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TOWARDS BIODIESEL BIOREFINERY: GLYCEROL VALORIZATION USING IMMOBILIZED BACTERIA

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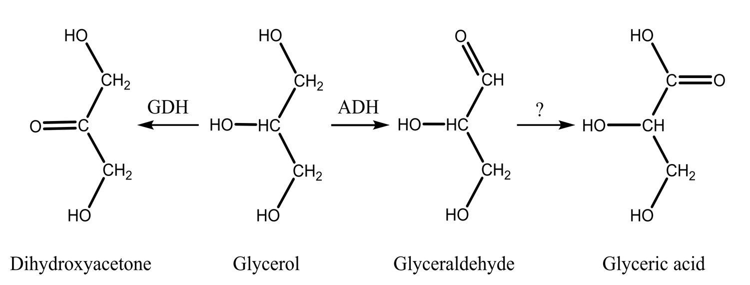
Introduction

New processes able to convert glycerol into novel value added products need to be developed to sustain the viability of the economy of biofuels (1,2). Biotransformations are performed under mild conditions with lower energy requirements, thereby, offering environmentally clean technologies. Microorganisms, which are becoming common industrial synthetic platforms, offer the possibility of reuse when immobilized or used as resting cells. Bacterial immobilization provides further operational advantages such as ease of separation, cost reduction, increase in the stability and productivity of the process and protection of the cells from the external environment. There are numerous examples in literature on microbiological conversion of glycerol (3,4).

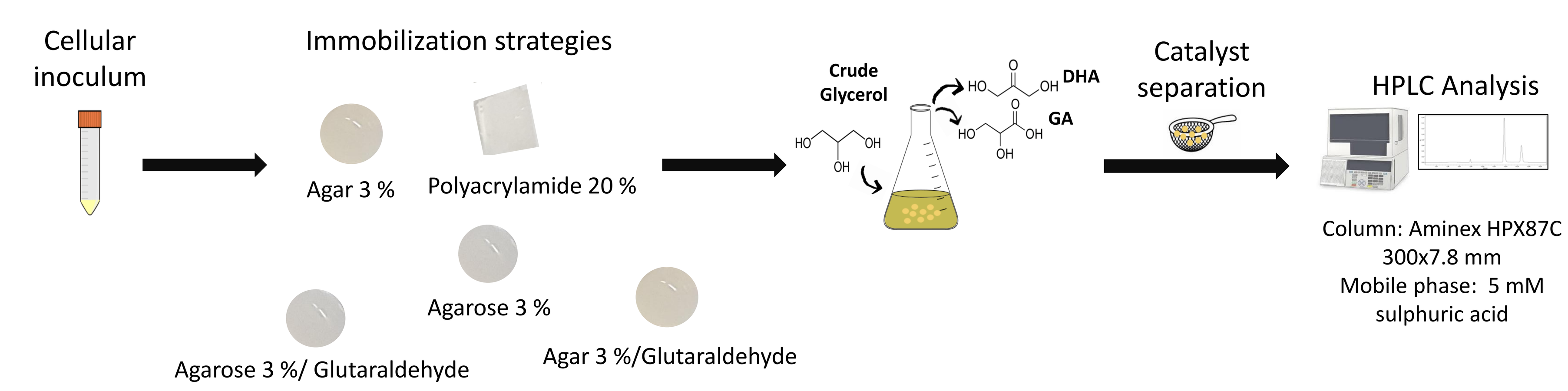


Scheme 1. DHA and GA production from glycerol.

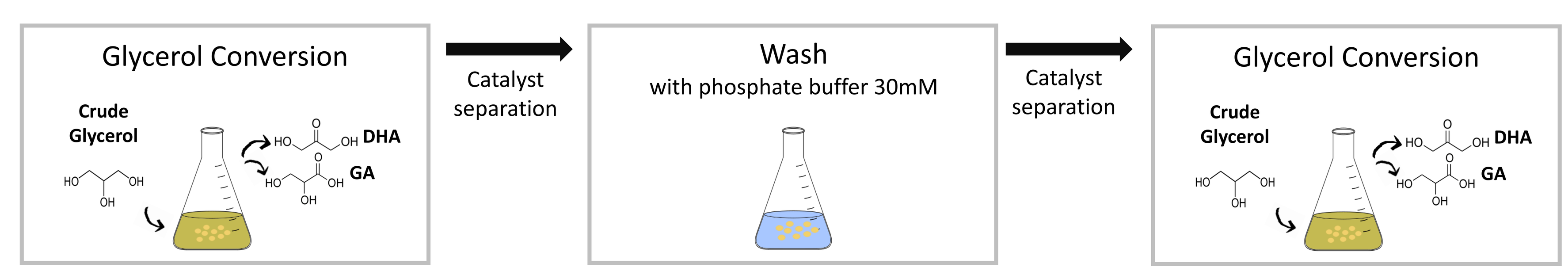
In this work, we have studied the biological transformation of glycerol to glyceric acid and dihydorxyacetone with immobilized *Gluconobacter frateurii* NBRC13465 (*G. frateurii*) (Scheme 1).

