High cell density cultivation of the chemolithoautotrophic bacterium *Nitrosomonas europaea* in a dialysis membrane bioreactor

[1]Levente Karaffa, [2]Tibor Török, [1]Antal Kökényesi, [1]István Kolláth, [3]Erzsébet Sándor, [1]Zoltán Németh, [1]Noémi Lipták, [1]Erzsébet Fekete and [1]Benedek Papp

[1] Department of Biochemical Engineering, University of Debrecen, Debrecen, Hungary
[2] TEVA Pharmaceutical Ltd., Safety and Environmental Department, Debrecen, Hungary
[3] Institute of Food Science, University of Debrecen, Debrecen, Hungary

HUNGARY'S RENEWAL
The project is supported by the European Union and co-financed by the European Social Fund.

INTRODUCTION

Nitrosomonas europaea **is a chemolithoautotrophic nitrifier Gram-negative bacterium that gains all of its energy for growth from the oxidation of ammonia into nitrite ions. These organisms are essential parts of the activated sludge used in domestic or industrial wastewater treatment systems. Moreover, the ammonia monooxygenase of these organisms is able to oxidize many non-physiological substrates, e.g. halogenated hydrocarbons and aromatic compounds as well, indicating that *N. europaea* may potentially be employed in a variety of biotechnological applications.**

However, fulfilling this potential requires the ability to produce viable, high cell density cultures.

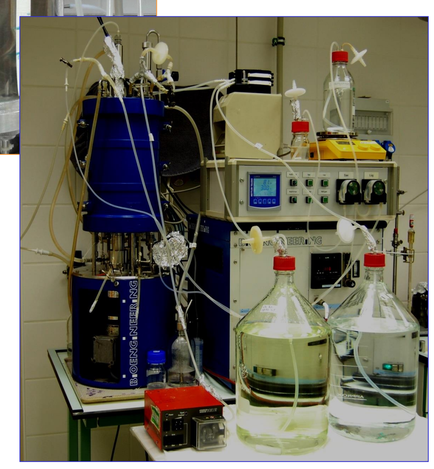
Growth of the culture

A major prerequisite to produce a high cell density *N. europaea* culture is to ensure that inhibitory metabolic by-products remain at minimal concentrations. The principal inhibitory metabolite being continuously formed during *N. europaea* fermentation is nitrite. As a consequence, N. europaea cannot grow into high cell density under conventional (non-dialysis)batch conditions.

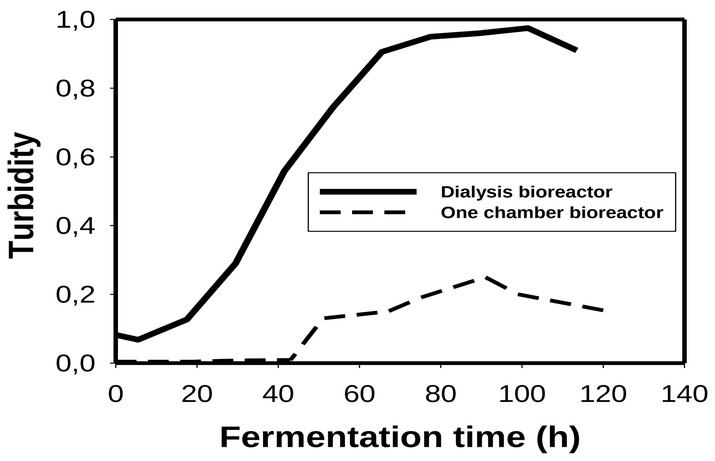
Fermentation conditions	Inner chamber	Outer chamber
Agitation	650 RPM	1200 RPM
Aeration	85 ml/min	No direct aeration
pH	Controlled 7.8 – 8.2	No feedback controll
Temperature	30 ºC	30 ºC
Light	Total darkness	Total darkness

