Biomass Conversion and Biorefinery (2024) 14:7981–7994
https://doi.org/10.1007/s13399-022-03025-8

ORIGINAL ARTICLE

Biovalorization of wastewater of fish canning process by *Yarrowia lipolytica* for biodiesel and animal feed supplement production

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Received: 16 March 2022 / Revised: 15 June 2022 / Accepted: 3 July 2022 / Published online: 8 July 2022

Abstract

Microbial treatment of industrial wastewater, following with the production of the valuable products using the biomass of the same cells, enhances the economic benefits of biorefineries. The current study aimed to explore the potential of the oleaginous yeast *Yarrowia lipolytica* for oily and saline fish canning wastewater biotreatment and to evaluate the features of the yeast biomass for biodiesel and animal feed supplement production. Using *Y. lipolytica* EBL13 in wastewater reduced chemical oxygen demand by 85% during 7 days. In addition, other parameters, including biological oxygen demand (59%), nitrate (51%), sulfite (50%), total suspended solids (85%), total hardness (15.6%), oil (68.2%), and total phosphorus (91%), were decreased significantly compared to untreated samples. However, pH and ammonia were increased. Amino acid and fatty acid composition analysis of *Y. lipolytica* biomass showed the presence of essential amino acids and fatty acids in significant quantities, respectively. From 50.2% of protein content derived from the yeast biomass, 16.5% was related to essential amino acids. In addition, linolenic acid (0.15%) and eicosapentaenoic acid (0.21%) as essential omega-3 fatty acids and linoleic acid (22.15%) as essential omega-6 fatty acid were observed in the fatty acid profile. Predicted biodiesel physicochemical parameters such as cetane number (55.7 min), iodine value (76.4 g Iod/100 g oil), and degree of unsaturation (83.54) met the EN 14214 specifications. *Y. lipolytica* can be introduced as an ideal candidate for fish canning wastewater treatment and production of biodiesel and animal feed supplements.

Keywords *Yarrowia lipolytica* · Fish canning process · Wastewater treatment · Feed supplement · Biodiesel · Fatty acid profile

1 Introduction

Voluminous amounts of water consumption and wastewater production in different steps of fish canning industries, including cooking, cleaning, cooling, and sanitization, are considered worldwide environmental challenges [1]. Approximately 10–40 m^3 of water is estimated to be required for processing each ton of raw seafood. Eurofish discharges at least 650 m^3 effluents/100 tons of tuna daily [2]. Fish canning wastewaters (FCWs) are known for their organic compounds and degradable proteins and lipids [3]. The fish variant, additive type, processing steps, and water source are effective parameters for the characteristics of the produced wastewater [4]. Currently, the wastewater treatment approaches include physicochemical, biological, or combinatory methods [1]. Physicochemical procedures such as sedimentation, acidity adjustment, and dissolved air flotation are mainly used for primary FCW treatment in the refinery systems. The process is followed by aerobic and anaerobic biological techniques to remove organic compounds. FCWs contain large amounts of saline (2–35 g L^{-1}), which cause problems in biological treatment and make desalination necessary [5, 6]. Common desalination methods are expensive and time-consuming techniques and lead researchers to find alternative strategies for removing organic compounds from saline FCWs [7]. Salt-resistant microorganisms have been investigated in a wide range of research. One group of the biotechnologically useful microorganisms with confirmed

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salinity tolerance behavior are some *Yarrowia lipolytica* strains. Also, the laboratory adaptation process is one of the strategies to improve the salt resistance phenotype in this yeast [8]. *Y. lipolytica*, as an oleaginous yeast, can accumulate fats in the form of intracellular lipids by more than 50% of its weight. To date, different strains of *Y. lipolytica* have been identified in various food matrices and natural environments with a broad spectrum of dry weight during growth on alkanes, lipids, and other fatty substrates [9], and physicochemical conditions (pH, temperature, metal ion concentrations, substrate, and nutrient limitations) [10]. *Y. lipolytica* has been studied as the cell factory for the synthesis of organic acids, recombinant enzymes, drug components, unsaturated fatty acids, biosurfactants, and polyhydroxyalkanoates. In addition, its bioremediation potentials of hydrocarbons, brominated organic compound pollutants, and heavy metal removal have been reported in many studies [11]. Effluents of olive oil mill, palm oil mill, vegetable oil refinery, waste cooking oil, and soybean oil refinery residue are the examples of matrixes treated by *Y. lipolytica* in recent studies coupled with the production of biosurfactants, SCO, lipase, citric acid, α-ketoglutaric acid, pyruvic acid, and antioxidant compounds as by-products [10]. Recently, *Y. lipolytica* has been used to treat crude and diluted tuna wash processing wastewater (TWPW) in Tunisia. After 7 days of incubation, chemical oxygen demand (COD) and total organic carbon levels were reduced by 69.8% in crude wastewater samples, respectively. Also, TWPW showed a reduction of 75% COD and a 74% reduction in total organic carbon [12]. Treatment of olive mill wastewater (DOMW)/TWPW mixture (75:25) by *Y. lipolytica* resulted in eliminating 97.49% and 98.90% of COD and phosphorus after 7 days, respectively [13]. Dunoyer et al. have also benefited from *Y. lipolytica* ATCC 9973 enzymatic features to treat dairy waste. Using the yeast enzymatic extract under optimal conditions, fat, BOD, COD, and total solids decreased by 82.88%, 43.32%, 44.3%, and 13.58%, respectively [14]. Data published on the production of ex novo and de novo bio-oil by *Y. lipolytica* using low-cost substrate have led scientists to study this yeast as a candidate for biodiesel production [15, 16]. Another biotechnologically valuable feature of *Y. lipolytica* is its capability to produce significant levels of proteins (48–54%) and lipids from low-cost substrates leading it to be introduced as a suitable SCP (single-cell protein) and SCO (single-cell oil) producer yeast [17, 18].

Considering the wide biotechnological applications of *Y. lipolytica*, the current study aimed to evaluate the following three features in a salt-adapted strain: (a) biotreatment efficiency of FCW; (b) biodiesel characteristics which can be produced from the biomass; and (c) capability of biomass for usage as the animal feed supplement. To the best of our knowledge, this is the first report aiming to improve the bio-based circular economy of wastewater treatment through achieving added-value by-products using *Y. lipolytica* in a cost-effective way.

2 Materials and methods

2.1 Microorganism and wastewater

In this study, *Y. lipolytica* EBL13 as the recently salt-adapted strain (up to 15% NaCl) developed from *Y. lipolytica* ATCC 18942 was used for the experiments. *Y. lipolytica* ATCC 18942 had been cultured under increasing NaCl concentration stress from 0.4 to 2.6 M in a stepwise manner for 200 serial transfers during 7 months and achieved constant growth in 2.6 M NaCl at the endpoint, which was deposited as *Y. lipolytica* EBL13 (data not shown). To prepare the pre-culture, *Y. lipolytica* EBL13 was grown in autoclaved wastewater (121 °C, 20 min) at 28 °C and 160 rpm for 72 h.

