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REVIEW

Use of Non-Conventional Yeast *Yarrowia lipolytica* in Treatment or Upgradation of Hydrophobic Industry Wastes

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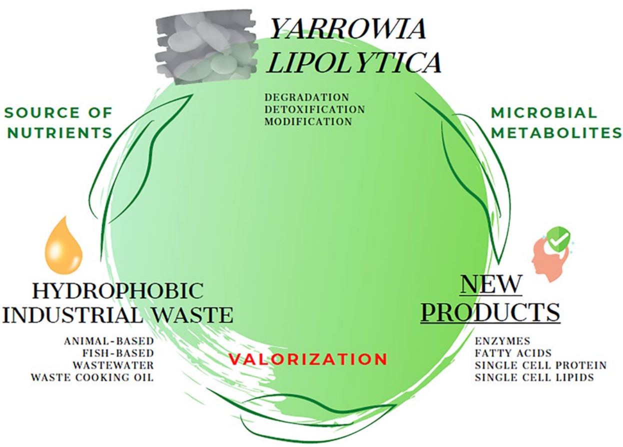
Abstract

The review aims to summarize the current knowledge on the possibility of using non-conventional yeast species *Yarrowia lipolytica* in the treatment and upgradation of industry wastes. Importantly *Y. lipolytica* yeast is argued as generally recognized as safe species, what indicates the high application potential of the reviewed technologies. Special emphasis in the paper was given on microbial processing of the food industry wastes, including fish and animals' wastes utilization. *Yarrowia*-based processing of waste cooking oil or oil-bearing plants wastewaters, such as palm oil mill effluents or olive mill wastewater was reviewed. Recent advances in biosynthesis of valuable metabolites (e.g. lipases or microbial oil) with simultaneous wastes utilization by *Y. lipolytica* are additionally discussed. The broad implications of the present paper are a part of sustainable development policy.

Graphic Abstract



Keywords Food industry wastes · Lipid waste · Sustainable bioprocess · Waste management · Non-pathogenic yeast · *Yarrowia lipolytica*

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Statement of Novelty

The article summarizes future perspectives of *Yarrowia lipolytica* yeast utilization in the context of hydrophobic waste management, especially in the food industry. The paper is a summary of current knowledge and scientific achievements. Currently, there is no article that comprehensively illustrates the use of *Y. lipolytica* yeast as a tool to valorize this type

of waste with the simultaneous obtaining new products. The issue is particularly significant due to the constantly growing population, which contributes to the vastness of food production and the vastness of production processes, the industrial waste produced could place a serious burden on the planet and thus on the people living there.

Introduction

In general, the composition of wastes from the food industries is heterogeneous. Above all, the type of the raw materials used in particular food industries determines the composition of the waste generated. Their production is often inevitable and their amount increases with the industry development. According to The World Bank 2.01 billion tonnes of municipal solid waste are generated annually worldwide [1]. Approximately 33% of the produced wastes are not managed, and about 0.74 kg of wastes per day are generated per capita. There are vivid opinions that the type and amount of waste, mainly organic residues of processed raw materials will remain unchanged if the quality of the final product is not changed. By-product management and waste disposal in the food industry pose problems in terms of sustainability and environmental protection [2].

In order to i.a. facilitate statistical processing and harmonization of data, the Food and Agriculture Organization of the United Nations (FAO) has introduced two distinct concepts: Food Loss and Food Waste. Losses cover the goods as a whole (including inedible parts) and occur during processing, storage and transport. Wastes are formed at the stages from the retail sale to the consumption. Nevertheless, in the current article, the authors use the term "waste" in the context of waste products from the industry. Growing attention to waste and loss of food was reflected in SDGs—Sustainable Development Goals. The SDGs call for reducing food losses along production and supply chains by 2030. This blueprint also opts for halving global per capita food waste at the retail and consumer levels [3].

It can be assumed that the problem of managing human-made waste will be aggravated. Scientists from the area of food technology, among others, are constantly trying to apply alternative methods of waste management. One of the strategies is using inexpensive substrates in microorganism cultivations. This is one of the methods to reduce production costs of microbiological metabolites [4]. Hydrophobic industrial wastes are mainly a carbon source for cells. There is widespread confidence that yeasts utilize lipids from the substrate for energy purposes via the fatty acid β-oxidation process [5]. Waste disposal with the simultaneous synthesis of added value products is a double benefit of this approach. Moreover, thanks to the use of cheap substrates such as waste from the food industry and the use of factor design approach, microbiological production of enzymes and other metabolites can be an economically viable process [4].

Yarrowia lipolytica—A Non-Conventional Yeast Species that Degrades Hydrophobic Substrates

Yarrowia lipolytica is an aerobic, non-pathogenic fungus, capable of effectively degrading hydrophobic substrates and thus many industrial and environmental applications of this organism were discussed in the literature [6]. *Yarrowia lipolytica* strains are part of the microflora of many food products and have been isolated from dairy products e.g. yoghurt, Rokpol and Camembert cheeses [7, 8], meat, dry fermented sausages, fish, soy sauce [9, 10], shrimp salad [11], poultry [12] and various environments with a high content of hydrocarbons or fats, for example oil-polluted soil, rancid margarine and sea water [13]. Food and Drug Administration has given the processes based on the yeast the GRAS status ("generally recognized as safe"). The majority of research regarding *Y. lipolytica* report that this species is non-pathogenic. In spite of that, the yeast can cause infections in critically ill and immunocompromised patients—like other fungal species. *Yarrowia lipolytica* demonstrate features that help them in invasion of the host cell, they produce hydrolytic enzymes, as well as biofilm allows them to protect their own cells [14].

Yarrowia lipolytica yeasts are classified as dimorphic species. Cells are capable of taking various forms, from those with a typical spherical shape to the form of pseudo-fungus, and even take the form characteristic of the septic mycelium [15, 16].

This yeast species have a high tolerance for low temperatures and the high salinity. They are also capable of growing in a wide range of pH. Due to their ability to produce extracellular proteolytic and lipolytic enzymes, proteins and lipids may be degraded [17]. *Yarrowia lipolytica* cells were present also in hypersaline and marine or oil-polluted environments [11]. Both the physiological and biochemical properties of *Y. lipolytica* yeasts, including high secretion capacity are the main reason for their wide use in biotechnological sciences [18] in processes such as biodegradation, biosynthesis or biotransformation of various organic compounds. At the turn of the last years *Y. lipolytica* yeasts could be found in a number of new applications. Some perspective processes have been developed to obtain a variety of products through biosynthesis with these microorganisms including erythritol, aromatic compounds e.g. γ-decalactone [19], carotenoids—e.g. β-carotene [20], organic acids—α-ketoglutaric, succinic, citric and pyruvic acids [21], lipases—enzymes used in food production, for water purification or as an ingredient in detergents [22], SCP (Single Cell Protein)—proteins of

microbiological origin that can be used as feed additives [23], SCO (Single Cell Oil)—microbiological oil which is a source of essential unsaturated fatty acids (EFA) with valuable properties for both humans and farm animals [5, 24].

The *Y. lipolytica* cells deal with the lipid components present in the medium by synthesis of lipolytic enzymes, which catalyze the hydrolysis of lipids to free fatty acids and glycerol molecules [25]. Moreover, lipid dissolving surfactants are excreted [6], as well as other additional cofactors for the removal of reactive oxygen species, which are formed by the process of β-oxidation of fatty acids [26, 27]. It was found that the microorganisms' cells form biofilms what increases the chance of cell survival in lipid rich environment [28, 29].

Among the various types of microorganisms next to microalgae, bacteria, moulds and yeasts, the latter has been subject of many studies due to their capability to grow on media with different types of waste generated from agricultural or industrial processes [10]. Yeast species *Y. lipolytica* produce a set of various metabolites when cultivated in the presence of various low-value carbon sources e.g. organic acids, enzymes (e.g. lipases and esterases [30], phosphatases [31], asparaginases [32], laccases [33], inulinase [34], mannosidase [35], single-cell proteins [36] and single-cell oils [37]. Furthermore, *Y. lipolytica* is oleaginous yeast species able to accumulate significant amounts of lipids in biomass and is considered to be an outstanding producer of lipids and has repeatedly been used as a model microorganism for fatty acid metabolism and lipid biosynthesis by ex novo route [38, 39], which is characterized by incorporation of intermediates or final products of β-oxidation into triacylglycerol molecules accumulated in the lipid bodies of yeast cells [24].

Yarrowia lipolytica in Wastewaters Treatment

Yarrowia lipolytica effectively degrades hydrophobic substrates and can therefore be successfully used to purify palm oil mill effluents (POME) and olive mill wastewater (OMW) [4]. OMW is the wastewater remaining after the olive oil pressing process [40] containing sugars, polyalcohols, polyphenols, tannins, lipids and pectins. These compounds cause high chemical oxygen demand (COD) of waste [41]. Oleaginous yeast species *C. rugosa, C. cylindracea* and *Y. lipolytica* produced biomass and other products such as organic acids or enzymes by consuming organic components from substrates with OMW [40, 41]. Lanciotti et al. [42] analyzed the potential of different strains of *Y. lipolytica* for growth in OMW media and the ability of yeast to reduce COD values (Table 1). Depending on the composition of the culture medium, it was possible to synthesize lipases of different specificity. Additionally, other strains, *Y. lipolytica* IMUFRJ 50,682 and W29, showed extracellular lipase production in culture media with OMW and there was presented that surfactant Tween 80 enhanced COD decreasing and cell growth. However, Tween 80 had a negative effect on lipase activity [43].

