and CON were also used to represent the fermentations carried out using RFH, WBH and CON, respectively, as carbon sources). Moreover, the effects of YE (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. PHB extraction and quantification

The *E. coli* cell biomass was harvested by centrifugation at 4000 rpm and 4 °C for 20 min. Then the pellets were stored in −80 °C freezer until completely frozen (for more than 12 h). Frozen samples were dried using a freeze-dryer (Labconco lyophilizer, Labconco Corporation, Kansas City, MO, USA). PHB quantification was carried out using ^{1}H nuclear magnetic resonance (NMR) spectrum based on the chloroform-sodium hypochlorite dispersion method as described previously with modifications [28]. Approximately 5–10 (±0.2) mg of lyophilized cells were dissolved by adding 0.7 mL of 5% sodium hypochlorite and 1 mL of $CDCl_3$ (0.03% TMS). The mixture was vigorously vortexed for 10 min and incubated in a shaker at 30 °C for 2 h while PHB standards were incubated at a high temperature (50 °C) for 2 h to facilitate dissolution. Afterwards, samples were centrifuged at 13,200 rpm for 10 min to induce phase separation. This resulted in three different layers (as shown in Fig. 1). The top layer was the aqueous hypochlorite solution, the middle layer included cells and other biological matters, and the bottom layer was PHB-containing chloroform phase. The chloroform phase was needled out carefully after centrifugation. The organic phase was transferred to a 5 mm NMR tube and was analyzed for PHB content in a Bruker 400 MHz NMR spectrometer. Data were averaged over 16 acquisitions. The spectrum was evaluated using Bruker uxnmr software (Bruker Biospin AG, Switzerland). The TMS inside $CDCl_3$ was used as an internal standard to minimize errors resulted from injection volume variations and adjust the retention time shifts.

2.7. PHB production and statistical analysis

All experiments and analyses were performed in triplicate. For each fermentation, PHB concentration (g/L, PHB produced per liter of culture) and PHB yield (g/g, defined as the ratio of PHB content to dry cell concentration) were quantified [29]. ANOVA General Linear Model (GLM) analysis ($\alpha = 0.05$) and mixed analysis ($\alpha = 0.05$) using SAS® (version 9. 1. 3 SP4) were applied to compare the different pretreatment methods for generating switchgrass hydrolysates for PHB production.

3. Results and discussion

3.1. Biomass pretreatment

The chemical compositions of switchgrass before and after alkaline pretreatment are illustrated in Table 1. Both WB and RF heating assisted alkaline pretreatment led to significant decrease in lignin content and increase of glucan fraction compared to raw feedstock. After enzymatic hydrolysis, NaOH pretreated switchgrass with RF heating generated a gulcan 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 glucose was produced from the WB heating pretreated hydrolysates (WBH), while 15.5 g/L was generated for RF heating pretreated hydrolysates (RFH). Acetic acid is usually generated during the pretreatment process due to the deacetylation of hemicellulose and part of lignin [30,31]. Higher level of acetic acid produced in RF heating pretreatment might result from the harsher condition in RF heating compared to WB heating, leading to severer acetylation.

3.2. Fermentation with switchgrass hydrolysates

The enzymatic hydrolysates of pretreated switchgrass were rather dilute and the total sugar concentration was only around 15 g/L. Fermentation was carried out in parallel using various carbon sources (supplemented with M9 medium), including RFH, WBH, and CON. Generally, cells grow rapidly in all the growth media with very short lag time. Interestingly, both RFH and WBH

