Process Biochemistry 161 (2026) 283–289

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

Valorization of polyester wastes into polyhydroxyalkanoates via a one-step microbial fermentation process

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ARTICLE INFO

Keywords:
Polyhydroxyalkanoates (PHA)
Poly(3-hydroxybutyrate) (PHB)
Paracoccus denitrificans
Polyesters
Monomers
Plastic bio-upcycling
Plastic waste

ABSTRACT

Polyhydroxyalkanoates (PHAs) are promising biodegradable alternatives to petroleum-based plastics, yet their large-scale application remains hindered by high production costs. Here, a direct and cost-effective microbial strategy to upcycle polyester waste into PHAs using *Paracoccus denitrificans* was investigated. Remarkably, the bacterium metabolized 10 out of 12 tested plastic-derived monomers and efficiently accumulated intracellular PHAs, even when fed with pretreated mixed polyester waste instead of purified substrates. More than 80 % of polyester monomers supported growth, and medium optimization through nitrogen reduction boosted PHA content up to 30 % of cell dry weight. This one-step process circumvents costly separation steps, enabling the valorization of heterogeneous plastic mixtures and significantly reducing both economic and processing burdens. By transforming post-consumer plastics into high-value biopolymers, this work positions *P. denitrificans* as a versatile platform for circular bioeconomy strategies, directly supporting global sustainability agendas and advancing sustainable bioprocessing for the green industry.

1. Introduction

Plastics are essential in modern consumer goods, yet the lack of biodegradability of conventional petroleum-derived polymers has led to severe environmental accumulation. Although aliphatic polyesters derived from succinic acid (SUC), lactic acid (LAC), adipic acid (ADI), or glycols have been developed as sustainable alternatives, their copolymerization with aromatic monomers hampers biodegradation [1]. In natural environments, plastic breakdown is slow and dependent on environmental factors [2], while physical and chemical treatments accelerate degradation at the expense of high energy demands and secondary pollution [3]. Biological approaches such as composting or anaerobic digestion remain ineffective for most conventional plastics [4], highlighting the urgent need for innovative valorization strategies.

Polyhydroxyalkanoates (PHAs) are natural microbial polyesters that combine biodegradability and biocompatibility with mechanical properties comparable to petrochemical plastics, enabling applications in packaging, agriculture, and medicine [5]. Their ability to undergo complete mineralization to CO_2 and H_2O under aerobic conditions, or to CH_4 and CO_2 under anaerobic methanogenic conditions, makes them attractive candidates for sustainable replacements [6,7]. Yet, PHA production remains costly, with carbon sources accounting for over half of total expenses [8]. Alternative bioprocesses have attempted to reduce costs through two-step conversions of organic waste into volatile fatty acids (VFAs) followed by PHA synthesis [7,9], closed-loop recycling [10,11], or upcycling hydrolyzed PHA-based plastics [12–15]. However, most of these studies targeted PHA-derived monomers and overlooked the complexity of mixed polyester streams.

Paracoccus denitrificans offers an attractive solution owing to its metabolic versatility. It can grow autotrophically on CO_2 and thiosulfate

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https://doi.org/10.1016/j.procbio.2025.12.019
Received 19 October 2025; Received in revised form 13 December 2025; Accepted 31 December 2025
Available online 1 January 2026

[16], as well as heterotrophically on a wide range of substrates, including C1 compounds, under aerobic and anoxic conditions [17]. Previous studies confirmed its ability to synthesize PHAs in diverse environments [18,19]. This study aims to investigate a one-step microbial process in which *P. denitrificans* converts polyester-derived monomers and pretreated polyester wastes into PHAs. First, the growth and PHA accumulation of *P. denitrificans* were systematically evaluated using twelve monomers that constitute common polyester wastes as the sole carbon and energy sources. Second, PHA production under anoxic, nitrate-respiring conditions was examined using SUC as a reference substrate, selected for its direct integration into central carbon metabolism and its established use as a physiological benchmark. Finally, the ability of *P. denitrificans* to grow and accumulate PHAs on hydrolysates obtained from mechanically and chemically pretreated polyester-based plastics was assessed.

Results showed that 10 out of 12 tested monomers supported bacterial growth and PHA accumulation, reaching up to 30 % of cell dry weight and producing copolymers 3-hydroxyvaleric acid (3HV). Although these yields are lower than those achieved with sugar- or oil-based substrates, the process provides a compelling proof-of-concept for valorizing post-consumer polyester waste. The novelty lies in demonstrating that a single microorganism can metabolize a broad spectrum of plastic-derived monomers under both aerobic and anoxic conditions, avoiding costly separation steps and advancing low-cost, circular bioeconomy solutions. While further optimization is needed to enhance yields and volumetric productivity, this study establishes a foundation for developing sustainable microbial platforms for plastic bio-upcycling.

2. Materials and methods

2.1. Bacterial strain and growth conditions

P. denitrificans (Pd1222 Rif^R, [20]) was cultivated in batch cultures at 37 °C with agitation at 200 rpm. Precultures were initiated by inoculating 20 μL of glycerol-frozen *P. denitrificans* stock cells into 20 mL of LB (Luria–Bertani) medium supplemented with rifampicin (50 μg·mL^{-1}). These precultures were incubated for 18 h. For each monomer tested, a specific secondary preculture was prepared by transferring 1 mL of the LB-grown culture into 50 mL of fresh mineral salts medium (MSM) [17] supplemented with 3 g·L^{-1} of the corresponding monomer as the sole carbon source (pH 7). Cultures were grown until an optical density (OD) at 600 nm (OD_{600}) of approximately 1.5 was reached.

Final cultures were conducted in 2.2 L gas-tight glass bottles, each containing 200 mL of MSM with 3 g·L^{-1} of the corresponding monomer (pH 7), inoculated with 5 mL of the secondary preculture. This corresponded to the following initial monomer concentrations: 25.4 mM SUC; 26.7 mM LAC; 48.3 mM 1,2-ethanediol (EDO); 39.4 mM 1,3-propanediol (PDO); 33.3 mM 1,4-butanediol (BDO); 20.5 mM ADI; 22.7 mM 6-hydroxycaproic acid (CAP); 28.8 mM 3-hydroxybutyric acid (3HB); 25.3 mM 3HV; 39.4 mM glycolic acid (GLY); 18.1 mM terephthalic acid (TER); and 13.9 mM 2,6-naphthalenedicarboxylic acid (NAP). All monomers were purchased from Sigma-Aldrich. Cultures were monitored until growth ceased, with measurements performed for biomass concentration, monomer consumption, and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) production.