Table 1 COD reduction [%] as a result of application *Y. lipolytica* yeast in various wastes treatment

Strain	Type of waste	COD reduction [%]	Other influencing factors	Other applications	Reference
62 diferent strains	OMW	1.47–41	–	Lipases, citric acid	[42]
ATCC 20,255	OMW	80	–	SCP, lipases	[104]
ATCC 9773	the dairy wastewater	37.93; 43.07	–	Lipases	[131]
ATCC 9773	the dairy waste	44.3	–	–	[55]
CBS 2073	OMW	22–52	Type of OMW	Lipases	[40]
CECT 1240	WCO	90	–	Lipases	[98]
CLIB 40	TWPW	75	Dilution (75:25)	SCP, SCO	[84]
		66	Crude waste		
IMUFRJ 50,682	OMW	23–62	Type of OMW	Lipases	[40]
IMUFRJ 50,682	OMW	80	12 g/l ammonium sulphate	Lipases	[43]
		75	6 g/l ammonium sulphate		
TISTR 5151	POME	72.9	Twofold dilluted effluent	Lipases, SCO	[46]
		64.2–93.4	Non dilluted effluent, pH 4.3–6.0		
W29	oil wastewater	67	Non-immobilized cells	–	[44]
		82	Immobilized cells in calcium alginat		
W29	OMW	22–52	Type of OMW	Lipases	[40]
W29	OMW	61	6 g/l ammonium sulphate	Lipases	[43]
		79	12 g/l ammonium sulphate		
		74	6 g/l ammonium sulphate, Tween 80		

OMW olive mill water, *POME* palm oil mill effluent, *TWPW* tuna wash processing wastewater, *WCO* waste cooking oil

The ability of *Y. lipolytica* strain to degrade grease and salad oil from food wastewater has been investigated by Wu et al. [44]. *Yarrowia lipolytica* was able to use salad oil as the only source of energy, carbon and nitrogen when the concentration did not exceed 3 g/l. Comparison of COD reduce and oil removal efficiencies by either immobilized or free cells indicated that the efficiency of removal of COD and oil by free cells was 66.95% and 93.3% respectively and by immobilized cells was 82.22% and 88.2% respectively. Moreover, immobilized cells saved physiological stability at the 12th reaction and the carrier had enough space to support cell growth. Three strains of *Y. lipolytica* yeasts have been tested for their potential for OMW remediation and bioproduct production. The treatment resulted in significant decoloration (63%) of the lipid substrate. The largest amount of citric acid (18.9 g/l) among the other strains was produced by ACA-YC 5033 strain after 144 h incubation in glucose medium with OMW with limited availability to nitrogen. However, the most efficient production of lipids (34%) was observed in the case of W29 strain after 48 h cultivation in the same medium. On the basis of the analysis of the fatty acids profile it was found that all strains in the OMW environment produced higher contents of oleic acid [45].

The study of Louhasakul et al. [46] aimed to utilization and valorization of POME into lipid and lipase by *Y. lipolytica.* From five strains cultivated in twofold diluted effluent, TISTR 5151 was selected, which proved to be the most promising strain in lipase and lipid production. All of the tested strains showed good growth on POME and at least 33% lipid production. In the case of *Y. lipolytica* TISTR 5151 the highest lipases production 610 U/l was recorded after 48 h of cultivation. After the same cultivation period the lipolytic activity of the remaining strains was much lower (61–245 U/l). When TISTR 5151 strain was cultured in undiluted effluent, at pH 5.0, lipase production reached 4081 U/l. However, raising the pH value to 6.0 decreased lipolytic activity to 534 U/l. The authors emphasized the potential of *Y. lipolytica* yeast in waste management and transesterification processes, which can contribute to environmentally friendly and economic biodiesel production. Biological reactions using a lipase catalyst is a green technology easier to perform than the chemical reaction. An additional advantage is the shorter purification step of final products. Yeast *Y. lipolytica* has been described as a species that allows obtaining satisfactory results in the production of both lipases and lipids [47–49].

In the research of Gao et al. [50] the supernatant from the anaerobic digestion of food waste was used as a substrate for the production of microbiological oils by *Y. lipolytica*. The food waste came from the solid waste treatment plant (Dongcun, Beijing), which collects waste from local restaurants. As shown in the study, the biomass concentration was similar for synthetic VFAs (volatile fatty acids) and VFAs from fermented food waste (DCW ranged 1.667–2.029 g/l). However, batch cultures in medium with fermented food, resulted in lower lipid content (from 16.3 to 18.5%) than these on synthetic VFAs. Gao et al. [51] used fermented food waste in their research. The wastes came from the restaurants in Beijing. What is more, the authors studied the effect of alkaline growing conditions on biomass yield and lipid production. The use of fermented food waste with an initial pH of 6, contributed to 48–72 lag phase and delayed growth of *Y. lipolytica* CICC 31,596. When the initial pH of the waste was increased to 7 and 8, growth was recorded with almost no lag phase. Production of lipids also improved significantly. As the results showed, at pH 6, the lipid content was 14.78%. However, in the case of cultivation under the alkaline condition in medium with fermented food waste reached a lipid content of 21.86%. In summary, an initial pH of 8 has been determined as the optimal for the conversion of high-content VFAs to microbial lipids by *Y. lipolytica* yeast.

A study was performed in which *C. curvatus* ATCC 20,509, *R. glutinis* ATCC 204,091, *Y. lipolytica* ATCC 20,460 were selected from well-known oleogenic yeast as showing good growth in YPG culture medium, in which food waste hydrolyzed broth was used instead of water. Food waste came from the cafeteria at Washington State University and was hydrolyzed with 3% (v/v) sulfuric acid and then the separated liquid phase was used in the medium. *Yarrowia lipolytica* produced more biomass when the substrate with food waste hydrolysate was used, but at this stage the lipid content in cells was not analysed. Nevertheless, the authors indicated these species as promising in the production of microbial oil and capable of growing in food waste. In the same paper, the authors describe the incubation of yeast in municipal wastewater used as water in the medium. When the substrate based on wastewater was supplemented with carbon and nitrogen sources, the biomass production by *Y. lipolytica* was 15.3 g/l. However, it was the lowest result compared to the other two strains. It was also checked whether municipal wastewater can be used as the only feedstock for the cultivation of oleogenic yeast. The selected strains were inoculated into wastewater without addition any nutrient supplementation. As it turned out, the wastewater environment was harmful to the yeast. The initial biomass (0.36 g/l) was higher than the final biomass (0.25 g/l), which contained low lipid content (11.5%). As a result of treatment of municipal wastewater, *Y. lipolytica* decreased phosphorus content by 92% and nitrogen content by 31%. In the experiment sterile and non-sterile cultures were conducted. In the sterile environment, COD has practically not been reduced. In case of non-sterile cultures, COD was reduced by 65%, which may be explained by the activity of bacteria present in the environment [52].

Johnravindar et al. [53] evaluated the efficiency of microbial oil production by selected oleogenic yeasts

Rhodotorula glutinis DSM 10,134, *Cryptococcus curvatus* DSM 70,022 and *Y. lipolytica* DSM 8218 using three types of food waste leachates as cultivation medium. One of the biological treatment methods taking into account dry anaerobic digesters were used to pretreat the leachates. Before inoculation of the yeast, the leachate was diluted to a desired carbon content of 25 g/l. For all tested yeast strains a satisfactory total VFAs removal was recorded. However, *Y. lipolytica* proved to be the most effective in removing VFAs and alcohols from the waste substrate in the range 72.28% to 94.71%. Just behind this strain was *C. curvatus* 67.14–73.93% and *R. glutinis* 30.14–90.25% with slightly worse results. *Yarrowia lipolytica* strain also showed the highest ability to accumulate lipids compared to other yeasts, reaching 48% of lipids in total biomass. The results prompted the authors of the study to define three strains as potential tools for the treatment of leachate from food waste under anaerobic conditions or other wastewater streams rich in VFAs [53].

Li et al. [54] used mixed food waste hydrolysate as a substrate. As described, *Y. lipolytica* PSA3.0 managed to produce succinic acid with a yield of 18.9 g/l with initial glucose concentrations 75.0 g/l and pH of 3.0. The experiment was conducted with the use of in situ fibrous bed bioreactor. When the authors used a glucose-based substrate, they obtained 19.3 g/l succinic acid. The results of the analyses prove the possibility of using cheap waste streams to produce the said acid [54].

Yarrowia lipolytica can not only use the waste to grow and produce valuable metabolites, but also contribute to reducing the BOD (biochemical oxygen demand) of such a waste. Dunoyer et al. [55] made some intriguing observations, when fatty effluent from dairy industry with high BOD, COD and high fat and dry matter content was treated by crude enzymatic extract (CEE) from *Y. lipolytica* ATCC 9773 cultivation. The strain decreased the lipid content in the waste by 82.88% and reduced the levels of BOD5 until reaching the values of 43.32% respectively. In another study Mendoza et al. [56] also used crude enzyme extract of *Y. lipolytica* ATCC 9773 yeast. The authors concentrated on the evaluation of lipolytic activity of the selected strain, which could improve the process of utilization of wastewater from the dairy industry using two breeding strategies (pH 5 and 6.5). Both pH treatments were economically viable, practical and most importantly effective. When using an environment with a pH of 5, the removal of oils and fats was recorded at a level of 83%. Apart from pH, the lipolytic capacity of CEE was also influenced by the used inoculum concentration of 8%, 12%, 16%. Maximum lipolytic activity was reached in 32 h of cultivation at 27 °C with an inoculum concentration of 16%, for pH 5. According to the removal percentage COD and BOD as parameters that prove the effectiveness of treatment. The best results correspond to: inoculum concentration 16%, pH: 5 (BOD removal—43.07%) and inoculum concentration 8%, pH: 6.5 (BOD removal–37.93%) [55].

