J Therm Anal Calorim (2012) 108:119–126
DOI 10.1007/s10973-011-1659-6

Energy efficiency evaluation of lipid production by oleaginous yeast *Rhodosporidium toruloides*

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Received: 31 March 2011 / Accepted: 11 May 2011 / Published online: 26 May 2011

Abstract Mass–energy balance of lipid fermentation by oleaginous yeast *Rhodosporidium toruloides* Y4 with glucose as sole carbon source was investigated. The elemental composition of the freeze-dried cell samples obtained at different time points was determined using a Vario EL3 analyzer to estimate the biomass energetic yield (η). Then this work established the biomass energetic yield (η) to sets of biochemical variables and a new equation was developed to determine η without elemental composition of biomass. Biomass energetic yield estimated by the new equation was highly in accordance with that based on elemental composition. Bomb-calorimetric measurements were shown to be a direct method of quantifying the energy content of oleaginous yeasts. Combustion heat (Q_c) of biomass determined experimentally was in consistent with those calculated according to its elementary contents. The relationship between lipid content and Q_c of the cells was simulated and a new practical equation was developed based on lipid content ($Y_{L/S}$) to evaluate Q_c of biomass. Biomass energetic yield of *R. toruloides* Y4 could reach higher than 0.8. Combustion heat of biomass obtained after 116 h was 33 (kJ g^{-1}) that was about 73% of the combustion heat of diesel. The results revealed that *R. toruloides* Y4 was an efficient "energy-converter" in lipid production with glucose as the substrate. The results also implied the approaches to estimating η of fermentation, and Q_c of single oil cells developed in this study should be valuable to address the overall techno-economical analysis of bio-energy production.

Keywords Mass–energy balance · Elementary analysis · Combustion heat · Biodiesel · Oleaginous yeast

List of symbols

C	Carbon
N	Nitrogen
L	Lipid
S	Substrate
X	Cell biomass
Mx	Molecular mass
γ_s	The reductance degree of substrate
γ_x	The reductance degree of cell biomass
σ_s	The reductance degree of substrate
σ_x	The reductance degree of cell biomass
$Y_{L/S}$	Lipid produced per g of consumed substrate (g g^{-1})
$Y_{X/S}$	Cell biomass produced per g of consumed substrate (g g^{-1})
η	Biomass energetic yield
Q_c	Combustion heat

Introduction

Microorganisms that could accumulate lipids at more than 20% of their biomass were named as oleaginous species [1]. Some yeast strains, such as *Lipomyces* sp., *Rhodosporidium* sp., and *Rhodotorula* sp. could accumulate intracellular lipids as high as 70% of dry weight of their biomass [2, 3]. The majority of those lipids were triacylglycerides with long-chain fatty acids that were comparable to conventional vegetable oils [4]. Microbial lipids

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have been recently considered as potential feedstock for the production of biodiesel [5–8]. Our early work demonstrated that oleaginous yeast *Rhodosporidium toruloides* Y4 produced notable quantity of intracellular lipid when cultivated in nitrogen-limited media with glucose as the sole substrate [9].

It was very important to investigate the energy and mass efficiency of the microbial production of biodiesel in the biotransformation process. For instance, calculations of energy capacity of algal biomass were applied to prove the advantage of microalgae biodiesel against the plant-oil-derived biodiesel and petroleum transport fuel [10, 11]. However, application of material and energy balances was usually not fully exploited in analysis of the fermentation data because biochemical processes were always complicated for including in cell growth and product formation. In some cases, material and energy balances were only roughly estimated in growth processes [12, 13]. One parameter to estimate the efficiency of microbial lipid synthesis was "fat coefficient" ($Y_{L/S}$), which characterized the lipid yield based on organic substrate, in g lipids synthesized/g organic substrate consumed. The $Y_{L/S}$ was analogous to other parameter of growth based on mass balance, such as $Y_{X/S}$ (g biomass/g organic substrate).

Other precise energy characteristics for growth efficiency and product formation were also important, including biomass energy yield (η) and combustion heat (Q_c) of biomass. Material and energy balances for fermentation process had been developed by previous study based on the facts that the heat of reaction per electron transferred to oxygen for a wide variety of organic molecules, the number of available electrons per carbon atom in biomass, and the weight fraction carbon in biomass were relatively constant [14]. For calculating the η, a precise elemental composition of the biomass was necessary. Combustion heat of dry biomass (Q_c) indicated energy content of the microbial cells intuitively and accurately, which was the most essential factor for the selection of promising biodiesel sources [15]. Nevertheless, a bomb-calorimeter was needed for the analysis of Q_c of the biomass, which was not available in many laboratories [16]. Another method to obtain the Q_c of biomass was based on a equation involving elemental composition of biomass as variables [17, 18]. That made energy analysis of fermentation more convenient.

