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The future of the climate: how can we tackle climate change?

Bioplastic from Methane

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1 Background

“Human influence on the climate system is clear. This […] is evident from the increasing greenhouse gas concentrations in the atmosphere (…). Continued emissions of greenhouse gases will cause further warming and changes in all components of the climate system. Limiting climate change will require substantial and sustained reductions of greenhouse gas emissions” [1].

After water vapour and carbon dioxide, methane has the greatest impact on climate change [2]. According to the report of the Intergovernmental Panel of Climate Change (1995) the atmospheric concentration of this methane has more than doubled since the pre-industrial times around 1750 A.D. [3]. The amount of methane released into atmosphere is 520 teragrams/year, which is 10^9 kg methane/year [4]. The major production is caused by human activities, including the use of fossil resources, landfills and agriculture [3].

Additionally, over 140 million tons of petroleum-based plastic is produced worldwide each year [5]. In contrast to their rapid manufacturing, plastics, such as polyethylene and polystyrene, remain 2,000 years or even longer in the environment [5]. The plastic production itself pollutesthe environment with hazardous chemicals and greenhouse gases (GHGs) [6].

Microorganisms also contribute to the release of GHGs by degrading 50% of the total carbon into methane [7]. But apart from organisms producing methane there are also bacteria utilizing methane as their carbon source to grow. These microorganisms are called methanotrophs, what might be translated into “getting its nourishment from methane”.

2 Characterization of methanotrophs

Methanotrophic bacteria are capable of using single-carbon compounds, such as methane, as their sole carbon source [8]. Methanotrophs depending on oxygen, the so-called aerobic methanotrophs, grow wherever single-carbon compounds and oxygen are available. Hence they can be found in forest soils, grasslands, landfills, rice paddies, marine soils and other environments with excess of methane [9]. Under aerobic conditions these bacteria oxidize methane to formaldehyde via four steps. Since methane is the most inert hydrocarbon the first step of methane oxidation requires a lot of energy [10]. The enzyme capable of breaking the bonds of this unreactive molecule is the methane monooxygenase (MMO). It occurs in two forms, either as a particulate enzyme (pMMO) bound to a membrane or as a soluble compound (sMMO) of the cell´s cytoplasm [8]. The expression of pMMO takes place in most methanotrophic bacteria and is induced by copper [8]. In contrast, sMMO is only expressed in some strains and the expression is favoured by the absence of copper [8] (**Figure 1**).

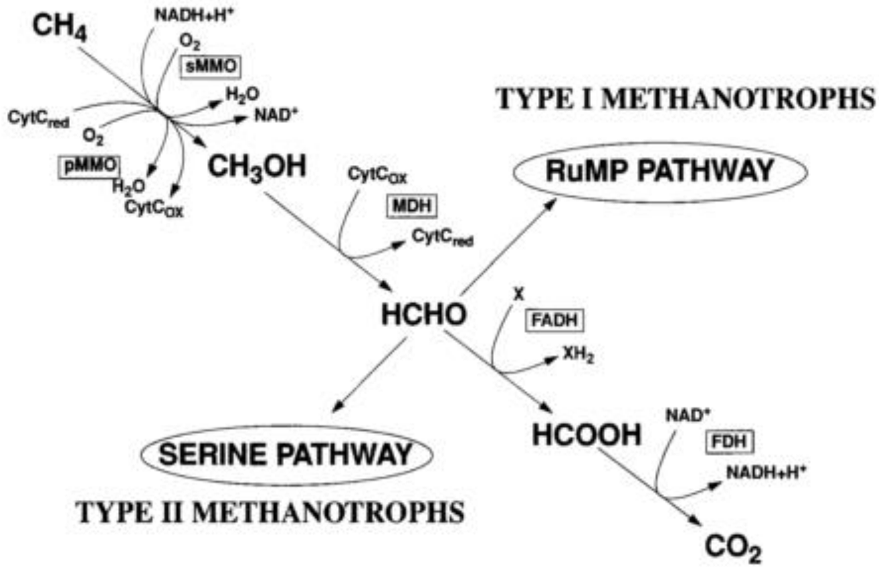

Figure 1 Pathways in methanotrophic bacteria to assimilate formaldehyde [9]

Formaldehyde is then incorporated into the microbial metabolism. At this point, methanotrophs can be distinguished into type I, employing the ribulose-monophosphate pathway (RuMP), and type II, which utilizes the serine pathway [9].

