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Lipid Production by Culturing Oleaginous Yeast and Algae with Food Waste and Municipal Wastewater in an Integrated Process

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Abstract Food waste and municipal wastewater are promising feedstocks for microbial lipid biofuel production, and corresponding production process is to be developed. In this study, different oleaginous yeast strains were tested to grow in hydrolyzed food waste, and growths of *Cryptococcus curvatus*, *Yarrowia lipolytica*, and *Rhodotorula glutinis* in this condition were at same level as in glucose culture as control. These strains were further tested to grow in municipal primary wastewater. *C. curvatus* and *R. glutinis* had higher production than *Y. lipolytica* in media made from primary wastewater, both with and without glucose supplemented. Finally, a process was tested to grow *C. curvatus* and *R. glutinis* in media made from food waste and municipal wastewater, and the effluents from these processes were further treated with yeast culture and phototrophic algae culture; 1.1 g/L *C. curvatus* and 1.5 g/L *R. glutinis* biomass were further produced in second-step yeast cultures, as well as 1.53 and 0.58 g/L *Chlorella sorokiniana* biomass in phototrophic cultures. The residual nitrogen concentrations in final effluents were 33 mg/L and 34 mg/L, respectively, and the residual phosphorus concentrations were 1.5 and 0.6 mg/L, respectively. The lipid contents in the produced biomass were from 18.7% to 28.6%.

Keywords Oleaginous yeast · Algae · Biodiesel · Municipal wastewater · Food waste

Introduction

Exploration of single cell oil (SCO) production by yeast fermentation began in the 1970s to produce SCO from microorganisms to substitute lipids from plants as a source of food for humans and feed for animals [1]. Cheese whey was used to culture oleaginous yeast *Cryptococcus curvatus* (*Candida curvata*) to produce cocoa butter substitute. Fermentation processes were developed and tested on both pilot scale (0.5-m^3 fermentor) and industrial scale (8.2-m^3 fermentor) [2]. Based on these studies, Davies has estimated the

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manufacturing cost of refined yeast oil to be 800 to 1,000 USD/ton [1, 2]. Beginning in the 1990s, a series of heterotrophic microalgae species were used to produce high-value polyunsaturated fatty acids, such as omega-3 fatty acids, to substitute for the traditional source fish oil [3]. Certain heterotrophic algae such as *Schizochytrium* sp. and *Cryptocodinium cohnii* were successfully used in industrial-scale docosahexaenoic acid (DHA) production processes, and commercially available algal DHA products have been used as supplements for infant formulae [4].

There has been increasing interest in seeking alternative lipid sources for biodiesel production. Although it is economically viable to use corn syrup for DHA production [4] and it was almost economically viable to use cheese whey for cocoa butter equivalent production [2], recent research on using glucose as feedstock for heterotrophic microalgae culture to produce biodiesel showed disappointing results due to high costs [5]. Biomass hydrolysates were tested and believed to be good feedstock for single cell lipid production [6], but this approach faces the same economical problem with cellulosic bioethanol production, which is how to produce sugar from cellulosic biomass at a sufficiently low cost. However, using organic waste should provide an option to greatly decrease production costs, since it has negative value. For example, monosodium glutamate wastewater has been determined to be a good feedstock [7, 8]. However, this single source is limited in quantity and thus similar waste streams need to be investigated.

More than 96 billion pounds of edible food was discarded in the USA in 1995 [9], and it can be assumed this amount is increasing. In Washington State alone, 246,000 dry tons of food waste is produced each year [10]. Food waste contains 46.8% of carbon in the dry matter [11], and the carbohydrate content in packed food and rice mixture was 67% and 66%, respectively [12]. If we assume that 80% of the carbohydrates present can be hydrolyzed, the produced sugar will be 53% of the food waste dry weight. In culture, 6 kg of sugar produce 1 kg of oil lipid in a yeast culture process [2]. Thus, the rough conversion rate from dry food waste to lipids is about 8.8%. Using Washington State as an example, 21,600 tons of lipids could be produced from the 246,000 dry tons of food waste per year. If processed into biodiesel, it could produce 6.2 million gallons of biodiesel.