2.2 FCW biotreatment and quality assessment

The wastewater sample was obtained from a local factory around Tehran in Iran and stored at 4 °C until the experiments. The provided pre-culture sample was amended (%5 v/v) to the biotreatment Erlenmeyer flasks containing 20 mL fresh wastewaters, and the flasks were incubated at 28 °C and 160 rpm for 7 days. To assess the potential of *Y. lipolytica* EBL13 in the reduction of COD [19], its reduction pattern was monitored at 24-h intervals using the colorimetric standard Hach Method 8000. Uninoculated wastewater samples were considered control flasks and were incubated in the same incubation conditions as test flasks [19]. The experiments were done in three independent experiments inside the lab.

The quality parameters of the wastewater sample including COD; pH; biological oxygen demand (BOD); total dissolved solids (TDS); total suspended solids (TSS); total hardness (TH); salinity; fat, oil, and grease (FOG); phosphate (PO_4^{3-}); chlorine (Cl); and sulfite (SO_3^{2-}) were measured by standard methods after 72 h in both test and control Erlenmeyer flasks. BOD (Standard Method 10099), COD (Standard Method 8000), TSS (Standard Method 8006), ammonia (Standard Method 10023), nitrate (Standard Method 8039), total phosphorus (Standard Method 8190), and total nitrogen (Standard Method 10072) were measured using Hach methods [20]. Total hardness (TH), phosphate, chlorine, and sulfite of the wastewater samples were measured using commercial kits.

2.3 Morphological analysis

To study the effect of salt on the yeast morphology using scanning electron microscopy (Tescan Company, Czech

Republic), *Y. lipolytica* EBL13 was cultured in both saline (containing 4% w v^{-1} NaCl) and non-saline wastewater for 48 h. Harvested cells were washed with dH_2O, fixed with glutaraldehyde 2%, and dehydrated by graded series ethanol [21]. Safranin and Sudan black were used to stain the produced lipid bodies.

2.4 Fatty acid profile analysis

To analyze the fatty acid profile, *Y. lipolytica* EBL13 was inoculated in the Erlenmeyer flask containing autoclaved wastewater and shaken at 28 °C and 160 rpm for 72 h. The cultures were centrifuged at 4000 rpm for 10 min, and the biomass was washed with NaCl solution (%0.9 w/v) and then lyophilized to extract fatty acids. To provide fatty acid methyl esters (FAMEs), the transesterification process was performed by acid catalysis. The yeast cells were sonicated at 90 W for 20 min in an anhydrous condition to lyse the cell wall. FAMEs were extracted using hexane and analyzed by gas chromatography (Agilent Technology 6890, USA) coupled with an FID detector. The retention time values obtained from GC were compared with those of the standard samples [22].

2.5 Prediction of biodiesel characteristics

The characteristics of fatty acid profiles are the factors affecting the quality of the produced biodiesel. In order to investigate the effective parameters in the quality of biodiesel production, the equations listed in Table 1 were used. Accordingly, the studied parameters included soap value (SV), iodine value (IV), cetane number (CN), degree of unsaturation (DU), long-chain saturated fatty acid (LCSF), cold filter plugging point (CFPP), cloud point, drop point, allelic position equilibrium (APE), base-allelic position equilibrium, higher heating value (HHV), kinematic viscosity (υ), and density (ρ) [23, 24].

2.6 Amino acid profile analysis

The extracted protein was hydrolyzed with hydrochloric acid 6 mol L^{-1} at 110 °C overnight. The sample was filtered using a 0.45-µm filter paper. To achieve the amino acid profile, the filtrate was subjected to high-performance liquid chromatography (HPLC) (YL9100 HPLC system, South Korea) with a binary eluent system and C18 column (5 µm, 4.6 mm × 250 mm). To prepare the elution buffer, 0.5 ml triethylamine was added to 1 L sodium acetate 140 mM pH 6.1 (adjusted by acetic acid and sodium hydroxide). Mobile phases A and B contained 96:4 v/v elution buffer:acetonitrile and 60:40 acetonitrile:dH_2O, respectively, and both degassed and filtered. The HPLC was performed at 38 °C with a flow rate of 1 ml min^{-1}, and the response was monitored at 254 nm.

2.7 Statistical analysis

SPSS statistic software version 24 was used for statistical analysis of the obtained data. The results were analyzed by

Table 1 Equations used to calculate the effective parameters in the quality of the predicted biodiesel from *Y. lipolytica* EBL13 biomass [23, 24]

Parameter	Equation	Abbreviations
Cetane number	$CN = 46.3 + (5458/SV) - (0.225 \times IV)$	CN: cetane number, SV: soap value, and IV: iodine value
Soap value	$SV = \sum(560 \times N)/M$	N: the percentage of each fatty acid and M_{wi}: the molecular mass of each component
Iodine value	$IV = \sum(254 \times D_i \times N)/M_{wi}$	D_i: the number of double bonds
Degree of unsaturation	$DU = MUFA + (n \times PUFA)$	MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid, and n: number of the double bond
Allelic position equilibrium	$APE = \sum(ap_n \times A_{cn})$	Acn: the amount (mass percent) of each fatty acid in the mixture
Base-allelic position equilibrium	$BAPE = \sum(bpn \times Acn)$	ap_n and bp_n: the numbers of allylic and bis-allylic positions in a specific fatty acid
Long-chain saturated fatty acid	$LCSF = (0.1 \times C16) + (0.5 \times C18) + (1 \times C20) + (1.5 \times C22) + (2 \times C24)$	C16: palmitic acid, C18: stearic acid, C20: arachidic acid, C22: behenic acid, and C24: lignoceric acid
Cold filter plugging point	$CFPP = (3.1417 \times LCSF) - 16.477$	LCSF: long-chain saturated fatty acid
Cloud point	$CP = (0.526 \times C16) - 4.992$	CP: cloud point
Kinematic viscosity	$\ln(\upsilon) = \sum Ni(-12.503 + (2.496 \times \ln Mwi) - 0.178 \times Di)$	υ: kinematic viscosity
Density	$\rho = \sum Ni(0.8463 + (4.9/Mwi) + 0.0118 \times Di)$	ρ: density
Higher heating value	$HHV = \sum Ni(46.19 - (1794/Mwi) - 0.21 \times Di)$	HHV: higher heating value

one-way ANOVA followed by the Tukey test. The values with $p < 0.05$ were considered a significant response.

3 Results and discussion

3.1 Wastewater treatment analysis

COD analysis results of the wastewaters samples treated with *Y. lipolytica* EBL13 indicated that the yeast was able to reduce about 85% of COD after 4 days compared to the control sample (Fig. 1). Lim et al. reported the reduction of soluble COD from 800 to 300 mg L^{-1} in FCW treated with *Candida rugopelliculosa* for 20 h [25]. Another study using *Oocystis* sp. (microalgae isolated from the lagoon) led to 70% COD decrease in FCW after 10 days [26]. Grgas et al. studied the effect of NaCl concentration on FCW treatment efficiency by activated sludge. Results showed that the presence of 2% salt in FCW caused 60%, 70%, and 100% decrease in COD, NH^{4+}, and PO_4^{3-}, respectively [27]. Here, the COD/BOD ratio in FCW was 2.35, which indicated the high biodegradable fraction, while the values of more than 3.5 show predomination of an inert fraction [28].

