# Production of biosurfactants from yeasts cultivated on glycerol

**A. Giannopoulos, A. Makri and G. Aggelis**

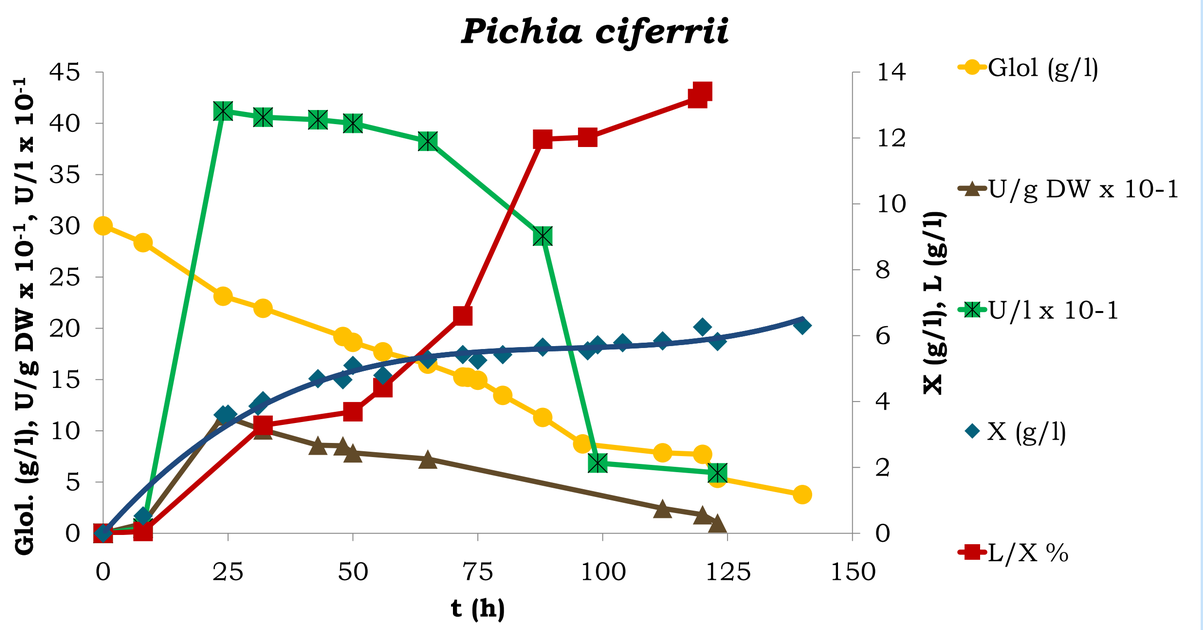
**Unit of Microbiology, Division of Genetics; Cell and Development Biology, Department of Biology; University of Patras; Patras; 265 00 – Greece**

