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YEAST CULTIVATION FOR SINGLE-CELL PROTEIN PRODUCTION USING THE CARBOHYDRATE HYDROLYSATE OF STEAM-EXPLODED EUCALYPTUS WOOD

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ABSTRACT

This study was aimed at producing single-cell (SCP) protein from the carbohydrate hydrolysate of steam-exploded eucalyptus (*Eucalyptus urophylla*) wood. Two yeast strains including *Saccharomyces cerevisiae* and *Candida utilis* 2.587 were used for batch fermentation. Results showed that the total reducing sugars (TRS), glucose and xylose in hydrolysate had concentrations of 17.52, 10.71, and 4.30 $g \cdot L^{-1}$, respectively. During fermentation, yeast strains of *S. cerevisiae* and *C. utilis* 2.587 used monosaccharides sequentially, and secondary growth occurred. The yeast biomass contained 43.59% crude protein and was rich in all essential amino acids such as lysine, leucine, and valine. Total amino acid reached 401.45 $g \cdot kg^{-1}$, and corresponded with the standard recommended by the Food and Agriculture Organization of the United Nations for amino acids, except sulfur-containing amino acids.

KEYWORDS: Carbohydrate hydrolysate, fermentation, single-cell protein, biomass, amino acids.

INTRODUCTION

The demand for livestock products increases with population growth worldwide, leading to protein deficiencies in feeds. As alternative resources, single-cell protein (SCP) production by microbial fermentation technology is employed (Anupama and Ravindra 2000). Also referred to as microbial protein, SCP is obtained by culturing microbial cells under suitable conditions with

the use of various substrates. Lignocellulosic resources mainly consist of carbohydrates (cellulose and hemicellulose) and lignin. The carbohydrate hydrolysate can be used as a substrate for SCP production.

Cellulose and hemicellulose in woody biomass can be hydrolyzed into several monosaccharides by using acids or enzymes, facilitating microbial fermentation to SCP (Grethlein and Converse 1991, Nigam 2000, Silva et al. 2003, Magalhaes et al. 2018, Sharma et al. 2018, Wu et al. 2018, Lapena et al. 2020, Mishra et al. 2020). It is important for high value-added utilization of lignocellulosic resources and addressing protein deficiency. Enzymatic hydrolysis is widely used because of its many advantages, such as mild conditions and manageable reactions. Moreover, the product solution can be directly used for fermentation.

Cellulase needs to be absorbed and has to come in contact with the carbohydrate substrate before hydrolysis. The accessibility of cellulase to carbohydrates is one of the key factors influencing the rate of hydrolysis. However, the enzymatic hydrolysis rate is lower than 20% because of the structural complexity of lignocellulose resources (Laser et al. 2002). The raw materials required pretreatment before hydrolysis to separate the cellulose and hemicellulose from lignin.

Steam explosion pretreatment can increase the enzymatic hydrolysis rate of woody biomass and is considered the most effective pretreatment method for hardwoods (Grethlein and Converse 1991, Alvira et al. 2010, Chiaramonti et al. 2012, Singh et al. 2015). The addition of sulfur dioxide during a steam explosion or the impregnation of sulfuric acid on exploded substrates before an explosion can improve enzymatic saccharification, and is effective for softwoods (Mackie et al. 1985, Clark and Mackie 1987). During steam explosion, the raw material is compressed under high-pressure steam and then fractured by explosive decompression in which acetyl groups on hemicellulose are broken down into acetic acid. Physical and chemical processes induce the relaxation of wood fibers and rupture of pit membranes (Zhang and Zhao 2008) or lead to fracture in bordered pit pairs between tracheids (Zhang et al. 2006), improving the chemical reaction performance and accessibility of cellulase (Laser et al. 2002). Simultaneously, cellulose solubility and glucose yield increase after steam explosion, which is attributed to the hydrolysis of hemicellulose to monosaccharides or oligosaccharides, and partial lignin is dissolved during this process (Grethlein and Converse 1991, Jacquet et al. 2012).