As far as organic carbon in municipal wastewater is concerned, a ten-state standard estimate of the biodegradable carbon produced by people is 0.17 lb/capita/day as 5-day biological oxygen demand (BOD5) or 0.22 lb/capita/day when garbage grinders are used [13]. Typical municipal wastewater contains 110–350 mg/L of BOD5 [14]. A significant amount of biofuel will be produced if all of this organic carbon can be converted into oil-enriched biomass. Research on this has been reported, but it was found that lipid content in the secondary sludge was only 2.5% [15]. The major cell mass in the secondary sludge was bacteria, which does not accumulate a high content of lipid. Thus, oleaginous microorganisms should be used in this process in order to obtain a higher lipid yield.

Converting organic waste into microbial lipids faces two major challenges. First, the oleaginous microbial strains that can grow in organic waste environment have to be screened. Second, the nutrients in the organic waste have to be completely removed during the cell culture processes. Otherwise, the wastewater stream from cell culture processes still contains very high residue carbon source concentrations [2] and chemical oxygen demand (COD) [7], which requires further treatment. Thus, a process is to be developed to completely remove the nutrients in food waste and wastewater, to convert them into microbial biomass and lipid. In this study, we tested if the oleaginous yeast strains can grow in food waste environment and proved that *C. curvatus*, *Yarrowia lipolytica*, and *Rhodotorula glutinis* can be used as effective strains in this process. Also, municipal wastewater was proved to be good water source for culture of *C. curvatus* and *R. glutinis*.

Finally, we tested to use second-step yeast culture and further phototrophic algae culture to treat the effluent stream to remove the residue COD, nitrogen, and phosphorus. The results showed that this process removed most residue COD, nitrogen, and phosphorus from the fermentation effluent and that oleaginous yeast and algae biomass were produced at the same time. This research provided a practical process to convert organic wastes into biofuel, with no waste or nutrients left in the final effluent stream.

Materials and Methods

Food Waste Processing and Hydrolysis

Food waste was collected from cafeteria system at Washington State University, Pullman, WA, USA. It was ground with a food processor, and the wet solids were mixed with water. Concentrated sulfuric acid 3.0% (*v*/*v*) was then added to the food waste broth to hydrolyze the polysaccharides and protein in the food waste. The mixture of food waste and wastewater was then placed in an autoclave at 121 °C for 30 min. The autoclaved mixture was neutralized with sodium hydroxide, and the liquid was separated from the solids by centrifugation. The sugar in the food waste hydrolyzed broth (FWHB) was analyzed by ion chromatography.

Sugar Analysis

The glucose, arabinose, xylose, and galactose concentrations in the hydrolysate were analyzed by a Dionex ICS-3000 ion chromatography system (Dionex Corporation) equipped with a CarboPac TM PA 20 (4×50 mm) analytical column and a CarboPac TM PA 20 (3×30 mm) guard column. Samples were filtered through a 0.2-μm-pore-size filter before injection and eluted with 0.01 M NaOH at a flow rate of 0.5 mL/min. The analytes were detected and quantified against standard carbohydrates by electrochemical detection in a pulsed amperometric detector.

Yeast Strains and Medium

Five strains of oleaginous yeast which can use a variety of carbon sources were obtained from the American Type Culture Collection (ATCC) and included: *C. curvatus* (ATCC 20509), *Y. lipolytica* (ATCC 20460), *Lipomyces starkeyi* (ATCC 12659), *Rhodosporidium toruloides* (ATCC 10788), and *R. glutinis* (ATCC 204091). The seed cells of these yeast strains were cultured in a medium consisting of 10 g/L glucose, 1 g/L peptone, and 1 g/L yeast extract. The pH of the medium was adjusted to 6.0.