2.2. Growth determination

Growth of *P. denitrificans* was measured spectrophotometrically using a SPECTROstar Nano spectrophotometer (BMG Labtech). OD was measured at 600 nm. These OD readings were used as a reproducible, high-throughput method of growth phenotyping. Conversion of OD measurements to an estimated biomass concentration value used a dry weight measurement for *P. denitrificans*. For dry weight determination, three culture samples (2 mL) were washed twice with MilliQ water and dried for 5 days at 80 ºC. Conversion of OD to biomass is shown as follows: Biomass (g L^{-1}) = OD_{600} × 0.37 g L^{-1}.

2.3. Determination of monomer concentration

Samples for monomers quantification were collected from 2 mL culture aliquots after centrifugation of the aliquots at 14000 rpm for 5 min (4 ºC) and filtering of the supernatant using Nylon syringe filters of 0.22 μm. 1,2-ethanediol; 1,3-propanediol; 1,4-butanediol; adipic acid; 6-hydroxycaproic acid and glycolic acid samples were analyzed using an Agilent 7820 A GC (gas chromatography) coupled with a 5977E mass selective detector (MSD; Agilent technologies, Santa Clara, USA) equipped with a DB-wax column (30 m × 250 μm × 0.25 μm). The detector and injector temperatures were kept constant at 250 °C and the oven temperature was increased from 50 °C to 220 °C at 10 °C min^{-1} and maintained at 220 °C for 2 min, before being increased again at 5 °C min^{-1} until reaching 240 °C. SUC, LAC, 3HB and 3HV samples were analyzed using an Alliance Waters HPLC equipped with an Aminex HPX-87H column (7.8 mm × 300 mm). The mobile phase was H_2SO_4 (25 mM), the column temperature was 75 °C and the eluent flow rate was 0.7 mL min^{-1}.

2.4. Determination of PHBV production

Quantitative determination of PHBV was carried out by adapting the method described in [21]. Samples of 1 mL were centrifuged (14000 rpm, 10 min) and the supernatant was discarded. Then, 1 mL of a solution of 1-propanol:HCl (80:20 v/v, 37 % HCl w/v), 50 μL of the internal standard (benzoic acid) in 1-propanol (25 g L^{-1}) and 2 mL of chloroform were added to the pellets and incubated for 4 h at 100 ºC in a Thermoreaktor TR 300 (Merck KGaA, Darmstadt, Germany). After digestion, 1 mL of deionized water was added, and the suspension was vortexed. The organic phase was collected and filtered through 0.22 μm fiber glass filters. The propyl esters of of 3HB and 3HV, the monomers constituting the copolyester PHBV were measured in a 7820 A GC coupled with a 5977E MSD (Agilent Technologies, Santa Clara, USA) and equipped with a DB-wax column (30 m × 250 μm × 0.25 μm). The detector and injector temperatures were maintained at 250 ºC. The oven temperature was initially maintained at 40 ºC for 5 min, increased at 10 ºC min^{-1} up to 200 ºC (maintained at this temperature for 2 min) and then increased up to 240 ºC at a rate of 5 ºC min^{-1}. Finally, PHBV values were calculated based on the standard curves corresponding to either 3HB or 3HV and the normalization with the benzoic acid internal standard.

2.5. Growth of P. denitrificans and PHBV production under anoxic conditions

Anoxic cultures of *P. denitrificans* were carried out in 120 mL serum bottles sealed hermetically with isoprene rubber septa and aluminum crimp caps. Each bottle contained 100 mL of MSM supplemented with SUC (3 g·L^{-1}) as the sole carbon and energy source (a monomer of polymers such as PBS (poly(butylene succinate)), PBSA ((poly(butylene succinate-co-adipate)), PBST (poly(butylene succinate-co-terephthalate)), and PBSTIL ((poly(butylene succinate/terephthalate/isophthalate)-co-(lactate)). For nitrogen assimilation and anoxic respiration, KNO_3 (50 mM) and Na_2SO_4 (5 mM) were provided in place of $(NH_4)_2SO_4$ (5 mM), with nitrate serving as the terminal electron acceptor. The inoculum was prepared as described for aerobic cultures, by transferring 20 μL of glycerol-frozen *P. denitrificans* stock cells into 20 mL of LB medium supplemented with rifampicin (50 μg·mL^{-1}). For the final cultures, 2.5 mL of this preculture was used to inoculate the serum bottles. Oxygen was subsequently removed by flushing the headspace with helium. Cultures were incubated for three days at 37 °C and 200 rpm. Sampling for OD_{600}, monomer concentration, and PHA content was performed aseptically using a needle and syringe to avoid oxygen contamination.

2.6. Production of PHAs from mechanically and chemically pretreated polymers

Six different polymers were subjected to mechanical and chemical pretreatment: two homopolymers (PLA (polylactic acid) and PHB poly (3-hydroxybutyrate)), two heteropolymers (PBS and PBAT (poly (butylene adipate-co-terephthalate)), the copolymer PHBV and the blend PLA-PCL (poly(ε-caprolactone); 80:20). The polymers were first shredded and sieved to a particle size of 500–1000 μm and subsequently treated with an alkaline solution (2 M NaOH), at an initial polymer concentration of 50 g L^{-1}, under continuous agitation (300 rpm) at 37 °C for 7 days, following the procedure described by [22]. After alkaline treatment, the pretreated polymers were neutralized to pH 7 using HCl. The neutralized polymer suspensions were then used as the sole carbon source in *P. denitrificans* MSM cultures. For this purpose, 10 mL of the pretreated polymer suspension was mixed with 90 mL of MSM lacking any additional carbon source and prepared under nitrogen-limiting conditions (0.5 mM ammonium sulfate instead of 5 mM) to promote PHA synthesis. Cultures were inoculated with 1 mL of an overnight *P. denitrificans* LB culture and incubated at 37 °C with orbital shaking at 200 rpm for several days. Biomass accumulation and PHBV content were periodically determined as described above.

