fermentation

MDPI

Article

Exploring the Impact of Lipid-Rich Food Industry Waste Carbon Sources on the Growth of *Candida cylindracea* DSM 2031

Bartłomiej Zieniuk *, Patrycja Mazurczak-Zieniuk and Agata Fabiszewska

Department of Chemistry, Institute of Food Sciences, Warsaw University of Life Sciences-SGGW, 159c Nowoursynowska St., 02-776 Warsaw, Poland; patrycja.mazurczak@op.pl (P.M.-Z.); agata_fabiszewska@sggw.edu.pl (A.F.)

* Correspondence: bartlomiej_zieniuk@sggw.edu.pl; Tel.: +48-22-59-37-621

Received: 13 November 2020; Accepted: 9 December 2020; Published: 11 December 2020

Abstract: The aim of this study was to evaluate the possibility of using several lipid-rich food industry wastes in the culture medium on the growth of *Candida cylindracea* DSM 2031 yeast strain. Four lipid wastes from the food industry: waste fish oil, rancid ghee, waste pork lard, and waste duck processing oil were investigated. It has been shown in the laboratory scale that the above-mentioned wastes can be used to obtain biomass and produce lipolytic enzymes by the tested strain and the *C. cylindracea* extracellular lipase is not constitutive. High yields of biomass (12.84, 12.75, and 12.24 g/dm^3) were obtained in media containing waste duck processing oil, olive oil, and waste pork lard, respectively. The highest lipolytic activity was obtained in the media containing waste fish oil and rancid ghee (0.050 and 0.047 U/cm^3). During 192-h flask cultures the highest extracellular lipase activity and biomass yield were observed in the late logarithmic phase. The study showed that there is a potential for waste management to produce lipolytic enzymes or to produce yeast biomass. The use of waste substrates may contribute to lowering the costs of commercial production, and such a solution is part of the sustainable development strategy.

Keywords: *Candida cylindracea*; lipases; lipid-rich food industry wastes; lipolytic activity

1. Introduction

Waste production is an integral feature of human activity and their proper management is an important problem. Wastes are burdensome for the environment, which is mainly associated with water, air and soil pollution. In 2018, 128 million tonnes of waste was generated in Poland, of which almost 10% was municipal waste and about 22% of the waste came from industrial processing [1]. A very interesting approach to waste management is the use of microorganisms that simultaneously produce many valuable metabolites. Biotechnological production of chemical compounds and biocatalysts in the form of enzymes with the use of waste substrates reduce the costs of obtaining valued metabolites. At the same time, enzymes are biodegradable, and their use in the industry contributes to the reduction of the use of chemical catalysts. The microorganisms described in the literature are both bacteria, moulds, and yeasts, and among them varied species from *Candida* genera.

The genus *Candida* is widespread in natural habitats. Up to 2014, three hundred fourteen species belonging to the genus *Candida* have been described [2]. These yeasts are widespread in the natural environment, especially in humid conditions, rich in organic compounds including organic acids and ethanol. The influence of *Candida* yeast is not without significance for the food industry. Various species are involved in both food and feed production, as well as, can be the cause of their spoilage. They are isolated from dairy products, sourdoughs, meats, and sausages, most often from

local, fermented products and snacks. Because of their uniqueness, *Candida* yeasts can be useful in miscellaneous biotechnological processes and selected enzymes produced by *Candida* yeasts, like lipases from *C. antarctica*, *C. rugosa*, or *C. cylindracea*, are of commercial and industrial interest [2,3].

The latter one, *C. cylindracea* is worth mentioning, but it should be clarified that yeast of the species *C. cylindracea* are often misled as synonymous with the species of yeast *C. rugosa*. Kurtzman and Robnett [4] tested the ribosomal DNA of both of these species and showed that *C. cylindracea* and *C. rugosa* are separate species representing the Ascomycota phylum. Subsequently, Boontham et al. [5] assigned *C. cylindracea* to the genus *Limtongozyma*. *C. cylindracea* was first isolated from soil in Japan in 1962 by Yamada and Machida [6]. The described yeast species was also isolated from the strelitzia flower in French Guiana, from the fruit of *Sapindus* sp. on one of the Hawaiian islands or from undercooked shrimps. They are non-pathogenic yeasts, which prefer aerobic growth and do not grow at 37 °C, therefore they do not pose a threat to human and animal health [3]. For many years, this species has been of great interest due to its ability to produce lipolytic enzymes and its potential use in the production of food and pharmaceuticals [7–11]. The analysis of the GenBank database reveals the known sequences of five triacylglycerol lipases and products of *LIP1*, *LIP2*, *LIP3*, *LIP4*, and *LIP5* genes.

The aim of the study was the possibility of using selected waste lipid substrates as the main carbon source in the culture of *C. cylindracea* DSM 2031. The work consists of two main stages. First—assessment of biomass yield and extracellular lipolytic activity of *C. cylindracea* DSM 2031 in shake flask cultures in media containing lipid-rich food industry wastes as carbon sources and olive oil as a control carbon source. In the second stage, the growth and activity of extracellular lipolytic enzymes of *C. cylindracea* DSM 2031 were evaluated during 192 h of shake culture in media containing waste fish oil.