The purpose of this article was to establish the mass–energy balance equations for lipid fermentation by oleaginous yeast *R. toruloides* Y4 with glucose as sole carbon source in order to establish a valid approach to estimate the energetic efficiency of microbial lipid synthesis by sets of biochemical variables obtained conveniently in most labs. The relationship between η, lipid yield ($Y_{L/S}$) and biomass yield ($Y_{X/S}$) was investigated. The connection between Q_c of the biomass and lipid content ($Y_{L/X}$) was simulated. It should be valuable to address the overall techno-economical analysis of bio-energy production.

Materials and methods

Organism, media, and chemicals

Yeast extract (containing 3% ammonium-N and 9.0% total nitrogen) and peptone (animal-tissue based containing 3% ammonium-N and 14.5% total nitrogen) were obtained from Aoboxing Bio-tech. Co. Ltd. (Beijing, China). All other chemicals and reagents were in analytical reagent (AR) grade.

Oleaginous yeast *R. toruloides* Y4 was a domesticated strain of *R. toruloides* AS 2.1389, originally from China general microbiological culture collection center [19]. The yeast strain was maintained at 4 °C on YPD agar slants with glucose 20 g l^{-1}, peptone 10 g l^{-1}, yeast extract 10 g l^{-1}, and agar 20 g l^{-1}.

Precultures were grown on YPD liquid medium including glucose 20 g l^{-1}, peptone 10 g l^{-1} and yeast extract 10 g l^{-1}, pH 6.0. Lipid production was done using the medium with (g l^{-1}): glucose 64, yeast extract 0.75, KH_2PO_4 0.4, $MgSO_4 \cdot 7\ H_2O$ 1.5, $(NH_4)_2SO_4$ 0.1, pH 7.0. All media were sterilized by autoclave at 121 °C for 20 min.

Batch fermentation was carried out in a 15-l stirred-tank bioreactor (FUS-15L (A), Guoqiang Bioengineering Equipment Co. Ltd., Shanghai, China) equipped with an on-line data acquisition and control system. Culture pH and dissolved O_2 were monitored with a pH meter (Mettler-Toledo, Switzerland) and an oxygen probe (Mettler-Toledo, Switzerland). The cultivation conditions were as follows: temperature 30 °C, pH 5.6 (by automatic control using 10.0 M NaOH), aeration at 0.9 vvm and dissolved oxygen at 40–50% saturation. Culture was initiated by inoculating sterilized medium (6300 ml) with 700 ml of 28-h-old preculture of *R. toruloides* Y4. Aliquots were released at different time intervals from the bioreactor to determine residual glucose, cellular biomass, lipid content, elemental composition and combustion heat. Culture was stopped when glucose concentration was dropped to less than 0.5 g l^{-1}.

Analytical methods

Total lipid was extracted according to known methods [20]. In brief, wet cells in 20-ml broth culture were harvested by centrifugation, washed with distilled water, and dried at 105 °C until to a constant weight to obtain biomass (g l^{-1}). Glucose concentration was determined using a SBA-50 B

glucose analyzer (Shandong Academy of Sciences, Jinan, China). Biomass yield ($Y_{X/S}$) was expressed as the quantity of biomass produced (g) per g of consumed substrate (S). Lipid yield ($Y_{L/S}$) was the quantity of lipid produced (g) per g of consumed substrate, while lipid content ($Y_{L/X}$) was expressed as g of total lipid per g of dried cellular biomass.

The elemental composition of the freeze-dried cell samples was determined using a Vario EL3 analyzer, (Elementar Instrument Co., Germany). Results for Q_c and the elemental composition were all presented in terms of ash- and water-free biomass. The residual water content of the freeze-dried cell samples was determined by drying for 24 h at 105 °C. The ash content was determined by heating to 600 °C for 6 h. Biomass combustion heat (Q_c) (kJ g^{-1}) was measured with an oxygen-bomb calorimeter established in the Thermochemistry Laboratory of Dalian Institute of Chemical Physics, CAS, China [16].

