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Microbial oil produced from biodiesel by-products could enhance overall production

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Abstract

Glycerol and rapeseed meal, two major by-products of biodiesel production, have been tested for possible use as low-cost raw materials for the production of microbial bio-oil using the oleaginous yeast *Rhodosporidium toruloides*. Using fed-batch fermentation with crude glycerol and a novel nitrogen rich nutrient source derived from rapeseed meal as feed, it was shown that 13 g/L lipids could be produced, compared with 9.4 g/L when crude glycerol was used with yeast extract. When 100 g/L pure glycerol was used, the final lipid concentration was 19.7 g/L with the novel biomedium compared to 16.2 g/L for yeast extract. The novel biomedium also resulted in higher lipid yields (0.19 g lipid/g glycerol consumed compared to 0.12 g/L) suggesting it provides a better carbon to nitrogen balance for accumulating lipids. FAMEs produced from the microbial lipids indicated a high degree of unsaturation confirming that the fatty acids produced from the novel biomedium have potential for biodiesel production.

Keywords: biodiesel; microbial bio-oil; rapeseed meal; crude glycerol; *Rhodosporidium toruloides*, solid state fermentation.

1. Introduction

Global production and use of biodiesel has increased dramatically in recent years and the fuel represents a promising alternative for use in compression-ignition (diesel) engines. It is generally produced by transesterification of vegetable oils such rapeseed oil. In the case of rapeseed, following extraction of the oil, a protein rich solid by-product, rapeseed meal, is generated, which is usually sold as organic fertilizer or animal feed. Global rapeseed production was 47 million tons in 2010 (FAO, 2010), resulting in the production of more than 25 million tons of rapeseed meal. However, the utilization of rapeseed meal as an animal feed is limited because it contains some anti-nutritional constituents such as phytic acid, erucic acid and fibre and precursors of toxic compounds such as glucosinolates and phenol (Koutinas et al., 2007; Pal Vig & Walia, 2001; Tranchino et al., 1983). As a consequence, the continued expansion of the biodiesel industry is likely to result in the production of greater quantities of rapeseed meal than the current demand can justify. Alternative uses for this by-product would therefore be desirable.

Another by-product of the biodiesel industry is crude glycerol, a mixture of the glycerol produced during the transesterification process along with residual methanol and salts of the reaction catalyst (sodium or potassium hydroxide). This can be purified but refining crude glycerol to high purities is expensive and energy-intensive and, again, the market is limited. The utilization of crude glycerol directly, without refining, could help to make biodiesel production more profitable and sustainable. There are many reports of the biological conversion of crude glycerol to value-added products such as animal feeds (Cerrate et al., 2006; Nitayavardhana & Khanal, 2011), 1,3-propanediol (Chatzifragkou et al., 2011; Mu et al., 2006; Tang et al., 2009; Willke & Vorlop, 2008), succinic acid (Vlysidis et al., 2011), single cell oil (André et al., 2010; Cui et al., 2010; Liang et al., 2010; Papanikolaou & Aggelis, 2009; Saenge et al., 2011) and citric acid (Papanikolaou & Aggelis, 2009;

Rymowicz et al., 2010; Rywinska et al., 2010). However, these studies have generally relied on the use of yeast extract or peptone as the nitrogen source to accompany the glycerol carbon source. Such materials are too expensive for use in a large biorefinery. In a sustainable, integrated biorefinery, it would make sense to utilise the available nitrogen source (rapeseed meal) alongside the crude glycerol for such processes. Furthermore, it would be very attractive to be able to convert these into additional oil using an oleaginous microorganism, such as *Rhodosporidium toruloides*. The objective of this study was, therefore, to investigate the production of additional lipids using biorefinery derived rapeseed meal and glycerol.

