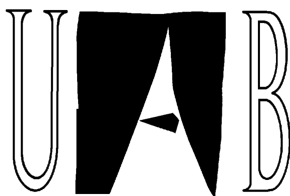

Universitat Autònoma de Barcelona
Dep. Enginyeria Química
08193 Bellaterra, Barcelona, Spain

MELISSA

Memorandum of Understanding

ECT/FG/MMM/97.012

Contract Number: ESTEC/CONTRACT11549/95/NL/FG

Technical Note: 43.62

R.rubrum biomass production for compartment I biodegradation tests

Version: 1

Issue: 0

ALBIOL, J.; CREUS, N., GODIA, F.

December 2000

Document Change Log

Version	Issue	Date	Observations
Draft	0	19/12/2000	Preliminary Version
1.0	1	15/1/2001	Final Version

Table of Contents

1 Introduction 4

2 Material and Metods 4

3 Results 5

4 REFERENCES 6

5 Appendix 1: Culture medium composition 7

1 INTRODUCTION

Research performed in compartment I at the laboratories of the University of Gent have reached a state were the biodegradation of biomass from other compartments is going to be assayed. For the first tests a considerable amount or *R. rubrum* biomass is necessary, grown in the culture conditions usually employed in the MELISSA connection tests. To this purpose it was decided to obtain biomass directly from the bioreactors used for the interconnection tests located at the MELISSA Pilot Plant.

This technical note reports on the culture conditions and first productivities obtained and supplied to the University of Gent.

2 MATERIAL AND METODS

The bacterial strain used was *Rhodospirillum rubrum* (ATCC 25903) and was obtained from the American Type Culture Collection (ATCC). The strain was received freeze-dried and was revived using R8AH medium (ATCC medium 550). This medium was also used for routinely subculture of the stock strain.

The culture medium used during the tests was based on the basalt salt mixture formulated by Segers & Verstraete and was modified in order to provide the required amount of nutrients to the compartments III and IV, which were connected to compartment II. Acetic acid was used as a carbon and electron source and biotin as the only vitamin. The culture medium composition is described in Appendix 1.

The photobioreactor (Applikon ADI 1030 Bio Controller) used consists on a 2.4 L cylindrical glass vessel stirred mechanically by a Rushton type propeller. The lateral surface of the reactor was completely illuminated by 15 halogen lamps (Sylvania professional BAB 38º 12V 20W, improved version, cool beam, UV filtered, green box, code type 215). The temperature was controlled by means of a thermostatic bath, which impelled the water through the glass jacket of the photobioreactor. The pH was maintained at 6.9 by means of the auxiliary control unit of the system, which added HCl (1.5 M) or NaOH (1.5 M) depending on the deviation from the set point value.

Biomass concentration was determined measuring the dry weight and the carbon source profiles were followed during the tests by liquid chromatography analysis.

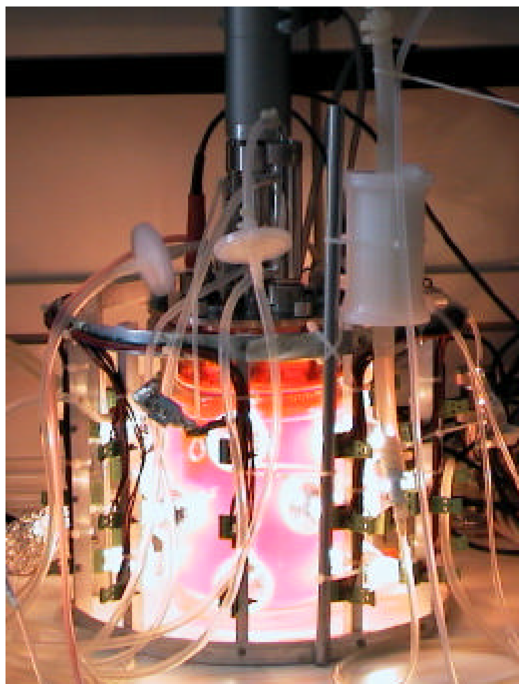

Figure 1:Applikon photobioreactor

3 RESULTS

Continuous cultures were set up according to the experimental protocol for the experiments performed in TN 37.7 (Cabello 2000). The output of the bioreactor was collected in a buffer tank and periodically centrifuged. The liquid phase obtained was used to feed compartment III bioreactors. The pellet containing the biomass, was resuspended in distilled water and centrifuged again. The supernatant was discarded, and the pellet resuspended in a small amount of distilled water. The suspension was introduced in an appropriated container and freezed by immersion in a bath containing dry ice and acetone. The freezed sample was introduced in a glass vacuum contained and connected to the freeze dryer during 24 hours. Freeze dried biomass was collected in a container and stored in a dry place.

Freeze dried biomass was collected until a total amount of 50g DW were obtained. This biomass was sent to University of Gent for biodegradation purposes. An agreement has been reached with this University to sent and equivalent amount of biomass every 6 months.

4 REFERENCES

Cabello, F.; Creus, N.; Albiol, J.; Gòdia, F. (2000) Photoheterotrophic compartment Light limitation growth cultures. Technical Note 37.7. ESTEC/CONTRACT11549/95/NL/FG.

5 APPENDIX 1: CULTURE MEDIUM COMPOSITION

Component	g/L medium
CH_3COOH	2.500
EDTA-Na· $2 H_2O$	0.100
$MnCl_2 \cdot 2 H_2O$	0.008
$FeSO_4 \cdot 7 H_2O$	0.033
KH_2PO_4	0.400
$NaHCO_3$	0.250
$MgSO_4$ $7 H_2O$	1.200
$CaCl_2$ $2 H_2O$	0.091
$(NH_4)_2SO_4$	2.728
$CuSO_4$ $5 H_2O$	$4.0 \cdot 10^{-6}$
Na_2HPO_4	0.489
$ZnSO_4$ $7 H_2O$	$4.3 \cdot 10^{-6}$
$(NH_4)_6Mo_7O_{27} \cdot 4 H_2O$	0.177
K_2SO_4	0.550
Trace elements solution	1.00 mL/L medium
Biotin solution	1.00 mL/L medium
A5 solution	1.00 mL/L medium
B6 solution	1.00 mL/L medium

Dissolutions	
A5 (g/L solution)	
H_3BO_3	2.860
$MnCl_2 \cdot 4 H_2O$	1.810
$ZnSO_4$ $7 H_2O$	0.222
$CuSO_4$ $5 H_2O$	0.079
MoO_3	0.015
B6 (g/L solution)	
NH_4VO_3	0.023
$KCr(SO_4)_2 \cdot 12 H_2O$	0.096
$NiSO_4 \cdot 7 H_2O$	0.048
$(NO_3)_2Co \cdot 6 H_2O$	0.049
$Na_2WO_4 \cdot 2 H_2O$	0.018
$Ti(SO_4)_2 + TiOSO_4$	0.048
Trace elements (g/L solution)	
$NiSO_4$ $6 H_2O$	0.500
$MnCl_2 \cdot 4 H_2O$	0.500
$FeSO_4$ $7 H_2O$	0.500
$ZnSO_4$ $7 H_2O$	0.100
$CoCl_2 \cdot 2 H_2O$	0.050
$CuSO_4$ $5 H_2O$	0.005
H_3BO_3	0.100
Na_2MoO_4 $2 H_2O$	0.050
Biotin solution (g/L solution)	
Biotin	0.015