Research Article

Biodiesel from *Saccharomyces cerevisiae*: fuel property analysis and comparative economics

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Abstract
The depletion of exhaustible underground petroleum resources has put the present civilization at stake, thereby warranting intense research on non-exhaustible fuel. With this energy crisis hitting the block, microorganisms such as yeasts are gaining wider importance as potential biofuel candidates. An indigenous yeast strain *Saccharomyces cerevisiae* isolated from laboratory-scale brewing was investigated for biodiesel production. Biodiesel was produced by in situ transesterification approach using 1,1,3,3-tetramethylguanidine as the catalyst. The fuel properties such as viscosity, density, calorific value and cetane number (CN) were determined to assess the fuel quality of *S. cerevisiae* biodiesel. Additionally, the investigation also focuses on theoretical studies considering the yeast de-oiled cake (low-value biomass refuse). Fatty acid methyl ester analysis revealed that biodiesel was primarily composed of tricosylic acid (C23:0, 28.71%), palmitoleic acid (C16:1, 28.96%) and oleic acid (C18:1, 18.13%). Eicosapentaenoic acid (C20:5, 2.01%), one of the most commonly known polyunsaturated fatty acid, was present in the yeast strain. The CN of yeast biodiesel was 71.58, which was much higher than petro-diesel. The theoretical findings suggest the competitiveness of yeast biomass conversion technologies with petroleum refining process economics. The overall study warrants the feasibility of co-production of biodiesel from *S. cerevisiae* and cracked biofuel products (from *S. cerevisiae* de-oiled cake) under the aegis of biorefining applications.

Keywords Yeast · *Saccharomyces cerevisiae* · Biodiesel · Biorefinery · Comparative economics

1 Introduction

The depletion of exhaustible geological reservoirs has created a worldwide concern and consequently has spiked up interest in alternative fuels. Production of biofuels from renewable carbon neutral feedstock has long been a research goal for future replacement of conventional petro-fuels. Although numerous terrestrial energy crops have been investigated in this regard, the conundrum of the food versus fuel debate, limited geographical availability, higher processing costs and slow growth rate impedes their commercialization [1–3]. A cost-effective approach much conducive to improving the process economics of biofuel production may well be addressed by targeting oleaginous microorganisms such as microalgae, yeasts, fungi and bacteria.

Oleaginous microbes are an attractive alternative to higher plants for lipid production since their production does not require agricultural land, thereby avoiding displacement of food production [4, 5]. Furthermore, these microorganisms have short life cycles, require less labor inputs and are less susceptible to environmental variations

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which further acquiesces their candidature as an ideal bioenergy feedstock. Among all these microorganisms today much of the scientific attention is focused on microalgae. Microalgae have been studied extensively on account of their various potential advantages for biofuel production when compared to terrestrial energy crops. They have been hypothesized to have tremendous theoretical potential as a renewable fuel source [6, 7]. The most prominent renewable biofuels from microalgae are methane produced by anaerobic digestion of algal biomass [8–10], biodiesel from microalgal oil [11–18], photobiologically produced biohydrogen [19–22], bioethanol [23] and pyrolytic bio-oil [24–27]. The production of renewable biofuels from algae is undeniably of considerable scientific interest; however, substantial technical barriers presently foil their commercial utilization. These include the scarcity of suitable land given the current levels of productivity, lack of adequate photosynthetically active radiation and the prohibitive costs of delivering supplementary light, fertilizer, controlling temperature and protection against invasive non-lipid-producing species [28–33].

Considering these lacunas in microalgae, yeasts may be a potentially attractive biofuel option. Oleaginous yeasts are considered as a promising alternative lipid source for biodiesel fuel production [34]. Yeasts offer credible candidature for biodiesel production owing to their unicellular high growth rate, short life cycle, easy scale-up and rapid lipid-accumulating ability in discrete lipid bodies [35, 36]. Furthermore, they are also capable of utilizing inexpensive fermentation media such as nutritional residues from agriculture and industry [37–39]. Several species of yeasts like *Rhodosporidium toruloides*, *Cryptococcus curvatus*, *Lipomyces starkeyi* and *Yarrowia lipolytica* accumulate more than 20% of lipids of their dry cell weight [40–44]. Oleaginous yeast species are highly productive on a per cell basis, with lipid yields of up to 65% dry weight and biomass yields of 10–100 g/L within 3–7 days [45]. Besides, majority of the yeast lipids are comprised of long-chain fatty acids which are comparable to conventional vegetable oils used in biodiesel production [46]. Application of yeasts such as *Saccharomyces cerevisiae* strain for alcoholic fermentation has also been reported in a recent investigation on bioethanol and biogas co-production by an integrated biorefinery approach using the green macroalgae Chaetomorpha linum [47]. Regardless of having tremendous biofuel potential, there is paucity of scientific literature pertaining to yeast-based biodiesel when compared to microalgae. As such, we attempted to investigate the feasibility of biofuel production from yeast using our laboratory strain *S. cerevisiae* 0A03.

