Using Black Liquor from the Soda Pulping Process for Protein Production by *Candida utilis*

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Black liquor produced from pulping with a high value of chemical oxygen demand (COD) and biological oxygen demand (BOD) is highly harmful if discharged into the environment directly. One possible way to decrease the damage to the soil and water is to reuse the organic substances contained in it to cultivate yeasts for producing single-cell proteins (SCP) while reducing the COD. With this in mind, this study is devoted to treatment technology and the comprehensive utilization of black liquor. Various parameters were evaluated, and the COD of black liquor, initial pH, and nitrogen sources had significant influences on biomass and crude protein production. The research resulted in the maximum values of COD removal rate and crude protein production with 78.78 ±3.21% and 1.18 ±0.02 g/L achieved, respectively, under the optimized condition of black liquor concentration (60%), the recruitment of urea (0.5 g/L), initial pH (6.0), temperature (34 °C), shaking speed (180 rpm), and incubation time (36 h). Furthermore, this study provided a potential viable treatment of black liquor and revealed a feasible way to make full use of black liquor for the economical production of SCP.

Keywords: Pulping; Black liquor; Yeast; Single-cell proteins (SCP); Candida utilis

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INTRODUCTION

The overall annual world production of black liquor was approximately 500 million tons in 2005 (Dafinov *et al.* 2005). Black liquor produced from the soda pulping process is composed of a great deal of residual soda, lignin derivatives, low molecular weight organics, and some poisonous substances (such as anthraquinone and sulfide). Because of the large quantity and toxicity problems, black liquor is an important source of environmental pollution, which causes serious damage to the soil and water if discharged without any proper treatment or disposal.

In the waste water treatment field, organic matter (substrate) has traditionally been measured as the total organic matter subject to chemical oxygen demand (COD) or biological oxygen demand (BOD), and they must be low enough to meet discharge limitations. Hence, COD or BOD, is an important parameter that has to be considered during the treatment of black liquor. The COD of black liquor varies from 10,000 to 120,000 mg/L, and the pH ranges from 10 to 13. This situation makes black liquor one of the most difficult materials to handle in wastewater treatment processes (Huang *et al.* 2006).

So far, the mainstream method to treat black liquor is causticizing combustion (Ai *et al.* 2003). However, this method is not very effective and causes secondary pollution in the meantime, due to high investment and high production of lime sludge in the process of alkali recovery.

Wastes and industrial by-products could be valuable materials as alternative resources for building, construction, and other applications (van Beers *et al*. 2009). In addition, current legislation and increased taxes have resulted in research on non-conventional methods for management or new uses (Monte *et al.* 2009). Following this perspective, the productive use of waste material represents a way of solving some problems of waste management (Davis and Cornweel 1998). Much of industrial waste water and residue—for instance, the residues of spirit and bioethanol production, ram horn hydrolysate, and rice polishing (Kurbanoglu and Algur 2002; Rajokaa *et al.* 2006; Silva *et al.* 2011)—has been treated with the fermentation method for production of single-cell proteins (SCP). The basic principle of this method is that the industrial waste water and residue contain certain organic carbon and nitrogen sources, which can be used to cultivate yeasts.

A few high quality yeast species, such as *Candida* and *Saccharomyces* spp., have been employed as a producer of microbial protein (referred to as SCP) to convert agro-industrial wastes, *e.g*., effluents from paper and olive mills, into a valuable amino acid supplement for animal feeds and plant nutrients over the past years (Gharsallah 1993; Ejiofor *et al.* 1996; Nigam 1998). The process is involved in both enhancing wastewater purification and increasing resource utilization (Zheng *et al.* 2005). It is generally thought to be an attractive way to deal with wastes from an economic point of view. On the other hand, *Candida utilis* is classified as one of the most promising microorganisms for their protein content, which can account for up to 50% of their dry weight (Ziino *et al.* 1999). Moreover, they can also supply feed with vitamins, minerals, and other nutrients (Raa 1990). *Candida utilis* has a relatively high concentration of essential amino acids in its proteins (Lawford *et al.* 1979) and possesses the ability to metabolize a wide range of saccharides (Shay and Wegner 1985). However, to date, there has been no publication that reports the use of *Candida utilis* in black liquor from the soda pulping process for the production of SCP.

Herein, we describe a novel technology for valuable SCP production by *Candida utilis* using black liquor produced from the soda pulping process, while investigating the effects of culture conditions on the production of microbial biomass, and examining the nutritional quality of the SCP. Therefore, this work provides a new finding for the treatment of black liquor.

EXPERIMENTAL

Materials

Microorganisms, *Candida utilis* 1314, were purchased from the China Center of Industrial Culture Collection, Beijing, China. Black liquor from a soda papermaking process (low alkaline pulping process) was obtained from the Beijing Insight Biotech New Material Technology Co., Ltd., Beijing, China. The bamboo, wheat straw, cornstalk, and wood are used for the process (Yin *et al.* 2012).

Methods

Pretreatment of microorganisms

A loopful of the isolates involved was inoculated into 100 mL of a seed culture medium containing different concentrations of glucose 15.0 g/L, yeasts extract 7.5 g/L, peptone 7.5 g/L, KH_2PO_4 0.5 g/L, and $MgSO_4$ 0.25 g/L. The pH of the seed culture was adjusted to 5.0, and incubation was carried out at 34 °C on an orbital shaker at 160 revolutions per minute (rpm) for 24 h. 10% of the seed culture was inoculated into black liquor, with changeable conditions when necessary.

