STUDY FOR POLY[(R)-3-HYDROXYBUTYRATE] PRODUCTION BY MICROORGANISMS FROM NATURAL WATER SAMPLES

A. M. Rodríguez[1], M. Koller[2],M. Calafell[3], M.S. Marqués-Calvo[1],

1 Departamento de Óptica y Optometría de Terrassa. Technical University of Catalonia,
2 Institute of Biotechnology and Bioprocess Engineering, Graz University of Technology,
3 Departament d' Enginyeria Química. Technical University of Catalonia

ABSTRACT

The main objective of the work was the study for an optimal production and extraction of PHB by isolated strains from natural water samples. A previous work was carried out in Barcelona (UPC-Terrassa) to isolate the bacteria from Bolvian water samples and a total of 49 different strains were isolated. The work carried out in Graz (TU Graz- Institut für Biotechnologie und Bioprozesstechnick) was the study for the optimization of the production, accumulation and extraction of biopolymer by two of these isolated bacteria (strains 46 and 2).

It was important to take into account, the fact that raw material claim the major part of biopolymer production cost, and that is why the study of optimal conditions for PHB production by strains 46 and 2 was carried out testing Küng media with different substrates as sucrose, fructose, glucose, xylose, maltose, starch, lactose, maltose, lactose and arabinose as carbon source, and nitrogen as growth-limited factor.

The results indicate that Küng media is a good media to produce and accumulate polymer in strain 46, especially by using xylose, maltose and glucose as carbon source. It was also found that Küng media is not the best one to obtain polymer from strain 2. Media HM, a salty media with 4,45% NaCl and sucrose as carbon source, was used in initial experiments and it was found that the bacteria from strain 2 is able to accumulate polymer in this conditions.

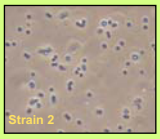
INTRODUCTION

Polyhydroxybutyrate (PHB) is one of the most important and studied PHAs, intracellular carbon and energy reserve materials accumulated by a variety of microorganisms. It is very promising as a biodegradable and biocompatible polymer and it has immense applications, especially in the biomedical field. These biopolymers are of great interest because they can be a good alternative to replace petrol-polymers.

The work at hand is a report of the first results obtained from the study carried out with two wild strains isolated from Bolivian water samples. Strains 46 and 2 were tested in order to study the optimal way to produce, accumulate and extract polymer from bacteria cells.

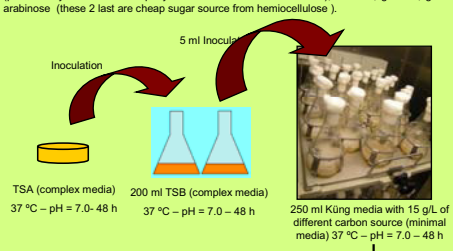
Picture of the Uyuni lake, where the strain 46 comes from.

Picture of the Laguna Colorada, where the strain 2 comes from.



MATERIALS AND METHODS

Küng media was used for fermentation strategy: I) phosphates (pH buffers and phosphate source), II) sulphates with (NH4)SO4 as limited nutrient, III) trace elements (salts as $CaCl_2.2H2O$, $NH_4Fe(III)$citrat among others), IV) nine different carbohydrates: Sucrose (provided by a Brazilian company and a economical raw material), fructose, glucose, galactose, maltose, lactose, starch, xylose and arabinose (these 2 last are cheap sugar source from hemiocellulose).



10 ml samples were taken at different times: immediately after inoculation, 22 and 48 hours after inoculation.

Optical density (OD)– pH

Freeze-Drier

Dried Biomass after lyophilize

Centrifuge 4000 rpm-10 min

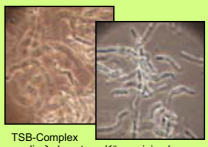
Media→ Determination of free amino groups (NH_4^+) and determination of carbohydrates concentration with HPLC.

Pellet→ Cell dry mass (CDM) determination and PHB analysis (transterification and GC measurement)

Isolation of PHA: The cells in media were centrifuged and the pellet was frozen (-70 ºC) and lyophilised for 24h. The polymer was extracted with chloroform and precipitated with methanol.

RESULTS AND COMENTS

Strain 46 :



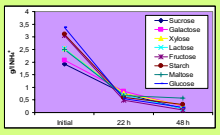
TSB-Complex media→ do not show polymer granules.

Küng-minimal media with sucrose after inoculation

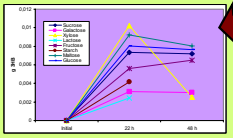
Küng-minimal media with sucrose after 48 h fermentation→ it has polymer granules (white cycles).

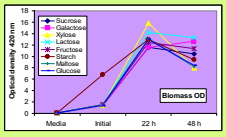
PHB production in Küng media with different carbon sources: +++ good production,++ normal production and + low production.

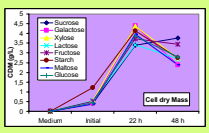
Sugar Source	Incubation time (h)	PHB Production
Sucrose	48	+++
Galactose	48	++
Xylose	24, 48	+++, +
Lactose	24	++
Fructose	48	+++
Starch	24	++
Maltose	48	+++
Glucosa	48	+++



The nitrogen was the limited compound and the bacteria consume it during the growth phase of the fermentation.





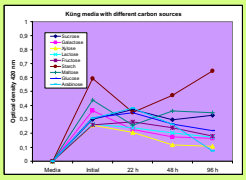


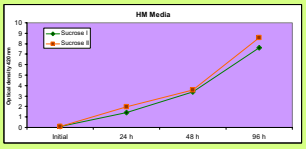
Evolution of the biomass during fermentation→ Biomass concentration and OD increase with fermentation time (OD is related with polymer accumulation)

Strain 2:

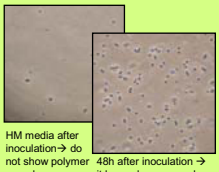
Bacteria did not grow properly in this media as OD results show.

New experiment was carried out using a salty media, HM (4,45% NaCl and sucrose as carbon source).



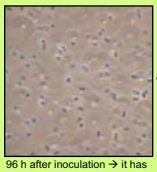


The increase on OD measurements show that the strain 2 grows properly in this media and although the OD do not demonstrate the formation of PHA, in following pictures it can seen the PHA granules and how they increase with fermentation time (C).



HM media after inoculation→ do not show polymer granules.

48h after inoculation → it has polymer granules.



96 h after inoculation → it has bigger polymer granules.

CONCLUSIONS

- The results indicate that Küng media is a good one to produce and accumulate polymer in strain 46, especially by using xylose, maltose and glucose as carbon source. It could be interesting go on studying the possibilities of PHA production with this media with cheaper carbohydrate subtracts, but it was found that this strain sporulates and it makes difficult the use of this strain on further studies of industrialization → sporulation and PHA formation are concurrent processes and spores are known to be very hard to be removed from the bioreactor equipment.
- It was also found that Küng media is not the best one to obtain polymer from strain 2. Media HM, a salty media with 4,45% NaCl and sucrose as carbon source, was used in initial experiments and it was found that the bacteria from strain 2 is able to accumulate polymer in this conditions, but further studies should follow to found the optimal way to produce and accumulate polymer

Proyecto financiado por el Ministerio de Educación y Ciencia de España (CICYT: MAT2006-05979)