Animal-Based Wastes Utilization by *Yarrowia lipolytica*

At the turn of the last decades, there has been a rapid increase in livestock production, particularly in the developing world. The increase in production comes mainly from industrial farms concentrated around major urban centers. Such a large territorial accumulation of both animals and animal waste close to dense clusters of the human population often causes significant pollution problems. Therefore, there is a need for an effective policy to regulate intensive livestock operations and to support economically and environmentally sustainable management of the waste generated [57]. The global agri-food market wastes many by-products and residues, contributing to the depletion of resources such as cereals. Approximately one third of global cereal production is used for livestock farming. The key to agroecological transformation is an integrated approach, which can be achieved, among other things, by recovering energy and nutrients from animal waste [58].

Over the past 35 years, the use of *Y. lipolytica* yeast for the management of animal fats and other animal waste has been discussed many times. Brabender et al. [59] showed that *Y. lipolytica* PO1f is capable of using urea as a source of nitrogen. It is noteworthy that with an ammonium sulphate concentration equivalent to urea nitrogen, cell growth was inhibited. This indicates the strain's potential for growth in nitrogen-rich animal waste.

A lot of research works concern the production of intracellular lipids by *Y. lipolytica.* Used animal fats are of particular interest, because the valorisation of the waste has considerable economic potential with low market value of the substrate [60]. Table 2 presents fatty acid composition of different animal fats or microbial oils produced by selected *Y. lipolytica* strains in the cultures with these waste lipids. Their nutritional values were calculated based on the methodology described by Ratusz et al. [61]: (a) Calculated Oxidizability Value (COX) connected with unsaturated fatty acids content in oils and is associated with oil's tendency to undergo oxidation; (b) Atherogenicity Index (AI) relating to the ratio of pro- and anti-atherogenic fatty acids; (c) Thrombogenicity Index (TI) related with the ratio of pro- and anti-thrombogenic fatty acids; and (d) Ratio of Hypocholesterolemic to Hypercholesterolemic Fatty acids (HH). The three latter indexes should be considered in determining the nutritional properties of oils, because consumption of oils with low AI and TI, and higher HH value is a reduction factor of incidence risk of cardiovascular diseases.

Table 2 Fatty acid compositions of different initial animal fats and mixtures or single-cell oils produced by selected *Y. lipolytica* strains with a comparison of their nutritional values

Animal fat or microbial oil samples	Additional information		Fatty acid composition [%]										Nutritional values				Reference
			C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	SFA[1]	MUFA[2]	PUFA[3]	COX[4]	AI[5]	TI[6]	HH[7]	
Stearin[9]	–		ND	ND	25.0	52.0	2.0	T[11]	ND	77.0	2.0	0.0	0.02	12.50	77.00	0.08	[36]
Y. lipolytica LGAM S(7)1 microbial oil	Changes in the fatty acid profile during culture [h]	14	ND	ND	22.0	52.0	7.0	ND	ND	74.0	7.0	0.0	0.07	3.14	21.14	0.32	
		21.5	ND	ND	17.0	68.0	3.0	ND	ND	85.0	3.0	0.0	0.03	5.67	56.67	0.18	
		38.5	ND	ND	16.0	79.0	1.0	ND	ND	95.0	1.0	0.0	0.01	16.00	190.00	0.06	
		62.5	ND	ND	18.0	77.0	1.0	ND	ND	95.0	1.0	0.0	0.01	18.00	190.00	0.06	
		72	ND	ND	13.0	78.0	1.0	ND	ND	91.0	1.0	0.0	0.01	13.00	182.00	0.08	
		84.5	ND	ND	13.0	83.0	3.0	ND	ND	96.0	3.0	0.0	0.03	4.33	64.00	0.23	
		108	ND	ND	14.0	80.0	3.0	ND	ND	94.0	3.0	0.0	0.03	4.67	62.67	0.21	
		116	ND	ND	13.0	78.0	2.0	ND	ND	91.0	2.0	0.0	0.02	6.50	91.00	0.15	
HORO[10]/Stearin 25/75	–		ND	ND	19.0	41.0	19.0	4.0	ND	60.0	19.0	4.0	0.60	0.83	5.22	1.21	
Y. lipolytica LGAM S(7)1 microbial oil			ND	ND	17.0	63.0	12.0	4.0	ND	80.0	12.0	4.0	0.53	1.06	10.00	0.94	
HORO/Stearin 50/50	–		ND	ND	15.0	26.0	38.0	10.0	ND	41.0	38.0	10.0	1.41	0.31	1.71	3.20	
Y. lipolytica LGAM S(7)1 microbial oil			ND	ND	12.0	64.0	18.0	3.0	ND	76.0	18.0	3.0	0.49	0.57	7.24	1.75	
HORO/Stearin 75/25	–		ND	ND	12.0	15.0	55.0	11.0	ND	27.0	55.0	11.0	1.68	0.18	0.82	5.50	
Y. lipolytica LGAM S(7)1 microbial oil			ND	ND	11.0	32.0	40.0	9.0	ND	43.0	40.0	9.0	1.33	0.22	1.76	4.45	
Stearin	–		ND	ND	25.0	52.0	2.0	T	ND	77.0	2.0	0.0	0.02	12.5	77.00	0.08	[68]
Y. lipolytica ACA-DC 50,109 microbial oil	Changes in the fatty acid profile during culture [h]	68	ND	ND	14.5	71.5	7.0	2.0	ND	86.0	7.0	2.0	0.28	1.61	19.11	0.62	
		89	ND	ND	15.5	72.0	6.5	2.5	ND	87.5	6.5	2.5	0.32	1.72	19.44	0.58	
		112.5	ND	ND	16.0	73.0	6.0	2.0	ND	89.0	6.0	2.0	0.27	2.00	22.25	0.50	
		113	ND	ND	16.0	72.0	5.0	1.5	ND	88.0	5.0	1.5	0.20	2.46	27.08	0.41	
		140	ND	ND	17.0	76.0	4.5	1.0	ND	93.0	4.5	1.0	0.15	3.09	33.82	0.32	
HORO/Stearin 30/70	–		7.0	7.0	20.1	37.7	22.2	4.5	1.5	71.8	22.2	6.0	1.01	1.95	3.56	1.04	[71]
Y. lipolytica ACA-DC 50,109 microbial oil	Changes in the fatty acid profile during culture [h]	40 h	ND	ND	18.1	77.8	4.1	ND	ND	95.9	4.1	0.0	0.04	4.41	46.78	0.23	
		maximum accumulated lipids	1.1	1.5	21.1	67.5	7.7	1.1	ND	91.2	7.7	1.1	0.19	3.20	20.48	0.39	
HORO/Stearin 40/60	–		6.1	6.2	16.2	32.5	29.4	7.1	2.5	61.0	29.4	9.6	1.57	1.21	2.10	1.74	
Y. lipolytica ACA-DC 50,109 microbial oil	Changes in the fatty acid profile during culture [h]	40	0.5	ND	12.5	77.5	5.5	0.5	ND	90.5	5.5	0.5	0.11	2.17	30.00	0.48	
		maximum accumulated lipids	1.0	ND	16.2	69.2	11.6	2.0	ND	86.4	11.6	2.0	0.32	1.26	12.56	0.84	
HORO/Stearin 60/40			4.1	3.0	13.1	22.1	44.2	9.0	4.5	42.3	44.2	13.5	2.34	0.51	0.94	3.58	
Y. lipolytica ACA-DC 50,109 microbial oil	Changes in the fatty acid profile during culture [h]	40	ND	ND	13.4	75.8	9.3	1.5	ND	89.2	9.3	1.5	0.25	1.24	16.52	0.81	
		maximum accumulated lipids	0.5	ND	15.6	57.8	22.6	3.5	0.5	73.9	22.6	4.0	0.69	0.61	5.00	1.71	

Table 2 (continued)

