Hindawi Publishing Corporation
Journal of Biomedicine and Biotechnology
Volume 2011, Article ID 141207, 8 pages
doi:10.1155/2011/141207

Research Article

In Situ Biodiesel Production from Fast-Growing and High Oil Content *Chlorella pyrenoidosa* in Rice Straw Hydrolysate

Penglin Li,[1,2] Xiaoling Miao,[1,2] Rongxiu Li,[1,2] and Jianjiang Zhong[1,2]

[1] *Key Laboratory of MOE for Microbial Metabolism and School of Life Science & Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China*
[2] *Biomass Energy Research Center, Shanghai Jiao Tong University, Shanghai 200240, China*

Correspondence should be addressed to Xiaoling Miao, miaoxiaoling@sjtu.edu.cn

Received 14 October 2010; Accepted 22 December 2010

Academic Editor: Rodomiro Ortiz

Rice straw hydrolysate was used as lignocellulose-based carbon source for *Chlorella pyrenoidosa* cultivation and the feasibility of *in situ* biodiesel production was investigated. 13.7 g/L sugar was obtained by enzymatic hydrolyzation of rice straw. *Chlorella pyrenoidosa* showed a rapid growth in the rice straw hydrolysate medium, the maximum biomass concentration of 2.83 g/L was obtained in only 48 hours. The lipid content of the cells reached as high as 56.3%. *In situ* transesterification was performed for biodiesel production. The optimized condition was 1 g algal powder, 6 mL *n*-hexane, and 4 mL methanol with 0.5 M sulfuric acid at the temperature of 90°C in 2-hour reaction time, under which over 99% methyl ester content and about 95% biodiesel yield were obtained. The results suggested that the method has great potential in the production of biofuels with lignocellulose as an alternative carbon source for microalgae cultivation.

1. Introduction

As a renewable, biodegradable, and environmentally friendly fuel, biodiesel has received more attention in the past few years. Biodiesel refers to fatty acids methyl esters (FAMEs) from vegetable oils or animal fats with high stability; low water and volatiles content; a low amount of polymers containing sulfur and nitrogen elements. Current sources of commercial biodiesel are primarily soybean oil, rapeseed oil, palm oil, corn oil, waste cooking oil, and animal fat, but these sources of biodiesel cannot meet even a slight fraction of present demand for fuels [1]. However, a major criticism against large-scale fuel production from agricultural crops, is that it will consume vast swaths of farmland, native habitats and drive up food prices [2]. For example, meeting only half the existing US transport fuel by biodiesel would require unsustainably 54% and 24% of the US cropping land using coconut and oil palm, respectively [1].

Microalgae have been considered as one of the most promising feedstocks for biodiesel production due to their short cell cycle (within 24 hours), high oil content (20%–50% normally and highest exceeding 80%), strong adaptive capacity to environment (high salinity, heavy metal ion, toxicants, high CO_2 concentration, etc.) and no occupation for cropping area [1]. However, price of algae-based biodiesel is still much higher than normal diesel with the price of US$1.25/lb and US$0.43/lb, respectively, [3, 4], which encumbers large-scale manufacturing and applications of algae-based biodiesel. Some attempts have been made to reduce the cost such as using cheap carbon sources for high oil microalgae production. For example, Jerusalem artichoke [5], cassava starch [6], and sugar cane juice [7] were introduced as new carbon supplies for microalgae cultivation and algae-based biodiesel production. The results showed that hydrolysate medium could provide an exceeding 15.2% to 42.3% biomass production leading to an 8.8% to 27.7% increase in algal oil production based on the carbon source. Then good quality biodiesel, whose FAME content reached as high as 93%, was produced from algal biomass [8]. These studies suggested that microalgae could be able to utilize such resources for high oil accumulation and better biodiesel production. However, such materials, mainly agricultural crops, have been also studied for bioethanol production for years [9, 10]. The conflict between food and

fuel still exists either for biodiesel or bioethanol. For further feasible study and sustainability, lignocellulose material has aroused people's interest for recent years [11]. Since the world's annual cereal production has been over 2 billion tons for years [12], straw, making up of 50% weight of cereal crops, is one of the largest lignocellulose resources as an agricultural byproduct. Additionally, biodegradable solid waste fraction from municipal refuse provides another alternative large source for discarded lignocellulose due to target for minimizing landfill use in many countries [13]. Based on these sources, microalgae cultivation could be coupled with wastes recovery, management, and reuse. Therefore biofuels derived from lignocellulose, especially straw and other nonfood feedstock offer a better option for food, energy, and environmental concerns [2].

The current algae-based biodiesel is mainly produced by conventional route: extraction of the lipids from the microalgal biomass followed by its conversion to FAMEs and glycerol [14–16]. However, such method is time consuming, costly, and difficult to be implemented in algae's crushing step because of the rigid cell walls [17]. Recently *in situ* transesterification method, in which the oil-bearing material contacts with alcohol directly instead of reacting with pre-extracted oil, has received serious attention for algae-based biodiesel production. As a method for biodiesel production, not only FAME content but also conversion efficiency from algal oil to biodiesel are imperative as quality and quantity standards in the process of transesterification [18]. However, current papers provide limited information about both quality and quantity studies of *in situ* transesterification from microalgal biomass. Johnson and Wen [17] reported a 63.5% of the FAME content using direct transesterification of *Schizochytrium limacinum* biomass with 100% biodiesel yield; Ehimen et al. [19] obtained a high-quality biodiesel with almost 93% FAME content from *Chlorella* strains, but no data was provided on yield from algal oil to biodiesel. Therefore, further research is needed to develop a feasible method for high yield and high quality *in situ* biodiesel production from microalgae.