2. Materials and Methods

2.1. Microorganism

The following yeast strain was used in the study: *Candida cylindracea* DSM 2031 from the German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, DSMZ, Braunschweig, Germany). The mentioned strain has been stored in vials containing cryobeads at −20 °C (Protect Select, Technical Service Consultants Ltd., Heywood, UK).

2.2. Materials

Four types of waste oils and fats were used in the experiments: waste fish oil obtained in the process of smoking fish carcasses from the fish processing plant in Poland; ghee, which went rancid and was purchased at retail in a local supermarket in Warsaw (Poland); waste pork lard obtained from smokehouse company located in Podlaskie Voivodeship (Poland); as well as waste duck processing oil from the household poultry roasting process. Moreover, olive oil also acquired in the local supermarket was used as a control carbon source in yeast cultures. Culture media components were purchased from BTL Sp. z o. o. (Łódź, Poland). Chemicals were purchased from Sigma-Aldrich (Poznań, Poland) and Avantor Performance Materials Poland S.A. (Gliwice, Poland), and *p*-nitrophenyl laurate was synthesized in the Department of Chemistry, Institute of Food Sciences, Warsaw University of Life Sciences-SGGW [12].

2.3. Determination of the Fatty Acid Composition of Oils and Fats Used

Fatty acid compositions of oils and fats were determined after derivatization to methyl esters according to Zieniuk et al. [13] and Agilent 7820A (Agilent Technologies, Santa Clara, CA, USA) with flame ionization detector (FID) was used. The fatty acid profiles of oils and fats are shown in Table 1.

Table 1. Profiles of fatty acids in olive oil and lipid-rich food industry wastes [%].

	Fatty Acid Profiles [%]				
	Olive Oil	**Waste Fish Oil**	**Rancid Ghee**	**Waste Pork Lard**	**Waste Duck Processing Oil**
SFA [1]	14.0	24.3	68.2	42.3	36.6
UFA	85.0	75.6	29.8	55.7	59.4
MUFA	78.2	51.0	27.5	41.9	45.4
PUFA	6.8	24.6	2.3	13.8	14.0

[1] SFA—saturated fatty acids; UFA—unsaturated fatty acids; MUFA—monounsaturated fatty acids; PUFA—polyunsaturated fatty acids.

2.4. Media and Culture Conditions

Inoculum cultures of *C. cylindracea* were carried out in flat-bottom flasks in 100 cm^3 of 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 (the temperature conditions were chosen on the basis of preliminary, which were not shown). In experimental cultures, glucose was replaced with lipid wastes or olive oil in control cultures. After 65 h of the culture biomass yield and extracellular lipase activity were determined.

2.5. Determination of Biomass Yield

Yeasts cells were harvested by centrifugation (8000 rpm, 10 min, 4 °C), washed in distilled water and dried at 105 °C until constant weight.

2.6. Determination of Extracellular Lipase Activity

Extracellular lipase activity of *C. cylindracea* was determined by spectrophotometric measurement of *p*-nitrophenyl laurate hydrolysis [14]. Briefly, yeast culture was centrifuged, then 15 cm^3 of supernatant was added to 0.3 mmol of *p*-nitrophenyl laurate dissolved in 2 cm^3 of heptane. After 15 min of stirring at 37 °C, absorbance was measured at 410 nm in UV/Vis spectrophotometer. Results were expressed as U/cm^3, i.e., units of activity (the enzyme quantity that liberated 1 µmol of *p*-nitrophenol per minute under the assay conditions at 37 °C) per 1 cm^3 of supernatant.

2.7. Determination of Selected Culture Parameters

For the comparison of flask cultures of *C. cylindracea* in media with lipid-rich wastes, the following parameters characterizing the growth and production of lipases were determined: product-biomass yield ($Y_{(P/X)}$), biomass-substrate yield ($Y_{(X/S)}$), specific production rate (q_p), and volumetric productivity (v_p).

2.8. Determination of the Growth Curve of C. cylindracea

Based on the first stage of the experiment, waste fish oil was selected for the next stage—determination of the growth curve of *C. cylindracea* in 192 h culture. Flasks with 100 cm^3 of YP medium (10 g/L yeast extract (Y), 20 g/L peptone (P), pH = 5) with the addition of waste fish oil (20 g/L) were prepared and inoculated with 0.1 cm^3 of the inoculum culture. Cultures were carried out in the same way as in the inoculum and in the first stage of the experiment, i.e., at 28 °C and 150 rpm on IKA KS 4000 ic control shaker (IKA, Königswinter, Germany). The following parameters were determined after 24, 41, 48, 65, 72, 89, 96, 113, 120, 144, 168, and 192 h: biomass yield, extracellular lipolytic activity, pH, and optical density (OD_{600}).