Analysis of black liquor

Black liquor was cooled, and subsequently frozen, until required for experiments. When operating experiments, a proper amount of black liquor was defrosted under room temperature for about 4 h, and then the pH value was adjusted to 6 by phosphoric acid and 25.3% of the lignin was removed through the process of precipitation after they were centrifuged at 5000 rpm for 10 min (Yin *et al.* 2012). Appropriate nutrient sources, such as carbon and nitrogen sources, were added to the resulting mixtures according to the corresponding experimental conditions. Finally, they were diluted with water until their concentration reached the indicated values, and 100 mL of them was injected to a 250 mL flask used as a fermentation medium after being autoclaved at 121 °C for 15 min.

Prior to the experiments, the component of black liquor was determined according to standard procedures. The parameters analyzed were reducing sugar (Nelson 1944), Oligosaccharide (Van Soest and Wine 1968), crude fiber (Van der Kamer and Van Ginkel 1952), total nitrogen (APHA 1992), phosphate (CMGMR 1993), pH, chemical oxygen demand (COD; APHA 1992), and soluble lignin (CSBTS 1995). The concentration of potassium was measured using a HI96750 instrument (Hanna, Italy).

Analysis of culture samples

Culture samples (25 mL) were centrifuged at 5000 rpm for 10 min. The supernatant fluids were used to analyze COD while the precipitates were washed twice with distilled water and then dried under a vacuum at 60 °C. Afterwards, the precipitates were used to analyze for nitrogen content, crude proteins, and amino acids. The nitrogen content and crude protein were estimated according to the Kjeldahl method (APHA 1992). The amino acid profile was determined by injecting the hydrolyzate of SCP into an amino acid analyzer (Evans Electro-Selenium Limited, UK). The dry mass of yeast biomass was measured after drying to constant weight under vacuum at 60 °C. The COD was determined using the potassium dichromate oxidation method with a HACH DR1010 COD-Analyzer.

Microscopic observation

Scanning electron microscopy (SEM) (Hitachi S-4800, Japan) was used to observe the morphologies of yeast cells. The samples were first ground to powder by a mortar, and sequentially dehydrated with 30, 50, 70, 85, and 95% ethanol, one time each for 20 min, and thereafter 100% ethanol, twice for 20 min. The dehydrated samples were mounted on a sample plate with an electrically conducting paster and sputter-coated with gold (Yu *et al.* 1990). At last, they were viewed with a scanning electron microscope.

Experimental design

Selected variables (concentration of black liquor, carbon sources, nitrogen sources, initial pH, shaking speed, temperature, incubation time, *etc.*) concerning physicochemical

parameters were investigated with single factor tests to evaluate how they affected the biomass and crude protein production, which provided fundamental support for the Plackett and Burman (PB) (1946) design and orthogonal design. In the following, the PB design and statistical analyses were carried out using software of Minitab 16.0. The model proposed by Plackett and Burman was used to find the significant variables associated with the biomass and crude protein production, with another three inert variables added to the experimental design for the calculation of standard errors. By means of this assessment method, a significant result was achieved through optimization of the cultivation medium ingredients. The results revealed that COD of black liquid, pH, and nitrogen sources have more influences on the production, and productivity of yeast biomass. Considering the cost, urea was chosen as the nitrogen source. The orthogonal test of four factors (concentration of black liquor, initial pH, urea addition, including vacancy) at three different levels was conducted based on a single-factor experiment. Finally, the amino acid composition and content were analyzed under optimized conditions.

RESULTS AND DISCUSSION

Physicochemical and Ingredient Analysis of Black Liquor

Physicochemical analysis of black liquor samples (Table 1) revealed the presence of nutrients (such as carbon sources, nitrogen sources, phosphorus sources, and potassium sources) for cultivating *Candida utilis* to produce SCP.

In preliminary experiments, it was found that the amount of potassium was enough for the yeast's growth, and the phosphate in the waste would meet the demand as well. Notably, the amount of carbon sources was abundant, but it seemed to be short of nitrogen sources comparatively, and an appropriate nitrogen source addition would enhance the production of biomass.

The importance of nitrogen sources has previously been demonstrated when using wastes to produce SCP (Nigam 1998; Zhang *et al*. 2005; Schultz *et al.* 2006), because they would supply balanced nutrients for yeast growth and raise the removal rate of COD, indicating that it would be necessary to add certain nitrogen sources to the culture medium on account of the lack of nitrogen in black liquor. Urea as a cheap nitrogen source may be the best choice.

Table 1. Components of Black Liquid Added to the Culture Medium

Parameter	Value
Reducing sugar (g/L)	3.97 ±0.06
Oligosaccharide (g/L)	0.12 ±0.01
Crude fiber (g/L)	0.52 ±0.03
Total nitrogen (g/L)	0.92 ±0.02
Potassium (g/L)	1.74 ±0.03
Phosphate (g/L)	0.19 ±0.01
COD (mg/L)	100500 ±100
pH	9.38 ±0.03
Soluble lignin (%, M/M)	2.33±0.01

Each value is a mean of three independent experiments;
± stands for standard deviation among replicates;
M/M: (mass of soluble lignin) / (total mass of black liquor).