3. Results and discussion

3.1. P. denitrificans metabolizes most of the monomers from the main polyesters

P. denitrificans is able to synthesize different types of PHAs, mainly PHB and PHBV, from a wide range of substrates [18]. To evaluate its ability to utilize the building blocks of common polyesters as sole carbon and energy sources, a comprehensive biodegradation analysis of 12 representative monomers was performed (Fig. 1A). These monomers account for the composition of at least 17 industrially relevant polyesters (Fig. 1A). The time-course profiles of growth, monomer concentration, and PHA production are shown in Fig. 1B-C.

P. denitrificans grew on all monomers tested except TER and NAP (Figs. 1A and 1B). Growth rates varied depending on the substrate, with the highest observed for SUC (0.2 h^{-1}) and the lowest for BDO (0.02 h^{-1}). Overall, two groups of growth behavior were identified: (i) monomers supporting growth rates above 0.05 h^{-1} (SUC, LAC, GLY, 3HB, 3HV), and (ii) monomers with growth rates below 0.05 h^{-1} (PDO, EDO, ADI, CAP, BDO). In nearly all cases, substrates were consumed to undetectable levels and growth ceased upon depletion of the carbon source (Figs. 1B and 1C). The only exception was BDO, for which consumption remained partial, suggesting a metabolic bottleneck that warrants further investigation.

In addition to supporting growth, all metabolized monomers led to PHA accumulation (see Section 3.1 for details). PHA concentration followed biomass accumulation trends, as expected, since PHA is not synthesized under carbon-limiting conditions and can be mobilized

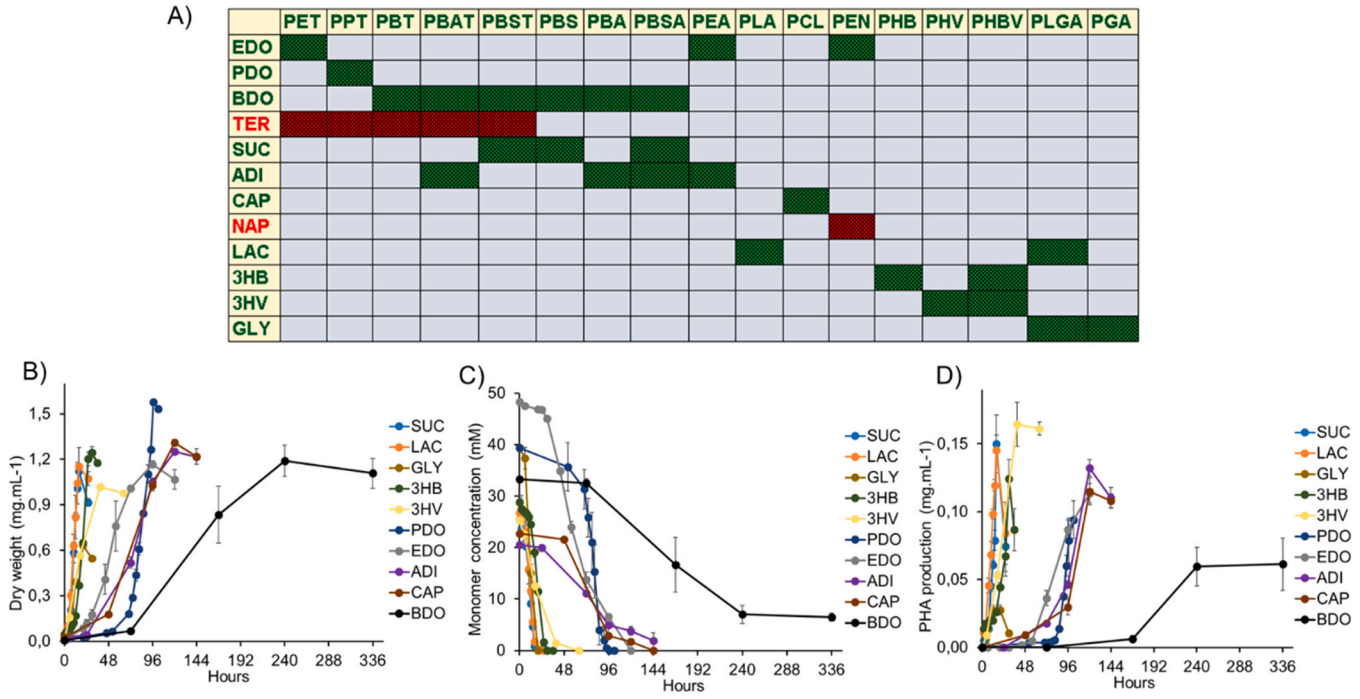

Fig. 1. Utilisation of polyester-derived monomers by *P. denitrificans* and associated growth and PHA production. A) Matrix showing the monomers (rows), corresponding to the polymer building blocks indicated above the table, that supported growth of *P. denitrificans* (green) and those that did not (red). The following abbreviations are used for monomers: EDO (1,2-ethanediol; ethylene glycol), PDO (1,3-propanediol), BDO (1,4-butanediol), TER (terephthalic acid), SUC (succinic acid), ADI (adipic acid; hexanedioic acid), CAP (6-hydroxycaproic acid; 6-hydroxyhexanoic acid), NAP (2,6-naphthalenedicarboxylic acid), LAC (lactic acid), 3HB (3-hydroxybutyric acid), 3HV (3-hydroxyvaleric acid), and GLY (glycolic acid). For some monomers, synonymous names are provided where relevant. The following abbreviations are used for polymers: PET (poly(ethylene terephthalate)), PPT (poly(propylene terephthalate)), PBT (poly(butylene terephthalate)), PBAT (poly (butylene adipate-co-terephthalate)), PBST (poly(butylene succinate-co-terephthalate)), PBS (poly(butylene succinate)), PBA (poly(butylene adipate)), PBSA (poly (butylene succinate-co-adipate)), PEA (poly(ethylene adipate)), PLA (polylactic acid), PCL (poly(ε-caprolactone)), PEN (poly(ethylene naphthalate)), PHB (poly(3-hydroxybutyrate)), PHV (poly(3-hydroxyvalerate)), PHBV (poly(3-hydroxybutyrate-co-3-hydroxyvalerate)), PLGA (poly(lactide-co-glycolide)), and PGA (polyglycolic acid). **B)** Growth of *P. denitrificans*, **C)** monomer concentration, and **D)** PHA production, using the listed monomers as the sole carbon and energy sources for the cultures. The error values represent the standard deviations calculated from three independent replicates. For interpretation of the references to colour in this Figure, the reader is referred to the web version of this article.

when the carbon source is exhausted. Collectively, these results show that *P. denitrificans* can grow and produce PHA on a wide range of polyester-derived monomers, with the exception of TER and NAP (Fig. 1A).