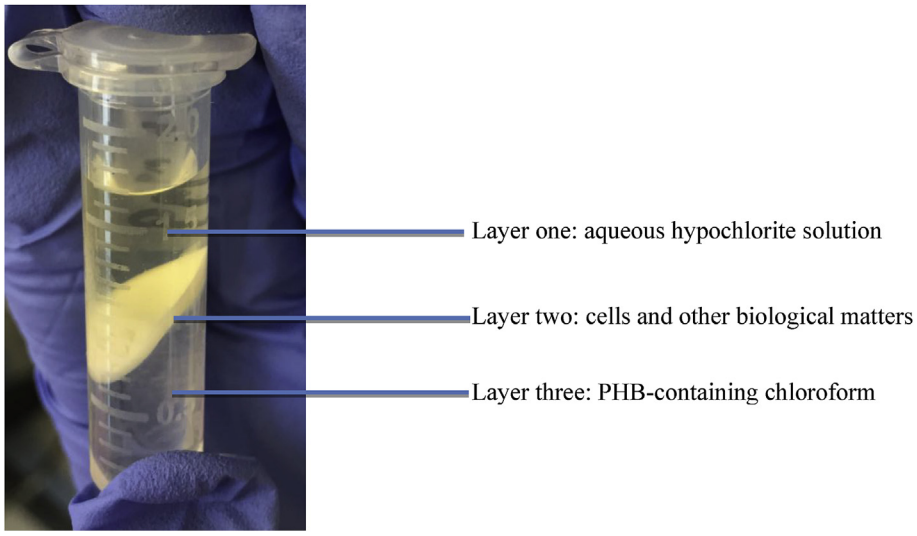

Fig. 1. Three different phases appeared after centrifugation.

Table 1
Chemical composition of alkaline pretreated switchgrass after using RF and WB heating (% dry basis) and sugar content of hydrolysates generated from RF (RFH) and WB (WBH) based pretreatments (g/L) [38].

Chemical composition	Untreated	WB	RF		RFH	WBH
Klason lignin	21.4 ± 0.79	11.2 ± 0.62	10.4 ± 0.14	Glucose	15.5 ± 0.11	14.9 ± 0.17
Acid-soluble lignin	2.4 ± 0.19	2.0 ± 0.42	1.9 ± 0.27	Xylose	5.6 ± 0.20	5.2 ± 0.18
Glucan	43.7 ± 0.23	58.4 ± 0.65	60.7 ± 0.55	Arabinose	0.2 ± 0.01	0.2 ± 0.04
Xylan	23.6 ± 0.24	18.6 ± 0.35	16.6 ± 0.19	Acetic acid	2.2 ± 0.33	1.6 ± 0.18
Galactan	1.1 ± 0.08	2.2 ± 0.67	2.5 ± 0.08			
Arabinan	3.0 ± 0.11	4.8 ± 0.09	5.5 ± 0.71			
Mannan	NA	0.5 ± 0.06	0.5 ± 0.15			
Extractives	1.0 ± 0.07	0.7 ± 0.05	0.7 ± 0.04			

reached higher maximum OD_{600} than CON did. The maximum OD_{600} for RFH was around 5.5 when stationary phase was reached at approximately 24 h post inoculation (Fig. 2A). WBH reached a maximum OD_{600} of around 5.0 which is 11% percent lower than that of RFH, but 20% higher than that of CON. As illustrated in Fig. 2B,C, PHB production was detected at ~10 h and reached the maximum levels at around 48 h for all fermentations. PHB usually accumulate to maximum levels during the late stationary phase rather than exponential growth phase. There is a hysteresis between carbon source utilization and PHB accumulation. PHB is a form of energy storage molecule employed by microorganisms to be metabolized when other energy sources are not available. The microorganisms start to accumulate PHB when they reach stationary phase and since there is a lack of energy sources, and thus the PHB storage reaches the maximum usually at the late stationary phase, as reported previously [27]. Corresponding to the cell growth profiles, RFH had the highest PHB production (2.3 g/L) which is 50% higher than that of WBH (1.1 g/L). While CON only generated 0.4 g/L of PHB which is less than 20% of that was produced by RFH.

The inhibitors presented in the hydrolysates caused almost no delay for the cell growth. Surprisingly, both RFH and WBH produced higher levels of PHB than CON although CON contained the same level of carbon sources (as RFH) and without inhibitors. It could be concluded that the dilute carbon sources in the switchgrass hydrolysates could be successfully fermented for PHB production without detoxification. Then it was hypothesized that in the hydrolysates (both RFH and WBH), other nutrients (such as nitrogen source) besides carbon sources generated during the pretreatment through the breakdown of the switchgrass likely made a huge difference for the cell growth and PHB production.

