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Aeration and Stirring in *Yarrowia lipolytica* Lipase Biosynthesis during Batch Cultures with Waste Fish Oil as a Carbon Source

Paulina Snopek¹, Dorota Nowak¹, Bartłomiej Zieniuk^{2,*} and Agata Fabiszewska²

- ¹ Department of Food Engineering and Process Management, Institute of Food Sciences, Warsaw University of Life Sciences-SGGW, 02-776 Warsaw, Poland; paulinka1504@gmail.com (P.S.); dorota_nowak@sggw.edu.pl (D.N.)
- ² Department of Chemistry, Institute of Food Sciences, Warsaw University of Life Sciences-SGGW, 02-776 Warsaw, Poland; agata_fabiszewska@sggw.edu.pl
- * Correspondence: bartlomiej_zieniuk@sggw.edu.pl; Tel.: +48-(22)-5937621

Abstract: *Yarrowia lipolytica* is one of the most studied non-conventional forms of yeast, exhibiting a high secretory capacity and producing many industrially important and valuable metabolites. The yeast conceals a great biotechnological potential to synthesize organic acids, sweeteners, microbial oil, or fragrances. The vast majority of bioprocesses are carried out in bioreactors, where suitable culture conditions are provided. In the current study, the effect of agitation speed (200–600 rpm) and air flow rate (0.0375–2.0 dm³/(dm³ × min)) on the biomass yield and lipase activity of *Y. lipolytica* KKP 379 is analyzed in a growth medium containing waste fish oil. The increase of aeration intensity limited the period of oxygen deficit in the medium. Simultaneously, an increase in lipolytic activity was observed from 2.09 U/cm³ to 14.21 U/cm³; however, an excessive agitation speed likely caused oxidative or shear stresses, and a reduction in lipolytic activity was observed. Moreover, it is confirmed that the synthesis of lipases is related to oxygen consumption, pH, and the yeast growth phase, and appropriate process selection may provide two advantages, namely, the maximum use of the waste carbon source and the production of lipolytic enzymes that are valuable in many industries.

Keywords: aeration; bioreactor; lipase activity; stirring; Yarrowia lipolytica

1. Introduction

Lipolytic enzymes (EC 3.1.1.3) belong to the hydrolase family and are responsible for the hydrolysis of triglycerides to di- and mono-glycerides, fatty acids, and glycerol. Lipases are widespread in nature and are synthesized by plants, animals, microorganisms, and even viruses [1,2].

Lipases play important physiological roles and show an ability to catalyze reactions opposite to hydrolysis, such as esterification and transesterification, including also aminolysis and thioesterification [3,4]. Due to their properties, particularly regioselectivity, chemoselectivity, and stereoselectivity, catalytic proteins have been widely exploited as attractive enzymes in industrial applications [5–8]. Interestingly, in the last 30 years, some reports have acknowledged lipase-catalyzed unconventional reactions, i.e., "lipase promiscuity". Alternative substrates, other than carboxylic acids and their esters, have been recognized to bind at the active site of lipase, resulting in aldol condensation, Cannizzaro reactions, Knoevenagel condensation, or Ugi reactions [9].

Both mold and yeast enzymes have been studied since the 1950s. Major filamentous fungi capable of synthesizing lipases belong to the *Rhizopus*, *Rhizomucor*, *Aspergillus*, and *Penicillium* genera, and, among yeasts, the following genera can be enumerated: *Candida*, *Pichia*, *Rhodotorula*, and *Yarrowia* [10]. Lipases of a microbial origin are often referred to as being more valuable in comparison with those extracted from plants or animals. Firstly, their production does not raise ethical concerns compared to animal proteins. Additionally, microorganisms provide high synthesis yields, their cultures are independent



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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). of seasons, and the waste substrates can be used for economical and environmentally friendly manufacturing [10,11].

The given culture conditions strongly affect the high activity of fungal lipases, which are inducible enzymes. The most important factors influencing the biosynthesis of these enzymes are the medium composition, including carbon and nitrogen sources, micro- and macronutrients, different inducers or inhibitors, and additionally various culture parameters, such as temperature, pH, aeration, or stirring intensity. Most of the environmental factors are species- or strain-dependent [12,13]. The best lipase activators are believed to be hydrophobic substrates, mainly selected fatty acids and triglycerides [14] e.g., vegetable oils such as rapeseed, corn, sunflower, or olive oil [15]. Nitrogen sources that are regarded as enzyme-inducing can be divided into organic (corn steep, peptone, tryptone, yeast extract, and urea) and inorganic compounds (ammonium or nitrate salts) [16]. Many other factors may influence the activity of lipases. Metal ions e.g., Ca²⁺ and Mg²⁺, often increase enzyme properties due to the stabilization of the active site. On the other hand, heavy metals like Hg²⁺, as well as EDTA or PMSF (phenylmethylsulfonyl fluoride), have been described as lipase inhibitors [17].

