Utilization of *Y. lipolytica* for Conversion of Crude Glycerol into Valuable Organic Acids

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Introduction

• The huge rise in the price of petroleum and its environmental concerns have turned the attention to alternative energy resources.

• Biodiesel with its lower environmental impact has been proposed as an alternative.

• The development of biodiesel production has led to a tremendous increase in production of crude glycerol, a by-product of bio-diesel, and a huge decline in its price[1].

• This project examines the conversion of crude glycerol obtained from a biodiesel manufacturing facility into valuable organic acids.

Methods

• Using high performance liquid chromatography (HPLC), glycerol present in the three samples-Technical grade glycerol, Crude Glycerol and Glycerol Pitch was identified.

• The standard calibration curve of glycerol can quantify the concentration of glycerol in given samples.

• The proper HPLC method was devised using HPLC optimizer software and comparison of the software prediction and HPLC results to separate α-ketogluterate, pyruvate, citrate, glycerol:
- **Eluent:** 10mM H_2SO_4
- **Flow rate:** 0.3ml/min
- **Column Temperature:** 36◦C
-**Run time:** 30min

• Citric acid production of the yeast, *Y. lipolytica* was examined in the auxotrophic strain PO1f (Leu- Ura-) and the prototrophic strain NRRL Y-423. Bioprocess optimization suggests two media for cultivation of *Y. lipolytica* on glycerol[2]:

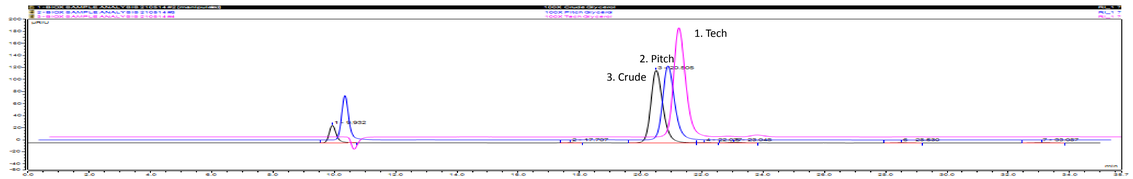
1) Citric acid producing medium (MP):
YNB* + 1g/L ammonium sulphate + 5% Glycerol
2) Growth medium (MG):
YNB + 3.5g/L ammonium sulphate + 2% Glycerol

• *Y. lipolytica* strains were grown on MP and MG media in 28◦C at 220rpm.

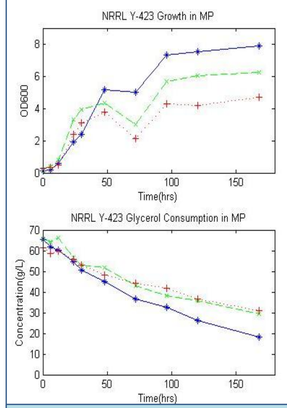
*YNB = Yeast Nitrogen Base, used as the defined medium. A defined medium has known composition of all the components.

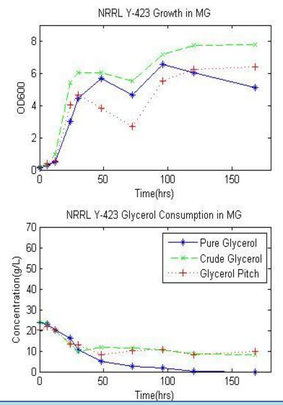
Results

The HPLC analysis of glycerol samples identify the concentration of glycerol and impurities :
1) Technical Grade Glycerol : 91.51% glycerol, highest concentration of glycerol with no impurities
2) Crude Glycerol : 60.85% glycerol, low concentration of impurities
3) Glycerol Pitch : 62.04% glycerol, highest concentration of impurities