Growth on substrates such as SUC and LAC is widely distributed among microorganisms, and similar occurs for 3HB and 3HV compounds [23,24], EDO [25], and ADI [26]. By contrast, the ability to metabolize CAP, PDO, BDO, GLY, or TER is less common and remains poorly studied. For instance, *Ideonella sakaiensis* is among the few bacteria capable of hydrolyzing polyethylene terephthalate (PET) and metabolizing TER [27], a monomer that *P. denitrificans* was not able to metabolize. On the other hand, the recently discovered β-hydroxyaspartate cycle in *P. denitrificans* has been shown to enable GLY catabolism via oxidation to glyoxylate, matching with results here observed [28]. Although microbial catabolism of BDO is not fully elucidated in *P. denitrificans* [19], species of *Pseudomonas* and *Cupriavidus* can also metabolize this monomer [29], 2008). For example, *Pseudomonas putida* KT2440 grows on BDO with a rate of 0.08 h^{-1} [30], slightly higher than the 0.02 h^{-1} rate observed in the present work with *P. denitrificans* under comparable conditions. In relation to CAP metabolism, two dehydrogenases—an alcohol dehydrogenase and an aldehyde dehydrogenase—appear central to CAP catabolism [31]. These enzymes have been characterized in *Acidovorax* sp. CHX100, a bacterium able to use CAP as its sole carbon and energy source [31,32]. The alcohol dehydrogenase (AdhP) oxidizes CAP to 6-oxocaproic acid, which is subsequently converted to ADI by the aldehyde dehydrogenase (Adh). BLAST analysis identified two candidate homologs in *P. denitrificans* (WP_011748650.1 and WP_011748649.1). Further work is required to confirm the roles of these enzymes in *P. denitrificans*, although a detailed molecular characterization lies beyond the scope of the present study.

3.2. Bio-upcycling of polyester monomers into PHA

The exploitation of plastic wastes through their bioconversion into bioplastics, together with strategies to reduce production costs, has become a major focus for achieving PHA production at the industrial scale. Therefore, it was important to quantify both the production yields and the composition of PHAs accumulated in *P. denitrificans* cells depending on the substrate used. These results are summarized in Table 1. The highest yield of PHA production per mol of substrate was obtained with the 3HV and ADI monomers (both 6.5 g mol^{-1}), followed by SUC (5.9 g mol^{-1}), LAC (5.4 g mol^{-1}), and CAP (5.1 g mol^{-1}). The remaining monomers supported PHA yields below 5 g mol^{-1}. Similarly, the highest proportion of accumulated PHA relative to total biomass was observed with 3HV (16.1 %), followed by SUC (13.4 %), LAC (12.6 %), ADI (10.5 %), and 3HB (10.0 %). The remaining monomers supported PHA contents below 10 %. In all cases, the composition of the accumulated PHA was dominated by 3HB (>90 %), with the exception of the polymer synthesized from the 3HV monomer, which consisted of > 90 % 3HV and < 10 % 3HB (Table 1). Among other factors, such as substrate catabolism and carbon chain length, the incorporation of 3HB units depends on the substrate specificity of PHA synthases, most of which preferentially polymerize short-chain-length monomers (scl-PHAs) [33]. Overall, the PHBV copolyester composition, excluding cultures with 3HV as substrate, ranged from 90.1 % to 95.4 % 3HB. These findings confirm that PHA production in *P. denitrificans* is robust across different substrates and consistently dominated by PHB accumulation. The predominance of this polymer is particularly relevant given its recognized biodegradability, biocompatibility, and favorable processing properties, which underpin its increasing use in fields such as sustainable packaging, biomedical implants, and controlled drug delivery. Consequently, the demonstration that PHB can be efficiently obtained from low-cost plastic wastes highlights a promising route for advancing circular bioeconomy strategies and reducing the environmental burden of synthetic polymers.

Table 1
PHA production yields and PHA composition obtained from the different monomers using a one-step microbial fermentation process with *P. denitrificans* cells.

Monomer	$Y_{PHA/substrate}$ ($g \cdot mol^{-1}$)	PHA/biomass (%)	3HB/PHA (%)	3HV/PHA (%)
3-Hydroxyvaleric acid	6.5 ± 0.41	16.1 ± 1.4	6.6 (± 1.9)	93.4 (± 1.9)
Adipic acid	6.5 ± 0.68	10.5 ± 0.5	93.2 (± 0.9)	6.8 (± 0.9)
Succinic acid	5.9 ± 0.28	13.4 ± 1.6	92.9 (± 0.8)	7.1 (± 0.8)
Lactic acid	5.4 ± 0.22	12.6 ± 0.4	95.4 (± 0.2)	4.6 (± 0.2)
6-Hydroxycaproic acid	5.1 ± 0.00	8.8 ± 0.6	91.8 (± 0.3)	8.2 (± 0.3)
3-Hydroxybutyric acid	4.3 ± 0.08	10.0 ± 0.8	92.2 (± 0.7)	7.8 (± 0.7)
1,2-Ethanediol	2.4 ± 0.01	9.0 ± 0.7	92.3 (± 0.4)	7.7 (± 0.4)
1,3-Propanediol	2.4 ± 0.04	5.8 ± 1.2	90.1 (± 0.1)	9.9 (± 0.1)
1,4-Butanediol	1.8 ± 0.02	5.0 ± 0.8	90.9 (± 3.1)	9.1 (± 3.1)
Glycolic acid	0.7 ± 0.11	5.7 ± 1.1	90.1 (± 0.5)	9.9 (± 0.5)
Terephthalic acid	0 ± 0	0 ± 0	0 ± 0	0 ± 0
2,6-naphthalenedicarboxylic acid	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Note: The error values represent the standard deviations calculated from three independent replicates.