Microorganisms that can be used as SCP include yeasts (Ghaly et al. 2003, Gao et al. 2007, Zhao et al. 2010, Gervasi et al. 2018, Mishra et al. 2020), molds (Silva et al. 2003, Rao et al. 2010, Pereira et al. 2016), bacteria (Kornochalert et al. 2014, Huelsen et al. 2018, Saejung and Chewapat 2020), and microalgae (Huelsen et al. 2018, Sharma et al. 2018). Owing to their large size, ease of recovery, high lysine content, and wide range of available carbon sources, yeast cells are preferable to other microorganisms (Tab. 1). *Saccharomyces cerevisiae* has high protein content but can only use hexose such as glucose. *Candida* spp. can use pentose, acetic acid, and uronic acid, in addition to hexose, as carbon sources (Nigam 2000, Rajoka et al. 2004, Wu et al. 2018, Razzaq et al. 2020).

Tab. 1: Yeast strains and substrates for single-cell protein production.

Yeast strains	Substrates	References
Hansenula sp.	Molasses stillage	Shojaosadati et al. 1999
Candida langeronii	Sugarcane bagasse hemicellulosic hydrolysate	Nigam 2000
Kluyveromyces fragilis	Whey	Deepen et al. 2002
Kluyveromyces fragilis	Cheese whey	Ghaly et al. 2003
Paecilomyces variotii	Eucalyptus hemicellulosic hydrolyzate	Silva et al. 2003
Cryptococcus aureus G7a	*Jerusalem artichoke* extract	Gao et al. 2007
Cryptococcus aureus G7a	Yacon extract	Zhao et al. 2010
Hanseniaspora uvarum KKUY-0084, *Zygosaccharomyces rouxii* KKUY-0157	wasted date fruits	Hashem et al. 2014
Kluyveromyces marxianus and *Candida krusei* (mixed culture)	Whey	Yadav et al. 2014
Saccharomyces cerevisiae PTCC5269	Mixed culture medium	Hezarjaribi et al. 2016
Candida intermedia FL023	Lignocellulosic hydrolysates	Wu et al. 2018
Candida tropicalis	Sugarcane bagasse hemicellulosic hydrolysate	Magalhaes et al. 2018
Saccharomyces cerevisiae	Food and agricultural waste	Gervasi et al. 2018
Candida utilis	Brown seaweed and spruce wood	Sharma et al. 2018
Cyberlindnera sp.	Banana peel hydrolysate	Jiru et al. 2018
Wickerhamomyces anomalus, Cyberlindnera jadinii, and *Blastobotrys adeninivorans*	Spruce sugars and poultry hydrolysate	Lapena et al. 2020
Saccharomyces cerevisiae	Wheat straw	Mishra et al. 2020
Saccharomyces cerevisiae	Date palm waste	Putra et al. 2020

In the present study, enzymatic hydrolysate is used to produce SCP by mixed cultivation of *S. cerevisiae* and *Candida utilis* 2.587 from steam-exploded *Eucalyptus urophylla* wood, and the contents of crude protein, ash, and amino acids in SCP product are determined.

MATERIALS AND METHODS

Materials and pretreatment

Three year-old eucalyptus (*E. urophylla*) woods were provided by Eucalyptus Research Center, China State Forestry Administration. The woods were cut into sections 30 cm in length, and the peeled sections were chipped into slices measuring 2–4 cm in length, 1–3 cm in width, and 3 mm in thickness. The chips were immersed in 0.2% sulfuric acid for 24 h without pressure before explosion, ratio 1:6. The raw chips were then soaked in water for 24 h. Steam explosion was then conducted with 2.2 MPa (temperature 218.5 ºC) for 4 min and then released. The exploded materials were dried at 60ºC for hydrolysis. The chemical compositions in steam-exploded wood and the control wood are presented in Tab. 2.

Tab. 2: Chemical compositions in eucalyptus wood.

Components	Cellulose	Pentosan	Lignin	Extract
Control sample (%)	43.32	29.86	27.65	4.35
Steam-exploded (%)	42.82	9.46	18.31	33.91

*Values are means of duplicate.

