Research Article

Reduction of Chemical and Biological Oxygen Demands from Oil Wastes via Oleaginous Fungi: An Attempt to Convert Food by Products to Essential Fatty Acids

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Background: The production of waste pollutants has become a major problem for many food and oil industries. However, oil wastes can provide alternative substrates for industry, which could help to solve environmental pollution problems. Furthermore, oil wastes can be used as substrates to produce unsaturated fatty acids, which are important for health.
Objectives: The production of fatty acids in fungi using oil wastes and renewable substrates were investigated.
Material and Methods: Oil waste sources were obtained from food factories and restaurants (F1, F2, F3, R1, and R2). *Cunninghamella echinulata* DSM1905 and *Rhizopus stolonifer* DSM2194 were used to treat the wastes. Changes in lipid and fatty acid contents, biomass, and pH were monitored.
Results: *C. echinulata* produced about 18.4 and 20.1% gamma linolenic acid (GLA) from the R1 and R2 oil wastes, respectively. It also produced 9.3% and 12.4% linolenate from the F2 and F3 wastes. *R. stolonifer* produced 21% GLA from R1 and 9.3% linolenate from F3. *C. echinulata* reduced biological oxygen demand (BOD) and chemical oxygen demand (COD) by 67%-74% and 50%-98%, respectively. *R. stolonifer* reduced BOD by 36%-74% and COD by 10%-78%.
Conclusions: This study emphasized the abilities of oleaginous fungi to utilize oil wastes as carbon sources to reduce BOD and COD of the wastes, producing essential fatty acids.

Keywords: BOD; COD; Fatty acid; Oil Waste

1. Background

Polyunsaturated fatty acids (PUFAs) are used as food and pharmaceutical additives for different biological activities. Omega-3 and -6 PUFAs are used to treat many diseases. Gamma linolenic acid (GLA) has beneficial impacts on health. Dihomogamma linolenic acid (C20:3) and arachidonic acid (C20:4) originate from GLA in n-6 pathway. These PUFAs are converted to eicosanoids, such as leukotrienes and prostaglandins, in humans. Omega-6 PUFAs have nutraceutical and medical applications in reduction of blood lipid levels, inflammation, and atherosclerosis. The lack of omega-6 PUFAs in the diet causes dry hair, hair loss, and poor wound healing (1). The role of docosahexaenoic acid (DHA) and other omega-3 PUFAs in improving cardiovascular diseases has been shown in many *in vivo* and *in vitro* experiments (2). Both eicosapentaenoic acid and DHA can improve blood pressure and prevent the development of hypertension (3). Some studies have converted renewable waste sources to edible products, such as enzymes, organic acids, carbohydrates, biodiesel, microbial lipids, and other products (4-10). Vegetable and animal oils can be replaced by microbial oil. Fungi can produce valuable oils containing PUFAs. Recently, microbial lipids have been validated as nutritious for infants (11-12). Several microorganisms, including *Cunninghamella echinulata*, *Mucor rouxii, Mortierella alpina*, *Mucor circinelloides, Mortierella isabellina*, *Cryptococcus curvatus, Crypthecodinium cohnii*, and *Yarrowia lipolytica,* produce popular high lipid products (13-17). They use a broad range of common substrates, such as glucose, xylose, lactose, starch, and cellulose, as well as some commercially important industrial and organic wastes. Various oils are used during food preparation in restaurants and factories and particularly in fast food. Oil wastes from oil factories and fried products cause serious environmental problems. For example, oil waste can increase biochemical oxygen demand (BOD) and chemical

oxygen demand (COD) in water resources, resulting in severe water pollution. Microbial degradation of oil wastewater has been investigated in earlier studies (5, 18, 19). Papanikolaou *et al*. (2007) used olive mill wastewater-based media to produce citric acid. Laconi *et al*. (2007) utilized the same substrate to culture a mixture of fungi to obtain a potentially useful microbial biomass and induce partial bioremediation of a particular waste. In present study, the efficacy of bioconversion various oil wastes to lipids via two oleaginous fungi, namely *Cunninghamella echinulata* DSM1905 and *Rhizopus stolonifer* DSM2194, were analyzed on Microscopic observations of the fungi showed the existence of lipid globules. Changes in total lipids, biomass, fatty acids, and pH were examined in a time course. The ability of these fungi to degrade oil wastes were examined by measuring BOD and COD.

