**Polyhydroxybutyrate (PHB) production**

**by methanotrophic consortia under high methane atmosphere**

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**Highlights**

* Selection of four methanotrophic consortia highly resistant to methane.
* Methane consumption confirmed through pMMO activity analyzed by PCR.
* PHB production of 15% (w/w) under 70% methane atmosphere

**1. Introduction**

Methane gas is abundantly present in the environment due to its both natural and anthropogenic production. However, methane is a harmful gas since it is one of the causes for the greenhouse effect. Its production has been increasing 10 ppb per year [1] and since methane has a greenhouse warming potential (GWP) 28 times higher than carbon dioxide [2], it is necessary to search for alternatives to mitigate it from the atmosphere. The use of methane as substrate for biodegradable plastics production would be an alternative to solve another issue of public awareness: the damage caused by the increasing production of non-biodegradable plastics. Methanotrophic bacteria are ubiquitous in the environment and have the ability of producing polyhydroxybutyrate (PHB) from methane under specific conditions [3]. PHB is a biodegradable plastic that still presents high production costs [4] and using methane as feedstock for biopolymer production could make the price competitive for the commercial market.

**2. Methods**

Mangrove sediment samples were collected and enriched with methane concentrations in the atmosphere increasing from 20% to 70% (v/v) during 40 days. The enrichment was performed using closed flasks coupled with needles and hoses to perform atmosphere change every seven days. From these samples, four different consortia were selected and analyzed by molecular biology through PCR reaction to confirm the methane monooxygenase (pMMO) activity and methane consumption. These consortia were tested for PHB production with N-free NMS during 30 days under 28 °C and 180 rpm. By the end of the experiment, PHB was extracted from inside bacterial cells as described in [5], collected and characterized by magnetic nuclear resonance (NMR).

**3. Results and discussion**

From mangrove samples, four consortia were selected and named MC, MG1 SED, MG2 and MG2 SUB. PCR analysis of the pmoA gene were performed and resulted positively for all the consortia. The gene pmoA is responsible for pMMO enzyme which oxidizes methane to methanol in the first step of methanotrophic metabolic pathway, confirming the methane consumption. PHB production was tested in closed flasks under 70% methane atmosphere in air (v/v). Atmosphere was changed every seven days to ensure the availability of high methane concentration inside the flasks. Results of PHB production reached nearly 15% of PHB (w/w) for all the consortia (Figure 1). Extracted PHB was collected and characterized by RMN to confirm the chemical characterization.

**Figure 1.** Biomass, PHB production and %PHB (w/w) of the consortia under 70% methane atmosphere after 30 days.

**4. Conclusions**

Four methanotrophic consortia highly resistant to methane were selected from environmental samples. All of them presented the pmoA gene, ability to consume methane and to produce PHB. PHB production reached 15% (w/w), which is a promising result when considering it has been made using a one-carbon molecule as feedstock.

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