Animal fat or microbial oil samples	Additional information		Fatty acid composition [%]										Nutritional values				Reference
			C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	SFA[1]	MUFA[2]	PUFA[3]	COX[4]	AI[5]	TI[6]	HH[7]	
Stearin	–		ND	ND	25.0	52.0	2.0	T	ND	77.0	2.0	0.0	0.02	12.50	77.00	0.08	[132]
Y. lipolytica ACA-DC 50,109 microbial oil	Stearin: glycerol initial concentrations [g/l: g/l]	10:10	ND	ND	15.0	76.0	5.0	2.0	ND	91.0	5.0	2.0	0.26	2.14	26.00	0.47	
		13:10	ND	ND	15.0	70.0	6.0	2.0	ND	85.0	6.0	2.0	0.27	1.88	21.25	0.53	
		10:11	ND	ND	15.5	72.5	5.0	1.5	ND	88.0	5.0	1.5	0.20	2.38	27.08	0.42	
		10:23	ND	ND	16.0	67.5	6.5	2.0	ND	83.5	6.5	2.0	0.27	1.88	19.65	0.53	
		10:34	ND	ND	14.0	67.0	9.5	2.5	ND	81.0	9.5	2.5	0.35	1.17	13.50	0.86	
		7:28	ND	ND	20.0	50.0	16.5	6.5	ND	70.0	16.5	6.5	0.83	0.87	6.09	1.15	
	Stearin: glucose initial concentrations [g/L: g/L]	10:10	ND	ND	20.0	47.5	18.5	6.5	ND	67.5	18.5	6.5	0.85	0.80	5.40	1.25	
		10:21	ND	ND	16.5	66.0	8.5	3.0	ND	82.5	8.5	3.0	0.39	1.43	14.35	0.70	
		10:34	ND	ND	14.0	67.0	8.5	2.5	ND	81.0	8.5	2.5	0.34	1.27	14.73	0.79	
		7:24	ND	ND	22.0	45.0	17.0	6.5	ND	67.0	17.0	6.5	0.84	0.94	5.70	1.07	
	Stearin: glycerol: glucose initial concentrations [g/L: g/L]	7:8:7	ND	ND	22.0	42.5	17.0	6.0	ND	64.5	17.0	6.0	0.79	0.96	5.61	1.05	
		7:11:7	ND	ND	19.0	48.5	15.5	5.5	ND	67.5	15.5	5.5	0.72	0.90	6.43	1.11	
Stearin	–		ND	ND	25.0	52.0	2.0	T	ND	77.0	2.0	0.0	0.02	12.50	77.00	0.08	[69]
Y. lipolytica LGAM S(7)1 microbial oil	Stearin: glucose initial concentrations [g/L: g/L] (Culture time [h])	10.5: 20.5 (72)	ND	ND	14.7	68.1	6.9	2.5	ND	82.8	6.9	2.5	0.33	1.56	17.62	0.64	
		10.5: 20.5 (140)	ND	ND	16.5	67.9	10.8	3.9	ND	84.4	10.8	3.9	0.51	1.12	11.48	0.89	
		8.5: 28.5 (71)	ND	ND	17.1	55.6	17.6	7.6	ND	72.7	17.6	7.6	0.96	0.68	5.77	1.47	
		8.5: 28.5 (160)	ND	ND	18.9	62.2	14.9	2.2	ND	81.1	14.9	2.2	0.38	1.11	9.49	0.90	
		10.5: 34.5 (46)	ND	ND	15.4	68.3	7.6	2.9	ND	83.7	7.6	2.9	0.37	1.47	15.94	0.68	
		10.5: 34.5 (140)	ND	ND	17.5	66.0	10.9	3.4	ND	83.5	10.9	3.4	0.46	1.22	11.68	0.82	
		7.1: 23.5 (66)	ND	ND	22.5	47.1	18.2	8.9	ND	69.6	18.2	8.9	1.10	0.83	5.14	1.20	
		7.1: 23.5 (138)	ND	ND	23.1	50.1	11.5	3.6	ND	73.2	11.5	3.6	0.49	1.53	9.70	0.65	

Table 2 (continued)

Pork lard	–	ND	ND	28.2	13.1	40.3	19.1	ND	41.3	40.3	19.1	2.37	0.47	1.39	2.11	[72]
Y. lipolytica W29 microbial oil	Taguchi method used in flask cultures of *Y. lipolytica* W29 to compare 4 factors (pH, lard concentration, arabic gum concentration and oxygen transfer rate) in lard utilization	ND	ND	34.9	11.9	43.3	9.9	ND	46.8	43.3	9.9	1.45	0.66	1.76	1.52	
		ND	ND	48.4	6.4	42.3	2.8	ND	54.8	42.3	2.8	0.71	1.07	2.43	0.93	
		ND	ND	31.8	2.7	46.4	19.0	ND	34.5	46.4	19.0	2.42	0.49	1.06	2.06	
		ND	ND	35.2	14.7	38.4	11.7	ND	49.9	38.4	11.7	1.59	0.70	1.99	1.42	
		ND	ND	25.2	2.9	53.2	18.7	ND	28.1	53.2	18.7	2.46	0.35	0.78	2.85	
		ND	ND	48.0	5.3	42.8	3.9	ND	53.3	42.8	3.9	0.83	1.03	2.28	0.97	
		ND	ND	35.3	21.0	34.7	9.1	ND	56.3	34.7	9.1	1.28	0.81	2.57	1.24	
		ND	ND	25.2	2.3	50.8	21.6	ND	27.5	50.8	21.6	2.73	0.35	0.76	2.87	
		ND	ND	30.5	1.9	50.8	16.7	ND	32.4	50.8	16.7	2.23	0.45	0.96	2.21	
	Bioreactor batch cultures	ND	ND	22.0	8.0	43.0	27.0	ND	30.0	43.0	27.0	3.21	0.31	0.86	3.18	
		ND	ND	21.0	6.0	48.0	25.0	ND	27.0	48.0	25.0	3.06	0.29	0.74	3.48	

1—Saturated Fatty Acids; 2—Monounsaturated Fatty Acids; 3—Polyunsaturated Fatty Acids; 4—Calculated Oxidizability Value, calculated based on Ratusz et al. [61]; 5—Atherogenicity Index, calculated based on Ratusz et al. [61]; 6—Thrombogenicity Index, calculated based on Ratusz et al. [61]; 7—Ratio of Hypocholesterolemic to Hypercholesterolemic Fatty acids, calculated based on Ratusz et al. [61]; 8—Not Detected; 9—An industrial lipid composed of free fatty acids of animal origin produced by alkaline hydrolysis of animal fat; 10—Hydrolyzed oleic rapeseed oil; 11—Traces

The possibility of using animal waste was reported, i.a., by Kamzolova et al. [62]. They determined the growth and production of lipases by *Y. lipolytica* yeast on rendered beef fat. A growth of biomass from 2.5 to 5.3 g/l DCW was shown for culture in medium with 10 g/l of peptone and 10 g/l of animal fat. Citric acid (18 g/l) and isocitric acid (5.2 g/l) were also produced.

Tan and Gill [63] concluded that hydrophobic solid carbon sources are not conducive to the growth of moulds and yeasts. The use of substrates with the addition of solid fats did not show good enough growth, which was explained by insufficient dispersion of the substrate in the liquid medium. The higher the degree of fat saturation, the more fat was left unused by fungi. Nevertheless, in the culture with 2.2 g/l of animal fat addition, 1200 rpm of stirring and temperature of 30 °C yeasts were able to consume almost all of the unsaturated fatty acids of lard, mutton tallow or beef tallow found in the culture media. Similarly, as a result of breeding *Y. lipolytica* in poultry fat, its content in the medium was reduced by an average of 34%, and in the case of beef tallow yeast cells consumed 18% of the lipid carbon source [64]. Chicken feather wastes were also used as a carbon source in intracellular lipids production by five strain of *Y. lipolytica* (NCIM 3229, NCIM 3450, NCIM 3472, NCIM 3589, NCIM 3590). Lipid yield coefficients ranged 0.03–0.06 g/g and these values were definitely lower than those obtained in the cultures with waste cooking oil or waste motor oil [65]. Besides, *Y. lipolytica* MTCC 9520 was also used by Radha et al. [66, 67] in the synthesis of single-cell oil utilizing slaughterhouse lipid waste, goat tallow, as well as chicken tallow.

Papanikolaou et al. [41, 68–70] and Papanikolaou and Aggelis [71] described the potential of *Y. lipolytica* yeast to grow on stearin (derivative of tallow consisting mainly of saturated fatty acids). Unused tallow and animal fats can become competitive substrates in microbiological industrial processes. Stearin supported the process of efficient biomass production. Yeast cells quickly drew this source of carbon from the culture medium. The use of stearin more abundant in stearic acid (thus more saturated) as a substrate for *Y. lipolytica* strains resulted in the production of significant amounts of biomass and SCO. By comparing the fatty acid compositions of stearin and microbial oils (Table 2), the ease of uptake of stearic acid from the medium compared to other fatty acids could be observed. During the culture, some of the fatty acids were metabolized and the microbial oil composition changed. It can be seen, that nutritional values of microbial oils obtained from stearin were very unfavorable, due to the high content of stearic acid [68]. In the same paper, scientists mixed stearin with HORO (hydrolyzed oleic rapeseed oil) in different ratios and used these mixtures in the culture of *Y. lipolytica* LGAM S(7)1. Similarly, nutritional values were still worse than initial oil, but

one of synthesized oil was characterized by an interesting composition. *Yarrowia lipolytica* LGAM S(7)1 microbial oil obtained from HORO/Stearin 75/25 mixture was composed of 11% palmitic acid, 32% stearic acid, 40% oleic acid and 9% linoleic acid. The aforementioned oil could find industrial application as a cocoa butter-substitute due to their similar compositions [49, 68].

Changing the culture conditions, the use of an emulsifier and the addition of a second carbon source—industrial glycerol allowed for the formation of microbial oil with increased content of unsaturated acids [69]. It is most likely related to the occurrence of two pathways of intracellular fat biosynthesis simultaneously, i.e. the ex novo (from stearin) and de novo (from glycerol) pathways. Despite the high content of stearic acid in the microbiological oil extracted during the entire culture (about 70%), the presence of unsaturated acids increases its nutritional value. Extending the culture favors the degradation of storage lipids, mainly unsaturated acids, therefore the calculated indexes also change their values, but are still better than in the initial fat [69].