2. Objectives

Although the capacity of oleaginous fungi to convert lipids and fatty acids has been investigated, the effects of lipid metabolism by fungi on changes in BOD and COD have not been evaluated. In this study, the ability of oleaginous fungi to utilize oil wastes as carbon sources to reduce BOD and COD of the wastes were investigated. Our results may be useful to investigate the abilities of *C. echinulata* and *R. stolonifer* to produce value-added lipids.

3. Materials and Methods

3.1. Chemicals

All chemicals were obtained from Sigma-Aldrich with analytical grade. The oil wastes from different industries including restaurants and fried food factories were collected in Isfahan, Iran. The samples were abbreviated as F1 and F2 for waste oils from fried food factory of Shilan Kish, F3 for the sample from Naz plant oil factory, and R1 and R2 from two different fast food restaurants.

3.2. Fungal Culture

Cunninghamella echinulat DSM1905 and *Rhizopus stolonifer* DSM 2194 were purchased from the German Collection of Microorganisms (DSMZ, Braunschweig, Germany). Preparation of media was carried out according to method described by Papanikolaou *et al*. (1). The inoculated culture medium contained (perliter of distilled water) 7.0 g KH_2PO_4, 2.5 g Na_2HPO_4, 1.5 g $MgSO_4$, 0.06 g $MnSO_4$, 0.15 g $CaCl_2$, 0.15 g $FeCl_3$, 0.5 g yeast extract, 0.5 g $(NH_4)_2SO_4$, pH 6.0, and 2% (V/V) oil waste as a carbon source. The media were inoculated by spores (1×10^7) and cultivated at 20ºC for 72 h with shaking at 180 rpm.

Cell dry weight was measured 72 h post inoculation, and mean values from 3 replicates were used for further analysis (14). *C. echinulat*a DSM1905 and *R. stolonifer* DSM 2194 were passed through Whatman No. 1 filter paper and washed with a large amount of distilled water. To remove unconsumed-oil, an additional washing was carried out using ethanol. The cells were dried down at 105ºC. The dry cell weight was measured gravimetrically.

3.3. Microscopic Observation of Lipid Droplets

Samples were obtained from production media after 72 h and stained by Sudan Black B, according to the method developed by Zhou (18) and visualized under light microscope.

3.4. Extraction and Modification of Lipids

Lipid extraction was performed according to the modified procedure of Bligh and Dyer (1959). Briefly, mycelia were obtained through Whatman No.1 filter paper, washed three times by distilled water and once by ethanol. HCl solution (10 mL of 4M) was added to 1 g mycelia, and incubated at 60ºC for 2 h. The hydrolyzed solution was shaken with 20 mL of chloroform/methanol (1:1) at room temperature for 3 h, and centrifuged at 2000 ×g for 5 min. The upper aqueous phase was separated by a Pasteur pipette and the lower phase containing lipids was evaporated under reduced pressure for 10 min (19).

The extracted fatty acids were modified to fatty acid methyl esters (FAMEs) according to the method of Christie (20). Lipid (50 mg) was dissolved in 1 mL toluene in a test tube fitted with a condenser. Sulfuric acid (2 mL) in methanol (1% v/v) was added to the lipid mixture and maintained at 50ºC for 24 h. The methylated fatty acids were separated from the aqueous layer by adding 5 mL of NaCl (5%) and dissolved in *n*-hexane. The separated *n*-hexane layer washed by 4 mL of water containing potassium bicarbonate (2%) and dried over anhydrous sodium sulfate. Solution is filtered and the solvent removed under reduced pressure in a rotary film evaporator.

3.5. FAMEs analysis by Gas Chromatography (GC)

GC was carried out using Agilent 19091J-413 Series Gas Chromatograph equipped with a FID and the capillary column DB-23 (USA). The temperatures of injector and detector were maintained at 260 and

300ºC, respectively. The oven was programmed as 100ºC: 2 min; 160°C: 3 min; 215ºC: 2 min; 217ºC: 2 min; 218ºC: 2 min;, and 260ºC for 2 min. Nitrogen was used as the carrier gas at a flow rate of 1.5 mL/min. The injection volume was 1 µL, with a split ratio of 50:1. The standard FAMEs (Sigma-Aldrich) including C18:2 (Linoleic acid), C18:3 (GLA), C18:3 (alpha-linolenic acid), C20:4 (ARA), C20:5 (EPA), C22:6 (DHA) were assayed by GC and the standard elution profile was obtained. The Production of FAMEs from the extractions of *C. echinulata* DSM1905 and *R. stolonifer* DSM 2194 were assayed by comparing retention time from extracts to those of standard FAMEs.