Methodology

Conversion using immobilized cells



Reuse of immobilized cells

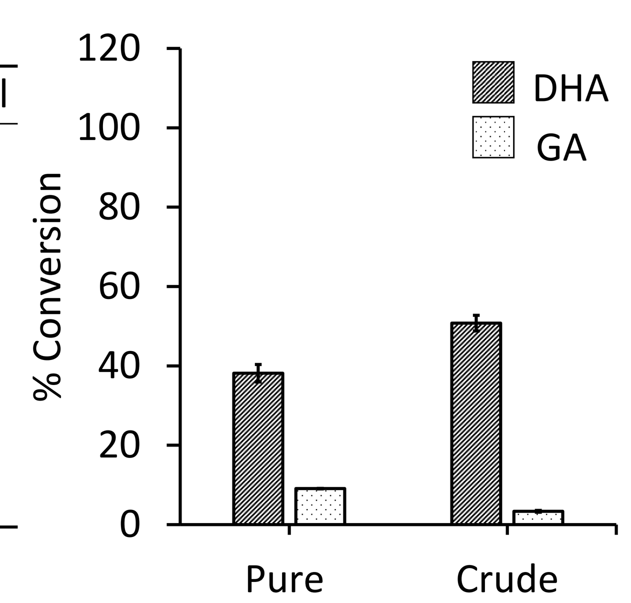


Results

Conversion of pure and crude glycerol using resting cells of *G. frateurii*

Glycerol composition

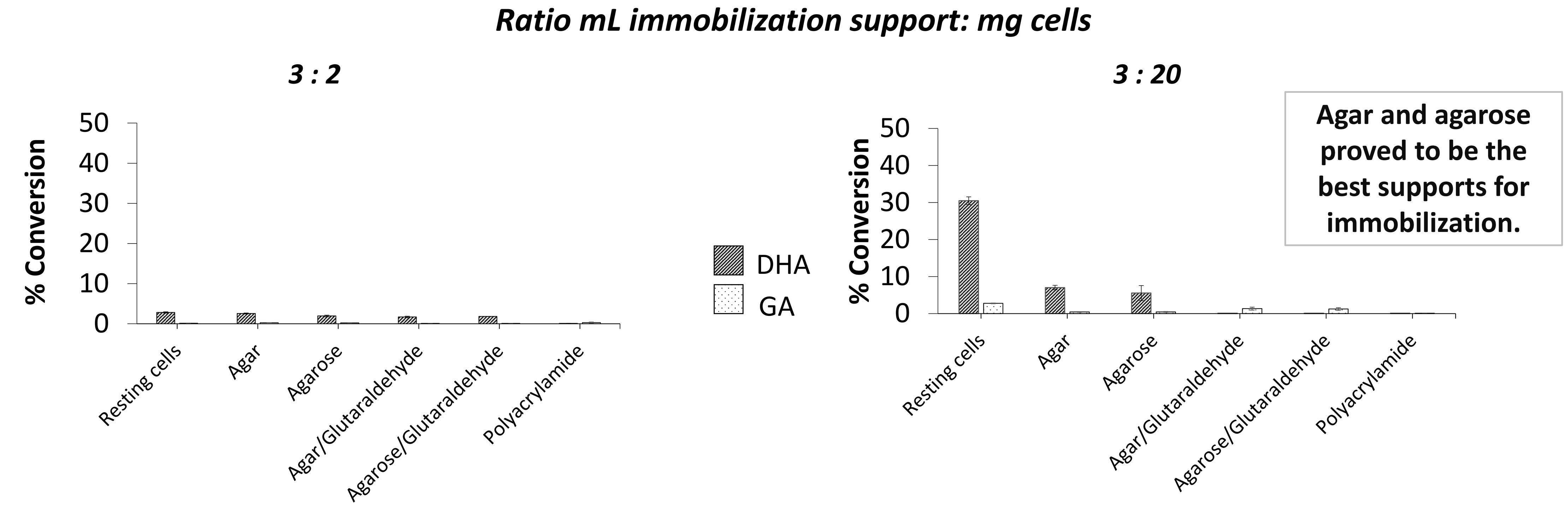
	Pure glycerol	Crude glycerol
glycerol (%)	99.50	70-80
water (%)	≤ 5	12
ashes (%)	not detected	7
fatty acids (%)	not detected	< 1
methanol (%)	not detected	< 5
chlorides (%)	not detected	< 3
heavy metals (ppm)	≤ 2	not detected