Effect of Variables Tested on Biomass and Protein Production

Assays were conducted according to the Plackett and Burman (1946) experimental model, in which the culture parameters were varied simultaneously (Table 3). Table 2 shows the maximal and minimal levels used for each variable tested. The influence of these parameters on the production of yeast biomass was estimated after fermentations.

Table 2. Conditions of Variables at Different Levels Using PB Experimental Design for Biomass Production from Black Liquor

V. No.	Variables	Low level (-)	High level (+)
X1	Initial pH	4	6
X2	Temperature (°C)	30	34
X3	Shaking speed (rpm)	180	220
X4	Straw powder hydrolyzed by phosphoric acid (g/L)	0	1
X5	Urea addition (g/L)	1	1.5
X6	Phosphoric acid addition (g/L)	0	1
X7	Concentration of black liquor added (V/V, %)	40	60

V. No.: Variables' number;
X1, X2, X3…: Symbols for different variables;
V/V: (volume of black liquor)/ (volume of culture medium.

Table 3. Results of Microbial Biomass and Crude Protein Determined over 36 h Incubation Using the PB Experimental Design

Assay	Variables							Biomass production (O)	Protein production (Y)
	X1	X2	X3	X4	X5	X6	X7		
P-1	+	-	+	-	-	-	+	10.875	3.555
P-2	+	+	-	+	-	-	-	**12.598**	3.868
P-3	-	+	+	-	+	-	-	6.612	3.425
P-4	+	-	+	+	-	+	-	8.526	3.293
P-5	+	+	-	+	+	-	+	9.244	3.490
P-6	+	+	+	-	+	+	-	3.668	1.648
P-7	-	+	+	+	-	+	+	6.442	3.456
P-8	-	-	+	+	+	-	+	10.220	**4.427**
P-9	-	-	-	+	+	+	-	4.360	2.109
P-10	-	-	-	-	+	+	+	7.034	3.415
P-11	-	-	-	-	-	+	+	9.788	4.728
P-12	-	-	-	-	-	-	-	4.846	2.135

P-1, P-2, P-3…: Assay number in PB experiments;
Maximum productivity values are bolded.

Table 4. Effect of Parameters on Biomass and Production (g/L) during Cultivation in Both Levels of Studies Factors

Variables	P	
	Biomass production (Y)	Protein production (O)
X1	**0.041**	**0.029**
X2	0.199	0.158
X3	0.876	0.987
X4	0.403	0.648
X5	**0.023**	**0.014**
X6	0.187	0.558
X7	**0.030**	**0.035**

The values significant at P≤0.05 are bolded.

The production of biomass ranged between 3.668 and 12.598 g/L, depending on the culture conditions, suggesting that the black liquor could be used to cultivate the yeasts. Furthermore, the initial pH, concentration of black liquor, and urea addition represented a statistically significant effect on both the biomass and crude protein production (Table 4).

COD removal rate and crude protein production change during treatment of black liquor

The optimizing experiment of incubation time during the treatment of black liquor was carried out on an orbital shaker (160 rpm) at 34 °C (Fig. 1). Samples were taken every 12 h. The crude protein production accumulated during fermentation time. The increasing rate became stable when the incubation time was 36 h. When the incubation time was above 36 h, production of crude protein was stable at about 1.2 g/L. The COD of black liquor decreased during fermentation time. The decreasing rate was stable when the incubation time was 36 h. Maximum COD removal rate was about 50%. Organic substances of black liquor, such as reducing sugar and nitrogen sources, could be used by yeasts. This is why the COD of black liquor decreased, and production increased. When organic substances of black liquor that could be utilized by yeasts were used up, the increasing rate of production and COD removal stabilized. As a result, the 36 h cultivation time was the most economical incubation time.