In this paper, we studied the feasibility of rice straw as the raw material of carbon source for *Chlorella pyrenoidosa*'s cultivation and the effects of solvent, methanol volume, temperature and reaction time on FAME content and biodiesel yield were also investigated in the process of *in situ* transesterification from algal biomass.

2. Methods

2.1. Preparation of Cellulose Enzymatic Hydrolysate. As reported by many papers, the barrier to production and recovery of valuable materials from lignocellulose was its resistance to degradation due to cross-linking between the polysaccharides (cellulose and hemicellulose) and the lignin via ester and ether linkages [20]. To utilize lignocellulose as a raw material, a pretreatment is needed [21]. Rice straw purchased from local market was selected as the lignocellulose material for hydrolysation as rice is one of three largest grain types produced in China. The straw was previously cut into 10-cm pieces and then pretreated in a two-step method according to our published paper [22]. First, 50 g dried rice straw was mixed with 1L 1% trifluoroacetate at 95°C for 5 hours. Then the samples after desiccation filled in a steel reaction kettle reacted with 60% alcohol at 210°C for 4 hours. After pretreatment, a commercially available cellulase, Accellerase 1000 from Genencor, was used for rice straw hydrolyzation. According to our pre-experiment and the enzyme protocol [23], 20 g/L pretreated rice straw and 20 mL/L enzyme were mixed with water (pH 5.0) in an Erlenmeyer flask. The reaction was done on the shaker (150 rpm) for 24 hours at a temperature of 50°C. Then the enzyme was deactivated (heating) and the solution was filtered to obtain rice straw hydrolysate (RSH).

2.2. Algae Strain and Cultivation Condition. The green microalga *Chlorella pyrenoidosa* was purchased from the algae strain laboratory of Aquatic Organism Research Institute of Chinese Academy of Science. A modified BG11 medium was used as the basic medium which contains 100 mg $NaNO_3$, 74.9 mg $MgSO_4 \cdot 7H_2O$, 30.5 mg KH_2PO_4, 27.18 mg $CaCl_2$, 20 mg Na_2CO_3, 8.9 mg $C_6H_5O_7Na_3 \cdot 2H_2O$, 6 mg ferric ammonium citrate, 1.04 mg $Na_2 \cdot$EDTA, 2.86 mg boric acid, 0.222 mg $ZnSO_4 \cdot 7H_2O$, 0.079 mg $CuSO_4 \cdot 5H_2O$, 1.81 mg $MnCl_2 \cdot 4H_2O$, 0.39 mg Na_2MoO_4, and 0.049 mg $Co(NO_3)_2 \cdot 6H_2O$ every liter. Glucose and RSH were used as carbon sources. 10 g/L glucose were selected for cultivation [15] and the same amount of glucose was used in RSH medium by adding appropriate volumes of RSH solution to the basic medium to achieve the concentration. The medium was adjusted to pH 7.5 and autoclaved; alga (OD600 = 0.5) was inoculated into an Erlenmeyer flask (250 mL, containing 50 mL medium) and cultivated on the shaker (110 rpm) at a temperature of 25 ± 1°C with continuous illumination at intensities of 40 μmol/(m^2s).

2.3. In Situ Transesterification for Biodiesel Production. A direct methanolysis of algal biomass was introduced for *in situ* biodiesel production due to its higher yield and time and cost saving. Algal powder, methanol, solvent, and 0.5 M sulfuric acid were reacted in a 20 mL-cylindrical tetrafluoroethylene reaction vessel and there was a steel shell outside the reactor for seal and high-temperature protection. With 1 g algal powder and 0.5 M sulfuric acid, different amounts of solvent (2.0 mL to 8.0 mL), methanol (0.5 mL to 10.0 mL), temperature (20°C to 110°C), and reaction time (0.25 hour to 4 hours) were studied. After the reaction, 2.0 mL water was added to the mixture. Samples were then cooled down to room temperature, and centrifuged at 8,228 ×g for 10 min. Three layers formed which were composed of a water phase for water and alcohol, a solid phase for algae residue, and an organic phase for solvent and biodiesel, respectively. The organic layer was collected and evaporated at 60°C to constant weight for analysis. The biodiesel yield from algal biomass was calculated by (1):

$$\text{Biodiesel yield (\%)} = \frac{\text{Biodiesel mass (g)}}{\text{algae mass (g)} \times \text{oil content (\%)}} \times 100\%. \quad (1)$$

2.4. Dry Cell Weight and Reducing Sugar Concentration. For biomass measurement, 5 mL culture medium was transferred to a preweighed centrifuge tube and centrifuged at 4,500 ×g for 5 min. After rinsing three times, the sample was lyophilized to constant weight and warmed up to room temperature in a desiccator, then the dry cell weight was determined.

