Feasibility of Lipid Production from Waste Paper by the Oleaginous Yeast *Cryptococcus curvatus*

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Waste paper was studied as a potential source for lipid production using the oleaginous yeast *Cryptococcus curvatus* for the first time. Three common types of waste paper, office paper, newspaper, and cardboard, were directly hydrolyzed by an enzyme cocktail to generate sugar-rich and nitrogen-limited hydrolysates. When these hydrolysates were used without any auxiliary nutrients by *C. curvatus*, the lipid content and lipid yield were higher than 50% and 200 mg/g, respectively. The nitrogen-rich enzyme cocktail exerted no negative effects on lipid production. Moreover, the integrated processes of enzymatic hydrolysis and lipid fermentation achieved comparable lipid yield to the separate hydrolysis and lipid production process. The resulting lipid samples had similar fatty acid compositional profiles to those of vegetable oils, which suggested their potential for biodiesel production. These findings strongly supported waste paper as appealing substrates for lipid production *via* oleaginous yeast, which provided cost-effective waste paper-to-lipids routes for sustainable biodiesel production.

Keywords: Cryptococcus curvatus; Waste paper; Nitrogen limitation; Microbial lipid; Biodiesel

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INTRODUCTION

As all major economies race to search for regenerative energy sources, biodiesel has been recognized as a sustainable and renewable substitute for petroleum diesel. However, large-scale biodiesel production is severely hampered by the high cost and limited supply of raw materials such as vegetable oils, animal fats, waste cooking oil, *etc*. Microbial lipids prepared from oleaginous species have captured much attention in recent years as perfect candidates for biodiesel production and substitutes for high value-added exotic fats such as polyunsaturated fatty acids (PUFA) and cocoa butter equivalents (Raltedge 1993; Papanikolaou and Aggelis 2010). However, the costs for microbial lipid production remain too high to be competitive. Techno-economic evaluation has demonstrated that the substrates requirement and the fermentation process are the two principal elements hindering the commercialization of lipid production (Koutinas *et al*. 2014).

To reduce the cost, considerable efforts have been devoted to exploring low-cost substrates for lipid production (Huang *et al*. 2013). Meanwhile, attempts have also been made to achieve higher titer, yield, and productivity, which are pivotal for better techno-economics of microbial lipid technology (Li *et al*. 2007; Gong *et al*. 2014).

Lignocellulose, annually produced in enormous quantities, is mainly composed of 40% to 50% cellulose, 25% to 30% hemicelluloses, 15% to 20% lignin, and 3% to 10% proteins (Chundawat *et al*. 2011; Menon and Rao 2012). Glucose and xylose, the two major sugars derived from lignocellulose, can be co-consumed for lipid biogenesis (Hu *et al*. 2011; Tsigie *et al*. 2011; Ruan *et al*. 2012; Zikou *et al*. 2013; Gong *et al*. 2016a). Various lignocellulosic residues, such as corn stover (Ruan *et al*. 2013; Gong *et al*. 2014; Xue *et al*. 2015), rice straw (Huang *et al*. 2009), wheat straw (Yu *et al*. 2011), corncob (Huang *et al*. 2012), rice hulls (Economou *et al*. 2011), sugarcane bagasse (Tsigie *et al*. 2011), and sorghum bagasse (Liang *et al*. 2012), have been investigated for lipid production. Moreover, acetic acid when present together with lignocellulosic sugars can serve as a building block for lipid production (Ruan *et al*. 2015; Gong *et al*. 2016a). However, there are at least three factors hindering the cost competitiveness of the lignocellulose-to-lipids routes. First, high-cost pretreatment technologies are generally required to break the rigid structure of lignocellulose (Himmel *et al*. 2007). Second, various inhibitors, such as furan aldehydes, aliphatic acids, and phenolic compounds, are inevitably generated during the pretreatment process, which are toxic to the oleaginous yeasts, hindering cell growth and lipid accumulation (Yu *et al*. 2014). Third, lignocellulose contains roughly 3% to 10% of proteins and minor amounts of other nitrogenous components, which disfavor lipid production, because lipid accumulation is commonly induced by nitrogen limitation (Ratledge and Wynn 2002; Chundawat *et al*. 2011; Huang *et al*. 2011).

Waste paper, derived from cellulosic biomass, is roughly composed of 40% to 80% cellulose, 5% to 15% hemicellulose, and negligible amounts of lignin and proteins (Ioelovich 2014). More than 400 million tons of waste paper are generated annually around the world (Shi *et al*. 2009). Recently, waste paper has been reintegrated for various valuable bio-products production (Nishimura *et al*. 2016). However, it is scarcely studied for lipid production. There are two distinct advantages of using waste paper over virgin lignocellulose for lipid production. On the one hand, energy-intense or corrosive reagents mediated pretreatments are not required to alleviate the biomass recalcitrance of waste paper because pretreatment has already been conducted during the pulping process (Elliston *et al*. 2013). In contrast, most lignocellulose-derived inhibitors and nitrogenous components have been removed through washing during the upstream papermaking processes. Therefore, waste paper may be amenable to generate sugar-rich and nitrogen-limited hydrolysates through direct enzymatic hydrolysis, which can potentially serve as appealing substrates for cost-effective microbial lipid production.