Data processing methods

According to the early thermodynamic analysis of microbial growth [21], sulfur and phosphorous were not considered in this work. The reductance degree (γ) of organic substance with a chemical formula $CH_pO_nN_q$ was calculated based on Eq. 1 [22].

$$\gamma_x = 4 + p - 2n - 3q \quad (1)$$

The biomass energetic yield (η) was the ratio of the heat produced by oxidation of the biomass to that of the substrate utilized in the fermentation process, where the oxidation results in the production of CO_2, H_2O and NH_3. According to Erickson et al. [23] microbial growth energetic yield was defined in Eq. 2.

$$\eta = \frac{\gamma_x \sigma_x}{\gamma_S \sigma_S} Y_{X/S} \quad (2)$$

where $Y_{X/S}$ was the biomass yield (g g^{-1} substrate), σ_x and σ_s were the weight fractions of carbon in biomass and substrate, respectively; and γ_x and γ_s were the reductance degree of biomass and substrate, respectively.

Combustion heat (kJ g^{-1}) of biomass could be determined not only by bomb-calorimetric, but by calculation based on the elements composition of the cells according to Eq. 3 [17].

$$Q_c = 33.5\,(C) + 142.3\,(H) - 15.4\,(O) - 14.5\,(N) \quad (3)$$

where C, H, O, N was the weight of corresponding element in per g biomass.

For simplicity, a small account of yeast extract in the substrate which mainly supplied N for the growth of the yeast was neglected when doing the calculation of γ_s and Q_c of substrate.

Results

Lipid fermentation by *R. toruloides* Y4

Lipid production by oleaginous microorganisms is attractive for obtaining fuel molecules with longer carbon chains from renewable biomass. In most cases, cells were cultured in a nutrient-deficient medium to ensure a higher lipid cellular content ($Y_{L/X}$). In our early work, we optimized the lipid fermentation conditions, and achieved high lipid yield and cell density [19]. Under these conditions, we cultured *R. toruloides* Y4 cells in a 15-l bioreactor with glucose as the sole carbon source. During different time intervals, samples were recovered to analyze. Figure 1 demonstrated the evolution of substrate, biomass and lipid. This profile was also quite similar to our early work [19]. As indicated, glucose concentration linearly decreased at an average rate of 0.48 g l^{-1} h^{-1}. Biomass and lipid were 21.6 and 15.0 g l^{-1}, respectively, at the end of the culture, which were slightly higher than the biomass and lipid of the fermentation (19.2 g l^{-1}, 14.2 g l^{-1}, respectively) conducted in 7-l bioreactor [19]. Biomass yield ($Y_{X/S}$) and lipid yield ($Y_{L/S}$) were 0.36 and 0.25 g g^{-1}, respectively, which indicated that higher yield could be obtained when the cultural scale was expended. It implied lipid production by *R. toruloides* Y4 has a great potential to be industrialized.

Biomass energetic yield of lipid fermentation process

Further, we performed elemental analysis of the fermentation samples, the results of which were summarized in Table 1. It was clear that there were dramatic shifts in the biomass elemental composition upon lipid accumulation. Generally, N and O contents continuously declined, while C and H contents increased. This was highly in accordance

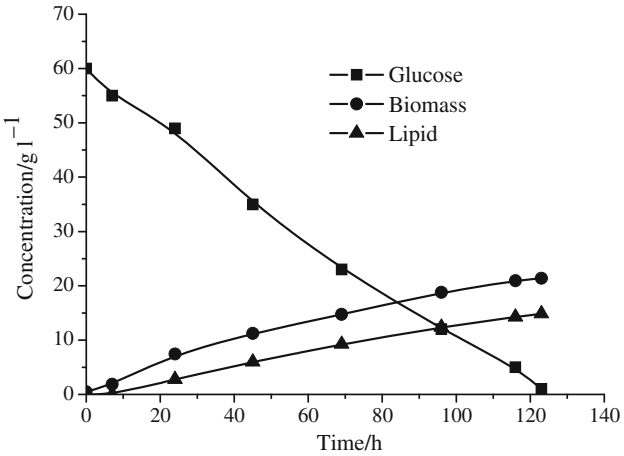

Fig. 1 Results of lipid fermentation by *R. toruloides* Y4 in optimized media in 15-l bioreactor

