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Research Paper

Biorefinery production of poly-3-hydroxybutyrate using waste office paper hydrolysate as feedstock for microbial fermentation

Neelamegam Annamalai[a,b], Huda Al-Battashi[a], Saif Al-Bahry[a], Nallusamy Sivakumar[a,*]

[a] Department of Biology, College of Science, Sultan Qaboos University, PO Box 36,Muscat - 123, Oman
[b] Hawaii Natural Energy Institute, University of Hawaii at Manoa, 1680, East-west Road, Honolulu – 96822, HI, USA

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ABSTRACT

Waste paper, a major fraction of municipal solid waste, has a potential to serve as renewable feedstock for the biorefineries of fuels, chemicals and materials due to rich in cellulose and abundant at low cost. This study evaluates the possibility of waste office paper (WOP) to serve as a potential feedstock for the biorefinery production of poly (3-hydroxybutyrate). In this study, the WOP was pretreated, enzymatically saccharified and the hydrolysate was used for PHB production. The hydrolysate mainly consists of glucose (22.70 g/L) and xylose (1.78 g/L) and the corresponding sugar yield was about 816 mg/g. Ammonium sulphate and C/N ratio 20 were identified as most favorable for high yield of PHB. The batch fermentation of *Cupriavidus necator* using the pretreated WOP hydrolysate resulted in cell biomass, PHB production and PHB content of 7.74 g/L, 4.45 g/L and 57.52%, respectively. The volumetric productivity and yield achieved were 0.061 g/L/h and 0.210 g/g sugar, respectively. The results suggested that WOP could be a potential alternative feedstock for the biorefinery production of bioplastics.

1. Introduction

Polyhydroxyalkanoates (PHAs) are synthesized under nutrient limiting conditions such as nitrogen, phosphorous, oxygen, sulphur or magnesium, in the presence of excess carbon source (Cesario et al., 2014; Kachrimanidou et al., 2014). Poly (3-hydroxybutyrate) (PHB) is a biodegradable and thermoplastic polymer accumulated intracellularly as carbon and energy storage granules that have the potential to replace the petrochemical based plastics (Kulpreecha et al., 2009). PHB production and commercialization has been intensified in recent years due to its potential applications in packaging, biomedicine, agriculture and many other fields (Rehm, 2006). The global production of microbial bioplastics was counted as 54 kt in 2014 where it is expected to be a 5 – fold increase by 2020 (Ferreira and Schlottbom, 2016). However, the production cost is the major problem that hinders the process since 45% of the total production cost drives for the carbon source (Kulpreecha et al., 2009; Kachrimanidou et al., 2014; Annamalai and Sivakumar, 2016). Several researchers have been intensively working to find a sustainable, renewable and cheaper alternative carbon source which serves as a bioplastic contender for petrochemical plastics market (Zhang et al., 2013; Saratale and Oh, 2015; Cerrone et al., 2015; Annamalai and Sivakumar, 2016).

Lignocellulosic biomasses and other industrial wastes are being considered as a promising renewable and sustainable feedstocks that is becoming rapidly developed and commercialized for production of polyhydroxyalkanoates as a substitute of fossil fuel derived plastics (Obruca et al., 2014, 2015). Waste paper is one among the major components of municipal and industrial solid wastes which accounts more than 35% of total lignocellulosic wastes (Dubey et al., 2012). Annually, more than 400 million tons of waste paper is generated and only about 50–65% is being recycled due to the constraints in recycling of paper fibers which turned into low quality paper products and also the difficult in the process when the paper mixed with other wastes (Wang et al., 2012). However, waste paper has a potential to be used as an excellent alternative feedstock for fermentable sugars production due to its high cellulose content (50–60%), relative abundance and low cost (average $52/ton) (Kadar et al., 2004). Utilization of waste paper as feedstock for production of other value added products is considered as a much valuable and an alternative route for waste management (Wang et al., 2013). There are several attempts have been made recently on utilization of waste paper and paper-derived materials for byproduct production for instance biofuel (Dubey et al., 2012; Wang et al., 2012; Wang et al., 2013; Brummer et al., 2014), however, there was no specific attempt has been made on the production of bioplastics. This study was aimed to explore the possibility of waste office paper to use as a feedstock for poly-3-hydroxybutyrate (PHB) production. The

* Corresponding author: Tel.: +968 24146891.
E-mail address: apnsiva@squ.edu.om (S. Nallusamy).