Compared to WBH, RFH performed much better for PHB fermentation. The total sugar content in RFH was slightly higher than that in WBH (Table 1); this could be the reason for the better performance for RFH. In order to testify this hypothesis, another fermentation was carried out with WBH supplemented with various sugars (glucose, xylose, and arabinose) and acetic acid to make the carbon sources in WBH equal to the values in RFH (new fermentation was termed as WBHS). As shown in Fig. 2A and Table 2, nevertheless, WBHS showed very similar fermentation profiles and generated around the same levels of PHB as WBH. The results indicated that the slightly higher level of carbon sources in

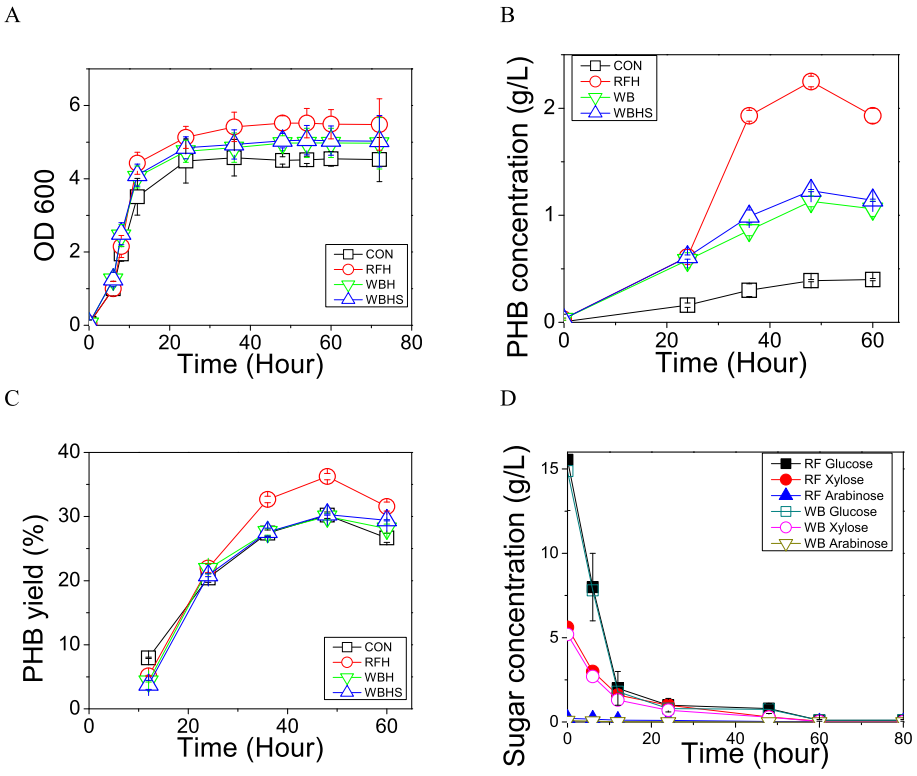

Fig. 2. PHB production profiles with different carbon sources (supplemented with M9 medium).

Table 2
Summary of fermentation results in different medium.

Media	PHB concentration (g/L)	PHB yield (%)
CON	0.4 ± 0.01	30.2 ± 1.10
RFH	2.3 ± 0.03	36.2 ± 1.76
WBH	0.9 ± 0.01	30.0 ± 1.69
WBHS	1.0 ± 0.11	30.3 ± 1.39
CON1YE	2.1 ± 0.19	44.7 ± 1.77
RFH1YE	3.9 ± 0.21	49.4 ± 1.49
WBHS1YE	3.6 ± 0.38	49.5 ± 2.60
CON2YE	3.2 ± 0.18	45.1 ± 1.48
RFH2YE	4.1 ± 0.22	49.4 ± 1.28
WBHS2YE	4.0 ± 0.23	48.8 ± 1.97
CON5YE	4.0 ± 0.38	50.6 ± 2.06
RFH5YE	4.5 ± 0.51	50.1 ± 2.15

RFH was not the determining factor for the better performance of RFH. Since better fermentation results for RFH than WBH did not likely result from the difference in carbon source concentrations, the effects of other nutrient sources on this matter were investigated further. Therefore, an investigation of nitrogen source was carried out. We studied the effects of yeast extract (YE) as a complex nutrient supplement on the fermentation performance. Results were reported in the following section.