Table 1 Results of the lipid fermentation by *R. toruloides* Y4 in optimized media in a 15-l bioreactor

Entry	Sampling time/h	$Y_{L/X}$/%	$Y_{X/S}$/g g^{-1}	Biomass elemental composition				Chemical formula	γ_x	η^a	η^b
				N/%	C/%	H/%	O/%				
1	7	1.00	0.27	6.75	43.87	6.74	42.64	$CH_{1.84}O_{0.73}N_{0.13}$	3.99	0.30	0.29
2	24	37.09	0.46	2.81	53.73	8.14	35.32	$CH_{1.82}O_{0.50}N_{0.04}$	4.70	0.73	0.76
3	45	53.23	0.43	2.10	59.54	9.11	29.25	$CH_{1.84}O_{0.37}N_{0.03}$	5.01	0.80	0.81
4	69	61.45	0.38	1.66	61.09	9.24	28.01	$CH_{1.82}O_{0.34}N_{0.02}$	5.06	0.73	0.77
5	96	65.99	0.38	1.14	65.39	10.14	23.33	$CH_{1.86}O_{0.27}N_{0.02}$	5.28	0.82	0.80
6	116	67.41	0.37	1.05	66.07	10.26	22.60	$CH_{1.87}O_{0.26}N_{0.01}$	5.31	0.82	0.79
7	123	70.16	0.35	0.92	66.12	9.98	22.99	$CH_{1.81}O_{0.26}N_{0.01}$	5.26	0.77	0.76

[a] Obtained using Eq. 2

[b] Obtained using Eq. 4

with the evolution profile of cellular lipid content. In *R. toruloides* Y4 sample cultured for 7 h, C and N content were 43.87 and 6.75%, respectively, similar to those of conventional microbial biomass [18]. Based on these elemental composition data, chemical formula of *R. toruloides* Y4 biomass at different time points were established. What's more, the reductive degree (γ) values were calculated using Eq. 1. Obviously, more reduced biomass was produced during fermentation.

Cell growth energetic yield (η) was also estimated according to Eq. 2. Compared with $Y_{X/S}$, η helped to distinguish microbial growth on substrates with different energy contents by considering the energy content of substrates in calculation. Our data showed that η values of *R. toruloides* Y4 in time points were higher than the maximum value of 0.7 proposed for heterotrophic microbial growth [14]. That probably resulted from additional energy produced in metabolism was stored by *R. toruloides* Y4 in its cells as lipid. Through the above analysis, *R. toruloides* Y4 could convert energy from glucose into biomass in higher efficiency than general heterotrophic microorganisms. The biomass yields of the oleaginous yeasts were consistently lower than that of non-oleaginous microorganisms, whereas their energetic yields were higher. Although Eq. 2 was very useful, a reliable elemental data was very important to calculate $\gamma_x\sigma_x$, and thus to acquire η value.

Lipids contain more energy than proteins or carbohydrates and this has been known for a very long time. In addition, oleaginous microorganisms can store more lipid than other compound. However, there is no report about using the relationship between lipid content and energy to estimate growth energy efficiency of lipid production. Previous data demonstrated that there was a good linear relationship between $\gamma_x\sigma_x$ and cellular lipid content ($Y_{L/X}$) for oleaginous yeast growth on ethanol, with the equation $\gamma_x\sigma_x = 0.0278 \times Y_{L/X} + 1.5655$ [23]. Thus, the item $\gamma_x\sigma_x$ that requires the elemental composition data can be estimated with lipid content ($Y_{L/X}$) as variables. This would be more practical in most labs. Indeed, when we plotted the $\gamma_x\sigma_x$ and $Y_{L/X}$ listed in Table 1, a straight line was obtained with an R^2 equaled to 0.9718 (Fig. 2). Our data further suggested that there was an intrinsic relationship between the energy content of yeast biomass and cellular lipid content. According to the linear relationship in Fig. 2 and Eq. 2, cell growth energetic yield (η) could also be written as Eq. 4.

$$\eta = \frac{0.0255Y_{L/X} + 1.6653}{\gamma_S\sigma_S} Y_{X/S} \quad (4)$$

When considering the relationship between $Y_{L/X}$ and $Y_{X/S}$, Eq. 5 could be also obtained.

$$\eta = \frac{0.0255Y_{L/S} + 1.6653Y_{X/S}}{\gamma_S\sigma_S} \quad (5)$$

Either Eqs. 4 or 5 should promise an easier estimation for cell growth energetic yield of lipid production by *R. toruloides* Y4. As did herein and by others with chemical defined substrates, such as glucose, measuring the residual substrate, cell biomass, and lipid contents were routine and essential to profile the lipid production process. Thus, energy efficiency could be obtained based on these experimental data, while the elemental composition of biomass was unnecessary. For example, according to Eqs. 4 or 5, overall energy efficiency of the lipid production shown in Fig. 1 was 0.76, which was comparable to that based on Eq. 2 (see Table 1).