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paper focuses about the pretreatment and enzymatic hydrolysis of waste office paper and subsequent production of PHB by *Cupriavidus necator* using WOP hydrolysate.

2. Materials and methods

2.1. Microorganisms and chemicals

The PHB producer strain, *C. necator* (Formerly known as *Ralstonia eutropha*) NCIMB 11599, a glucose-utilizing mutant (*nagE_G265RΔnagR*) of wild type strain *C. necator* H16 was procured from the National collection of industrial food and marine bacteria (NCIMB) and was maintained in yeast peptone meat extract (YPM) agar slants [yeast extract −10; peptone − 10; meat extract −5; $(NH_4)_2SO_4$ −5; agar −15 (g/L)] at 4 °C.

The chemicals and materials used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA) or as indicated.

The enzymes used in this study such as cellulase from *Trichoderma reesei* ATCC 26921 (C2730) and β– glucosidase from *Aspergillus niger* (49291) were purchased from Sigma-Aldrich (MO, USA). The activity of cellulase and β – glucosidase were estimated as 185 FPU/mL and 500 CBU/mL respectively. The cellulase activity was determined by standard filter paper assay (Ghose, 1987). One unit of enzyme activity (FPU) is defined as the amount of enzyme required to liberate 1 mmol of glucose from filter paper in 60 min at 50°C and pH 4.8. The β – glucosidase activity was determined by measuring the amount of *p*-nitrophenol released from *p*-nitrophenyl – β – D-glucopyranoside (*p*NPG) (Parry et al., 2001). One unit of enzyme activity (CBU) is defined as the amount of enzyme required to produce 1 mmol of *p*-nitrophenol from *p* −nitrophenyl-b-D-glucopyranoside (*p*NPG) per minute at 50 °C and pH 5.0.

2.2. Preparation of waste office paper

Waste office paper (WOP) collected from the local market were shredded into small pieces (2 × 5 mm) using a mechanical shredder (Atlas, China). The shredded WOP was soaked with distilled water (5%, w/v), wet milled, filtered using cloth sheath and dried at 60 °C for 24 h. Further, the dried pulp was then milled again and used for further study.

2.3. Pretreatment of waste office paper

The pretreatment was carried out in a screw cap bottle with 0.5% hydrogen peroxide and waste office paper (5% w/v) and then autoclaved at 121 °C for 30 min. The solid residues were collected by filtration using cloth sheath and washed several times with distilled water until neutral pH. The pulp dried at 60 °C for 24 h was milled again and used as a substrate for enzymatic hydrolysis.

2.4. Enzymatic hydrolysis of pretreated waste office paper

Enzymatic hydrolysis of pretreated WOP was carried out in 500 mL hydrolysis flask containing 100 mL of 50 mM citrate buffer (pH 4.8) with 3 % (w/v) solids loadings. The enzyme mixture such as cellulase (37 FPU/g solid) and β-glucosidase (25 CBU/g solid) was used for hydrolysis. After addition of solids and enzymes, the flasks were incubated at 50 °C for 120 h at 160 rpm. Aliquots were withdrawn at regular intervals (24 h), centrifuged at 10,000 ×*g* for 10 min and the supernatant was subjected to sugar analysis. The hydrolysate obtained from the enzymatic hydrolysis (10,000 ×*g*, 10 min) was used for PHB production. The percentage of hydrolysis (%) was calculated based on the amount of sugars in the initial substrate and the sugars released from hydrolysis using the following formula:

$$\text{Percentage of hydrolysis (\%)} = \frac{\text{Sugar released (g/mL)} \times \text{Total volume (mL)} \times 0.9}{\text{Sugar in the initial subtract (mg/mL)}} \times 100$$