- **Reaction media:** glycerol 50 g/L, KH_2PO_4 0.9 g/L, K_2HPO_4 0.1 g/L, $MgSO_4.7H_2O$ 1 g/L, pH 8
- **Reaction conditions:** 30 mL in 250 mL flask, 30°C, 160 rpm, 45 h

Conversion of crude glycerol to DHA with heterogeneous catalysts of *G. frateurii* using different immobilization supports

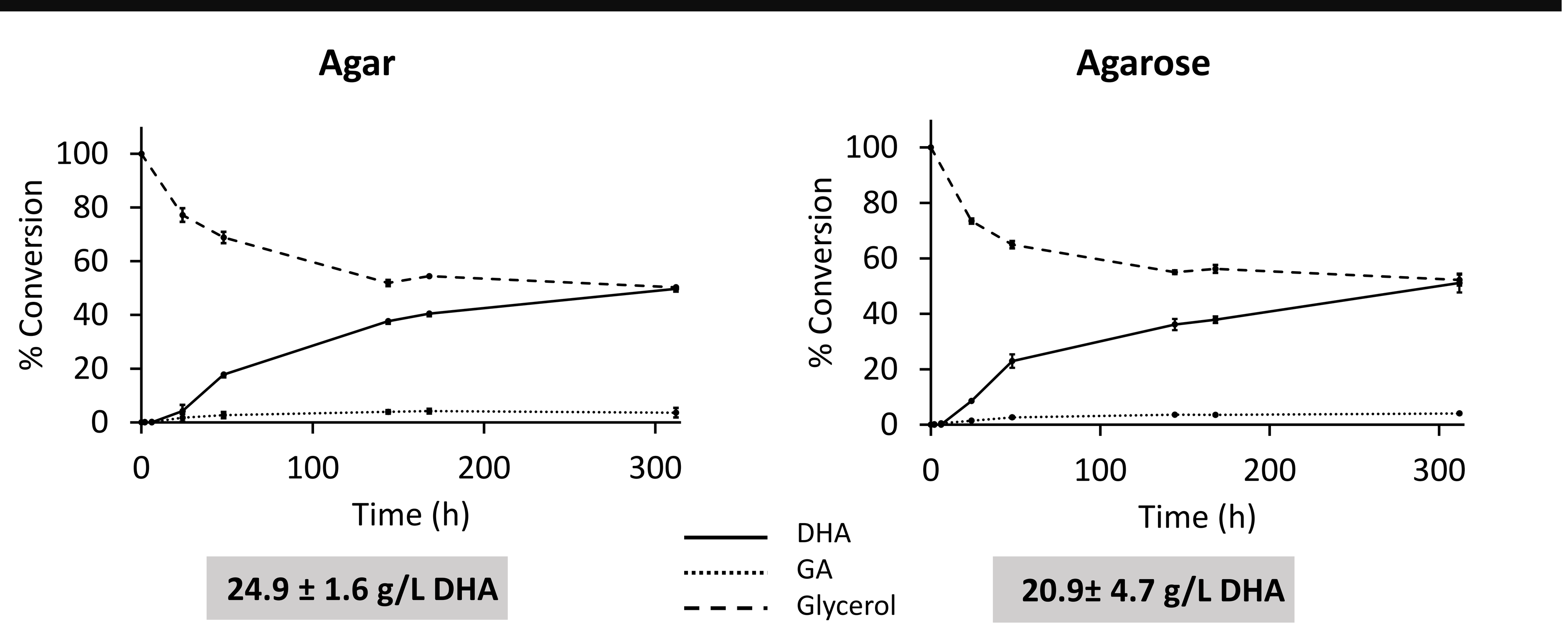
Ratio mL immobilization support: mg cells



Agar and agarose proved to be the best supports for immobilization.

- **Reaction media:** glycerol 50 g/L, KH_2PO_4 0.9 g/L, K_2HPO_4 0.1 g/L, $MgSO_4.7H_2O$ 1 g/L, pH 8
- **Reaction conditions:** 30 mL in 250 mL flask, 30°C, 160 rpm, 20 h