Cryptococcus curvatus has several highly desirable features for lipid production, such as broad feedstock spectrum, high tolerance to lignocellulose derived inhibitors, and high lipid accumulation under a relatively low carbon-to-nitrogen (C/N) molar ratio (Yu *et al*. 2011; Gong *et al*. 2016b). Various low-cost lignocellulosic residues have been applied for lipid production by *C. curvatus* (Yu *et al*. 2011; Liang *et al*. 2012; Gong *et al*. 2016a,b). The goal of this study is to explore the possibility of waste paper as preferable substrates for lipid production. Three typical categories of waste paper; office paper, newspaper, and cardboard, were evaluated for lipid production by *C. curvatus*. The enzymatic hydrolysates, without auxiliary nutrient supplementation, were investigated for lipid production. Further simultaneous saccharification and lipid production (SSLP) on office paper was investigated. To the authors' knowledge, this is the first report on the feasibility of waste paper-to-lipids routes by oleaginous species, which provides valuable information for cost-effective lipid production.

EXPERIMENTAL

Materials

Strain and medium

Cryptococcus curvatus ATCC 20509 was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA), stored at 4 °C and propagated twice a month on yeast peptone dextrose (YPD) agar slants (yeast extract 10 g/L, peptone 10 g/L, glucose 20 g/L, agar 15 g/L, pH 6.0).

Preparation of waste paper enzymatic hydrolysates

Shredded office paper with an average size of 2 mm × 8 mm and moisture content of 5.0% (w/w) was supplied by the Dean's office of Wuhan University of Science and Technology (Wuhan, China). Cardboard and newspaper, with moisture contents of 8.7% and 7.5%, respectively, were obtained from the local reclamation depot (Wuhan, China). The samples were shredded into pieces with comparable sizes to the office paper. Cellulase, β-glucosidase, and xylanase were used as described (Gong *et al*. 2016a). Waste paper was adjusted to a pH of 4.8 with sulphuric acid (H_2SO_4), loaded at 10% (w/v) solid loading and hydrolyzed at 50 °C for 72 h in the presence of 15 FPU/g cellulase, 30 CBU/g β-glucosidase, 5 mg/g xylanase, and 50 μg/mL of ampicillin. The enzymatic hydrolysates were boiled for 10 min before vacuum filtration to remove the residual solids and precipitated proteins. The liquid hydrolysates, without any auxiliary nutrient supplementation, were adjusted to a pH of 5.5 and sterilized by autoclaving at 121 °C for 20 min before used as culture media.

Lipid production on waste paper enzymatic hydrolysates

Pre-cultures were grown in 50 mL YPD liquid medium (yeast extract 10 g/L, peptone 10 g/L, glucose 20 g/L, pH 6.0) at 30 °C for 24 h unless otherwise specified. Lipid production cultures were initiated by adding 5 mL of pre-culture to 45 mL of the waste paper enzymatic hydrolysates in 250-mL unbaffled conical flasks, and incubated at a rotary rate of 200 rpm at 30 °C. The cultivation pH was adjusted to 5.5 in 12 h time intervals. Experiments were conducted in triplicates and data were presented as mean value ± standard deviation.

SSLP on waste paper

Experiments were conducted in 250-mL conical flasks with 5.0 g of office paper at 10% (w/v) solid loading. The culture pH was adjusted to 5.2 with H_2SO_4 and sterilized by autoclaving at 121 °C for 20 min. Next, an enzyme cocktail containing 15 FPU/g cellulase, 30 CBU/g β-glucosidase, and 5 mg/g xylanase was then supplemented. Cultures were initiated by adding 5.0 mL of the pre-culture, and held at 30 °C, 200 rpm for 120 h. The cultivation pH was adjusted to 5.2 in 12 h time intervals.

Methods

Glucose was determined using a glucose analyzer (SBA-40E, Shandong Academy of Sciences, Jinan, China). Xylose was measured by a K-XYLOSE assay kit from Megazyme (Wicklow, Ireland). The total reducing sugars (TRS) were quantified according to the dinitrosalicylate (DNS) method with glucose as the standard (Miller 1959).

Nitrogen was determined according to the Kjeldahl method (Morgan *et al*. 1957). Total nitrogen concentration and C/N ratio were calculated according to the published equations (Gong *et al*. 2016b). The crude proteins content was roughly calculated by multiplying Kjeldahl nitrogen by a factor of 6.25.

Structural carbohydrates, lignin, and ash contents of waste paper were analyzed according to the procedures of the National Renewable Energy Laboratory (NREL) (Sluiter *et al*. 2008a, 2008b).