The growth of *Y. lipolytica* EBL13 and consumption of fatty acids and protein compounds in the effluent, which led to ammonia production, can be a possible reason for increasing wastewater pH after yeast treatment (Table 2). Mansour et al. have shown that growing a strain of *Y. lipolytica* isolated from cheese in the lactate-containing medium with high concentrations of amino acid resulted in a dramatic pH increase due to amino acid degradation and ammonia production [29]. Also, the ammonia concentration in the media increases by a functional nitrate reductase in *Y. lipolytica*, which converts the absorbed nitrate to nitrite as the intermediate metabolite. Finally, ammonia is synthesized by nitrite reductase. Therefore, the nitrate reductase function results in decreasing nitrate concentration and increasing ammonia levels [30]. The reduction of total nitrogen is associated with microbial growth and biomass production and also BOD and COD levels. Phosphorous is one of the essential elements for the synthesis of yeast cellular components, including phospholipids, proteins, and nucleic acids. Phosphorous uptake is known as a limiting growth factor and is regulated by the phosphate signal transduction pathway (PHO pathway) in *Saccharomyces cerevisiae* through sensing phosphorous concentration signals [31]. One of the key enzymes in sulfur metabolism is sulfite reductase in *Y. lipolytica*, which catalyzes the reduction of sulfite to sulfide, which then converts to cysteine in combination with O-acetyl-L-serine by cysteine synthase [32]. BOD analysis of FCW showed that after treatment, wastewater BOD decreased by about 60%. In addition, reduction in other wastewater quality indexes, including FOG, nitrate, total nitrogen, total phosphor, sulfite, TH, and TSS, confirmed the efficiency of biological treatment with *Y. lipolytica* EBL13. Various hydrolytic enzymes of *Y. lipolytica*, such as lipase, protease, and esterase, are responsible for the organic matter degradation [33] and a significant reduction in the BOD, COD, and FOG levels. Also, it should be considered that the low total nitrogen amount indicated the high levels of carbon/nitrogen (C/N) ratio in the studied FCW. Lopes et al. showed induction of lipase production by *Y. lipolytica* W29 and IMUFRJ 50682 in the high C/N ratios [34]. In addition, this condition is in favor of citric acid and intracellular lipid accumulation, and de novo lipid biosynthesis [33]. The significant capacity of *Y. lipolytica* in biosurfactant production from the various oily substrates such as glycerol, cottonseed oil, soapstock, corn oil, ground-nut oil refinery residual waste, crude glycerin, and canola oil has been confirmed by several studies [35]. The high adsorption propensity of biosurfactants to suspended solids leads to an increase in their biodegradability and, hence, a decrease in TSS amounts [36].

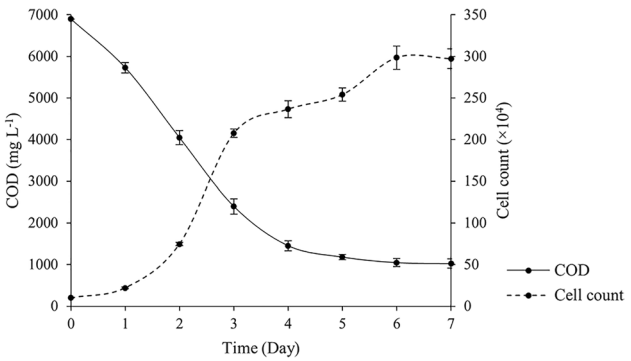

Fig. 1 Changes in COD levels and *Y. lipolytica* EBL13 cell growth in fish canning wastewater at 28 °C and 160 rpm. Error bars represented standard deviations and were calculated from three independent replications

Even though the COD, BOD, oil, and phosphate have decreased by 59.06%, 59.04%, 68.2%, and 91.34, respectively, they have not met the 1989 standards of Law 93/1962 for the specifications of liquid wastes to be disposed of in public sewerage [37]. Therefore, further treatments are necessary to achieve the standard levels. The quality parameters of some FCWs published in the literature are summarized in Table 3.

3.2 Morphological assessment

Y. lipolytica is a biotechnologically beneficial yeast strain with multiple valuable physiological and metabolic properties [9]. Various changes in environmental factors such as pH, nutrient starvation, temperature, oxygen availability, heavy metal concentration, and mechanical parameters, including pressure and mixing, can lead to stressful conditions for the cells. In addition, carbon and nitrogen source

Table 2 Characteristics of fish canning effluent before and after biotreatment with *Y. lipolytica* EBL13. Different superscript letters indicate significant difference ($p < 0.05$)

Parameter	Unit	Untreated wastewater	Treated wastewater in 3rd day	Treated wastewater in 6th day	Increase/decrease percentage
pH	-	5.7^{b}	$8.15^{a} \pm 0.38$	$8.45^{a} \pm 0.22$	+48.25
Oil	g/L	$1.32^{a} \pm 0.05$	$0.42^{b} \pm 0.12$	$0.25^{b} \pm 0.03$	−81.06
BOD	mg/L	$2932^{a} \pm 38$	$1201^{b} \pm 163$	$342^{c} \pm 65$	−88.34
COD	mg/L	$6900^{a} \pm 127$	$2825^{b} \pm 266$	$1048^{c} \pm 68$	−84.81
TSS	mg/L	$1050^{a} \pm 21$	$157^{b} \pm 18$	$153^{b} \pm 7$	−85.43
TH	mg/L	$650^{a} \pm 8$	$540^{b} \pm 19$	$507^{b} \pm 11$	−22
FOG	mg/L	$460^{a} \pm 11.8$	$97^{b} \pm 32.12$	$52^{b} \pm 8.5$	−88.7
Ammonia	mg/L	$47.25^{b} \pm 2.5$	$160.5^{a} \pm 20$	$190^{a} \pm 10.6$	+302.12
Nitrate	mg/L	$23^{a} \pm 0.52$	$11.1^{b} \pm 1.23$	$2.7^{c} \pm 0.28$	−88.26
Total phosphorus	mg/L	$201^{a} \pm 2.89$	$17.4^{b} \pm 4.25$	$9.4^{c} \pm 1.23$	−95.32
Total nitrogen	mg/L	$1210^{a} \pm 12.7$	$121^{b} \pm 7.67$	$87^{c} \pm 2.5$	+93
SO_3^{2-}	mg/L	$8^{a} \pm 0.5$	$4^{b} \pm 0.33$	$2.48^{c} \pm 0.21$	−69