The content of reducing sugars in medium was determined by the 3,5-dinitrosalicylic acid method [22]. The reducing sugar concentration was measured using an ST360 biosensor. The sugar components of the hydrolysate were analyzed by HPLC. The enzymatic hydrolysis yield was calculated by

$$\text{Enzymatic hydrolysis yield (\%)} = \frac{\text{Reducing sugar (g)} \times 0.9}{\text{Carbohydrate (g)}} \times 100\%. \quad (2)$$

2.5. Lipid Content. Lipid in the algal cells was extracted according to a modified method from Xu et al. [14]. 0.2 g dry algae were triturated in the liquid nitrogen for cell fragmentation. Then the algae were blended with 3 mL chloroform/methanol (2 : 1), shaken for 20 min and centrifuged (4,500 ×g) for 10 min. The supernatant was collected in a pre-weighed tube, and the same process was repeated twice. All the supernatant was collected together, evaporated and dried to constant weight at 60°C, and finally weighed to calculate the total lipid content.

2.6. Composition Analysis of Biodiesel. The composition of the biodiesel produced from *in situ* transesterification of algal biomass was analyzed by GC-linked mass spectrometry (GC-MS) equipped with a DB-5MS column (30 m × 0.25 mm ID DF = 0.25 μm) and with a flow rate of 1.0 mL/min.

3. Results and Discussion

3.1. Enzymatic Hydrolysis of Rice Straw. To obtain a higher glucose concentration for algae cultivation, time of enzymatic hydrolysis was modified. 20 g/L pretreated rice straw mixed with 20 mL/L cellulase was hydrolyzed for 24 hours at 50°C. According to our pre-experiments, a dosage of 1mL enzyme to 1 g straw was essential for fast hydrolyzation (within one day) and other conditions were followed by the protocol from the enzyme. The time course of hydrolyzation was shown in Figure 1. The reducing sugar concentration increased notably in the first 8 hours, reaching over 12 g/L (Figure 1). Then its concentration remained almost constant between 12 to 14 g/L in the following time. The highest sugar concentration (about 13.7 g/L) appeared in the 20th hour (Figure 1). The enzymatic hydrolysis yield was about 61.7% calculated by (1). This data was close to a 67% yield of wheat straw hydrolyzation pretreated by an aqueous glycerol method at the same temperature [24]. The HPLC analysis of the hydrolysate showed that the glucose took up over 98% of the total reducing sugar and the rest was mainly xylose. Other compounds such as acetic acid and glycerol was also detected in the hydrolysate, but their concentration was very low (<0.05 g/L). This suggests that higher percentage of glucose could be obtained from rice straw through our present method. For further algae cultivation, the hydrolysate was prepared in a 20-hour hydrolysis.

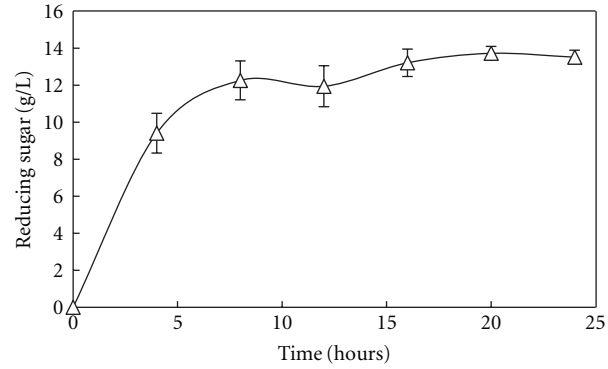

Figure 1: Time course of rice straw hydrolyzation. Reaction condition: 20 g pretreated rice straw and 20 mL enzyme in 1 L volume on the shaker (150 rpm) at 50°C Error bars = mean ± standard deviation.

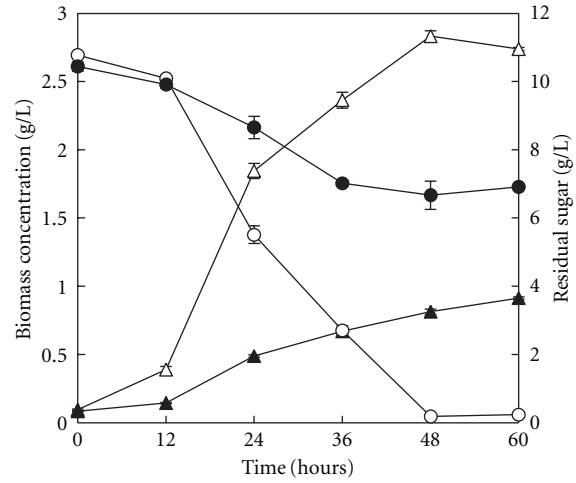

Figure 2: Cell growth and sugar consumption of *Chlorella pyrenoidosa* in the glucose medium and the rice straw hydrolysate (RSH) medium. Biomass concentration (▲) and residual sugar concentration (•) in the glucose medium; biomass concentration (△) and residual sugar concentration (○) in the RSH medium. Error bars = mean ± standard deviation.

3.2. Shake-Flask Cultivation of C. pyrenoidosa with Rice Straw Hydrolysate. To improve *C. pyrenoidosa*'s utilization of rice straw hydrolysate in the presence of probable inhibitors, algae adaptation had been done in the RSH medium for generations. After adaptation, comparison experiments in shake-flask cultivation using glucose medium and RSH medium were conducted. The effects of glucose and RSH medium on the growth and sugar consumption were shown in Figure 2. The algae showed rapid growth in RSH medium; the maximum biomass concentration of 2.83 g/L was obtained in only 48 hours. The lipid content of the cells reached as high as 56.3% (Table 1). The biomass productivity

Table 1: The effects of different carbon sources on cell growth and lipid accumulation of *Chlorella pyrenoidosa*.