Y. lipolytica is an obligate aerobe, and intensive aeration with an optimized agitator speed has been shown to positively affect lipase production [18]. A striking approach was proposed by Amaral et al. [19], who applied perfluorodecalin in a culture of *Y. lipolytica* that consequently benefited from better gas dissolution and increased growth and lipase synthesis [19]. Nevertheless, few researchers have addressed the issue of oxygen availability on lipase secretion yield in batch cultures of lipolytic yeast. The current study aims to analyze the effect of bioreactor culture conditions on the growth of biomass and the ability to biosynthesize extracellular lipolytic enzymes for *Yarrowia lipolytica* KKP 379. The intensity of stirring and the flow rate of air supplied to the culture medium were selected as the test variables.

This paper also provides an interesting look into the problem of microbial enzyme synthesis in waste substrate media. Growing care for the environment by designing sustainable processes has implied the replacement of conventional medium components used for biosynthesis of enzymes with agri-food industry wastes. Recently, some waste substrates have been used for lipase production in order to reduce the costs of microbial media and provide valorization for industrial by-products e.g., waste cooking oil [18], waste fish oil, and other waste lipids [20], sugarcane molasses, crude glycerol [21,22], soybean meal [17], or olive mill wastewater [23]. With this in mind, we replaced the commonly used olive oil lipase inducer with a fishery industry waste oil.

2. Materials and Methods

2.1. Materials

Chemicals were purchased from Avantor Performance Materials Poland S.A. (Gliwice, Poland) and Sigma-Aldrich (Poznań, Poland). *p*-Nitrophenyl laurate for lipase activity measurements was synthesized in the Department of Chemistry, Institute of Food Sciences, Warsaw University of Life Sciences-SGGW [24].

The waste fish oil after the smoking process was collected from the fish processing plant located in the Podlaskie Voivodeship (Poland). Full characteristic of the waste oil was presented by Fabiszewska et al. [25]. The other culture media components were purchased from BTL Sp. z o. o. (Łódź, Poland).

2.2. Microorganism

The yeast strain *Y. lipolytica* KKP 379 was purchased from the Collection of Industrial Microorganisms, Prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology—State Research Institute (Warsaw, Poland) and used for the experiments.

2.3. Media and Culture Conditions

Inoculum cultures of *Y. lipolytica* KKP 379 were carried out in 100 cm³ of a YPG medium (10 g/L yeast extract (Y), 20 g/L peptone (P), 20 g/L glucose (G), pH = 5) for 24 h at 28 °C on a rotary shaker. All media were sterilized at 121 °C for 20 min.

Cultures were produced in a BIOFLO 3000 laboratory bioreactor (New Brunswick Scientific, Edison, NJ, USA) in 4 dm³ of a YPF medium (glucose was replaced with waste fish oil) at 28 °C, mixed using a Rushton turbine and aerated with compressed air for about 42–48 h. The bioreactor vessel with the waste fish oil medium was sterilized at 121 °C for 20 min. The medium was inoculated with 0.025% v/v of a yeast inoculum culture. A silicon anti-foaming agent (Avantor Performance Materials Poland S.A.) was used to inhibit foaming.

During the culture process, the following parameters were monitored: dissolved oxygen (DO₂) in the medium, agitation speed, air flow, temperature, and pH. Different variants of oxygenation and stirring were used in five yeast cultures according to Table 1.

Culture No.	Mark of Culture	Agitator Speed (rpm)	Air Flow (dm ³ /(dm ³ \times min))
1	200 C 0.375	200	0.375
2	400 C 1.0	400	1.0
3	400 C 2.0	400	2.0
4	600 C 2.0	600	2.0
5	(200–600) V 1.75	Variable agitator speed dependent on the medium aeration (200–600)	1.750

Table 1. Cultures conditions regarding medium aeration applied in the current study.

In the culture (200–600) V 1.75, a variable aeration profile was used while referring to maintaining the aeration of the medium at the set level by changing the agitator speed in the range of 200–600 rpm, where the four previous cultures were carried out within the agitator speed range mentioned above. The set aeration levels are presented in Table 2.

Culture Time (h)	Set Aeration Level (%)		
0.0–3.0	80		
3.0-6.5	60		
6.5–12.0	40		
12.0–18.5	20		
18.5–27.5	10		
Above 27.5	5		

 Table 2. Medium aeration profile of the fifth culture applied in the current study.