Apart from the carbon source another important factor for the growth of methanotrophs is nitrogen, which is essential for proteinbiosynthesis and other metabolic conversions. In particular, the presence of nitrogen in form of nitrate or ammonia favors the microbial growth since their uptake is easy for the microorganisms [11]. In addition to the two pathways for formaldehyde inclusion, methanotrophs also differ in their ability to use different nitrogen sources. Type II methanotrophs possess the genes encoding for nitrogenase which enables them to fix atmospheric nitrogen, whereas the genome of type I does not contain these genes [11]. In conclusion, type II is far less dependent on easily accessible nitrogen sources than type I.

Under excess of carbon and limitation of another nutrient some methanotrophs are capable of carbon accumulation in form of polyhydroyxalkanoates (PHAs). PHAs as a carbon storage is a huge advantage over other microorganisms during a shortage of nutrients [12]. Those enormous intracellular molecules can make up to 80% of the cell´s dry weight [13] and are even visible under a microscope (**Figure 2**).

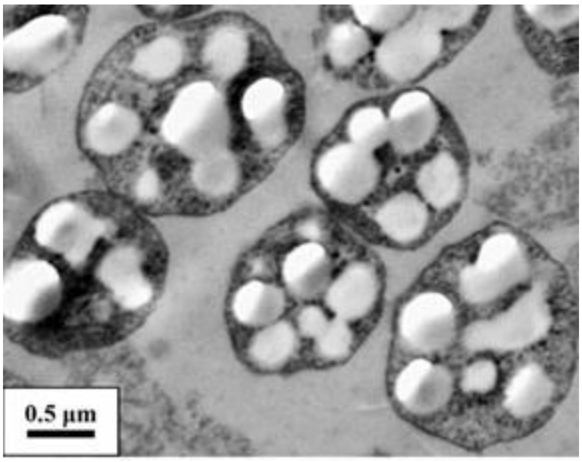

Figure 2 PHA granules accumulated in cells
(http://www.im.ac.cn/ketizu/XiangH/PHA.jpg, 02.07.15)

Additionally to their importance for the bacteria, PHAs belong to biologically produced polymers, which can be used as a bioplastic.

3 Polyhydroxyalkanoates

3.1 Characterization of polyhydroxyalkanoates

PHAs are polymers, large molecules composed of single units also referred to as monomers. These monomers are (*R*)-hydroxy fatty acids consisting of different numbers of carbon atoms (C atoms). Up to 30,000 fatty acids can be connected to a PHA by ester bonds [13] (**Figure 3**). The length and the functional groups of the side chains effect the physical properties of the entire polymer to a great extent [6]. More than 150 PHA-monomers exist and depending on their structure and their alignment the properties of the large polymer differ completely [13].

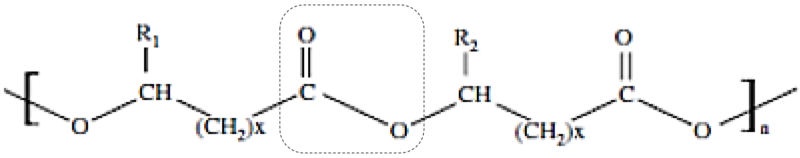

Figure 3 general molecular structure of PHA [6]
R = side chains, $C_1 – C_{13}$; X = 1 – 4; n = 100 – 30,000
frame signalizes an ester bond connecting two fatty acids (inserted by autor)

A very well studied PHA is poly(3-hydroxyburyrate) (PHB), composed of butyric acid monomers. It was firstly discovered in 1926 by the French chemist and bacteriologist Maurice Lemoigne [5]. PHB shows useful properties such as the insolubility in water, a high degree of polymerization and a melting point at 180 °C. Furthermore it is non-toxic to mammalian cells and due to its intracellular synthesis it shows stereoregularity and high purity [6]. But PHB is also brittle, has poor elastic properties and is thermally unstable due to its decomposition temperature, which is close to its melting temperature [6].

