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Production of Microbial Lipids from Rice Straw Hydrolysates by *Lipomyces starkeyi* for Biodiesel Synthesis

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Abstract

To minimize the cost of biodiesel production, massively available biomass, rice straw was selected as starting material for fermentation by oleaginous yeast, *Lipomyces starkeyi*. Acid hydrolysis was carried out to obtain Rice Straw Hydrolysates (RSH). Maximum amount of glucose was liberated with 3.5% H_2SO_4. Highest levels of biomass (~12 g/L) and lipid content (~36%) of *L. starkeyi* were obtained through fermentation of RSH produced with 3.5% H_2SO_4. The optimum pH for biomass yield and lipid accumulation of *L. starkeyi* was 6.0. Periodic supplementation of fresh RSH increased lipid content and lipid yield approximately15% and 40%, respectively. The growth and lipid accumulation of *L. starkeyi* were enhanced when carbon and nitrogen sources were supplemented with RSH. Gas chromatographic analysis revealed that the lipid obtained from *L. starkeyi* cultivated with RSH was composed of saturated and unsaturated fatty acids. The fatty acid composition of this microbial lipid is similar to that of vegetable oils. The results reported herein indicate that rice straw might be a valuable alternative feedstock for microbial lipids production by *L. starkeyi* for biodiesel synthesis.

Keywords: Rice straw; Microbial lipids; *Lipomyces starkeyi*; Fatty acids; Biodiesel

Introduction

Due to limited supply, exhaustion in the foreseeable future and negative environmental impacts of fossil fuel, biodiesel has attracted the attention lately as reliable alternatives. As a matter of facts, biodiesel is a nontoxic, degradable and clean energy that can be obtained from a wide range of renewable sources. Usage of biodiesel reduces greenhouse gas emission, deforestation and pollution, and thus let a balance to be sought between agriculture, economic development and the environment [1]. It is reported that biodiesel produced from soy oil and waste grease can reduce the emission of greenhouse gas by 57% and 87%, respectively when compared to petroleum diesel [2]. Demand for biodiesel has increased significantly due to the instability of petroleum prices and the development of government measures in many countries around the world that a minimum proportion of biodiesel must be used in parallel of petroleum diesel. The European Union established a minimum content of 5.75% of biodiesel by 2010 (European Union Directive 2003/30/EC) and the United States plans to increase the amount of bioethanol and biodiesel to 36 billion gallons by 2022 (Energy Independence and Security Act of 2007). But, the high cost of biodiesel, of which raw material amounts to about 75%, has become one of the major obstacles to its wide application [3].

Traditionally, vegetable oil, animal fat and cocking waste oil were used for biodiesel production [4]. However, plant based feedstock have some limitations for biodiesel production due to long time and large amount of lands for plant growth. Moreover, using of plant based feedstock encounters a problem that their use for biodiesel production would turn away agricultural lands from their original purpose that is food production and compete with edible oils, thus leading to food insecurity and raise in food price. The increasing demand of biodiesel makes in search of alternative cheap sources from which good quality biodiesel could find, and agro-industrial wastes are notable [5]. Different agro-industrial wastes showed remarkable capacity to be used for microbial lipid production i.e. glycerol, molasses, whey, olive mill wastes, and plant waste hydrolysates [6,7]. Sewage sludge was used very recently for biodiesel production [8,9]. To convert agro-industrial wastes to microbial lipids that represent a valuable suitable feedstock for biodiesel production, a large number of oleaginous microorganisms involving bacteria, yeasts, molds and microalgae are regularly used, which have the capability to accumulate lipids with the similar fatty acid composition to that of vegetable oils [3,10,11]. *Lipomyces starkeyi*, the oleaginous yeast, could utilize a variety of carbon sources (i.e. xylose, glucose) and accumulates a high amount of intracellular lipids [12]. Amongst various agro-industrial waste materials, rice straw, whose hydrolysate mainly contains glucose, xylose, and arabinose [13], is the most abundant in Bangladesh as well as in South Asia and therefore, it was chosen as the raw material. Moreover, fermentation inhibitors in Rice Straw Hydrolysates (RSH) is much lower than in other lignocellulosic hydrolysates [3,13]. In Bangladesh, currently rice straw is used only as feed of cattle or left in the land. The RSH was tested for lipid production previously by *Trichosporon fermentans* [3]. In this study, the possibility of microbial lipid production from RSH by *L. starkeyi* was explored and production conditions were optimized. Furthermore, lipid production by *L. starkeyi* from the RSH medium supplemented with different carbon and nitrogen sources was studied. Finally the fatty acid profile of the microbial lipid was determined.