2.9. Measurement of the Yeast Optical Density

The optical density of the yeast cultures was determined by spectrophotometry at a wavelength of 600 nm (OD_{600}), using a Rayleigh UV-1601 spectrophotometer (BRAIC, Beijing, China). For this purpose, 1 cm^3 of the culture medium was taken and centrifuged in Eppendorf miniSpin plus centrifuge (Eppendorf AG, Hamburg, Germany) for 2 min at 10,000 rpm. The yeast biomass pellet was then

suspended in 1 cm^3 of distilled water and the absorbance was measured. Appropriate dilutions of the yeast suspension were made when measuring the optical density of the culture.

2.10. Measurement of the pH of the Culture Medium

The pH values of the cultures were determined using a CP-551 pH meter (Elmetron, Zabrze, Poland).

2.11. Statistical Analysis

Statistical analysis was performed using Statistica 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA). The results were analyzed using one-way analysis of variance (ANOVA) and Tukey's post-hoc test. The significance level was $\alpha = 0.05$.

3. Results and Discussion

3.1. The Use of Lipid-Rich Food Industry Wastes in the Cultivation of C. cylindracea Yeast

Most of the lipases are inductive enzymes. Microorganisms that produce lipases need selected substances that induce the synthesis of these enzymes. A common example of a lipolytic enzyme inducer reported in the literature is olive oil [15,16], which also served as a control carbon source in the current study. Therefore, before starting the cultivation, the analysis of fatty acid profiles was performed in triacylglycerols of waste lipid substrates and in olive oil. The results of the analyses are presented in Table 1.

It has been shown that the fatty acid compositions in wastes are diversified. Lipid wastes were characterized by higher content of saturated fatty acids (SFA) than olive oil, and the highest content of SFA was found in ghee (68.2%). Waste pork lard, duck oil, and fish oil were composed of 42.3, 36.6, and 24.3% of SFA, respectively. Olive oil contained the highest amount of unsaturated fatty acids (UFA)—85%, and fish oil had a similar content (75.6%), where one third of UFA were polyunsaturated fatty acids (PUFA).

High yields of biomass (12.84, 12.75, and 12.24 g/dm^3) were obtained in media containing waste duck processing oil, olive oil, and waste pork lard, respectively (Figure 1). In the medium where the carbon source was waste fish oil, biomass yield was 10.58 g/dm^3. The lowest biomass yield was obtained in the medium containing ghee (4.93 g/dm^3) and in the YP medium (1.89 g/dm^3).

Growth of yeast in the YP medium, despite the lack of the addition of a carbon source, was possible because peptides contained in peptone and yeast extract were used to build cells and for energy processes. Although peptone and yeast extract are added to the medium to provide amino acids, vitamins and minerals, yeast also uses these substrates for energy purposes and as building components, but it is an insufficient source of carbon, therefore in YP medium the lowest yield of biomass was observed.

Figure 2 shows the dependence of extracellular lipolytic activity on the waste carbon source used in the medium in yeast culture. YP medium (containing no lipid carbon source) served as a control to determine the activity of constitutively produced lipolytic enzymes, regardless of the presence of hydrophobic molecules.

The highest lipolytic activity was obtained in the media containing waste fish oil and rancid ghee (0.050 and 0.047 U/cm^3). Almost two times lower activities were obtained in the media with the other substrates, i.e., 0.028 U/cm^3 (waste pork lard), 0.027 U/cm^3 (olive oil), and 0.024 U/cm^3 (waste duck oil). No lipolytic activity was observed in the YP medium. The obtained results suggest that the lipases produced by *C. cylindracea* are not constitutive enzymes as there was observed a need of lipid carbon source presence in the culture medium. Moreover, no relationship was found between extracellular lipolytic activity and biomass yield. Waste fish oil and rancid ghee were characterized by the most diverse fatty acids profile (Table 1). Waste fish oil contained 24.3% of SFA (almost the lowest amount apart from olive oil) and the highest amount of PUFA (24.6%). On the other hand, rancid ghee showed the highest SFA and the lowest PUFA concentration, but still, both waste lipids influenced the secretion of lipolytic enzymes to the greatest extent.

