

Using a cyanobacteria *Synechocystis* sp. to produce the biopolymer polyhydroxyalkanoate (PHA) by mixotroph cultivation

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Highlights

A cyanobacterium uses mixotrophic pathway to capture CO₂ & biosynthesis PHA. PHA prod. peaked (26% w/v) at 8 days, [Ac] = 0.3% w/v & [P] = 10 mg L⁻¹. More acetate & shorter times enhance PHA yield.

Abstract

Polymers, a widely used material in modern society, continue to heavily rely on petroleum and its derivatives, which are highly polluting and challenging to decompose. To address this issue, the world needs to adopt greener alternatives, such as polyhydroxyalkanoates (PHA). These polymers degrade in a matter of months in soil and can be used as standalone materials or blended with other environmentally-friendly polymers.¹ The widespread adoption of PHA could reduce the accumulation of waste from disposable plastics and packaging, thus promoting a cleaner environment. The present work focuses on a newly isolated cyanobacteria, *Synechocystis* sp. B12, which was obtained from a contaminated mangrove in Santos, SP.² Using cyanobacteria are particularly attractive for its ability to produce PHA through mixotrophic pathways, making it a promising option for capturing CO₂ (via photo-autotrophic) and converting it into a biopolymer. Additionally, this cyanobacterium could be cultivated using agro-industrial waste as a nutrient medium. To optimize PHA production, the study utilized two strategies combined: *feast and famine* and experimental planning. The *feast and famine* approach involved growing the cyanobacteria in two phases: an abundance of nutrients (*feast*) and nitrogen-free medium with limited phosphate availability (*famine*), both under atmospheric aeration and artificial light (fig 1). The experimental planning (table 1 and 2) involved manipulating three factors: incubation period (2, 8, and 14 days), sodium acetate (Ac) concentration (0.2%, 0.3%, and 0.4% w/v), and potassium phosphate (P) concentration (0, 10, and 20 mg L⁻¹). The best conditions for PHA production were determined based on the yield relative to total dry mass. The optimal results were found to be at the center of the experimental design (8 days, sodium acetate concentration of 0.3%, and potassium phosphate concentration of 10 mg L⁻¹), with a yield of 26.0 ± 3.6%. The study also noted two trends: increased acetate concentration and shorter incubation periods resulted in higher PHA production. Further investigation is underway to consider other factors, such as light intensity and alternative substrates.

Table 1. Experimental Design

#	Acetate	Potassium phosphate	Incubation	Yield
1	-1	-1	-1	9%
2	1	-1	-1	15%
3	-1	1	-1	5%
4	1	1	-1	18%
5	-1	-1	1	4%
6	1	-1	1	6%
7	-1	1	1	3%
8	1	1	1	3%
9	0	0	0	23%
10	0	0	0	30%
11	0	0	0	25%

Table 2. Experimental Factors

Variable	Level		
	-1	0	1
Acetate (% w/v)	0,2	0,3	0,4
K ₃ PO ₄ (mg L ⁻¹)	0	10	20
Incubation (days)	2	8	14



Fig. 1 – Incubation system

¹Carpine R, Olivieri G, Hellingwerf K, et al; *Processes*. 2020, 8, 1-23.

²Gracioso L, Bellan A, Karolski B, Cardoso L, et al; *Bioresource Technol.* 2021, 320, 124379.

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