3.3. Growth of P. denitrificans and PHA production under anoxic conditions

An industrially relevant PHA-producing microorganism should combine rapid growth on inexpensive carbon sources with high substrate-to-PHA conversion efficiency. In addition, the ability to thrive under anoxic conditions is highly desirable, as it reduces the need for costly aeration in large-scale fermentations—a major limitation in current processes. As suggested by its name, *P. denitrificans* is capable of growing in the absence of oxygen, using nitrate as a terminal electron acceptor. However, the capacity of this bacterium to accumulate PHAs under anoxic conditions, while maintaining the same carbon and energy inputs, has not been characterized to date. To address this question, the culture medium was reformulated by substituting the nitrogen source—using KNO_3 and Na_2SO_4 instead of $(NH_4)_2SO_4$—to enable nitrate reduction. Oxygen was also removed from the headspace of the incubation bottles. Biomass formation and PHA accumulation under these conditions, compared with fully aerobic cultures supplied with SUC as the sole carbon and energy source, are presented in Fig. 2. The comparison clearly reveals distinct physiological responses depending on the electron acceptor available. *P. denitrificans* exhibited, on average, a 33 % lower biomass production with nitrate compared to oxygen and accumulated approximately 42 % less PHA (12.3 vs. 5.2 % of cell dry weight). Here, SUC was used as a reference substrate to establish a physiological baseline for comparison with other plastic-derived monomers. In this regard, it is worth noting that while the aerobic-anoxic differences observed are expected to be broadly applicable, monomer-specific metabolic behaviour should be examined in future studies. Nonetheless, the results demonstrate that the bacterium is capable of producing PHAs under anoxic conditions, which represents a valuable trait for its consideration as an industrial PHA producer. Importantly, these findings open a promising avenue for future studies focused on optimizing anoxic fermentation conditions, which could further improve yields and process efficiency while expanding the versatility of *P. denitrificans* as a platform for sustainable PHA production.

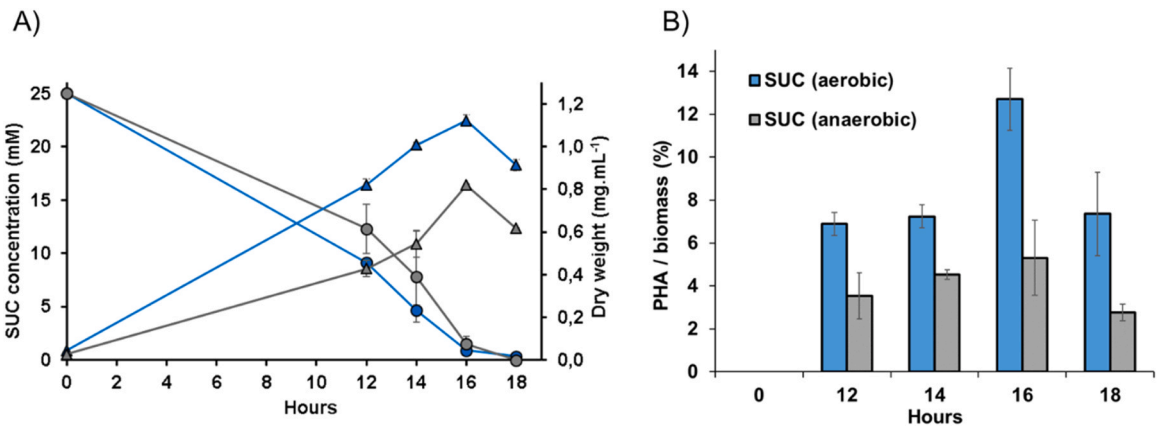

Fig. 2. Growth of *P. denitrificans* and PHA production under anoxic conditions using succinic acid as the sole carbon and energy source. A) Comparison of growth (triangles) and succinic acid consumption (circles) by *P. denitrificans* under aerobic (blue) and anoxic (grey) conditions. **B)** Comparison of PHA production (% of cell dry weight) in *P. denitrificans* cultures under aerobic (blue) and anoxic (grey) conditions. Error values represent the standard deviations of three independent replicates. For interpretation of the references to colour in this Figure, the reader is referred to the web version of this article.

3.4. Growth of P. denitrificans and PHA accumulation on bioplastic polymer hydrolysates

The growing demand for sustainable PHA production has been accompanied by increasing interest in strategies to upcycle bioplastic waste [2]. In this context, the development of processes enabling the direct conversion of polymeric plastics into new bioplastics represents a highly attractive goal. To explore this potential, six different polyesters were mechanically and chemically pretreated to facilitate depolymerization. These included two homopolymers (PLA and PHB), two heteropolymers (PBS and PBAT), one copolymer (PHBV) and a PLA-PCL blend.

Many PHA-producing bacteria repress polymer accumulation in the presence of sufficient nitrogen [34]. To enhance PHA synthesis, the nitrogen concentration in the culture medium was reduced ten-fold (see Section 2.6), a strategy widely reported to boost microbial PHA production across various bacterial models [35–37]. The experimental approach comprised two key steps: (i) mechanical shredding and chemical pretreatment of the polymer substrates; and (ii) cultivation of *P. denitrificans* in MSM containing the hydrolyzed polymers as the sole carbon and energy source under previously described growth conditions. Results demonstrated that *P. denitrificans* grew (Fig. 3A) and accumulated PHA (Fig. 3B) on all polymer hydrolysates tested. The highest PHA accumulation, reaching up to 30 % of cell dry weight, was observed when PHB or PHBV hydrolysates were used as substrates (Fig. 3B). PLA and the PLA-PCL blend supported intermediate PHA contents (~15 %), whereas PBS and PBAT hydrolysates resulted in lower PHA accumulation (~6 %) (Fig. 3B). Biomass formation followed similar trends across the different polymers (Fig. 3A).

The differences in biomass formation and PHA accumulation observed across the different polymer hydrolysates can be partially explained by polymer hydrolysis behavior (i.e., the extent of carbon solubilization) as well as by the chemical nature and bioavailability of the solubilized hydrolysis products. In this context, under the same hydrolysis conditions applied in this study (2 M NaOH, particle size 500–1000 μm, 7-day hydrolysis), PLA and the PLA-PCL (80:20) blend have been reported to exhibit high apparent alkaline hydrolysis efficiencies (92–98 %), as inferred from total organic carbon (TOC) solubilization, whereas lower values were observed for PHAs (80–93 %) [22]. For PBS and PBAT, TOC solubilization data under these exact conditions are not available; however, independent experiments conducted by our research group using 1 M NaOH achieved TOC solubilization efficiencies of 57.8 % and 40.9 %, respectively, after 12 days of hydrolysis. On this basis, higher solubilization efficiencies would be expected at increased alkaline strength. Although the efficiency of alkaline hydrolysis was not quantified for each polymer in this study, the