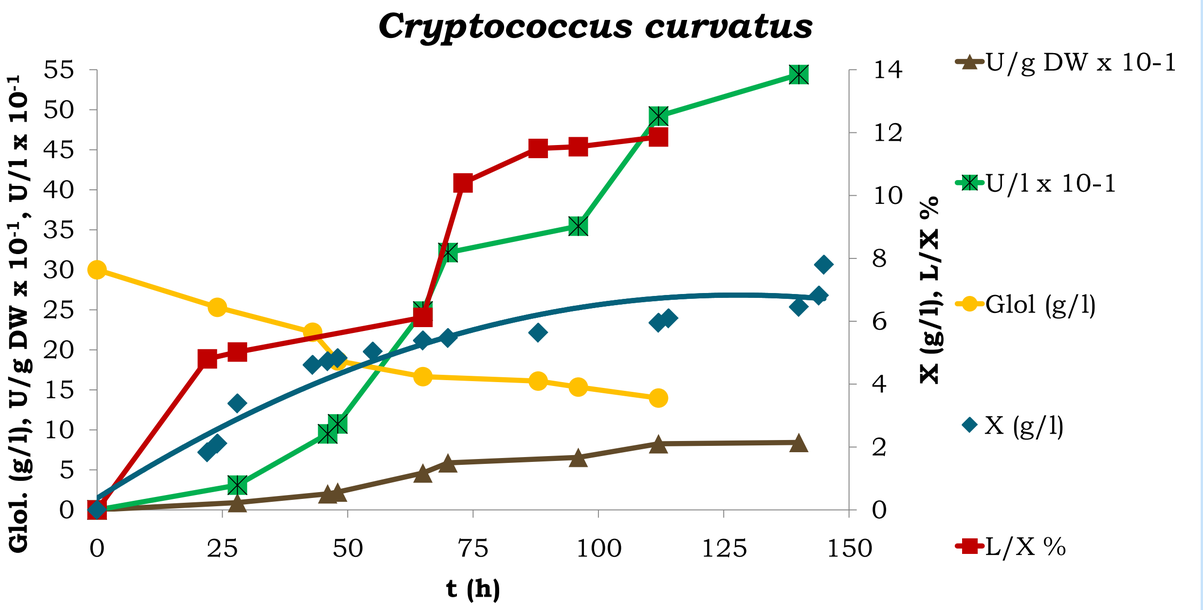
## Introduction

Surfactants are organic molecules that usually consist of a hydrophobic and a hydrophilic part. The hydrophilic part makes surfactants soluble in water, while its hydrophobic part makes them tend to concentrate at interfaces **[1]**. Depending on the nature of the hydrophilic group, four types of surfactants can be distinguished: anionic, cationic, zwitterionic, and non-ionic surfactants. The major classes of biosurfactants include glycolipids, phospholipids, fatty acids, lipopeptides/lipoproteins, and biopolymeric surfactants **[2]**. The hydrophobic part of the molecule consist of long-chain fatty acids, hydroxy fatty acids or α-alcyl-β-hydroxy fatty acids. The hydrophilic portion can be a carbohydrate, amino acid, cyclic peptide, phosphate, carboxylic acid or alcohol **[3]**. Surfactants are used in several fields, such as in industrial cleaning, agriculture, construction, food, paper and metal industries, textiles, cosmetics, pharmaceutical, and petroleum and petrochemical industries, including applications in environmental bioremediation. They are capable of a broad range of functional properties that include wetting, foaming, emulsification, viscosity reduction, phase separation, and solubilization. Typical desirable properties include solubility enhancement, surface tension reduction, the critical micelle concentrations, wettability and foaming capacity **[4]**. The production of biosurfactants by microorganisms grown on glycerol has been studied before. The yeast strain *Pseudozyma antarctica* produces mannosylerythritol lipids **[5]**, while the bacterial strain *Pseudomonas aeruginosa* produces rhamnolipids during cultivation on glycerol **[6]**. Many more microorganisms have also been studied during the past years **[7]**.

## Materials and Methods

**Microorganisms:** *Cryptococcus curvatus* (NRRL Y-1511), *Candida tropicalis* (NRRL Y-12968), *Pichia ciferrii* (NRRL Y-1031), *Candida guilliermondi* (NRRL Y-2075), *Candida diddensiae* (NRRL Y-7589) **Culture conditions:** Microorganisms were cultivated in batch cultures at T=28 °C, with glycerol being the only carbon and energy substrate. **Lipid extraction:** According to Folch protocol **[8]**. **GC analysis:** Fatty acid analysis of the various lipid fractions was performed after trans-methylation according to the AFNOR method **[9]**, in an Agilent Technologies 7890 A device equipped with a HP-88 (J&W scientific) column (60 m x 0.25 mm). Conditions: carrier gas helium, flow rate 1 ml/min, oven T=200 °C, injector T=250 °C, detector (FID) T=280 °C. **HPLC analysis:** Glycerol was determined in filtered (through 0.2 μm pore size bacteriological filter, Whatman) aliquots of the culture by an HPLC apparatus (Ultimate 3000, Dionex, Germering, Germany) equipped with an HPX-87H column and a R.I. detector. Conditions: eluant $H_2SO_4$ 0.004 N, flow rate 0.9 ml/min, T =55°C. **Emulsification activity:** Determination of biosurfactants' emulsification activity was performed according to Cirigliano & Carman and Van Dyke et al. **[10, 11]**. Broth samples were collected after centrifugation of the cultures at 15.000 g for 15 min, at T=4 °C. The samples were filtrated through 0,4 μm pore size bacteriological filters (Whatman). 2 ml of the filtrate were added to 2 ml of sodium acetate buffer 0,1 M (pH=3) and 1 ml of olive oil. The mixture was shaken on vortex for 30 sec. The resulting emulsion was then incubated for 30 min. after which its absorbance was measured at 540 nm. The blank used contained 2 ml of sterile medium containing glycerol.