Table 3 The quality parameters of some FCWs

Country	pH	BOD (mg L^{-1})	COD (mg L^{-1})	TSS (mg L^{-1})	Total N (mg L^{-1})	PO_4^{-3} (mg L^{-1})	Salinity (mg L^{-1})	Ref
Tunisia	7.4 ± 0.1	-	139.15 ± 8	-	-	-	21200 ± 2000	[12]
Portugal	6.1–7.1	463–4569	1147–8131	324–3150	21–471	0–9	-	[1]
Portugal	5.6–9.6	2420–13626	3314–17048	740–12093	131–1385	0.002–633	3364–36371	[4]
Malaysia	6–7	5100	6000–9000	2000	750	-	-	[56]
Spain	6.5	-	8000–26000	1100–2100	1200–4000	-	2000–15000	[57]
Spain	-	-	7400–10423	-	1687–18500	-	-	[58]

types can be considered determining parameters for yeast morphology [38, 39]. Braga et al. had observed that *Y. lipolytica* W29 and MTLY40-2P showed mycelium form in the presence of olive oil, while they showed yeast form when castor oil was used as the carbon source [40]. Published literature has proven the influence of environmental factors on the morphologic alterations in *Y. lipolytica* cells from yeast to mycelium form. To evaluate the role of salt in morphologic features of *Yarrowia*, saline (containing 4% NaCl) and non-saline FCW were used. Here, *Y. lipolytica* EBL13 morphology has changed from cocci shape in non-saline conditions to elongated shape in saline conditions (Fig. 2). In parallel, Andreishcheva et al. adapted *Y. lipolytica* to 9% salt in a basal medium enriched with yeast extract, glycerol, and vitamins. The adapted cells were observed in a more round shape with decreased size compared to the control strain [8]. Figure 2(B) shows the lipid content in *Y. lipolytica* in FCW.

3.3 Amino acid and fatty acid profile

Amino acid profile analysis showed that 50.2% w/w of the produced biomass in FCW contained proteins (Table 4). The most presented amino acids were Ala (77 mg/g biomass, 153.39 mg/g protein), Glu (70 mg/g biomass, 139.44 mg/g protein), Asp (65 mg/g biomass, 129.48 mg/g protein), Gly (58 mg/g biomass, 115.54 mg/g protein), and Ser (50 mg/g biomass, 99.6 mg/g protein). In addition, all essential amino acids were presented in amino acid composition, which comprised 32.9% of the profile.

Amino acid composition is affected by several parameters such as physical culture conditions, substrate type, and substrate concentration [41]. Figure 3 shows a comparison of some amino acid content in total cell proteins in *Y. lipolytica* EBL13 and other strains. As represented in Fig. 3, *Y. lipolytica* EBL13 showed higher levels of Trp and sulfur-containing amino acids (Met and Cys) when growing on FCW as the substrate compared with other strains cultured on glycerol or glucose-containing substrates.

Currently, about 97% of the soybean meal produced in the world is used as animal feed. In contrast, plant-based protein sources need to be supplemented with essential amino acids to achieve optimal performance. Since Lys content is low in cereals (for example, 2.8 mg g^{-1} in wheat), the current yeast-based SCP with 22 mg/g biomass can be a suitable food supplement alternate for poultry. In addition, Lys, Met, and Thr are the first three limiting amino acids for broilers, respectively [42–44]. Lys content of *Y. lipolytica* has

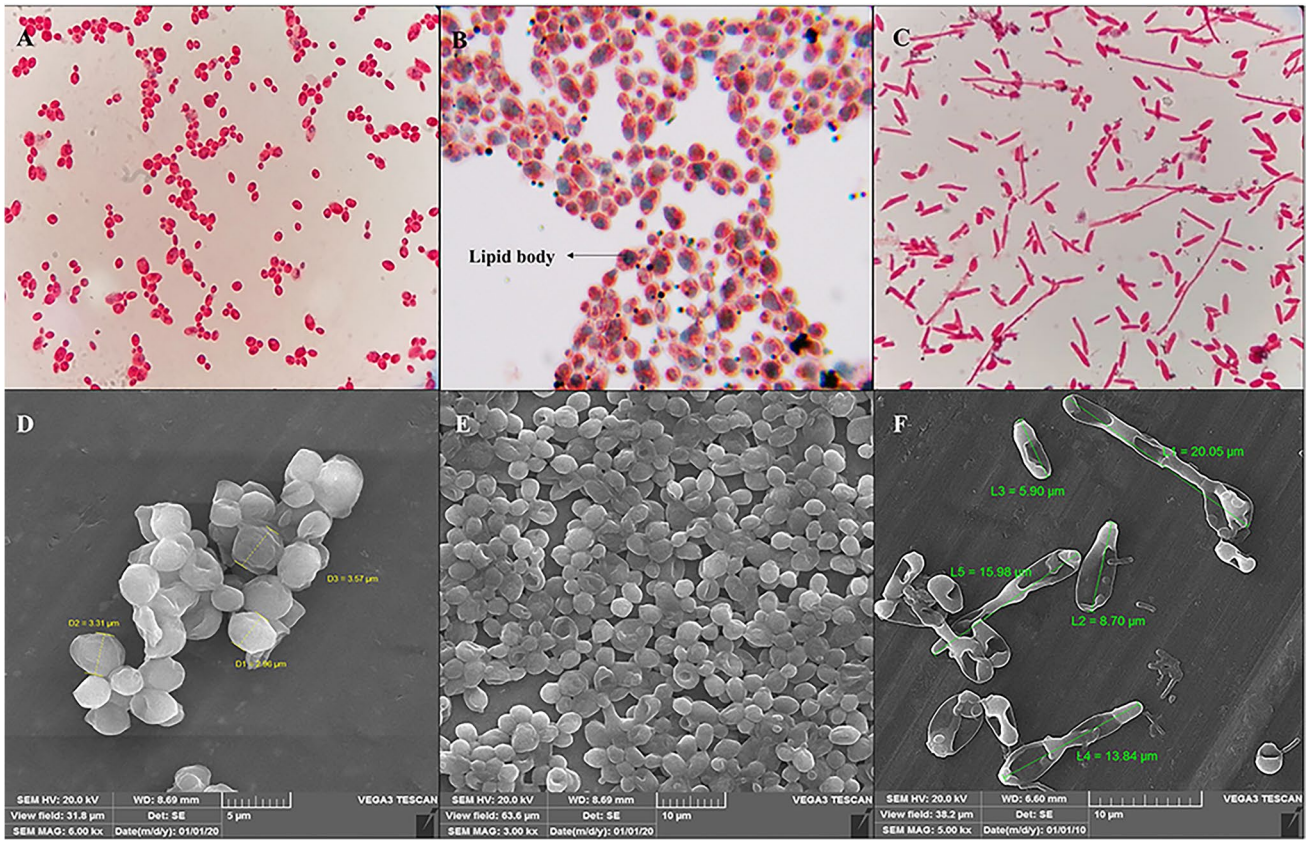

Fig. 2 Light microscopy and SEM images of *Y. lipolytica* EBL13 in saline and non-saline wastewater. (A, D, E) Yeast cells in non-saline wastewater (×800, ×6000, and ×3000). (B) Lipid content in yeast cells. (C, F) Yeast cells in saline wastewater (×800 and ×5000)