Cells from 30 mL of the culture broth were harvested by centrifugation and washed twice with distilled water. The cell mass was determined gravimetrically after drying the wet cells at 105 °C overnight.

Lipid extraction was performed according to a previously published procedure (Gong *et al*. 2012). Specifically, dried cells were digested with 4 M HCl at 78 °C for 1 h and extracted twice with chloroform/methanol (1:1, v/v). The extracts were washed with 0.1% NaCl, dried over anhydrous sodium sulphate (Na_2SO_4) and then evaporated in a vacuum. The residue was dried at 105 °C overnight to a constant weight. For the SSLP process, lipids were obtained *via* extraction according to the above method followed by a petroleum ether partition step (Gong *et al*. 2014). Lipid content was expressed as gram lipid per gram lipid-containing dry cell weight. Lipid yield was calculated as gram lipid per gram sugars consumed.

The fatty acid compositional profiles of lipid samples were analyzed using a Shimadzu 2010 plus gas chromatography (GC) instrument (Shimadzu, Kyoto, Japan) after transmethylation according to a published procedure (Gong *et al*. 2016a).

Statistical analysis

An analysis of variance (ANOVA) and multiple comparisons were performed to identify the significant differences. Tukey's post hoc test was conducted to analyze significant differences among the experimental data of various substrates. A *P* value of less than 0.05 was considered statistically significant. All statistical analyses were conducted using SPSS software (SPSS Inc., Version 17.0, Chicago, USA).

RESULTS AND DISCUSSION

Compositional Analysis and Enzymatic Hydrolysis of Waste Paper

The chemical composition of the three types of waste paper was measured according to the procedures of the NREL (Sluiter *et al*. 2008a, 2008b). As shown in Table 1, the office paper consisted of 60.3% glucan, 11.7% xylan, 1.4% lignin, 0.4% crude proteins, and 23.4% ash. It contained high amounts of sugar polymers (72%) and minimal lignin, as alkaline delignification during papermaking was conducted to concentrate cellulose and remove lignin (Elliston *et al*. 2013). The nitrogen content was negligible, which indicated nitrogen-limited hydrolysates could have been generated. The relative high ash content was mainly due to kaolin and calcite used as mineral fillers and $CaCO_3$ used as white pigment during papermaking (Ioelovich 2014). Similarly, newspaper and cardboard also contained large amounts of sugar polymers and negligible amounts of proteins (Table 1), which could also favor the generation of hydrolysates with high C/N ratios. However, lignin exceeding 10% was observed in both newspaper and cardboard, which were noticeably higher than that in office paper.

Table 1. Chemical Composition of Three Common Types of Waste Paper

Entry	Substrates	Relative Contents (%, w/w)				
		Glucan	Xylan	Lignin	Crude Proteins	Ash
1	Office paper	60.3 ± 1.5	11.7 ± 0.6	1.4 ± 0.1	0.4 ± 0.0	23.4 ± 0.9
2	Newspaper	55.6 ± 0.9	10.1 ± 0.5	10.3 ± 0.3	0.7 ± 0.0	15.2 ± 0.5
3	Cardboard	56.8 ± 0.8	6.2 ± 0.6	13.1 ± 0.9	1.3 ± 0.0	12.5 ± 0.3

Office paper, newspaper, and cardboard were directly hydrolyzed using an enzyme cocktail containing 15 FPU/g cellulase, 30 CBU/g β-glucosidase, and 5 mg/g xylanase, respectively. The hydrolysis was conducted for 72 h at 10% (w/v) solid loading. The calcium carbonate ($CaCO_3$) exerted a strong inhibitory effect on cellulase, while calcium sulfate ($CaSO_4$) caused no negative effects (Wang *et al*. 2011). Thus, H_2SO_4 was used to adjust the pH of the waste paper slurries. The results are shown in Fig. 1. A one-way ANOVA followed by Tukey's post hoc test demonstrated that TRS yield was decreased significantly ($P < 0.05$) among different substrates following the order of office paper, newspaper, and cardboard (Fig. 1). For office paper, glucose and xylose was 37.3 g/L and 7.3 g/L, which corresponded to 55.7% and 54.9%, respectively, of the theoretical yields. The formation of 49.2 g/L TRS suggested that roughly 61% of the polymeric carbohydrates were hydrolyzed to soluble reducing sugars. Thus, office paper could be remarkably hydrolyzed by an enzyme cocktail, as pretreatment had already been conducted during the pulping process. Glucose and xylose in newspaper was 29.0 g/L and 6.5 g/L, respectively, which was inferior to those in office paper. The hydrolytic processes of cardboard generated 27.7 g/L glucose, 4.9 g/L xylose, and 35.8 g/L TRS. This data was 25.7%, 32.9%, and 27.2% lower than those in office paper. It was indicated that cardboard was the most recalcitrant substrate for enzymes accessibility, probably because of the lower levels of delignification. Overall, the sugar polymers that ranged from 50% to 60% were deconstructed into monomeric sugars.