This research aims to highlight the feasibility of biodiesel production from an indigenously isolated yeast strain *S. cerevisiae* 0A03 isolated from laboratory-scale brewing. Relevant fuel properties such as viscosity, density, calorific value and cetane number (CN) were determined to assess the fuel quality. Further, a theoretical approach based on a previous study [6] has been discussed to study the competitiveness of yeast biomass with current petroleum prices.

2 Materials and methods

2.1 Isolation, purification, growth medium and culture conditions

Yeast isolation was done from the starter culture cake used by the Ahom community, Assam, India, for preparation of rice beer. The starter culture cake was ground into fine powder. One gram of powder was homogenized in 5 mL sterile distilled water and serially diluted to 10^{-5} dilutions with Millipore water. Fifty microliters of aliquot from each of the dilutions was spread on YM agar plates (yeast extract 3 g/L, malt extract 3 g/L, peptone 5 g/L, glucose 10 g/L, agar 18 g/L and pH 6.6). The plates were incubated at 25 °C for 48 h. The growing yeast colonies were divided on the basis of their morphological characteristics. Pure cultures were obtained from the distinct single colonies by quadrant streaking. The isolated strains were maintained in 20% glycerol at − 20 °C. Among the different isolates obtained, the fastest and dominantly growing isolate was used for the present investigation. For further experimentation, the isolated pure colonies were maintained at 28 °C for 48 h in YM culture media (in triplicate in a batch) before harvesting the cells for lipid extraction.

2.2 Molecular characterization

2.2.1 DNA extraction and PCR amplification

Yeast genomic DNA was extracted using a HIMEDIA yeast DNA extraction kit (HiPurA™ 96 Yeast Genomic DNA Purification kit). DNA amplification was performed with GeneAmp PCR system (Applied Biosystems, USA). The PCR mixture was prepared by mixing 1.5 µL of 15 mM $MgCl_2$, 1.5 µL 10XTaq buffer, 1.2 µL of 2 mM dNTPs, 0.2 µL 1 Unit Taq polymerase, 1 µL of each forward and reverse primer (5 picomolar concentration) and nuclease-free water to obtain a final volume of 15 µL. To the reaction mixture, 1 µL genomic DNA was added as the template. The sequences of the ITS primers used were as follows: ITS1 forward: 5′-TCCGTAGGTGAACCTGCGG-3′ and ITS4 reverse: 5′-TCC TCCGCTTATTGATATGC-3′. PCR was run for 40 cycles, and the conditions used for the amplification of 18S rDNA gene were: initial denaturation (94 °C for 2 min), denaturation (94 °C for 1 min), annealing (45 °C for 40 s), elongation

(72 °C for 2 min) followed by a final elongation step (72 °C for 10 min).

2.2.2 DNA sequencing

Following resolution in agarose gel, the PCR product was purified using gel extraction kit (Bangalore Genei) and sequenced. Sequencing reaction was performed using a PRISM BigDye Terminator v3.1Cycle sequencing kit. The DNA samples containing the extension products were added to Hi-Di formamide (Applied Biosystems, Foster City, CA). The mixture was incubated at 95 °C for 5 min, followed by 5 min on ice and then analyzed by ABI Prism3730XL DNA analyzer (Applied Biosystems, Foster City, CA).

2.2.3 Nucleotide sequence analysis

Consensus sequence of 18S rRNA gene was generated from forward and reverse sequence data using aligner software. The 18S rRNA gene sequence of the yeast strain 0A03 was submitted to NCBI GenBank and was assigned the accession number KF650433. The 18S rRNA gene sequence obtained was used to carry out BLAST analysis [48]. Based on maximum identity score, the sequences of other yeast isolates and strains were selected and the respective sequences were retrieved from the online database. The retrieved sequences of the selected organisms were aligned using CLC Main Workbench and clustered using neighbor-joining method to generate the phylogenetic tree.

2.3 Lipid extraction

Total lipid was determined by the method of Bligh and Dyer [49].