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Material and Methods

Preparation of RSH

Rice straw was collected from local farm land and cut into smaller pieces. After washing in running tap water, straw was heated at 60°C for overnight. Dried rice straw particles were then grinded by a grinder machine to generate rice straw dusts. Dust particles were mixed with diluted H_2SO_4 with ratio of 1:10 (w/v). The mixture was then autoclaved at 121°C for 2 h and filtered through Whatman no. 1 filter paper. Saturated NaOH was added to the filtrate to adjust the desired pH and that was the RSH. The effects of H_2SO_4 concentrations on glucose liberation from rice straw were investigated by using different concentrations of H_2SO_4 to prepare RSH.

RSH was divided into two parts; one part was kept as it was. The other part was used to modify the RSH to investigate different parameters. To make low nitrogen content of RSH, 2M H_2SO_4 was added to adjust the pH at 3.0 and the mixture was centrifuged for 20 min at 4500 rpm and 4°C. The precipitate was removed by filtration through Whatman no. 1 filter paper. The pH of filtrate was adjusted to 9.0 with saturated NaOH and the mixture was centrifuged for 20 min at 4500 rpm and 4°C. The precipitate was removed by filtration through Whatman no. 1 filter paper and finally the pH was adjusted to 6.0 with 2M H_2SO_4 and this was the lower nitrogen containing RSH, RSHLN. The higher nitrogen content, RSHHN was prepared by adding 1% of NH_4NO_3 (RSHHN1%NH_4NO_3), $(NH_4)_2SO_4$ (RSH HN1% $(NH_4)_2SO_4$), peptone (RSHHNHN1% Peptone), yeast extract (RSHHN1% Yeast Extract) and malt extract (RSHHN1% Malt Extract) separately in RSH. One gram of glucose, sucrose and maltose were added separately to 100 ml of RSH to make RSH1% glucose, RSH1% sucrose and RSH1% maltose, respectively.

RSH were also adjusted to four different pH values 5.0, 5.5, 6.0 and 6.5 to optimize the microbial growth. Three different dilutions (RSH100%, RSH50%, RSH25%) of RSH were made by adding sterile distilled water accordingly. Glucose concentration of RSH medium was measured by DNS method [14].

Microorganism and fermentation in shaken flasks

L. starkeyi previously used by Yousuf et al. [7] was obtained by the collection of Department of Chemical Engineering and Polymer Science, Shahjalal University of Science and Technology, Bangladesh. The strain was maintained on potato dextrose agar at 4°C as described previously [7].

The effect of the different compositions of RSH on the growth of *L. starkeyi* was checked by growth curve analysis. The fermentation was carried out in the conical flask of 500 ml. A 100 ml liquid medium was inoculated with 3 ml of *L. starkeyi* suspension, obtained by dissolving 10 loops of solid culture in 10 ml of physiological solution and adjusted the OD600 to 2. OD600 of 1.0 equals approximately1.5×10^7 yeast cells/ml. Inoculum size at 0 h was determined by measuring the difference of OD600 of liquid medium before and after inoculation. The flasks were then incubated in a rotary shaker at an agitation rate of 160 rpm and an incubation temperature 30°C. The *L. starkeyi* cultured in different RSH media were taken periodically to measure OD600. Un-inoculated RSH medium was used as a control. Difference of OD600 between the sample and control was recorded for making graph up to 72 h at 8 h intervals to investigate the growth kinetics. Samples were also collected at 24 h intervals, in triplicates, for determination of biomass and lipid accumulation.