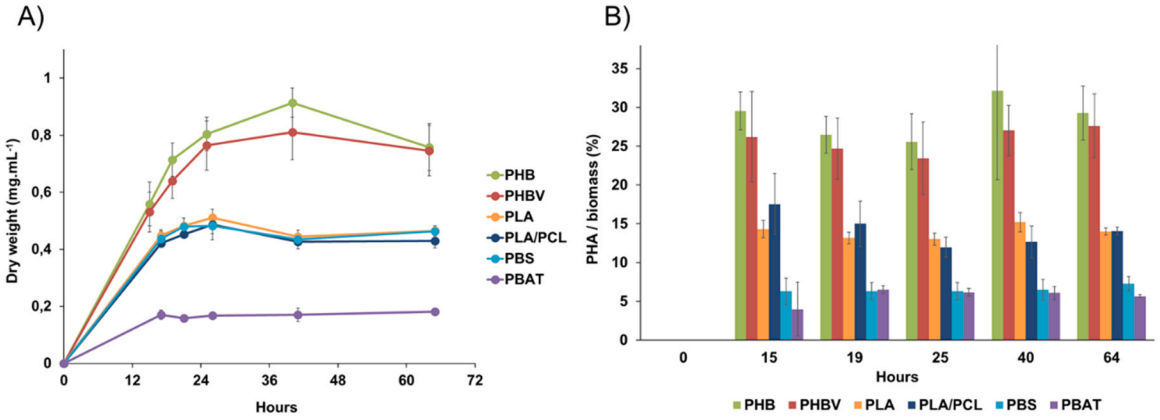

Fig. 3. Growth of *P. denitrificans* and PHA accumulation on bioplastic polymer hydrolysates. A) Growth of *P. denitrificans* using mechanically and chemically pretreated polymers as sole carbon source. **B)** Production of PHAs from the mechanically and chemically pretreated polymers used as sole carbon source of the cultures. PHB: (poly(3-hydroxybutyrate)), PHBV: (poly(3-hydroxybutyrate-co-3-hydroxyvalerate)); PBS: (poly(butylene succinate)); PBAT: (poly(butylene adipate-co-terephthalate)); PLA: (polylactic acid); PLA-PCL (80:20). The error values represent the standard deviations calculated from three independent replicates. For interpretation of the references to colour in this Figure, the reader is referred to the web version of this article.

overall results clearly demonstrate the ability of *P. denitrificans* to biologically upcycle hydrolyzed polymers, highlighting its versatility beyond the use of pure monomeric substrates. Future work should therefore consider not only the extent of polymer solubilization, but also the characteristics of the resulting hydrolysates, as these factors are expected to influence carbon partitioning toward PHA storage.

These findings provide compelling evidence that *P. denitrificans* is capable of converting diverse bioplastic wastes into valuable PHAs, highlighting its potential as a robust microbial platform for sustainable plastic upcycling. The approach opens avenues for future optimization of hydrolysis conditions and culture strategies to further improve yields, establishing a promising framework for integrating microbial bioconversion into circular bioeconomy initiatives. To translate these insights into industrial practice, several critical steps are now needed. First, optimization of pretreatment strategies is essential to ensure consistent liberation of monomers from diverse polymer waste streams at scale, while minimizing energy input and chemical use. Second, bioprocess engineering efforts must focus on increasing volumetric productivity, improving carbon conversion yields, and developing robust fed-batch or continuous cultivation strategies compatible with mixed and variable waste streams. Third, strain engineering could enhance key metabolic pathways to broaden substrate specificity, accelerate monomer uptake, or shift polymer composition beyond PHB toward tailored PHA copolymers with higher market value. In parallel, techno-economic and life-cycle assessments are needed to benchmark this microbial upcycling route against existing recycling technologies and to identify the conditions under which it becomes economically and environmentally competitive. Finally, industrial implementation will require the integration of this bioprocess into existing waste-handling infrastructures, addressing scalability, regulatory frameworks, and supply-chain logistics. Taken together, these next steps will be essential for moving from laboratory-scale proof-of-concept to a commercially viable bio-upcycling platform capable of contributing meaningfully to a circular plastics economy.

4. Conclusions

This study demonstrates that *P. denitrificans* is a promising candidate for the bio-upcycling of polyester waste into valuable PHAs. Efficient growth was achieved on 10 of the 12 tested monomers, all of which are constituent building blocks of different polyesters, leading predominantly to PHB accumulation. *P. denitrificans* was able to grow and accumulate PHA under both aerobic and anoxic conditions, providing significant operational flexibility. Successful bio-upcycling was also achieved using mechanically and chemically pretreated polymeric plastics, resulting in PHA contents of up to 30 % of the cell dry weight. Overall, these findings highlight the potential of *P. denitrificans* as a versatile microbial platform for sustainable PHA production, offering a viable route for integrating bioplastic upcycling into circular bioeconomy frameworks.

Funding

This work was financed by Société des Produits Nestlé S.A. The support from the Department of Education of the Regional Government of Castilla y León and co-financed by the European Union through the European Regional Development Fund (ERDF) (References: CLU-2025–2–06, UIC 379) is also acknowledged.

CRediT authorship contribution statement

Santos Beneit Fernando: Writing – original draft, Investigation, Conceptualization. **Muñoz Torre Raúl:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization. **Aragao Boerner Rosa:** Writing – review & editing, Resources, Conceptualization. **Boerner Tim:** Writing – review & editing, Resources, Conceptualization. **Garcia Depraect Octavio:** Writing – review & editing, Supervision. **Carlos de la Fuente:** Writing – review & editing, Investigation. **Martin Gonzalez Diego:** Writing – review & editing, Investigation. **Sergio Bordel:** Writing – review & editing, Investigation, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Raul Munoz reports financial support was provided by Nestlé Research & Development. Raul Munoz reports a relationship with Nestlé Research & Development that includes: funding grants. Raul Munoz has patent #WO2024083888A2 issued to Rosa Aragao. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Acknowledgements

We thank Dr. Rob van Spanning (Vrije Universiteit Amsterdam) for providing the *P. denitrificans* Pd1222 strain. Octavio García-Depraect acknowledges Grant RYC2021–034559-I, funded by MCIN/AEI/10.13039/501100011033 and by the European Union NextGenerationEU/PRTR.

Data Avail ability Statement

The authors declare that all data obtained have been included into the manuscript, its additional files and/or repositories.