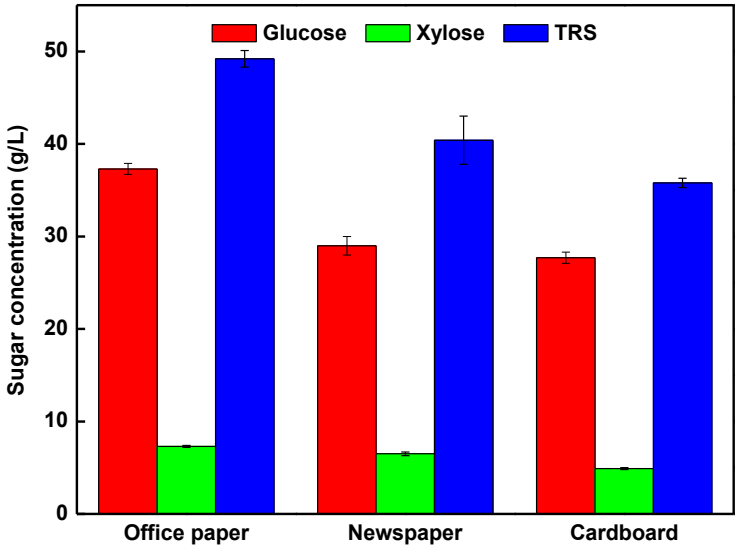

Fig. 1. Results of enzymatic hydrolysis on three common types of waste paper

Lipid Production on Waste Paper Enzymatic Hydrolysates by *C. curvatus*

Office paper, newspaper, and cardboard enzymatic hydrolysates were then investigated for their feasibility as sole nutrient sources for lipid production. The hydrolysate was boiled for 10 min and filtered to remove precipitated enzymes before use. The results are summarized in Fig. 2 and Table 2. The liquid hydrolysates of office paper contained 33.3 g/L glucose and 6.6 g/L xylose. The C/N ratio was calculated as 45.9, which was significantly lower than expected. This was because some nitrogenous components were introduced into the hydrolysates along with the enzyme cocktail. When the hydrolysates, without any auxiliary nutrients, were used to culture *C. curvatus*, glucose and xylose were both below the limit of detection within 48 h (Fig. 2A). The overall sugar consumption rate reached 0.83 g/L/h, which was even significantly ($P < 0.05$) higher than that on 40 g/L of glucose or xylose (Gong *et al*. 2016a). The cell mass, lipid titre, lipid content, and lipid productivity was 17.3 g/L, 9.1 g/L, 52.5%, and 4.4 g/L/d, respectively (Table 2). The lipid yield reached 201.4 mg/g, which indicated that *C. curvatus* favored lipid biosynthesis rather than cell growth. As shown in Fig. 3, the lipid yield was equal to 98.2 g lipid per kg raw office paper supplied, which was identical to that from corn stover through simultaneous saccharification and an enhanced lipid production process (Gong *et al*. 2014). Furthermore, significantly better lipid production results, *i.e.*, lipid content, lipid yield, and lipid productivity, were achieved on the office paper hydrolysates than those on other low-cost substrates rich in glucose and xylose such as rice straw hydrolysates (Huang *et al*. 2009), wheat straw hydrolysates (Yu *et al*. 2011), corn stover hydrolysates (Hu *et al*. 2011; Huang *et al*. 2011; Ruan *et al*. 2013), corncob hydrolysates (Huang *et al*. 2012), and corncob residues hydrolysates (Gao *et al*. 2014). These superior results might have been attributed mainly to the nitrogen limited condition and the absence of lignocellulose-derived inhibitors.