Microbial lipid extraction

After fermentation, yeast cells were separated by centrifugation at 8000 rpm for 15 minutes then dried and weighted. Dry cells were suspended to potassium phosphate buffer (KP buffer), pH 7.0 at the ratio of 1:5 (w/v). Cells were disrupted with a sonicator (UP50H, Hielscher, Germany) at 30 kHz for 5 min. Lipids were then extracted from the disrupted cell suspension in accordance with the method described by Bligh and Dyer (1959). A 3.75 ml of $CHCl_3$ with ratio of 1:2 (v/v) $CHCl_3$: MeOH was added to 1 ml disrupted cell sample. Finally, 1.25 ml distilled H_2O was added and vortex well. Then the mixture was centrifuged at 1000 rpm for 5 min to provide a two-phase system (aqueous top, organic bottom). Bottom phase was recovered by inserting Pasteur pipette through the upper phase with gentle positive-pressure (i.e., gentle bubbling) so that the upper phase does not get into the pipette tip. Recovered bottom phase was kept overnight at 45°C and the solvent was evaporated as previously described [15].

Determination of fatty acid composition

The composition of fatty acids in the extracted lipids was determined by gas chromatography (GC-14B, Shimadzu Corporation, Japan) as described previously [16]. The GC was equipped with a flame ionization detector and a capillary column (Packed Fame wax by Restek, USA). The column temperature was programmed to increase from 80°C to 360°C at 10°C/min and kept at 360°C for 10 minutes for thermal stability before use. The temperature of both the injector and the detector was maintained at 370°C. A 20 mg of sample was dissolved in 1 ml of ethyl acetate and 5 µl of sample was taken and injected into the GC. Nitrogen gas was used as the carrier gas at a flow rate of 0.80 ml/min. Fatty acids were identified by comparison of their retention times with those of standard ones, quantified based on their respective peak areas and normalized.

Statistical analysis

All experiments were carried out twice with sample size of at least n=3. Statistical analysis and data presentation was done as described previously [17].

Results and Discussion

Effects of H_2SO_4 concentrations on rice straw pretreatment

Hydrolysis was conducted with 10%, 5%, 3.5% and 0.0% (w/v) of H_2SO_4 to optimize the condition at which maximum amount of rice straw was transformed into fermentable sugars. Amongst sugars, we measured only the amount of released glucose. Formation of glucose from rice straw was the highest at 3.5% of H_2SO_4 in the hydrolysis process (Table 1). Hydrolysis was also performed with concentrations of H_2SO_4 lower than 3.5% and 3.5% of H_2SO_4 was the optimum one that liberated maximum level of glucose. Acid hydrolysis converts complex polysaccharides into simple sugar. The cellulose and hemicellulose portions are broken down by diluted acid into fermentable sugar that

RSH medium	Glucose concentration (g/L)		Dry weight of biomass (g/L)	Lipid content (% of dry cell weight)	Lipid yield (g/L)
	Before inoculation	After 72 h incubation			
$RSH_{(0.0\%\ H2SO4)}$	12.07 ± 0.41	2.53 ± 0.09	5.72 ± 0.11	16.50 ± 0.59	0.94 ± 0.04
$RSH_{(3.5\%\ H2SO4)}$	29.50 ± 1.41	4.75 ± 0.12	12.76 ± 0.14	35.65 ± 1.67	4.55 ± 0.13
$RSH_{(5\%\ H2SO4)}$	22.69 ± 1.21	3.56 ± 0.11	12.06 ± 0.14	32.70 ± 1.49	3.94 ± 0.11
$RSH_{(10\%\ H2SO4)}$	19.75 ± 1.03	3.15 ± 0.13	10.43 ± 0.12	28.39 ± 0.99	2.96 ± 0.08

Table 1: Effects of H_2SO_4 concentrations on glucose release from rice straw[a]. [a]Dry weight of biomass and lipid content were determined after 72 h of fermentation.

can be up taken by microorganisms. When hemicellulose is degraded, xylose, mannose, acetic acid, galactose and glucose are liberated [13]. Cellulose is hydrolyzed to glucose. When hydrolysis takes place at severe conditions such as higher temperatures and higher acid concentrations or at prolonged period of pretreatment, xylose is further degraded to furfural. Similarly, 5-hydroxymethyl furfural is formed from hexose degradation and these by-products are considered as fermentation inhibitors [18].