Papanikolaou et al. [41, 70] focused on the possibility of using mixtures of glycerol and stearin, as well as glucose and stearin and their ternary mixture in various proportions in the synthesis of microbial oil by *Y. lipolytica* ACA-DC 50,109 (LGAM S(7)1). Concentration-dependent and mixture ratio-dependent effects were observed. Similarly to the above-cited papers addition of second non-lipid carbon source increased the unsaturated fatty acids content in microbial oils. In the case of glycerol addition, it looked like it should be necessary to present in the culture medium for increasing the unsaturation of microbial oil. For glucose, its concentration is crucial, because increasing its amount also increases the quantity of stearic acid.

Lopes et al. [72] used statistical methods for simultaneous optimization of 4 factors, i.e. pH of the culture, lard concentration, arabic gum concentration and oxygen transfer rate in the utilization of lard in flask cultures of *Y. lipolytica* W29. Taguchi method with orthogonal arrays was applied and 4 factors in three level were compared in nine designed experiments. Oxygen transfer rate was the most influential parameter in the culture. Experiment no. 8, where a pH of 7.2, 50 g/l of lard, 5 g/l of arabic gum and 192 mg/l/h were used, turned out to be the best attempt, where the highest microbial lipid content (57.9%) and cell density (8.6 g/l) were achieved. Modification of initial lard composition was observed in all experiments, and again in the experiment no. 8 the most valuable fatty acid composition was obtained, and microbial oil consisted of 25.2% palmitic acid, 2.3% stearic acid, 50.8% oleic acid and 21.6% linoleic acid. Nutritional value of obtained oil significantly increased compared to initial animal fat and atherogenicity index, thrombogenicity index and the ratio of hypocholesterolemic to hypercholesterolemic fatty acids were 0.35, 0.76, 2.87, respectively, which can indicate their usefulness in the food technology and industry. Furthermore, the simultaneous production of lipase and citric acid was also observed.

As can be seen, animal waste can be used in many ways in oleaginous yest cultures. There is a clear trend in the use of animal fats for the production of microbial oils, as well as yeast biomass and other valuable metabolites (organic acids or lipases). Despite the fact that yeasts do a great job of recycling troublesome fatty waste, not all oils obtained are suitable for human consumption but intracellular lipids can be used in other ways, such as the production of substitutes for different vegetable oils, where the sourcing of them can be time-consuming and costly. On the other hand, it is possible to control the culture process in such a way as to obtain the most valuable oil, e.g. for food purposes.

Fish Processing Wastes and Methods of its Utilization

Over the last decades, with globalization expanding, the maritime sector becomes more and more important. The maritime industry already accounts for 90% of international trade [73]. The fish market is regulated because the supply of most fish species depends primarily on catch limits and the potential of the fishing fleet [74]. In 2018, global capture fisheries production reached 96.4 million tonnes. while for the aquaculture production it was 82.1 million tonnes. Over the last few years it was the record result. Maritime economy is not only fishing but also fish processing. On a global scale, live, fresh and chilled fish were the preferred form and also the cheapest available. Frozen fish were the second in order. The interest in preserves, prepared and cured products was lower [3].

Global fish production in 2018 was estimated at 179 million tons, of which nearly 22 were used for non-food purposes, such as fish oil and fishmeal [3]. Fish oil is a source of dietary omega-3 fatty acids, this is why the food industry needs this product for food enrichment. Fishmeal is used on farms as a component of livestock feed and in aquafeed sector, in which is widely used as a supplemental source of protein for culturing fish [75, 76]. Fish meal used as a feed supplement should have a high protein content. Another important parameter is the lipid content, whose low content determines the acceptable quality of fishmeal. Of the 12 strains tested in the Yano et al. [77] study, the researchers selected *Y. lipolytica* NRBC-10073, which was found to be the most effective in reducing lipid content from anchovy mince in solid-state fermentation. Neutral lipids were analyzed using the TLC method. The yeast strain was able to decompose and reduce lipids in the minced anchovy samples as evidenced by the fatty acid bands, which were very small before incubation and after

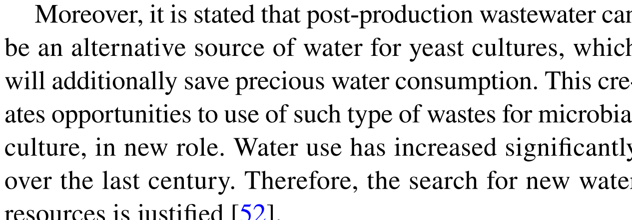
incubation it was one of the dominant bands. The efficiency of lipid reduction by the yeast strain was affected by the water content of minces, inoculum cell density and ratio of surface area to weight [77]. During β-oxidation, fatty acids are formed as a result of lipid degradation and this process demands a much of oxygen [78]. Importantly, *Y. lipolytica* NRBC-10073 was found to consume protein at an efficiency lower than 1%, although this species is known for its proteolytic activity. Incubating and material conditions were indicated as factors affecting the production of proteases [77].

The progressive development of fish processing contributes to the generation of significant amounts of waste, which poses a challenge to industry and the scientific community in terms of their disposal. Generated by-products could represent up to 70% of initial raw [3]. The ratio of the weight of fish for consumption to the weight of by-products varies according to the season, fishing zone, species or size of fish [79]. The by-products of fishing include backbones, fish fins, belly flaps, gills, liver, head, skin, viscera among others [80]. Due to their rich microflora and endogenous enzymes, they are susceptible to rapid degradation [81].

It has been proven that waste from fish processing can be effectively used for biogas production as the only carbon source due to its lipid content. The experiment used waste from common carp (*Cyprinus carpio*), including fish oil extracted from its viscera. The waste was delivered by a fish processing company from Roca Sales, RS, Brazil [82].

Fat in fish accumulates mainly in muscles, under the skin and in gonads and liver. Fish fat is a source of polyunsaturated fatty acids (PUFA), necessary for the proper functioning of the body. The most common PUFA in fish are omega-3 fatty acids—eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). For example, in herring oil, EPA is 14% and DHA is up to 11% in relation to the sum of all fatty acids in this oil [83].

In another study, Hamimed et al. [84] used *Y. lipolytica* CLIB 40 strain for biological treatment wastewater remaining after the tuna washing process (TWPW). After 7 days yeasts incubation, reduction of 100% phosphorus, 69.8% organic source of carbon, 66% COD and 66% salinity has been noticed. The assessment of the phytotoxicity of analysed wastewater has given promising results. The fenugreek seeds could sprout on the treated and diluted TWPW. The results have shown *Y. lipolytica* is helpful in reducing tuna wash process wastewater toxicity. The discoloration and purification of the waste took place with simultaneous intensive production of biomass *Y. lipolytica* with high protein and lipid content. The treated TWPW was safe for endotrophic bacteria. Endotrophic bacteria are very useful as they participate in activities promoting plant growth [85]. This confirms the possibility of using this kind of processing in agriculture [86].



Moreover, it is stated that post-production wastewater can be an alternative source of water for yeast cultures, which will additionally save precious water consumption. This creates opportunities to use of such type of wastes for microbial culture, in new role. Water use has increased significantly over the last century. Therefore, the search for new water resources is justified [52].

Yeasts capable of accumulating exogenous DHA and EPA fatty acids from crude fish oil were analyzed for use as feed for fish, hens and other animals. Based on the studies of Guo et al. [87], *Y.lipolytica* FO726A was found to be the most efficient strain in the production of EPA and DHA rich biomass. What can be added, FO726A was incapable of synthesizing these unsaturated fatty acids. This shows that all EPA and DHA that were accumulated in the cells came from added fish oil.

Akpinar and Uçar [88] chose three different lipids (olive oil, tributyrin and fish oil) and tested them for the growth of *Y. lipolytica* strains and production of lipolytic enzymes. It was determined that, in this work, all analyzed *Y. lipolytica* strains isolated from different environments produced lipases, but TEM TAN 46 strain proved to be the best producer in fish oil medium. Moreover, all lipid-related substrates supported lipase production, but fish oil (1%) showed maximum specific activity in the supernatant.

Fish oil is undeniably rich in polyunsaturated fatty acids [67]. Fabiszewska et al. [89] made an attempt to manage some selected waste from the fish industry: fish leach, sludge and oily waste from fish smoking process. Oils after smoking were rich in omega 6 (DPA—docosapentaenoic) and omega 3 (EPA, DHA) fatty acids, but also in palmitic, myristic and oleic acids. Although the above mentioned experience was preliminary research, the results definitely indicate the potential of *Y. lipolytica* KKP 379 yeast in fish waste management. The highest biomass yield (13.02 g/dm^3) was obtained for cultivation in medium with solid waste (sludge), and the highest extracellular lipase activity was in medium with oil after the fish smoking process.

Various strains of *Y. lipolytica* were analyzed for lipid accumulation as a result of culturing in different local renewable carbon sources. One of them was fish oil, whose 1% addition to the medium resulted in lipid production at the level of 5% for the NCIM 3229 strain to 14% for NCIM 3589 after 72 h of incubation. Microbiological oils from yeast cultivation in a medium with fish oil were characterized by a high content of polyunsaturated fatty acids up to 60% of total fatty acids for strain 3589. In this way, strain 3589 was found to be the most efficient producer of SCO from lipid carbon sources [65].