	Carbon source	
	Glucose	Rice straw hydrolysate
Maximum biomass concentration (g/L)	0.92 ± 0.01	2.83 ± 0.04
Lipid content (%, w/w)	50.3 ± 0.9	56.3 ± 1.4
Biomass productivity (g/L/day)	0.37 ± 0.00	1.10 ± 0.02
Lipid productivity (g/L/day)	0.19 ± 0.00	0.62 ± 0.01

and lipid productivity of the cells growing in RSH medium were about 3.0 times and 3.3 times higher than that of the cells growing in the glucose medium, respectively (Table 1). This is probably because the *C. pyrenoidosa* could fully utilize carbon sources in the RSH medium. Larson and Rees [26] reported that lipid accumulation could be triggered in a low nitrogen medium (0.1 g/L in our medium). Under such a condition, cell division ceases while respiration does not decrease, so lipid could be accumulated for energy storage if cells absorb additional carbon sources. Almost all the sugars in the RSH medium had been used up after 48 hours, while about two-thirds of the glucose in the glucose medium was not consumed (Figure 2). The maximum biomass concentration of the cells growing in the glucose medium was only 0.92 g/L after 60 hours (Table 1). Another possible reason for these phenomena reported by many papers [8, 25] was that there were probably some beneficial growth factors for cell growth and oil accumulation in the hydrolysate system. Compared with other research, the *C. pyrenoidosa* growing in RSH medium also showed higher biomass productivity and higher lipid content (Table 2). As reported, different microalgal biomass concentrations were obtained at relatively longer culture period in other research such as 3.92 g/L [14], 7.2 g/L [6], 4.26 g/L [25], and 5.1 g/L [8] in 6 day, 10 days, 125 hours, and 120 hours, respectively (Table 2). Our present research suggests that higher biomass and higher lipid content microalgal cells could be obtained in a shorter culture period (48 hours) by using RSH medium, which would have a better application in industrial production.

3.3. In Situ Transesterification for Biodiesel Production

3.3.1. Effect of Solvent. Generally, methanol plays the roles of both reactant and extractant in *in situ* transesterification, but it was proved to be a poor solvent for oil extraction [27]. This would probably lead to low conversion efficiency from algal biomass to biodiesel by *in situ* transesterification. One of the possible solutions to this problem is to introduce a good oil extraction solvent such as *n*-hexane or chloroform in the process of *in situ* biodiesel production. According to our previous experiments, adding these two kinds of solvents to the *in situ* reaction system could lead to a higher biodiesel yield. Therefore, these two kinds of widely used oil extraction solvent, chloroform, and *n*-hexane, were selected for further *in situ* transesterification studies. Reaction was carried out at 90°C with 1 g algal powder, 4 mL methanol and 0.5 M sulfuric acid. Solvent volume was set at 2, 4, 6, and 8 mL, respectively. The effects of different volume of chloroform and *n*-hexane on biodiesel yield and FAME content were shown in Figure 3. All the samples' FAME content maintained over 98% suggesting that high-quality biodiesel could be prepared by our *in situ* transesterification method. For solvent volume from 2 mL to 6 mL, biodiesel production increased with the increase of solvent amount for both groups and approximately 10% higher biodiesel yield was obtained in *n*-hexane group compared with that in chloroform group. While at solvent volume of 8 mL, chloroform showed a better result with a more 5% conversion ratio. The highest biodiesel yield was obtained under the conditions of 6.0 mL *n*-hexane and 8.0 mL chloroform where the conversion efficiency from algal biomass to biodiesel was 94.3% and 95.7%, respectively. Although highest yield and FAME content were almost equal to each other at 6.0 mL *n*-hexane, and 8.0 mL chloroform conditions, the colour of the biodiesel was different. A light yellow sample was made with no solid residue in the *n*-hexane group. In contrast, there were some solid residues in the chloroform group where the samples' color was brown. As reported by Kulkarni et al. [28] and Goff et al. [29], oxidization of fatty acid and formation of sulfones or sultones from sulfuric acid are two of the possible reasons for dark products in biodiesel production. That protein has a better solubility in higher polarity solvent such as chloroform might be one of the reasons to solid residue in the samples. Dark products could have a smaller cetane number representing a lower power take off and efficiency of biodiesel and the additional residue in the biodiesel could be harmful for engine's lifespan. This suggested that in *in situ* transesterification, nonpolarity solvents should be a better choice than polarity ones. Therefore, 6 mL *n*-hexane was chosen for following experiments considering both biodiesel yield and quality levels.

3.3.2. Effect of Methanol Volume. Apart from the positive effect of *n*-hexane on *in situ* transesterification, the amount

Table 2: Comparison of maximum biomass concentration, biomass productivity, and lipid content of *Chlorella* strains growing in different hydrolysate medium.

Hydrolysate material	Maximum biomass concentration (g/L)	Biomass productivity (g/L/day)	Lipid content (%, w/w)
Rice straw	2.83	1.10	56.3
Corn powder[a]	3.92	0.65	55.3
cassava starch[b]	7.20	0.72	28.9
Cassava[c]	4.26	0.82	50.2
Sweet sorghum[d]	5.10	1.02	53.3

[a] Xu et al. [14]; [b] Wei et al. [6]; [c] Lu et al. [25]; [d] Gao et al. [8].