L. starkeyi was cultured (initial pH and temperature were 6.0 and 30°C, respectively) with RSH prepared by different H_2SO_4 concentrations to investigate the effect of glucose concentrations on its growth. The biomass that indicated the growth of *L. starkeyi* was proportional to the concentration of glucose (Table 1). Therefore, in the subsequent experiments 3.5% H_2SO_4 was used to prepare RSH. The growth kinetics expressed as biomass and OD600 (Figure 1 and Figure 2, respectively) revealed that the growth was almost saturated after 48 h of fermentation. However, after 48 h of fermentation, only ~60% of glucose was assimilated while ~85% of the glucose was assimilated after 72 h of fermentation (Figure 1). Up to 24 h of fermentation, lipid accumulation was very low compared to that at subsequent stages (Figure 1). However, after 24 h of fermentation, the lipid accumulation gradually increased up to 72 h of cultivation. As the lipid content was maximum after 72 h of fermentation, all the data of biomass and lipid accumulation shown herein was of 72 h of cultivation.

Effects of pH on yeast growth and lipid accumulation

Fermentation of hydrolysates is significantly affected by initial pH of the media. The RSH in the present study was categorized into four types depending on initial pH values- RSHpH5, RSHpH5.5, RSHpH6 and RSHpH6.5. Firstly, the influence of pH on growth was determined by taking OD600 at regular interval. It was observed that microbial growth was faster and maximum in RSHpH6 (Figure 2A) and the growth kinetics was almost the same as was observed in Figure 1. After 72 h of incubation, dry weight of biomass and lipid yield at each pH treatment were measured. Table 2 showed that pH 6.0 was the most favorable condition for the biomass yield and lipid accumulation of *L. starkeyi*. It was shown previously that fermentation of RSH was inhibited at pH 4.5 due to inhibitory effect of acetic acid that could be overcome by working at pH 6.0 [13]. For subsequent experiments, pH 6.0 was maintained for RSH broth.

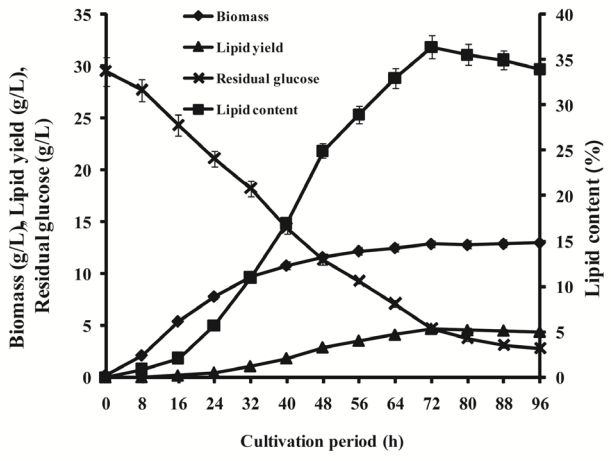

Figure 1: Time course of cell growth and lipid accumulation of *L. starkeyi* cultivated in RSH prepared with 3.5% of H_2SO_4.

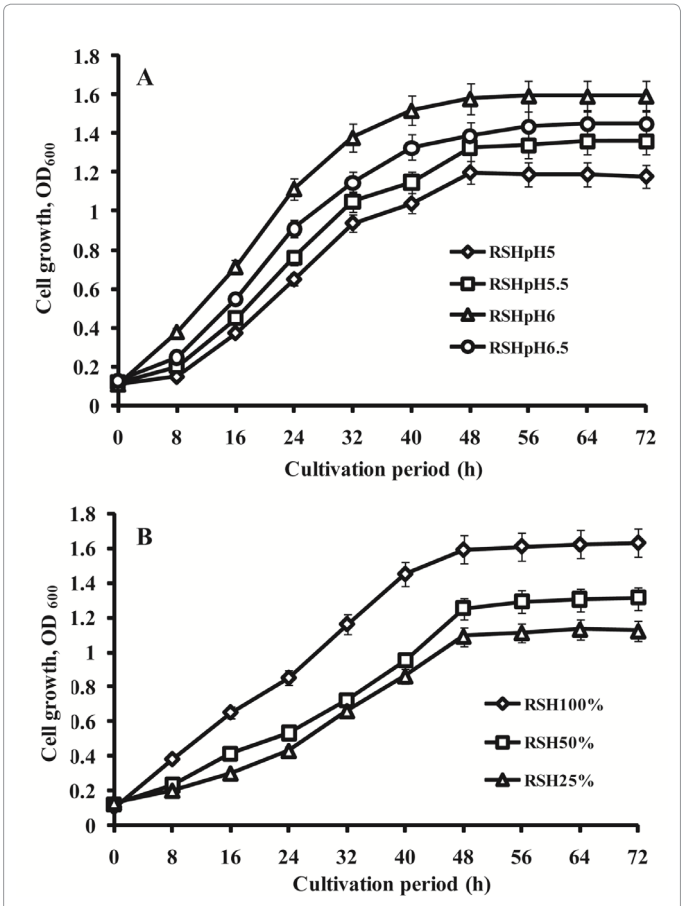

Figure 2: Effects of initial pH of the medium (A) and RSH dilution (B) on growth kinetics of *L. starkeyi*.