Oleaginous Yeast Culture with Food Waste Hydrolyzed Broth

The FWHB supernatant was used as culture media for the yeast strains. The medium prepared with water, rather than with FWHB, was used as the control medium, which consisted of 5 g/L peptone, 5 g/L yeast extract, and 20 g/L glucose. For each culture, 5 mL of the sub-cultured cells was used as seed cells to inoculate 50 mL culture medium placed into a 250-mL Erlenmeyer flask. The flask was incubated at 25 °C in an orbital shaker set to170 rpm for 5 days. Yeast biomass was harvested by centrifugation, and the supernatant was assayed for chemical oxygen demand (COD), total nitrogen, and total phosphorus.

Oleaginous Yeast Culture with Municipal Wastewater

Primary wastewater was collected from the Pullman Wastewater Treatment Plant at Pullman, Washington (USA). In addition to wastewater, three nutrient supplemented culture conditions were tested. To the first group, 10 g/L of glucose was added; to the second group, 100 g/L of glucose; and to the third group, 100 g/L of glucose, 10 g/L peptone, and 10 g/L yeast extract. All of the cultures were sterilized by autoclaving at 121 °C for 15 min. For the wastewater culture without nutrient supplementation, 50 mL of primary wastewater was aliquoted into a 250-mL Erlenmeyer flask. Five milliliters of sub-cultured cells was inoculated into each culture with or without sterilization. After inoculation, 5 mL of sample was removed and centrifuged, and the supernatant was analyzed for COD, total nitrogen, and total phosphorus. The flasks were then incubated at 25 °C for 3 days. Yeast biomass was then harvested by centrifugation, and the supernatant was analyzed for COD, total nitrogen, and total phosphorus.

Yeast and Algae Integrated Process

A schematic drawing of the integrated process is shown in Fig. 1. The FWHB was mixed with primary wastewater with a ratio of 1:4 (*v*/*v*) and used as the culture media for *R. glutinis* and *C. curvatus*. Five milliliters of sub-cultured cells was used to inoculate into 50 mL culture medium in a 250-mL Erlenmeyer flask. The flask was incubated at 25 °C in an orbital shaker set to170 rpm for 6 days. The initial pH in the medium was 6.0, and it was very stable during the culture process. After this incubation, 90% of the cell suspension was harvested and the produced biomass was separated by centrifugation. The supernatant was inoculated with the 10% remaining cell suspension and further grown with nutrients under the same culture conditions. The effluent from this second step was used to support growth of the phototrophic algae *Chlorella sorokiniana* (UTEX 1602), which was possible since the effluent still contained nitrogen and phosphorous. The seed cells of *C. sorokiniana* were maintained in phototrophic culture in Kuhl's medium [16], and the inoculation rate of the yeast culture to the effluent was 1% (*v*/*v*). The pH of Kuhl's medium was 6.0, and no pH adjustment is necessary before and after the inoculating. The culture was maintained for

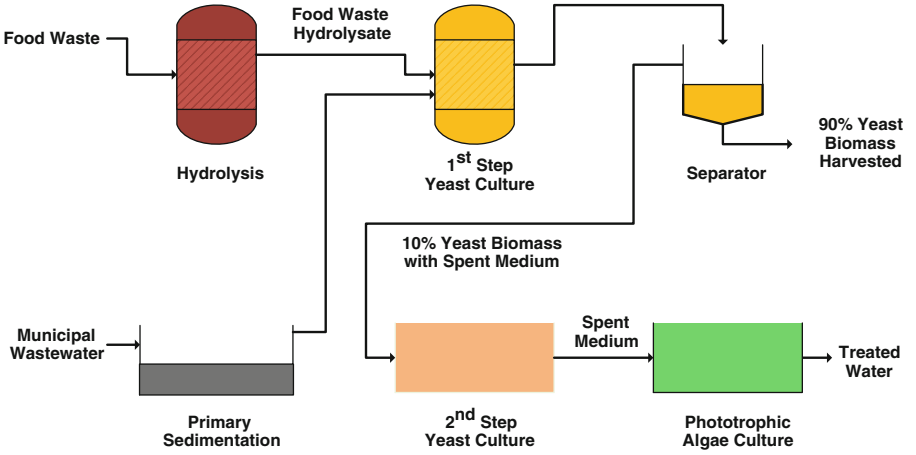

Fig. 1 Schematic drawing of integrated process of two-step yeast culture and phototrophic algae culture