2. Materials and methods

2.1. Rapeseed meal

Rapeseed meal was kindly supplied by the Oilseeds Processing Division of Cargill Plc (Liverpool, UK). Its composition was determined and reported in a previous publication (Wang et al., 2010). The rapeseed meal was kept in an air-tight plastic container and stored at room temperature. Rapeseed meal proteins are not easily accessible for microorganisms and a pre-treatment is therefore required to obtain a suitable N source for the subsequent microbial oil fermentation (Wang et al., 2010). *Aspergillus oryzae,* an excellent protease producer was used to break down proteins present in the rapeseed meal into peptides and amino acids. Firstly, rapeseed meal was moistened with the required amount of tap water to obtain a 65 % moisture content in a 1 L bottle then sterilized at 121°C for 15 min. The meal was allowed to cool to room temperature before inoculating with approximately 10^6 spores of *A. oryzae*$\cdot g^{-1}$ meal. The content was mixed by stirring with a sterile aluminium rod and vigorous shaking. After mixing, approximately 10-13 g of content was distributed into each 9-cm Petri dish and incubated at 30°C for 3 days. Following the fermentation, autolysis of the filamentous fungus was initiated by mixing the required amount of distilled water with the fermented solids to

obtain approximately 55-60 g·L^{-1} solid concentration. The content was homogenised using a kitchen blender then incubated at 55°C for 3 days in a tightly capped bottle. Afterwards, the autolysate was centrifuged at 12,000 g for 10 minutes and the supernatant was filtered through a 0.2 µm filter and kept at -20ºC until required.

2.2. Synthetic crude glycerol

In addition to real crude glycerol, obtained from a local biodiesel plant, a synthetic crude glycerol was also prepared based on the composition 65% glycerol, 27% water, 4% methanol and 4% salts (Gonzalez-Pajueloa et al., 2005). Chemicals were supplied by Sigma.

2.3. Microbial-oil production

The oleaginous yeast *Rhodosporidium toruloides* Y4, which has previously been shown by Li et al. (2007) to be capable of high cell density culture was kindly provided by Professor Zongbao (Kent) Zhao of the Dalian Institute of Chemical Physics in China and was used throughout this study. It was kept at 4°C on Petri dishes containing 3 g.L^{-1} malt extract, 10 g.L^{-1} yeast extract, 10 g.L^{-1} peptone, 10 g.L^{-1} NaCl, 50 g.L^{-1} glycerol and 15 g.L^{-1} agar. Microbial-oil production was carried out using the solid state fermentation autolysate (SSFA) as the sole N source and pure or crude glycerol as the C source. In control experiments, yeast extract and pure glycerol were used as N and C sources, respectively.

The effect of initial glycerol concentration on yeast growth was investigated in flasks. For the inoculum preparation, *R toruloides* Y4 was grown for 3 days in 100 mL liquid medium composed of: 3 g.L^{-1} malt extract (Sigma), 10 g.L^{-1} yeast extract (Fisher), 10 g.L^{-1} peptone (Oxoid), 10 g.L^{-1} NaCl (Sigma) and 50 g.L^{-1} glycerol (Sigma). For experiments in 500 mL erlenmeyer flasks, the fermentation medium (100 mL) was prepared using 90 mL water containing different concentrations of pure or crude glycerol and the components above. The flasks were autoclaved at 121°C for 20 minutes, and then inoculated aseptically with 10 mL of inoculum. A control without glycerol was also carried out in parallel.

Fermentations were carried out in triplicate for 72 hours at 30°C on a 200 rpm rotary shaker.

For bioreactor (1 L, Electrolab) experiments, the fermentation medium was prepared using 900 mL filtered SSFA as nitrogen source and diluted to a Free Amino Nitrogen (FAN) concentration of 300 $mg.L^{-1}$. The medium was supplemented with 0.215 $g.L^{-1}$ $(NH_4)_2SO_4$, 0.4 $g.L^{-1}$ KH_2PO_4, 1.5 $g.L^{-1}$ $MgSO_4.7H_2O$. Glycerol was autoclaved separately, and added at the beginning of the fermentation to obtain concentrations of 100 $g.L^{-1}$ and 50 $g.L^{-1}$ for pure and crude glycerol, respectively. The inoculum (100 mL) was transferred aseptically and the fermentation was performed at pH 6 and 30°C. Samples were taken at regular intervals to follow the yeast growth, glycerol and FAN consumption. For batch fermentations the bioreactor was operated for 72 hours, after which, for fed-batch mode, glycerol was added every 24 hours in order to maintain a C/N ratio, in the range 70-90, for lipids accumulation.