	RSH Medium	Dry weight of biomass (g/L)	Lipid content (% of dry cell weight)	Lipid yield (g/L)
Initial pH of the medium	RSH_{pH5}	11.32 ± 0.16	26.08 ± 1.33	2.95 ± 0.06
	$RSH_{pH5.5}$	11.39 ± 0.17	28.47 ± 1.36	3.24 ± 0.09
	RSH_{pH6}	12.59 ± 0.14	36.45 ± 1.55	4.59 ± 0.15
	$RSH_{pH6.5}$	12.05 ± 0.12	32.31 ± 1.49	3.89 ± 0.13
RSH dilution	$RSH_{100\%}$	12.42 ± 0.17	36.89 ± 1.66	4.58 ± 0.14
	$RSH_{50\%}$	8.74 ± 0.19	27.33 ± 1.38	2.39 ± 0.11
	$RSH_{25\%}$	6.30 ± 0.11	21.17 ± 0.92	1.33 ± 0.04

Table 2: Effects of initial pH of the medium and RSH dilution on biomass yield and lipid accumulation of *L. starkeyi*.

Effects of RSH dilutions

Dilution would help to reduce the concentration of antimicrobial agent if present in the broth. Growth rate was observed in 100% RSH (RSH100%), 50% RSH (RSH50%) and 25% RSH (RSH25%). In the case of RSH100%, growth rate, biomass yield and lipid content were maximum (Figure 2B and Table 2). This was due to the presence of higher amount of fermentable glucose in RSH100% than that of RSH50% and RSH25%. Again, the biomass and lipid content decreased proportionally to RSH dilutions. This result further indicated that at this experimental condition there was no fermentation inhibition. Due to higher cell growth and maximum yield of biomass and lipid content, RSH100% media were used for all subsequent experiments.

Fed batch fermentation increases biomass yield and lipid accumulation

To measure the effect of periodic supplementation of fresh RSH medium into old culture, RSH was categorized into three flasks of 500 ml containing 100 ml of RSH. RSHS0 remained as it was for 72 h after inoculation with *L. starkeyi*. In RSHS1, fresh 50 ml RSH was added after 24 h of inoculation, whereas RSHS2 got an additional RSH supplementation at 48 h after inoculation. The effect of supplementation of fresh RSH into old culture was measured in terms of growth curve analysis, biomass production and lipid content (Figure 3; Table 3). Results showed that supplementation of fresh medium enhanced the growth of *L. starkeyi* due to additional nutritional requirements such as nitrogen and carbon. The RSHS1 and RSHS2 increased the total biomass (~9% and ~21%, respectively), lipid content (~9% and ~15%, correspondingly) and lipid yield (~19% and 40%, respectively) compared to those with RSHS0. The results suggested that RSH could be used in fed-batch fermentation of *L. starkeyi*.

Effects of supplementation of carbon and nitrogen sources

The effect of the additional carbon in RSH was studied to evaluate the growth pattern of *L. starkeyi* as shown in the Figure 4. Results of this experiment revealed that the additional carbon in RSH enhanced the growth of *L. starkeyi* along with the lipid accumulation (Table 4). Table 4 showed that the maximum level of biomass and lipid content was obtained by *L. starkeyi* when 1% glucose was supplemented in RSH. This is occurred due to the increase of free glucose content in the medium and *L. starkeyi* can uptake glucose more readily than that of sucrose and maltose. The increase in lipid content by glucose supplementation was more than 2- and 3- fold in compared to that by sucrose and maltose supplementation, respectively. In addition, RSH 1% Glucose had increased total biomass yield by ~23% whereas RSH 1% Sucrose and RSH 1% Maltose had increased that by ~9% and ~8%, respectively.