Table 4 Amino acid content of the produced *Y. lipolytica* EBL13 biomass in fish canning wastewater

Essential amino acid	mg/g biomass	mg/g protein	Non-essential amino acid	mg/g biomass	mg/g protein
Hydrophobic amino acids			Hydrophobic amino acids		
Valine (Val)	12	23.9	Glycine (Gly)	58	115.54
Proline (Pro)	24	47.8	Alanine (Ala)	77	153.39
Isoleucine (Ile)	17	33.86	Hydrophilic amino acids		
Leucine (Leu)	29	57.77	Glutamine (Gln)	70	139.44
Phenylalanine (Phe)	13	25.9	Asparagine (Asn)	65	129.48
Methionine (Met)	9	17.93	Serine (Ser)	50	99.6
Tryptophan (Trp)	10	19.92	Tyrosine (Tyr)	7	13.94
Hydrophilic amino acids			Threonine (Thr)	21	41.83
Arginine (Arg)	29	57.77	Cysteine (Cys)	2	3.98
Lysine (Lys)	22	43.82	Histidine (His)	7	13.94
Total	165	328.68	Total	337	671.31

been reported to be as much as its content in a whole egg [10]. Synthesis pathways of the essential amino acids in *Y. lipolytica* extracted from the KEGG database are presented in Fig. 4.

Gly and Ser are not generally considered essential in the diet of poultry. However, they are necessary for young birds' growth [45]. Cys and Tyr that are synthesized from Met and Phe, respectively, are classified as semi-essential amino acids [46].

Currently, various companies use yeast biomass to produce SCP, including Unilever, Tangshan Top Bio-technology Co. (*Saccharomyces* sp.), Skotan S.A. (*Y. lipolytica*),

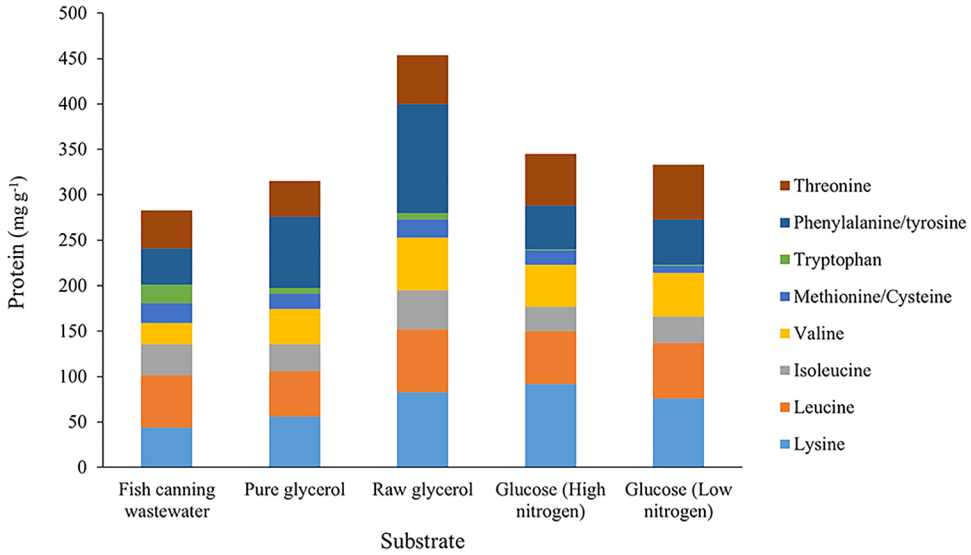

Fig. 3 Comparison of essential amino acid composition in *Y. lipolytica* in different carbon sources and fish canning wastewater

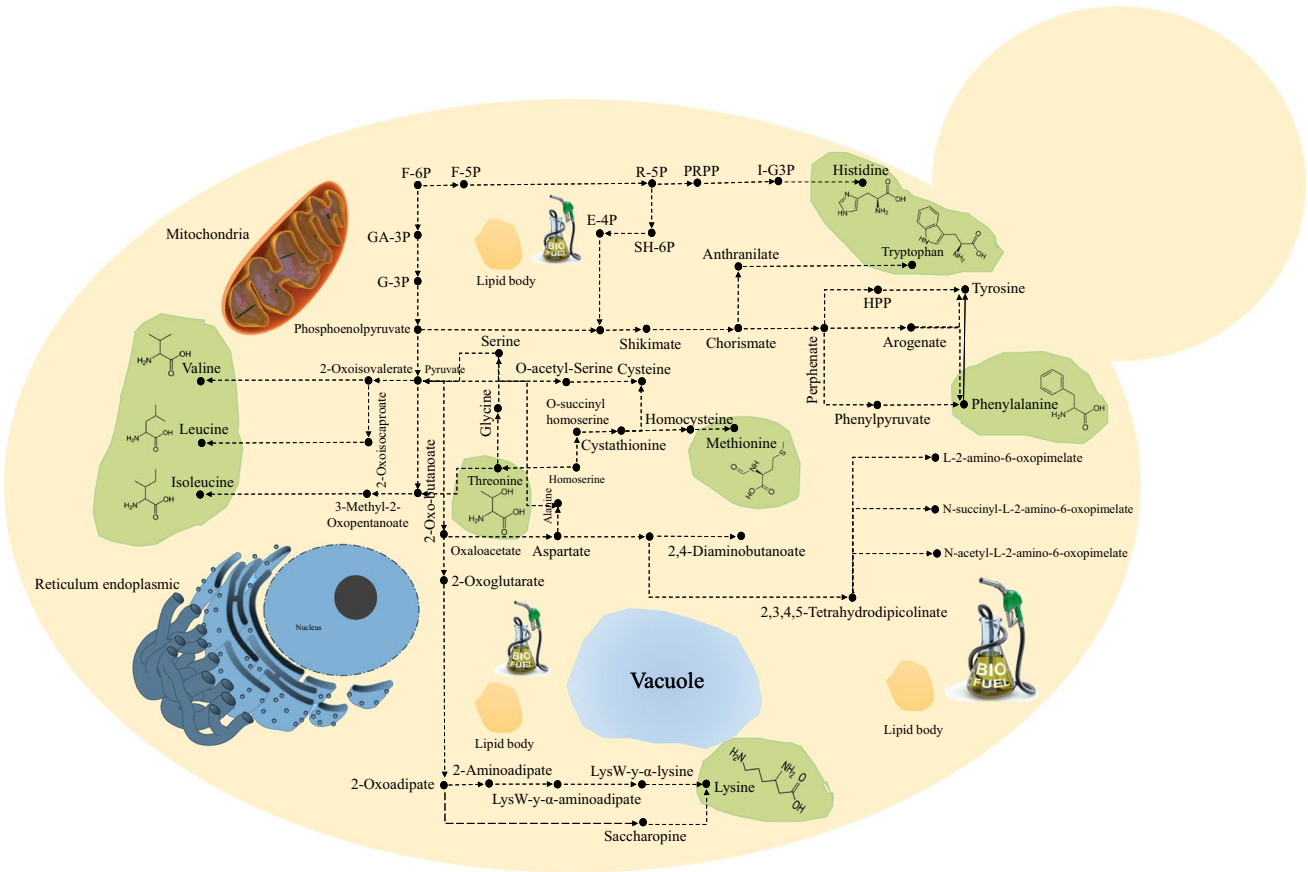

Fig. 4 Metabolic pathways for the synthesis of essential amino acid in *Y. lipolytica*