**Figures 1 and 2:** Kinetics of *P. ciferrii* and *C. curvatus* cultivated on glycerol (30 g/l) as sole carbon source.
Biomass (X, g/l), cellular lipids (L, g/l), glycerol (Glol, g/l), biosurfactants' emulsification activity (U/g DW x $10^{-1}$; 1U=1 unit of change in absorbance at 540 nm)

| Yeasts | t (h) | Biomass (g/l) | Emuls. activity (U/g DW x $10^{-1}$) |
|---|---|---|---|
| ***C. guilliermondi*** | 90 | 5,79 | 0.00 |
| | 119 | 6,46 | 0.00 |
| ***C. tropicalis*** | 70 | 1.04 | 0.00 |
| | 119 | 1.63 | 0.00 |
| ***C. diddensiae*** | 68 | 5,74 | n.d. (*) |
| | 114 | 7,41 | 1.82 |

**Table 1:** Indicative evidence from the kinetics of *C. guilliermondi*, *C. tropicalis* and *C. diddensiae* cultivated in media of glycerol (30 g/l) as sole carbon source. *: non defined

| Yeasts | t (h) | C16:0 | C16:1 | C18:0 | C18:1 | C18:2 | C18:3 alpha | Others |
|---|---|---|---|---|---|---|---|---|
| ***Pichia ciferrii*** | 50 | 35.37 | 10.68 | 2.49 | 30.65 | 14.65 | 2.78 | 3.38 |
| | 56 | 36.10 | 10.49 | 2.29 | 30.71 | 14.63 | 2.86 | 2.93 |
| | 76 | 33.23 | 10.07 | 2.12 | 28.27 | 15.28 | 3.09 | 7.96 |
| | 80 | 35.98 | 10.05 | 2.19 | 28.82 | 15.20 | 2.75 | 5.03 |
| | 99 | 31.63 | 7.98 | 2.74 | 27.22 | 15.62 | 3.14 | 11.67 |
| | 104 | 32.84 | 8.51 | 2.53 | 27.50 | 15.62 | 2.59 | 10.41 |
| | 120 | 34.06 | 9.30 | 1.99 | 27.41 | 17.88 | 2.95 | 14.33 |
| | 123 | 33.08 | 8.40 | 1.94 | 26.78 | 17.10 | 3.04 | 9.66 |
| ***Cryptococcus curvatus*** | 43 | 25.65 | 0.87 | 12.90 | 43.36 | 13.04 | 2.79 | 1.39 |
| | 48 | 25.95 | 0.78 | 12.36 | 42.73 | 12.20 | 2.50 | 3.04 |
| | 65 | 25.83 | 0.74 | 11.01 | 43.93 | 13.00 | 2.66 | 2.82 |
| | 72 | 25.82 | 0.79 | 12.10 | 45.83 | 11.77 | 2.22 | 1.48 |
| | 96 | 24.26 | 0.55 | 13.90 | 46.24 | 11.79 | 2.29 | 0.43 |
| | 112 | 25.95 | 0.82 | 11.84 | 47.04 | 11.05 | 2.38 | 0.92 |
| | 120 | 26.08 | 0.85 | 11.58 | 47.37 | 11.24 | 2.06 | 0.82 |

**Table 2:** Fatty acid composition in *P. ciferrii* and *C. curvatus*.

## Results and discussion

Growth of a variety of yeast strains was studied on nitrogen-limited media. All strains studied, except for *C. tropicalis*, show a significant growth ability (biomass produced up to 7 g/l). Growth of *C. tropicalis* was limited (up to 2 g/l) on the present media. The ability of *P. ciferrii* and *C. curvatus* to assimilate glycerol was determined by HPLC analysis. *P. ciferrii* shows greater ability of assimilating glycerol consuming almost all of the quantity of glycerol, whereas, *C. curvatus* consumes almost half of the initial quantity. Fatty acid composition of *P. ciferrii* and *C. curvatus* was determined by GC analysis. Palmitic (C16:0) and oleic acids (C18:1) are the major fatty acids accumulated in the cells of these yeasts, followed by linoleic acid (C18:2). *P. ciferrii* also accumulates palmitoleic acid (C16:1) in greater quantities than *C. curvatus*, which tends to concentrate more stearic acid (C18:0) than *P. ciferrii*. While *P. ciferrii* and *C. curvatus* demonstrate emulsification activity of about 10 U/g DW x $10^{-1}$ and *C. diddensiae* about 2 U/g DW x $10^{-1}$, *C. tropicalis and C. guilliermondi* shows no emulsification activity at all, when grown on glycerol as sole carbon source.

## References

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