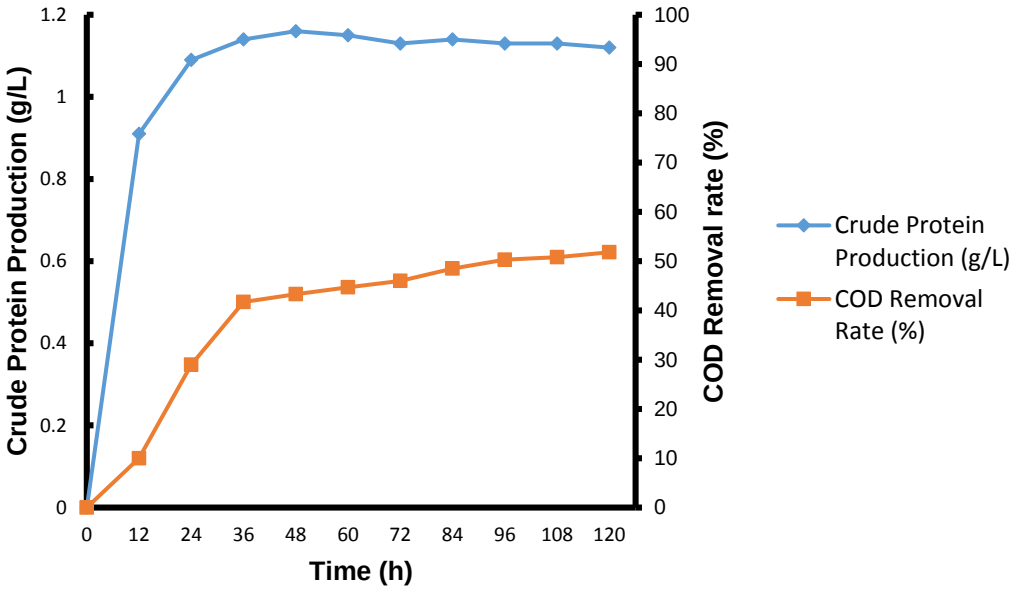

Fig. 1. Effect of fermentation time on the crude protein production and COD removal rate (urea 3 g/L, 34 °C, initial pH 6.0, 180 rpm, concentration of black liquor 60%)

Effect of the concentration of black liquor on biomass and protein production

The concentration of black liquor added to the fermentation cultures could represent the most nutrient abundance for the yeasts. Thus, the effect of the concentration of black liquor was assessed depending on different values, as shown in Fig. 2. The removal rates of COD in different tests were close to each other, while the biomass and protein production rose at first and then declined, resulting in a peak value. The abundant substance affected the production, and high concentrations would hamper microbial growth attributed to some constituents, such that they are either not readily metabolized, or deleterious to the assimilation of toxic ones.

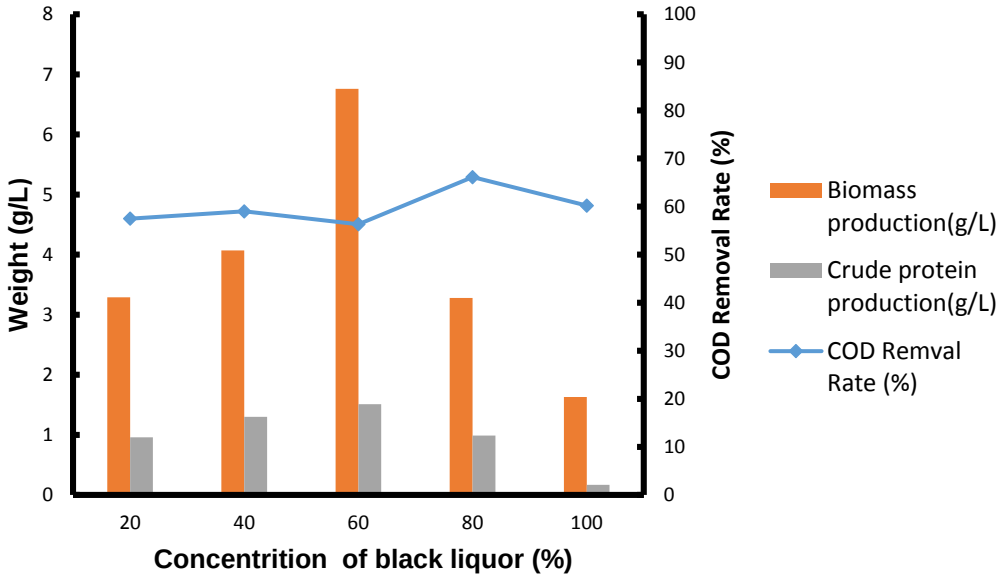

Fig. 2. Effect of the biomass, crude protein production and COD removal rate (urea 3 g/L, 34 °C, initial pH 6.0, 180 rpm, 48 h)

Effect of different temperatures on crude protein production

It is well known that temperature can affect positively and/or negatively all the biological reactions and mechanisms or functions. The temperature of the fermentation medium is one of the critical factors that have a profound influence on production of crude protein. The optimizing experiment for different temperatures (28 to 36 °C) was carried out on an orbital shaker (160 rpm) (Fig. 3).

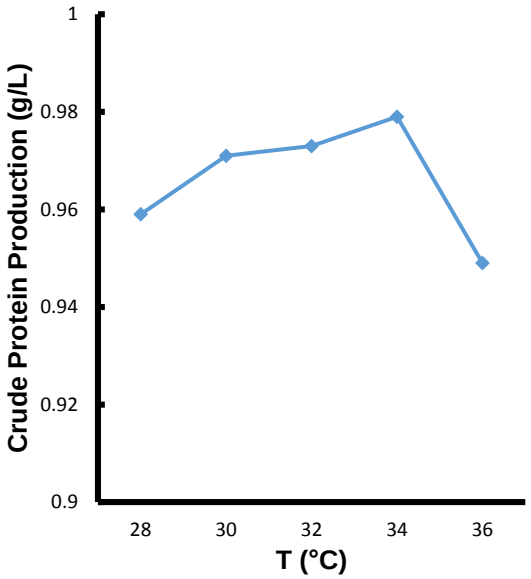

Fig. 3. Effect of different temperatures on crude protein production (urea 3 g/L, initial pH 6.0, 180 rpm, concentration of black liquor 60%, 48 h)

The crude protein production was gradually promoted with the increase of temperature due to the flow enhancement of nutrient across cell membrane and low demand

for maintenance energy according to the reports of Roels (1983), and Converti and Dominguez (2001). However, the crude protein production declined when the temperature was above 34 °C, since a high temperature can lead to inactivation of enzymes (Aiba *et al.* 1973; Roels 1983; Converti and Dominguez 2001). In this study, it was observed that when the fermentation temperature was maintained at 34 °C, maximum production of crude protein reached 0.979 g/L. Therefore, the optimal temperature was judged to be 34 °C.