Shanghai Tramy Green Food Co. (*S. cerevisiae* and *Trichoderma* sp.), Phillips Petroleum Company USA (*Pichia* sp. and *Torula* sp.), Nucelis (*Y. lipolytica*), Mondelez (yeast), Liquichemica (*Candida maltosa*), LeSaffre (yeast), Lallemand Inc. (Yeast), IFP (*Candida tropicalis*), Flint Hills Resources (*S. cerevisiae*), Cangzhou Tianyu Feed Additive Co. (yeast powder), Belly Yeast (*Kluyveromyces*), Bega Cheese (*Saccharomyces*), and Amoco (*Candida utilis*). In the industrial production processes of SCP, companies have tried to use the cheapest substrates such as carbon dioxide,

alkanes, methanol, natural gas, and industrial effluents [18]. In the list published by the International Dairy Federation (IDF), the European Food and Feed Cultures Association (EFFCA), and the American Food and Drug Administration (FDA), *Y. lipolytica* is seen as Generally Regarded As Safe (GRAS) for food applications [10, 17]. Safety of *Y. lipolytica* yeast biomass as a novel food pursuant to Regulation (EU) 2015/2283 was declared by the European Food Safety Authority (EFSA) in 2019 [47]. A comparison of essential amino acid percentages in *Y. lipolytica* EBL13 and *S. cerevisiae* strains showed more agreement with FAO/WHO/UNU recommendations in *Y. lipolytica* EBL13 (Table 5).

In addition to essential amino acids, the presence of essential fatty acids (EFAs) in food is of special importance. For example, EFA deficiency in cystic fibrosis patients has been observed. Furthermore, EFA shortage can cause liver and kidney disorders which lead to growth defects, impaired immune system function, and skin dryness, particularly in children. EFAs are classified into two groups, omega-3 and omega-6, which cannot be biosynthesized by a human. According to dietary guidelines for poultry, 4–5% of daily food content should be fat, from which 1% should be omega-6. *Y. lipolytica* is an oleaginous microorganism and is used as a model for the production of bio-oils. Regarding the importance of essential fatty acids in oils, the fatty acid profile of *Y. lipolytica* EBL13 was analyzed to find essential fatty acid content (Table 6). Linolenic acid (0.15%) and eicosapentaenoic acid (0.21%) as essential omega-3 fatty acids and linoleic acid (22.15%) as essential omega-6 fatty acid were observed in the fatty acid profile.

Y. lipolytica biomass has previously been used as a substitute for eicosapentaenoic acid supplied by cornmeal, fish meal, and rapeseed oil during salmon farming. Feeding salmon fish using biomass containing 6% eicosapentaenoic acid and 2% oil increased the weight of fish from 180 to 400 g in 95 days. These results showed a significant increase in growth compared to control samples fed rapeseed oil and a mixture of rapeseed and fish oil [48]. The fatty acid composition in *Y. lipolytica* EBL13 biomass is compared with some widely used plant oils in Table 7.

3.4 Prediction of biodiesel characteristics

Several physical and chemical parameters determine the quality of produced biodiesel from various feedstocks. Prediction of these properties using fatty acid profiles may lead to selecting an appropriate and cost-effective oily source candidate for large-scale applications [49]. Viscosity is considered an index of fuel resistance against shear and tensile forces and shows an inverse relationship with biodiesel fluidity. Therefore, in

Table 5 Comparison of the essential amino acid (mg/g protein) in *Y. lipolytica* EBL13, *S. cerevisiae* strains, and FAO/WHO/UNU recommendation

Essential amino acids	*Y. lipolytica* EBL13	*S. cerevisiae* [59]	*S. cerevisiae* [60]	FAO/WHO/UNU recommendation
Valine (Val)	23.9	71.3	25.7	39
Proline (Pro)	47.8	18.7	22.8	-
Isoleucine	33.86	30.9	90.1	30
Leucine	57.77	102.5	70.2	59
Phenylalanine (Phe)	25.9	-	38.5	-
Methionine	17.93	50.5	14.6	16
Tryptophan (Trp)	10	67.2	50.9	6
Arginine	57.7	38.5	-	-
Lysine	43.82	60.1	29.5	45

Table 6 Fatty acid profile of *Y. lipolytica* EBL13 grown in fish canning wastewater

Concentration (%)	Fatty acid	Concentration (%)	Fatty acid
Capric acid (C10:0)	0.16	Oleic acid (18:1)	31.39
Lauric acid (C12:0)	0.36	Linoleic acid (18:2)	22.15
Myristic acid (C14:0)	2.37	Linolenic acid (18:3)	0.15
Myristoleic acid (C14:1)	0.05	Arachidic acid (C20:0)	0.62
Pentadecanoic acid (C15:0)	1.27	Gadoleic acid (C20:1)	0.55
Palmitic acid (C16:0)	26.53	Eicosadienoic acid (C20:2)	0.39
Palmitoleic acid (C16:1)	3.53	Behenic acid (C22:0)	0.65
Margaric acid (C17:0)	1.21	Eicosapentaenoic acid (C20:5)	0.21
Heptadecenoic acid (C17:1)	2.22	Lignoceric acid (C24:0)	0.59
Stearic acid (C18:0)	5.51	Other	0.09

Table 7 Comparison of fatty acid profile in vegetable oil methyl esters [61] and *Y. lipolytica* EBL13

	Fatty acid	*Y. lipolytica* EBL13	Castor	Lesquerella	Rapeseed	Soybean
C14:0	Myristic	2.37	-	-	-	0.56
C14:1	Myristoleic	0.05	-	-	-	0.18
C16:0	Palmitic	26.53	0.86	1.00	2.7	14.17
C16:1	Palmitoleic	3.53	1.01	0.6	-	1.27
C16:2	Hexadecanoic	-	0.7	-	-	0.24
C18:0	Stearic	5.51	2.63	1.7	0.9	5.19
C18:0, OH	Densipolic	-	89.54	-	-	-
C18:1	Oleic	31.39	4.10	16.7	12.6	48.2
C18:1, OH	Ricinoleic	-	0.36	0.5	-	-
C18:2	Linoleic	22.15	0.29	6.8	12.1	22.19
C18:3	Linolenic	0.15	0.16	11.4	8	1.45
C18:4	Octadecatetraenoic	-	0.35	-	-	-
C20:0	Arachidic	0.62	-	0.8	-	0.28
C20:1	Eicosenoic	0.55	-	-	7.4	-
C20:1, OH	Lesquerolic	-	-	56.3	-	-
C20:2, OH	Auricolic	-	-	3.5	-	-
C22:0	Behenic	-	-	-	0.7	-
C22:1	Erucic	-	-	-	49.8	-

the case of high-density fuels, injectors do not exhibit accurate performance, especially in cold starting engine conditions [50]. The high degree of unsaturation in the oily feedstocks leads to the production of low viscosity biodiesel. Here, the calculated value of viscosity according to Table 1 was 1.33 mm^2/s. According to biodiesel specifications established by ASTM D6751 and EN 14214, kinematic viscosity should be 1.9–6.0 mm^2/s and 3.5–5.0 mm^2/s, respectively.