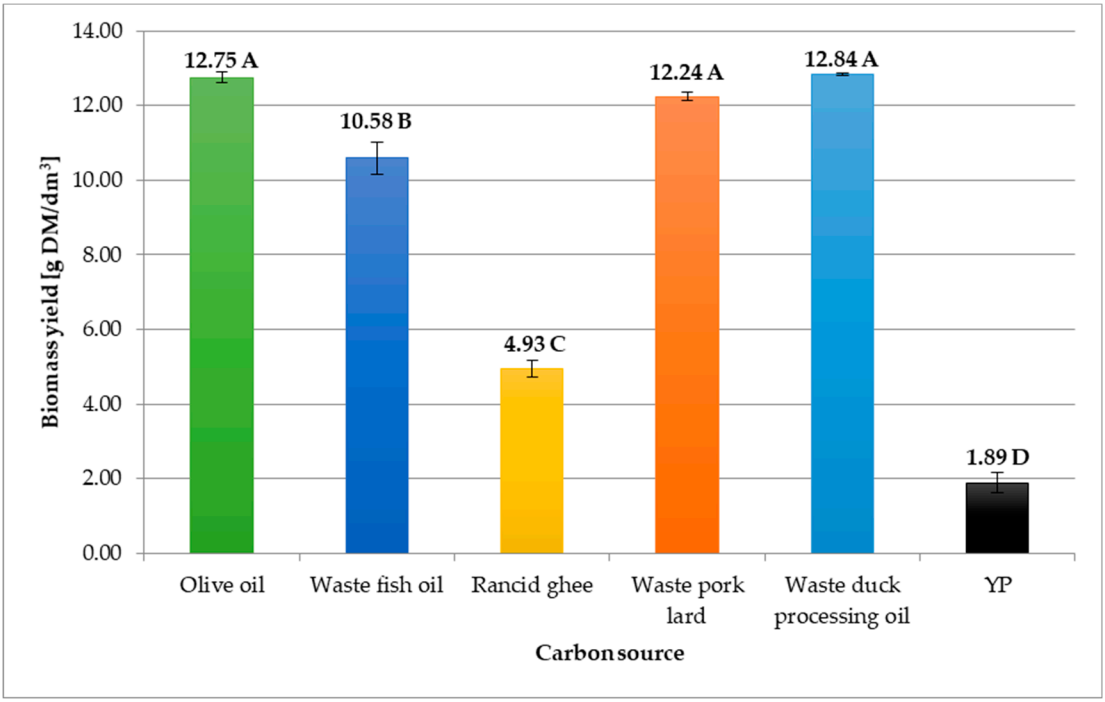

Figure 1. Effect of lipid-rich wastes on the biomass yield of *C. cylindracea* DSM 2031 after 65 h cultures. YP—control medium (10 g/L yeast extract (Y), 20 g/L peptone (P), pH = 5). Means with the same letter did not differ significantly ($\alpha < 0.05$).

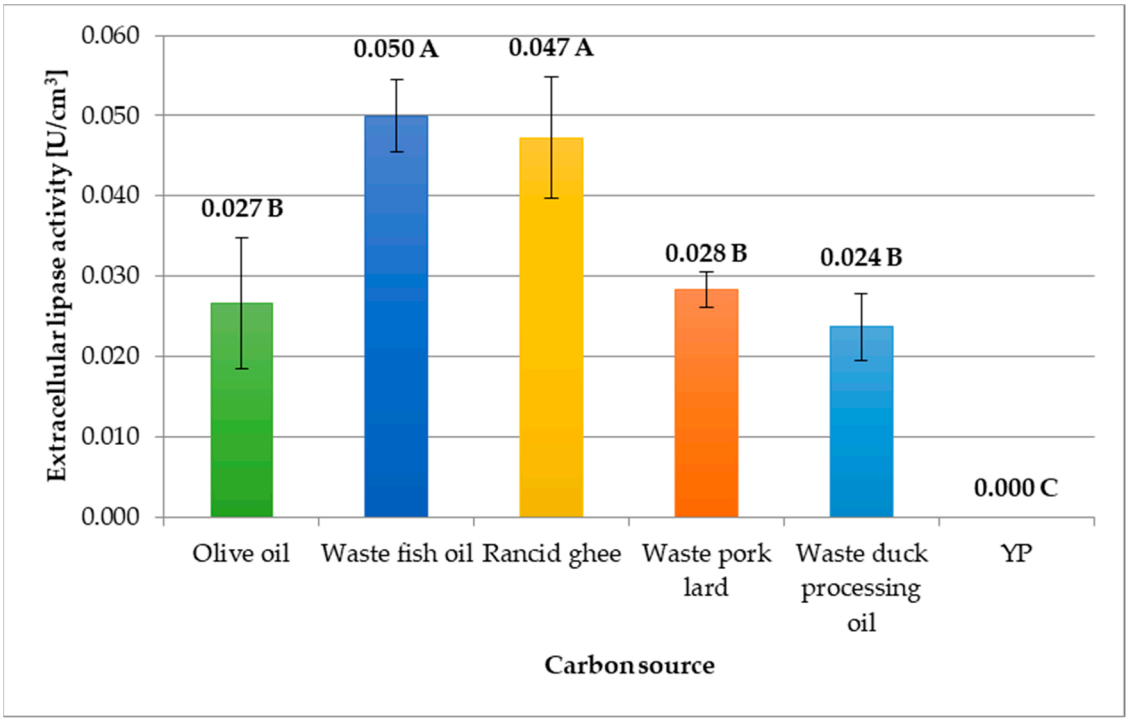

Figure 2. Effect of lipid-rich wastes on the extracellular lipase activity of *C. cylindracea* DSM 2031 after 65 h cultures. YP—control medium (10 g/L yeast extract (Y), 20 g/L peptone (P), pH = 5). Means with the same letter did not differ significantly ($\alpha < 0.05$).