2.4. Analytical methods

FAN concentration was analysed by the ninhydrin colorimetric method (Lie, 1973). Glycerol concentration was measured in triplicate using an Analox GL6 analyser (Analox, England). Fungal spores and yeast cells were quantified microscopically using a haemacytometer (Improved Neubauer, Weber England, Depth 0.1mm, 1/400mm^2). For dry cell biomass determination, 5 mL fermentation broth was filtered through a 0.2 μm filter and dried at 60°C overnight. Oil content of dried cells was determined by chloroform:methanol (1:1 v/v) extraction in a Soxtec-HT6 system (Höganäs, Sweden). The extraction time was 2 hours at 140°C, followed by 20 minutes of rinsing. Oil content was determined in triplicate.

Fatty acid analysis of lipids was conducted by gas chromatography (GC). For this, 1 mg of lipid was subjected for 10 h to methanolysis at 50°C in the presence of 4g/L NaOH with 1:20 biomass:methanol ratio. The resulting fatty acid methyl esters were analysed by GC on a Varian CP-3800 equipped with a DB-23 capillary column (60 m by 0.25 mm; film thickness of 150 nm) and a flame ionization detector (Agilent Technologies). A 2 μL portion

of the organic phase was analysed after split injection (1:50); helium (constant flow of 0.2 ml min^{-1}) was used as a carrier gas. The temperatures of the injector and detector were 250°C. The following temperature program was applied: 50°C for 1 min, increase of 25°C min^{-1} to 175°C, increase of 4°C min^{-1} to 230°C, and 230°C for 5 min. Substances were identified by comparison of their retention times with those of standard fatty acid methyl esters.

3. Results and discussion

Pure and crude glycerols were used, in separate sets of flask experiments, as the carbon source in order to determine the effect of glycerol concentration on *Rhodosporidium toruloides* Y4 growth. It can be seen from Figure 1 that the optimum concentration for pure glycerol was 70 $g.L^{-1}$ while for growth on crude glycerol, it was 50 $g.L^{-1}$. Beyond these concentrations growth started to decrease and above 100 $g.L^{-1}$ growth was markedly affected.

In 1 L bioreactor studies, the effect of agitation was investigated using an initial glycerol concentration of 100 $g.L^{-1}$. Since *R. toruloides* Y4 is an obligate aerobe, it grows better in oxygen rich media. Hence, cell growth should be enhanced by using a high mixing speed. It was indeed observed that greater specific growth rates (up to 0.085 h^{-1}) were obtained when fermentations were carried out at high agitation speeds of up to 1200 rpm. The maximum available speed (1200 rpm) was therefore chosen for subsequent experiments.

The highest lipid yield (0.26 g lipid/g glycerol) was obtained at an initial glycerol concentration of 100 $g.L^{-1}$. Beyond this level, growth was reduced even at high mixing speeds (Table 1). Thus, 100 $g.L^{-1}$ glycerol was chosen for subsequent experiments.

For batch fermentations in flasks, dry cell weights of 13 and 9 $g.L^{-1}$ were obtained using 100 $g.L^{-1}$ pure and crude glycerol, respectively. By contrast, values of 33 and 19.3 $g.L^{-1}$ were reached in bioreactor experiments with the same substrate concentrations. Cell growth obtained with crude glycerol was consistently lower than with pure glycerol due to the inhibitory effects of impurities in the crude glycerol such as methanol and salts. However, the

consumption profile of FAN in the crude glycerol medium showed that the yeast could assimilate the nitrogen sources derived from rapeseed meal in the presence of crude glycerol. The SSFA used to produce the rapeseed nutrient broth therefore appears to be a suitable fermentation medium for the production of lipids.

Fed-batch fermentations were carried out to increase biomass and lipid yields. Results using crude glycerol at 50 and 100 $g.L^{-1}$ are shown in Table 1. When 50 $g.L^{-1}$ crude glycerol was used, a final dry biomass concentration of 12.1 $g.L^{-1}$ and a lipid concentration 6.1 $g.L^{-1}$ were obtained in batch culture. These values reached 23.1 $g.L^{-1}$ and 9.4 $g.L^{-1}$ in fed-batch culture. Although yeast growth and lipid production were improved, the lipid content of the cells was lower in fed-batch culture compared to batch culture. This indicated that the cells consumed more glycerol to grow rather than storing more lipids in the fed-batch culture. Saenge et al. (2011) also demonstrated that biomass and lipid yield increased 1.41 and 1.48 folds, respectively, when *Rhodotorula glutinis* was grown on crude glycerol as a carbon source in a fed-batch culture compared with those of the batch culture.