Combustion heat of the biomass

Based on the definition of cell growth energetic yield, there were more options to obtain η values. Using the elemental data, the combustion heat (Q_c) of yeast biomass was estimated according to Eq. 3 and showed in Table 1. Equation 3 was based on Dulong's formula and usually gives the HHVs within 5% error [24]. Alternatively, the

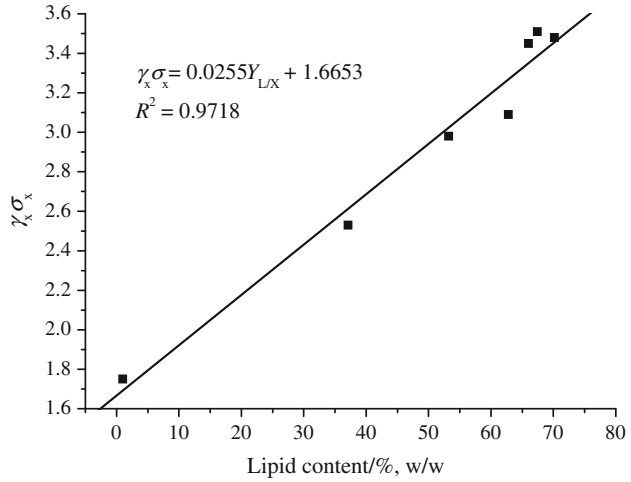

Fig. 2 Correlation between the energy content of cell biomass ($\gamma_x\sigma_x$) and lipid content ($Y_{L/X}$). Data was obtained from lipid fermentation results in Table 1. Solid line indicated linear regression $\gamma_x\sigma_x = 0.0255\ Y_{L/X} + 1.6653$, $R^2 = 0.9718$

combustion energy of cell biomass was determined using oxygen-bomb calorimeter. The Q_c values for cell samples obtained at time points were experimentally determined. However, differences between the experimental data and calculated data were obvious and interesting. For the 45-h sample with a lipid content of 51%, the experimental data was slightly more than the calculated. Besides, for the 123-h sample (Entry 3), the experimental Q_c was about 5% less than the calculated. Because the 123-h sample was extremely fatty, i.e., with a cellular lipid content of 70%, lipid loss occurred when the mixing and handling of the sample in the calorimetric analysis process. Specifically, the sample must be pressed into a crucible of about 4 cm^3 before hanging in the bomb, as could lead to lipid leakage. Thus, similar result was found for other samples after 69-h. The energy content of *R. toruloides* Y4 cells increased because of an increased amount of intracellular lipids. According to our knowledge, the Q_c value 33 (kJ g^{-1}) of growth obtained after 116 h up to 73% of the energy content of diesel oil (45.4 (kJ g^{-1})) was higher than the combustion heat of most microorganisms, which indicated the potential of biomass of oleaginous yeasts used as biodiesel fuel [15, 18, 25]. When we plotted the Q_c and $Y_{L/X}$ listed in Table 2, a straight line was obtained with an R^2 equaled to 0.9713 (Fig. 3). Thus, Eq. 6 was developed to estimate Q_c of biomass based on $Y_{L/X}$.

$$Q_c = 0.2387Y_{L/X} + 15.857 \quad (6)$$

Obviously, the Q_c calculated by Eq. 6 fit the experimental data quite well.

Discussion

As mentioned above, two methods were developed to estimate the energy content of oleaginous yeast samples with different lipid content and elemental composition. By comparing the η values in Tables 1 and 2, the results obtained by the two approaches were in accordance with each other very well (relative errors < 5%). When we extend the application of the two equations into the estimation of growth energy efficiency of other oleaginous microorganisms base on other substrate, the results could fit very well. For example, Table 3 showed the biomass energetic coefficient (η) and combustion heat (Q_c) of several different ethanol-growth microorganisms were estimated by Eqs. 5 and 6 [23].