Determination of chemical compositions

The materials were crushed into wood power of 40–60 mesh for the determination of chemical components. The content of cellulose was determined by nitric acid ethanol method. The materials were treated by nitric acid ethanol mixture (ratio 1:4) 4 times in boiling water bath, 1 h at a time. The remaining cellulose was dried at 105ºC. The content of cellulose was calculated by the "dry weight of cellulose (g)/dry weight of materials (g)". Dibromide method was used to determine the content of pentosan. The materials were boiled with 12% hydrochloric acid. The content of furfural produced in this process was used to calculate the content of pentosan. The extract was the content of substances extracted from the mixture of benzene and ethanol (ratio 2:1) for 6 h. The content of lignin was determined by Klason method, widely used internationally. The materials extracted from benzene ethanol mixture (ratio 2:1) were hydrolyzed by 72% sulfuric acid for 2 h. The concentration of sulfuric acid was then reduced to 3% and hydrolysis continued for 4 h. The remaining lignin was dried at 105ºC. The content of lignin was calculated using the ratio "dry weight of lignin (g)/dry weight of materials (g)".

Preparation of the hydrolysate

The dried, smashed, and grained exploded materials (less than 60 mesh) were hydrolyzed by cellulase in shaker bottles. The cellulase was solid power, produced by Beijing AOBOX Technology Co., Ltd. The enzyme activity was 1:1000 (1000 μ mol of glucose was produced by hydrolyzing cellulose using 1 g cellulase within 1 h). The substrate concentration and cellulase dosage were 4% and 15 $FPIU \cdot g^{-1}$ substrate, respectively. The pH of 4.8 was adjusted with 0.05 $mol \cdot L^{-1}$ citric acid–sodium citrate buffer. The shaker bottle was sealed with two plastic films and placed on the table for hydrolysis at 50ºC and rotational speed of 150 $r \cdot min^{-1}$ until the 48 h time point was reached. Subsequently, the solid residue was separated by filtration, and the hydrolysate was collected for yeast cell production. Total reducing sugars (TRS), glucose, xylose, disaccharide, and acetic acid of the resulting hydrolysate were determined.

Hydrolysate composition analysis

The concentration of TRS was determined using 3,5-dinitrosalicylic acid colorimetry (the DNS method). High-performance liquid chromatography (HPLC) (Sugar-park 1 column with a differential detector) was conducted to determine the concentrations of glucose, xylose, arabinose, and disaccharide at 70ºC, with pure water as the mobile phase (flow rate 0.6 $mL \cdot min^{-1}$). Acetic acid was analyzed by HPLC (20 μL of the sample injected) using a C18 column, with pure water as the eluent, under the following conditions: flow rate of the eluent, 0.8 $mL \cdot min^{-1}$; temperature, normal. An ultraviolet detector was used.

Sources of yeast strains

S. cerevisiae was provided by Prof. Bolin Zhang, College of Biology, Beijing Forestry University. *C. utilis* 2.587 was purchased from Strain Preservation and Management Center, Institute of Microbiology, Chinese Academy of Science.

Yeast strains cultivation

Test tube slant strains: Yeast strains preserved by freeze-drying were grown on potato agar slant for 72 h at 28ºC and then stored at 4ºC with subculturing every 2 months. Flask liquid strains: Yeast strains from a stock culture on a potato agar slant were transferred into 250 mL flasks containing 60 mL of the potato glucose medium previously sterilized at 121ºC for 20 min. The flasks were incubated for 18–20 h at 31ºC on a rotary shaker with a speed of 150 $r \cdot min^{-1}$. A 10% inoculum of active cells in all experiments was used.