Zieniuk et al. [19] compared four strains of *Y. lipolytica* in growth and intracellular lipases production in medium containing waste fish oil. Two of them, KKP 379 and W29 were able to grow and produce lipases at a satisfactory level,

and the latter was used in bioreactor culture to obtain whole-cell biocatalyst with lipolytic properties. Approximately 90% of the waste fish oil in the medium was used, and the obtained biomass served as a biocatalyst in the esterification of phenolic compounds with antimicrobial and/or antioxidant activities. Moreover, Fabiszewska et al. [5] provided evidence for high nutritional value of the microbial oil obtained in *Y. lipolytica* KKP 379 yeast culture in waste fish oil medium resulting from the high content of unsaturated fatty acids including DHA and EPA and high polyphenols concentration, what makes the lipid-rich yeast biomass a promising source of beneficious nutrients.

Other post-process fish waste of a lipid nature also requires attention. There has been a search for microorganisms capable of growing and disposing of oil waste, including those from the fishing industry. Bialy et al. [90] isolated a strain *Y. lipolytica* NC-1 yeast showing such skills. The composition of crude and fermented waste oil from frying fish has been analysed. As a result of yeast culture in medium with waste oil, the fatty acid composition of the waste oil has changed. According to Papanikolaou and Aggellis [71] cells preferentially accumulated selected fatty acids. For example, the linoleic acid content has decreased from 48.38 to 18.90% in the waste substrate. The authors have proven that *Y. lipolytica* yeast cells take more oleic acid (C18:1) and linoleic acid (C18:2) from the medium. However, these preferences depend on the composition of the substrate used.

Waste Cooking Oil as a Substrate in the Biosynthesis of Valuable Metabolites of *Y. lipolytica*

Waste Cooking Oil (WCO) is the waste produced after frying food in vegetable oil (sunflower, palm tree, coconut, rapeseed, olive, etc.). Approximately 1 million tonnes of WCO are produced annually worldwide [91]. The WCO comes from the household, as well as from the catering and hotel industry. Valorization of WCO, also with the use of *Yarrowia lipolytica* yeast, is a sustainable approach to transform processed waste into new valuable products using biotechnological processes [39].

Oil wastes are primarily disposed of in two ways. They are often poured directly into the sink or, better still, collected in containers designed for this type of waste. Both are problematic. The first one causes clogging of the pipelines, the second one is problematic when it comes to storage [90]. In order to reduce costs and thus increase profits, a large part of the oil waste is used again in catering services. These activities are illegal and should be stigmatized [92].

Table 3 presents metabolites which may be produced by different strains of *Y. lipolytica* yeast in media with WCO. Liu et al. [11] used WCO from containers designed for this type of waste to produce citric acid by the *Y. lipolytica* SWJ-1b yeast. As indicated in the literature, *Y. lipolytica* yeasts are capable of producing organic acids, including citric acid. This acid is a by-product of the synthesis of lipids by de novo in culture media with a source of carbon in the form of glucose as well as ex novo, in the presence of hydrophobic carbon sources [93]. Maximum citric acid production was achieved when nitrogen was added to the medium, which means that the waste oil used was poor in nitrogen and rich in carbon. Moreover, WCO concentration higher than 80 g/dm^3 reduced citric acid production efficiency [11]. Moreover, the process of citric acid synthesis by *Y. lipolytica* became an alternative to the process of using *Aspergillus niger* [20].

WCO can find potential use as a substrate ingredient for *Y. lipolytica* in the production of erythritol. Xiaoyan et al. [94] used WCO for microbiological synthesis of this sugar alcohol with *Y. lipolytica* yeast. Commercially, erythritol is produced using glucose obtained by enzymatic hydrolysis of polysaccharides such as starch. It is assumed that by using non-sugar waste streams such as WCO, it is possible to reduce the costs of such a complex procedure as biological glucose production [29]. Liu et al. [95] investigated that erythritol could be also produced from WCO by *Y. lipolytica* under different cultivation conditions. The critical parameters in the process were the substrate pH and osmotic pressure. Under conditions of high osmotic pressure of 2.76 osmol/l and low pH of 3 the high erythritol yield (21.8 g/l) with simultaneous low citric acid production of 2.5 g/l was observed. By contrast, under conditions of low osmotic pressure of 0.75 osmol/l and higher pH of 6, a high level of biosynthesis of citric acid of 12.6 g/l was recorded, while erythritol yield (4.0 g/l) was low [95].

The Pang et al. [96] study is the first report on the development of new and efficient processing of WCO into l-limonene and d-limonene through the use of metabolically developed *Y. lipolytica* strains. The experiment showed that both l-limonene and d-limonene were successfully produced at levels of 2.723 mg/l and 2.514 mg/l under optimal growing conditions, where beside glucose 70% of the added carbon source was WCO.

It has also been proven that the use of WCO as a carbon source favors the synthesis of lipases by *Y. lipolytica*, more than olive oil or pure sunflower oil [91, 94]. Based on these results, it can be assumed that WCOs are alternative to expensive edible oils, namely olive oil, a well-known lipase synthesis activator [22]. However, according to Nunes et al. [97], the use of olive oil provides higher extracellular lipase production than fried soybean oil. In some cases WCO is only treated as an additional source of carbon for *Y. lipolytica* [98, 99]. Interestingly, Nunes et al. [100] used waste soybean frying oil to synthesize cell-wall-associated

Table 3 Metabolites production by different *Y. lipolytica* strains in media with waste cooking oils

Y. lipolytica Strain	WCO origin	WCO concentration in medium [g/l]	Metabolite	Maximum yield	Maximum activity [U/ml]	Additional information	Reference
Y. lipolytica SWJ-1b	The WCO obtained from a waste oil recycle bin in Huaian, Jiangsu Province, China	80	Citric acid	31.7 g/l	–	94.6% WCO utilization by *Y. lipolytica* SWJ-1b after 336 h in 10 l bioreactor	[11]
			Isocitric acid	6.5 g/l	–		
			Lipases	–	8.30		
			SCO	42.1 g of SCO/100 g of cell dry weight	–		
Y. lipolytica W29	The WCO collected from a public school canteen	10	Lipases	-	12.00	–	[91]
			SCO	48 g of SCO/100 g of cell dry weight	–		
Y. lipolytica Po1g KdHR	The WCO collected from a local kitchen	700 (ml/l)	D-Limonene	2.514 mg/l	–	Engineered *Y. lipolytica* strains (D-limonene synthase from *Citrus limon* and L-limonene synthase from *Mentha spicata* with ten homologous genes involved in the mevalonate pathway were overexpressed)	[96]
Y. lipolytica Po1g KlHR			L-Limonene	2.723 mg/l			
Y. lipolytica CECT 1240	The WCO collected from a local restaurant	30	Lipases	–	9.27	–	[98]
Y. lipolytica 50,682	Waste soybean frying oil	10	Extracellular Lipases	–	0.033	–	[97]
			Intracellular Lipases	–	1.33 (U/g)		
			Cell-bound Lipases	–	1.30 (U/g)		
		25	Extracellular Lipases	–	257.30	–	[100]
			Released Lipases		204.13 (U/g_{DCW})		
			Cell-bound Lipases		178.78 (U/g_{DCW})		
Y. lipolytica M53	–	30	Lipases	–	12.70	–	[94]
			Erythritol	22.1 g/L	–		
Y. lipolytica NCIM 3229	–	10	SCO	33 g of SCO/100 g of cell dry weight	–	–	[65]
Y. lipolytica NCIM 3450				45 g of SCO/100 g of cell dry weight			
Y. lipolytica NCIM 3472				33 g of SCO/100 g of cell dry weight			

Table 3 (continued)

Y. lipolytica Strain	WCO origin	WCO concentration in medium [g/l]	Metabolite	Maximum yield	Maximum activity [U/ml]	Additional information	Reference
Y. lipolytica NCIM 3589				24 g of SCO/100 g of cell dry weight			
Y. lipolytica NCIM 3590				28 g of SCO/100 g of cell dry weight			
Y. lipolytica YlB6	The WCO obtained from a local eatery in Pune, India	100	SCO	55 g of SCO/100 g of cell dry weight	–	Mutants of *Y. lipolytica* NCIM 3589	[101]
Y. lipolytica YlC7				60 g of SCO/100 g of cell dry weight			
Y. lipolytica YlE1				67 g of SCO/100 g of cell dry weight			
Y. lipolytica NC-I	Waste oil from frying fish	5	SCO	45.49 g of SCO/100 g of cell dry weight	–	–	[90]
	Waste oil from frying vegetables	5		57.89 g of SCO/100 g of cell dry weight			
Y. lipolytica LFMB 20	Waste butter	8.5	SCO	20 g of SCO/100 g of cell dry weight	–	29.0% WCO utilization after 25 h, 88.0% after 191 h	[102]
	Waste olive oil			24 g of SCO/100 g of cell dry weight		42.6% WCO utilization after 20 h, 90.6% after 220 h	

WCO waste cooking oil, *SCO* single cell oil

lipases by *Y. lipolytica* and the treatment with acoustic waves was applied for lipases activation and release from the cell wall. According to some scientists, the production of lipases by *Y. lipolytica* yeast is dependent on the WCO concentration used. An increase in concentration from 10 to 50 g/dm^3 caused a 3.5-fold improvement in the synthesis [94]. However, the increase to 140 g/dm^3 significantly impeded the production of lipases [11]. In the case of *Y. lipolytica* SWJ-1b and *Y. lipolytica* W29 the WCO concentration was not affected and the dose significantly affected enzyme production and the dose of 10 g/dm^3 was sufficient to achieve maximum lipase activity [94].