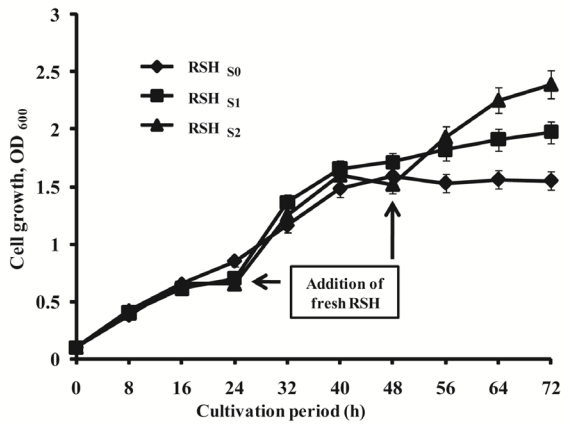

Figure 3: Effects of periodic supplementation of fresh RSH on the growth of *L. starkeyi*.

RSH was categorized into three types depending on the nitrogen content- higher nitrogen (RSHHN), normal nitrogen (RSH) and lower nitrogen (RSHLN). It was observed that microbial growth was maximum in RSHHN 1% Yeast extract (Figure 5 and Table 4). The somewhat lower biomass yield by RSHHN 1% Malt extract (Table 4) might be due to lower nitrogen content in comparison with yeast extract and peptone. RSH supplemented with both organic and inorganic nitrogen sources enhanced the growth and biomass yield of *L. starkeyi*. However, supplementation of organic nitrogen source favored the increased biomass yield than the inorganic nitrogen source (Table 4). Nevertheless, although supplementation of organic nitrogen sources increased lipid accumulation, that of inorganic nitrogen sources did not change the accumulation of lipid. In contrast, RSHLN had decreased the total biomass yield by ~40% but increased the lipid content by ~6%. This result indicated that the lipid accumulation by the oleaginous *L. starkeyi* was triggered by nitrogen limitation. Before nitrogen limiting condition, culture might have been in exponential phase and biomass might be increased. In batch fermentation, nitrogen exhaustion stops the growth of oleaginous microorganisms and induces cascades of biochemical events for lipid accumulation [19,20]. Therefore, the high C/N ratio might have channelized the extra carbon into lipid accumulation after the arrival of nitrogen limiting condition. Indeed, the level of lipid accumulation depends on the concentration of biomass constituted during the growth phase [19] which in turn depends on the initial concentrations of carbon and nitrogen added.

In terms of total biomass and lipid production by *L. starkeyi*, carbon and nitrogen supplementation in RSH medium could increase both of them. These results suggested that pretreated cellulosic and

RSH medium	RSH supplementation period			Dry weight of biomass (g/L)	Lipid content (% of dry cell weight)	Lipid yield (g/L)
	0 h	24 h	48 h			
RSH_{S0}	100 ml	-	-	12.52 ± 0.10	36.14 ± 1.55	4.52 ± 0.14
RSH_{S1}	100 ml	50 ml	-	13.64 ± 0.17	39.45 ± 1.59	5.38 ± 0.15
RSH_{S2}	100 ml	50 ml	50 ml	15.25 ± 0.23	41.45 ± 1.68	6.32 ± 0.17

Table 3: Effects of periodic RSH supplementation on biomass yield and lipid accumulation of *L. starkeyi*.

	RSH Medium	Dry weight of biomass (g/L)	Lipid content (% of dry cell weight)	Lipid yield (g/L)
Carbon sources	RSH	12.46 ± 0.18	36.82 ± 1.43	4.59 ± 0.16
	$RSH_{1\%glucose}$	15.39 ± 0.11	39.47 ± 1.66	6.07 ± 0.18
	$RSH_{1\%sucrose}$	13.58 ± 0.13	38.05 ± 1.53	5.17 ± 0.17
	$RSH_{1\%maltose}$	13.50 ± 0.12	37.71 ± 1.49	5.09 ± 0.16
Organic nitrogen source	RSH_{LN}	7.42 ± 0.11	38.89 ± 1.66	2.89 ± 0.07
	$RSH_{HN1\%peptone}$	14.84 ± 0.19	37.91 ± 1.48	5.63 ± 0.19
	$RSH_{HN1\%yeast\ extract}$	16.14 ± 0.18	38.61 ± 0.92	6.23 ± 0.18
	$RSH_{HN1\%malt\ extract}$	14.25 ± 0.14	37.73 ± 1.59	5.38 ± 0.17
Inorganic nitrogen source	$RSH_{HN1\%NH4NO3}$	13.91 ± 0.17	36.96 ± 1.61	5.14 ± 0.15
	$RSH_{HN1\%(NH4)2SO4}$	13.28 ± 0.13	37.19 ± 1.45	4.94 ± 0.14

Table 4: Effects of supplementation of carbon and nitrogen sources on biomass yield and lipid accumulation of *L. starkeyi*.