6 days under light from florescent lamps at room temperature and was bubbled with air/2% (*v*/*v*) CO_2 mixed gas. After the culture period, the algae biomass was separated by centrifugation, and the supernatant was collected for COD, N, and P tests.

Cell Density, Cell Dry Weight, and Fatty Acids Analysis

Cell density was determined by microscopically counting the cells with a hemocytometer. To determine cell dry weight, a 5-mL cell suspension sample was centrifuged at 2,500 rpm for 5 min. The cell pellet was then washed twice with distilled water and dried in a pre-weighed aluminum dish at 105 °C for 3 h. For the fatty acids analysis, yeast cells were harvested and freeze-dried overnight. The method of fatty acid methyl ester preparation and gas chromatography analysis are the same as described in our previous publication [17].

Analysis Methods for COD, Nitrogen, and Phosphorus

All of the analysis kits for COD, total nitrogen, and total phosphorus were purchased from Hach Company (Loveland, CO). The Low Range (3 to 150 mg/L) COD testing kit (#2125825) was used in the COD analysis, and Hach Method 8000 was followed. The total nitrogen Test'N Tube Reagent Set (#26722-45) was used in the total nitrogen analysis, and Hach Method 10071 was followed. Total Phosphorus (0.06 to 3.50 mg/L PO_4^{3-}) Test'N Tube Reagent Set (#27426-45) was used in the total phosphorus analysis with Hach Method 8190. All of the samples were diluted with distillated water to the desired concentration range for the test kits, and the results were obtained with a digital reader model 2400 from the Hach Company (Loveland, CO).

Results

1. The composition analysis of food waste hydrolyzed broth (FWHB)

   The FWHB was analyzed to determine the nutrient compositions in the food waste. The result showed that 92.4±1.1% of the total sugar in the FWHB was glucose, which is a readily used carbon source for most microorganisms. The total sugar in FWHB was 51.2±9.7 g/L, and the total COD was 106.3±3.3 g/L. Also, there was sufficient nitrogen and phosphorus for microorganism growth in FWHB (Table 1).

Table 1 Composition of food waste hydrolyzed broth (n=3)

Components		Concentration	% of total sugar
Total sugar (g/L)		51.2±9.7	
	Arabinose	0.8±0.2	1.6±0.5
	Galactose	1.3±0.3	2.6±0.1
	Glucose	47.4±9.6	92.4±1.1
	Mannose/xylose	1.0±0.2	2.0±0.4
	Fructose	0.8±0.3	1.5±0.8
Total COD (g/L)		106.3±3.3	N/A
Total nitrogen (g/L)		4.28±0.75	N/A
Total phosphorus $\left(g/LPO_4^{3-}\right)$		1.68±0.23	N/A

2. Oleaginous yeast species selection

Five well-known oleaginous yeast strains obtained from ATCC were tested with the media made from FWHB. The yeast biomass productions from the cultures are shown in Fig. 2. Except for *L. starkeyi* and *R. toruloides*, the yeast strains produced comparable biomass to the control culture. *C. curvatus* and *Y. lipolytica* produced more biomass when cultured with FWHB than when cultured in the control medium. The lipid content in the biomass was not measured, since the major purpose of this experiment was to screen for yeast species that could grow in a food waste environment. These results indicate there is no inhibition in the cultures of *R. glutinis*, *C. curvatus*, and *Y. lipolytica*. Thus, these three yeast strains were selected as promising oil producers when grown on food waste.