Yarrowia lipolytica is the most frequently studied species for the accumulation of intracellular lipids using WCO as a carbon source [39]. Triacylglycerols as the main component of waste cooking oil are transferred to the cells of microorganisms as a result of extracellular lipases [62]. Selected strains of *Y. lipolytica* yeast were investigated in order to evaluate their potential in biodiesel production, cultivating them on inexpensive waste like WCO. Katre et al. [65] selected two strains NCIM 3479 and NCIM 3589 as effective in using WCO for microbial lipid production. The results of the study showed that NCIM 3479 strain achieved the highest lipid production efficiency (47%) in the medium with 30 g/l WCO. On the other hand, strain 3589 was able to use larger amounts of WCO up to 100 g/l and then achieve a yield of 43%. For this strain there was no inhibitory effect of higher waste doses on growth or lipid production. This indicates that SCO from *Y. lipolytica* 3589 can be considered a potential feedstock for biodiesel production. The fatty acid product of extracted SCO corresponds to international biodiesel standard specifications. Katre et al. [101] generated 800 mutants of NCIM 3589 strain and then also conducted an experiment on ten mutants assessing the biomass yield, lipid content and lipid yield to finally select the three with the highest capacity to produce microbiological lipids (YlB6, YlC7 and YlE1). These strains were able to grow in WCO and accumulated oils with yields of 55, 60 and 67% respectively.

Although the experience of Lopes et al. [91] was conducted under optimal conditions for the production of lipases by *Y. lipolytica* W29 (10 g/l WCO, kLa = 16, 24 h^{-1} of fermentation), it achieved a satisfactory yield of microbiological oils (48%) rich in unsaturated fatty acids (linoleic and oleic acid). Under these culture conditions the amount of microbial lipids was 3 g/l and it was comparable to the results of other authors research. For example, in the experiment Liu et al. [11], the *Y. lipolytica* SWJ-1b yeast strain achieved a lipid yield of 42% as a result of 336 h of culture in a medium with 80 g/l WCO. The biomass yield reached 5.9 g/l. Interestingly, as a result of the described culture, only 4.3 g/l of waste lipid carbon source remained in the medium. As a consequence, it suggests that *Y. lipolytica* SWJ-1b yeast cells used as much as 94.6% of WCO present in the culture medium.

Bialy et al. [90] assumed the use of WCO as a substrate for *Y. lipolytica*, but it was not the oil obtained from waste oil recycling bin, in which different types of the waste are stored mixed together. The authors obtained WCO from local entrepreneurs differentiating: WCO from frying fish and WCO from frying vegetables. For WCO from frying fish the biomass yield was 7.40 g/l of substrate and the lipid content was 45.49%, while for WCO from frying vegetables it was 7.56 g/l and 57.59%. As a result of the treatment of lipid waste by *Y. lipolytica*, their fatty acid profiles were changed. The raw WCO remaining after frying consisted of C16:0—17.13% (fermented WCO—22.87%), C18:0—1.12% (5.15%), C18:1—28.76% (35.46%), C18:2—48.38% (18.90%). Raw and fermented WCO after frying vegetables also differed in composition, analogically: C12:0—0.16%, C14:0—0.92%, C16:0—41.88% (19.87%), C18:0—4.42% (5.52%), C18:1—42.38% (50.48%), C18:2—48.38% (16.63%). Fatty acids C12:0 and C14:0 were undetected in WCO from frying vegetables after *Y. lipolytica* treatment. The use of different types of WCO allowed to extend the conclusion about the effect of nitrogen on the effectiveness of microbial oils production. The accumulation of intracellular lipids is supported by excess sugars in the culture medium and low pool of nitrogen compounds. Therefore, when the used oil waste is rich in a source of nitrogen (e.g. nitrogen seeping from fish into frying oil), as a consequence, the overall content of accumulated lipids may decrease [90].

Microbial degradation of waste cooking butter and waste cooking olive oil was carried out by strain *Y. lipolytica* LFMB 20. The distribution of these fats by yeasts indicates their ability to effectively utilize FOGs (fats, oils, greases) present in various types of waste. When *Y. lipolytica* grew in the medium with the addition of waste cooking olive oil, 42.6% (20 h of cultivation) to 90.6% (220 h of cultivation) of the substrate fat was removed. The initial fatty substrate concentration was 8.5 ± 1.5 g/l. In 20 h of cultivation the highest efficiency (24%) of lipid production was achieved. However, after 220 h, apart from the best effect of removing fat from the medium, the highest biomass yield of 8 g/l was obtained. In case of cultivation in medium with waste cooking butter, 29–88% of fat was removed within 25–191 h. The highest biomass yield was achieved after 100 h of yeast cultivation and it was 7.5 g/l. Similarly to olive oil, the highest production of lipids (20%) was achieved in the initial phase of growth, it was 20% in 25 h. Microbiological lipids with changed profile of fatty acids arouse interest in biotechnology, industry and ecology, taking into account simultaneous solution of problems of fatty food waste disposal [102].

Bioremediation and Toxic Contaminants Removal

Yarrowia lipolytica is an exceptional example of yeast species with various biotechnological applications [6]. The yeast has been used i.a. in the bioremediation process, which is a technique using microorganisms to accelerate the degradation of contaminants found in the environment into less toxic form or to reduce the level of these contaminants to acceptable levels [103]. This technique has been used to reclamation of environments contaminated with oils. Oil-pollutions occur in both marine and freshwater environments as well as on land [4]. Moreover, they are the main cause of environmental and ecological damage. Over a decade ago, bioremediation became the main method of wastewater treatment in the oil industry [104]. Many studies have been carried out on the skills of the *Y. lipolytica* yeast species, which could also be used in biotechnological waste disposal processes. Planning the management of waste such as production wastewater must take into account the presence of various toxic compounds (Table 4) such as catechol. This compound has a strong inhibitory effect on the respiratory processes of *Y. lipolytica* yeasts [40]. In spite of this, *Y. lipolytica* NCIM 3589 yeast was able to grow in media with catechol and phenol—bromobenzene degradation products and used them as a source of carbon. The growth of yeast biomass was much higher in catechol, compared to that of phenol. These yeasts tolerated phenol at concentrations of up to 5 mM, and good growth in a catechol environment indicates effective and fast compound utilization. In conditions where phenol and catechol were the only sources of carbon, the authors were unable to detect any activity of dehalogenase—an enzyme catalyzing the reaction of elimination of atoms from the halogen group, e.g. bromine. The dehalogenation reaction precedes the aromatic ring cleavage [105]. *Yarrowia lipolytica* W29 reduced total phenol content in cultivation media with OMW by 70% [40]. There have been also studied that *Y. lipolytica* Y103 strain degraded 4-chlorophenol, component of herbicides and pesticides to catechol [106].

Dil et al. [107] investigated the possibility of using live *Y. lipolytica* 70,562 yeast cells as a biosorbent for waste decoloration. The study focused on the simultaneous biosorption of dyes: Brilliant Green (BG) and Crystal Violet (CV) from wastewater. To assess the impact of parameters such as: contact time (4-20 h), dye concentration (6–14 mg/l) and solution pH (4.0–8.0), as well as to develop a model and optimization of the biosorption process, the response surface method (RSM) was used. Contact time of 16 h, initial BG concentration of 10 mg/l and CV of 8 mg/l under pH 7.0 were indicated as the most optimal operating parameters. The above mentioned conditions led to a maximum biosorption of 99.927% and 98.823% for BG and CV dyes respectively.

The ability of *Y. lipolytica* NBRC 1658 strain to decolorize Reactive Black 5 by biodegradation was confirmed. This strain discolored 97% of the pigment within 24 h at a concentration of 50 mg/l. The yeast was able to tolerate dye up to 300 mg/l. The whole process of discoloration took place in the exponential growth phase. Aerobic batch culture in medium with glucose (5 g/l), ammonium sulphate (1 g/l) and pH = 7 proved to be the most optimal conditions [108].

Interestingly, Romero et al. [60] proved that strain *Y. lipolytica* LPS605 is able to degrade dibenzofuran. However, they did not use it as a carbon source. Yeast cells oxidized the compound, converting it to less toxic derivatives. Other strains of *Y. lipolytica* were able to hydroxylate phenol and aromatic hydrocarbons, which is a process of reducing the toxicity of these compounds. The same authors have proven that *Y. lipolytica* LPS 605 strain can degrade biphenyl to 4-hydroxybiphenyl, as well as 3,4-dihydroxybiphenyl, which is a hydroxylated product within 24 h [109]. It has been shown that *Y. lipolytica* yeast species can detoxify 2,4,6-trinitrotoluene (TNT) by reducing nitrate groups and aromatic ring [110]. The study of Żogała et al. [111] showed the capacity of *Y. lipolytica* to detoxify petrol contaminated soils. What is more, strains of *Y. lipolytica* have high crude oil degrading activity due to cell hydrophobicity and high emulsifying activity. The strains PG-32 and PG-20 degraded 58 and 68% of crude oil, respectively. They could be used for the bioremediation process and decreasing oil pollution in the marine ecosystem like Persian Gulf [13].

Yarrowia and *Candida* strains when grown on hydrocarbons were able to produce mannosylerythritol lipids, sophorolipids, carbohydrate-protein complexes, carbohydrate-protein-lipid complexes or fatty acids [112–114].