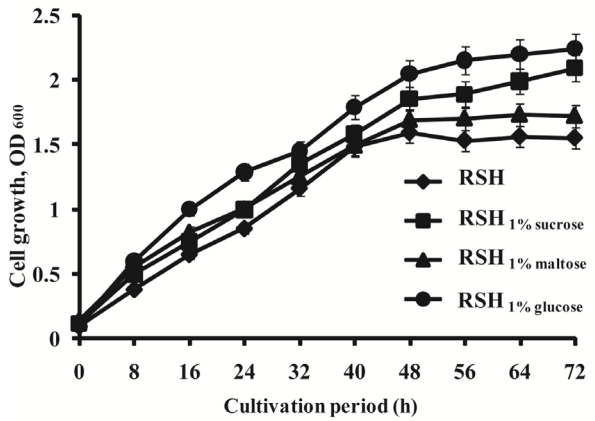

Figure 4: Effects of carbon source supplementation in RSH on the growth of *L. starkeyi*.

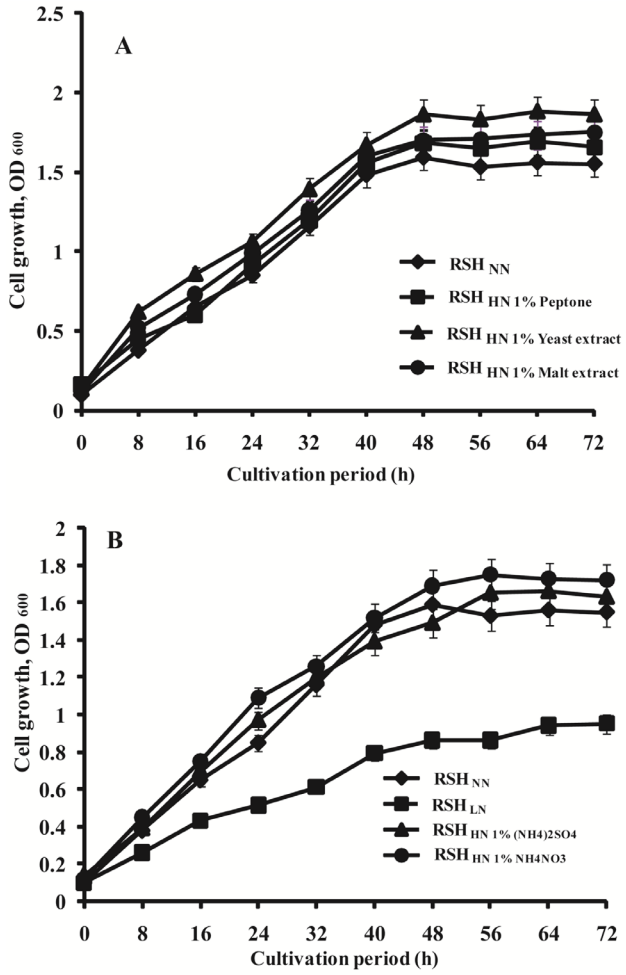

Fig. 5

Figure 5: Effects of supplementation of organic (A) and inorganic (B) nitrogen sources in RSH on the growth of *L. starkeyi*.

proteinous agro-industrial wastes and/or organic municipal wastes as supplement in RSH might have tuned up lipid accumulation by *L. starkeyi*. Currently we are performing study to compare the growth kinetics and lipid accumulation of *L. starkeyi* by using RSH, sugarcane baggage hydrolysates and municipal wastes hydrolysates in batch and fed-batch fermentation.