Lipid yields obtained using 50 g/L crude glycerol in fed-batch systems were higher than those using 100 g/L crude glycerol in batch indicating that increasing the initial concentration to 100 g/L remains a challenge. In the study of Li et al. (2007), there was little inhibitory effect on the culture of *R. toruloides* Y4 with a glucose concentration up to 150 g/L. The growth was enhanced and the lipid content was increased by nearly 48% by using fed-batch culture compared to batch culture. Accordingly, yeast growth and lipid production can be improved by maintaining optimal substrate concentration in a fed-batch system. Although the lipid contents of the cells growing on pure and crude glycerol were similar, lipid yield obtained with crude glycerol was lower than that with pure glycerol. This may have resulted from growth inhibition due to the presence of inhibitors in crude glycerol.

In fed-batch fermentation using the SSF autolysate (SSFA) as N source, conventional

yeast extract was replaced by the nitrogen sources obtained from fungal treatment of rapeseed meal. Interestingly, cell growth was enhanced using SSFA compared to the results obtained with yeast extract in both pure and crude glycerol containing fermentation media (Table 1). It was found that lipid yields based on the glycerol consumed were increased from 0.12 to 0.2 when SSFA was used instead of yeast extract. Moreover, lipid yields based on the glycerol consumption were nearly equal (0.20 and 0.19) when 100 g/L pure or 50 g/L crude glycerol were used, respectively. The theoretical yield of glycerol is about 0.3 g/g glycerol (Ratledge, 1988), however, only 0.1 to 0.2 g/g or even lower have been reported in the literature regardless of the oleaginous strain used (André et al., 2010; Fakas et al., 2008; Liang et al., 2010; Meesters et al., 1996; Papanikolaou & Aggelis, 2009; Rymowicz et al., 2010). According to Makri et al. (2010) and Papanikolaou and Aggelis (2009) this may be due to the poor regulation of the enzymes involved in glycerol assimilation (e.g. glycerol kinase, 3-P-glycerol dehydrogenase).

The lipid yields (g lipid/ g glycerol consumed) obtained in this study are higher than the lipid yields obtained in previous studies in which pure or crude glycerol were utilized as C sources. Although the initial FAN concentration of SSFA and yeast extract containing media were identical, SSFA contained more nitrogen in other forms than yeast extract. As a result, the overall lipid concentration increased from 9.4 g/L to 13 g/L using 50 g/L crude glycerol. When 100 g/L pure glycerol was used, the final lipid concentration increased from 16.2 to 19.7 g/L when yeast extract was replaced by SSFA showing that the SSFA results in a better carbon to assimilable nitrogen ratio for accumulating lipids. However, as expected, the growth and lipid production using crude glycerol was lower than that obtained with pure glycerol due to inhibitory compounds found in crude glycerol. Still, the cell growth and lipid yields obtained with crude glycerol were much higher than other results found in the literature.

In a report by Saenge et al. (2011), crude glycerol was used as the sole C source with ammonium sulphate to produce lipids using *Rhodotorula glutinis*. Using batch fermentation with 95 g/L crude glycerol, the highest biomass concentration, lipid content and lipid concentration were 4.3 g/L, 53% and 2.28 g/L, respectively. By conducting fed-batch fermentation, the biomass concentration increased to 10.1 g/L with a lipid content and lipid concentration of 60.7% and 6.1 g/L, respectively. Chatzifragkou et al. (2011) reported that the lipid production using 90 g/L crude glycerol with *T. elegans* resulted in 11.6 g/L cell concentration with a lipid content of 71.1%.