The fuel fluidity is also affected directly by DU as another key fuel feature. While increasing DU levels improves fluidity, more unsaturated fuels suffer from more NOx emissions. The maximum DU in Europe and Spain are met at 137 and 160, respectively [23]. The calculated DU for the predicted biodiesel from *Y. lipolytica* EBL13 was 83.54.

The IV provides a direct indication of unsaturation degree and is described as the mass of iodine (I_2) in grams that is

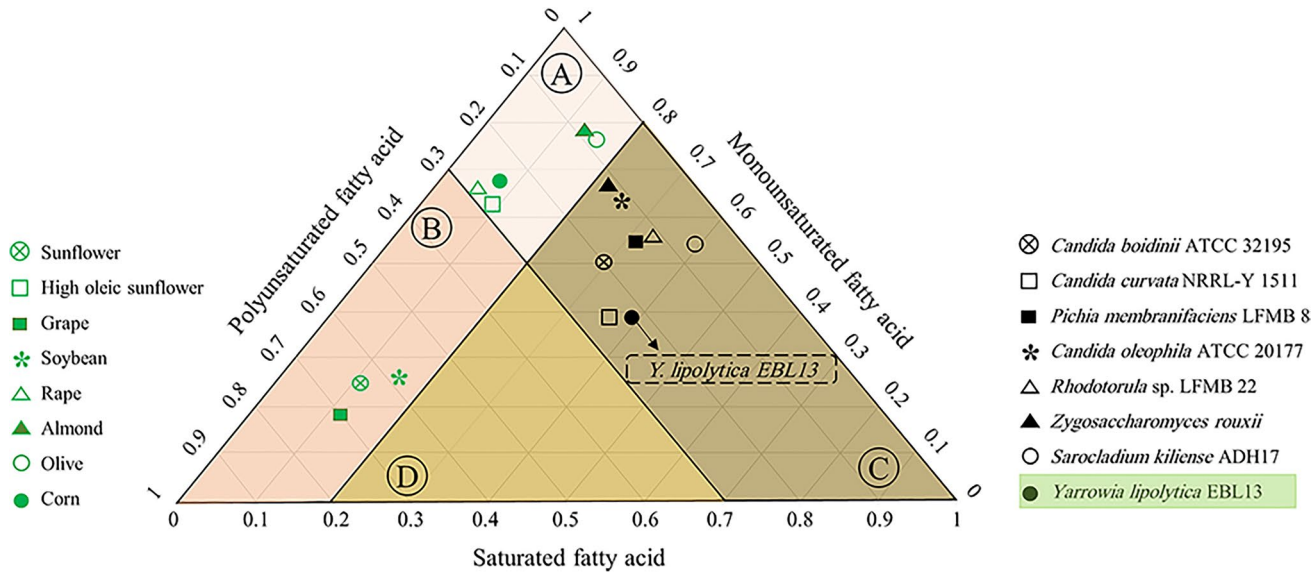

Fig. 5 Comparison of the different parameters in biodiesel produced from yeast and plant oils in different studies [55] and this study. (A) Biodiesel that satisfied EN 14214. (B) Appropriate CFPP. (C) Appropriate CN, and IV

Table 8 Comparison of the different parameters in produced biodiesel from different oleaginous yeasts

Strain	Substrate	SFA/USFA	CN	IV	DU	CFPP	HHV	ρ	υ	SV	Ref
Meyerozyma caribbica	Glycerol	1.27	60.51	39.01	-	-	39.15	-	4.98	-	[62]
	Vegetable oil	0.53	54.3–55.2	98–107	-	-	40.5–40.7	-	4.4	-	[62]
Sarocladium kiliense ADH17	GYP medium	0.71	57.94	60.92	62.67	3.89	40.6	0.9	4	215.35	[22]
Trichosporon cutaneum	Lignocellulosic residues	0.7	59.9	57.9	-	-	-	1.38	-	204.1	[63]
Rhodotorula mucilaginosa IIPL32	Modified sugarcane bagasse	0.98	57.97	-	-	-	-	0.88	4.11	-	[64]
Rhodococcus sp. YHY 01	Empty fruit bunch hydrolysate	0.89	64.96	37.18	-	19.47	37.51	0.83	3.63	201.9	[65]
	Glucose	1.32	63.46	41.2	-	24.94	38.7	0.86	3.83	206.4	[65]
Cryptococcus curvatus	Optimized waste office paper hydrolysate	0.34	53.09	79.06	85.02	−4.13	41.61	0.92	-	-	[66]
Pichia kudriavzevii	Rice husk	-	70.73	0	-	−6.3	39.03	0.87	1.19	223.37	[67]
	Bagasse	-	57.85	0	-	−16.48	30.41	0.88	1.19	472.54	
	Glucose	-	69.97	15.61	-	−9	34.78	0.77	1.22	200.78	
Chlorella vulgaris	Moh202 media	0.35	44	135.26	116.6	4.6	-	-	-	194	[46]
Chlorella salina	Moh202 media	0.5	49.93	117.92	99.78	2.58	-	-	-	180.97	[46]
Chlorella protothecoides	Moh202 media	0.41	54.57	11.75	91.6	-0.99	-	-	-	163.37	[46]
Rhodococcus sp. YHY01	Glucose	1.27	63.46	41.2		24.94	38.7	0.85	3.83	206.4	[65]
	Empty fruit bunch hydrolysate		64.96	37.18		19.47	37.51	0.83	3.63	201.9	[65]
Yarrowia lipolytica	Fish canning wastewater	0.65	55.75	76.4	83.54	9.22	39.48	1.12	1.33	204.9	This study

necessary to completely saturate the molecules of 100 g of oil. Here, the IV was measured as 76.4 g Iod/100 g, which is in accordance with EN 14214 specifications that require the maximum value of biodiesel IV as 120 g Iod/100 g. In addition, the SFA to USFA ratio in the fatty acid profile was 0.65. Darvishi et al. have reported the SFA to USFA ratio of 0.56 in *Y. lipolytica*–treated vegetable oil refinery wastewater [51].