3.3. Effect of yeast extract on PHB production

First, fermentations were carried out using various carbon sources (RFH, WBHS and CON) supplemented with 1 g/L YE, which were termed as RFH1YE, WBHS1YE and CON1YE, respectively. As shown in Fig. 3, the addition of 1 g/L YE significantly increased the cell growth compared to the fermentation without YE. The OD_{600} of RH1YE reached maximum of 8.4, while that of WBHS1YE attained to the similar level of around 8.3. The addition of YE did not improve PHB production significantly in RFH1YE than that in RFH. Comparatively, with 1 g/L YE supplementation, the PHB production in CON1YE increased by ~400% than that in CON, and that in WBHS1YE increased by ~190% than that in WBHS. As a result, the addition of 1 g/L YE narrowed down the gap of performances between the fermentations based on RF and WB generated hydrolysates.

In order to further elucidate the effects of YE on the fermentation performance, the addition of 2 g/L YE based on various carbon sources (RFH, WBHS, and CON; termed new fermentations as RFH2YE, WBHS2YE and CON2YE, respectively) were further carried out. As shown in Fig. 4, the OD_{600} was further enhanced for all fermentation performance. The maximum OD_{600} of RFH2YE reached around 8.7 which was slightly higher than that of WBHS2YE (around 8.5). The maximum OD_{600} of CON2YE followed similar profile and reached the similar level of maximum OD_{600}. Compared to fermentations without YE supplementation, the PHB concentration and yield increased significantly for fermentations with all carbon sources. PHB concentrations in RFH2YE and WBH2YE increased to 4.1 g/L (~80% improvement compared to those of RFH) and 4.0 g/L (~200% improvement of those of WBH). PHB concentration in CON2YE reached 3.2 g/L, which was ~720% increase compared to that of CON. Therefore, supplementation of 2 g/L YE further increased cell growth and PHB production for all fermentation conditions and diminished the difference among fermentations based on three different carbon sources. Nevertheless, PHB production of CON2YE was still 20% less than that of RF2YE or WBHS2YE.

Supplementation of YE (1 g/L and 2 g/L) enhanced the PHB production and meanwhile gradually reduced the difference among the performances for fermentations with three different carbon sources. However, with 2 g/L YE supplementation, CON2YE still produced significantly less PHB than the fermentations with