The yeast *Y. lipolytica* has been intensively studied in view of its highly efficient degradation of hydrophobic substrates such as alkanes [13]. Matatkova et al. [115] conducted research to evaluate the ability of *Y. lipolytica* CCY 30-26-36 strain to grow in media containing glucose with odd n-alkanes (pentadecane/heptadecane) or odd alkanes as the only source of carbon. Microbial oil from yeast biomass contained about 80% of unsaturated fatty acids. The authors also analyzed the influence of rhamnolipids on the use of alkanes by yeast cells and their possible influence on the fatty acid profile and lipid yield. For the substrate with glucose and alkanes a higher lipid content was recorded than for the substrate with alkanes as a single carbon source. The use of rhamnolipids resulted in a 15% increase in lipid content for cultures with glucose and alkane and 30% for the medium with alkane only. Other researchers report that the addition of rhamnolipids to the hydrophobic culture medium in *Y. lipolytica* CCY 30-26-36 culture caused dissolution and

Table 4 Summary of chemical compounds which may be degraded by *Yarrowia lipolytica* yeasts

Aromatic compounds	Alkanes and Alkenes	
phenol, catechol, dibenzofuran, biphenyl	decane, dodecane, tetradecane, hexadecane, octadecane, hexadecene	
	Nitroaromatic compounds	**Halogenated compounds**
	2,4,6-trinitrotoluene	4-chlorophenol, bromobenzene
Industrial dyes		
Brilliant Green (HSO_4^-)	Crystal Violet (Cl^-)	Reactive Black 5

formation of a stable emulsion. Rhamnolipids by reducing the hydrophobicity of yeast cells contributed to a reduction in substrate absorption [116].

Yarrowia lipolytica is a dimorphic yeast species. It is considered that the ability of specific yeast transformation may be a key factor for effective degradation of alkanes. After transition from the mycelium to yeast form, strain NCIM 3589 degraded pure alkanes (*n*-decane 40%, *n*-dodecane 40%, tetradecane 50%, hexadecane 60%, octadecane 45%) as well as aliphatic crude oil fractions under aerobic conditions within 24 h. In another study, immobilized in porous agar beads cells of NCIM 3589 strain degraded to 92% of aliphatic fractions of Bombay High crude oil. The cells in free form degraded 78% of aliphatic fractions [117–119].

Fabiszewska et al. [22] reported that dodecane might be used in microbiological production of lipases by *Y. lipolytica* KKP 379. However, the condition that must be fulfilled for the process is the presence of olive oil as an enzyme stimulator. Wei et al. [120] used dodecane in the role of cosolvent. Addition of dodecane to the *C. rugo*sa culture resulted in higher lipolytic activity with glycerol trioleate. However, after 3 days of incubation neither growth nor lipolytic activity was noticed. It is claimed that dodecane can be used in two ways, as a carbon source and to solubilize the hydrophobic substrate [22].

Yarrowia lipolytica Metabolic Engineering in Treatment Industry Wastes

Since a long time, wild-type *Yarrowia* strains have been distinguished for the high secretion yield of numerous proteins [121]. The use of *Y. lipolytica* as a host for heterologous expression was initiated, 30 years ago, by the almost simultaneous development of transformation methods by Pfizer Inc. (USA) and a French INRA team [122]. Recently, approaches including metabolic engineering, have been applied to increase potential applicability of *Yarrowia* yeast in waste management. The common target of genetic modifications are the key steps required for *Y. lipolytica* to grow well on low-cost substrates what could have a tremendous impact on the production of industrially relevant compounds (proteins and lipids for food, feed and energy purposes or other biotechnological products such as organic acids, aromas, polyalcohols, emulsifiers and surfactants) potentially reducing the cost of synthesis and making these biotechnological processes more competitive in the market [123]. Usually, the best microbial producers are often unable to bioconvert the cheapest or most convenient substrates and at the same time the substrate costs must be reduced by employing industrial wastes. Synthetic biology and metabolic engineering could be used to modify the best-suited microorganisms to use target substrates [124]. Synthetic review was prepared by Ledesma-Amaro and Nicaud [124] on metabolic engineering which expanded the substrate range of *Y. lipolytica*. Some newest communications or examples directly related to lipid waste utilization has been reviewed in the chapter emphasizing the possibilities to produce valuable metabolites in waste substrates media via genetic modification approach.

Yarrowia lipolytica is unable to fully use the most abundant and inexpensive carbon source in nature—sucrose and as a consequence hexoses such as glucose and fructose as a product of its hydrolysis. Interesting study was performed by Rakicka et al. [125] who modified the yeast strain *Y. lipolytica* Wratislavia K1 by overexpression of the *Saccharomyces cerevisiae* invertase *SUC2* gene and overexpression of the native glycerol kinase *GUT1* gene. The engineered yeast strain efficiently utilized sucrose and rapidly assimilated glycerol from industrial raw molasses and crude glycerol in order to produce 100.65 ± 3.75 g/l polyols—sugar alcohols and sweeteners [125]. In the work by Yan et al. [126], homologous overexpression of extracellular lipase *LIP2* gene was performed along with the heterologous expression of *S. cerevisiae* invertase *SUC2* gene. Among different low cost agro-industrial substrates (sugarcane molasses, waste cooking oil and crude glycerol from biodiesel production) sugarcane molasses appeared as the most effective substrates for simultaneous production of lipases (16,420 U/ml) and single cell protein (151.2 g/l) as excellent feed additives [126].

Because *Y. lipolytica* is also unable to consume xylose, the major pentose in lignocellulosic hydrolysates, which are low cost carbon sources for bioprocesses and could be used as inexpensive carbon sources in place of glucose, the mutant strain has been engineered to produce lipids and citric acids in media with xylose. The overexpression of xylose reductase and xylitol dehydrogenase from ascomycetous yeast *Scheffersomyces stipitis* and the additional overexpression of the endogenous xylulokinase enabled this mutant to produce up to 80 g/l of citric acid from xylose [123].

Increased interest in sustainable production of renewable diesel and other valuable bioproducts is redoubling efforts to improve economic feasibility of microbial-based oil production. Katre et al. [101] used the chemical mutagenesis strategy to successfully isolate three stable high SCO yielding *Y. lipolytica* mutants. The mutants exhibited an increase in lipid contents when grown on 100 g/l waste cooking oil than the parental yeast strain. The fatty acid methyl ester (FAME) profiles of all three mutants determined the strains to be suitable for biodiesel synthesis along with the management of waste cooking oil [101]. Blazeck et al. [127] optimized the oleaginous yeast to create a strain with significant lipid accumulation and lipids conversion into biodiesel. Acyl-CoA:diacylglycerol acyltransferases isozymes I and II overexpression was reported for their potential in catalysing the ultimate step in triglyceride synthesis [127].

Lindquist et al. [128] irradiated *Y. lipolytica* NRRL YB-567 with UV-C to enhance ammonia (for fertilizer) and lipid (for biodiesel) production in low-cost coffee waste medium. The mutant strain produced 0.12 g/l ammonia and 0.20 g/l 2-phenylethanol, a valuable fragrance, in addition to acylglycerols containing predominantly C16 and C18 residues. This approach revealed that it was possible to provide bioprocess aiming independently two goals.

The overexpression of the genes involved in lipid uptake and degradation may enhance the efficiency of bioconversion of oil-containing wastes into desired products [124]. The simplest way seems to be overexpression of extracellular lipase genes. Furthermore, due to heterogeneity of waste materials, an innovative approach might be constructing of mutant strain able to degrade a wide range of substrates. Another trend could focus on improving metabolite yield, increasing tolerance to metabolites or toxins present in waste substrates what can be expanded in the future.

Conclusion and Future Perspectives

Many publications refer to the use of industrial and agricultural waste to obtain new products with simultaneous valorization of wastes. However, the production of microbiological metabolites is efficient and cost-effective, it is crucial to define the influencing factors, e.g. used waste substrates. Generally, wastes from food processing can be characterized as follows: (1) depending on their origin, they contain high levels of organic compounds: carbohydrates, fats or proteins, (2) differing on chemical oxygen demand (COD) and biochemical oxygen demand (BOD), (3) containing suspended solids [129].

Given the constantly growing population, which contributes to the vastness of food production and the vastness of production processes, the industrial waste produced could place a serious burden on the planet and thus on the people living there. Therefore, the key role of scientists, technologists and entrepreneurs in the development of practical, realistic, pro-environmental techniques for the management of various types of waste, especially oily waste and those associated with animal production is pointed out.

Appropriate selection and characteristics of the waste used are essential. This will allow researchers to assess the real impact on the effectiveness of biotechnological processes. Many different types of waste are available. Bücker et al. [82] pointed out that such analysis is insufficient. The use of waste materials would result in products with higher added value. However, this does not mean that the use of such waste would be economically justified. It is necessary to determine i.a. local availability and transport possibilities. More research is needed on the selection of substrates and microorganisms. It is assessed that the carbon source used in the production of microbiological oils accounts for up to 60–75% of the total cost. Therefore the use of waste substrates is an interesting option [130].

Yarrowia lipolytica yeast has found a place in environmental applications related to the management of oil waste due to a wide range of different beneficial features such as reduction of chemical oxygen demand and production of different types of added value metabolites. Therefore, as long as oily waste poses a serious hazard to the environment, the development of practical and valuable "green" processing methods is necessary. Apart from the optimization of cultivation parameters, strain selection is an important element of effective microbial detoxification of selected oil waste. Appropriate selection and characteristics of the waste used are essential. This will allow scientists to assess their real impact on the effectiveness of biotechnological processes. However, a deeper analysis is advisable.

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