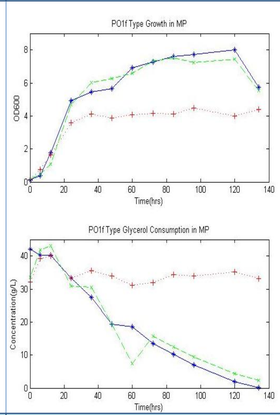


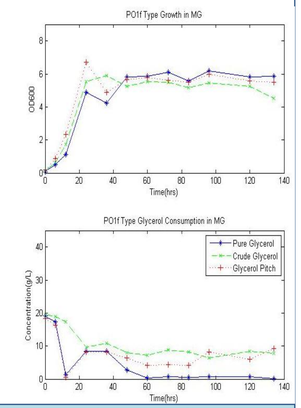
Growth on Glycerol and Glycerol Consumption of NRRL Y-423





Growth on Glycerol and Glycerol Consumption of PO1f strain





Growth and Consumption Rate of PO1f and NRRL Y-423

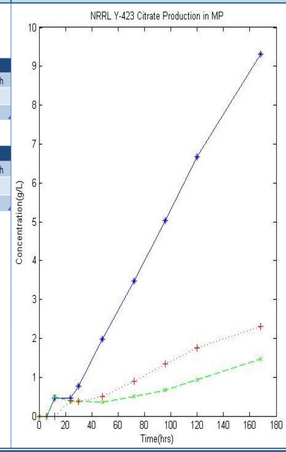
PO1f	MP Pure Glycerol	MP Crude Glycerol	MP Glycerol Pitch	MG Pure Glycerol	MG Crude Glycerol	MG Glycerol Pitch
Specific Growth Rate (h^{-1})	0.15	0.13	0.12	0.16	0.15	0.14
Specific Consumption Rate(mmol/gDCW/hr)	7.93	6.81	9.58	10.96	8.63	6.73

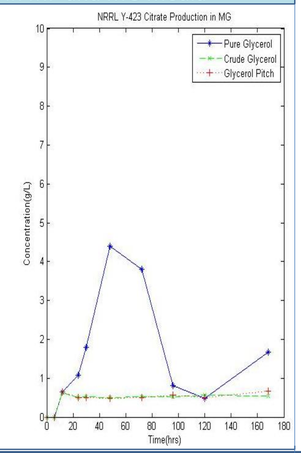
NRRL Y-423	MP Pure Glycerol	MP Crude Glycerol	MP Glycerol Pitch	MG Pure Glycerol	MG Crude Glycerol	MG Glycerol Pitch
Specific Growth Rate (h^{-1})	0.04	0.10	0.11	0.12	0.11	0.12
Specific Consumption Rate(mmol/gDCW/hr)	3.32	11.73	11.62	8.85	6.06	7.13

• Citric acid is produced from NRRL Y-423 strain. MP medium indicates up to approximately 2g/L citrate production from crude and 10g/L from pure glycerol.

• MG medium shows higher rate of growth with low concentration of citric acid. MG medium with pure glycerol sample converts mixture of citric and isocitric acid to α-ketogluterate. Hence, the concentration of citric acid reduces.

Citric Acid Production in MP and MG Media





Discussion

• The prototrophic strain, PO1f shows a higher growth rate on glycerol than the auxotrophic strain, NRRL Y-423.

• The trends in growth and glycerol consumption between the 'technical Grade' and 'crude glycerol' samples are close for PO1f strain.

• The NRRL Y-423 strain in MP medium is suitable for the secretion of citrate and isocitrate despite its slightly slower growth rate.

Conclusion

• *Y. lipolytica* with high glycerol assimilation is a viable option for conversion of crude glycerol into a variety of organic acids.

• In general, yeasts such as *Y. lipolytica* have a higher tolerance to acidic environment relative to bacteria. This is illustrated in NRRL Y-423 strain to reach a titer of up to 10g/L concentration in MP medium.

•The PO1f strain with higher growth rate relative to NRRL Y-423 can be engineered with serial gene deletions or gene overexpression to produce other valuable organic acids.

References

[1]K. Souza, R.Schwan, D.Dias, "Lipid and citric acid production by wild yeasts grown in glycerol", *Microbiology and Biotechnology*, vol. 24, pp.497-506, Jan. 2014

[2]W. Levinson, C. Kurtzman, T. Kuo, "Characterization of Yarrowia lipolytica and related species for citric acid production from glycerol", *Enzyme and Microbial Technology*, vol. 41, pp. 292-295, Feb. 2007