2.4 In situ transesterification

In situ transesterification was carried out with modifications [50]. 1,1,3,3-Tetramethylguanidine (TMG) was used as the catalyst. In short, transesterification reactions were carried out with 100 mg of lyophilized yeast biomass. The sample biomass was reacted with 2 ml of CH_3OH containing 25% (v/v) TMG for 30 min at 60 °C. Following completion, the reaction was quenched by adding $CHCl_3$ (1 mL) whereby forming a single-phase solution with CH_3OH. Phase separation was achieved by washing the methanol–chloroform solution with 5 ml distilled water. This was followed by centrifugation at 2000 rpm for 2 min. The upper phase was comprised of TMG and CH_3OH with water, whereas FAME, TAG and other lipids partitioned with $CHCl_3$ in the lower organic phase. The remnant biomass formed a layer at the boundary between two phases. The $CHCl_3$ phase was removed by a syringe and collected in a 10-mL centrifuge tube. The remnant biomass was twice washed with 2 mL of $CHCl_3$ to recover residual FAMEs and lipids. The total $CHCl_3$ volume was brought to 5 ml and mixed by inversion following which 100 µl aliquot was used for GC analysis.

2.5 GC-FID analysis

The fatty acid profile of *Saccharomyces cerevisiae* was determined by GC of the corresponding methyl ester on a TRACE™ 1300 (Thermo Scientific) gas chromatograph equipped with FID detector and TRACE™ TR-FAME GC column. The column temperature was held at 50 °C for 2 min, raised to 200 °C at 10 °C/min, followed by 300 °C at 5 °C/min and finally held for 10 min at the same temperature. Helium was used as the carrier gas at a flow rate of 1 ml/min. The identification of individual components was done by comparison of retention time with the standard FAME mix. The wt% of the individual fatty acids was calculated based on the internal standard.

2.6 Determination of fuel properties

For comparison, the fuel properties of FAMEs (biodiesel) were determined using a set of empirical relations [51]. The following general expression is used to estimate the properties of biodiesel from the properties of individual FAMEs:

$$f_b = \sum_{i=1}^{n} z_i \cdot f_i \tag{1}$$

where f is a function that represents any physical property (i.e., viscosity, density or calorific value), the subscripts b and i refer to the biodiesel and the pure ith FAME, respectively), z_i is the mass or mole fraction of the ith FAME. The function f_b is replaced by the variables $\ln \eta_i$, ρ_i and δ_i in order to specify the CN, natural logarithm of kinematic viscosity, density and higher heating value (calorific value) of biodiesel, whereas the function f_i is interchanged by the variables $\ln \eta_i$, ρ_i and δ_i of the individual ith FAME [51].

The individual FAME properties were estimated using the following mathematical relations:

The kinematic viscosity expressed as a function of M_i and N is given as,

$$\ln \eta_i = -12.503 + 2.496 \ln M_i - 0.178N \tag{2}$$

where η_i is the kinematic viscosity at 40 °C of the ith FAME in mm^2/s.

The general expression used for the determining the density of saturated and unsaturated FAMEs is:

$$\rho_i = 0.8463 + \frac{4.9}{M_i} + 0.0118N \quad (3)$$

where ρ_i is the density at 20 °C of the *i*th FAME in g/cm^3.

The calorific value of methyl esters can be calculated from:

$$\delta_i = 46.19 - \frac{1794}{M_i} - 0.21N \quad (4)$$

where δ_i is the calorific value of the *i*th FAME in MJ/kg.

2.7 Economics of yeast biofuel

In the present investigation, economics of producing of yeast biofuel was estimated based on a previous study [6] on algal biomass.

The yeast de-oiled cake following lipid extraction was assumed as a feedstock for thermal/catalytic cracking. The quantity of yeast biomass (*M* tons), which is the energy equivalent of a barrel of crude petroleum, can be estimated as follows:

$$M = \frac{E_{\text{Petroleum}}}{ywE_{\text{biodiesel}} + (1-w)\left(E_{\text{bio-oil}}x_{\text{bio-oil}} + qE_{\text{syngas}}x_{\text{syngas}} + E_{\text{biochar}}x_{\text{biochar}}\right)} \quad (5)$$

where $E_{\text{Petroleum}}$ (~ 6100 MJ) is the energy content in the crude petroleum, *w* is the oil content of the biomass in weight percentage and *y* is the biodiesel yield from yeast biomass. $E_{\text{biodiesel}}$, $E_{\text{bio-oil}}$, E_{syngas} and E_{biochar} are the energy contents of biodiesel, bio-oil, syngas and biochar, respectively. Similarly, $x_{\text{bio-oil}}$, x_{syngas} and x_{biochar} are the fractions of bio-oil, syngas and biochar, which are obtained as the products of cracking process and *q* is (m^3/ton) of syngas obtained from the cracking unit.