CN is one of the biodiesel quality determining parameters that result in long combustion delay in high values. However, decreased levels of CN cause more noise radiation during fuel ignition. The predicted CN in the current study was 55.7 min which satisfied the minimum CN of ASTM D6751 and EN 14214 standards which are 47 min and 51 min, respectively.

The calculated cold flow parameters, CP and CFPP, were 8.96 °C and 9.22 °C, respectively. Since CP and CFPP values of diesel depend on the climate, they are not considered in ASTM D6751 and EN 14214 standards.

In Fig. 5, the predicted biodiesel from *Y. lipolytica* EBL13 and plant and yeast oils in published literature are grouped in each area of the triangular graph. According to this graph, a comparison of fatty acid profiles in plant and yeast species demonstrated a significant compositional difference. However, yeast oils of various genera showed more similar properties to each other when compared to plant ones. To achieve biodiesel which satisfies the EN 14214 specifications completely, the fatty acid composition must fit part A in the triangular graph (Fig. 5). Table 8 indicates a comparison of the different parameters in biodiesel produced from different oleaginous yeasts.

Considering the importance of biological fuels and animal food supplements, developing a microbial strain with multiple biotechnological applications may be of interest. Investigation of *Y. lipolytica* EBL13 fatty acid and amino acid composition indicates the high potential of this yeast strain in food and environmental biotechnology. In addition, the by-products in the wastewater treatment process lead to cost reduction of the treatment process. Eurofish uses 1300 m^3 of wastewater produced during fish processing to produce 1300 m^3 of biogas per day [52].

Nowadays, sustainable management of waste streams through secondary material recovery from fully treated and semi-treated wastewaters is an eco-friendly strategy that maintains the circular flow of water, waste, material, and energy [53, 54]. Here, producing biodiesel from the remaining biomass of *Y. lipolytica*

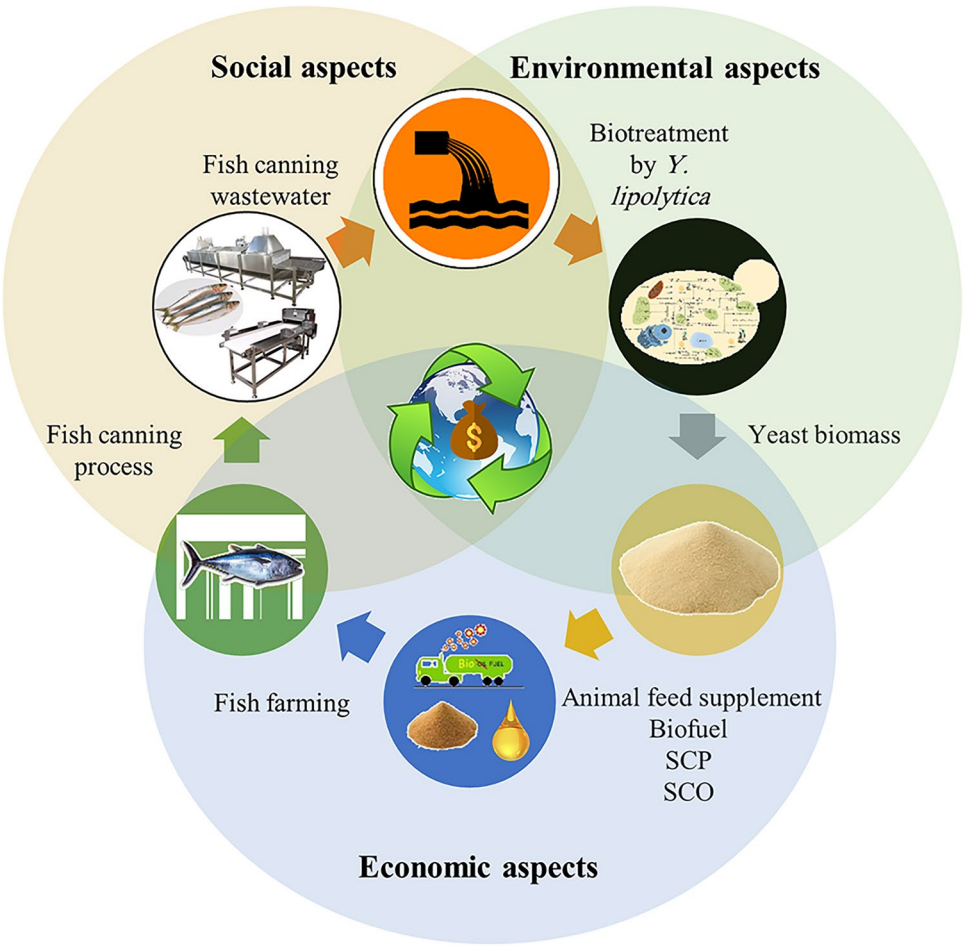

Fig. 6 The circular economy flow in FCW management using *Y. lipolytica* EBL13

following the biotreatment of FCW can lead to a decrease in the demand for fossil fuel and also minimize the CO_2 emission into the environment. In addition, SCP production as the animal food supplement from the biomass can reduce agricultural demand to supply animal feed and also reduce the water and energy consumption. Figure 6 shows a circular economy flow in FCW management using *Y. lipolytica* EBL13. Accordingly, followed by FCW treatment by *Y. lipolytica* EBL13 in a fish canning operational unit, the extracted SCP from the yeast biomass can be consumed by fish as a food supplement, and the extracted SCO can be used for biodiesel production to provide the required fuel in the same unit.

4 Conclusion

Freshwater scarcity and the growing world population have highlighted the need to access low-cost food and water through wastewater recycling and microbial SCP production. In the current study using FCW as the carbon source, *Y. lipolytica* EBL13 showed an 85% decrease in COD levels. It should be noted that autoclaving the FCW sample may lead to evaporating volatile compounds and therefore result in fewer levels of COD compared with actual values, which is a limitation in the accurate evaluation of treatment efficiency. In addition, the yeast produced 50.2% protein as the by-product, from which 16.5% was composed of essential amino acids. Also, essential omega-3 fatty acids and omega-6 fatty acids composed 25.15% and 22.15% of the fatty acid profile, respectively. According to our obtained results, *Y. lipolytica* EBL13 may be a potent SCP and SCO producer strain for animal feed applications. Furthermore, predicted parameters of the biodiesel produced from *Y. lipolytica* EBL13 fatty acids such as cetane number, iodine value, and unsaturation degree met the EN 14214 specifications. However, it is helpful to produce biodiesel from yeast lipids and then measure its parameters to obtain a more precise understanding of biodiesel quality. Finally, it should be noted that applying fatty acids of *Y. lipolytica* EBL13 can be used as biofuel, and other yeast components such as its amino acids may be useful in animal feed supplement production. Nevertheless, it is suggested to test the produced SCP as the animal feed supplement in laboratory animal models in further experiments.

Funding This work is based upon research funded by the Iran National Science Foundation (INSF) under project no. 4002334.

Declarations

Conflict of interest The authors declare no competing interests.

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