2.4. Determination of Biomass

Yeasts cells were separated from the culture medium by centrifugation ($6000 \times g$, 10 min), washed in distilled water, and dried at 105 °C until of a constant weight.

2.5. Determination of Extracellular Lipase Activity

The extracellular lipase activity of *Y. lipolytica* was determined by a spectrophotometric method based on the hydrolysis of *p*-nitrophenyl laurate [26]. After the centrifugation 1 cm³ of the cell-free supernatant, it was diluted up to 15 cm³ with a potassium phosphate buffer (0.1 M, pH = 7) and the aqueous solution was added to 0.3 mmol of *p*-nitrophenyl laurate dissolved in 2 cm³ of heptane. A hydrolysis reaction was performed on a magnetic stirrer at 37 °C, and, after 15 min, absorbance was measured at 410 nm using a Rayleigh UV-1601 spectrophotometer (BRAIC, Beijing, China). The conditions used are consistent with the pH and temperature optimum for *Y. lipolytica* extracellular lipase [27]. One unit of

enzyme activity corresponded to the enzyme quantity present in 1 cm^3 of supernatant that liberated 1 µmol of *p*-nitrophenol per minute under the assay conditions.

2.6. Determination of Selected Culture Parameters

The following parameters characterizing the growth and production of lipases were determined: product-biomass yield $(Y_{(P/X)})$, biomass-substrate yield $(Y_{(X/S)})$; calculated based on the initial concentration of the substrate), specific production rate (q_p) , and volumetric productivity (v_p) . For the calculation, the highest lipase activities, the culture times when were achieved and corresponding biomass yields were taken into account. The parameters were calculated according to López-Fernández et al. [28] and Nooh et al. [29].

2.7. Statistical Analysis

Cluster analysis was performed using the Statistica 13.3 software package (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results and Discussion

In the current research, five different bioreactor cultures with varying agitation speeds were produced. Changes in the biomass yields, lipase activities, dissolved oxygen concentrations, and pH values are presented in Figures 1–5. The last two parameters were measured on-line and may indicate the intensity of multiplication of microorganisms and suggest changes in the lipolytic enzyme activity.

A characteristic pH plot shape was observed in all cultures, except culture 1 (Figure 1a). The starting value was kept constant for the first 15 h, while between 22 and 36 h, depending on the cultivation conditions, a sharp rise in pH above 8 was observed over a period of approximately 6 h. In the case of the first culture, the pH plot changed in a narrow range of 1.5 pH, from about 3.7 to approximately 5.2. The final increase in the medium pH might be a consequence of the utilization of acidic compound or proteolysis [30]. In media with peptone and a yeast extract, as in the current study, exogenous amino acids were present and utilized for yeast growth, where the ammonia resulting from their metabolism alkalized the culture environments [31].



Figure 1. Kinetics of dissolved oxygen and pH (**a**) and biomass yield and extracellular lipase activity (**b**) of the yeast strain *Y. lipolytica* KKP 379 in the culture with an agitation speed of 200 rpm and air flow of 0.375 dm³/(dm³ × min).



Figure 2. Kinetics of dissolved oxygen and pH (**a**) and biomass yield and extracellular lipase activity (**b**) of the yeast strain *Y. lipolytica* KKP 379 in the culture with an agitation speed of 400 rpm and air flow of $1 \text{ dm}^3/(\text{dm}^3 \times \text{min})$.



Figure 3. Kinetics of dissolved oxygen and pH (**a**) and biomass yield and extracellular lipase activity (**b**) of the yeast strain *Y. lipolytica* KKP 379 in the culture with an agitation speed of 400 rpm and air flow of 2 dm³/(dm³ × min).



Figure 4. Kinetics of dissolved oxygen and pH (**a**) and biomass yield and extracellular lipase activity (**b**) of the yeast strain *Y. lipolytica* KKP 379 in the culture with an agitation speed of 600 rpm and air flow of 2 dm³/(dm³ × min).



Figure 5. Kinetics of dissolved oxygen and pH (**a**), biomass yield and extracellular lipase activity (**b**), and agitation speed (**c**) of the yeast strain *Y. lipolytica* KKP 379 in the culture with a variable agitator speed dependent on the medium aeration and air flow of 1.75 dm³/(dm³ × min).

A significant decrease in the dissolved oxygen in the medium has always been correlated with an increase in biomass growth, and often with an increase in enzyme activity. A very similar trend of changes in the level of oxygen consumption was observed in all experiments. Initially, the oxygen content in the medium was high, and then, as the number of microorganisms increased, it decreased rapidly between 8 and 20 h of cultivation. In the case of second (400 C 1.0) and third (400 C 2.0) cultures (Figures 2a and 3a), increases in the oxygen level was noticed at 42 and 35 h of cultivation, respectively, due to the end of the logarithmic growth phase of microorganisms and a decrease in oxygen consumption.