Assuming that the cost involved in converting a barrel of crude petroleum to various transport fuels is equivalent to converting M tons of biomass to bioenergy, the maximum acceptable price that could be compensated for the biomass would be the same as the price of a barrel of crude petroleum; thus,

Acceptable price of biomass ($/ton) can be calculated as follows:

$$\text{Acceptable price of biomass} = \frac{\text{Price of a barrel of petroleum}}{M} \quad (6)$$

3 Results and discussion

3.1 Isolation and molecular characterization

The yeast strain under investigation was isolated from rice cakes (locally known as 'pitha') which are used as starter culture by the Ahom community of Assam, India. The rice cakes have been in use since centuries for the production of rice beer from a kind of sticky rice locally known as the 'bora rice.' Among all the isolated strains, the one which exhibited marked degree of ecological success (as evident from faster growth rate) was selected for the present investigation. Morphological characterization and subsequent molecular analysis revealed the strain as *S. cerevisiae*. It is noteworthy to mention here within that these microorganisms have been traditionally used for fermentation processes; however, their potential with regard to biodiesel production has not been scientifically assessed properly.

Figure 1 shows the partial DNA sequence of the conserved region of 18S rRNA gene of *S. cerevisiae* 0A03. BLAST analysis (Table 1) revealed up to 96% homology of 18S rDNA sequence of strain 0A03 to 18S rDNA sequences of other known strains of *S. cerevisiae*. The sequence was deposited in the NCBI GenBank database and was assigned the GenBank accession No. KF650433.

Fig. 1 Partial DNA sequence of the conserved region of 18S rRNA gene of *S. cerevisiae* 0A03

AAAATTTTAATAATTTTGAAATGGATTTTTTTTGTTTTGGCAAGAGCATGAGAGCTTTTACTGGGCA
AGAAGACAAGAGATGGAGAGTCCAGCCGGGCCTGCGCTTAAGTGCGCGGTCTTGCTAGGCGTAA
GTTTCTTTCTTGCTATTCCAAACGGTGAGAGATTTCTGTGCTTTTGTTATAGGACAATTAAAACCGTT
TCAATACAACACACTGTGGAGTTTTCATATCTTTGCAACTTTTTCTTTGGGCATCAGCAATCGGGGCC
CAGAGGTAACAAACACAAACAATTTTATTTATTCATTAAATTTTTGTCAAAAAACAAGAATTTTCGTA
ACTGGAAATTTTAAAATATTAAAAACTTTCAACAACGGATCTCTGGTCTCGCATCGATGAAGAACGC
AGCGAAATGCGATACGTAATGTGAATTGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTG
CGCCCCTTGGTATTCCAGGGGGCATGCCTGTTTGAGGTCTTTCCTTCTCAAACATTCTGTTTGGTAG
TGAGTGATACTCTTTGGAGTTAACTTGAAATTGCTGGCCTTTTCATTGGATGTTTTTTTTTTTCCAAA
GAGAGGTTTCTCTGCGTGCTGGAGGTAAATGAATTACGGTCGTTTTAGGTTTTCCCAACTGCGGCT
AATCTTTTTTTATACCGGAGCGTATTGAACCGTTATCGAATAAGAAGAGAGCGTTCTAGGCGACCAA
TGTTCTTAAAGTTTGACTTCATCAGTAGAAGTACCCGCTGACTTAGCATATCATAACGCGGA

Table 1 BLAST analysis

Description	Max. score	Total score	Query cover (%)	*E* value	Identity (%)	Accession
Saccharomyces cerevisiae strain 0A03 18S ribosomal gene, partial sequence	1421	1421	100	0.0	99	KF650433.1
Saccharomyces cerevisiae isolate YN2 18S ribosomal gene, partial sequence: internal transcribed spacer 1.5 8S ribosomal RNA gene	1288	1288	99	0.0	96	KJ502661.1
Saccharomyces cerevisiae strain W36 18S ribosomal gene, partial sequence: internal transcribed spacer 1.5 8S ribosomal RNA gene	1286	1286	98	0.0	96	DQ167471.1
Saccharomyces cerevisiae strain X14-3 18S ribosomal gene, partial sequence: internal transcribed spacer 1.5 8S ribosomal RNA gene	1284	1284	99	0.0	96	JN837099.1
Saccharomyces cerevisiae strain X68-2 18S ribosomal gene, partial sequence: internal transcribed spacer 1.5 8S ribosomal RNA gene	1282	1282	99	0.0	96	JN837100.1
Saccharomyces cerevisiae strain P1 18S ribosomal gene, partial sequence: internal transcribed spacer 1.5 8S ribosomal RNA gene	1280	1280	99	0.0	96	KU131578.1
Saccharomyces cerevisiae isolate YN3 18S ribosomal gene, partial sequence: internal transcribed spacer 1.5 8S ribosomal RNA gene	1280	1280	99	0.0	96	KJ502662.1
Saccharomyces cerevisiae isolate AUS-LFB-MA-YC6 18S ribosomal gene, partial sequence: internal transcribed spacer 1.5 8S ribosomal RNA gene	1280	1280	98	0.0	96	JN093147.1
Saccharomyces cerevisiae strain PMM08-412-AL isolate ISHAM-ITS ID MITS2754 18S ribosomal gene, partial sequence: internal transcribed spacer 1.5 8S ribosomal RNA gene	1279	1279	97	0.0	96	KP132597.1
Saccharomyces cerevisiae strain Sc20 18S ribosomal gene, partial sequence: internal transcribed spacer 1.5 8S ribosomal RNA gene	1279	1279	97	0.0	96	KC515374.1
Saccharomyces cerevisiae strain Chicha 08 18S ribosomal gene, partial sequence: internal transcribed spacer 1.5 8S ribosomal RNA gene	1279	1279	99	0.0	95	KC183729.1