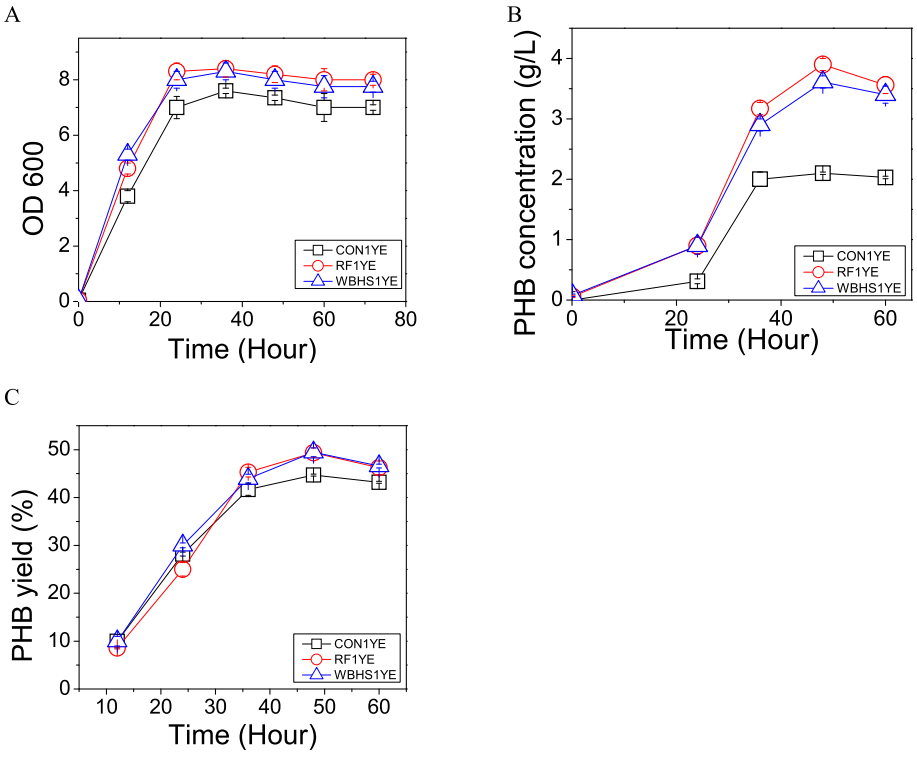

Fig. 3. PHB production profiles with different carbon sources (supplemented with M9 medium and 1 g/L yeast extract).

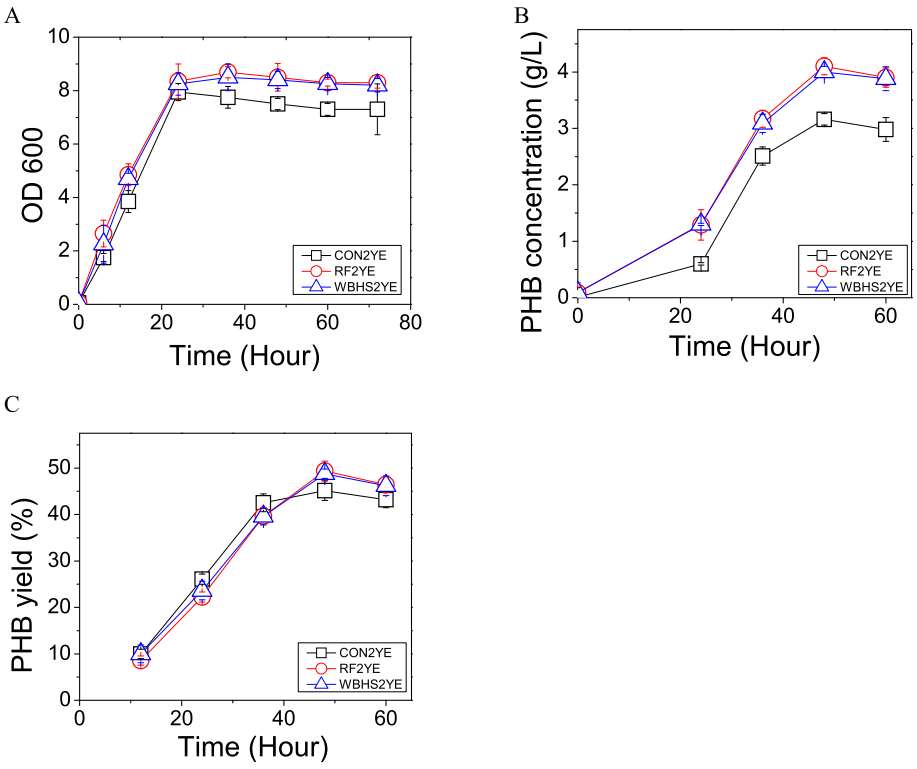

Fig. 4. PHB production profiles with different carbon sources (supplemented with M9 medium and 2 g/L yeast extract).