Single-cell protein production

Yeast biomass production was performed in a 5 L glass fermentor (Eastbio GBCS-5 B-1, Zhenjiang East Biotech Equipment and Technology Co., Ltd.) by batching and adding the following to the hydrolysate: 4.0 $g \cdot L^{-1}$ of $(NH_4)_2HPO_4$, 0.5 $g \cdot L^{-1}$ of yeast extract, 1.0 $g \cdot L^{-1}$ of KH_2PO_4, and 0.3 $g \cdot L^{-1}$ of $MgSO_4 \cdot 7H_2O$. The fermentor was automatically stirred, and the working volume was 2.5 L. The fermentation system was sterilized under the following conditions: temperature, 121°C for 20 min; initial pH, 4.5; stirring speed, 200 $r \cdot min^{-1}$; air flow, 1.0 v/v/m. The system was then cooled down to 31°C. The mixed inoculation concentration reached 10^7 $cells \cdot L^{-1}$ for 10% of the inoculation amount. The *S. cerevisiae* to *C. utilis* 2.587 ratio was 1: 3. Subsequently, 5.0 mL of aliquots were taken every 3 h to determine the pH values, concentrations of the cell biomass, TRS, glucose, xylose, and acetic acid. The assay was performed in triplicates.

Yeast biomass samples were centrifuged at 3000 $r \cdot min^{-1}$ for 30 min and then washed twice with distilled water. The biomass was determined as dry weight at 105ºC until constant weight.

Calculation of kinetic parameters

Yeast biomass productivity (Y_{BP}, $g \cdot L^{-1} \cdot h^{-1}$) was calculated using the ratio "maximum biomass concentration ($g \cdot L^{-1}$)/time of cultivation (h)". Biomass yield (Y_B) and Y_P were determined by the "maximum biomass or protein divided by the mass of reducing sugar consumed", expressed in $g \cdot g^{-1}$ (Magalhaes et al. 2018). The rate of substrate consumption (Q_s) was calculated by the "reducing sugar consumed in the concentration ($g \cdot L^{-1}$) divided by the time of cultivation (h)". The specific rate of cell growth (μ_x, h^{-1}) was determined from the relationship $\mu_x t = \ln X_t/X$. The specific rate of substrate consumption (μ_y, $g_s/g_x \cdot h$) was calculateded from the relation $\mu_y = ds/dt/X_t$, (Zhang 2010).

Nutritional analysis of the SCP product

The crude protein content was determined using the Kjeldahl method, expressed as total nitrogen multiplied by 6.25. Amino acid analysis was conducted on an automatic amino acid analyzer after hydrolysis of the sample in 6N HCl for 20 h.

The ash content was measured by burning the SCP to constant weight in a muffle furnace at 550ºC. The iron, zinc, and manganese contents in biomass were determined by atomic absorption spectrometry (AAS ZEEnit 700P). The ash was dissolved with 6N HCl, the solution was then introduced into the air-acetylene flame of the atomic absorption spectrophotometer. Absorbance values of the iron, zinc, and manganese at the wavelength of 248.3 nm, 213.8 nm and 279.5 nm

were measured respectively, and compared with the absorbance value of the same element calibrating solution for quantification. The content of calcium was evaluated using the potassium permanganate ($KMnO_4$) method. Dissolving the ash with 6N HCl, 0.1% ammonium oxalate was then added to the solution, calcium ions formed calcium oxalate precipitation, which was filtered and washed and then dissolved in 10% sulfuric acid. The content of calcium was determined indirectly by titrating oxalic acid (1:1 binding with calcium ion) in the solution with the potassium permanganate standard solution.

The spectrophotometry was used to evaluate the content of phosphorus. The product was dissolved with 6N HCl, phosphorus formed a yellow complex compound after chromogenic agent of ammonium vanadate-molybdate was added in the solution. The complex content was determined by colorimetry at the wavelength of 400 nm.

RESULTS AND DISCUSSION

Compositions in hydrolysate

The concentration of TRS determined using the DNS method was 17.52 $g \cdot L^{-1}$. The glucose, xylose, arabinose, disaccharide, and acetic acid concentrations in the hydrolysate, as determined by HPLC, are listed in Tab. 3. Glucose was identified as the most abundant sugar in hydrolysate, higher than xylose, and the concentrations were 10.71 and 4.30 $g \cdot L^{-1}$, respectively. Glucose originated from the hydrolysis of cellulose and small portions of hemicellulose, xylose and arabinose were hydrolysates of the hemicellulose. In addition, a small amount of arabinose, acetic acid, and incompletely hydrolyzed disaccharide were present in the hydrolysate.