3.6. BOD and COD Determinations

BOD was determined using the modified iodometric method and COD was determined by the standard closed reflux method according to the procedures described by Clesceri *et al.* (1998). The BOD and COD degradation efficiency was defined as the reducing amount of BOD and COD versus the amount of initial values (21).

3.7. Statistical Analysis

All the experiments were performed in triplicate, and data presented as means ± SE. A one-way ANOVA was used toevaluatewhethertherewasastatistically significant difference between data. Statistical analysis was performed using SPSS 15.0 (SPSS Inc., USA), and tests were considered significant at $p < 0.05$.

4. Results

4.1. Cell Culture and Lipid Assay

The fungal strains of *C. echinulata* DSM 1905 and *R. stolonifer* DSM2194 were cultured under nitrogen-limited conditions in flasks containing five different oil wastes (2%), and the dry cell weights were measured gravimetrically. Oil particles were observed in both strains and appeared as black droplets after Sudan Black B staining (Figure 1).

All oil wastes were subjected to assays for total lipids, biomass, and pH at the indicated time intervals (Figure 2). The maximum yields of all oil wastes were achieved 72 h after inoculation.

4.2. Fatty Acid Determination and Gas Chromatography (GC)

A fatty acid analysis of cellular lipids was performed in both strains. The fungal cells were disrupted, and the lipids were extracted to assay the types and quantities of lipids. The lipid-containing fractions were separated and modified to obtain fatty acid methyl esters (FAMEs). The FAMEs were subjected to GC analysis. The GC results were analyzed based on the standard peaks obtained from FAME standards (Table 1).

Both *R. stolonifer* and *C. echinulata* produced lipids. The yield of *C. echinulata* lipid production was about 65% in F3 oil waste medium, but in F1, F2, and R1 wastes was less than that of *R. stolonifer*. Linoleate and GLA were the typical omega-6 fatty acids produced by the two strains in all oil waste media. *C. echinulata* cultured in F3 oil waste medium demonstrated maximum linoleate content (46.7%), but minimum GLA content (0.63%) of total FAMEs. Linolenic acid was produced by both strains in F3 and R1, and DHA appeared in F1 for the two strains. ARA concentration varied in relation to media and strain. Initial pH was 6.1, but decreased to 3.9 after 72 h and remained at this value until 192 h (Figure 2).

4.3. BOD and COD Assay

BOD and COD in all oil wastes decreased significantly by both strains. The changes in BOD over 5

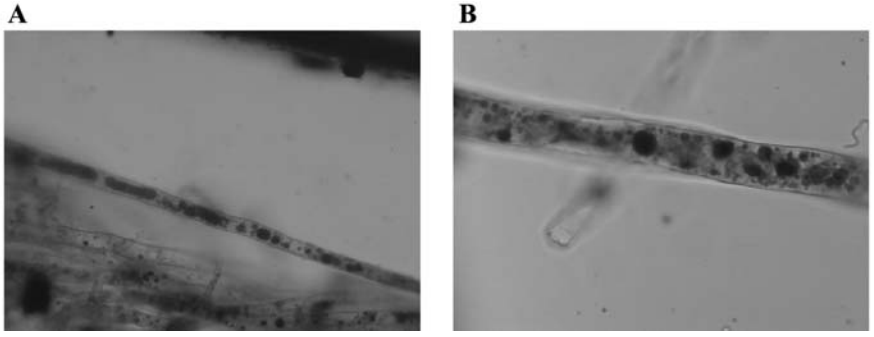

Figure 1. Mycelium with lipid globules by staining using Sudan Black B, A: *Cunninghamella echinulata* DSM1905 and B: *Rhizopus stolonifer* DSM2194

Table 1. Comparison of lipid, biomass, yields (%) lipid/ biomass w/w), omega 6 and omega 3 production (mg.L^{-1}) and BOD and COD reduction (%) by two strains in medium contains different oil wastes