Ghee, pork lard, and waste duck oil contained a relatively high amount of saturated fatty acids, which determined their solid or semi-solid consistency, unlike other fats. The aggregate state of fats may be of significant importance in the cultivation of microorganisms, because their solid form

is not conducive to homogeneous distribution in a liquid medium, and thus reduces the contact surface of cells or their enzymes with the substrate, and consequently, fat hydrolysis and assimilation may be difficult. Similar observations were made by Tan and Gill [17] who showed that the higher content of saturated fatty acids in fats, the more of them was left unused by fungi. On the other hand, Papanikolaou and Aggelis [18] showed that *Yarrowia lipolytica* exhibited growth and accumulation of intracellular lipids when cultivated on stearin as a carbon source, which is a stearic acid-rich waste, but emulsifiers and use of disperser were necessary for better fat dispersion in the aqueous phase.

Sokolovska et al. [15] conducted an experiment comparing the growth and lipolytic activity of the yeast *C. cylindracea* CBS 6330 (DSM 2031) in media with oleic acid or olive oil. In both cultures, comparable lipolytic activities were obtained, however, they significantly differed in the obtained biomass yields. The authors presumed that the discussed species of yeast prefers glycerol released by triacylglycerols hydrolysis as a carbon source rather than free fatty acids [15]. *C. cylindracea* yeasts were able to assimilate lipid wastes contained in the media, as evidenced by the significantly higher yields of biomass compared to the control culture in the YP medium. Other studies indicate that *C. cylindracea* lipases exhibit greater substrate specificity for triacylglycerols in which the fatty acid residues contain from 14 to 18 carbon atoms. However, there is no preference for the degree of triacylglycerols saturation [19].

Fabiszewska and Białecka-Florjańczyk [20] revised and compared the results of different authors related to the extracellular lipolytic activity of the *Y. lipolytica* non-conventional yeast species and concluded that the lipase secretion is a resultant of at least several factors and it is most likely mistaken to correlate it only with the oleic acid content of the plant oil used as a carbon source in culture medium [20]. The authors agree with the hypothesis, but further investigation should be taken including *C. cylindracea* species.

The presented results of the experiments showed that it is possible to use lipid wastes in the cultivation of *C. cylindracea*. Goncalves et al. [21] in their research tried to valorize olive mill wastewater. The following yeast strains were selected for the experiment: *Candida rugosa* PYCC 3238, *C. rugosa* CBS 2275, *C. cylindracea* CBS 7869, *Yarrowia lipolytica* W29, *Y. lipolytica* CBS 2073, and *Y. lipolytica* IMUFRJ 50682. All the strains used showed lipolytic activity in the medium with the mentioned substrate, and the most effective lipase producer turned out to be *C. cylindracea* CBS 7869 [21].

For better comparison of flask cultures of *C. cylindracea* in media with lipid-rich wastes, culture parameters such as product-biomass yield ($Y_{(P/X)}$), biomass-substrate yield ($Y_{(X/S)}$), specific production rate (q_p), and volumetric productivity (v_p) were determined (Table 2).

Table 2. Selected culture parameters of *C. cylindracea* in media with lipid-rich food industry wastes as carbon sources.

	$Y_{(P/X)}$ [U/g] [1]	$Y_{(X/S)}$ [g/g]	q_p [U/g × h]	v_p [U/L × h]
Olive oil	2.09	0.64	0.03	0.41
Waste fish oil	4.72	0.53	0.07	0.77
Rancid ghee	9.57	0.25	0.15	0.73
Waste pork lard	2.31	0.61	0.04	0.44
Waste duck processing oil	1.85	0.64	0.03	0.36
YP	0.00	0.09	0.00	0.00

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

Obtained results indicated that waste fish oil was the best carbon source for simultaneous production of high biomass yield and high lipolytic activity. In case of product-biomass yield higher value was found for rancid ghee, due to the lower biomass yield. Values of biomass-substrate yield for olive oil, waste fish oil, waste pork lard, and waste duck processing oil were comparable and ranged 0.53–0.64. Specific production rate and volumetric productivity were also highest for cultures with waste fish oil or ghee as carbon sources. Despite similar v_p values in cultures with ghee and fish oil and a higher $Y_{(P/X)}$ value in culture with ghee as a carbon source, the waste fish oil was applied in

the next stage of the experiment due to the better use of the waste for the simultaneous production of both biomass and enzymes. An important factor in the microbiological disposal of waste should be its maximized use, i.e., the processing of more lipid waste into yeast biomass and other valuable metabolites that can be used then in different industries.

3.2. Investigation of Changes in Biomass Yield, Lipolytic Activity, and pH Value During the Culture of C. cylindracea in a Medium with Waste Fish Oil

Obtaining a relatively high yield of biomass and enzymatic activity in the medium with waste fish oil allowed for the next part of the experiment, where it was decided to check and determine the optimal time for culturing *C. cylindracea* DSM 2031 to obtain the highest possible extracellular lipolytic activity.