Effect of the initial pH on protein production

As an important parameter of fermentation, the pH of the culture medium plays a large part in the growth of yeasts and the accumulation of protein, as it not only affects the activation of enzymes in microorganisms and changes their cytomembrane but also influences the dissociation of certain ingredients in a culture medium and metabolic intermediates. As seen in Fig. 4, the protein production was gradually enhanced with the increase of the initial pH at the beginning stage and then decreased, similar to the effect of the temperature in accordance with the discipline of most fermentation. When the initial pH value was 6, the maximum protein production was obtained up to 1.29 g/L.

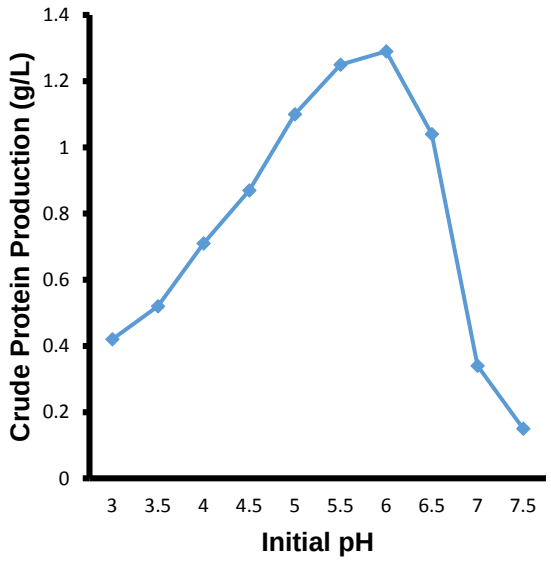

Fig. 4. Effect of the initial pH on crude protein production (urea 3 g/L, 34 °C, 180 rpm, concentration of black liquor 60%, 48 h)

Effect of shaking speed on the crude protein

Shaking speed mainly affects the dissolution of oxygen in culture medium, since the increase of shaking speed could promote the gas-liquid mass transfer. In aerobic fermentation processes, dissolved oxygen is an essential ingredient for yeasts, and oxygen supply is one of the most important factors affecting physicochemical property of culture medium, nutrients uptake, cell growth and the production of biomass. Therefore, to obtain high biomass yield in mushroom cultivation, investigation on the influence of oxygen supply is necessary. Thus, the effect of shaking speed on production of crude protein was evaluated as shown in Fig. 5. The maximum production (1.01 g/L) was obtained at the shaking speed (180 rpm). Besides, it was observed here that shaking speed did not have a significant effect on the production, which was also verified in the PB experiments (Table 4).

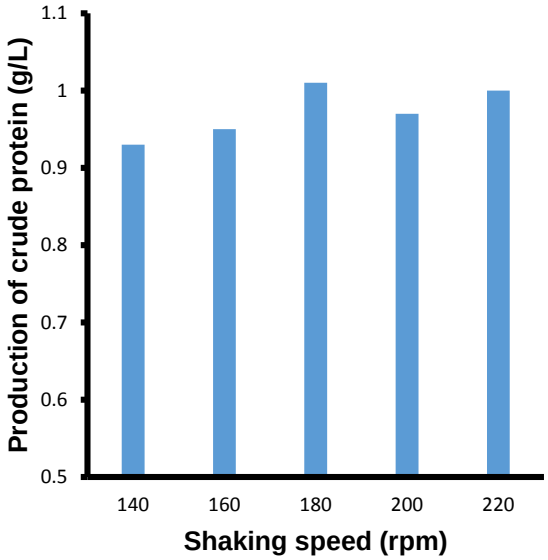

Fig. 5. Production of crude protein at different shaking speed (urea 3 g/L, initial pH 6.0, 34 °C, 180 rpm, concentration of black liquor 60%, 48 h)

Results of Optimizing Experimental Design

To maximize the total crude protein production, and minimize the cost of black liquor treatment, the experiment was designed according to four factors and three levels (orthogonal table, Table 5).The bioprocess was optimized in shake flasks. As such, the degree of effect of these three parameters on the production of yeast biomass could be estimated, and this allowed the optimum medium composition to be determined.

Table 5. Factors and Levels of Orthogonal Experiment

Variables	Level		
	1	2	3
X0	1	2	3
X1	4	5	6
X5	0.5	1.0	1.5
X7	40	60	80

X0: Vacancy, means adding nothing to it, but it is needed for the software of Minitab 16.0 to perform further statistical analyses.