The highest dry biomass (43 g/L) and lipid yield (19.7 g/L) were obtained by using pure glycerol and rapeseed meal hydrolyzate in this study. When crude glycerol was used with rapeseed meal hydrolyzate, 31.1 g/L dry biomass with a final lipid concentration of 13 g/L was obtained. Lipid contents of the cells were within the range of 41-46%. Although the lipid contents of the cells were lower than those reported in the literature, dry biomass levels and lipid concentrations obtained in this study were significantly higher. The reasons for that might be related to increased oxygen uptake of the cells because of high mixing. Depending on the glycerol content in crude glycerol (40-90%) (Gonzalez-Pajueloa et al., 2005; Kerr et al., 2009; Yazdani & Gonzalez 2008), the overall biodiesel production for an 800,000 tons/year biodiesel plant can be increased by 1-2.6% if the crude glycerol is converted to lipids using microbial fermentation. Also, significant savings and improved lipid concentration could be obtained if rapeseed meal was used as the nitrogen source for fermentation instead of yeast extract.

3.3. Characterization of the lipids

Long chain fatty acids were produced in each experiment and the corresponding Fatty Acid Methyl Esters (FAMEs) are listed in Table 2. Oleic (53.7-66.6 %), palmitic (15.1-20.1%), linoleic (4.4-19.2%) and stearic (8.1-12.7%) acids were the major fatty acids

produced in this study. This is very close to rapeseed oil, which contains mostly linoleic (23.3%) and oleic acids (64.4%) (O'Brien, 1988). This indicates that lipids from *R. toruloides* Y4 growing on crude glycerol and SSFA have great potential as biodiesel feedstocks. In this study, slightly higher stearic acid content but lower oleic acid content were observed using crude glycerol compared to pure glycerol. It was also found that lipids produced in the SSFA containing media included higher linoleic (C18:2) but lower palmitic acid (C16:0) compared to those obtained using yeast extract. In other words, higher unsaturated fatty acid content was obtained using SSFA as nitrogen source. It has been shown before that unsaturated fatty acid containing lipids are desirable for several fuel properties, including viscosity, specific gravity, cetane number, iodine value, and low temperature performance metrics (Hoekman et al., 2012). Therefore, our results show that growing the yeast in SSFA potentially improves the quality of the biodiesel as shown by a better average degree of unsaturation in Table 2.

4. Conclusions

Crude glycerol and a novel nitrogen stream derived from rapeseed meal were used as carbon and nitrogen sources, respectively, for the growth of the oleaginous yeast *R. toruloides* Y4 in order to produce microbial lipids. The novel biomedium resulted in higher lipid yields compared to yeast extract medium suggesting it provides a better carbon to nitrogen balance for accumulating lipids. FAMEs produced from the microbial lipids indicated a high degree of unsaturation confirming that the fatty acids produced in the novel biomedium have potential for biodiesel production.

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6. References

André, A., Diamantopoulou, P., Philippoussis, A., Sarris, D., Komaitis, M., Papanikolaou, S. 2010. Biotechnological conversions of bio-diesel derived waste glycerol into added-value compounds by higher fungi: Production of biomass, single cell oil and oxalic acid. *Industrial Crops and Products*, **31**(2), 407-416.

Cerrate, S., Yan, F., Wang, Z., Coto, C., Sacakli, P., Waldroup, P.W. 2006. Evaluation of glycerine from biodiesel production as a feed ingredient for broilers. *Int J Poult Sci*, **5**, 1001-1007.

Chatzifragkou, A., Makri, A., Belka, A., Bellou, S., Mavrou, M., Mastoridou, M., Mystrioti, P., Onjaro, G., Aggelis, G., Papanikolaou, S. 2011. Biotechnological conversions of biodiesel derived waste glycerol by yeast and fungal species. *Energy*, **36**(2), 1097-1108.

Cui, Y., Blackburn, J.W., Liang, Y. 2010. Identifying the optimal parameters for yeast fermentation on crude glycerol for lipid production. *AIChE annual meeting*, November 7-12, Salt Lake City, UT.

Fakas, S., Papanikolaou, S., Galiotou-Panayotou, M., Komaitis, M., Aggelis, G. 2008. Organic nitrogen of tomato waste hydrolysate enhances glucose uptake and lipid accumulation in *Cunninghamella echinulata*. *J Appl Microbiol*, **105**(4), 1062-1070.

FAO. 2010. Food Outlook Global Market Analysis.

Gonzalez-Pajueloa, M., Meynial-Salles, I., Mendesa, F., Andradea, J.C., Vasconcelosa, I., Soucaillec, P. 2005. Metabolic engineering of Clostridium acetobutylicum for the industrial production of 1,3-propanediol from glycerol. *Metabolic Engineering*, **7**, 329-336.