Referring to Figure 4a, despite the use of antifoams, foaming of the medium disturbed DO₂ reading, as well as ending the culture process earlier when compared to other experiments. Foaming often occurs in aerated and agitated bioreactors. Many factors affect the stability and pattern of foam formation, such as the medium composition (particularly high protein content), metabolite production, the introduction of gas or the presence of surface-active agents [32]. The described phenomenon often leads to a decrease of the oxygen transfer rate and a working volume of the reactor and may also lead to the removal of cells or metabolites and pose a risk of contamination [33].

Stirring promotes the dissolution of oxygen and subsequently it can be used more efficiently by microorganisms. In the last culture ((200–600) V 1.75, Figure 5a), the oxygen consumption of yeast increased, and thus the oxygen content in the medium decreased, and

the agitation speed was increased in order to maintain the oxygenation at the appropriate level (Table 2). The aeration profile was established based on the observation of the cultures 1–4. Up to 3 h of cultivation, the oxygenation level was supposed to be kept above 80%, which caused an increase in the stirrer speed, and then, up to 15 h of cultivation, the agitation was at the minimum level of 200 rpm, due to the sufficient oxygenation of the substrate (Figure 5c). At the beginning of the logarithmic growth phase, the dissolved oxygen level in the medium dropped significantly, which resulted in a further increase in the agitator rotation intensity until the maximum value was reached after 21 h of cultivation (600 rpm). At that time, the oxygenation of the medium was kept at about 5%.

Aeration is a very important parameter affecting both biomass growth and lipolytic activity due to the fact that *Y. lipolytica* is an aerobic microorganism. Compared to flask cultures, cultivation in the bioreactor provides an opportunity to control the oxygenation level. The agitation speed increases oxygen dissolution in the medium, but excessive stirring may cause oxidative and mechanical stresses or produce shear forces [34]. The most remarkable observations of the presented study are in the agreement with this statement. In the first culture (200 C 0.375, Figure 1b), where the lowest values of culture parameters were applied, the biomass yield reached 5.39 g/dm³ and the highest lipase activity was achieved at 26 h of the culture and was 0.123 U/cm^3 . Such low results testified to the insufficient amount of oxygen supplied, limiting the growth of yeast and the production of lipolytic enzymes.

In the remaining cultures (cultures 2–5), both yeast growth and enzyme activity were much higher than in the first one. Increasing both examined parameters led to an improvement in the growth and production of the described metabolite. According to Figure 2b, the highest lipase activity was 2.09 U/cm³ (24 h), and, after 28 h of cultivation, a biomass yield of 22.61 g/dm³ was obtained. An increase in the air flow rate from $1 \text{ dm}^3/(\text{dm}^3 \times \text{min})$ to $2 \text{ dm}^3/(\text{dm}^3 \times \text{min})$ resulted in an almost 2.5-fold increase in the yeast lipolytic activity (5.16 U/cm^3) , but it did not have such a big impact on the biomass yield, which was 21.07 g/dm³ (Figure 3b). A slight decrease in the activity of lipolytic enzymes in relation to culture 400 C 2.0 was observed in the 600 C 2.0 culture (Figure 4b). The maximum value of activity was 4.89 U/cm^3 at 30 h of the cultivation, which may be a result of earlier described mechanical, oxidative or shear stresses caused by the high intensity of the stirrer's rotation [34]. Moreover, no further measurements were made due to the high foaming of the culture. Interestingly, the use of a variable speed of the stirrer depending on the set level of medium aeration and air flow in the amount of $1.75 \text{ dm}^3/(\text{dm}^3 \times \text{min})$ allowed for the highest enzyme activity among all cultures, namely, 14.21 U/cm³ (Figure 5b). This may indicate the adverse effects of a large shear stress on the production of lipase.

For all cultures variants, with the exception of culture 200 C 0.375, a characteristic shape of the microorganism growth curve was observed. Initially, an increase in biomass yield was insignificant in the adaptation phase, then a transition to the logarithmic growth phase was observed. After the stationary phase, there was usually a slight decrease in the amount of biomass, which represented the death and lysis phase of yeast cells, and lipases were the most active in the logarithmic growth phase. Dissolved oxygen, aeration level, and stirring are in correlation. It is well-known that *Y. lipolytica* is strictly aerobic yeast, hence an increase in agitation speed influence the dissolved oxygen level in favor of biomass growth. Similar results have been described by Stolarzewicz et al. [35] in a culture of the same yeast strain.