The results (Table 6) showed that which levels at different factors could maximize biomass and protein production. These levels were the concentration of black liquor (60%), initial pH (6.0), and urea addition (0.5 g/L). Under these conditions, the optimal biomass production, protein production, and COD removal rate could reach 5.19 ±0.04, 1.18±0.02g/L, and 78.78 ±3.21%, respectively. The remaining black liquor, the COD of which was approximately 13000 mg/L after protein extraction, can be recycled or treated using the activated sludge method until it reaches the standard of discharge. The range analysis of the orthogonal experiment (Table 7) revealed the degree of effect on the consequence of different factors: pH＞urea addition＞concentration of black liquor. The results (Table 8) showed that the effect of pH on the consequences was significantly under the 95% credibility.

Table 6. Results of Production of Microbial Biomass and Productivity Determined over 12 h using Orthogonal Experimental Design

Assay	Variables				Biomass Production /g·L^{-1}(O); Protein Production / g·L^{-1}(Y); COD Removal Rate /% (C)		
	X0	X1	X5	X7	O	Y	C
O-1	1	1	1	1	0.84±0.02	0.33±0.03	56.75±1.35
O-2	2	2	2	1	2.68±0.05	1.07±0.02	65.50±2.34
O-3	3	3	3	1	2.47±0.03	0.84±0.02	27.38±2.45
O-4	3	1	2	2	0.67±0.04	0.18±0.04	64.17±3.43
O-5	1	2	3	2	3.08±0.03	1.10±0.01	67.83±2.87
O-6	2	3	1	2	**5.19**±0.04	**1.18**±0.02	**78.18**±3.21
O-7	2	1	3	3	0.96±0.01	0.18±0.03	66.00±2.17
O-8	3	2	1	3	3.06±0.03	1.07±0.04	73.88±1.98
O-9	1	3	2	3	6.01±0.06	1.05±0.03	37.63±2.33

O-1, O-2, O-3…: Assay number in orthogonal experiments;
Each value is a mean of three independent experiments;
± Stand for standard deviation among replicates;
Maximum productivity values are bolded.

Table 7. Range Analysis of Orthogonal Experiment

Level	Variables			
	X0	X1	X5	X7
1	0.827	0.230	0.860	0.747
2	0.810	1.080	0.767	0.820
3	0.697	1.023	0.707	0.767
Range	0.130	0.850	0.153	0.073

Table 8. Variance Analysis of Orthogonal Experiment

Variables	DEVSQ	DF	F-ratio	F Critical Values	Significance
X1	1.355	2	45.167	19.000	O
X5	0.036	2	1.200	19.000	O
X7	0.009	2	0.300	19.000	O
Error	0.03	2			

DEVSQ: Sum of Square of Deviations;
DF: Degree of Freedom;
O: Significant.

Microscopic Observation and Potential Value of the Black Liquor Treatment

Different cell shapes exhibited dissimilar physiological characteristics. Such versatility might facilitate a fungal adaptation to environmental fluctuations. The cell size and shape therein was similar to those reported by Zhang *et al.* (2005) and Silva *et al.* (2011). The result is shown in Fig. 6. The yeast cells had good viability. The most prominent morphological characteristic was the presence of cluster-like cell agglomerates and multiple sprouting spots, indicating that they were entering the stationary phase.

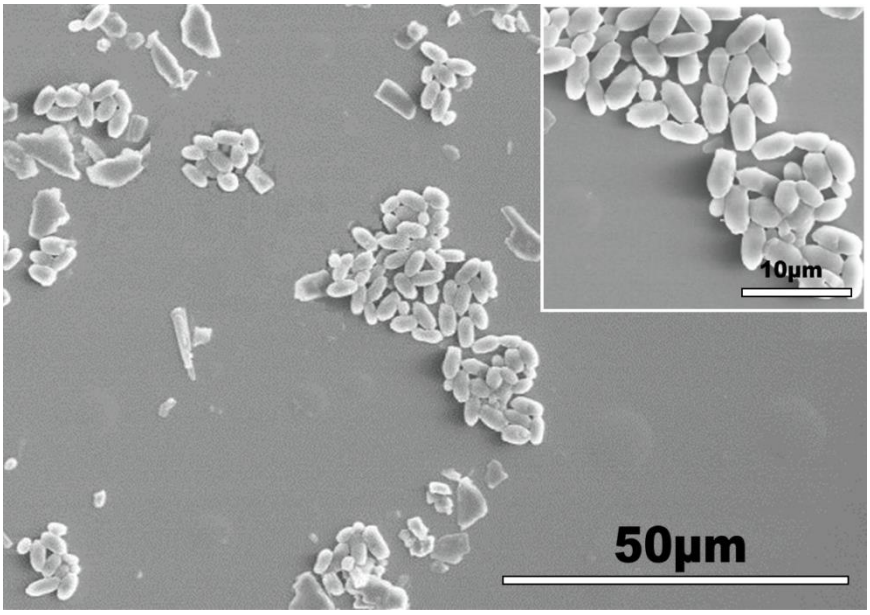

Fig. 6. Scanning electron photomicrographs (with a magnification of 3000 times) of *Candida utilis* 1314 under optimal conditions after cultivating for 36 h

Table 9. Comparative Analysis of Essential Amino Acids between the Fermentation Products in this Study and Beer Yeast Feed