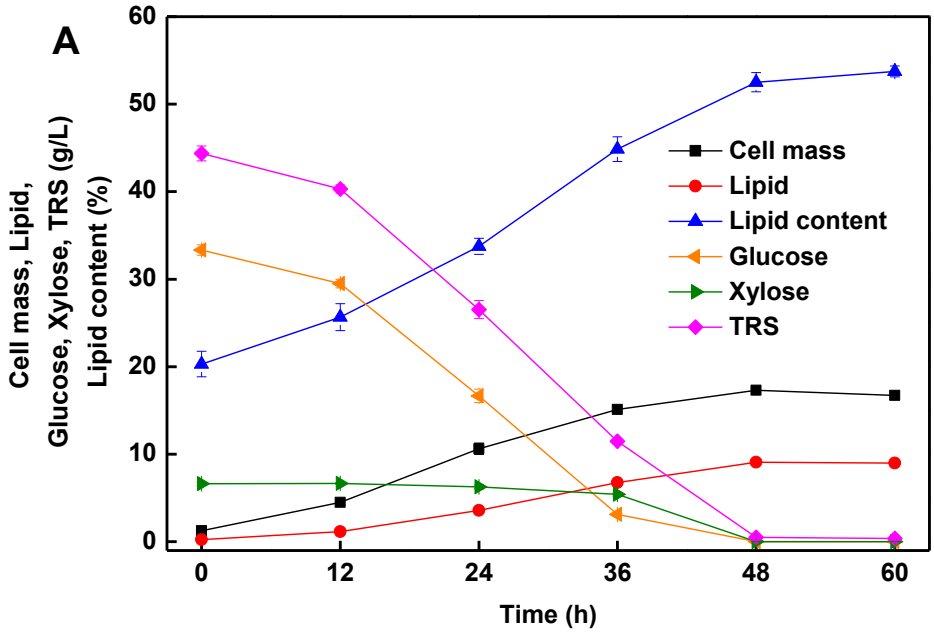

Fig. 2A. Profiles of substrates consumption, cell growth, and lipid production on enzymatic hydroylsates of office paper (A), newspaper (B), and cardboard (C) by *C. curvatus*

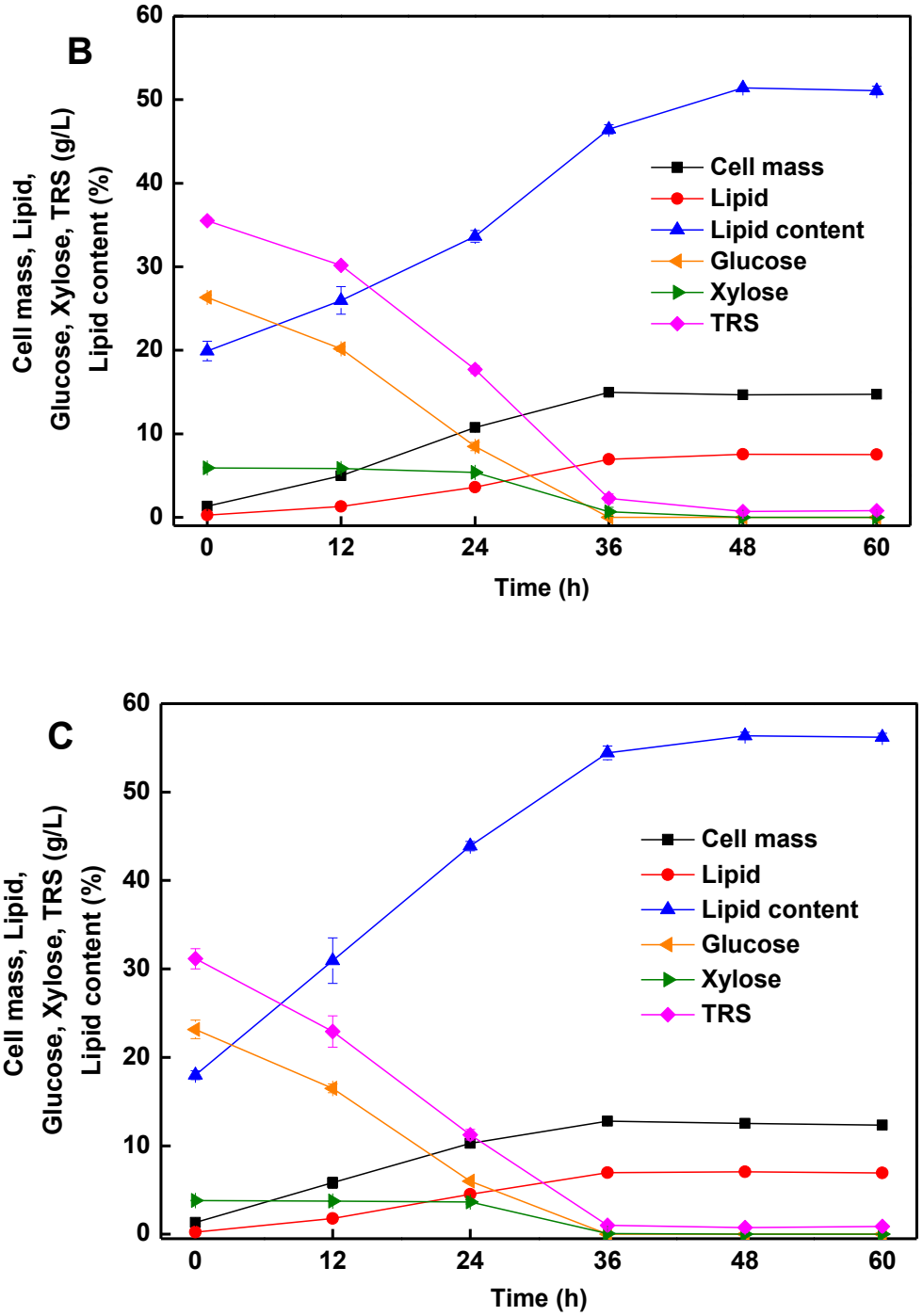

Fig. 2B & 2C. Profiles of substrates consumption, cell growth, and lipid production on enzymatic hydroylsates of office paper (A), newspaper (B), and cardboard (C) by *C. curvatus*

Similarly, newspaper and cardboard hydrolysates contained few amounts of nitrogenous components, which was beneficial for lipid production. The C/N ratios reached 47.2 and 53.7, respectively. When the culture was initiated on the newspaper hydrolysates containing 26.3 g/L glucose and 5.9 g/L xylose, all glucose was consumed and residual xylose was only 0.7 g/L within 36 h (Fig. 2B).