A significant decrease in the lipase activity was observed when the pH value reached value about 8. Some authors have observed that the most likely cause of a lipase activity decrease is the presence of alkaline extracellular proteases (AEP). Shu et al. [36] acknowl-edged that *Y. lipolytica* simultaneously synthesizes lipases and proteases and is capable of digesting proteins, including those with catalytic properties. Furthermore, Najjar et al. [37], similarly to the current studies, noticed an increase in the pH values of the medium in the stationary phase and in proteolytic enzyme activity, which finally caused a sharp decrease

of lipases activity. This phenomenon was also observed in the culture of another yeast species, *Pichia pastoris*, but controlling the pH value contributed to the inhibition of proteases and led to a 2-fold increase in the production of a cellulose-binding domain-lipase fusion protein [38].

The obtained results allow formulation of the statement that the higher the dissolved oxygen concentration in the medium, the higher the activity of lipases. These results were confirmed in the studies by Lopes et al. [39]. The authors claimed that *Y. lipolytica* W29 adapts quickly to hyperbaric conditions and conducting experiments at a pressure of 6 bar resulted in a 5-fold higher increase in biomass and higher lipolytic activity in comparison with cultures carried out at atmospheric pressure; however, studies by Alonso et al. [34] showed that the optimal agitation speed was only 200 rpm, along with an air flow rate of 1–2 dm³/min (0.8–1.7 vvm) for *Y. lipolytica* IMUFRJ 50682.

Additionally, for better comparison of bioreactor cultures of *Y. lipolytica* KKP 379, the following culture parameters were determined: product-biomass yield $(Y_{(P/X)})$, biomass-substrate yield $(Y_{(X/S)})$, specific production rate (q_p) , and volumetric productivity (v_p) (Table 3). The coefficient of product to biomass formation $(Y_{(P/X)})$ was highest in the (200–600) V 1.75 culture (677.41 U/g), where the lipase activity peaked at 14.21 U/cm³. The 200 C 0.375 culture was the lowest and the coefficient reached 37.50 U/g. Comparing cultures 400 C 2.0 and 600 C 2.0 confirmed the earlier results, where lower lipase activity was obtained when higher agitation speed was applied. Moreover, these differences were more noticeable when comparing the specific production rates which took into account the culture time and volumetric productivity, which describes metabolite production in 1 dm³ of the medium in a time unit. Biomass-substrate yield ranged from 0.16–1.19, but after discarding the value for the first culture the range was narrower, i.e., 0.78–1.19. For these cultures, the parameters presented are promising, especially due to the use of waste fish oil as the main carbon source. The bioutilization of wastes may reduce environmental pollution and reduce the costs of metabolite production [40–42].

Culture Variant	$Y_{(P/X)}$ (U/g) ¹	Y _(X/S) (g/g)	q_p (U/g $ imes$ h)	v_p (U/dm ³ × h)
200 C 0.375	37.50	0.16	1.44	4.73
400 C 1.0	134.58	0.78	5.61	86.92
400 C 2.0	290.55	0.89	10.76	191.22
600 C 2.0	205.85	1.19	6.86	163.10
(200–600) V 1.75	677.41	1.05	25.09	526.37

Table 3. Selected culture parameters of *Y. lipolytica* in a medium with waste fish oil.

¹ $Y_{(P/X)}$ —product-biomass yield; $Y_{(X/S)}$ —biomass-substrate yield; q_p —specific production rate; v_p —volumetric productivity.

The aim of the cluster analysis (Figure 6) was to summarize all the differences between the analyzed cultures. It is worth mentioning that the 200 C 0.375 culture exhibited the highest dissimilarity in comparison with the rest of the cultures, what was acknowledged along with other results presented in the current study, and the separated cluster was distinguished. Moreover, cultures 400 C 1.0 and 400 C 2.0 were grouped into next cluster, and these cultures differed only in air flow rate. The (200–600) V 1.75 culture also exhibited high dissimilarity, particularly due to the highest lipolytic activity.



Figure 6. The results of the cluster analysis.

4. Conclusions

In summary, the evidence from this study suggests that both the level of aeration in the medium and the intensity of stirrer rotation significantly affect the activity of extracellular lipolytic enzymes of *Y. lipolytica* yeast. In the current study, the use of a variable agitator speed dependent on the medium aeration (200–600 rpm) and an air flow rate of $1.75 \text{ dm}^3/(\text{dm}^3 \times \text{min})$ allowed a very high lipase level of 4.21 U/cm^3 to be obtained in a medium with waste fish oil. Due to their various properties, lipases are enzymes that are used in many industries, and microbial production enables a wider and more practical application of these biocatalysts. The selection of optimal culture parameters, including aeration and stirring intensity, significantly affects the growth of microorganisms and thus the amounts and activities of enzymes produced by them.