2.5. Effect of various nitrogen sources and C/N ratio on cell biomass production and PHB accumulation

The effect of various nitrogen sources on cell biomass and PHB accumulation was studied by growing *C. necator* in mineral salt medium (MSM) [2.4 − KH_2PO_4; 2.5 − Na_2HPO_4; 0.5 − $MgSO_4$ and 0.05- ferric ammonium citrate (g/L)] (pH − 6.8) prepared with WOP hydrolysate (20 g/L) was supplemented with various nitrogen sources (2 g/L) to be investigated. The effect of C/N ratio [10,20, 40, 60, 80 and ∞ (N-free)] was investigated by cultivating in WOP hydrolysate with different concentrations of ammonium sulphate. No ammonium sulphate was added to the medium for N-free condition. The flasks were seeded with 1% inoculum (OD – 1.0 at 600 nm), incubated at 30 °C for 72 h at 200 rpm and the cell biomass and PHB content was estimated. The cell biomass was estimated gravimetrically by centrifuging the culture broth (5 mL) at 5000 ×*g* for 10 min at 4 °C, washed with deionized water and dried at 50 °C for 24 h and expressed in g/L.

2.6. PHB production using WOP hydrolysate

C. necator was precultured in YPM broth at 30 °C for 24 h and the cells with low PHB content were harvested by centrifugation at 5000 ×*g* for 10 min, suspended in sterile distilled water and used as an inoculum (OD – 1.0 at 600 nm). For PHB production, batch fermentation was carried out in a 500 mL Erlenmeyer flasks containing 100 mL of MSM [2.4 − KH_2PO_4; 2.5 − Na_2HPO_4; 0.5 − $MgSO_4$; 0.05- Ferric ammonium citrate and 2.2 − NH_2SO_4 (g/L)] prepared with WOP hydrolysate (24.48 g/L total sugar/22.7 g/L glucose) was filtered through pre-sterilized membrane filter (0.2 mm). The flasks were seeded with 1% (v/v) inoculum and incubated at 30 °C for 96 h with 200 rpm. The dry cell weight (DCW) (g/L), PHB content (%) and residual sugars (g/L) were determined from the aliquots withdrawn at regular interval (24 h).

2.7. Analytical methods

2.7.1. Compositional analysis

The moisture and ash content of waste office paper were determined using the method of NREL/TP – 510 – 42621 (Sluiter et al., 2008a) and NREL/TP – 510 – 42622 methods (Sluiter et al., 2008b), respectively. The structural carbohydrates (cellulose and hemicellulose), acid soluble (ASL) and insoluble lignin of waste office paper were determined by the method of NREL/TP – 510 – 42618 (Sluiter et al., 2012).

2.7.2. Sugars and inhibitors analysis

The glucose, xylose, hydroxymethylfurfural (HMF) and furfural were analyzed using HPLC (Shimadzu; LC10AD) equipped with Aminex HPX − 87H (300 mm × 7.8 mm; Bio-Rad, USA) column at 65 °C using 5 mM sulfuric acid as mobile phase at 1 mL/min flow rate with refractive index detector (Shimadzu; RID10A). The sugars were quantified by external calibration with standards. Total phenolics were determined by Folin – Ciocalteu method (Singleton et al., 1999).

2.7.3. Poly(3-hydroxybutyrate) quantification

The PHB content of dry cell was estimated by gas chromatography (GC) analysis as described earlier (Annamalai and Sivakumar, 2016). Briefly, 50 mg of dried cells were added with 2 mL chloroform and 2 mL acidic methanol (2.8 M H_2SO_4 in methanol) containing octanoic acid (10 mL/L) as an internal standard and heated at 100 °C for 4 h. After cooling, 1 mL of distilled water was added to the mixture, vortex vigorously for 3 min and then centrifuged at 2000 ×*g* for 1 min. After that,