hydrolysates (RFH2YE and WBHS2YE). Then the YE concentration was further increased to 5 g/L to ulteriorly investigate the effects of YE on the fermentation results based on various carbon sources (RFH, WBHS and CON; termed the new fermentations as RFH5YE, WBHS5YE and CON5YE, respectively). Cell growth was further improved in all fermentations, and CON5YE demonstrated a little bit higher maximum OD_{600} (the maximum OD_{600} increased to 8.7 for RFH5YE and WBHS5YE, and 8.9 for CON5YE) (Fig. 5). PHB production in RFH5YE and WBHS5YE increased to ~4.5 g/L, while it was still a little bit lower in CON5YE (~4.0 g/L). Results demonstrated that the supplementation of 5 g/L YE further enhanced the PHB production, and brought the final PHB concentration to be very close to each other for all three fermentation conditions (Fig. 5). It is reported that final PHB concentration reached 3.52 g/L in *E. coli* DH5α harboring pBHR68 after 24–48 h [32]. Moreover, it has been demonstrated that PHB can accumulate in larger quantities in *E. coli* when using larger volume bioreactors compared to using shaker flasks [33,34]. Therefore, our PHB production based on switchgrass hydrolysates could be further enhanced at a larger scale fermentation.

Without the addition of YE, RFH showed much better fermentation performance than WBH, WBHS and CON. Although inhibitors might present in biomass hydrolysates, RFH, WBH and WBHS worked much better compared to CON in terms of PHB production. This demonstrated that hydrolysates generated through RF or WB heating assisted pretreatment provided extra nutrient sources (even though various fermentation inhibitors might also present) and enhanced fermentation performance than CON. However, although hydrolysates provided more nutrients for the PHB fermentation (than CON), the nutrients in the media were not sufficient for good PHB production. YE, as an organic nitrogen source, can provide various amino acids, vitamins, minerals and growth factors that can promote the growth of microorganisms [35]. The supplementation of YE in this study enhanced the fermentation performance for all conditions, and meanwhile diminished the difference of fermentation performance among different conditions based on various carbon sources. The positive effects of nutrient supplementation on PHB production have also been reported by many other researchers. Borah et al. reported that the addition of organic nitrogen source to medium containing sucrose promoted PHB yield and productivity [36]. The increase of PHB accumulation may be due to the presence of amino acids and peptides in YE. Song et al. reported that the PHB content of the cells was enhanced significantly by any following supplement tested: nutrient broth, YE, peptone, or casein acid hydrolysate (0.5 g/L for each) [37]. In addition, the authors inferred that YE, as a supplement, seemed to be by far the best option to obtain high amounts of PHB. Lee and Chang reported that PHB synthesis was generally promoted by supplementing the medium with a small amount of complex nitrogen sources [34]. Supplementation with 0.2% (w/v) tryptone, casamino acids, and YE or casein hydrolysates promoted PHB synthesis to a greater extent.

On the other hand, comparatively, RFH performed much better than WBH for PHB production under similar conditions without or with YE supplementation (1 or 2 g/L). We speculated that the RF pretreatment had broken down the biomass more completely and thus generated more nutrients (such as nitrogen sources and others) than the WB pretreatment. RF heating, is a type of dielectric heating (in contrast to the traditional convection- or conduction-based heating), transforming electromagnetic energy into heat between RF electromagnetic field and the object being heated. When switchgrass was being heated through RF, the more polar parts would absorb more energy and create a hot spot. This special heating mechanism resulted in an enhanced disruption of the recalcitrant structures of switchgrass, as well as accelerated the destruction of the crystallinity structure [38]. Recently, the ionic-liquid-based biomass pretreatment technology has attracted increasing attentions. It was reported that the ionic liquid such as