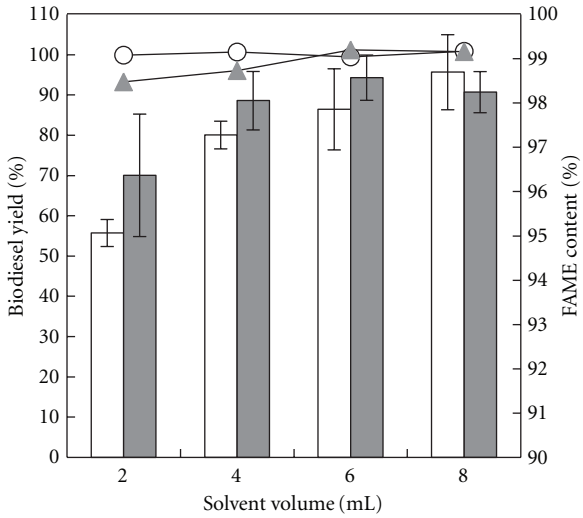

Figure 3: Effects of chloroform and *n*-hexane volume on the biodiesel yield and FAME content by *in situ* transesterification of *C. pyrenoidosa*. Reaction condition: 1 g algal powder with 4 mL methanol and 0.5 M sulfuric acid at 90°C. Solvent volume: 2, 4, 6, and 8 mL. Biodiesel yield (□) and FAME content (○) in the chloroform group, biodiesel yield (■) and FAME content (▲) in the *n*-hexane group. Error bars = mean ± standard deviation.

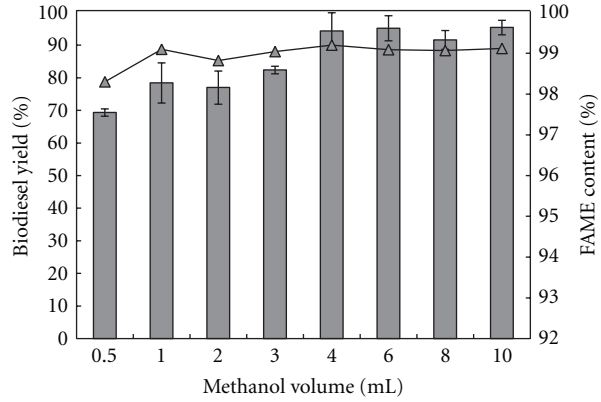

Figure 4: Effects of methanol volume on the biodiesel yield (■) and FAME content (▲) by *in situ* transesterification of *C. pyrenoidosa*. Reaction condition: 1 g algal powder with 6 mL *n*-hexane and 0.5 M sulfuric acid at 90°C. Methanol volume: 0.5, 1, 2, 3, 4, 6, 8, and 10 mL. Error bars = mean ± standard deviation.

of methanol is one of the most important factor affecting quality and quantity of biodiesel production. As supported by many papers [18, 30], an exceeding amount of methanol is essential for *in situ* biodiesel production. In order to know the appropriate volume for algae's *in situ* transesterification, a series of methanol volume (0.5, 1, 2, 3, 4, 6, 8, 10 mL) was selected for biodiesel production with 1 g algal powder, 6 mL *n*-hexane and 0.5 M sulfuric acid at 90°C. The methanol volume from 0.5 to 10 mL represented a reacting alcohol to oil molar ratio from about 21 : 1 to 413 : 1 (calculated with a methanol density of 0.792 g/mL). The results were shown in Figure 4. Biodiesel yield was enhanced from 69.4% to 94.3% when increasing methanol volume from 0.5 mL to 4 mL and the FAME content remained at over 98% level. For further increase of methanol amount, there was no significant difference on the biodiesel yield (about 95%) and FAME content (>98%). Moreover, the excess of methanol quantities also slows down the separation process [15]. Therefore, 4 mL methanol was suggested to be a suitable volume.

Miao and Wu [15] reported a 63% biodiesel yield from algal oil transesterification using an optimized 56 : 1 methanol to oil molar ratio. In our experiments, when methanol to oil molar ratio was 41 : 1 (1.0 mL methanol to 1 g algae), about 78.4% biodiesel yield from algal biomass was obtained, which was 24% higher than a 63% biodiesel production. Such situation could be attributed to introduction of *n*-hexane as an effective solvent in the reaction. The highest biodiesel yield of 94.7% was obtained under the optimized methanol to oil molar ratio of about 165 : 1 (4 mL methanol to 1 g algae). Adequate amount of methanol is required for high yield of *in situ* biodiesel production because methanol plays the role of both reactant and substance to submerge algal powder [19, 31]. These results suggested that *in situ* transesterification for biodiesel production from microalgal biomass could not only save the time and cost, but also have high conversion efficiency.

For the comparison of *in situ* transesterification of algal biomass, Johnson and Wen [17] reported an about 100% yield crude biodiesel using a proportion of 3.4 mL methanol/g algae from *Schizochytrium limacinum* cells. However, the FAME content of their biodiesel was only 63.5%, while it was about 98% in our samples under a 4 mL methanol condition. Ehimen et al. [19] prepared the algae-based biodiesel from *Chlorella* strains that contained 93% FAME. However, the optimized methanol to oil molar ratio they used was about two times as high as that of our results (315 : 1 compared with 165 : 1). This was probably due to the introduction of solvent in our *in situ* transesterification system. The addition of *n*-hexane could help oil extraction so that half of the methanol could be saved. The results suggested that 4 mL methanol to 1 g algal powder was a reasonable ratio for *in situ* biodiesel production, but for specific strains, the suitable reaction condition should be modified.