The determination of the growth curve of *C. cylindracea* was based on the determination of biomass yield, extracellular lipolytic activity, pH and optical density. The results of 192-h culture are shown in Figures 3 and 4.

The highest yield of biomass (16.06 g/dm^3), as well as OD_{600} were obtained in 120 h of shake culture and the resulting biomass yield and optical density curves were very comparable. During the first day of culture, the cells adapted to the environmental conditions, and then a logarithmic growth phase started, in which yeast cells were dividing intensively, and the biomass yield increased. After 120 h of cultivation, the yeast clearly slowed down their growth, which was related to entering the stationary phase.

An increase in pH value was also noted during the cultivation (Figure 4). A sudden increase in pH was observed during the first two days from 4.85 to 6.40, and then the medium environment was relatively stable and finally, pH of 7.25 was obtained. According to Vylkova et al. [22] the increase in pH occurs in media with the presence of exogenous amino acids and in the current study peptone and yeast extract as sources of proteins and amino acids were used. Amino acids are metabolized and used for cell growth, and the resulting products of nitrogen metabolism, e.g., in the form of ammonia, alkalize the culture environment [22].

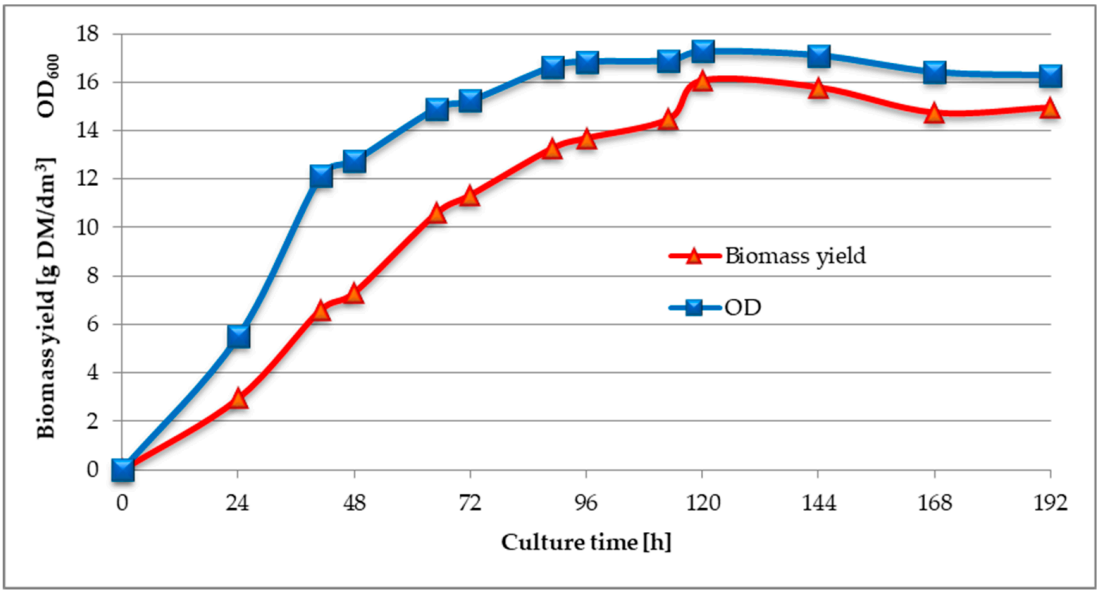

Figure 3. Changes in biomass yield and optical density (OD_{600}) during 192-h culture of *C. cylindracea* DSM 2031 in a medium with waste fish oil.

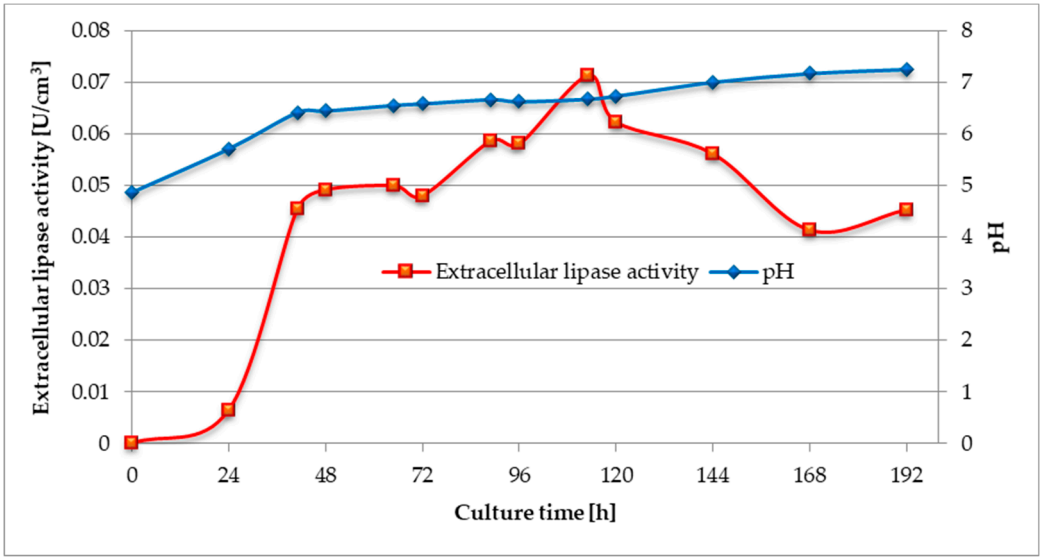

Figure 4. Changes in extracellular lipase activity and pH value during 192-h culture of *C. cylindracea* DSM 2031 in a medium with waste fish oil.

