Development of a closed-loop control system for production of medium-chain-length poly(3-hydroxyalkanoates) (mcl-PHAs) from bacteria

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Abstract

Large scale availability of bacterial polyhydroxyalkanoates (PHAs) is still limited to a few types of short-chain-length PHAs, namely poly(3-hydroxybutyrate) (PHB) and its copolymer Biopol™, consisting of 3-hydroxybutyrate and 3-hydroxyvalerate repeating units. In order to increase the number of available medium-chain-length PHA (mcl-PHA) copolymers a flexible high-cell-density fed-batch process was developed. Continuous process monitoring and substrate control were achieved by coupling on-line gaschromatography (on-line GC) to a software-based Proportional Integral (PI) substrate controller. System development time and continuous system upgrading were considerably shortened by using *LABV*iew™, a powerful graphical programming environment. The control of octanoic acid and 10-undecenoic acid at 1.5 and 0.5 gL^{-1} respectively, enabled the production of high levels of biomass (30 gL^{-1}) and mcl-PHA (10.5 gL^{-1}) by avoiding substrate limitations or toxicities. The resulting mcl-PHA was an amorphous copolyester consisting of 37 mol% unsaturated monomers. The present system represents a valuable tool for the production of tailor-made mcl-PHAs, where the desired monomer composition is determined by the ratio of added cosubstrates.

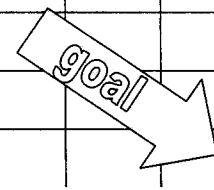
Introduction

Medium-chain-length poly(3-hydroxyalkanoates) (mcl-PHAs) are bacterial polyesters currently receiving much attention because of their potential application as biodegradable and biocompatible elastomers [1]. Furthermore, due to the broad substrate specificity of its enzymatic polymerization system, mcl-PHAs offer unique opportunities for the synthesis of tailor-made copolymers. In this respect, the monomer composition can be controlled by the addition of substrates to the culture medium [2]. However, the scarce availability of these mcl-PHAs at gram and kilogram scales may represent a major bottleneck for testing and development of novel materials (Table 1). Therefore, our research focuses on the development of a

flexible bioprocess for the production of mcl-PHA copolyesters from bacteria. Special attention should be paid to control the monomer composition and thus the resulting material properties.

Tab.1. Availability of bacterial PHAs

Amount Type	Analytical Scale (mg)	Test Scale (10 g)	Application Scale (100 g)	Pilot Scale (1 Kg)	Technical Scale (tons)
Short-chain PHAs (PHB, BIOPOL™)	√	√	√	√	√
Aliphatic mcl-PHAs	√	√	√	√	
Olefinic mcl-PHAs	√	√	√		
Substituted mcl-PHAs	√	√			
Halogenated mcl-PHAs	√				
4-, 5-, and 6-OH-mcl-PHAs	√				



Process development

In fed-batch fermentation processes control of the supply rate of carbon sources is very important, especially when two or more carbon sources are supplied concurrently, in order to tailor the PHA monomer composition and the resulting physical properties. In a first step, we monitored substrate levels in the culture supernatant by on-line GC. This provided the basis for the development of sophisticated process control strategies. In a second step, we used the graphical programming environment *LAB*View™ (National Instruments, Austin, Texas) to program a PI based control loop and to automatically compensate for substrate depletion, maintaining substrate levels within the desired values. The process set-up is described in Figure 1 [3].

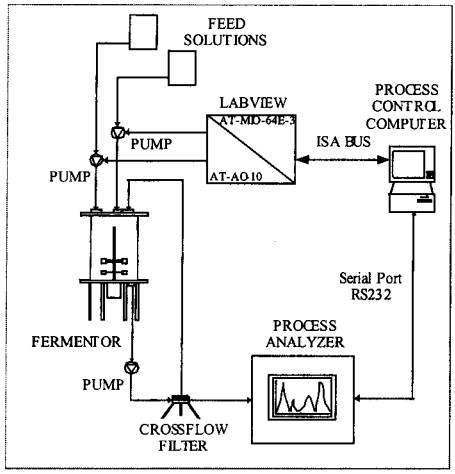


Fig. 1. Schematic process layout for the production of tailor-made mcl-PHA copolymers. The process analyzer and the process computer communicate through the RS232 serial port. The sampling rate is set to 10 minutes.

Production of tailor made mcl-PHAs

The process outlined above was used for the production of mcl-PHAs at laboratory scale. *Pseudomonas putida* KT2442 was cultivated in a 3 L bioreactor using a fed batch process in which the feed rates were controlled by closed loop control of the substrate concentration in the culture supernatant. Octanoic acid (OA) and 10-undecenoic acid (UE) concentrations fluctuated around 1.5 and 0.5 gL^{-1} respectively, preventing the culture from undesired substrate limitations and toxic effects due to overfeeding. The application of this feeding strategy to this particular PHA production process resulted in high mcl-PHA and biomass concentrations of 10.4 and 30 gL^{-1}, respectively. The resulting PHA monomer composition was analyzed by Gas-Chromatography/Mass-Spectrometry (GC-MS) and reflected the incorporation of both substrate molecules. Closed loop controlled feeding of the second cosubstrate UE, which was started after 25 h of cultivation, resulted in an

increasing amount of mono-unsaturated repeating units over time (Table 2). At the end of the cultivation UE accounted for 39 mol% of the total substrate feed, which resulted in 37 mol% unsaturated monomers in the PHA.

Tab.2. Monomer composition and fermentation data at different times [3].

	[PHA][a]	PHA Monomer composition [mol%][b]						C=C feed[c]	C=C PHA[d]
Time [h]	gL^{-1}	C6	C8	C10	C7:1	C9:1	C11:1	mol%	mol%
21.75	0.4	11	88	1	0	0	0	0	0
26.5	1.4	9	74	2	9	4	2	13	15
36.75	6.0	8	61	2	7	16	6	25	29
48.75	10.4	6	54	3	4	21	12	39	37

[a] volumetric PHA concentration
[b] C6: 3-hydroxyhexanoic acid; C8: 3-hydroxyoctanoic acid; C10: 3-hydroxydecanoic acid; C7:1: 3-hydroxy-6-heptenoic acid; C9:1: 3-hydroxy-8-nonenoic acid; C11:1: 3-hydroxy-10-undecenoic acid
[c] molar ratio UE in substrate feed
[d] molar ratio of monounsaturated units in polymer

Conclusions

It has been shown that closed-loop-control of the carbon sources is a very valuable tool for the production of mcl-PHAs in high-cell-density fed-batch processes. An automated process feeding strategy was developed which enabled the production of mcl-PHAs containing unsaturated repeating units. The content of unsaturated monomers reflected the fraction of 10-undecenoic acid which was fed to the reactor. The system will be applied for the production of a wide range of tailor-made PHAs.

References

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