3.3.3. Effect of Temperature and Reaction Time. Temperature plays an important role in the acid-catalyzed synthesis of biodiesel. To investigate the influence of temperature and reaction time, 1 g algal powder, 6 mL *n*-hexane, 4 mL methanol, and 0.5 M sulfuric acid were mixed for *in situ* transesterification. Reaction temperature was set as 20°C (room temperature), 50°C, 70°C, 90°C, and 110°C. For each temperature, we chose 0.25 hour, 0.5 hour, 1 hour, 2 hours, and 4 hours as sampling time. The data was shown in Figure 5. Our data agreed with the conclusion that higher temperature could produce biodiesel with high FAME content in much shorter time [32]. Higher yield of biodiesel was produced with higher temperature, especially in the first hour of the reaction. Interestingly, we found that there was

Table 3: Components of biodiesel from *Chlorella pyrenidosa* biomass under the conditions of 1 g algal powder, 6 mL *n*-hexane, and 4 mL methanol with 0.5 M sulfuric acid at 90°C.

Components	Molecular formula	Content (%)
10-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	70.18 ± 3.07
9,12-Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	14.53 ± 5.28
Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	10.48 ± 1.36
Octadecanoic acid methyl ester	$C_{19}H_{38}O_2$	3.21 ± 1.24
Methyl tetradecanoate	$C_{15}H_{30}O_2$	0.68 ± 0.07
9-hexadecenoic acid methyl ester (z)	$C_{17}H_{32}O_2$	0.31 ± 0.01
Eicosanoic acid methyl ester	$C_{21}H_{42}O_2$	0.24 ± 0.06
	Total	99.63 ± 0.36

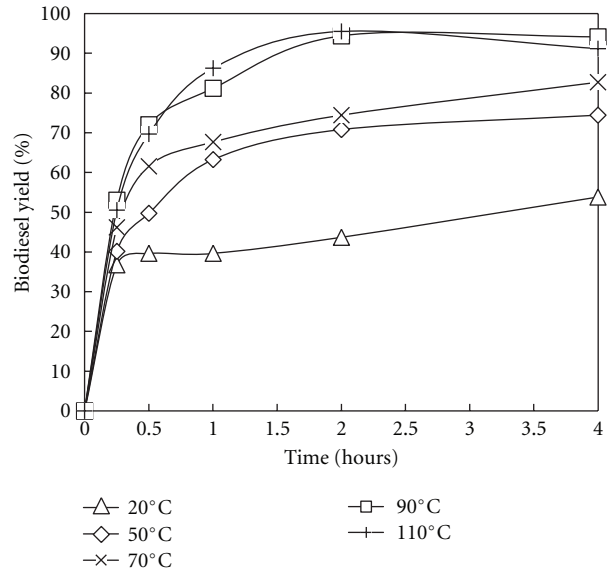

Figure 5: Effects of temperature and reaction time on the biodiesel yield by *in situ* transesterification of *C. pyrenoidosa*. Reaction condition: 1 g algal powder with 6 mL *n*-hexane, 4 mL methanol and 0.5 M sulfuric acid. Temperature: 20°C, 50°C, 70°C, 90°C, and 110°C.

no distinction on the FAME content (>98%) of samples from 0.25 hour to 4 hours, which suggests that the key point of *in situ* transesterification would be how to efficiently extract algal oil from biomass rather than transesterification itself. The largest amount of biodiesel is obtained at both 90°C and 110°C with about 95% yield in 2 hours. At 20°C, 50°C, and 70°C conditions, the yield was lower and still increasing after 4 hours (Figure 5). The reason for more biodiesel production at higher temperature (90°C, 110°C) is that high temperature could lead to a fast transesterification rate. Moreover, high temperature may also improve cell disruption in the *in situ* transesterification system so that oil in the algal biomass could contact the reactants more easily, resulting in a higher biodiesel yield. It was found that there was a slight decrease of biodiesel from 2 hours to 4 hours at 110°C (Figure 5). This is probably caused by that high temperature and long reaction time could burn some of the oil [15]. Based on the quality and quantity standard of biodiesel, the optimized temperature and the reaction time for *in situ* biodiesel production from microalgal biomass were 90°C and 2 hours.

3.3.4. Composition of In Situ Biodiesel. The main FAME components of biodiesel produced from microalgal biomass (1 g) under the optimized conditions of 6 mL *n*-hexane, 4 mL methanol, and 0.5 M sulfuric acid at 90°C with 2 hours reaction time was shown in Table 3. The total FAME content was over 99%. There were seven FAMEs derivatized in the biodiesel, and the most abundant composition was octadecenoic acid methyl ester with the content of 70.18%. Octadecenoic acid methyl ester, octadecadienoic acid methyl ester, hexadecanoic acid methyl ester and octadecanoic acid methyl ester are the dominating acid methyl esters, and the total content of these four FAMEs was over 98%. This could result in the high quality of the biodiesel.