the organic phase (methylated monomers dissolved in chloroform) of each sample was filtered and analyzed through GC − FID. The gas chromatographic separation was performed using an Agilent J & W HP-5 capillary column (30 × 0.32 mm × 0.25 mm). The temperature of the injector was kept at 250 °C. One mL of the sample was injected (split ratio – 1: 20) and helium (99.999%) was used as the carrier gas with a constant flow rate of 2 mL/min. The oven temperature was programmed to increase from 100 °C (1 min) to 250 °C (4 min) at a heating rate of 15 °C/min. The flame ionization detector (FID) was kept at 270 °C (air 300 mL/min, hydrogen 30 mL/min, nitrogen 30 mL/min). The PHB content was estimated using calibration with commercial PHB (Sigma, MO, USA) and expressed as percentage of dry cell weight (%).

2.7.4. Statistical analysis

The mean values and standard deviations were calculated from the data obtained from three independent experiments. Analysis of variance was performed by one way ANOVA followed by Tukey's HSD Post Hoc multiple comparison analysis using IBM SPSS statistics package, version 21. Statistical differences at $p < 0.05$ were considered as a significant.

3. Results and discussion

3.1. Compositional analysis of waste office paper

The components of the untreated and pretreated WOP were analyzed and presented in Table 1. The cellulose, hemicellulose and lignin in untreated and pretreated office paper was about 52.16 ± 1.63, 9.54 ± 0.63 and 15.32 ± 1.16, and 73.28 ± 1.24, 5.62 ± 0.24 and 2.61 ± 0.28%, respectively. It is suggested that the pretreatment with hydrogen peroxide significantly increased the cellulose and reduced the hemicellulose, lignin and ash content of waste office paper ($p < 0.05$). Likewise, Kim et al. (2000) reported that the pretreatment with 5% hydrogen peroxide removed 60% of lignin in addition to the considerable increase in cellulose from the waste paper. The untreated waste office paper contains relatively high content of ash (18.52 ± 1.32 %) which mainly composed of fillers and coating materials during paper making such as calcite ($CaCO_3$) and talc; however, the pretreatment significantly lowered the ash content of WOP (10.56 ± 1.26%). Kemppainen et al. (2014) suggested that the pretreatment greatly reduces the ash content and its impact during enzymatic hydrolysis of biomass. Thus, the significant increase in cellulose content in addition to higher removal of lignin with minimum effect on hemicellulose suggested that the hydrogen peroxide pretreatment would be the most suitable for WOP for biorefinery production of bioplastics.

3.2. Enzymatic hydrolysis of pretreated WOP

The results on enzymatic hydrolysis of pretreated WOP suggested that the sugars produced from pretreated WOP were increased continuously up to 72 h and there was no significant increase afterwards ($p < 0.05$) (Fig. 1). Likewise, the hydrolysis of WOP was also increased significantly up to 96 h and no further increase till 120 h

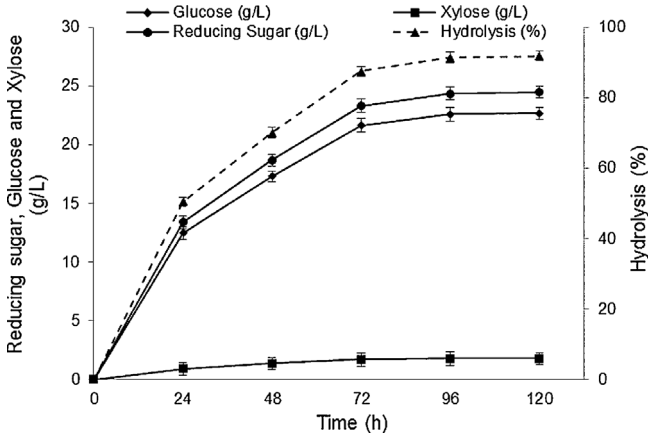

Fig. 1. Hydrolysis and sugar yield of pretreated waste office paper. The results were presented in mean ± SD, n = 3.