Depending on the application the polymers´ characteristics can be changed by blending PHB with other monomers, for instance with 3-hydroxyvalerate (3HV) or 3-hydroxyhexanoate (3HHx). The best known blend is composed of 3-hydroxybutyrate (3HB) and 3HV leading to the copolymer poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) [14]. Subject to the condition that the bacteria show a natural ability to accumulate 3HB and 3HV, the intracellular synthesis of PHBV can be achieved by growing the bacteria on specific substrates and under certain conditions, which favour PHBV production [14]. Not every PHA producing methanotrophic strain possesses the genes for 3HB synthesis, but most of type II methanotrophs are capable of PHB accumulation.

3.2 Applications of polyhydroxyalkanoates

Due to their broad spectrum of chemical components the applications of PHAs are widespread. Depending on the physical properties these biopolymers can be used in packaging and for medical purposes as well as in agriculture and fishing industry [6].

To state a few applications, PHAs can be utilized for paper coatings, foils and films and also for disposable products such as shampoo bottles or other cosmetic articles. Moreover, these biopolymers can either be applied as a hot-melt adhesive, a latex paint or even a fabric. Since PHAs are small biodegradable granules they can serve as carrier molecules releasing chemicals slowly into their environment. In terms of dispensing herbicides and insecticides in agriculture this application is potentially very useful [6].

Particularly due to their non-toxicity to mammalian cells and their biodegradability, PHAs have various applications in medical and pharmaceutical industries. Interestingly, the monomer 4-hydroxybutyrate (4HB) is a natural intermediate in the human metabolism present in brain, heart, lung, liver, kidney and muscle [15]. That is why 4HB can be used to treat cataplexy attacks in people having narcolepsy [16]. Other medical applications of PHAs include the utilization for sutures, heart valves, bone fixations and bone marrow scaffolds [6]. Fabrics serving for wound dressing can be produced from PHB fibres, which are also considered as cartilage tissue for implantation [6]. In combination with other polymers, such as collagen, polylactide (PLA) and polyglycolide (PGA), PHAs is even applied in regenerative medicine [6].

In summary, PHAs can replace many petrochemical products in various areas holding the great advantages of being biologically produced, biocompatible and biodegradable.

4 Fermentation of methanotrophs

4.1 Advantages of methane as a feedstock

Natural carbon sources for PHA producing bacteria are wheat bran, whey, molasses, starch, vegetable waste and oil, ethanol, methanol, sucrose, glucose, organic acids etc. [17]. All these materials are food- or oil-based and are commonly very expensive. The cost for the carbon source in a fermentation of methanotrophs can be up to 40% of the total operating cost, which makes it very inefficient for companies to produce PHA [18]. Apart from the elimination of a GHG, another huge advantage of growing PHA producing methanotrophs is the possibility to use a cheap feedstock. Methane, as an end product of many natural and industrial processes, is plenteously available and costs only about \$0.15 – \$0.30/kg [19]. The conversion of methane to PHA, costing US\$4 – 6/kg [6], holds great potential for establishing large-scale processes for industrial PHA production.

4.2 Conditions of cultivation

Methanotrophs growing on methane are cultivated in the aqueous phase of bioreactors providing them with the required nutrients in the growth medium as well as in the gaseous headspace of the reactor.