3.4. Possible Methods for Further Utilization of Residue and Byproducts. Chlorella pyrenoidosa was able to accumulate high content of lipids in the cells with fast growth in the rice straw hydrolysate medium. Apart from the cellulose part, other parts of straw could also be used for energy production. Kaparaju et al. [33] reported that the liquid hemicellulose fraction from straw pretreatment could be used for biohydrogen production by dark fermentation and the effluents from biohydrogen process could also be converted to methane. Thus, the processes would be energetically most efficient compared to production of monofuel from straws in such cases. The present results of *in situ* transesterification demonstrated that approximately 95% algal oil was converted to high-quality biodiesel. This suggested that *in situ* biodiesel production from microalgal biomass would be an alternative way for the conversional two-step method. In addition to the biodiesel, glycerol and algae waste, as two main byproducts in algae-based biodiesel production, can be made for different uses. Crude glycerol can be transformed into many high value chemical compounds such as 1,3-propanediol [34], poly-3-hydroxybutyrate [35] and D-glyceric acid production [36]. For algae residue treatment, Sialve et al. [37] suggested that methane production from algae waste after lipid recovery is viable especially for higher lipid content (>40%). All of the above methods can be used to make the best and sustainable use of raw materials in the whole process from straw to algae-based biodiesel. Based on these studies, the idea of biodiesel production from microalgae by using lignocellulose material might be attractive and promising.

4. Conclusions

The study investigated the feasibility of using lignocellulose material, rice straw, as carbon sources for microalgae

cultivation. Enzymatic hydrolyzation of pretreated rice straw produced glucose available for microalgae cultivation. *Chlorella pyrenoidosa* showed a fast growth rate and a high lipid content growing in the hydrolysate medium. Good quality and high yield of biodiesel could be obtained from algal biomass by adding *n*-hexane in *in situ* transesterification system. This method for *in situ* biodiesel production showed a better performance than the traditional two-step method. The strategy provides a combined idea of recovering and reusing discarded lignocellulose and utilizing fibrous biomass for biodiesel production. Further research should be focused on biorefinery of lignocellulose and algal biomass and cost analysis.

Acknowledgments

This work was financially supported by Shanghai Municipal Committee of Science and Technology (08DZ1204400). It was also supported by the 863 Key Program (2007AA100503) and by China National Offshore Oil Corporation.

References

[1] Y. Chisti, "Biodiesel from microalgae," *Biotechnology Advances*, vol. 25, no. 3, pp. 294–306, 2007.

[2] V. Patil, K. -Q. Tran, and H. R. Giselrød, "Towards sustainable production of biofuels from microalgae," *International Journal of Molecular Sciences*, vol. 9, no. 7, pp. 1188–1195, 2008.

[3] J. O. B. Carioca, J. J. Hiluy Filho, M. R. L. V. Leal, and F. S. Macambira, "The hard choice for alternative biofuels to diesel in Brazil," *Biotechnology Advances*, vol. 27, no. 6, pp. 1043–1050, 2009.

[4] EIA, "Gasoline and diesel fuel update," March 2010, http://tonto.eia.doe.gov/oog/info/gdu/gasdiesel.asp.

[5] Y. Cheng, W. Zhou, C. Gao, K. Lan, Y. Gao, and Q. Wu, "Biodiesel production from Jerusalem artichoke (*Helianthus Tuberosus L.*) tuber by heterotrophic microalgae *Chlorella protothecoides*," *Journal of Chemical Technology and Biotechnology*, vol. 84, no. 5, pp. 777–781, 2009.

[6] A. Wei, X. Zhang, D. Wei, G. U. Chen, Q. Wu, and S. T. Yang, "Effects of cassava starch hydrolysate on cell growth and lipid accumulation of the heterotrophic microalgae *Chlorella protothecoides*," *Journal of Industrial Microbiology and Biotechnology*, vol. 36, no. 11, pp. 1383–1389, 2009.

[7] Y. Cheng, Y. Lu, C. Gao, and Q. Wu, "Alga-Based biodiesel production and optimization using sugar cane as the feedstock," *Energy and Fuels*, vol. 23, no. 8, pp. 4166–4173, 2009.

[8] C. Gao, Y. Zhai, Y. Ding, and Q. Wu, "Application of sweet sorghum for biodiesel production by heterotrophic microalga *Chlorella protothecoides*," *Applied Energy*, vol. 87, no. 3, pp. 756–761, 2010.

[9] A. Margaritis and P. Bajpai, "Continuous ethanol production from Jerusalem artichoke tubers. II. Use of immobilized cells of Kluyveromyces marxianus," *Biotechnology and Bioengineering*, vol. 24, no. 7, pp. 1483–1493, 1982.

[10] C. Martín and A. B. Thomsen, "Wet oxidation pretreatment of lignocellulosic residues of sugarcane, rice, cassava and peanuts for ethanol production," *Journal of Chemical Technology and Biotechnology*, vol. 82, no. 2, pp. 174–181, 2007.

[11] C. E. Wyman, "Potential synergies and challenges in refining cellulosic biomass to fuels, chemicals, and power," *Biotechnology Progress*, vol. 19, no. 2, pp. 254–262, 2003.

[12] FAO, "Global cereal production brief," 2009, http://www.fao.org/docrep/012/ai484e/ai484e04.htm.

[13] A. Li, B. Antizar-Ladislao, and M. Khraisheh, "Bioconversion of municipal solid waste to glucose for bio-ethanol production," *Bioprocess and Biosystems Engineering*, vol. 30, no. 3, pp. 189–196, 2007.

[14] H. Xu, X. Miao, and Q. Wu, "High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters," *Journal of Biotechnology*, vol. 126, no. 4, pp. 499–507, 2006.

[15] X. Miao and Q. Wu, "Biodiesel production from heterotrophic microalgal oil," *Bioresource Technology*, vol. 97, no. 6, pp. 841–846, 2006.

[16] K. Vijayaraghavan and K. Hemanathan, "Biodiesel production from freshwater algae," *Energy and Fuels*, vol. 23, no. 11, pp. 5448–5453, 2009.