Data availability

The data that has been used is confidential.

References

[1] F. Santos-Beneit, L.M. Chen, S. Bordel, R. Frutos de la Flor, O. García-Depraect, R. Lebrero, S. Rodriguez-Vega, R. Muñoz, R.A. Börner, T. Börner, Screening enzymes that can depolymerize commercial biodegradable polymers: heterologous expression of *Fusarium solani* cutinase in Escherichia coli, Microorganisms 11 (2) (2023) 328, https://doi.org/10.3390/microorganisms11020328.

[2] O. García-Depraect, S. Bordel, R. Lebrero, F. Santos-Beneit, R.A. Börner, T. Börner, R. Muñoz, Inspired by nature: microbial production, degradation and valorization of biodegradable bioplastics for life-cycle-engineered products, Biotechnol. Adv. 53 (2021) 107772, https://doi.org/10.1016/j.biotechadv.2021.107772.

[3] Roohi, K. Bano, M. Kuddus, M.R. Zaheer, Q. Zia, M.F. Khan, G.M. Ashraf, A. Gupta, G. Aliev, Microbial enzymatic degradation of biodegradable plastics, Curr. Pharm. Biotechnol. 18 (5) (2017) 429–440, https://doi.org/10.2174/1389201018666170523165742.

[4] L. Vargas-Estrada, O. García-Depraect, J. Zimmer, R. Muñoz, Analysis of organic recycling technologies, their present infrastructures and suitability for biodegradable food packaging: a review, J. Environ. Manag 376 (2025) 124395, https://doi.org/10.1016/j.jenvman.2025.124395.

[5] R.P. Mahato, S. Kumar, P. Singh, Production of polyhydroxyalkanoates from renewable resources: a review on prospects, challenges and applications, Arch. Microbiol 205 (5) (2023) 172, https://doi.org/10.1007/s00203-023-03499-8.

[6] B. Comesaña-Gándara, O. García-Depraect, F. Santos-Beneit, S. Bordel, R. Lebrero, R. Muñoz, Recent trends and advances in biogas upgrading and methanotrophs-based valorization, Chem. Eng. J. Adv. 11 (2022) 100325, https://doi.org/10.1016/j.ceja.2022.100325.

[7] O. García-Depraect, R. Lebrero, S. Rodriguez-Vega, S. Bordel, F. Santos-Beneit, L. J. Martínez-Mendoza, R. Aragão Börner, T. Börner, R. Muñoz, Biodegradation of bioplastics under aerobic and anaerobic aqueous conditions: kinetics, carbon fate and particle size effect, Bioresour. Technol. 344 (Pt B) (2022) 126265, https://doi.org/10.1016/j.biortech.2021.126265.

[8] D. Martín-González, C. de la Fuente Tagarro, A. De Lucas, S. Bordel, F. Santos-Beneit, Genetic modifications in bacteria for the degradation of synthetic polymers: a review, Int. J. Mol. Sci. 25 (10) (2024) 5536, https://doi.org/10.3390/ijms25105536.

[9] S. Riaz, K.Y. Rhee, S.J. Park, Polyhydroxyalkanoates (PHAs): biopolymers for biofuel and biorefineries, Polymers 13 (2) (2021) 253, https://doi.org/10.3390/polym13020253.

[10] O. García-Depraect, R. Lebrero, S. Rodriguez-Vega, R.A. Börner, T. Börner, R. Muñoz, Production of volatile fatty acids (VFAs) from five commercial