($p < 0.05$). The sugar yield and percentage hydrolysis achieved through the enzymatic hydrolysis was about 13.38 g/L and 50.4 % at 24 h and 24.49 g/L and 91.5% at 120 h, respectively with the corresponding sugar yield of 816 mg/g WOP. The results suggested that the hydrolysis rate and production of sugars were significantly higher at the initial up to 24 h and decreased afterwards ($p < 0.05$). Furthermore, sugars in the hydrolysate were mainly composed of glucose (22.70 ± 0.32 g/L) and xylose (1.78 ± 0.32 g/L). Rocha et al. (2016) achieved about 15 g/L of glucose and 2.5 g/L of xylose from untreated WOP; however, the yield was significantly decreased with 1% sulphuric acid pretreated WOP. The sugar yield and hydrolysis achieved from this study was comparatively higher than the previous studies which is due to the less lignin and $CaCO_3$ in pretreated waste office paper (Wu et al., 2014; Zhou et al., 2017). Earlier studies were also suggested that the $CaCO_3$ not only raise the pH of the suspension, it is also an inhibitor to enzymatic saccharification of cellulose (Wang et al., 2012; Brummer et al., 2014).

Further, the pretreated WOP hydrolysate contains no detectable amount of 5 – hydroxymethylfurfural (HMF) and furfural; however, there was considerable amount of phenolics (0.14 ± 0.04 g/L) due to the degradation of lignin during the pretreatment (Table 2). Earlier reports suggested that the hydrolysates of lignocellulosic biomass contains inhibitors like furfural, hydroxylmethylfurfural, acetic acid, phenolic compounds, and water soluble lignin which are greatly affects PHB production. Hence, it is essential to detoxify the hydrolysate of lignocellulosic biomasses in order to increase the PHB yield (Silva et al., 2004; Pan et al., 2012). But, the WOP hydrolysate obtained in this study does not require any detoxification since it contains relatively low amount of fermentation inhibitors. Likewise, Rocha et al. (2016) also reported that there was no furfural and 5-HMF detected in 1% H_2SO_4 pretreated office paper.

3.3. Effect of nitrogen source and C/N ratio on cell biomass and PHB accumulation

The effect of various organic and inorganic nitrogen sources on cell

Table 1
Components of untreated and pretreated waste office paper. The results were presented as mean ± SD, n = 3.

Components	Untreated (%)	**Pretreated (%)**
Cellulose	52.16 ± 1.63[b]	73.28 ± 1.24[a]
Hemicellulose	9.54 ± 0.63[a]	5.62 ± 0.24[b]
Lignin	15.32 ± 1.16[a]	2.61 ± 0.28[b]
Ash	18.52 ± 1.32[a]	10.56 ± 1.26[b]
Moisture	4.24 ± 1.16[b]	6.54 ± 1.42[a]

Values with different alphabets in the same column are significantly different ($p < 0.05$).

Table 2
Inhibitors in the hydrolysate of pretreated waste office paper. The results were presented in mean ± SD, n = 3.

Inhibitors	Amount in the hydrolysate (g/L)
HMF	BDL
Furfural	BDL
Total phenolics	0.14 ± 0.04

*BDL- Below Detectable Level (Detectable Level − 0.02 g/L).

Table 3
Effect of various nitrogen sources on cell biomass and PHB production by *R. eutropha* using WOP hydrolysate. .

