Producing of microbial oil using waste glycerol from biodiesel production
From by-product to raw material

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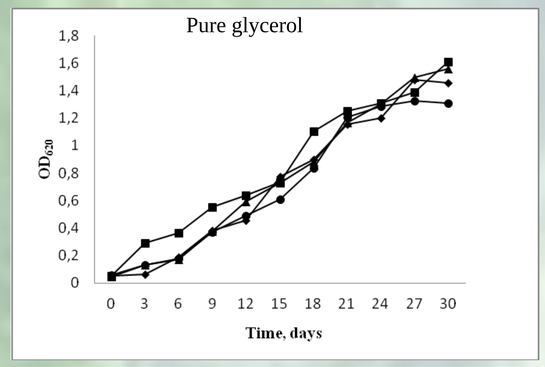
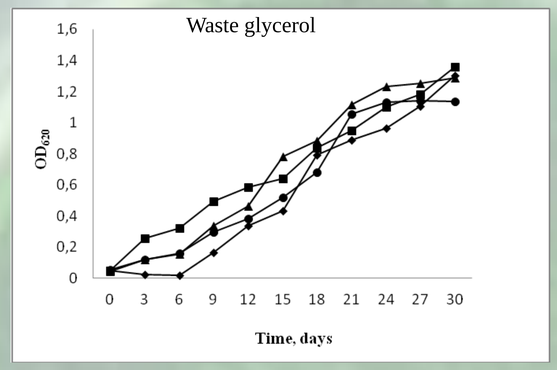
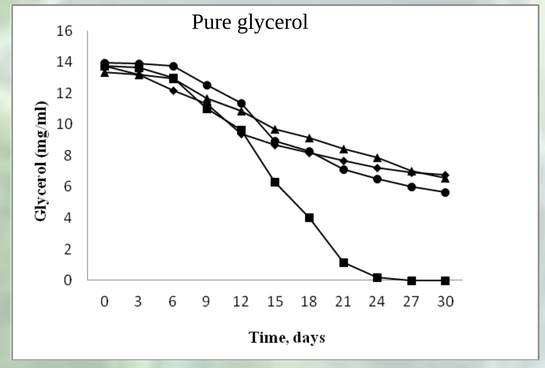
Comparatively, with the great increase in the industrial production of biodiesel worldwide in the last decade, huge reserves of glycerol have been created and the price of pure and waste glycerol in the world market has fallen significantly. While the pure glycerol is an important commercial raw material with wide application in the pharmaceutical, cosmetic, chemical and food industry, waste glycerol presents a promising raw material for some new processes. One of the potential possibilities is to use it as a carbon source in nutrient media for microbial growth in industrial fermentation and the production of commercially important products. Results of studying the possibility of waste glycerol, obtained in sunflower oil-based biodiesel production, utilization by microalgae in order to produce microbial oil as a raw material for biodiesel are presented in this paper.

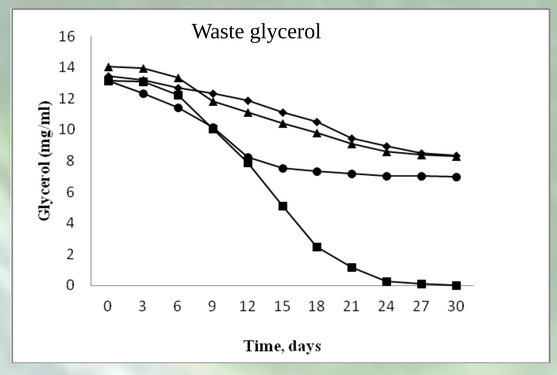
Four isolated strains of microalgae, identified as members of the *Chlorococcum*, *Chlorella*, *Desmodesmus* and *Scenedesmus* genera, were used for the experiments. The strains were isolated from the samples of South Serbian swamps and ponds in the Laboratory for Microbiology and Food Technology of the Faculty of Technology in Leskovac. Media used for growing of microalgae were as follows: Bolds Basal Medium (pH 6.6) ($NaNO_3$ 0.249 g/l; $CaCl_2$ x 2 H_2O 0.025 g/l; $MgSO_4$ x 7 H_2O; 0.075 g/l; K_2HPO_4 0.072 g/l; KH_2PO_4 0.175 g/l; NaCl 0.025 g/l; EDTA 0.16 g/l; KOH 0.077 g/l; $FeSO_4$ x 7 H_2O 0.012 g/l; H_3BO_3 0.028 g/l; $ZnSO_4$ x 7 H_2O 0.019 g/l; $MnCl_2$ x 4 H_2O 0.004 g/l; MoO_3 0.002 g/l; $CuSO_4$ x 5 H_2O 0.004 g/l; $Co(NO_3)_2$ x 6 H_2O 0.001 g/l). Pure (Sigma Aldrich, 99.5 %) and waste glycerol, obtained in sunflower oil-based biodiesel production, were added to the BBM medium in quantity of 15 g/l. Waste glycerol was obtained in Laboratory for chemical engineering of Faculty of Technology in Leskovac, (University of Niš). Under the constant light, during the 30 days, isolated microalgae were grown in 1000 ml modified BBM media, on a rotary shaker (140 min-1) at a temperature of 22 °C. After inoculation, an absorbance value was approximately 0.05 (measured at λ = 620 nm). The growth of algae was monitored spectrophotometrically by measuring optical density (OD_{620}), and the concentration of dry biomass was determined at the end of the process, after the entry of the algae into the stationary phase. The change in glycerol concentration was determined using the HPLC method. Dry algal biomass content was determined gravimetrically. The content of algal oil was determined using the Bligh-Dayer method.