3. Oleaginous yeast culture with municipal wastewater medium

To determine if the selected yeast can grow in the municipal wastewater environment, the cultures were supplemented with glucose and nitrogen sources (Fig. 3). The results showed that if provided with sufficient carbon and nitrogen, all three species had good growth in the wastewater environment. The final biomass production for *C. curvatus*, *Y. lipolytica*, and *R. glutinis* was 17.0, 15.3, and 21.4 g/L, respectively. Also, both *C. curvatus* and *R. glutinis* grew to more than 10 g/L in the culture with 100 g/L glucose without additional nitrogen, suggesting they were able to utilize the nitrogen source in the wastewater. These results indicate that the selected oleaginous yeast strains are able to grow in municipal wastewater and that municipal wastewater can be used as the water source for these strains of yeast.

4. Oleaginous yeast culture with municipal wastewater only

To test if municipal wastewater can be used as solely feedstock for oleaginous yeast culture, the selected yeast strains were inoculated into primary wastewater without any nutrient supplementation. Both non-sterile and sterilized culture experiments were conducted. The biomass concentration introduced into the culture from inoculation was tested and subtracted from the final biomass production. The result showed that *C. curvatus* and *R. glutinis* exhibited minimal growth in the wastewater in either sterile or non-sterile cultures, indicating they were able to utilize the nutrients in the wastewater, but that the nutrients were limited in concentration. Final biomass of *Y. lipolytica* was lower than the initial biomass, indicating the wastewater environment was actually harmful to it. Also, all of the produced biomass had low lipid content (Table 2).

Besides oleaginous yeast biomass production, the other objective of this process was to remove the organic carbon, nitrogen, and phosphorus from wastewater. The analysis of COD showed that only a minor amount of the COD in the wastewater was consumed by pure yeast culture. However, non-sterile cultures removed much more

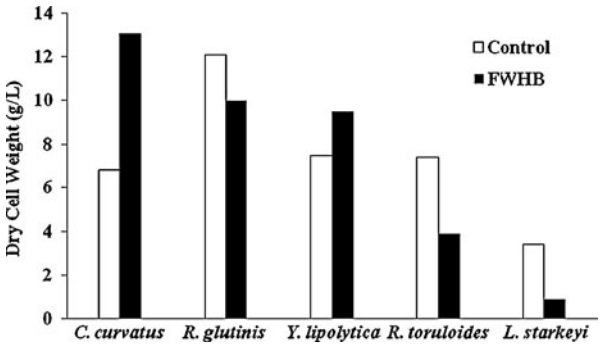

Fig. 2 Oleaginous yeast culture with FWHB

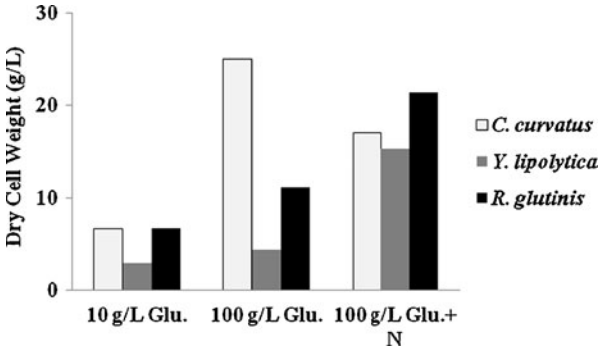

Fig. 3 Oleaginous yeast culture with glucose supplemented municipal wastewater

COD than sterile cultures (Table 3). This probably resulted from bacteria playing a major role in COD removal in the non-sterile cultures. It can be noted that the COD in the original primary wastewater was less than 0.4 g/L. According to Monod's equation [18], low substrate concentration leads to low growth rate. This should be the main reason why the yeast exhibited poor growth in wastewater alone. These results indicate that there is not a sufficient concentration of organic carbon in the wastewater and not enough nutrients to support satisfactory growth of the yeast.