The present study has only investigated a wild strain of lipolytic yeast species. Although intensive genetic engineering projects for microbial strains are ongoing, the culture conditions tremendously influence the bioprocess yield. Due to the species and strain dependence on those factors, culture optimization is still a bothering issue.

The tremendous application possibilities of microbial lipases encourage the search for new strains that are capable of synthesizing enzymes with high activity from waste and cheap substrates. In this paper, further evidence of the utilization of waste carbon sources in *Y. lipolytica* cultures with its simultaneous valorization to enzymatic proteins has been provided. This can significantly reduce production costs and have a positive impact on the environment. Additionally, further works should be applied to research waste sources of organic proteins, amino acids, and microelements to replace the peptones or yeast extracts used in microbiological media.

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References

- 1. Gupta, P.; Chaubey, A.; Mahajan, N.; Anand, N. A review on Arthrobacter sp. lipase: A versatile biocatalyst for the kinetic resolution to access enantiomerically pure/enriched compounds. *Chirality* **2021**, *33*, 209–225. [CrossRef]
- Kamil, J.P.; Tischer, B.K.; Trapp, S.; Nair, V.K.; Osterrieder, N.; Kung, H.J. vLIP, a Viral Lipase Homologue, Is a Virulence Factor of Marek's Disease Virus. J. Virol. 2005, 6, 6984–6996. [CrossRef]
- Zeng, S.; Liu, J.; Anankanbil, S.; Chen, M.; Guo, Z.; Adams, J.P.; Snajdrova, R.; Li, Z. Amide Synthesis via Aminolysis of Ester or Acid with an Intracellular Lipase. ACS Catal. 2018, 8, 8856–8865. [CrossRef]
- 4. Zhou, N.; Shen, L.; Dong, Z.; Shen, J.; Du, L.; Luo, X. Enzymatic Synthesis of Thioesters from Thiols and Vinyl Esters in a Continuous-Flow Microreactor. *Catalysts* **2018**, *8*, 249. [CrossRef]
- 5. Mhetras, N.; Mapare, V.; Gokhale, D. Cold Active Lipases: Biocatalytic Tools for Greener Technology. *Appl. Biochem. Biotechnol.* **2021**. [CrossRef]
- Coelho, A.L.S.; Orlandelli, R.C. Immobilized microbial lipases in the food industry: A systematic literature review. *Crit. Rev. Food* Sci. Nutr. 2021, 61, 1689–1703. [CrossRef] [PubMed]
- Vishnoi, N.; Dixit, S.; Mishra, J. Microbial Lipases and Their Versatile Applications. In *Microbial Enzymes: Roles and Applications in Industries. Microorganisms for Sustainability*; Arora, N., Mishra, J., Mishra, V., Eds.; Springer: Singapore, 2020; Volume 11, pp. 207–230. [CrossRef]
- 8. Mehta, A.; Guleria, S.; Sharma, R.; Gupta, R. 6-The lipases and their applications with emphasis on food industry. In *Microbial Biotechnology in Food and Health*; Ray, R.C., Ed.; Academic Press: Cambridge, MA, USA, 2021; pp. 143–164. [CrossRef]
- Dwivedee, B.; Soni, S.; Sharma, M.; Bhaumik, J.; Laha, J.K.; Banerjee, U.C. Promiscuity of Lipase-Catalyzed Reactions for Organic Synthesis: A Recent Update. *ChemistrySelect* 2018, 3, 2441–2446. [CrossRef]
- 10. Chandra, P.; Enespa; Singh, R.; Arora, P.K. Microbial lipases and their industrial applications: A comprehensive review. *Microb. Cell Factories* **2020**, *19*, 169. [CrossRef]
