The utilization of waste materials from biofuel production by lactic acid bacteria isolated from traditional milk products

Jovan Ćirić[1, 2], Nataša Joković[3], Nikola Stanković[3], Marko Živković[2], Đorđe Lazarević[2]

[1] Faculty of Technology, University of Niš, Leskovac, Serbia

[2]Faculty of Electronic Engineering, University of Niš, Niš, Serbia,

[3]Faculty of Sciences and Mathematics, University of Niš, Niš, Serbia

joca.ciric@gmail.com

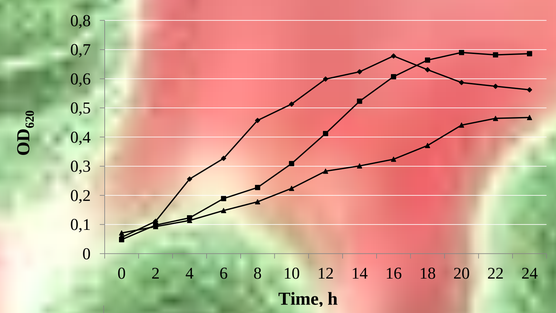
The production of ecological renewable fuel is a topic that attracts the attention of a large number of researches in the last few decades. Biodiesel is one of the most studied. Also, a lot of researches are focused on the use of waste glycerol as a by-product in biodiesel production. It was confirmed that waste glycerol can be a valuable raw material for the production of valuable products, even microbial metabolites. The majority of lactic acid bacteria members can assimilate glycerol and produce lactic acid by using it. There is published research about the conversion of waste glycerol to lactic acid and some of them confirmed that certain strains of *Enterococcus faecalis* can produce lactic acid on waste glycerol media. The results of studying the possibility of microbial conversion of waste glycerol as a by-product obtained in biodiesel production, by lactic acid bacteria isolated from traditional milk products, to produce lactic acid as a valuable product are presented in this paper.

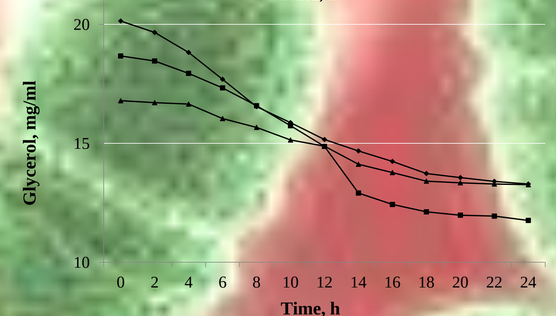
In this study, strain *Enterococcus faecalis* MK3-10A was used in performed fermentations. The studied strain was isolated from fermented milk spread (kaymak).

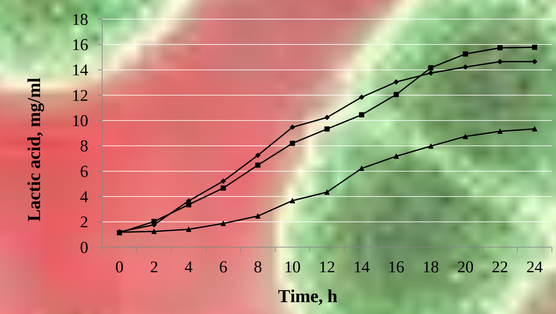
E. faecalis MK3-10A were cultivated in 300 ml of MRS (de Man, Rogosa, Sharpe) broth without carbon source (10 g/l peptone; 10 g/l meat extract; 5 g/l yeast extract; 2 g/l K_2HPO_4; 5 g/l CH_3COONa; 2.5 g/l $MgSO_4$ x 7 H_2O; 0.2 g/l $MnSO_4$ x 7 H_2O; pH 6.4). Pure glycerol (Sigma Aldrich) and waste glycerol were added afterward (15 g/l). Waste glycerol from sunflower oil and rapeseed oil-based biodiesel production was obtained in the Laboratory for Chemical engineering of Faculty of Technology in Leskovac, University of Niš. Therefore, in this study are used MRS medium with pure glycerol as a carbon source (PG medium) and media with waste glycerol obtained in sunflower oil-based biodiesel production (SfOG medium) and rapeseed oil-based biodiesel production (RsOG medium).