Chlorella (♦) *Chlorococcum* (■) *Desmodesmus* (▲) *Scenedesmus* (●)





CHARACTERISTICS OF BIOPROCESS

Isolated strain	Pure glycerol					Waste glycerol				
	DBM g/l	Δglyc mg/ml	%	Oil $Y_{P/X}$ g/l	%	DBM g/l	Δglyc mg/ml	%	Oil $Y_{P/X}$ g/l	%
Chlorella sp.	1.1	6.97	51	0.4	34	1.0	5.11	38	0.3	35
Chlorococcum sp.	1.7	13.77	100	0.3	17	1.4	13.14	100	0.2	15
Desmodesmus sp.	1.4	6.80	51	0.3	22	1.2	5.76	41	0.3	22
Scenedesmus sp.	1.0	8.31	59	0.3	27	0.9	6.20	47	0.3	31

The highest concentration of biomass (1.7 g/l) was obtained with the *Chlorococcum* strain, followed by the *Desmodesmus* and *Chlorella* strains (1.4 g/l and 1.1 g/l), while the lowest concentration of biomass (1 g/l) was obtained with *Scenedesmus* strain. Glycerol had a stimulating effect on *Chloroccocum* growth, neutral effect on *Scenedesmus* strain and slightly negative effect on the *Desmodesmus* and *Chlorella* strains. The lower growth was achieved by growing microalgae in media with waste glycerol. The obtained values of dry biomass content of isolated strains *Chlorella* and *Scenedesmus* were 10 % lower and the values for the *Chlorococcum* and *Desmodesmus* strains were 18 % and 15 % lower, respectively. The highest concentration of biomass was achieved with *Chlorella* (1.4 g/l) followed by *Desmodesmus* and *Chlorella* (1.2 g/l and 1 g/l).

During the cultivation of algae, it was observed that all isolated strains consumed glycerol, that is, the concentration of glycerol decreased during the process. The greatest glycerol consumption, with some variations, is observed in pure glycerol substrates. Compared to the other studied strains, *Chlorococcum* sp. consumes the most glycerol in both media. Until the end of the process, it spends all available quantity. Isolated strain *Scenedesmus* sp. has also consumed a higher amount of pure glycerol (59 %) compared to waste glycerol (47 %). In pure glycerol medium, *Chlorella* and *Desmodesmus* strains consumed approximately the same amount of glycerol, 51 % of the total available amount.

Isolated strains produce different amounts of oil during the growth in glycerol media, unrelated to the microbial growth rate. It is well known that algae accumulate oil reserves in unfavorable growth conditions. Despite the weaker growth in all media, compared to *Chlorococcum* sp. and *Desmodesmus* sp., *Chlorella* sp. produced the highest amount of oil. The oil content of the biomass obtained in pure glycerol medium was 34 %, slightly higher (35 %) was obtained in medium with waste glycerol. In these two cases, the productivity of the oil was 0.4 and 0.2 g/l.

During the study of the microbial utilization possibility of waste glycerol obtained in sunflower oil-based biodiesel production using the microalgae for oil production, it was concluded that the obtained results are encouraging for further research and they are consistent with the data reported in the literature.

The isolated strains, members of the *Chlorella* and *Scenedesmus* genera, have grown weaker and consumed less glycerol, but they have produced a significant amount of oil (27 – 35 % in DBM) by growing in media with waste glycerol, thus confirming that under less favorable conditions algae produce greater amount of oil.

In general, waste glycerol obtained in sunflower oil-based biodiesel production has proved to be suitable for the microbial processes and can be used without purification.

ACKNOWLEDGMENT
This work has been funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Projects III 44006 and III 45001).