Unlike the COD, a large amount of total nitrogen was removed by both sterile and non-sterile cultures by all three species (Table 4), and the non-sterile cultures removed more nitrogen than the sterile cultures. The total nitrogen remaining in the treated wastewater ranged from 24.7 to 40.7 mg/L.

It is interesting to note that most of the phosphorus in the wastewater was removed during the yeast culture process, in both sterile and non-sterile culture conditions. The total phosphorus remaining in the treated wastewater ranged from 0.8 to 7.7 mg/L PO_4^{3-} (Table 5).

5. Integrated yeast and algae culture

Since the municipal wastewater supported sufficient cell growth if supplemented with organic carbon, it was used as the water source and mixed with FWHB to make culture media for the oleaginous yeasts *R. glutinis* and *C. curvatus*. The concentration of nutrients in the FWHB (Table 1) was diluted five times its volume with the municipal wastewater. After 6 days of culture, the biomass yield of *C. curvatus* and *R. glutinis* was 7.5 g/L and 5.2 g/L, respectively, and *C. curvatus* consumed more COD than *R. glutinis* (Table 6). The total fatty acid content in the produced biomass was 28.6% and 19.6%, respectively.

Table 2 Cell dry weight and lipid content of yeast biomass cultured with wastewater (n=3)

Strain inoculated		Initial biomass (g/L)	Final biomass (g/L)	Biomass increased (g/L)	Total fatty acids content (%)
C. curvatus	Sterilized	0.47±0.04	0.58±0.03	0.11±0.06	11.1±1.0
	Non-sterile	0.42±0.03	0.51±0.02	0.09±0.04	9.1±0.8
R. glutinis	Sterilized	0.36±0.03	0.40±0.04	0.04±0.05	6.2±2.1
	Non-sterile	0.36±0.03	0.40±0.04	0.04±0.05	6.3±0.4
Y. lipolytica	Sterilized	0.36±0.03	0.25±0.02	−0.11±0.04	11.5±5.2
	Non-sterile	0.36±0.03	0.23±0.02	−0.14±0.04	6.6±2.3

Table 3 COD removal from the culture with wastewater (n=3)

Strain inoculated		Initial soluble COD (mg/L)	Final soluble COD (mg/L)	Soluble COD removed (mg/L)
C. curvatus	Sterilized	369.7±20.9	259.0±12.8	110.7±20.5
	Non-sterile	326.0±7.2	132.0±7.2	194.0±10.2
R. glutinis	Sterilized	357.3±11.7	300.7±8.3	56.7±14.4
	Non-sterile	357.3±11.7	132.0±8.7	225.3±14.6
Y. lipolytica	Sterilized	317.3±18.9	314.0±6.9	3.3±20.1
	Non-sterile	317.3±18.9	110.0±9.2	207.3±21.0

There are still high concentrations of COD, N, and P in the effluent of the first step culture, and so the second-step culture is actually a wastewater treatment process, with lipid production at the same time. After the second-step culture, the organic carbon was further converted into biomass, but the efficiency was lower than the first step. The total fatty acid content in the biomass was even lower, which was about 20.0% and 18.7%, respectively, for the two yeast strains. The effluent from the second step was then used to support phototrophic growth of the green algae *C. sorokiniana*. The algae biomass produced in the two culture media contained 22.9% and 20.0% lipids, respectively. Some COD was removed in the second step and also in the phototrophic algae culture step, but much less than during the first step.

The initial COD in the culture medium was about 21.3 g/L (calculated from Table 1), and this was reduced to 3,800 mg/L and 8,300 mg/L after the first step culture, respectively (Table 6). This was further reduced in the second-step culture, to 2,500 and 3,200 mg/L, respectively. It is notable that *C. sorokiniana* has the ability to utilize glucose, and this may have contributed to the consumption of COD in the last step. Bacteria may also contribute to this consumption, since the second-step culture was non-sterile and bacteria were also growing in that the culture. Thus, although the final COD in the effluent was still 1,030 mg/L and 1,200 mg/L, respectively, it is resistant to removal by the organisms present and thus would not cause a rapid growth of bacteria and a subsequent pollution of the environment. The phototrophic culture process effectively absorbed all of the nitrogen and phosphorous (Table 6). We also analyzed the concentrations of nutrients in the effluent from Pullman Wastewater Treatment Plant. Compared to this commercial process, the total nitrogen concentration from our wastewater treatment process was lower than that of Pullman's water treatment plant effluent, and our total phosphorous was much lower (Table 6).