Tab. 3: Composition analysis of the hydrolysate of steam-exploded eucalyptus wood.

Components	TRS[a]	Glucose[b]	Xylose[b]	Arabinose[b]	Disaccharide[b]	Acetic acid[b]
Concentration ($g \cdot L^{-1}$)	17.52	10.71	4.30	0.11	0.35	0.54
Proportion of TRS[c] (%)		61.10	24.54	0.63	1.99	

[a] The value of TRS concentration was assayed using the DNS method.
[b] The concentration values of glucose, xylose, arabinose, disaccharide, and acetic acid were assayed by HPLC.
[c] The proportion of TRS of every sugar was the value of its concentration divided by TRS concentration.

Grethlein and Converse (1991) reported that steam explosion pretreatment increased the pore volume of the wood by removing the hemicellulose that increased the surface area available to the cellulase enzyme, leading to an increase in the rate of hydrolysis. The current study found that the percentage of pentosane (comprising the majority of hemicellulose) in eucalyptus wood decreased by 68.3% (from 29.86% to 9.46%) after steam explosion, and a saccharification rate of 82.43% ± 0.017% under optimal conditions coincide with the result of Grethlein and Converse (1991). Moreover, the high saccharification rate was close to that of the steam-exploded *Pinus radiata*, as reported by Clack et al. (1987).

Glucose, xylose, arabinose, and disaccharide comprised 61.17%, 24.54%, 0.69%, and 2.25% of total reducing sugars presented in the hydrolysate, respectively. Other monosaccharides in small amounts, such as mannose and galactose, might have existed in the hydrolysate but were not detected owing to low contents. The content of acetic acid in

the hydrolysate was 0.54 $g \cdot L^{-1}$, as determined by HPLC. The acetic acid originated from hydrolysis of acetyl groups on hemicellulose, which was reported by Pessoa et al. (1996) and Nigam (2000) as an available carbon source for some yeasts during SCP production.

Fermentation process assay

Biomass, TRS, cellulose, xylose, acetic acid, and pH values are presented in Fig. 1. As shown in the figure, glucose is used immediately and fully consumed 9 h after inoculation, and its concentration most rapidly decreases within the 6-9 h range. Xylose and acetic acid were used immediately before glucose depletion and completely consumed at the 15 h and 18 h time points, respectively. The pH values decreased slowly during fermentation for 12 h but rapidly rose in the few subsequent hours (Fig. 1). Changes in acetic acid concentration and pH with fermentation indicated that acetic acid was one of the carbon sources for *S. cerevisiae* and *C. utilis* 2.587, which finding was consistent with the reports by Pessoa et al. (1996) and Nigam (2000).

According to the literature (Pessoa et al. 1996, Nigam 2000), when yeasts were grown in a medium containing multiple carbon sources, rapid glucose consumption was observed, followed by xylose and acetic acid consumption. The differential utilization of the monosaccharides was attributable to metabolic differences, mutual competition for a transporter, and capture of induction enzymes.