- 11. Gurung, N.; Ray, S.; Bose, S.; Rai, V. A Broader View: Microbial Enzymes and Their Relevance in Industries, Medicine, and Beyond. *BioMed. Res. Int.* 2013, 329121. [CrossRef] [PubMed]
- 12. Kebabci, O.; Cihangir, N. Comparison of three *Yarrowia lipolytica* strains for lipase production: NBRC 1658, IFO 1195, and a local strain. *Turk. J. Biol.* **2012**, *36*, 15–24.
- Lopes, M.; Araujo, C.; Aguedo, M.; Gomes, N.; Goncalves, C.; Teixeira, J.A.; Belo, I. The use of olive mill wastewater by wild type *Yarrowia lipolytica* strains: Medium supplementation and surfactant presence effect. *J. Chem. Technol. Biotechnol.* 2009, 84, 533–537. [CrossRef]
- 14. Fickers, P.; Nicaud, J.M.; Gaillardin, C.; Destain, J.; Thonart, P. Carbon and nitrogen sources modulate lipase production in the yeast *Yarrowia lipolytica*. J. Appl. Microbiol. **2004**, *96*, 742–749. [CrossRef] [PubMed]
- 15. Fabiszewska, A.U.; Białecka-Florjańczyk, E. Factors influencing synthesis of extracellular lipases by *Yarrowia lipolytica* in medium containing vegetable oils. J. Microbiol. Biotechnol. Food Sci. 2014, 4, 231–237. [CrossRef]
- Bharathi, D.; Rajalakshmi, G. Microbial lipases: An overview of screening, production and purification. *Biocatal. Agric. Biotechnol.* 2019, 22, 101368. [CrossRef]
- de Souza, C.E.C.; Ribeiro, B.D.; Coelho, M.A.Z. Characterization and Application of *Yarrowia lipolytica* Lipase Obtained by Solid-State Fermentation in the Synthesis of Different Esters Used in the Food Industry. *Appl. Biochem. Biotechnol.* 2019, 189, 933–959. [CrossRef]
- 18. Lopes, M.; Miranda, S.M.; Alves, J.M.; Pereira, A.S.; Belo, I. Waste Cooking Oils as Feedstock for Lipase and Lipid-Rich Biomass Production. *Eur. J. Lipid Sci. Technol.* **2019**, *121*, 1800188. [CrossRef]
- Amaral, P.F.F.; Freire, M.G.; Rocha-Leao, M.H.M.; Marrucho, I.M.; Coutinho, J.A.P.; Coelho, M.A.Z. Optimization of oxygen mass transfer in a multiphase bioreactor with perfluorodecalin as a second liquid phase. *Biotechnol. Bioeng.* 2008, 99, 588–598. [CrossRef] [PubMed]
- 20. Zieniuk, B.; Mazurczak-Zieniuk, P.; Fabiszewska, A. Exploring the Impact of Lipid-Rich Food Industry Waste Carbon Sources on the Growth of Candida cylindracea DSM 2031. *Fermentation* **2020**, *6*, 122. [CrossRef]
- Yan, J.; Han, B.; Gui, X.; Wang, G.; Xu, L.; Yan, Y.; Madzak, C.; Pan, D.; Wang, Y.; Zha, G.; et al. Engineering Yarrowia lipolytica to Simultaneously Produce Lipase and Single Cell Protein from Agro-industrial Wastes for Feed. Sci. Rep. 2018, 8, 758. [CrossRef]
- 22. Sarris, D.; Sampani, Z.; Rapti, A.; Papanikolaou, S. Valorization of Crude Glycerol, Residue Deriving from Biodiesel-Production Process, with the Use of Wild-type New Isolated *Yarrowia lipolytica* Strains: Production of Metabolites with Pharmaceutical and Biotechnological Interest. *Curr. Pharm. Biotechnol.* **2019**, *20*, 881–894. [CrossRef] [PubMed]
- Sarris, D.; Stoforos, N.G.; Mallouchos, A.; Kookos, I.K.; Koutinas, A.A.; Aggelis, G.; Papanikolaou, S. Production of added-value metabolites by Yarrowia lipolytica growing in olive mill wastewater-based media under aseptic and non-aseptic conditions. *Eng. Life Sci.* 2017, 17, 695–709. [CrossRef]