Additionally, the nucleotide sequences of other similar organisms from BLAST analysis were retrieved from the GenBank database. Their nucleotide sequences were aligned using CLC main workbench, and a tree was created based on neighbor-joining method which is shown in Fig. 2 along with the respective bootstrap values. The phylogenetic tree revealed the strain 0A03 to be closely related to *S. cerevisiae* strain X68-2 and SC20 belonging to the same cluster with a high bootstrap value of 100 and 98, respectively. A bootstrap value of 70% or higher indicates reliable groupings and shows the measure of phylogenetic accuracy and repeatability of the phylogenetic trees [52]. Thus, it is evident that the isolated yeast species, with a bootstrap value of 100, is *S cerevisiae* strain 0A03.

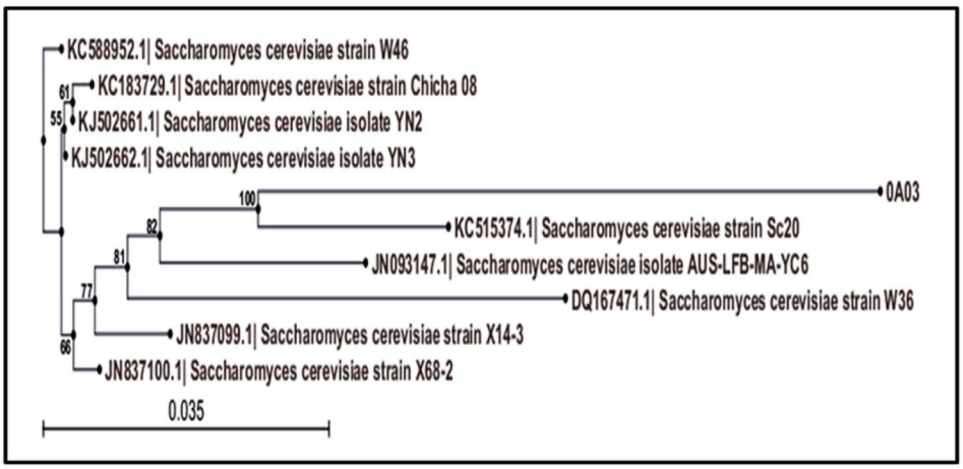

Fig. 2 Phylogenetic relationship of *S. cerevisiae* 0A03 and other closely related *Saccharomyces* species based on 18S rDNA sequencing

3.2 Fatty acid analysis of *S. cerevisiae* 0A03

Oil accumulation in yeasts has been known since many years. These oils, generally referred to as Single Cell Oils (SCOs), are triacylglycerols and can constitute up to 70% of the weight of the yeast cells [53]. The fatty acyl components of the triacylglycerols are the same as those that occur in plants such as soybean and rapeseed oil [53]. Fatty acid analysis in yeast is important not only from the biofuel standpoint, but also from the perspective of distinguishing between closely related yeast species both at the generic and at the species levels. Acceptable proportion of saturated and unsaturated fatty acids is crucial for investigating yeast as a biodiesel feedstock. Yeasts usually have an average lipid content ranging from 5 to 15% dw [54], and *S. cerevisiae* has been reported to have a lipid content of 35–147 mg/g dw [55]. In this study, *S. cerevisiae* 0A03 had a lipid content of 12.8% dw. The fatty acids present in *S. cerevisiae* 0A03 (Table 2) were C12:0 (13.35%), C16:1 (28.96%), C16:0 (5.03), C18:2 (2.01), C18:1 isomer (18.13), C18:0 (1.76), C20:5 (2.01) and C23:0 (28.71), respectively. The fatty acids of *S. cerevisiae* 0A03 were similar to previous reports on oleaginous yeasts [53]. In addition, two additional long-chain fatty acids, viz. C20:5 (eicosapentaenoic acid) and C23:0 (tricosylic acid), were present in *S. cerevisiae* 0A03. Halim et al. (2011) reported the lipid content for the dry biomass of microalgae *Chlorococcum* sp. as 7.1% [56]. Biodiesel from the microalga *Monoraphidium* sp. is comprised of palmitic acid (C16:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3), with palmitic acid (C16:0) and linoleic acid (C18:2) being the major fractions [57]. A comparison of the fatty acid composition of *S. cerevisiae* and microalgae is presented in Table 3. A representative gas chromatogram of the FAME mix is presented in Fig. 3.