The medium supplies various nutrients including vitamins, salts and trace elements, such as copper. Since the pMMO is only active in the presence of copper, this metal can be utilized as a factor controlling the expression of pMMO or sMMO. Depending on the culture the nitrogen source can also act as a selection pressure. As mentioned previously, type I methanotrophs are not capable of fixing atmospheric nitrogen. Hence the supply of dissolved nitrogen is essential for them, which can be implemented by adding ammonia or nitrate to the medium [9]. If PHB accumulation is to be obtained, type II methanotrophs need to be enriched, which can be achieved by supplying atmospheric nitrogen [20].

Around 78% of air consists of atmospheric nitrogen, hence flushing the reactor´s headspace with air once in a while ensures enough nitrogen for the microbial community. Since methanotrophs are aerobic bacteria, they require oxygen, which is also available in air [21]. The gaseous substrate methane can be added additionally into the headspace. Thus the essential substrates methane, oxygen and in some cases nitrogen are gaseous with low solubility in the aqueous phase [8]. In conclusion, a high diffusion rate of these gases from the headspace into the medium needs to be ensured. This can be done by providing a sufficiently large surface area of the liquid and shaking the reactor at a low mode.

PHA accumulation can be achieved by limiting one nutrient apart from methane. During the process of fermentation the nutrients provided will be utilized by the bacteria. The amount of required nutrients can be calculated once the amount of biomass in the reactor and the biochemical equations of PHA synthesis and biomass synthesis are known. Running out of one nutrient while still having the other nutrients available, will cause the bacteria to start accumulating PHA. Thereby, it is extremely important to ensure a continuous supply of methane.

5 Biodegradation of polyhydroxyalkanoates

In terms of sustainability the most attractive property of PHAs is their biodegradability. Various aerobic and anaerobic bacteria as well as fungi capable of degrading PHAs have been isolated, excreting the enzyme responsible for degradation [22]. The extracellular depolymerase can split the ester bonds between the single units, generating water-soluble monomers and oligomers. It should be mentioned that extracellular depolymerases differ from intracellular depolymerases. Inside the cells PHAs remain mobile and non-crystalline, but as soon as the polymers are recovered they denature and become more crystalline. Hence, intracellular depolymerases can only degrade non-crystalline PHAs, whereas extracellular depolymerases can break down polymers outside the cells. The monomers can then be used by the bacteria as a carbon source.

The degradation rate of PHB and PHBV was investigated by various groups. PHBV was entirely degraded after 6, 75 and 350 weeks in anaerobic sewage, soil and sea water, respectively [22]. Hence, this bioplastic can be removed completely from the environment, whereby the degradation rate depends on the microbial population, the environmental factors, the temperature and the polymer properties [22].

6 Conclusion and future prospects

According to Akaraonye et al. (2010) the production of biodegradable polymers is still less than 1% of the world´s annual production of polymers [6]. But the establishment of large-scale processes using cheap feedstocks makes it more attractive for industry to produce biopolymers. Methane is plenteously available and much cheaper than other carbon sources. The capability of some methanotrophs to incorporate methane into their metabolism and accumulate the carbon in form of PHA is an environmentally friendly way to produce plastic. The use of an infinite and renewable carbon source is not only sustainable but will also help to mitigate the greenhouse effect by removing one of the GHGs from the atmosphere.

In summary, the production of PHA by methanotrophs with methane as a feedstock holds great potential in terms of both eliminating GHGs and finding a permanent alternative to petrochemically produced plastics for various applications.

Although methanotrophs were discovered over a century ago, their biodiversity is enormous and a lot of research needs to be done in terms of their genomics and proteomics to fully understand their pathways, requirements and capabilities [8]. This provides work for a constructive cooperation of biotechnologists, microbiologists, geneticists, chemists, chemical engineers, polymer scientists, medical scientists and government officials [22]. But the rapid development of novel biotechnological methods will improve the utilization of these organisms to a much wider extent and might also reveal totally new engineering applications.

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