Hoekman, S.K., Broch, A., Robbins, C., Ceniceros, E., Natarajan, M. 2012. Review of biodiesel composition, properties, and specifications. *Renewable and Sustainable Energy Reviews*, **16**(1), 143-169.

Kerr, B.J., Weber, T.E., Dozier, W.A., Kidd, M.T. 2009. Digestible and metabolizable energy content of crude glycerin originating from different sources in nursery pigs. *J. Animal Science*, 4042-4049.

Koutinas, A.A., Wang, R.-H., Webb, C. 2007. The biochemurgist - Bioconversion of agricultural raw materials for chemical production. *Biofuels Bioproducts & Biorefining-Biofpr*, **1**(1), 24-38.

Li, Y., Zhao, Z., Bai, F. 2007. High-density cultivation of oleaginous yeast Rhodosporidium toruloides Y4 in fed-batch culture. *Enzyme Microb Technol*, **41**(3), 312-317.

Liang, Y., Cui, Y., Trushenski, J., Blackburn, J.W. 2010. Converting crude glycerol derived from yellow grease to lipids through yeast fermentation. *Bioresource Technology*, **101**(19), 7581-7586.

Lie, S. 1973. The EBC-ninhydrin method for determination of free alpha amino nitrogen. *J Inst Brewery*, **79**, 37-4.

Makri, A., Fakas, S., Aggelis, G. 2010. Metabolic activities of biotechnological interest in Yarrowia lipolytica grown on glycerol in repeated batch cultures. *Bioresource Technology*, **101**(7), 2351-2358.

Meesters, P.A.E.P., Huijberts, G.N.M., Eggink, G. 1996. High-cell-density cultivation of the lipid accumulating yeast <i>Cryptococcus curvatus</i> using glycerol as a carbon source. *Applied Microbiology and Biotechnology*, **45**(5), 575-579.

Mu, Y., Xiu, Z.L., Zhang, D.J. 2006. Microbial production of 1,3-propanediol by klebsiella pneumoniae using crude glycerol from biodiesel preparations. *Biotechnology Letters*, **28**(21), 1755-1759.

Nitayavardhana, S., Khanal, S.K. 2011. Biodiesel-derived crude glycerol bioconversion to animal feed: A sustainable option for a biodiesel refinery. *Bioresource Technology*, **102**(10), 5808-5814.

O'Brien, R.D. 1988. *Fats and oils*. Lancaster Technomic Publishing Co., United Kingdom.

Pal Vig, A., Walia, A. 2001. Beneficial effects of Rhizopus oligosporus fermentation on reduction of glucosinolates, fibre and phytic acid in rapeseed (Brassica napus) meal. *Bioresour Technol*, **78**(3), 309-312.

Papanikolaou, S., Aggelis, G. 2009. Biotechnological valorization of biodiesel derived glycerol waste through production of single cell oil and citric acid by *Yarrowia lipolytica*. *Lipid Technology*, **21**(4), 83-87.

Ratledge, C. 1988. Biochemistry, stoichiometry, substrates and economics. in: *Single cell oil*, (Ed.) R.S. Moreton, Longman. London, pp. 33.71.

Rymowicz, W., Fatykhova, A.R., Kamzolova, S.V., Rywinska, A., Morgunov, I.G. 2010. Citric acid production from glycerol-containing waste of biodiesel industry by Yarrowia lipolytica in batch, repeated batch, and cell recycle regimes. *Applied Microbiology and Biotechnology*, **87**(3), 971-979.

Rywinska, A., Rymowicz, W., Zarowska, B., Skrzypinski, A. 2010. Comparison of citric acid production from glycerol and glucose by different strains of Yarrowia lipolytica. *World Journal of Microbiology and Biotechnology*, **26**(7), 1217-1224.

Saenge, C., Cheirsilp, B., Suksaroge, T.T., Bourtoom, T. 2011. Potential use of oleaginous red yeast *Rhodotorula glutinis* for the bioconversion of crude glycerol from biodiesel plant to lipids and carotenoids. *Process Biochem*, **46**(1), 210-218.

Tang, S., Boehme, L., Lam, H., Zhang, Z. 2009. Pichia pastoris fermentation for phytase production using crude glycerol from biodiesel production as the sole carbon source. *Biochem Eng J*, **43**, 157-162.