[17] M. B. Johnson and Z. Wen, "Production of biodiesel fuel from the microalga schizochytrium limacinum by direct transesterification of algal biomass," *Energy and Fuels*, vol. 23, no. 10, pp. 5179–5183, 2009.

[18] S. Siler-Marinkovic and A. Tomasevic, "Transesterification of sunflower oil *in situ*," *Fuel*, vol. 77, no. 12, pp. 1389–1391, 1998.

[19] E. A. Ehimen, Z. F. Sun, and C. G. Carrington, "Variables affecting the *in situ* transesterification of microalgae lipids," *Fuel*, vol. 89, no. 3, pp. 677–684, 2010.

[20] G. Y. S. Mtui, "Recent advances in pretreatment of lignocellulosic wastes and production of value added products," *African Journal of Biotechnology*, vol. 8, no. 8, pp. 1398–1415, 2009.

[21] B. C. Saha, L. B. Iten, M. A. Cotta, and Y. V. Wu, "Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol," *Process Biochemistry*, vol. 40, no. 12, pp. 3693–3700, 2005.

[22] D. Dong, J. Sun, F. Huang, Q. Gao, Y. Wang, and R. Li, "Using trifluoroacetic acid to pretreat lignocellulosic biomass," *Biomass and Bioenergy*, vol. 33, no. 12, pp. 1719–1723, 2009.

[23] Genencor, "Accellerase™ 1000," 2007, http://www.genencor.com/wps/wcm/connect/d23ac9804fa2d4cdaec6be4895e32-24e/ACCELLERASE+1000+prod+info+sheet.pdf?MOD=AJPERES%CACHEID=d23ac9804fa2d4cdaec6be4895e3224e.

[24] F. Sun and H. Chen, "Enhanced enzymatic hydrolysis of wheat straw by aqueous glycerol pretreatment," *Bioresource Technology*, vol. 99, no. 14, pp. 6156–6161, 2008.

[25] Y. Lu, Y. Zhai, M. Liu, and Q. Wu, "Biodiesel production from algal oil using cassava (*Manihot esculenta Crantz*) as feedstock," *Journal of Applied Phycology*, vol. 22, no. 5, pp. 573–578, 2010.

[26] T. R. Larson and T. A. V. Rees, "Changes in cell composition and lipid metabolism mediated by sodium and nitrogen availability in the marine diatom Phaeodactylum tricornutum (Bacillariophyceae)," *Journal of Phycology*, vol. 32, no. 3, pp. 388–393, 1996.

[27] G. Kildiran, S. Ö. Yücel, and S. Türkay, "In-situ alcoholysis of soybean oil," *Journal of the American Oil Chemists' Society*, vol. 73, no. 2, pp. 225–232, 1996.

[28] A. S. Kulkarni, R. R. Khotpal, R. L. Mehta, and H. A. Bhakare, "Studies in sulfation and sulfonation of some semi-drying oils," *PaintIndia*, vol. 41, pp. 41–42, 1991.

[29] M. J. Goff, N. S. Bauer, S. Lopes, W. R. Sutterlin, and G. J. Suppes, "Acid-catalyzed alcoholysis of soybean oil," *Journal of the American Oil Chemists' Society*, vol. 81, no. 4, pp. 415–420, 2004.

[30] J. Qian, F. Wang, S. Liu, and Z. Yun, "*In situ* alkaline transesterification of cottonseed oil for production of biodiesel and nontoxic cottonseed meal," *Bioresource Technology*, vol. 99, no. 18, pp. 9009–9012, 2008.

[31] S. Özgül-Yücel and S. Türkay, "Variables affecting the yields of methyl esters derived from *in situ* esterification of rice bran oil," *Journal of the American Oil Chemists' Society*, vol. 79, no. 6, pp. 611–614, 2002.

[32] X. Miao, R. Li, and H. Yao, "Effective acid-catalyzed transesterification for biodiesel production," *Energy Conversion and Management*, vol. 50, no. 10, pp. 2680–2684, 2009.

[33] P. Kaparaju, M. Serrano, A. B. Thomsen, P. Kongjan, and I. Angelidaki, "Bioethanol, biohydrogen and biogas production from wheat straw in a biorefinery concept," *Bioresource Technology*, vol. 100, no. 9, pp. 2562–2568, 2009.

[34] P. A. Selembo, J. M. Perez, W. A. Lloyd, and B. E. Logan, "Enhanced hydrogen and 1,3-propanediol production from glycerol by fermentation using mixed cultures," *Biotechnology and Bioengineering*, vol. 104, no. 6, pp. 1098–1106, 2009.

[35] C. Zhu, C. T. Nomura, J. A. Perrotta, A. J. Stipanovic, and J. P. Nakas, "Production and characterization of poly-3-hydroxybutyrate from biodiesel-glycerol by Burkholderia cepacia ATCC 17759," *Biotechnology Progress*, vol. 26, no. 2, pp. 424–430, 2010.

[36] H. Habe, T. Fukuoka, D. Kitamoto, and K. Sakaki, "Biotechnological production of d-glyceric acid and its application," *Applied Microbiology and Biotechnology*, vol. 84, no. 3, pp. 445–452, 2009.

[37] B. Sialve, N. Bernet, and O. Bernard, "Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable," *Biotechnology Advances*, vol. 27, no. 4, pp. 409–416, 2009.