bioplastics via acidogenic fermentation, Bioresour. Technol. 360 (2022) 127655, https://doi.org/10.1016/j.biortech.2022.127655.
[11] Y. Jin, K.D. de Leeuw, D.P.B.T.B. Strik, Microbial recycling of bioplastics via mixed-culture fermentation of hydrolyzed polyhydroxyalkanoates into carboxylates, Materials (Basel) 16 (7) (2023) 2693, https://doi.org/10.3390/ma16072693. PMID: 37048987; PMCID: PMC10096456.
[12] S. Kaihara, Y. Osanai, K. Nishikawa, K. Toshima, Y. Doi, S. Matsumura, Enzymatic transformation of bacterial polyhydroxyalkanoates into repolymerizable oligomers directed towards chemical recycling, Macromol. Biosci. 5 (7) (2005) 644–652, https://doi.org/10.1002/mabi.200500030.
[13] J. Myung, N.I. Strong, W.M. Galega, E.R. Sundstrom, J.C.A. Flanagan, S.G. Woo, R. M. Waymouth, C.S. Criddle, Disassembly and reassembly of polyhydroxyalkanoates: recycling through abiotic depolymerization and biotic repolymerization, Bioresour. Technol. 170 (2014) 167–174, https://doi.org/10.1016/j.biortech.2014.07.105.
[14] C. Samorì, G.A. Martinez, L. Bertin, G. Pagliano, A. Parodi, C. Torri, P. Galletti, PHB into PHB: recycling of polyhydroxybutyrate by a tandem thermolytic distillation–microbial fermentation process, Resour. Conserv. Recycl 178 (2022) 106082, https://doi.org/10.1016/j.resconrec.2021.106082.
[15] L. Zoghbi, C. Argeiti, D. Skliros, E. Flemetakis, A. Koutinas, C. Pateraki, D. Ladakis, Circular PHB production via *Paraburkholderia sacchari* cultures using degradation monomers from PHB-based post-consumer bioplastics, Biochem. Eng. J. 191 (2023) 108808, https://doi.org/10.1016/j.bej.2023.108808.
[16] S.L. Jordan, I.R. McDonald, A.J. Kraczkiewicz-Dowjat, D.P. Kelly, F.A. Rainey, J. C. Murrell, A.P. Wood, Autotrophic growth on carbon disulfide is a property of novel strains of *Paracoccus denitrificans*, Arch. Microbiol 168 (3) (1997) 225–236, https://doi.org/10.1007/s002030050492.
[17] N. Harms, G.E. de Vries, K. Maurer, E. Veltkamp, A.H. Stouthamer, Isolation and characterization of *Paracoccus denitrificans* mutants with defects in the metabolism of one-carbon compounds, J. Bacteriol. 164 (3) (1985) 1064–1070, https://doi.org/10.1128/jb.164.3.1064-1070.1985.
[18] A. Maehara, S. Ueda, H. Nakano, T. Yamane, Analyses of a polyhydroxyalkanoic acid granule-associated 16-kilodalton protein and its putative regulator in the PHA locus of *Paracoccus denitrificans*, J. Bacteriol. 181 (9) (1999) 2914–2921, https://doi.org/10.1128/JB.181.9.2914-2921.1999.
[19] S. Bordel, D. Martín-González, T. Börner, R. Muñoz, F. Santos-Beneit, Genome-scale metabolic model of the versatile bacterium *Paracoccus denitrificans* Pd1222, mSystems 9 (2) (2024) e0107723, https://doi.org/10.1128/msystems.01077-23.
[20] G.E. de Vries, N. Harms, J. Hoogendijk, et al., Isolation and characterization of *Paracoccus denitrificans* mutants with increased conjugation frequencies and pleiotropic loss of a (nGATCn) DNA-modifying property, Arch. Microbiol 152 (1989) 52–57, https://doi.org/10.1007/BF00447011.
[21] C. Zúñiga, M. Morales, S. Le Borgne, S. Revah, Production of poly-β-hydroxybutyrate by Methylobacterium organophilum in a two-phase partition bioreactor, J. Hazard. Mater. 190 (1–3) (2011) 876–882, https://doi.org/10.1016/j.jhazmat.2011.04.011.
[22] O. García-Depraect, R. Lebrero, L.J. Martínez-Mendoza, S. Rodriguez-Vega, R. A. Börner, T. Börner, R. Muñoz, Enhancement of biogas production rate from bioplastics by alkaline pretreatment, Waste Manag 164 (2023) 154–161, https://doi.org/10.1016/j.wasman.2023.04.009.
[23] C. Feller, R. Günther, H.J. Hofmann, M. Grunow, Molecular basis of substrate recognition in D-3-hydroxybutyrate dehydrogenase from *Pseudomonas putida*, Chembiochem 7 (9) (2006) 1410–1418, https://doi.org/10.1002/cbic.200600167.
[24] D. Mountassif, P. Andreoletti, M. Cherkaoui-Malki, N. Latruffe, M.S. El Kebbaj, Structural and catalytic properties of the D-3-hydroxybutyrate dehydrogenase from *Pseudomonas aeruginosa*, Curr. Microbiol 61 (1) (2010) 7–12, https://doi.org/10.1007/s00284-009-9568-7.
[25] M.A. Franden, L.N. Jayakody, W.J. Li, et al., Engineering *Pseudomonas putida* KT2440 for efficient ethylene glycol utilization, Metab. Eng. 48 (2018) 197–207, https://doi.org/10.1016/j.ymben.2018.06.003.
[26] D. Parke, M.A. Garcia, L.N. Ornston, Cloning and genetic characterization of dca genes required for β-oxidation of straight-chain dicarboxylic acids in *Acinetobacter* sp. strain ADP_1, Appl. Environ. Microbiol 67 (10) (2001) 4817–4827, https://doi.org/10.1128/AEM.67.10.4817-4827.2001.
[27] S. Yoshida, K. Hiraga, T. Takehana, et al., A bacterium that degrades and assimilates poly(ethylene terephthalate), Science 351 (6278) (2016) 1196–1199, https://doi.org/10.1126/science.aad6359.
[28] L. Schada von Borzyskowski, F. Severi, K. Krüger, et al., Marine Proteobacteria metabolize glycolate via the β-hydroxyaspartate cycle, Nature 575 (7783) (2019) 500–504, https://doi.org/10.1038/s41586-019-1748-4.
[29] A.A. Amirul, A.R. Yahya, K. Sudesh, M.N. Azizan, M.I. Majid, Biosynthesis of poly (3-hydroxybutyrate-co-4-hydroxybutyrate) by *Cupriavidus* sp. USMAA1020, Bioresour. Technol. 99 (11) (2008) 4903–4909, https://doi.org/10.1016/j.biortech.2007.09.040.
[30] W.J. Li, T. Narancic, S.T. Kenny, P.J. Niehoff, K. O'Connor, L.M. Blank, N. Wierckx, Unraveling 1,4-butanediol metabolism in *Pseudomonas putida* KT2440, Front. Microbiol 11 (2020) 382, https://doi.org/10.3389/fmicb.2020.00382.
[31] D. Salamanca, K. Bühler, K.H. Engesser, A. Schmid, R. Karande, Whole-cell biocatalysis using *Acidovorax* sp. CHX100 Δ6HX for the production of ω-hydroxycarboxylic acids from cycloalkanes, N. Biotechnol. 60 (2021) 200–206, https://doi.org/10.1016/j.nbt.2020.10.009.
[32] D. Salamanca, K.H. Engesser, Isolation and characterization of two novel strains capable of using cyclohexane as carbon source, Environ. Sci. Pollut. Res. 21 (22) (2014) 12757–12766, https://doi.org/10.1007/s11356-014-3206-z.
[33] M. Knoll, T.M. Hamm, F. Wagner, V. Martinez, J. Pleiss, The PHA depolymerase engineering database: a systematic analysis tool for the diverse family of polyhydroxyalkanoate depolymerases, BMC Bioinforma. 10 (2009) 89, https://doi.org/10.1186/1471-2105-10-89.
[34] S. Bordel, R.J.M. van Spanning, F. Santos-Beneit, Imaging and modelling of poly(3-hydroxybutyrate) synthesis in Paracoccus denitrificans, AMB Express 11 (2021) 113, https://doi.org/10.1186/s13568-021-01273-x.
[35] R. Durner, M. Zinn, B. Witholt, T. Egli, Accumulation of poly[(R)-3-hydroxyalkanoates] in *Pseudomonas oleovorans* during batch and chemostat culture with different carbon sources, Biotechnol. Bioeng. 72 (3) (2001) 278–288.
[36] T. Egli, M. Zinn, The concept of multiple-nutrient-limited growth of microorganisms and its application in biotechnological processes, Biotechnol. Adv. 22 (1–2) (2003) 35–43, https://doi.org/10.1016/j.biotechadv.2003.08.006.
[37] S. Shahid, R. Mosrati, J. Ledauphin, et al., Impact of carbon source and variable nitrogen conditions on bacterial biosynthesis of polyhydroxyalkanoates: evidence of an atypical metabolism in Bacillus megaterium DSM 509, J. Biosci. Bioeng. 116 (3) (2013) 302–308, https://doi.org/10.1016/j.jbiosc.2013.02.017.