Biodiesel from *S. cerevisiae* 0A03 is expected to have poor oxidative stability owing to the presence of long-chain unsaturated fatty acid, which may restrict its utilization. Biodiesel from highly unsaturated sources oxidizes more rapidly than conventional diesel, resulting in formation of insoluble sediments which interferes with engine performance [58]. One strategy to deal with the oxidative stability would be to extract the PUFA prior to biodiesel synthesis. Very-long-chain PUFA is well known for their

Table 2 Fatty acids of *S. cerevisiae* 0A03

Sl nos.	Retention time	Fatty acid	wt (%)
1	17.443	C12:0	13.3501
2	25.155	C16:1	28.9673
3	25.642	C16:0	5.03778
4	28.513	C18:2	2.01511
5	28.685	C18:1 isomer	18.136
6	29.248	C18:0	1.76322
7	31.22	C20:5	2.01511
8	37.183	C23:0	28.7154

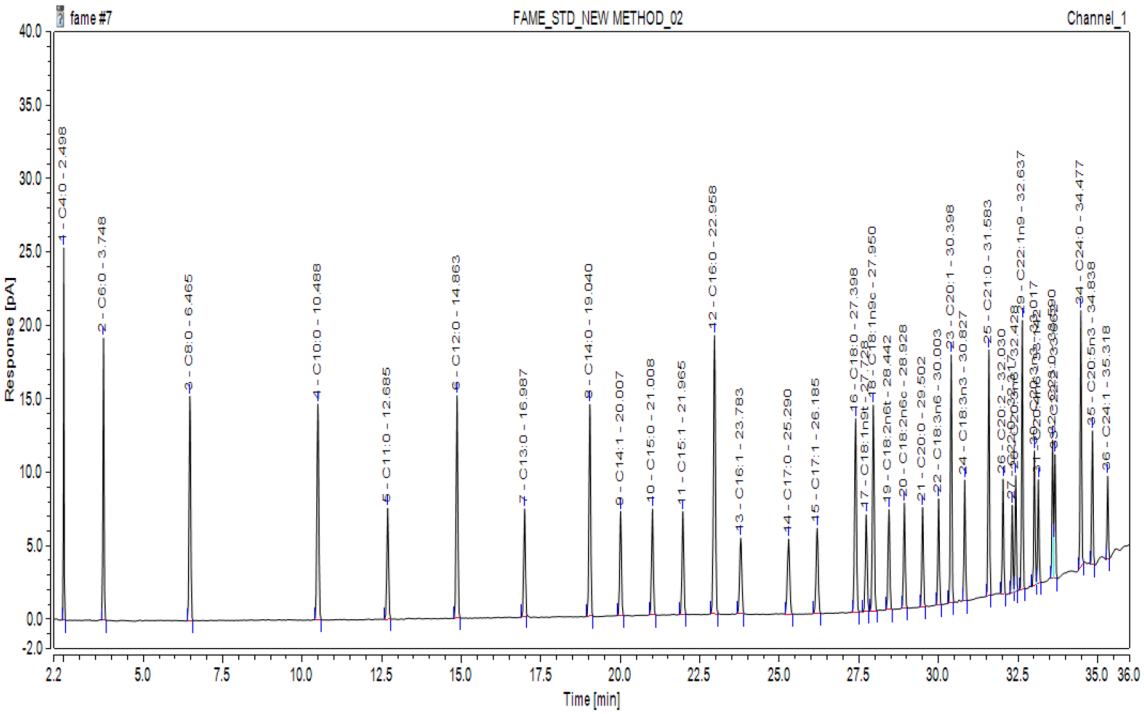

Fig. 3 Representative gas chromatogram of the FAME mix

Table 3 Fatty acids profile of *S. cerevisiae* and microalgae *Chlorococcum* sp. and *Monoraphidium* sp

