Cultivation of a slow-growing bacterium strain into high cell density

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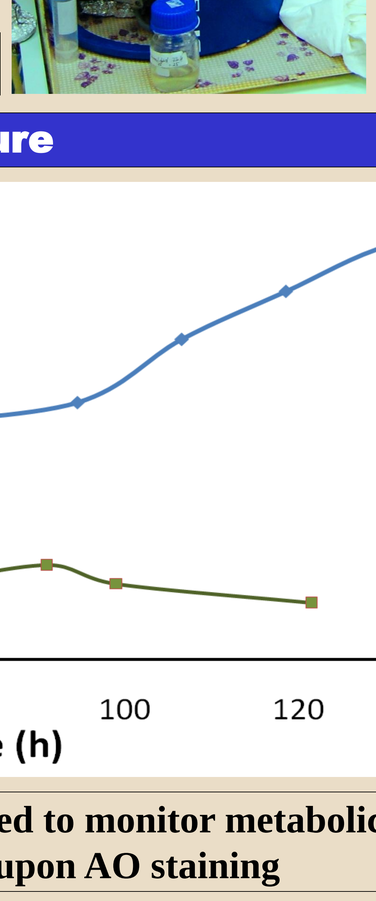
INTRODUCTION

Nitrosomonas europaea **is an autotrophic Gram-negative bacterium that gains all of its energy for growth from the oxidation of ammonia into nitrite ions (nitrification). *N. europaea* (ATCC 19718) cultures are relatively easy to grow, although several factors may have a negative influence on biomass formation.**
To achieve high cell density under submerged (batch) conditions is therefore not trivial [1]. A major prerequisite to achieve high cell density in batch culture is to ensure that inhibitory metabolic by-products remain at minimal concentrations. In case of *N. europaea*, the principal inhibitory metabolite being continuously formed during fermentation is nitrite. As a consequence, this bacterium cannot grow into high cell density under conventional batch conditions.



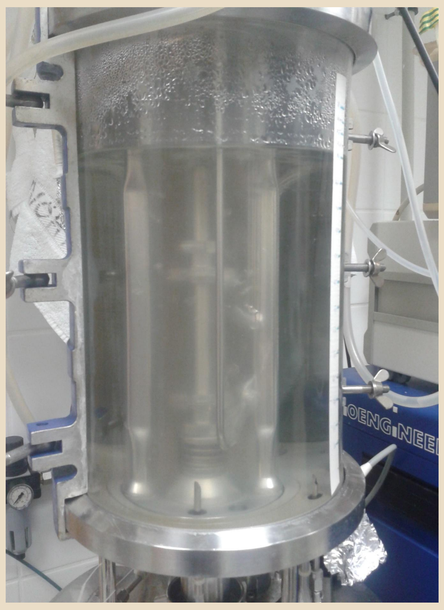
One suitable method to overcome this problem is the application of a single-vessel dialysis membrane bioreactor system [2]. Dialysis membrane fermentors are highly efficient for the continuous removal of inhibitory or toxic products during cultivations, and bacterial cells can also be supplied with substrate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

CONCLUSIONS

Growth of *N. europaea* was monitored via cell density determinations and by the measurement of nitrite formation. Maximal cell density achieved was over 3 times of the value achieved under concentional batch conditions using the same fermentation technology.

RESULTS

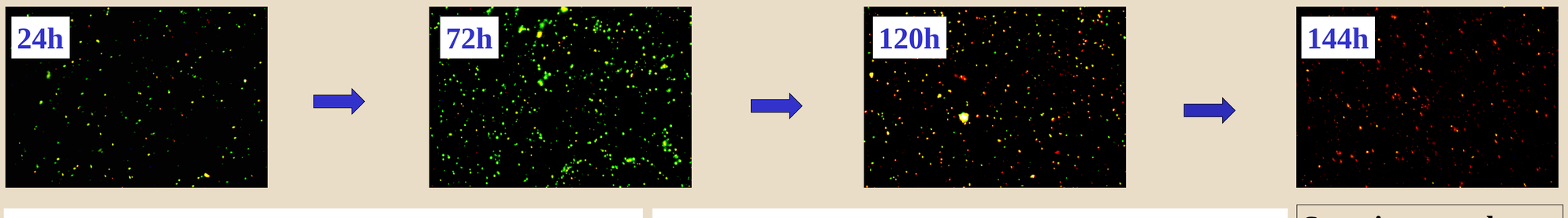
0. h — **Growth of the culture** — **144. h**