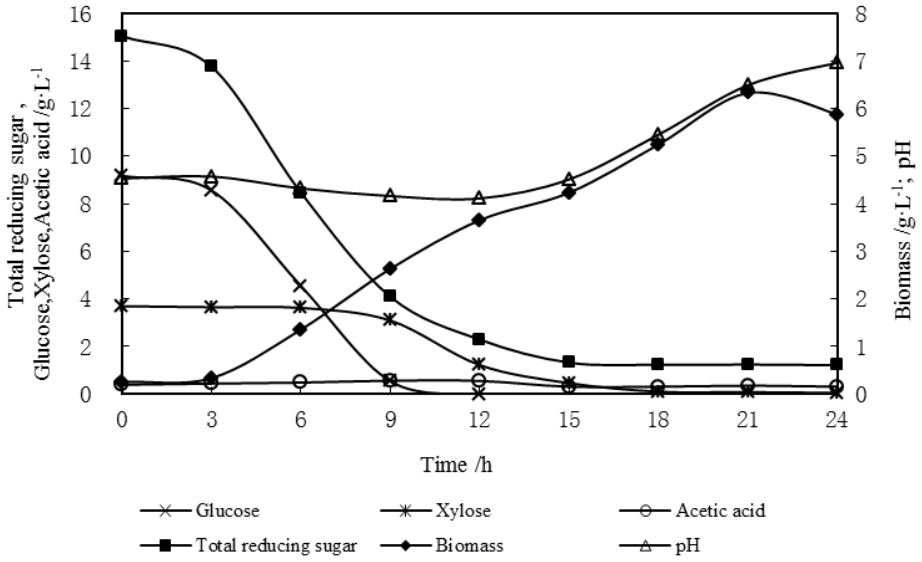

Fig. 1: Biomass, total reducing sugar, glucose, xylose, arabinose, acetic acid, and pH along fermentation assay of carbohydrate hydrolysate.

In addition to sequential substrate utilization, diauxic growth occurred, as at the 12 h and 21 h time points of the fermentation assays. Maximum biomass with a concentration of 6.72 ± 0.13 $g \cdot L^{-1}$ was produced after cultivation for 21 h, and Y_{BP} was 0.320 ± 0.006 $g \cdot L^{-1} \cdot h^{-1}$. The percentage of protein in yeast biomass was 43.59%, reflecting a total of 2.93 $g \cdot L^{-1}$ of yeast SCP. Thus, Y_B and Y_P were 0.415 ± 0.008 $g \cdot g^{-1}$ and 0.181 ± 0.005 $g \cdot g^{-1}$, respectively. The kinetic parameters are listed in Tab. 4. The specific rate of yeast growth (μ_x) and specific rate of

substrate consumption (μ_s) were 0.165 ± 0.004 h^{-1} and 0.115 ± 0.016 $g \cdot g^{-1} \cdot h^{-1}$, respectively.

Tab. 4: Kinetic parameters of Saccharomyces cerevisiae and Candida utilis 2.587 fermented on hydrolysate of steam-exploded eucalyptus wood.

Parameters	Values
X_{max} (Maximum yeast biomass concentration, $g \cdot L^{-1}$)	6.72 ± 0.130
Y_{BP} (Yeast biomass productivity, $g \cdot L^{-1} \cdot h^{-1}$)	0.320 ± 0.006
μ_x (Specific rate of yeast growth, h^{-1})	0.165 ± 0.004
Y_B (Biomass yield coefficient, $g \cdot g^{-1}$)	0.415 ± 0.008
Y_P (Protein yield coefficient, $g \cdot g^{-1}$)	0.181 ± 0.005
S_{min} (Remaining substrate, $g \cdot L^{-1}$)	1.33 ± 0.040
Q_s (Rate of substrate consumption, $g \cdot L^{-1} \cdot h^{-1}$)	0.771 ± 0.002
μ_s (Specific rate of substrate consumption, $g \cdot g^{-1} \cdot h^{-1}$)	0.115 ± 0.016

*Values are means of triplicate analyses ± standard deviation.

The maximum biomass production was lower than that obtained by Sharma et al. (2018) for *C. utilis* production using enzymatically hydrolyzed spruce wood as a carbon source and enzymatically hydrolyzed brown seaweed as a nitrogen source, given that the latter had a high initial glucose concentration. The Y_{BP} obtained was greater than that observed for *C. utilis* by Sharma et al. (2018) and *Candida tropicalis* by Magalhaes et al. (2018) and Pessoa et al. (1996) when cultured in sugarcane bagasse hemicellulose hydrolysate.

Notably, the Y_B and productivity values for SCP production are difficult to compare because of their strong dependence on culture medium composition, type of yeast strains, and environmental conditions, including the incubation temperature, medium pH, dissolved oxygen, aeration rate, and fermentation mode. However, the conversion efficiency of the 5 L experiment may still be compared with the results obtained for *Candida langeronii* production by Nigam (2000). The Y_B and Y_P reached at 1.0 v/v/m aeration were 0.36 $g \cdot g^{-1}$ and 0.17 $g \cdot g^{-1}$, lower than that in this study (0.415 and 0.181 $g \cdot g^{-1}$, respectively). The Y_B was in the range of yields reported for the aerobic growth of yeasts, typically in the range of 0.4-0.5 g biomass per g of sugar (Ugalde and Castrillo 2002).