Under the aseptic conditions, liquid samples (10 ml) were sampled after every 2 h of fermentation. Microbial growth was measured spectrophotometrically (λ = 620 nm, Cole Parmer 2100 UV/VIS spectrophotometer). The concentrations of glycerol and produced lactic acid were determined using an HPLC method (Agilent 1100 Series chromatograph, Aminex HPX-87H column).

All experiments were performed in triplicate. Results for microbial growth, glycerol consumption, and product concentration were used for calculation of consumption rate glycerol Δ_{glyc} (mg/ml/d) and production rate yield of lactic acid $Y_{P/S}^{LA}$ (mg/ml/d) and the percentile of consumption was calculated in the relation between the decrease of carbon source with the starting concentration.







PG (♦) SfOG (■) RsOG medium (▲)

It was noticed that *E. faecalis* MK3-10A showed relatively good and constant growth on glycerol media with pure glycerol and SfOG media, while on the RsOG medium microbial growth was a bit different, it was weaker. The maximum biomass growth was achieved on the SfOG medium (OD_{620}^{max}=0.642) and the slightly lower on the PG medium (OD_{620}^{max} =0.622). On PG medium the sustained growth (lag phase) was noticed in the first 2 h of fermentation and other media, it was noticed in the first 4 h of fermentation. This was followed by an exponential growth phase which lasts up to 16 h (PG medium), 20 h (SfOG medium) and 22 h (RsOG medium).

The increase in biomass resulted in the decrease (consumption) of glycerol. The highest glycerol consumption rate of 6.96 mg/ml/day was achieved during the fermentation process on the SfOG medium while 37 % of the total available amount of glycerol was consumed. Compared to the PG medium, where 34 % of glycerol was consumed, glycerol consumption rate on SfOG medium was 7 % higher. During the fermentation of RsOG medium, the achieved consumption of 21 % was the lower (3.60 mg/ml/day) similar to it was the case with microbial growth. In all three fermentation media, the concentration of glycerol decreased constantly until the 16 h of fermentation. It can be concluded that the growth of *E. faecalis* MK3-10A was the effect of glycerol consumption as the sole carbon source.

Lactic acid production followed the microbial growth and glycerol consumption which is under the definition of primary metabolism and production of primary metabolites. In all studied media it can be noticed that lactic acid concentration increased until 20 h of fermentation. The maximum lactic acid concentration (15.78 mg/ml) and production rate yield (14.64 mg/ml/day) was obtained on SfOG medium which was 8 % and 9 % higher than was obtained on PG medium. Same as it were cases with growth and glycerol consumption, the lowest lactic acid concentration and production rate yield were obtained on RsOG medium (9.34 mg/ml and 8.16 mg/ml/day, respectively). This can be explained by the harmful and inhibitory effects of impurities contained in waste glycerol from rapeseed oil-based biodiesel production.

The lactic acid bacteria *E.faecalis* MK3-10A did not use all available amounts of glycerol. The production of lactic acid inhibited bacterial growth and it is known that lactic acid in a concentration higher than 1-2 % can inhibit further microbial growth.

In this performed fermentations, the strain *E. faecalis* MK3-10A showed the best growth and lactic acid production (15.78 mg/ml or 14.64 mg/ml/day, respectively) on media containing glycerol obtained in sunflower oil-based biodiesel production. Therefore, this waste glycerol has a great potential as a low price carbon source for the further research of lactic acid production on the laboratory and semi-industrial level.

In general, this could be a very perspective possibility of microbiological treatment of waste material that obtained in the production of green fuels.

CHARACTERISTICS OF BIOPROCESS

Carbon source (Glycerol)	(ΔOD_{620}^{max})		Δ_{glic}			C_{max}^{LA}		$Y_{P/S}^{MK}$
	-		(mg/ml/day)		(%)	mg/ml		(mg/ml/day)
PG	0,622	±0,021	6,48	±0,33	34	14,65	±0,43	13,44
SfOG	0,642	±0,026	6,96	±0,24	37	15,78	±0,51	14,64
RsOG	0,396	±0,017	3,60	±0,36	21	9,34	±0,11	8,16

PG - Pure glycerol
SfOG -Waste glycerol obtained in sunflower oil-based biodiesel production.
RsOG -Waste glycerol obtained in rapeseed oil-based biodiesel production.

ACKNOWLEDGMENT

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