***Cunninghamella echinulata* DSM1905**								
				Omega 6/3 concentrations in total lipid (mg.L^{-1})				
Oil waste In media	**Biomass (g.L^{-1})**	**Total lipid (g.L^{-1})**	**Yeild (w/w %)**	**C18:2 Linoleate (n-6 %)**	**C18:3 (GLA) (n-6 %)**	**C 18:3 Linolenate (n-3 %)**	**C20:3 ARA (n-6 %)**	**C22:6 DHA (n-3 %)**
F1	12.3	4.2	34	36.1	11.42	-	8.9	8.37
F2	13.78	5.1	37	24.2	9.4	8.3	-	-
F3	9.69	6.3	65	48.06	5.1	12.01	-	-
R1	13.63	4.5	33	32.5	18.4	-	-	-
R2	13.71	4.8	35	30/01	20.1	-	3.4	-
***Rhizopus stolonifer* DSM 2194**								
				Omega 6/3 concentrations in total lipid (mg.L^{-1})				
Oil Waste In media	**Biomass (g.L^{-1})**	**Total lipid (g.L^{-1})**	**Yeild (w/w %)**	**C18:2 Linoleate (n-6 %)**	**C18:3 (GLA) (n-6 %)**	**C 18:3 Linolenate (n-3 %)**	**C20:3 ARA (n-6 %)**	**C22:6 DHA (n-3 %)**
F1	10.38	5.4	52	34.2	3.4	19.9	12.1	11.3
F2	11.04	4.97	45	51.2	19.9	12.4	6.3	-
F3	10.54	5.8	55	52.5	3.8	9.3	-	-
R1	11.35	5.9	52	58.9	21.1	21.1	7.3	-
R2	16.92	4.23	25	22.1	21.6	2.1	-	-

days are shown in (Figure 3).

Figure 4 shows the reduction of BOD_5 in all oil wastes, and the final BODs were 67%-75% and 37%-55% of the initial values for *C. echinulata* and *R. stolonifer,* respectively. About 98% reduction in COD was achieved by *C. echinulata* in F5 and other decreases in COD were remarkable. In contrast, *R. stolonifer* decreased COD in F2, F3, and R2 (Figure 5).

5. Discussion

Oil wastes from factories and restaurants may provide suitable carbon sources for microorganisms to produce lipids. Sewage sludge has been used to produce oil by Papanikolaou and Aggelis (2002). Fungi and yeasts can grow on waste products, such as glycerol, which is a biodiesel byproduct (22). Hydrophilic materials and molasses have been applied to single *Cunninghamella echinulata* cells to make oil (23). Tomato hydrolyses waste was used by this fungi to produce fatty acids (15). Food waste, olive oil mill wastewater, industrial fats, whey, and starch hydrolysates are other wastes that have been used by oleaginous fungi to produce lipids (24-27). The filamentous fungus *Mortierella alliacea* collects arachidonic acid, particularly in the form of triglycerides, in its mycelia and yields about 46.1 g.L^{-1} dry biomass. In the present study, oil wastes were obtained from food factories and restaurants. Lipid and FAME analyses of *C. echinulata* and *R. stolonifer* showed that these wastes were good substrates to produce unsaturated fatty acids, such as omega-3 and omega-6 PUFAs. The best results were found when R1 and R2 oil wastes were used as the carbon sources, as about 19-21% GLA was obtained from the R1 and R2 wastes by *C. echinulata*, respectively. Our results showed when linoleate and linolenate increased, GLA concentration decreased, particularly when using R2 and F3 oil wastes as substrates. Our data confirmed the results of Fakes *et al*. (2008) who determined lipids from *C. echinulata* with emphasis on the γ-linolenic acid distribu-

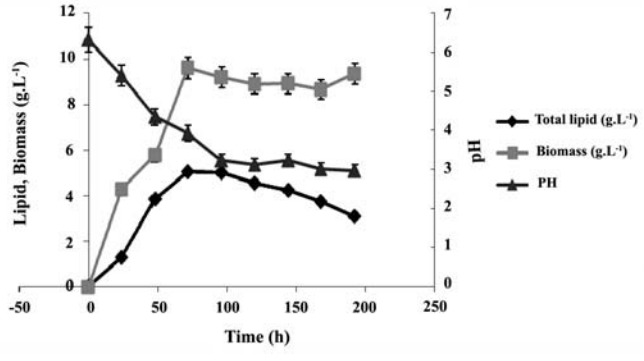

Figure 2. The changes of total lipid (g.L^{-1}), biomass (g.L^{-1}), and pH during the time (0-192 h)