Table 2. Results of Lipid Production on Various Waste Paper Enzymatic Hydrolysates by *C. curvatus*

Entry	Substrates[a]	C/N Ratio	Sugar Consumption (g/L)	Cell Mass (g/L)	Lipid Titre (g/L)	Lipid Content (%, w/w)	Lipid Yield (mg/g)
1	OPEH	45.9	43.9 ± 0.8	17.3 ± 0.2	9.1 ± 0.1	52.5 ± 1.1	201.4 ± 4.4
2	NPEH	47.2	34.8 ± 0.3	14.7 ± 0.1	7.5 ± 0.1	51.4 ± 0.1	208.9 ± 3.4
3	CBEH	53.7	30.4 ± 1.1	12.5 ± 0.0	7.1 ± 0.1	56.4 ± 0.4	224.4 ± 9.3

[a]: Office paper, newspaper, and cardboard enzymatic hydrolysates are abbreviated as OPEH, NPEH, and CBEH, respectively

Cell mass, lipid content, and lipid yield was 14.7 g/L, 51.4%, and 208.9 mg/g, respectively (Table 2, Entry 2). Similarly, when cardboard hydrolysate was used to culture *C. curvatus*, glucose and xylose was consumed within 36 h. Lipid content and lipid yield reached 56.4% and 224.4 mg/g, respectively, which was the highest of the three (Table 2, Entry 3). However, the lipid yield corresponded to 75.7 kg per ton raw cardboard, which was significantly ($P<0.05$) lower than that on office paper, due to lower sugar yield during enzymatic hydrolysis (Fig. 3). It was crucial to further improve the sugar yields during the waste paper hydrolysis process, which should provide hydrolysates with more building blocks and higher C/N ratios to advance lipid production. Higher sugar yield is expected through the demineralization of the waste paper (Ioelovich 2014).

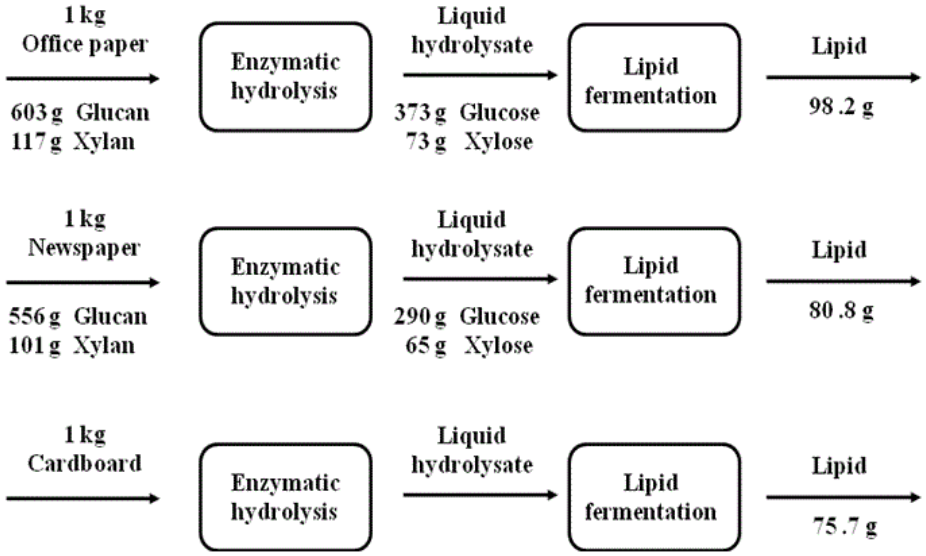

Fig. 3. The overall process illustrating the lipid yields from 1 kg raw waste paper

As shown in Fig. 2, *C. curvatus* assimilated glucose quickly from 0 h to 24 h, whereas xylose almost kept constant, regardless of the variations in substrates. Xylose consumption started until the glucose concentration dropped below 10.0 g/L, suggesting that the yeast strongly prefers glucose over xylose (Kim *et al*. 2010). This phenomenon was in line with the results of *C. curvatus* and *Mortierella isabellina* on lignocellulosic hydrolysates (Ruan *et al*. 2015; Gong *et al*. 2016b). However, this finding was inconsistent with results reported for other oleaginous species such as *Trichosporon cutaneum*, *Trichosporon fermentans*, *Thamnidium elegans*, *etc*., probably due to the strain diversities

(Hu *et al*. 2011; Zikou *et al*. 2013; Liu *et al*. 2015). The sequential manner of sugars assimilation was probably because glucose can repress the utilization of xylose *via* a catabolite repression mechanism or allosteric competition for sugar transporters (Kawaguchi *et al*. 2006).