Nitrogen Source	Time (h)	Biomass (g/L)	PHB production (g/L)	PHB Content (%)	R_{glu} (g/L)	R_{xyl} (g/L)	Productivity (g/L/h)	Y_{PHB} (g/g sugar)
$(NH_4)_2SO_4$	72	5.68 ± 0.18[a]	2.38 ± 0.16[a]	41.90 ± 0.58[a]	0	3.02 ± 0.58	0.033 ± 0.002[a]	0.11 ± 0.002[a]
NH_4Cl	72	4.80 ± 0.13[b]	1.72 ± 0.11[b]	35.84 ± 1.28[b]	0	3.03 ± 0.58	0.024 ± 0.003[b]	0.08 ± 0.001[b]
NH_4NO_3	72	4.96 ± 0.15 [b]	1.60 ± 0.18[b]	32.25 ± 2.94[b]	0	3.02 ± 0.58	0.022 ± 0.002[b]	0.07 ± 0.003[a]
Yeast extract	72	2.16 ± 0.08 [d]	0.36 ± 0.12[c]	16.66 ± 2.73[c]	0	3.04 ± 0.58	0.005 ± 0.001[c]	0.02 ± 0.002[d]
Peptone	72	4.19 ± 0.05[c]	1.83 ± 0.26[b]	43.67 ± 2.57[a]	0	3.01 ± 0.58	0.025 ± 0.002[b]	0.08 ± 0.003[b]

Values with different alphabets in the same column are significantly different ($p < 0.05$).

biomass production and PHB accumulation were investigated and the results were presented in Table 3. The results suggested that the cell biomass and PHB production were significantly higher with $(NH_4)_2SO_4$; however, the PHB content of the dry cell (%) was significantly higher with peptone than other organic and inorganic nitrogen tested ($p < 0.05$). Thus, the results clearly evidenced that the $(NH_4)_2SO_4$ greatly enhances both the new cell formation and PHB accumulation; however, peptone mainly promotes the PHB accumulation rather than new cell production. The productivity (g/L/h) and PHB yield (g/g sugar) were about 0.033 and 0.11, 0.024 and 0.08, 0.022 and 0.07, 0.005 and 0.02, and 0.025 and 0.09 with $(NH_4)_2SO_4$, NH_4Cl, NH_4NO_3, yeast extract and peptone, respectively. It is suggested that the productivity and yield obtained in this study was significantly higher with $(NH_4)_2SO_4$ than the other nitrogen sources tested ($p < 0.05$). Based on the results, it is clearly evidenced that nitrogen sources highly influenced the cell biomass production and PHB accumulation and $(NH_4)_2SO_4$ was the most suitable for high yield of PHB. Similarly, several other studies also suggested that the inorganic nitrogen such as ammonium sulphate and ammonium nitrate were most suitable nitrogen sources for better cell growth and PHB production (Gouda et al., 2001; Sreekanth et al., 2013); however, Pal et al. (2009); Zhang et al. (2013) were reported that tryptone (organic) as a suitable nitrogen source for high yield of PHB.

The effect of C/N ratio on cell biomass and PHB accumulation was investigated and the results were presented in Fig. 2. The cell biomass production was 5.79, 4.94, 2.82, 1.87, 1.63 and 0.33 g/L with the C/N ratio of 10, 20, 40, 60, 80 and N – free condition, respectively. The results suggested that the cell biomass was significantly decreased with increasing C/N ratio ($p < 0.05$) and the reduction in cell mass was about 50, 70 and 75% while the C/N ratio was increased to 40, 80 and N-free condition, respectively. The higher C/N ratio caused N – deficiency and imbalance distribution of carbon and nitrogen in the medium which leads to significant decrease in cell biomass. Similarly, Johnson et al., (2010) suggested that the high C/N limitation in the production medium significantly affects the cell biomass production. The PHB content of the dry cell was 24.7, 50.7, 60.2, 68.4, 75.5 and 32.6 % with the C/N ratio of 10, 20, 40, 80 and N-free condition, respectively. The accumulation of PHB was significantly increased with increasing the C/N ratio from 10 to 80; however, N – free condition (∞) resulted decrease in PHB content ($p < 0.05$). The results confirmed that the *C. necator* more preferred high N – deficient condition (C/N −80) than the N-free condition (∞) and the high C/N deficient is not suitable for cell biomass production, but most favorable for the accumulation of PHB. Lee et al., (2008); Kulpreecha et al., (2009) were also suggested that the high C/N limitation promotes the PHB accumulation and significantly affects the cell biomass production. Several other studies were also investigated on the effects of C/N ratio for the better production of PHB and concluded that the nitrogen limitation favors the PHB accumulation while minimizing the cell growth (Yu and Stahl, 2008; Johnson et al., 2010; Annamalai and Sivakumar, 2016).