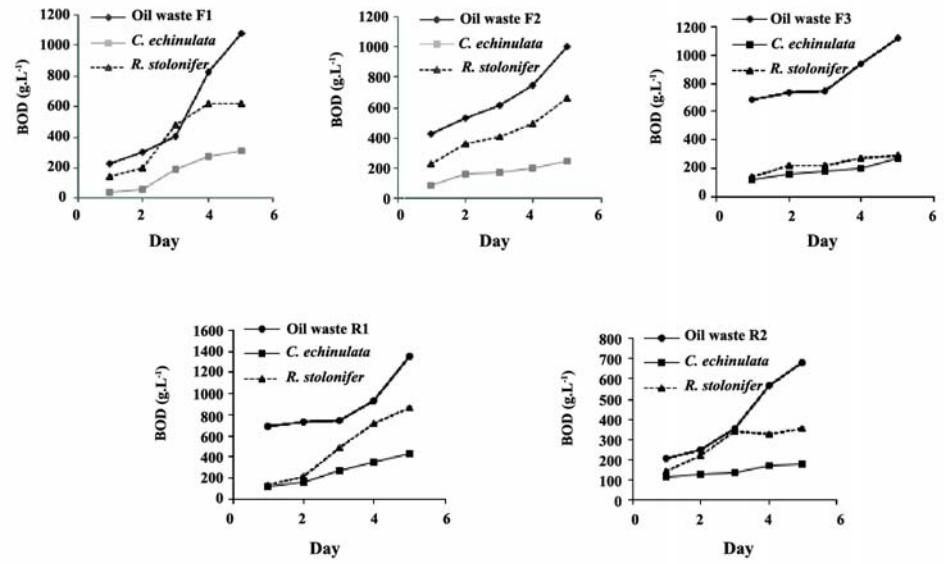

Figure 3. BOD values (mg.L^{-1}) during 5 days by two strains *C. echinulata* DSM1905 and *R. stolonifer* DSM2194 in different oil wastes

tion (27). Linoleate production by the two strains was remarkable from all oil wastes, but *R. stolonifer* yielded more alpha linolenate. DHA was produced only when C22:0 was present in the oil wastes, and arachidonic acid appeared when C20:0 was in the substrate mixture. The two strains produced GLA, even though this fatty acid was not found in the wastes. Chen and Liu reported that *C. echinulata* cultivated on various carbon sources produces high biomass quantities but that the fungus did not accumulate large amounts of stored lipids (19, 28, 29). The same zygomycetes strain, *C. echinulata* ATHUM 4411, produced 7.8 g.L^{-1} SCO (single cell oil) and about 800 mg.L^{-1} GLA when cultivated on tomato hydrolyzate enriched with glucose (27), whereas growth on potato starch was accompanied by production of 540 mg.L^{-1} GLA (28). Additionally, *C. echinulata* strain CCRC 31840 produced 964 mg/g GLA when grown on starch, whereas it produced 1,349 mg.g^{-1} GLA after optimizing the inoculum (27, 29). Production of arachidonic acid by *Mortierella alpina* CBS 754.68 was evaluated in submerged fermentation (30). Our data showed that the lipids produced were related to the substrate type and indicated that PUFA biosynthesis is strongly associated with mycelial growth. The economic restrictions regarding application of microbial lipids as renewable sources for fatty acid production can be overcome by using a cheap feedstock. Finally, the impacts of lipid metabo-

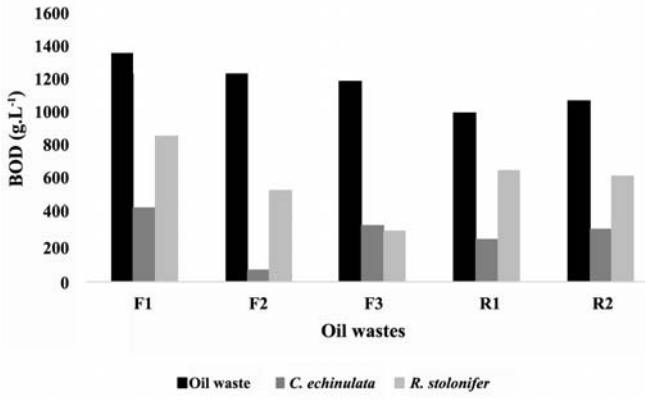

Figure 4. BOD_5 values (g.L^{-1}) in different oil wastes and oil wastes treatment by oleaginous fungi *Cunninghamella echinulata* DSM1905 and *Rhizopus stolonifer* DSM2194