Fatty acid	*S. cerevisiae* wt (%)	*Chlorococcum* sp. [56] wt (%)	*Monoraphidium* sp.* [57] wt (%)
C12:0	13.3501	–	–
C16:1	28.9673	~04	–
C16:0	5.03778	~19	32.83
C18:3	–	–	19.31
C18:2	–	–	29.85
C18:1	2.01511	~04	18.01
C18:1 isomer	18.136	~63	
C18:0	1.76322	~03	–
C20:5	2.01511	–	–
C23:0	28.7154	–	–

Table 4 Fuel properties of *S. cerevisiae* 0A03 biodiesel

Fuel properties	Yeast biodiesel	ASTM standard
Density (g/cm^3 at 15 °C)	0.870	0.86–0.9
Viscosity (mm^2/s at 40 °C)	5.797	3.5–5
Calorific value (MJ/kg)	40.142	–
Cetane number (CN)	71.58	–

nutritional importance. PUFA has been reported to confer flexibility, fluidity and selective permeability properties to cellular membranes [59]. This is crucial for brain development, advantageous for the cardiovascular system and for other important pharmaceutical targets [59]. It is desirable that the extraction of pharmaceutical-grade PUFA should be coupled with biodiesel production from yeast to reduce the economics of feedstock utility. *S. cerevisiae* 0A03 biodiesel is composed primarily of tricosylic acid (C23:0, 28.71%), palmitoleic acid (C16:1, 28.90%) and oleic acid (C18:1, 18.13%). High percentage of saturated fatty acids and monounsaturated fatty acids were also observed in the yeast strain. This may be considered optimal from a fuel quality standpoint because fuel polymerization during combustion would be substantially less than with PUFA-derived fuel [11].

3.3 Fuel properties of yeast biodiesel

The fatty acid profile of the feedstock oil influences the biodiesel fuel properties. Calorific value, cetane numbers (CN), cold flow properties, oxidative stability, viscosity, etc., are some of the important fuel properties that are being influenced. Table 4 presents the fuel properties of the biodiesel as against ASTM specifications. In this study, we have only considered four fuel properties, viz. density, kinematic viscosity, calorific value and CN. In general, the properties of biodiesel are quite similar to conventional petro-diesel. Density of the yeast biodiesel was 0.870 g/cm^3, which was well within the ASTM range (0.86–0.90 g/cm^3). Conversely, yeast biodiesel exhibited a slightly higher kinematic viscosity (5.797 mm^2/s). Unlike the CN, the kinematic viscosity increases with the number of carbon and decreases with the degree of unsaturation [60]. The presence of C23:0 (28.71%) in yeast biodiesel can be attributed to the rise in kinematic viscosity of yeast biodiesel. This higher kinematic viscosity is likely to create engine problems such as engine deposits [61]. The use of biosurfactants may be helpful in reducing this high viscosity of the yeast biodiesel. Biosurfactants are much preferable in comparison with chemical surfactants in this respect, owing to their environmentally benign nature. The calorific value of yeast biodiesel was 40.142 MJ/kg. In general, the calorific value of petro-diesel ranges from 39 to 41 MJ/kg [59]. The calorific values for yeast and microalgal biodiesel were in close proximity. A Chinese study reported microalgal *Chlorella protothecoides* biodiesel with calorific value of 41 MJ/kg [62]. CN is one of the most important properties of biodiesel fuel. CN has been extensively used to assess the ignition quality of fuels. The CN for yeast biodiesel was 71.58, which was much higher than that for conventional petro-diesel fuels. The CN of petro-diesel fuels generally fall within the range of 47 to 51 [63]. According to a previous study, this value for microalgal biodiesel ranges from 39 to 54 [64]. A CN value of 70 was previously reported for biodiesel from the microalga *Spirulina platensis* [65]. High CN in yeast biodiesel can be attributed to increase in saturated FAMEs content and its chain length [65]. Higher CN results in higher combustion efficiency, improved engine performance and cleaner emissions and can be recommended for use in the high-speed engine (speeds above 800 rpm) [65]. Conversely, fuels with low CN will cause difficulty in engine starting, generate higher noise and exhaust smoke [65].