Experimental evidence has shown that microbial lipid accumulation capacity can be tuned up by application of complex organic nitrogen source [21]. *L. starkeyi* can accumulate lipid ~60% of its dry weight under optimal conditions of carbon source, yeast extract and ferrous sulphate, and lipid production was maximal when glucose and xylose ratio was 2:1 [12]. Two enzymes namely, citrate lyase and malic enzyme are exclusively responsible for the lipid accumulation of oleaginous microbes and the regulation of the gene for malic enzyme in oleaginous Mucor circinelloides leads to a 2.5-fold increase in lipid accumulation [22,23]. This study suggests the further research for finding the correlation between RSH and the expression patterns of these genes in *L. starkeyi*.

Fatty acid composition of the lipid

The GC analysis revealed that the lipid extracted from *L. starkeyi* after 72 h of cultivation in RSH was composed of three main types of fatty acids that could be present in a triglyceride: saturated (Cn:0), monounsaturated (Cn:1) and polyunsaturated with two to four double bonds (Cn:2,3,4) (Table 5). The fatty acid composition of the lipid is similar to that of vegetable oils, and so the lipid with this fatty acid composition is a promising feedstock for biodiesel production through transesterification [10,24]. Considering the amount of monounsaturated and polyunsaturated fatty acids present in this microbial lipid, the degree of unsaturation (DU) was calculated in accordance with the empirical equation [DU=(monounsaturated Cn: 1; wt:%)+2 (polyunsaturated Cn: 2;3;4; wt:%)] described by Ramos et al. [24]. The DU, which was calculated as 61.39, might have influenced the cetane number of the biodiesel to be synthesized with the extracted lipid [24]. The higher the cetane number, the better the ignition properties of the biodiesel [1]. The higher cetane number is associated with lower DU and vice versa [25], and higher DU than 137 makes lipids unsuitable to meet the European Standard for the cetane number. Again, the abundance of lignoceric acid, the longer saturated fatty acid including other saturated fatty acids might be associated with higher cetane number [25,26]. Furthermore, the lower DU indicated the higher oxidation stability of the biodiesel to be synthesized with the microbial lipid produced from rice straw by *L. starkeyi*. However, further research is necessary to synthesize biodiesel with this microbial lipid and to study its physico-chemical properties.

The results described above indicate that without the detoxification treatment of RSH which was done by Huang *et al.* for fermentation by *T. fermentans* [3], the lipid content (~36%) and yield (`4.6 g/L) of *L. starkeyi* during batch fermentation was much higher (~244% and 268%, respectively) than those of *T. fermentans* although the detoxification treatment of RSH made *T. fermentans* accumulate

Fatty acids composition	%
Tridecanoic acid C13:1	0.47
Palmitic acid C16:0	5.42
Stearic acid C18:0	3.86
Oleic acid C18:1	28.26
Linolenic acid C18:3	9.14
Arachidonic acid C20:4	7.19
Behenic acid (22:0	3.08
Lignoceric acid C24:0	41.56

Table 5: Distribution of fatty acids in the lipid accumulated in *L. starkeyi* grown on RSH.

lipid 40.1% that corresponded to lipid yield of 11.5 g/L. However, the capability of utilizing RSH may vary with microbial strains [3]. Although *L. starkeyi* accumulated the highest lipid content (68%) when grown on sewage sludge [8], similar lipid yield was obtained by fed-batch fermentation of RSH (Table 3). The results further indicate that periodic supplementation (fed-batch) of fresh RSH was sufficient to achieve more lipid content and yield compared to the supplementation of commercial carbon and nitrogen sources to the RSH (Tables 3 and 4). Therefore, RSH might be more potential raw material for large scale production of microbial lipids by *L. starkeyi* for biodiesel synthesis due to its availability and much lower cost without detoxification treatment.

Conclusion

As of abundance, RSH was chosen to determine its suitability to microbial lipid production. The RSH prepared with 3.5% H_2SO_4 liberates optimum level of glucose for maximum growth and lipid accumulation of *L. starkeyi*. It grows well with efficient lipid accumulation on the RSH without any detoxification treatment of RSH. Periodic feeding of RSH increases lipid content and yield, indicating that pretreated cellulosic and proteinous agro-industrial and/or organic municipal wastes can be supplemented to RSH for boosting up the lipid accumulation by *L. starkeyi*, and so *L. starkeyi* is a promising strain for economic production of microbial lipids from RSH.

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