Though the maximum of combustion of those ethanol-growth microorganisms could reach up to 36.23 (kJ g^{-1}), η values of ethanol-growth microorganisms were very low,

Table 2 Relationship between the enthalpy of combustion (Q_c) and growth energetic yield (η)

Entry	Sampling time/h	$Y_{L/X}$/%	Q_c^a/kJ g^{-1}	Q_c^b/kJ g^{-1}	Q_c^c/kJ g^{-1}	η^d	η^e	η^f
1	7	1.00	16.74	16.83	16.10	0.31	0.31	0.30
2	24	37.09	23.74	23.79	24.70	0.74	0.75	0.77
3	45	53.23	28.10	28.81	28.55	0.82	0.84	0.84
4	69	61.45	29.06	30.24	30.51	0.75	0.78	0.79
5	96	65.99	32.58	32.63	31.59	0.84	0.85	0.82
6	116	67.41	33.10	33.04	31.93	0.83	0.83	0.81
7	123	70.16	32.68	31.08	32.58	0.78	0.74	0.78

[a] Calculated data based on Eq. 3

[b] Experimental data based on calorimeter

[c] Obtained using the Eq. 6

[d] Based on Q_c^a

[e] Based on Q_c^b

[f] Based on Q_c^c

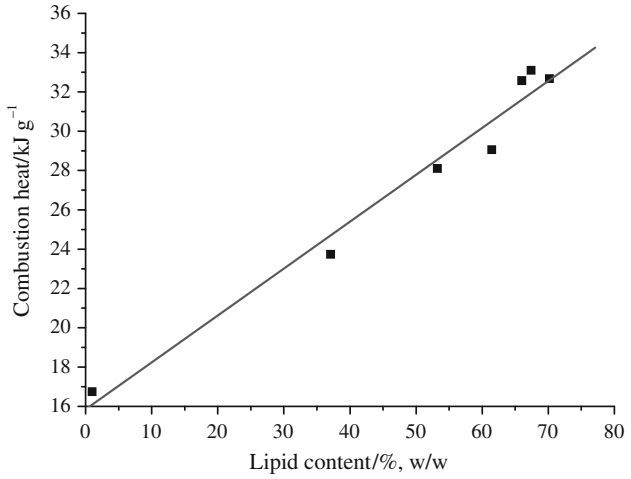

Fig. 3 Relationship between combustion heats of biomass and lipid content ($Y_{L/X}$) of the cells. Combustion heats of biomass were calculated by Eq. 3. *Solid line* indicates linear regression $Q_c = 0.2387\ Y_{L/X} + 15.857$, $R^2 = 0.9713$

of which the maximum one was 0.46. In addition, ethanol could be used as fuel directly. Therefore, it seemed that supplement of the material of biodiesel production by microbial lipid based on ethanol-growth microorganisms was uneconomic. From Table 3, it could be seen that maximum and minimum of $Y_{X/S}$ and $Y_{L/X}$ of these microorganisms were ranging from 0.16 to 0.39 and 19.5 to 66.5, respectively. Nevertheless, when Eq. 5 established by our data was utilized to estimate the η of the microbes, similar values were obtained with those calculated based on elemental composition with the relative errors less than 11.26%. Similar situation happened for comparing the Q_c estimated by Eq. 6 and that based on Eq. 3 (relative error $< 12.5\%$). As a result, Eqs. 4 and 6 could be exploited to investigate efficiency of energy conversion of other microbial growth processes cultured in media with different energy content, such as fungus and microalgaes for lipid production. It would be useful for understanding the process for lipid fermentation. For instance, Table 4 showed the maximum lipid content and combustion heat of several common oleaginous microorganisms. Obviously, most microalgaes, yeasts and fungis could store more energy in cells than bacteriums for their ability of accumulating more intracellular lipid. According to the data reported in literature, the maximum combustion heat of biomass of microalgae, yeast and fungi were 34.24, 35.67, and 36.39 (kJ g^{-1}), respectively. It implied that oleaginous microorganisms might be a rich "material storehouse" of biodiesel production. If the energy content of substrates utilized by these oleaginous microorganisms was determined, η of the biomass could also be estimated, which helped to choose the optimum cultural media for economic production of lipid.