DHA and GA production kinetics using heterogeneous catalysts of *G. frateurii*



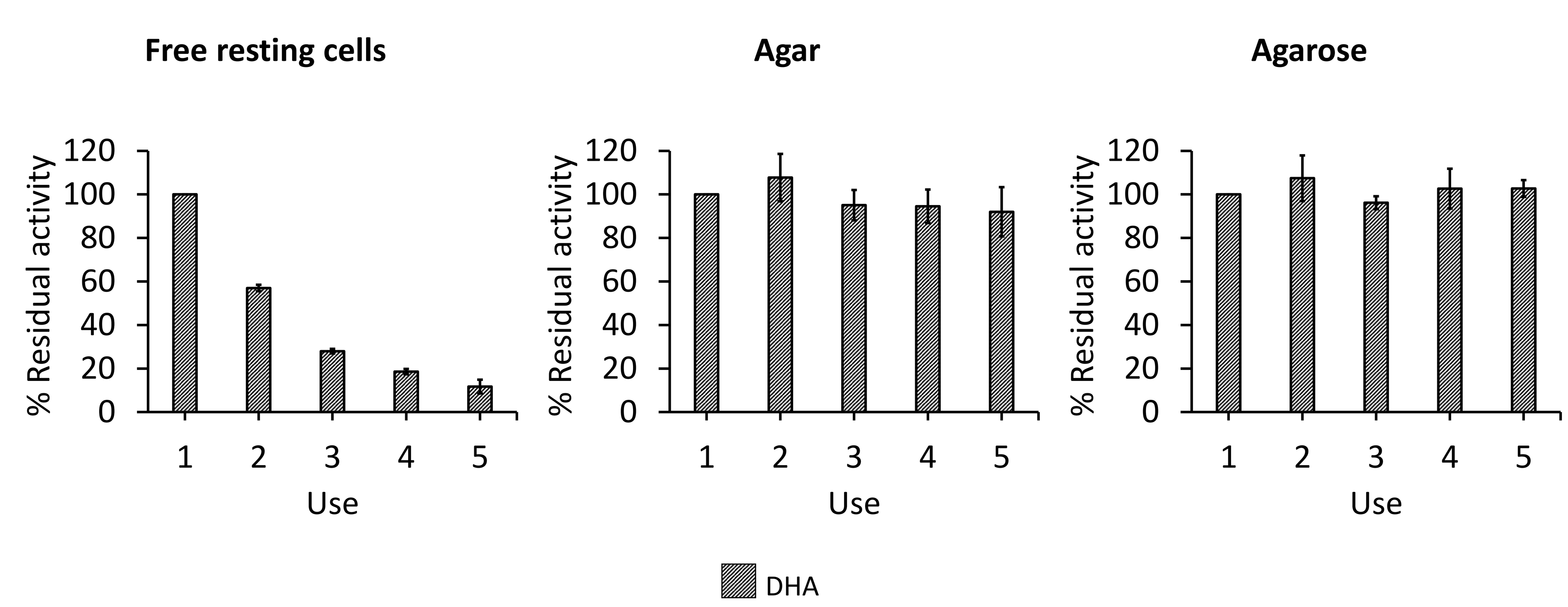
24.9 ± 1.6 g/L DHA

20.9± 4.7 g/L DHA

No significant differences in DHA and GA production were observed between the two immobilization supports

- **Inoculum:** 20 mg dry cell weight immobilized in 3% agar or agarose (with and without glutaraldehyde treatment) or in 20% polyacrylamide
- **Reaction media:** glycerol 50 g/L, KH_2PO_4 0.9 g/L, K_2HPO_4 0.1 g/L, $MgSO_4.7H_2O$ 1 g/L, pH 8
- **Reaction conditions:** 30 mL in 250 mL flask, 30°C, 160 rpm, 20 hours.

Reuse of resting and immobilized cells in glycerol transformation to DHA



When immobilized, *G. frateurii* cells maintained its ability to convert glycerol for at least 5 uses.

- **Inoculum:** 20 mg dry cell weight in resting cells form or immobilized in 3% agar or agarose
- **Reaction media:** glycerol 50 g/L, KH_2PO_4 0.9 g/L, K_2HPO_4 0.1 g/L, $MgSO_4.7H_2O$ 1 g/L, pH 8
- **Reaction conditions:** 30 mL in 250 mL flask, 30°C, 160 rpm, 20 hours per use.

References

(1) Ayoub and Abdullah, 2012
(2) Yang et al., 2012
(3) Andreeßen and Steinbüchel, 2012
(4) Szymanowska-Powałowska and Białas, 2014

Acknowledgements

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Conclusions

- Resting cells *G. frateurii* were able to convert successfully pure and crude glycerol.
- *G. frateurii* preserved the ability to convert glycerol using unprecedented immobilization strategies for this genus.
- Agar and agarose resulted in the best immobilization supports in the production of DHA.
- Under the studied conditions, immobilized preparations of *G. frateurii* converted up to 24.9 ± 1.6 g/L of DHA. No significant GA was observed under this conditions
- Immobilization allowed the reuse of the heterogeneous catalysts which maintained their full ability to convert glycerol to DHA for at least 5 uses.