The extension of the cultivation time also had a positive effect on lipolytic activity. The highest lipase activity was obtained at 113 h of cultivation (0.07 U/cm^3) in the late logarithmic phase, which indicates the need for longer cultures in order to obtain more biomass and extracellular enzymes, as well as more efficient waste management. The obtained activity was 1.4-fold higher than reported in the first stage of the experiment (0.05 U/cm^3). Similar observations have been reported in the literature, both Sokolovska et al. [15] and Krastanov et al. [23] observed that the highest lipase activity is obtained near the end of the logarithmic phase and alike to the cited papers, lipolytic activity decreased in the stationary phase. The results presented in the paper are also in accordance with Fabiszewska et al. [24] who observed a different pattern of extracellular and cell-bound lipase production, which was the highest in the early exponential phase of model non-conventional yeast species *Yarrowia lipolytica*. The extracellular lipase activity increased in the late exponential phase due to the lower accumulation of lipase molecules in cell walls [24]. The observations seemed to be similar for both species.

The production of lipases by *Candida cylindracea* has also been investigated by other scientists. Salihu et al. [25] optimized production of lipase by *C. cylindracea* ATCC 14830 in shake flask cultures with palm oil mill effluent (POME) as a basal medium. They suggested that amounts of peptone, Tween-80, and inoculum significantly influenced extracellular lipase production. Scientists provided also that statistical experimental design allowed to utilize POME along with lipase production on a considerable level, and compared to the unoptimized medium, use of 0.45% (*w/v*) peptone, 0.65% (*v/v*) Tween-80, and 2.2% (*v/v*) inoculum resulted in 5.19-fold higher activity of lipolytic enzymes [25].

A statistical approach was also applied by Muralidhar et al. [26]. The central composite design was employed to optimize five components of the medium. Besides yeast extract, malt extract, peptone, and Tween-80, two different carbon sources—glucose and olive oil—were investigated. In optimized media, similarly to the current research, biomass yield and lipase activity increased until 4–5-day of the cultivation. Significantly higher enzymatic activity was achieved in medium with olive oil as a carbon source, what was proved also were results of the current study, where the presence of lipid molecules in the medium affect the lipase production.

4. Conclusions

In the current study, the ability of *C. cylindracea* DSM 2031 to utilize several lipid wastes from the food industry on a laboratory scale was assessed. Lipid-rich wastes can serve as a valuable source of carbon in the media used for the cultivation of lipolytic microorganisms. Thus, there is a potential for

their use for the production of lipolytic enzymes or the production of yeast biomass for animal feed. The use of waste substrates can contribute to lowering the costs of commercial production, and such a solution is part of the sustainable development strategy. The presented work is preliminary research and it is justified to conduct further research on the use of waste materials in the microbiological synthesis of enzymes in order to obtain higher yields and possibly the application of such technology on an industrial scale.