When fed-batch culture in 30-l bioreactor with glucose as the sole carbon source was conducted, *R. toruloides* Y4 could accumulate lipid as much as 83% of the dry weight of biomass after 142 h fermentation, and the $Y_{X/S}$ was 0.35 g g^{-1} [26]. Biomass energetic yield of the lipid fermentation process was 0.83, which could also reveal the fact that *R. toruloides* Y4 was an efficient "energy-converter" in lipid production with glucose as the substrate. Moreover, it had the potentials to use other cheap sugars as substrate for lipid production, such as xylose and arabinose the main components of cellulose hydrolyzate [27]. From

Table 3 Biomass energetic coefficient (η) and combustion heat (Q_c) of ethanol-growth microorganisms

Microorganism[a]	$Y_{X/S}$/g g^{-1}	$Y_{L/X}$/%	η^b	η^c	Re[d]/%	Q_c^e/kJ g^{-1}	Q_c^f/kJ g^{-1}	Re[g]/%
Lipomyces lipofer IBPhM y-281	0.29	37.1	0.27	0.24	9.10	27.90	24.70	11.47
Lipomyces starkey IBPhM y-282	0.16	43.1	0.15	0.14	8.13	29.55	26.13	11.57
Zygolipomyces lactosus IBPhM y-695	0.39	66.5	0.46	0.42	9.79	36.23	31.71	12.47
Cryptococcus albidus var. albidus IBPhM y-706	0.28	53.4	0.27	0.27	−0.61	29.55	28.59	3.27
Cryptococcus albidus IBPhM y-415	0.33	29.4	0.25	0.25	−0.56	23.44	22.87	2.46
Torulopsis kestonii IBPhM y-901	0.27	20.4	0.19	0.19	−0.04	22.52	20.72	7.97
Trichosporon fermentans IBPhM y-481	0.34	19.5	0.25	0.23	4.66	22.13	20.51	7.32
Endomyces magnusii IBPhM y-261	0.26	27.5	0.21	0.20	5.29	24.43	22.41	8.25
Candida methylica IBPhM y-670	0.39	19.7	0.24	0.27	−11.26	19.19	20.55	−7.11

[a] From literature [23]

[b] Calculated from Eq. 2

[c] Calculated from Eq. 5

[d] Relative error between η^b and η^c

[e] Calculated from Eq. 3

[f] Calculated from Eq. 6

[g] Relative error between Q_c^e and Q_c^f

Table 4 Combustion heat of several oleaginous microorganisms

Microorganism	$Y_{L/X}$/%	Q_c^a/kJ g^{-1}
Microalgae		
Botryococcus braunii[b]	75	33.76
Cylindrotheca sp.[b]	37	24.69
Nitzschia sp.[b]	47	27.08
Schizochytrium sp.[b]	77	34.24
Yeast		
Candida curvata[b]	58	29.70
Cryptococcus albidus[b]	65	31.37
Lipomyces starkeyi[b]	64	31.13
Rhodotorula glutinis[b]	72	33.04
Rhodosporidium toruloides Y4[c]	83	35.67
Bacterium		
Arthrobacter sp.[b]	>40	>25.41
Acinetobacter calcoaceticus[b]	38	24.93
Rhodococcus opacus[b]	25	21.82
Bacillus alcalophilus[b]	24	21.59
Fungi		
Aspergillus oryzae[b]	57	29.46
Mortierella isabellina[b]	86	36.39
Humicola lanuginosa[b]	75	33.76
Mortierella vinacea[b]	66	31.61

[a] Calculated by Eq. 6

[b] From literature [2]

[c] From previous experiments [26]

the advantages mentioned above, *R. toruloides* Y4 was hoped to play an important role in the microbial lipid production.

Conclusions

This article provided the analysis on biomass yields and energetic yields of the oleaginous yeasts *R. toruloides* Y4. The relationship between biomass energetic yield coefficient (η), biomass yield on substrate ($Y_{X/S}$) and lipid yield on substrate ($Y_{P/S}$) was established. Combustion heat of *R. toruloides* Y4 biomass determined experimentally, were in good agreement with those calculated based on its elementary contents. A new equation (Eq. 6) was developed to calculate combustion heat (Q_c) of biomass by lipid content ($Y_{L/X}$), by which energy content of the biomass could be obtained without the elements composition of the cells. It should be valuable to address the overall techno-economical analysis of bio-energy production.

Acknowledgements We thank Prof. Tan ZC of DICP, CAS for his assistance on calorimetric analysis.

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