- 24. Zieniuk, B.; Fabiszewska, A.; Białecka-Florjańczyk, E. Screening of solvents for favoring hydrolytic activity of *Candida antarctica* Lipase B. *Bioproc. Biosyst. Eng.* **2020**, *43*, 605–613. [CrossRef]
- Fabiszewska, A.U.; Zieniuk, B.; Kozłowska, M.; Mazurczak-Zieniuk, P.M.; Wołoszynowska, M.; Misiukiewicz-Stępień, P.; Nowak, D. Studies on Upgradation of Waste Fish Oil to Lipid-Rich Yeast Biomass in *Yarrowia lipolytica* Batch Cultures. *Foods* 2021, 10, 436. [CrossRef]
- Kapturowska, A.; Stolarzewicz, I.; Krzyczkowska, J.; Białecka-Florjańczyk, E. Studies on lipolytic activity of sonicated enzymes from Yarrowia lipolytica. Ultrason. Sonochem. 2012, 19, 186–191. [CrossRef] [PubMed]
- 27. Dervishi, F.; Destain, J.; Nahvi, I.; Thonart, P.; Zarkesh-Esfahani, H. High-level production of extracellular lipase by *Yarrowia lipolytica* mutants from methyl oleate. *New Biotechnol.* **2011**, *28*, 756–760. [CrossRef] [PubMed]
- 28. López-Fernández, J.; Barrero, J.J.; Benaiges, M.D.; Valero, F. Truncated Prosequence of *Rhizopus oryzae* Lipase: Key Factor for Production Improvement and Biocatalyst Stability. *Catalysts* **2019**, *9*, 961. [CrossRef]
- 29. Nooh, H.M.; Masomian, M.; Salleh, A.B.; Mohamad, R.; Ali, M.S.M.; Rahman, R.N.Z.R.A. Production of Thermostable T1 Lipase Using Agroindustrial Waste Medium Formulation. *Catalysts* **2018**, *8*, 485. [CrossRef]
- 30. Corzo, G.; Revah, S. Production and characteristics of the lipase from *Yarrowia lipolytica* 681. *Bioresour. Technol.* **1999**, *70*, 173–180. [CrossRef]
- 31. Vylkova, S.; Carmar, A.J.; Danhof, H.A.; Collete, J.R.; Zhou, H.; Lorenz, M.C. The fungal pathogen *Candida albicans* autoinduces hyphal morphogenesis by raising extracellular pH. *mBio* **2011**, *2*, e00055-11. [CrossRef]
- 32. Vardar-Sukan, F. Foaming: Consequences, prevention and destruction. Biotechnol. Adv. 1998, 16, 913–948. [CrossRef]
- Bouchedja, D.N.; Danthine, S.; Kar, T.; Fickers, P.; Sassi, H.; Boudjellal, A.; Blecker, C.; Delvigne, F. pH level has a strong impact on population dynamics of the yeast *Yarrowia lipolytica* and oil micro-droplets in multiphasic bioreactor. *FEMS Microbiol. Lett.* 2018, 365, fny173. [CrossRef] [PubMed]
- Alonso, F.O.M.; Oliveira, E.B.L.; Dellamora-Ortiz, G.M.; Pereira-Meirelles, F.V. Improvement of lipase production at different stiring speeds and oxygen levels. *Braz. J. Chem. Eng.* 2005, 22, 9–18. [CrossRef]
- 35. Stolarzewicz, I.A.; Zaborniak, P.; Fabiszewska, A.U.; Białecka-Florjańczyk, E. Study on the properties of immobilized biocatalysts with lipase activity produced by *Yarrowia lipolytica* in batch culture. *Chem. Biochem. Eng. Q.* **2017**, *31*, 251–259. [CrossRef]
- 36. Shu, Z.Y.; Jiang, H.; Lin, R.F.; Jiang, Y.M.; Lin, L.; Huang, J.Z. Technical methods to improve yield, activity and stability in the development of microbial lipases. *J. Mol. Catal. B Enzym.* **2010**, *62*, 1–8. [CrossRef]
- Najjar, A.; Robert, S.; Guerin, C.; Violet-Asther, M.; Carriere, F. Quantitative study of lipase secretion, extracellular lipolysis, and lipid storage in the yeast *Yarrowia lipolytica* grown in the presence of olive oil: Analogies with lipolysis in humans. *Appl. Microbiol. Biotechnol.* 2011, 89, 1947–1962. [CrossRef] [PubMed]
- Jahic, M.; Gustavsson, M.; Jansen, A.K.; Martinelle, M.; Enfors, S.O. Analysis and control of proteolysis of a fusion protein in *Pichia pastoris* fed-batch processes. *J. Biotechnol.* 2003, 102, 45–53. [CrossRef]
- Lopes, M.; Gomes, N.; Mota, M.; Belo, I. Yarrowia lipolytica Growth Under Increased Air Pressure: Influence on Enzyme Production. Appl. Biochem. Biotechnol. 2009, 159, 46–53. [CrossRef]
- 40. Kaur, P.; Ghoshal, G.; Jain, A. Bio-utilization of fruits and vegetables waste to produce β-carotene in solid-state fermentation: Characterization and antioxidant activity. *Process. Biochem.* **2019**, *76*, 155–164. [CrossRef]
- Morgunov, I.G.; Kamzolova, S.V.; Lunina, J.N. Citric Acid Production by Yarrowia lipolytica Yeast on Different Renewable Raw Materials. *Fermentation* 2018, 4, 36. [CrossRef]
- Sarris, D.; Rapti, A.; Papafotis, N.; Koutinas, A.A.; Papanikolaou, S. Production of Added-Value Chemical Compounds through Bioconversions of Olive-Mill Wastewaters Blended with Crude Glycerol by a Yarrowia lipolytica Strain. *Molecules* 2019, 24, 222. [CrossRef]