Tranchino, L., Costantino, R., Sodini, G. 1983. Food grade oilseed protein processing-sunflower and rapeseed. *Qualitas Plantarum-Plant Foods Hum Nutr*, **32**(3-4), 305-334.

Wang, R., Shaarani, S.M., Godoy, L.C., Melikoglu, M., Vergara, C.S., Koutinas, A., Webb, C. 2010. Bioconversion of rapeseed meal for the production of a generic microbial feedstock. *Enzyme Microb Technol*, **47**(3), 77-83.

Willke, T., Vorlop, K. 2008. Biotransformation of glycerol into 1,3-propanediol. *European Journal of Lipid Science and Technology*, **110**(9), 831-840.

Yazdani, S.S., Gonzalez , R. 2008. Engineering *Escherichia coli* for the efficient conversion of glycerol to ethanol and co-products. *Metabolic Engineering*, **10**, 340-351.

Table 1. Dry biomass, lipid content (% of dry biomass) and lipid concentration (g lipids.L^{-1}) obtained at the end of the fermentation in a 1L bioreactor with different carbon and nitrogen sources and concentrations. (PG = pure glycerol, CG = crude glycerol, YE = yeast extract, SSFA = solid state fermentation autolysate).

C source *(g/L)*	***N source***	***Dry biomass*** *(g cell/L)*	***Lipid content*** *(%) (g lipid/g biomass)*	***Lipid conc.*** *(g lipid/L)*	***Lipid yield*** *(g lipid/g glycerol consumed)*	***Time*** *(h)*	***Mode of operation***
PG (100 g/L)	YE	35.3	46.0	16.2	0.26	120	batch
PG (200 g/L)	YE	16.2	53.1	8.6	0.23	120	batch
PG (300 g/L)	YE	4.0	50.6	2.0	0.16	120	batch
CG (50 g/L)	YE	12.1	50.9	6.1	0.12	72	batch
CG (50 g/L)	YE	23.1	40.9	9.4	0.12	120	fed-batch
CG (100 g/L)	YE	19.3	43.0	8.9	0.08	120	batch
PG (100 g/L)	SSFA	43.0	45.8	19.7	0.20	120	batch
CG (50 g/L)	SSFA	25.8	36.6	9.5	0.19	72	batch
CG (50 g/L)	SSFA	31.1	41.7	13.0	0.19	120	fed-batch

Table 2. Fatty acids composition of fatty acid methyl esters (FAME) derived from lipids of *R. toruloides* Y4 using different C and N sources. (PG = pure glycerol, CG = crude glycerol, YE = yeast extract, SSFA = solid state fermentation autolysate, NRS = nitrogen rich stream of yeast extract + peptone).

FAME Content (%, w/w)	***C + N source***				
	PG+YE	**CG+YE**	**PG+SSFA**	**CG+SSFA**	**CG+NRS**[a]
Myristic acid (C14:0)	0.9	0.0	1.4	0.0	0.9
Palmitic acid (C16:0)	18	20.1	7.2	15.1	16.8
Palmitoleic acid (C16:1)	1.2	0.0	0.0	0.0	0.8
Heptadecanoic acid (C17:0)	0.1	0.0	0.0	0.0	1.2
Stearic acid (C18:0)	8.1	12.7	10.2	12.0	3.7
Oleic acid (C18:1)	66.6	55.3	64.8	53.7	45.8
Linoleic acid (C18:2)	4.4	11.9	13.6	19.2	17.9
Linolenic acid (C18:3)	0.4	0.0	2.8	0.0	4.3
Arashidic acid (C20:0)	0.1	0.0	0.0	0.0	0.0
Behenic acid (C22:0)	0.2	0.0	0.0	0.0	0.7
Average degree of unsaturation[b]	0.778	0.791	1.004	0.921	0.953

[a] Data obtained from *R glutinis* (Saenge et al., 2011).

[b] Values computed by multiplying the mass fraction of each fatty acid constituent by the associated number of carbon–carbon double bonds, then summing over the entire profile.

Figure 1. *R. toruloides* Y4 dry cell weight after 72 hours at various initial concentrations of pure and crude glycerol. Data points show the averages from triplicate fermentations.