Author Contributions: Conceptualization, B.Z., A.F. and P.M.-Z.; methodology, B.Z., P.M.-Z. and A.F.; formal analysis, B.Z. and P.M.-Z.; investigation, B.Z. and P.M.-Z.; writing—original draft preparation, P.M.-Z. and B.Z.; writing—review and editing, B.Z., A.F. and P.M.-Z.; visualization, B.Z.; supervision, A.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Domańska, W. *Environment 2019*; Statistics Poland: Warsaw, Poland, 2019; pp. 148–160.
2. Hommel, R.K. CANDIDA Introduction. In *Encyclopedia of Food Microbiology*, 2nd ed.; Batt, C.A., Tortorello, M.L., Eds.; Academic Press: Cambridge, MA, USA, 2014; pp. 367–373.
3. Lachance, M.A.; Boekhout, T.; Scorzetti, G.; Fell, J.W.; Kurtzman, C.P. Candida Berkhout (1923). In *The Yeast, a Taxonomic Study*, 5th ed.; Kurtzman, C.P., Fell, J.W., Boekhout, T., Eds.; Elsevier: Amsterdam, The Netherlands, 2011; pp. 978–1278.
4. Kurtzman, C.P.; Robnett, C.J. Indentification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Anton. Leeuw.* **1998**, *73*, 331–371. [CrossRef] [PubMed]
5. Boontham, W.; Angchuan, J.; Boonmak, C.; Srisuk, N. *Limtongozyma siamensis* gen. nov., sp. nov., a yeast species in the Saccharomycetales and reassignment of *Candida cylindracea* to the genus *Limtongozyma*. *Int. J. Syst. Evol. Microbiol.* **2020**, *70*, 199–203. [CrossRef] [PubMed]
6. Yamada, K.; Machida, H. Studies on the production of lipase by microorganisms. *Nippon Nogeikagaku Kaishi* **1962**, *36*, 858–860. [CrossRef]
7. Kim, B.S.; Hou, C.T. Production of lipase by high density fed-batch culture of *Candida cylindracea*. *Bioprocess Biosyst. Eng.* **2006**, *29*, 59–64. [CrossRef] [PubMed]
8. Zalcain, I.; Zapalena, M.J.; Astiasaran, I.; Bello, J. Dry-fermented Sausages Elaborated with Lipase from *Candida cylindracea*. Comparison with Traditional Formulations. *Meat Sci.* **1995**, *40*, 55–61. [CrossRef]
9. Belkacemi, F.Z.; Merabet-Khelassi, M.; Aribi-Zouioueche, L.; Riant, O. Productionof l-mentyl acetate through kinetic resolution by *Candida cylindracea* lipase: Effect of alkaloids as additives. *Res. Chem. Intermed.* **2018**, *44*, 6847–6860. [CrossRef]
10. Alcântara, T.D.A.P.; Oliveira, J.M.; Evangelista-Barreto, N.S.; Marbac, P.A.S.; Cazetta, M.L. Aerobic decolorization of azo dye orange g by a new yeast isolate *Candida cylindracea* SJL6. *Biosci. J.* **2017**, *33*, 1340–1350. [CrossRef]
11. Tang, A.; Zhang, Y.; Wei, T.; Wu, J.; Li, Q.; Liu, Y. Immobilization of *Candida cylindracea* Lipase by Covalent Attachment on Glu-Modified Bentonite. *Appl. Biochem. Biotechnol.* **2019**, *187*, 870–883. [CrossRef]
12. 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]
13. Zieniuk, B.; Wołoszynowska, M.; Białecka-Florjańczyk, E. Enzymatic synthesis of biodiesel by direct transesterification of rapeseed cake. *Int. J. Food Eng.* **2020**, *16*, 3. [CrossRef]
14. 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]
15. Sokolovska, I.; Albasi, C.; Riba, J.P.; Bales, V. Production of extracellular lipase by *Candida cylindracea* CBS 6330. *Bioprocess Biosyst. Eng.* **1998**, *19*, 179–186. [CrossRef]
16. Fickers, P.; Marty, A.; Nicaud, J.M. The lipases from *Yarrowia lipolytica*: Genetics, production, regulation, biochemical characterization and biotechnological applications. *Biotechnol. Adv.* **2011**, *29*, 632–644. [CrossRef] [PubMed]
17. Tan, K.H.; Gill, C.O. Batch growth of *Saccharomycopsis lipolytica* on animal fats. *Appl. Microbiol. Biotechnol.* **1985**, *21*, 292–298. [CrossRef]

18. Papanikolaou, S.; Aggelis, G. Selective uptake of fatty acids by the yeast *Yarrowia lipolytica*. *Eur. J. Lipid Sci. Technol.* **2003**, *105*, 651–655. [CrossRef]
19. Lie, O.; Lambertsen, G. Fatty Acid Specificity of *Candida cylindracea* Lipase. *Eur. J. Lipid Sci. Technol.* **1986**, *88*, 365–367. [CrossRef]
20. 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]
21. Goncalves, C.; Lopes, M.; Ferreira, J.P.; Belo, I. Biological treatment of olive mill wastewater by non-conventional yeasts. *Bioresour. Technol.* **2009**, *100*, 3759–3763. [CrossRef]
22. 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]
23. Krastanov, A.; Govindarajan, A.; Daniel, D. Studies on lipase fermentation using *Candida cylindracea* NRRL Y-175061N a stirred tank bioreactor. *Bulg. J. Agric. Sci.* **2008**, *14*, 290–299.
24. Fabiszewska, A.U.; Stolarzewicz, I.; Zamojska, W.; Białecka-Florjańczyk, E. Carbon source impact on *Yarrowia lipolytica* KKP 379 lipase production. *Appl. Biochem. Microbiol.* **2014**, *50*, 404–410. [CrossRef]
25. Salihu, A.; Alam, Z.M.; AbdulKarim, M.I.; Salleh, H.M. Optimization of lipase production by *Candida cylindracea* in palm oil mill effluent based medium using statistical experimental design. *J. Mol. Catal. B Enzym.* **2011**, *69*, 66–73. [CrossRef]
26. Muralidhar, R.V.; Chirumamila, R.R.; Marchant, R.; Nigam, P. A response surface approach for the comparison of lipase production by *Candida cylindracea* using two different carbon sources. *Biochem. Eng. J.* **2001**, *9*, 17–23. [CrossRef]