Minerals, crude protein, and amino acid composition of SCP

Mineral elements, particularly trace elements, are indispensable nutrients for animal life activities. Mineral element deficiencies lead to metabolic disorders or abnormal growth and reproduction. Manganese, the important component of arginine kinase and pyruvate carboxylase, can promote growth, strengthen immune response, and improve animal reproductive performance. The iron, zinc, and manganese contents in biomass produced in the current study were 0.216 $g \cdot kg^{-1}$, 0.353 $g \cdot kg^{-1}$, and 16.7 $mg \cdot kg^{-1}$, respectively, which exceeded those reported by Deepen (2002) and Sharma et al. (2018) (Tab. 4). The results indicated that the minerals also contributed to the overall nutritional value of this biomass SCP product.

The yeast biomass produced from *S. cerevisiae* and *C. utilis* 2.587 was rich in protein and minerals (Tab. 5). The percentage of crude protein in the biomass was 43.59%, which was greater than that produced from *Kluyveromyces fragilis* using whey (Deepen et al. 2002) and that from *Candida utilis* using brown seaweed and spruce wood hydrolysate (Sharma et al.

2018). The ash content in the biomass produced was 7.68%, which was close to that (7.9% ± 0.4%) from *S. cerevisiae* using sugar beet bagasse, as observed by Razzaq et al. (2020), but lower than that (16%) from *K. fragilis*. The high ash content could be attributed to the higher mineral content in whey permeate (Deepen et al. 2002). According to the literature (Shojaosadati et al. 1999, Deepen et al. 2002), the contents of crude protein and ash in SCP were usually in the range of 40 – 50% and 7.6 – 10.4%, respectively, which agreed with the results of this study.

Tab. 5: Contents of dry matter, crude protein, ash, and mineral elements in SCP (Saccharomyces cerevisiae and Candida utilis 2.587) fermented on the hydrolysate of steam-exploded eucalyptus wood.

	***Saccharomyces cerevisiae* and *Candida. utilis* 2.587 ***	***Kluyveromyces fragilis*** [a]	***Candida utilis*** [b]
Dry matter (%)	92.82	100	96.4
Crude protein (%)	43.59	37.0	33.3
Ash (%)	7.68	16.0	9.8
Mineral elements			
Ca ($g \cdot kg^{-1}$)	1.5	—	2.4
P ($g \cdot kg^{-1}$)	0.9	—	3.7
Fe ($g \cdot kg^{-1}$)	0.216	0.04	0.2
Zn ($g \cdot kg^{-1}$)	0.353	0.18	0.1
Mn ($mg \cdot kg^{-1}$)	16.7	10	7.7

*Values are means of duplicate.
[a] Contents of dry matter, crude protein, ash and mineral elements were taken from Deepen et al. (2002).
[b] Contents of dry matter, crude protein, ash and mineral elements were taken from Sharma et al. (2018).

The amino acid content in the dried biomass of *S. cerevisiae* and *C. utilis* 2.587 and the amino acid compositions of protein are listed in Tab. 6. The biomass contained a broad spectrum of amino acids essential for animal feed, such as lysine (31.69 $g \cdot kg^{-1}$), leucine (29.38 $g \cdot kg^{-1}$), valine (21.80 $g \cdot kg^{-1}$), and threonine (21.49 $g \cdot kg^{-1}$). High contents of nonessential amino acids such as glutamic acid (60.90 $g \cdot kg^{-1}$), aspartic acid (42.89 $g \cdot kg^{-1}$), and arginine (26.20 $g \cdot kg^{-1}$) were also found. The amino acid composition profile is consistent with the findings for *C. utilis* produced from brown seaweed and spruce wood hydrolysate (Sharma et al. 2018) and *Wickerhamomyces anomalus* produced from spruce sugars and poultry hydrolysate (Lapena et al. 2020). The total essential amino acid and total amino acid contents in dried biomass were 156.53 and 401.45 $g \cdot kg^{-1}$, that is considerably higher than that for the strains of previously mentioned yeasts.