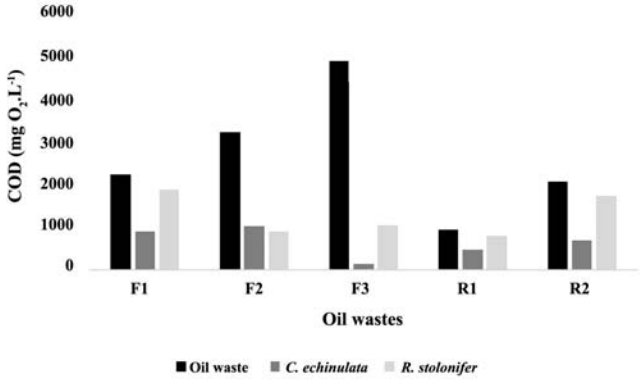

Figure 5. COD values (mg O_2.L^{-1}) in different oil wastes and oil wastes treatment by oleaginous fungi *Cunninghamella echinulata* DSM1905 and *Rhizopus stolonifer* DSM2194

lism on changes in BOD and COD were significant in the presence of *C. echinulata* and *R. stolonifer*. Therefore, our results may help to overcome some environmental problems.

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References

1. Jang HD, Yang SS. Polyunsaturated fatty acids production with a solid-state column reactor. *Bioresource Technol*. 2008; **99**(14):6181-6189. DOI: 10.1016/j.biortech.2007.12.024
2. Connor WE. Importance of ω-3 fatty acids in health and disease. *Am J Clin Nutr*. 2000;**71**(1):171-175.
3. Narayan B, Miyashita K, Hosakawa M. Physiological effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)-A review. *Food Rev Int*. 2006;**22**(3):291-307.
4. Papanikolaou S, Galiotou-Panayotou M, Fakas S, Aggelis G. Lipid production by oleaginous Mucorales cultivated on renewable carbon sources. *Eur J Lipid Sci Technol*.2007;**109**(11):1060-1070. DOI: 10.1002/ejlt.200700 169
5. Vamvakaki AN, Kandarakis I, Kaminarides S, Komaitis M, Papanikolaou S. Cheese whey as a renewable substrate for microbial lipid and biomass production by Zygomycetes. *Eng Life Sci*. 2010;**10**(4):348-360. DOI: 10.1002/elsc.201000063
6. Davery A, Pakshirajan K, Sangeetha P, Sophorolipids. Production by *Candida bombicola* using synthetic dairy wastewater. *IJCEE*. 200;p.1- 4.
7. Sayadi S, Ellouz R. Screening of white rot fungi for the treatment of olive mill waste-waters. *Chem Technol Biotechnol*. 1993;**57**(2):141-146. DOI: 10.1002/jctb.280570208
8. Laconi S, Giovanni M, Antonio C, Raffaello P. Bioremediation of olive oil mill wastewater and production of microbial biomass. *Biodegrad*. 2007;**18**(5):559-566.
9. Mafakher L, Mirbagheri M, Darvishi F, Nahvi I. Isolation of lipase and citric acid producing yeasts from agro-industrial wastewater. *New Biotechnol*. 2010;**27**(4):337-340. DOI: 10. 1016/j.nbt.2010. 04.006
10. Ratledge C, Wilkinson SJ. eds, Microbial Lipids. Academic Press, London, U.K;1989. p.121-151.
11. Ratledge C. Fatty acid biosynthesis in microorganisms being used for single cell oil production. *Biochimie*. 2004;**86**(11). 807-815. DOI: 10.1016/j.biochi.2004.09.017
12. Mishra D, Singh S. Microbial production of g-linolenic acid: An Overview. *J Pharmacy Res*. 2011;**4**(2):199-203.
13. Jangbua P, Laoteng K, Kitsubun P, Nopharatana M, Tongta A. Gamma-linolenic acid production of *Mucor rouxii* by solid-state fermentation using agricultural by-products. *Lett Appl Microbiol*. 2009;**49**(1):91-97. DOI: 10.1111/j.1472-765X.20 09.02624.x
14. Papanikolaou S, Sarantou S, Komaitis M, Aggelis G. Repression of reserve lipid turnover in *Cunninghamella echinulata* and *Mortierella isabellina* cultivated in multiple-limited media. *J Appl Microbiol*. 2004;**97**(4):867-875. DOI: 10. 1111/j.1365-2672.2004.02376.x