Table 4 Total nitrogen removal from the culture

Strain inoculated		Initial nitrogen (mg/L)	Final nitrogen (mg/L)	Nitrogen removed (mg/L)
C. curvatus	Sterilized	63.1±0.7	33.4±0.7	29.7±1.0
	Non-sterile	63.1±0.7	37.4±0.6	25.7±0.9
R. glutinis	Sterilized	53.2±0.8	24.7±0.8	28.5±1.1
	Non-sterile	53.2±0.8	28.3±0.6	24.9±1.0
Y. lipolytica	Sterilized	47.6±0.6	32.9±0.7	14.8±0.9
	Non-sterile	47.6±0.6	40.7±0.4	6.9±0.7

Table 5 Total phosphorus removal from yeast cultures

Strain inoculated		Initial phosphorus (mg/L)	Final phosphorus (mg/L)	Phosphorus removed (mg/L)
C. curvatus	Sterilized	21.4±0.3	4.6±0.1	16.9±0.3
	Non-sterile	21.4±0.3	0.8±0.2	20.6±0.2
R. glutinis	Sterilized	19.9±0.3	1.8±0.1	18.1±0.3
	Non-sterile	19.9±0.3	2.1±0.4	17.8±0.4
Y. lipolytica	Sterilized	19.8±0.3	1.6±0.3	18.3±0.4
	Non-sterile	19.8±0.3	7.7±0.2	12.1±0.2

Discussion

Food waste and municipal wastewater were used as initial feedstock in this process, and the products produced were oil-enriched biomass and clean water that did not require any further treatment for nitrogen or phosphorous removal. A two-step oleaginous yeast culture was used in this process because a high-density culture is preferred for an industrial-scale fermentation process (first step), but wastewater produced in the fermentation process usually needs further treatment before discharge, the problem of which is addressed in the second step. Theoretically, using both of these steps will result in higher overall efficiency than a one-step process in which the culture remains in the same fermentor until the residue sugar is depleted. Incidentally, no sterilization is needed for the second culture step, and this culture does not have to be finely controlled, since this process is actually a wastewater treatment process. Phototrophic algae culture is also involved in this process because it sequesters residual nitrogen and phosphorus efficiently, and it can produce oil-enriched biomass at the same time. The results of this study demonstrated the concept of completely converting an organic waste stream into valuable products.

Food waste actually contains all of the nutrients from our food. The acid hydrolysis process used in this study not only hydrolyzed carbohydrate but also protein. The sugars

Table 6 Integrated yeast and algae culture process with food waste and municipal wastewater as initial feedstock

	Initial	1st step culture	2nd step culture	Culture of *C. sorokiniana*	Effluent from treatment plant
Cryptococcus curvatus					
Biomass (g/L)	N/A	7.5±0.3	1.1±0.1	1.53	N/A
TFA/Biomass (%)	N/A	28.6±2.2	20.0±2.5	22.9±2.4	N/A
COD (mg/L)	21,630±660	3,800±600	2,500±200	1,030±60	37
N (mg/L)	916±150	420±100	190±70	33±4	45
P (mg/L of PO_4^-)	356±50	22±2	19±6	1.5±0.5	12.8
Rhodotorula glutinis					
Biomass (g/L)	N/A	5.2±1.2	1.5±0.3	0.58	N/A
TFA/Biomass (%)	N/A	19.6±0.2	18.7±9.0	20.0±0.7	N/A
COD (mg/L)	21,630±660	8,300±1200	3,200±200	1,200±170	37
N (mg/L)	916±150	540±30	140±10	34±6	45
P (mg/L of PO_4^-)	356±50	25±5	14±7	0.6±0.2	12.8