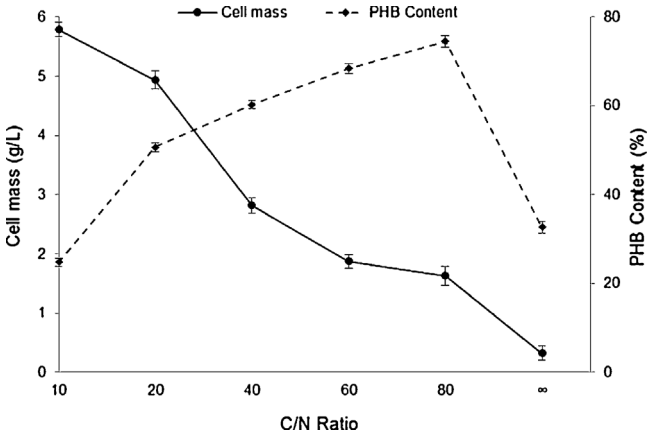

Fig. 2. Effect of various C/N ratio on cell biomass and PHB accumulation by *R. eutropha* using WOP hydrolysate. The results were presented in mean ± SD, n = 3.

3.4. PHB production using WOP hydrolysate

For production of PHB, the strain *C. necator* was grown in mineral salt medium (MSM) prepared with pretreated WOP hydrolysate and the results were presented in Fig. 3. The initial sugar concentration in the hydrolysate was considered as 22.7 (g/L) since *C. necator* does not utilize xylose and $(NH_4)_2SO_4$ was added accordingly to maintain C/N ratio of 20. The cell biomass production was significantly increased with increasing fermentation time, reached maximum at 72 h and no further significant increase afterwards ($p < 0.05$). The cell biomass production was 3.42 g/L at 24 h, reached to 7.74 g/L at 72 h and decreased to 7.45 g/L at 96 h. The PHB production was about 0.4 g/L at 24 h, reached a maximum of 4.45 g/L at 72 h and decreased to 3.17 g/L at 96 h. It reveals that the rate of production of cell biomass and PHB was relatively lower at the beginning till 24 h due to the log phase and increased significantly up to 72 h and decreased afterwards ($p < 0.05$). Van-Thuoc et al. (2008) achieved about 8 and 4 g/L of biomass and PHB using wheat bran hydrolysate, respectively. Gouda et al. (2001) reported that the cell biomass and PHB production were about 3.6 and 2.2 g/L by *B. megaterium* using sugarcane molasses and

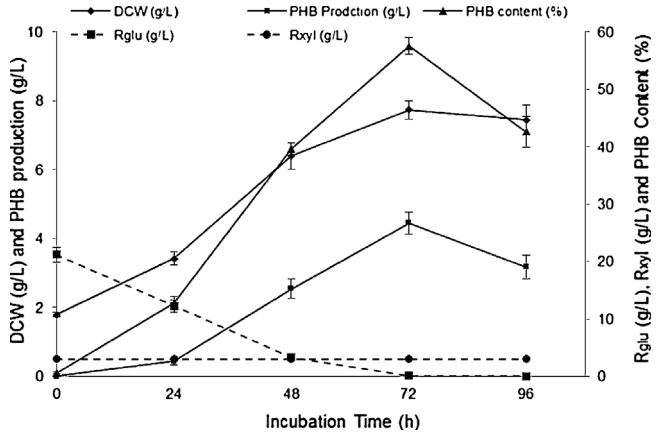

Fig. 3. Cell biomass, PHB production, PHB content and residual sugars during batch fermentation of *R. eutropha* using pretreated WOP hydrolysate as a feedstock. The results were presented in mean ± SD, n = 3.

Table 4
Cell Biomass, PHB yield and productivity of *R. eutropha* utilizing various lignocellulosic biomasses.

