MELISSA

PHOTOHETEROTROPHIC SUB COMPARTMENT BIOMASS PRODUCTION

PROCESS OF PHOTOBIOREACTION

"Materials and methods and subsequent analysis"

TN 29.1

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INTRODUCTION

Photoheterotrophic microorganismes, such as *Rhosdospirillum rubrum,* could be used in closed ecological system (CES) used to provide life support systems for survival of man in space. The ability of this bacteria to grow with organic acids, such as lactic acid, justify its incorporation in one of the subcompartments of the MELISSA loop. The aim of the present work is to provide biomass of *Rhodospirillum rubrum* for further studies of its suitability to human consumption. As the corresponding biomass was to be produced as soon as possible, there was not enough delay to determine the optimal conditions for continuous culture. This production of *Rhodospirillum rubrum* has to be produced by successive batch cultures.

I - DEFINITION OF THE METHODOLOGY

a - Strain used

The strain of *Rhodospirillum rubrum,* used in this work, was obtained as freeze dried cells from The American Type Culture Collection (ATCC 25903).

b - Culture media

Freeze dried cells were revived using R8AH medium, as recommended by ATCC (Appendix 1).

The bacterial biomass was produced in the Segers and Verstraete medium (Appendix 2). Lactic acid was used as carbon source with pH adjusted to 6.9+/- 0.1 with 3 M NaOH as described by Segers and Verstraete (1983). Vitamins were added to the medium after sterilization at 121°C during 20 minutes.

c - Culture conditions

Bioreactors

Precultures were performed in two bioreactors (SGS-INCELTECH and LSB-BIOLAFITTE) each with 1,8 litres working volume. Cultures were performed in bioreactors with 20 litres working volumes (pilot reactors developped by the CNAM-INTECHMER). Agitation was performed by magnetic stirrer.
All culture vessels were sparged with filter-sterilized argon during start-up.

Temperature

Stable temperature was obtained by growing the bacteria in a thermostated room with ISO 6 walls (ISOCAB), refrigerating unit, electric resistance and ventilation (Froid ElectroConfort, Tourlaville, France). We have checked that temperature inside the illuminated reactor was actually at 30°C +/- 1°C.

pH

pH was continuously controlled and adjusted through automatic (peristaltic pumps) addition of a 1 M NaOH solution. The pH was regulated at 6.8+/- 0.1 with a biocontroller ADI 1030 equiped with four pH sensor interface (APPLIKON). Sensors were combined INGOLD electrodes sterilized by autoclave.

Illumination

Illumination was provided by fluorescent lamps (TLE 32W and TF30 MAZDAFLUOR) installed symetrically arround the culture vessels. Photon flux density (PFD) of 300 µmoles quant.m-2.s-1 was obtained by adjusting the number and the proximity of the fluorescent tubes. PFD was controlled and adjusted at the set-up of the cell culture unit. Measures were done in the middle of the culture vessels in the presence of the call free culture medium. PFD was determinated with the Li 193 spherical sensor. The light path between the point of the measure and the light source was 20 cm.

Anaerobiose

Anaerobiose was obtained by flushing the medium with Argon during 10 minutes and was maintained during all the batch culture.

d - Culture parameters control.

The pH of the medium was controlled before sterilization with a SCHOTT CG 825 pH meter.

Temperature of the bioreactor room was recorded by ORPHY GTS unit connected to SONDOR software within personnal computer (DELL 316SX)

Photon Flux Densities (PFD) in the bioractors were measured with a spherical LI-193 4Ò sensor (LI CORR) for Photosynthetic Active Radiations (PAR 400-700 nm). The sensor connected to a LI 1000 data loger (LI CORR) was placed in the middle of reactor and the PFD were measured when full of culture medium but free of bacterial cells.

Biomass in the bioreactors was controlled by measuring the absorbance of samples at 700 nm with a U 2000 spectrophotometer (HITACHI).

II - ANALYTICAL METHODS

Biochemical composition of the final biomass will be characterized by different methods.

a - Total proteins

Protein analysis will be performed according the method of Lowry *et al.* (1951) modified as described by Stoscheck (1990).

b - PHB

PHB content was determined using the Law and Slepecky method modified according Albiol (1994).

c - Glycogen

Glycogen will be extracted using the Palmsternia modified method and the glucose obtained analysed according the phenol method (Albiol, 1994).

d - DNA

DNA content will be determined in bacterial extracts according the colorimetric method of Burton (1956).

III - REFERENCES

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APPENDIX 1 : R8AH medium (g/l)

$Mg\ SO_4\ 7H_2O$	0,2
$CaC_{l2}\ 2H_2O$	0,07
EDTA	0,02
KH_2PO_4	0,6
K_2HPO_4	0,9
$(NH_4)^2SO_4$	1,25
Yeast extract	1
Malic acid	2,5
Fe-citrate	0,01
Agar	20
Trace elements	1 ml
Vitamines	1 ml

Trace elements:

ZnSO4 7H2O	0,001
$CoC_{l2}\ 2H_2O$	0,02
$CuSO_4\ 5H_2O$	0,001
H3BO3	0,001
$MnSO_4\ H_2O$	0,002
$(NH_4)6Mo7O27$	0,002
EDTA	0,05

Vitamines:

Nicotinic acid	0,2
Nicotinamide	0,2
Thiamine HCl	0,4
Biotin	0,008

APPENDIX 2 : Segers and Verstraete medium.

$Mg\ SO_4\ 7H_2O$	0,2
$CaC_{l2}\ 2H_2O$	0,05
$FeSO_4$	0,02
$MnC_{l2}\ 4H_2O$	0,01
EDTA	0,02
KH_2PO_4	1,2
$K2HPO_4$	1,8
$(NH4)2SO_4$	0,5
$CH3CH_2COOH$	2,7
Trace elements	1 ml
Vitamines	1 ml

Trace elements:

$ZnSO_4\ 7H_2O$	0,1
$CoCl2\ 2H_2O$	0,05
$CuSO_4\ 5H_2O$	0,005
$H3BO_3$	0,1
$MnCl2\ 4H_2O$	0,5
$(NH_4)6Mo7O27$	0,002
EDTA	0,05
$NiSO_4\ 6H_2O$	0,5
$Na2MoO_4\ 2H_2O$	0,05

Vitamines:

Nicotinic acid	1
Thiamine HCl	1
Biotin	0,015