15. Fakas S, Papapostolou I, Papanikolaous S, Georgiou DC, Aggelis G. Susceptibility to peroxidation of the major mycelia lipids of *Cunninghamella echinulata*. *Eur J Lipid Sci Technol*. 2008;**110**(11):1062-1067. DOI: 10.1002/ejlt.200800025
16. Mendes A, Reis A, Vasconcelos R, Guerra P, da Silva TL. Fatty acids in the species of several zygomycete taxa *Crypthecodinium cohnii* with emphasis on DHA production. *J Appl Phycol*. 2009;**21**(2):199-214.
17. Ratledge C. *Microbial production of Gamma-linolenic acid,* in Handbook of Functional Lipids. edited by C. Akoh, CRC Press, Boca Raton, FL, USA. 2005; p.19.
18. Zhou DQ. *Microbiology Lab Manual*. Shanghai Scientific and Technical Publishers. Shanghai. PR China. 1983.
19. Pan L, Yang D, Shao L, Li W, Chen G, Liang Z. Isolation of oleaginous yeasts. *Food Technol Biotechnol*. 2009;**47**(2):215-220.
20. Christie WW. *Preparation of ester derivatives of fatty acids for chromatographic analysis*. In: Christie WW, editor. Gas Chromatography and Lipids: Oily Press. 1993; p.69-111.
21. Clesceri LS, Greenberg AE, Eaton AD. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association (APHA), Washington. DC. 1998.
22. Papanikolaou S, Aggelis G. Lipid production by *Yarrowia lipolytica* growing on industrial glycerol in a single-stage continuous culture. *Bioresource Technol*. 2002;**82**(1):43-49. DOI: 10.1016/S0960-8524(01)00149-3
23. Chatzifragkou A, Fakas S, Galiotou-anayotou M, Komaitis M, Aggelis G, Papanikolaou S. Commercial sugars as substrates for lipid accumulation in *Cunninghamella echinulata* and *Mortierella isabellina* fungi. *Eur J Lipid Sci Technol*. 2010; **112**(1):1048-1057. DOI: 10.1002/ejlt.201000027
24. Amaral C, Marcos L, Sampaio Ana, Peres J, Dias A A, Peixoto F, Anjos M, Pais C. Biodegradation of olive mill wastewaters by a wild isolate of *Candida oleophila*, *Int Biodeterior Biodegrad*. 2012;**68**(6):45-50. DOI: 10.1016/j.ibiod.2011.09. 013
25. Zhu M, Yu L, Wu Y. An inexpensive medium for production of arachidonic acid by Mortierella alpina. *J Ind Microbiol Biotechnol*. 2003;**30**:75-59.
26. Fakas S, Certik M, Papanikolaou S, Aggelis G, Komaitis M, Galiotou- Panayotou M. γ-Linolenic acid production by *Cunninghamella echinulata* growing on complex organic nitrogen sources. *Bioresour Technol*. 2008;**99**(13):5986-5990. DOI: 10.1016/j.ibiod.2011.09.013
27. Chen HC, Liu TM. Inoculum effects on the production of gamma- linolenic acid by the shake culture of *Cunninghamella echinulata* CCRC 31840. *Enzyme Microb Tech*. 1997;**21**(2):137-142. DOI: 10.1016/j.ibiod.2011.09.013
28. Subramaniam R, Dufrech S, Zappi M, Bjapai R. Microbial lipids from renewable resources. production and characterization. *J Ind Microbiol Biotechnol*. 2010;**37**(12):1271-1287.
29. Rocky-Salimi K, Hamidi-Esfahani Z, Abbasi S. Statistical optimization of arachidonic acid production by *Mortierella alpina* CBS 754.68 in submerged fermentation. *Iran J Biotech.* 2011;**9**(2):87-93.