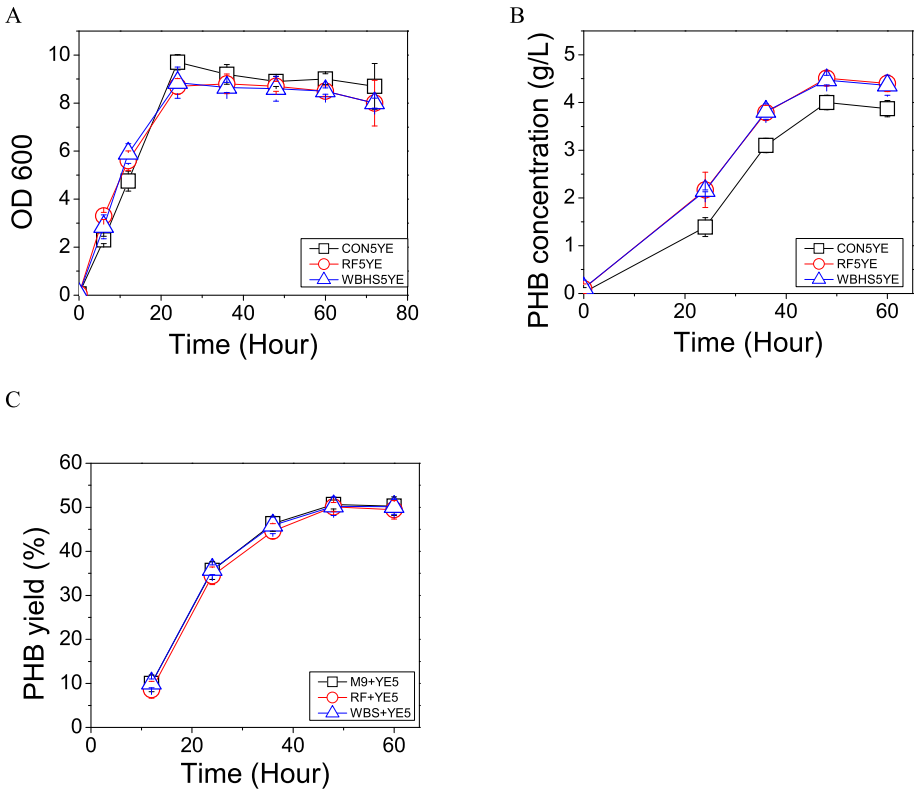

Fig. 5. PHB production profiles with different carbon sources (supplemented with M9 medium and 5 g/L yeast extract).

[C_4mim] Cl could effectively break the extensive network of intra- and intermolecular hydrogen bonds in cellulose, thus boosting cellulose dissolution in the ionic liquid [39,40]. Here, we believe that there may be similarities between RF heating based pretreatment and ionic liquids based pretreatment technologies. However, the reactions of ionic liquid are based on the chemical-ionic activity, while the RF pretreatment is based on the 'physical' ionic activity induced by the electromagnetic field.

3.4. Elemental analysis

In order to confirm our hypothesis that RF heating assisted alkaline pretreatment led to higher nutrient content in the switchgrass hydrolysates, we performed a CHNS analysis on the pretreated switchgrass, as well as switchgrass hydrolysates generated from RF or WB heating based pretreatment approaches.

For the pretreated switchgrass, the nitrogen content in the RF heating was 27% higher than that in the WB heating (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). This illustrated that RF heating assisted pretreatment generated more nutrients (especially nitrogen sources) compared to WB pretreatment and thus led to better cell growth and PHB production. This was in well concert with the fermentation results as we discussed above.

Moreover, the GC-MS result showed that some specific compounds including hydroxyl-acetaldehyde, propanoic acid, 2-methoxy-4-vinyl phenol, 2-methoxy phenol and 1,2-benzenediol (catechols) were observed in RFH, but not in WBH (data not shown). These compounds were also reported to be presented in the 'bio-oils' generated through fast pyrolysis (experiencing super high temperatures) of biomass [38,41–43]. This indicated that even the same temperature (90 °C) was applied, due to a different heating mechanism, the RF pretreatment provided a much harsher heating condition than WB pretreatment and led to different composition of nutrients (higher levels of nutrients along with slightly higher levels of carbon sources) for better PHB fermentation performance.

4. Conclusion

In this study, RF assisting pretreatment of switchgrass to generate hydrolysates for PHB production using recombinant *E. coli* strain was evaluated. Hydrolysates generated through RF pretreatment exhibited much better performance than those generated through WB pretreatment. The supplementation of YE improved fermentation performance under all conditions, and simultaneously narrowed down the difference of fermentations using hydrolysates generated through RF and WB heating. Taken together, this work demonstrated that RF-based dielectric heating pretreatment is an efficient and promising procedure for lignocellulosic biomass processing for biochemical/biomaterial production purposes.

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