Type of Biomass	Fermentation	DCW (g/L)	PHB production (g/L)	PHB (%)	Productivity (g/L/h)	Y_{PHB}(g/g sugar)	References
Sugarcane Bagasse	Batch	6.0	3.9	65.0	0.08	ND	Yu and Stahl, 2008
Water hyacinth	Batch	12.0	7.0	58.0	0.09	0.240	Radhika and Murugesan, 2012
Sunflower	Batch	10.9	7.8	72.5	0.13	0.395	Kim et al., 2016
Wheat bran	Batch	24.5	14.8	62.5	0.25	0.319	Annamalai and Sivakumar, 2016
Sargassum	Batch	5.3	3.93	74.4	0.10	0.470	Azizi et al., 2017
Waste office Paper	Batch	7.7	4.45	57.5	0.06	0.210	This study

ND-Not Determined.

corn steep liquor.

Further, the PHB content of the dry cell was about 12.83% at 24 h, increased to 39.6% at 48 h and reached a maximum of 57.52% at 72 h. The results revealed that the PHB content of the dry cell was significantly increased with cultivation time up to 72 h and decreased afterwards ($p < 0.05$). It seems that the sugars were mainly utilized for new cell production at the beginning up to 24 h and then subsequently used for accumulation of PHB. The decrease in PHB content after 72 h may be due to indigenous utilization of PHB by PHB depolymerase for bacterial metabolism because of carbon deficiency in the fermentation medium. The PHB content of *C. necator* obtained from the present study utilizing WOP hydrolysate was comparatively higher than the previous studies (Gouda et al., 2001; Van-Thuoc et al., 2008). Table 4 summarized the cell biomass, PHB yield and productivity of *C. necator* utilizing various lignocellulosic biomass. It is suggested that the cell biomass and PHB production from *R. eutropha* obtained in this study was comparatively higher than the previous studies. Yu and Stahl (2008) achieved the dry cell weight, PHB production and productivity of 6 g/L, 3.9 g/L and 0.08 g/L/h, respectively. Azizi et al. (2017) obtained the cell biomass, PHB production and productivity of 5.3 g/L, 3.93 g/L and 0.010 g/L/h, respectively. However, the PHB content of the dry cell obtained in this study was quite comparable with other studies utilizing lignocellulosic biomass by *C. necator* (Radhika and Murugesan, 2012; Annamalai and Sivakumar, 2016).

The residual sugar analysis suggested that glucose was significantly consumed during the whole course of cultivation and almost utilized completely at 72 h; however, xylose remains unconsumed as *C. necator* was not able to utilize xylose ($p < 0.05$) (Fig. 3). Although xylose remains unutilized while consuming glucose, there was no carbon catabolite repression (CCR) occurred during PHB production since the hydrolysate contains only fewer amounts of pentose sugars (xylose). The PHB productivity and yield achieved in this study was about 0.061 g/L/h and 0.210 g/g sugar, respectively which was lower than the yield of other studies utilizing lignocellulosic biomasses. Radhika and Murugesan (2012) obtained the productivity and yield of 0.09 (g/L/h) and 0.240 (g/g sugar) from the hydrolysate of water hyacinth and Kim et al. (2016) achieved the P (3HB) productivity and yield about 0.13 (g/L/h) and 0.395 (g/g sugar) from sunflower stalk hydrolysate.

4. Conclusion

The results of the study suggests that the pretreatment with hydrogen peroxide resulted in better removal of lignin, increases enzymatic accessibility and high sugar yield would be the most suitable for waste office paper. The resulting WOP hydrolysate was used as feedstock for the production of poly (3-hydroxybutyrate) without any detoxification as it does not have any fermentation inhibitors like HMF and furfurals. The cell biomass and PHB content achieved from this study using WOP hydrolysate were about 7.74 g/L and 57.52% at 72 h, respectively. Thus, the study proved that the waste office paper could be efficiently utilized as a potential feedstock for industrial production of bioplastics at reduced cost. Further studies to increase the productivity and PHB yield using high solids loadings, high density cultivation and scale up are in progress.

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