RESULTS

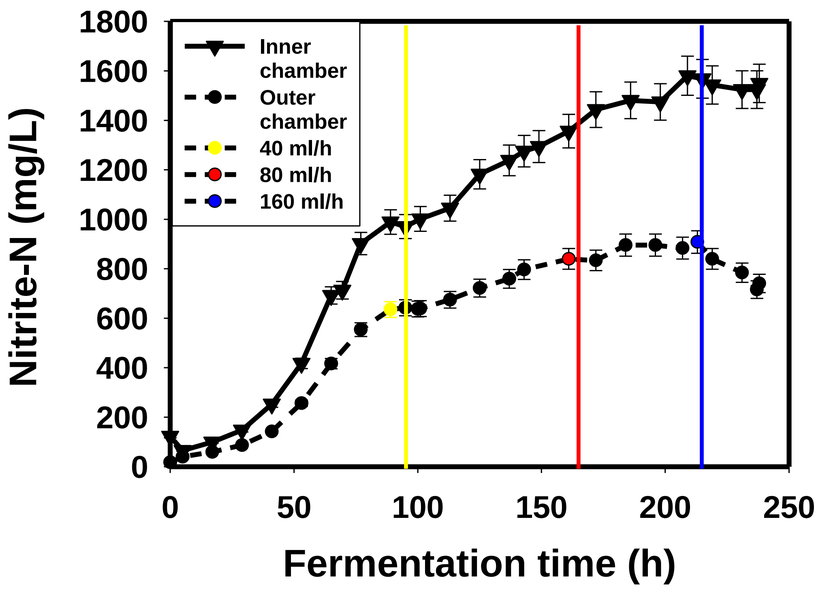
One suitablemethod to overcome the problem of low cell density is the application of a single-vessel dialysis membrane bioreactor system [2].
Dialysis membrane fermentors are highly efficient for the continuous removal of inhibitory or toxic products during cultivations, and bacterial cells can also be supplied with substrate via the membrane. The two chambers are separated with a cellulose-ester based dialysis membrane.



Batch fermentation profiles



Time-profile of nitrite concentration during standard and continuously freshed dialysis fermentations

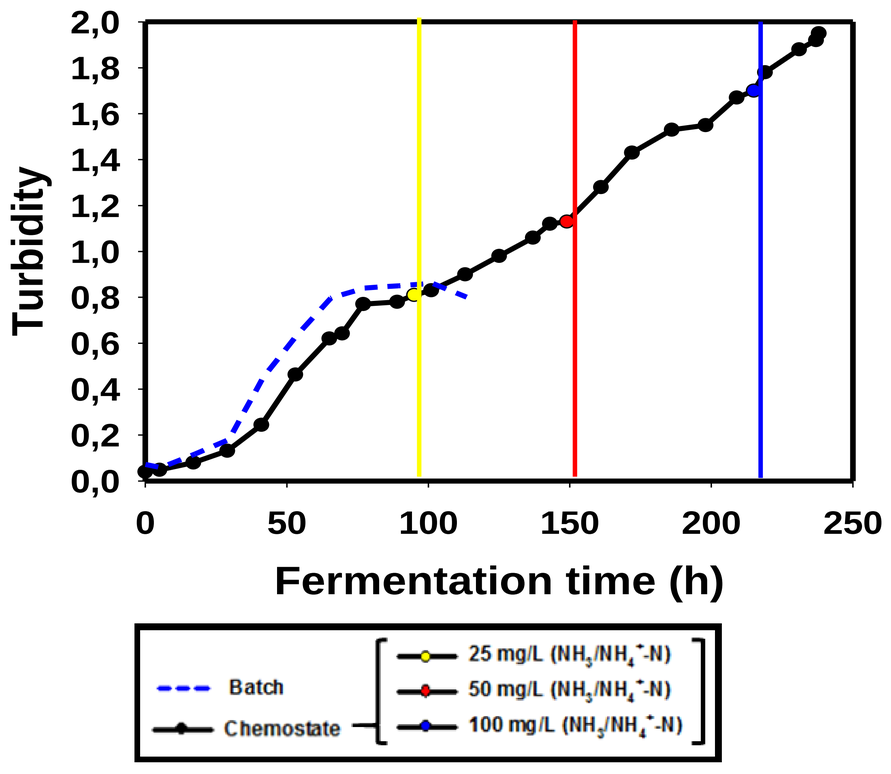


CONCLUSIONS

Growth of *N. europaea* was monitored via cell density determinations and by the measurement of nitrite formation. Maximal cell density achieved was over 5 times of the value achieved under conventional batch conditions using the same fermentation technology.

Cell density was further increased by continously replacing the spent medium in the outer chamber with fresh medium. Feed rate after the conclusion of the batch phase was set first at 40 ml/h and was later increased to 80 ml/h and further to 160 ml/h. The highest achived cell density (determined by Bürker-chamber) was $2*10^9$ cell/ml.

Time-profile of the turbidity of cultures during batch as well as continuously freshed dialysis fermentation



Acknowledgement:
The research was supported by the EU and co-financed by the European Social Fund under the project ENVIKUT (TÁMOP-4.2.2.A-11/1/KONV-2012-0043), and also by the Hungarian Scientific Research Fund (OTKA Grant K1006600 to Dr. Erzsébet Fekete).

[1] Sándor E., Szentirmai A., Biró S., Karaffa L. (1999): Specific cephalosporin C production of *Acremonium chrysogenum* is independent of the culture density. Biotechnology Techniques, 13: 443-445.
[2] Karaffa L. (2000): Membrane Fermentors. In: *Integration of Membrane Processes into Bioconversions* (eds: Bélafi-Bakó, K.; Gubicza, L.; Mulder, M.), pp. 223-229. Kluywer Academic / Plenum Publishers, New York, NY, U.S.A.