The theoretical lipid yields on glucose and xylose by oleaginous species are 330 mg/g and 340 mg/g, respectively (Ratledge 1988). However, the experimental results were rarely higher than 220 mg/g (Papanikolaou and Aggelis 2011). The high lipid yields that ranged from 201.4 mg/g to 224.4 mg/g suggested that carbon sources in the hydrolysates were mainly channeled into lipid biosynthesis. These data were comparable to those obtained by *Yarrowia lipolytica* and *Rhodosporidium toruloides* on glucose (Qiao *et al.* 2015; Tchakouteu *et al.* 2017). Overall, the authors' results indicated that waste paper hydrolysates could serve as appealing substrates for lipid production.

Enzymes were retained in the liquid hydrolysates when the enzymatic hydrolysates were directly filtered to remove residual solids without boiling. The cell mass, lipid titre, lipid content, and lipid yield reached 17.6 g/L, 9.2 g/L, 52.2%, and 204.1 mg/g, respectively, when hydrolytic enzymes were present in the office paper hydrolysates (Fig. 4). These data were comparable to those on the hydrolysates with enzymes removed, which demonstrated that proteins exerted no negative effect on lipid accumulation. Lipid accumulation by *C. curvatus* generally occurs under nutrients limitation, preferably nitrogen starvation (Ratledge and Wynn 2002). It indicated that proteins were invalid nitrogen sources, probably because *C. curvatus* was unable to secrete proteases, which might be the major reason that lipid accumulation by *C. curvatus* could be triggered under relatively lower C/N molar ratios than other oleaginous yeasts (Li *et al*. 2007; Angerbauer *et al*. 2008; Gong *et al*. 2016b). Overall, there was no need to remove the hydrolytic enzymes of the hydrolysates, which could simplify the processes.

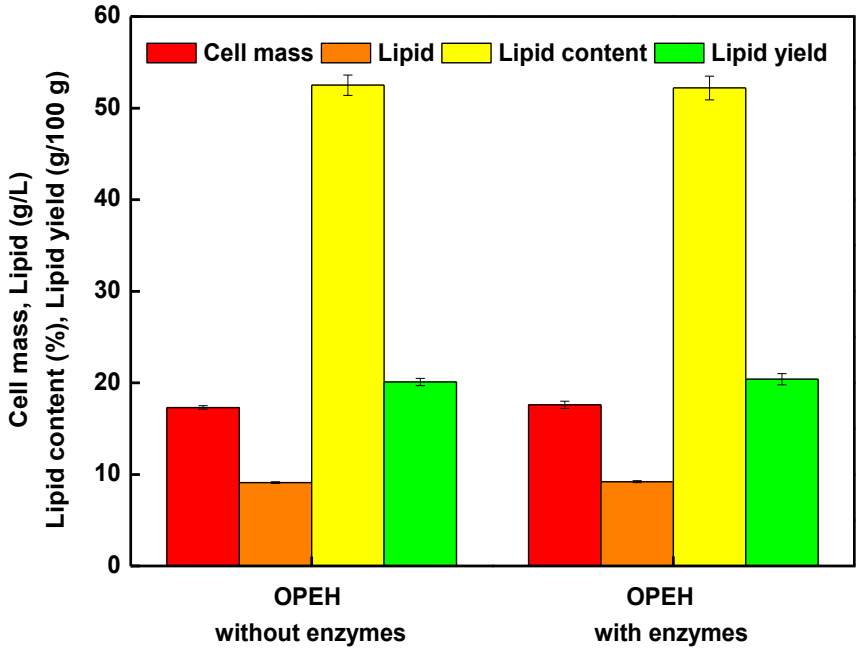

Fig. 4. Results of lipid production on different processed office paper enzymatic hydrolysates; Hydrolytic enzymes were either removed from or remained in the hydrolysates (Office paper enzymatic hydrolysates are abbreviated as OPEH)

SSLP on Waste Paper by *C. curvatus*

Integrating the processes of enzymatic hydrolysis and lipid production seemed to have great potential to avoid product inhibition, reduce enzyme usage, and simplify the processes for the conversion of cellulosic biomass into lipids. In fact, SSLP has been investigated for lipid production from lignocellulose by oleaginous species, such as *C. curvatus* and *T. cutaneum* (Liu *et al*. 2012; Gong *et al*. 2014). In this study, waste office paper was directly used according to the SSLP process by *C. curvatus* without auxiliary nutrients. As shown in Fig. 5, glucose and TRS rapidly accumulated up to 6.7 g/L and 11.4 g/L, respectively, within 6 h, which indicated that the substrates consumption was rate-limiting. The values then both rapidly dropped to 0.6 g/L and 3.1 g/L at 24 h. Xylose increased continuously and reached a plateau concentration of 1.5 g/L at 24 h, probably because of the glucose repression. Glucose and xylose were both below the limit of detection after 48 h, which suggested that the sugars became limiting for yeast assimilation. Lipid production increased over time. Lipid titre and lipid yield were 9.6 g/L and 96.2 mg/g, respectively, when the culture was terminated at 120 h. These values were three times higher than that of corn stover by *T. cutaneum* using the SSLP process (Liu *et al*. 2012), probably because less nitrogenous components existed in waste paper than in corn stover. Moreover, the lipid yield was comparable to that obtained from the separate hydrolysis and lipid production (SHLP) process. Thus, SSLP could simplify the processes while achieving comparable lipid production results. However, lipid productivity was only 1.9 g/L/d, which was significantly lower than that by the SHLP process, probably because of the limited supply of sugars during culture. The coordination of waste paper enzymatic hydrolysis and sugars assimilation is essential for further improving the efficiency of the SSLP process.