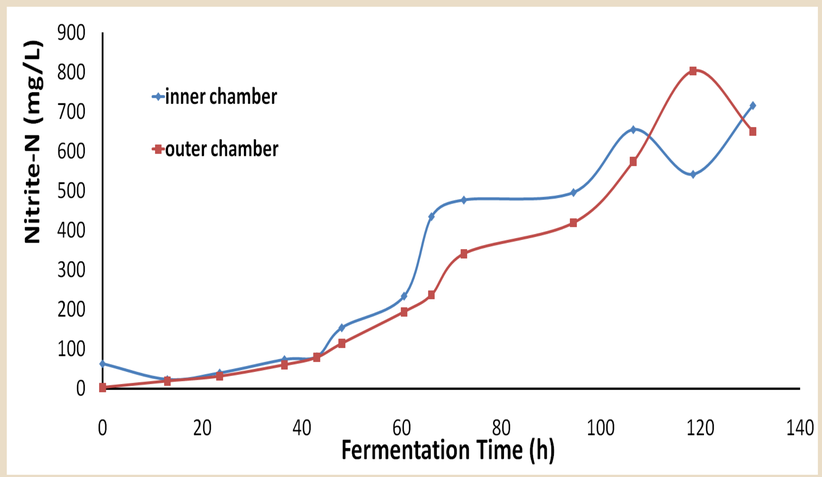


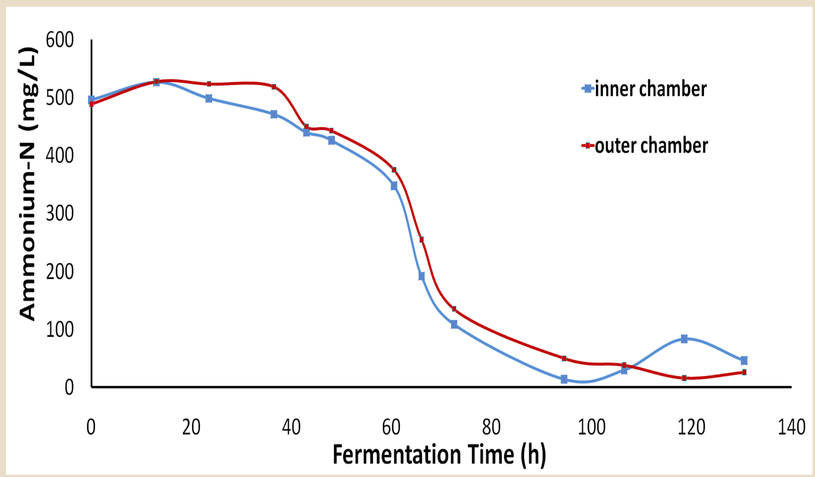
FERMENTATION CONDITIONS

The growth medium contained inorganic salts and ammonium-sulphate. At pH 7.8, the level of free ammonia is only approximately 4 % of that of the ammonium ion. The pH of the medium was kept above 7.8 value by the automatic addition of concentrated (2 M) sodium carbonate. Because oxidation of ammonia is light-sensitive, cultivation was performed in total darkness.

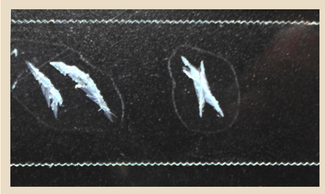
The fluorescent dye Acridine Orange (AO) was employed to monitor metabolic activity of the cultures. Active cells displayed green, inactive ones displayed orange colour upon AO staining







Struvit crystals (NH_4MgPO_4 $6H_2O$) were formed in the fresh medium. During the fermentation , this precipitate was solved back.



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