3.4 Economics of yeast biofuel

Let us consider in Eq. (5), y = 0.90, $E_{\text{bio-oil}}$ = 27,216 MJ/ton, E_{syngas} = 15 MJ/m^3, E_{biochar} = 27,216 MJ/ton, $x_{\text{bio-oil}}$ = 0.25, x_{syngas} = 0.45, x_{biochar} = 0.30 and q = 1050 m^3/ton. From the experimental results, $E_{\text{biodiesel}}$ was obtained as 35,742 MJ/ton. $E_{\text{Petroleum}}$ = 6100 MJ. Accordingly, Eq. (5) becomes

$$M = \frac{6100}{32167.8w + 22056.3(1 - w)} \tag{7}$$

The predictable price of yeast biofuel calculated on the basis of Eqs. (5–7) is shown in Fig. 4 for crude petroleum prices up to $1000/barrel and for biomass with 20–80% oil content. With $100/barrel of petroleum yeast biomass with an oil content of 80% needs

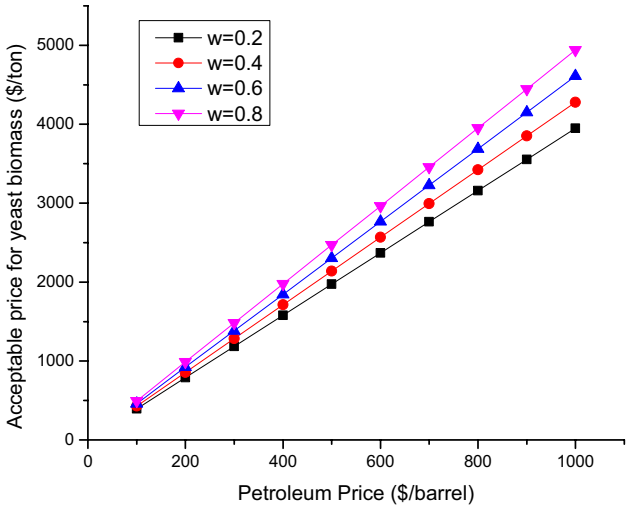

Fig. 4 Competitiveness of yeast biomass with petroleum prices

to be produced at < \$495 ton^{-1} to be competitive with petroleum. Consequently, taking into account the yeast biomass production cost as \$1500 ton^{-1}, the price of producing yeast biomass needs to decline by a factor of ~ 3. Strain improvement in yeast (genetic and metabolic engineering-based approaches) coupled with advances in biomass conversion technologies and downstream processing can be instrumental in curbing down overall yeast biomass production cost.

Economic favorability is unlikely to make any sense if the focus is only on the final product (biodiesel here). A biorefinery model for yeast necessitates the use of low-cost substrates, co-production of high value-added products [66]. As such, the current investigation also takes into account possible income generation from biomass residues (yeast de-oiled cake) through theoretical approaches. Several biofuel research studies have focused on producing biodiesel from microorganisms such as microalgae. Similarly for yeasts, such a production scheme will definitely produce an enormous amount of de-oiled cake as a low-value biomass refuse. Material and energy recovery from these biomass refuses is undeniably a challenge for the forthcoming biorefineries. One viable option in this regard would be to couple the production of biodiesel with cracked biofuel products. This integrated approach would be much favorable in reducing the economics of feedstock usefulness besides favoring the need for greater reliance of mankind on a bio-based economy. With greater research capacities, the conversion technologies involved in biofuel production from yeast may prove economically competitive with petroleum refining. However, yeast biofuel research is still at its infantile stage, and concept to commercialization of these biofuels can only be appraised through critical analysis of the process economics of large-scale production.

Considering the fatty acid profile, fuel properties and theoretical approach, the yeast strain *S. cerevisiae* 0A03 necessitates further research for sensible biodiesel production. The study also warrants superior research competence (strain improvement, oxidative stability, cold flow properties, exhaust emissions, viscosity reduction, lubricity, etc.) for a better comprehensive understanding.

4 Conclusion

Yeasts have always captivated the attention of the scientific community as model organisms for fermentation processes (bioalcohol production). However, in this study we have specifically emphasized on an entirely new perspective, viz. biodiesel production from yeast. Our findings advocate that the yeast strain *S. cerevisiae* 0A03 exhibits several interesting features for bioenergy research. Elevated proportions of saturated fatty acids and monounsaturated fatty acids in the yeast strain may be considered optimal from a fuel quality standpoint. High energy content (40.142 MJ/kg) and high CN (71.58) of yeast biodiesel are indicative of improved engine performance, higher combustion efficiency and cleaner emissions. Furthermore, our findings necessitate the need for coupling biodiesel production with cracking to improve biofuel process economics. Theoretical studies suggest that yeast biomass conversion technologies are likely to prove economically competitive with petroleum refining. We conclude that microorganisms like yeasts with untapped biological diversity should be harnessed for bioenergy–biorefinery-based applications in lines parallel with microalgae. However, this work warrants greater research capacities such as life-cycle assessment (LCA) for a better comprehensive understanding of yeast biorefinery concept.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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