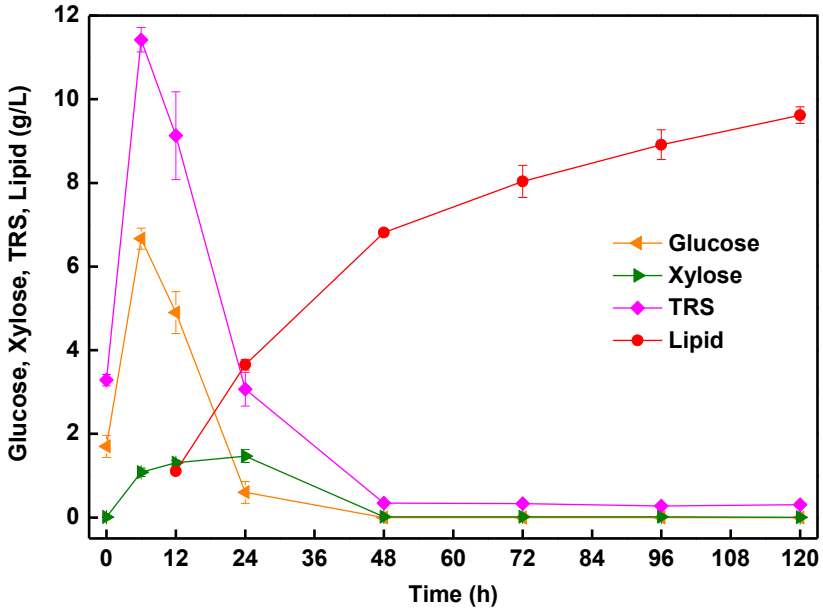

Fig. 5. Time course of lipid production by *C. curvatus* on office paper using the SSLP process

Fatty Acid Compositional Profiles of Lipid Samples

Lipid samples originated from the three waste paper types were transmethylated, and the resulting fatty acid methyl esters were analyzed by GC. The results are shown in Table 3. It was clear that those samples consisted mainly of long-chain fatty acids with 16

and 18 carbon atoms. The four major fatty acids were palmitic acid, stearic acid, oleic acid, and linoleic acid, regardless of the variations in substrates. Specifically, linoleic acid accounted for around 50% of the total fatty acids. It was demonstrated that these lipids could neither serve as substitutes for PUFA nor cocoa butter equivalents (Raltedge 1993). However, the fatty acid compositional profiles were comparable with those of vegetable oils, indicating that microbial lipids produced from waste paper could be excellent precursors for biodiesel production (Liu and Zhao 2007).

Table 3. Fatty Acid Profiles of Lipid Samples of *C. curvatus* on Various Types of Waste Paper

Entry	Substrates[a]	Relative Fatty Acid Contents (%, w/w)					
		Myristic Acid	Palmitic Acid	Palmitoleic Acid	Stearic Acid	Oleic Acid	Linoleic Acid
1	OPEH	0.6 ± 0.1	32.9 ± 2.6	0.6 ± 0.0	7.6 ± 0.9	52.3 ± 1.1	4.7 ± 2.4
2	NPEH	0.7 ± 0.0	32.6 ± 0.5	0.6 ± 0.0	6.8 ± 0.2	51.5 ± 0.6	6.8 ± 0.3
3	CBEH	0.6 ± 0.0	30.2 ± 0.4	0.7 ± 0.0	6.0 ± 0.0	55.2 ± 0.2	5.8 ± 0.2
4[b]	Office Paper	0.7 ± 0.0	30.7 ± 0.5	0.3 ± 0.1	8.7 ± 0.2	49.7 ± 0.2	8.3 ± 0.3

[a]: Office paper, newspaper, and cardboard enzymatic hydrolysates are abbreviated as OPEH, NPEH, and CBEH, respectively; [b]: Lipid samples were derived from the SSLP process

CONCLUSIONS

1. Three common types of waste paper hydrolysates were found to be appealing substrates for lipid production by *C. curvatus*.
2. A nitrogen-rich enzyme cocktail exerted no negative effects on lipid production.
3. The SSLP process could simplify the processes and achieve comparable lipid yield to the SHLP process.
4. Microbial lipids from waste paper could serve as excellent precursors for biodiesel production.

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