and amino acids which were produced proved to be an excellent nutrient source for yeast culture. *C. curvatus* can be cultured on many different carbon sources like glycerol [19], ethanol, and a variety of sugars including glucose, sucrose, lactose, and xylose [20]. It can also be cultured on waste streams like whey [21] and tomato juice [22]. The capability of *C. curvatus* to utilize a wide range of carbon sources and grow in a wastewater environment resulted in abundant cell biomass and consumption of food waste hydrolysates. *R. glutinis* has been previously grown on monosodium glutamate wastewater [7, 8], and it grew well in our culture systems also. These results demonstrate that *R. glutinis* is a strain that can flourish in different complex wastewater environments. *Y. lipolytica* can be grown on glycerol [23] and industrial fats [24]. It also had satisfactory growth on the food waste utilized in this study but had exhibited a significantly lower yield in municipal waste water than *C. curvatus* and *R. glutinis*. Subsequently, it was not tested in the final integrated culture process. *R. toruloides*, on the other hand, showed poor growth on food waste in this study, although it could reach 106.5 g/L when cultured with glucose [25], indicating that it was not an ideal strain for organic waste utilization. Although *L. starkeyi* grew on sewage sludge without inhibition [26], it demonstrated poor growth in the food waste environment of this study.

The sole use of primary wastewater for oleaginous yeast culture was not satisfactory because wastewater alone does not contain sufficient nutrients. Additionally, there are many challenges to using wastewater as feedstock on an industrial scale, since it may be impossible to sterilize the wastewater to maintain oleaginous yeast as the dominant cultured species. However, it was demonstrated in this study that municipal wastewater can be used as water source for yeast culture. This would save precious clean water and also reduce the production cost. Supplementing the wastewater, an organic carbon enriched waste stream such as food waste can be used as the major carbon source for this process. In an integrated system, a food waste treatment facility could be built in a wastewater treatment plant, and this combination could produce biofuel. Additionally, the effluent from this tandem process can be used as feedstock for phototrophic algae culture, as shown in this study. The results obtained in this study have demonstrated that phototrophic algae culture was an effective method for removing nitrogen and phosphorous and for producing an oil-enriched algae biomass at the same time.

The lipid contents of *C. curvatus* and *R. glutinis* biomass produced in the first culture step were 28.6±2.2% and 19.6±0.2%, respectively, which were close to the lipid content of soybean. However, *C. curvatus* is able to accumulate lipid up to 50% of its dry biomass when cultured on a nitrogen-deficient medium [20]. The low lipid content in this study could be caused by the low C/N ratio in food waste, which was 10.6/1 (calculated from Table 1). The average C/N ratio of food waste collected from 500 sources, as reported in Zhang's study, was 14.8/1 and, in other studies as cited, ranged from 14.7/1 to 36.4/1 [11]. Generally, oleaginous yeast does not accumulate a high content of lipid in a medium with a C/N ratio less than 20/1, whereas a C/N ratio from 40/1 to 80/1 is preferred for lipid accumulation in most oleaginous microorganisms [1]. The low C/N ratio in food waste may be improved by supplementation with an extra carbon source. For example, crude glycerol from the biodiesel industry [17] or biomass hydrolysate [6] contains no nitrogen, or very low nitrogen, and is thus good candidate to be used as supplemental carbon sources for this culture process.

The lipid productivity of the integrated system in this study was relatively low, but that was not its major purpose. Instead, this study demonstrated the feasibility of completely converting organic waste to valuable products. The productivity of the first step culture can be significantly improved by optimization of the culture conditions and control strategies.

The effluent from the first culture step can be treated with both yeast culture and algae culture, as we did in this study, to completely remove undesirable nutrients in wastewater and produce biofuel feedstock at the same time.

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