Amino Acid (AA)	AA content (%) of SCP[a]	AA content (%) of beer yeast feed[b]
Aspartic acid	3.94	---[c]
Threonine	2.45	2.33
Serine	2.19	---[c]
Glutamic acid	4.70	---[c]
Glycine	2.00	---[c]
Alanine	2.58	---[c]
Cystine	0.45	0.05
Valine	2.72	---[c]
Methionine	0.36	0.83
Isoleucine	2.15	2.86
Leucine	3.79	4.76
Tyrosine	1.66	0.12
Phenylalanine	2.00	4.07
Lysine	3.16	3.38
Histidine	1.15	1.11
Arginine	2.14	2.67
Proline	2.38	---[c]
Total	39.82	22.63

[a]: Essential amino acids detected by the instrument; The asparagine and glutamine could have been hydrolyzed to aspartate and glutamate during sample preparation, and Tryptophan is not abundant; As a result, they might be under the detection of the instrument;
[b]: The Chinese feed number of beer yeast feed is 7-15-0001, and it is provided for the reference of amino acids essential for poultry feed;
[c]: Not provided.

In addition, the protein production was 1.18±0.02 g/L under the optimal conditions. 17 sorts of essential amino acids (AA) were detected by the instrument, and the total amino acid content was up to 39.82% (Table 9). In contrast to beer yeast feed provided by the *Chinese Food*, AA composition of crude protein in the biomass was various, and the total AA content was higher as well. The results indicated that it almost contained amino acids essential for poultry feed, and possessed better values than beer yeast food (Xiong *et al.* 2011). Due to these advantages, this SCP has great potential for use as a feed supplement.

In this study, 1 ton of black liquor and 0.5 kg urea afforded an average yield of about 1.18 kg of crude protein; additionally, black liquor could be treated effectively. Thus, the potential profit of fermentation was obvious. Due to various AA compositions and high AA content, SCP produced from pulping of soda in the papermaking industry might be used as a supplement for poultry feed and fertilizer (Yin *et al.* 2011).

CONCLUSIONS

1. *Candida utilis* produces SCP using black liquor from the pulping of soda in the papermaking industry as nutrients, without any detoxification. A high yield of 1.18 ±0.02 g SCP could be obtained per kg of black liquor under the optimized conditions, demonstrating that black liquor has shown excellent potential as a carbon and energy source for yeasts.
2. The maximum value of COD removal rate was 78.78 ±3.21%, clearly indicating that the process of cultivating yeasts might be an effective step in the overall process of black liquor treatment.
3. The biomass protein contains sorts of potential AA, and the total AA content was up to 39.82%, suggesting that the SCP generated could be useful for poultry feed and the fertilizer industry.

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REFERENCES CITED

Aiba, S. H., Humphrey, A. E., and Millis, N. F. (1973). *Biochemical Engineering* (2nd ed.), Academic Press, New York, pp. 92-127. DOI: 10.1002/jobm.19760160311

Ai, T., Jiang, Z., Qiu, Y., Dong, X., and Cui, S. (2003). "New technology advance in soda recovery of pulp black liquors," *Hubei Papermaking* (4), 36-39. (in Chinese)

APHA (1992). *Standard Methods for the Examination of Water and Waste Water* (18th ed.), American Public Health Association (APHA), Washington, USA. DOI: 10.1016/0048-9697(94)90332-8

CMGMR (1993). *Standard Methods for the Phosphate Examination of Groundwater*, Dz/T 0064.1-0064.80-9. Chinese Ministry of Geology and Mineral Resources

(CMGMR), Guangdong, China. (in Chinese)

Converti, A., and Dominguez, J. M. (2001). "Influence of temperature and pH on xylitol production from xylose by *Debarryomyces hansenii*," *Biotechnology and Bioengineering* 75(1), 39-45. DOI: 10.1002/bit.1162

CSBTS (1995). Pulp-Determination of chlorine consumption (Degree of delignification), GB/T 2678.3-1995(National Standard of the People's Republic of China). State Bureau of Technical Supervision (CSBTS), Beijing, China. (in Chinese)

Dafinov, A., Font, J., and Garcia-Valls, R. (2005). "Processing of black liquors by UF/NF ceramic membranes," *Desalination* 173(1), 83-90. DOI: 10.1016/j.desal.2004.07.044

Davis, M. L., and Cornweel, D. A. (1998). *Introduction to Environmental Engineering*, 3rd Ed., McGraw-Hill, WCB.

Ejiofor, A. O., Chisti, Y., and Moo-Young, M. (1996). "Culture of *Saccharomyces cerevisiae* on hydrolyzed waste cassava starch for production of baking-quality yeast," *Enzyme and Microbial Technology* 18(7), 519-525. DOI: 10.1016/0141-0229(95)00166-2

Gharsallah, N. (1993). "Production of single cell protein from olive mill wastewater by yeasts," *Environmental Technology* 14(4), 391-395. DOI: 10.1080/09593339309385305

Huang, G., Shi, J. X., and Langrish, T. A. G. (2006). "A new pulping process for wheat straw to reduce problems with the discharge of black liquor," *Bioresource Technology* 98(15), 2829-2835. DOI: 10.1016/j.biortech.2006.09.029

Kurbanoglu, E. B., and Algur, O. F. (2002). "Single-cell protein production from ram horn hydrolysate by bacteria," *Bioresource Technology* 85(2), 125-129. DOI: 10.1016/S0960-8524(02)00094-9

Lawford, G. R., Kligeman, A., and Williams, T. (1979). "Production of high-quality edible protein from *Candida* yeast grown in continuous culture," *Biotechnology and Bioengineering* 21(7), 1163-1173. DOI: 10.1002/bit.260210707

Monte, M. C., Fuente, E., Blanco, A., and Negro, C. (2009). "Waste management from pulp and paper production in the European Union," *Waste Management* 29(1), 293-308. DOI: 10.1016/j.wasman.2008.02.002

Nelson, N. A. (1944). "A photometric adaptation of the Somogyi method for the determination of glucose," *Journal of Biological Chemistry* 135, 375-380.