Lysine, leucine, valine, and threonine contents in protein were 7.27%, 6.74%, 5.00%, and 4.93%, respectively, which far exceeded the standards set by the Food and Agriculture Organization (FAO) (Pessoa et al. 1996, Nigam 2000) (Tab. 6). However, sulfur-containing amino acids such as methionine (Met) and cysteine (Cys) were lower than the FAO reference pattern. Sulfur-containing amino acids commonly occur at low levels for almost all yeast strains, and their use as the sole protein source in feed is normally restricted (Lapena et al. 2020). The high lysine and threonine contents indicated that the biomass SCP could be used as a feed supplement, particularly in diets based on cereals (Pessoa et al. 1996, Nigam 2000).

The literature reviews (Tab. 1) showed the presence of 16 different yeast species and

substrates used to produce SCP worldwide. In the current study, we used two yeast species, *S. cerevisiae* and *C. utilis* 2.587, as fermentation seeds to produce SCP. The high percentage (43.59%) of total protein and reasonable amino acid composition in the hydrolysate of wood as substrate indicates that the strains can be potentially used for SCP production.

Tab. 6: Contents of amino acids in single-cell protein (Saccharomyces cerevisiae and Candida utilis 2.587) and amino acid components of proteins from different sources.

Amino acids[a]	***Saccharomyces cerevisiae* and *Candida utilis* 2.587***		***Candida utilis***[b]	***Wickerhamomyces anomalus***[c]	**FAO**[d]
	In dry matter ($g \cdot kg^{-1}$)	Percentage of protein (%)	In dry matter ($g \cdot kg^{-1}$)	In dry matter ($g \cdot kg^{-1}$)	Percentage of protein (%)
EAAs[a]					
Methionine	6.80	1.56	4.5	3.27	2.2
Threonine	21.49	4.93	20.6	18.91	2.8
Valine	21.80	5.00	20.9	19.52	4.2
Isoleucine	20.09	4.61	17.4	18.41	4.2
Leucine	29.38	6.74	25.8	28.96	4.2
Lysine	31.69	7.27	22.8	30.61	4.2
Tryptophan	3.88	0.89	5.3	5.20	1.4
Histidine	21.40	4.91	6.3	11.19	—
Total EAAs	156.53	38.94	123.5	136.07	—
NEAAs[a]					
Cystine/Cysteine	5.80	1.33	2.8	3.27	2.0
Phenylalanine	17.22	3.95	15.0	16.33	2.8
Aspartic acid	42.89	9.84	34.1	40.51	—
Serine	19.31	4.43	18.7	20.19	—
Glutamic acid	60.90	13.97	43.3	76.50	—
Proline	15.39	3.53	14.4	17.67	—
Glycine	19.09	4.38	15.9	22.18	—
Tyrosine	13.12	3.01	10.6	11.20	—
Arginine	26.20	6.01	17.3	25.71	—
Alanine	25.20	5.78	21.4	24.11	—
Total NEAAs	245.12	56.23	193.4	257.67	—
Total AAs	401.45	95.17	316.9	395.8	—

*****Values are means of duplicate.
[a] EAAs-Essential amino acids; NEAAs -Non-essential amino acids.
[b] Contents of amino acids in *Candida utilis* were taken from Sharma et al. (2018).
[c] Contents of amino acids in *Wickerhamomyces anomalus* were taken from Lapena et al. (2020).
[d] FAO-Food and Agriculture Organization of the United Nations.

CONCLUSIONS

The present results demonstrate that the steam-exploded *E. urophylla* wood can be easily hydrolyzed (82.43% saccharification rate) using an enzyme, and the resulting sugar solution can act as a cultivation medium for *S. cerevisive* and *C. utilis* 2.587 to produce SCP with a Y_B of 0.415 ± 0.008 $g \cdot g^{-1}$. The SCP product contains 43.59% of total protein and 18 amino acids. The amino acid profile meets the requirements recommended by FAO, except for sulfur-containing amino acids. Subsequent efforts can be employed to increase the Y_B and conduct animal feeding.

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