Nigam, J. N. (1998). "Single cell protein from pineapple cannery effluent," *World Journal of Microbiology & Biotechnology* 14(5), 693-696. DOI: 10.1023/A:1008853303596

Plackett, R. L., and Burman, J. P. (1946). "The design of optimum multifactorial experiments," *Biometrika* 33(4), 305-325. DOI: 10.2307/2332195

Raa, J. (1990). "Biotechnology in aquaculture and the fish processing industry: A success story in Norway," in: *Advances in Fisheries Technology and Biotechnology for Increased Profitability*, Voigt, M. N., and Botta, J. R. (eds.), Technomic Publishing Co, Inc, Lancaster, PA, USA.

Rajoka, M. I., Khan, S. H., Jabbar, M. A., Awan, M. S., and Hashmi, A. S. (2006). "Kinetics of batch single cell protein production from rice polishing with *Candida utilis* in continuously aerated tank reactors," *Bioresource Technology* 97(15), 1934-1941. DOI: 10.1016/j.biortech.2005.08.019

Roels, J. A. (1983). *Energetics and Kinetics in Biotechnology*. Elsevier Biomedical Press, Amsterdam. DOI: 10.1016/0141-4607(85)90047-2

Schultz, N., Chang, L., Hauck, A., Reuss, M., and Syldatk, C. (2006). "Microbial

production of single-cell protein from deproteinized whey concentrates," *Applied Microbiology and Biotechnology* 69(5), 515-520. DOI:10.1007/s00253-005-0012-z

Shay, L. K., and Wegner, G. H. (1985). "Improved fermentation process for producing Torula yeast," *Food Technology* 39(10), 61-66.

Silva, C. F., Arcuri, S. L., Campos, C. R., Vilela, D. M., Alves, J. G. L. F., and Schwan, R. F. (2011). "Using the residue of spirit production and bio-ethanol for protein production by yeasts," *Waste Management* 31(1), 108-114. DOI: 10.1016/j.wasman.2010.08.015

Van Beers, D., Bossilkov, A., and Lund, C. (2009). "Development of large scale reuses of inorganic by-products in Australia: The case study of Kwinana, Western Australia," *Resources Conservation and Recycling Journal* 53(7), 365-378. DOI: 10.1016/j.resconrec.2009.02.006

Van der Kamer, J. H., and van Ginkel, L. (1952). "Rapid determination of crude fiber in cereals," *Cereal Chemistry* 29(4), 23-25.

Van Soest, P. J., and Wine, R. H. (1968). "Determination of lignin and cellulose in acid detergent fiber with permanganate," *Journal of the Association of Official Analytical Chemists* 51, 780-785.

Xiong, B. H., Pang, Z. H., Luo, Q. Y. (2011). "The standards of Chinese feed compositions and nutritive value," *China Feed* 21, 32-37. (in Chinese)

Yin, Y., Yu, H., Li, H., Lai, Y., Zhang, S., Zhang, L., Zhang, Y., Gou, C., Ji, G., Sun, R., and Wan, P. (2012). "A new pulping technology to obtain high-performance fibers efficiently from the plants," Chinese Patent No. CN 102337687A. (in Chinese)

Yin, Y., Zhang, L., Wang, Z., Wan, P., Sun, R., Zhang, S., Fan, Y., Li, Y., Zhao, Y., and Chen, Y. (2011). "Amino acid used as phosphate release formulation in the soil," Chinese Patent No. CN102351612A. (in Chinese)

Yu, Y., Wu, G., and Meng, X. (1990). *Inspection Handbook of Environmental Engineering Microbiology*. Chinese Environmental Science Press, Beijing, pp. 8-51. (in Chinese)

Zhang, Y., Rittmann, B. E., Wang, J., Sheng, Y., Yu, J., Shi, H., and Qian, Y. (2005). "High-carbohydrate wastewater treatment by IAL-CHS with immobilized *Candida tropicalis*," *Process Biochemistry* 40(2), 857-863. DOI: 10.1016/j.procbio.2004.02.010

Zheng, S., Yang, M., and Yang, Z. (2005). "Biomass production of yeast isolate from salad oil manufacturing wastewater," *Bioresource Technology* 96(10), 1183-1187. DOI: 10.1016/j.biortech.2004.09.022

Ziino, M., Lo Curto, R. B., Salvo, F., Signorino, D., Chiofalo, B., and Giuffrida, D. (1999). "Lipid composition of *Geotrichum candidum* single cell protein grown in continuous submerged culture," *Bioresource Technology* 67(1), 7-11. DOI: 10.1016/S0960-8524(99)00102-9

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