



Microbial community-based polyhydroxyalkanoates (PHAs) production from wastewater: Techno-economic analysis and ex-ante environmental assessment



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HIGHLIGHTS

- Fermentation of wastewater towards polyhydroxyalkanoates is scaled-up.
- Intracellular polyhydroxyalkanoates release by three downstream processes.
- Process design, techno-economic and life cycle assessment are developed.
- Key features of the industrial process are identified prior to commercialisation.
- Sensitivity analysis of the costs and environmental impacts is performed.

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ABSTRACT

This work investigates the potential for polyhydroxybutyrate (PHB) production from wastewater, from a techno-economic and an environmental perspective, examining scale-up opportunities and bottlenecks prior to commercialisation. Conceptual process design, economic, environmental impacts and sensitivity analysis are developed for one fermentation process and three downstream processing routes, based on alkali, surfactant-hypochlorite and solvent treatments. Environmentally and cost-wise, the alkali treatment is the most favourable with production costs of 1.40 €/kg PHB, global warming potential of 2.4 kg CO₂-eq/kg PHB and non-renewable energy use of 106 MJ/kg PHB. The solvent-based process yields the highest costs and environmental burdens: 1.95 €/kg PHB, 4.30 kg CO₂-eq/kg PHB and 156 MJ/kg PHB. The production of PHB from wastewater is identified as an interesting alternative to pure culture-polyhydroxyalkanoates production from sugars. However, these results are not yet competitive with those for the petrochemical counterparts. Additional performance improvements may be possible, through process integration and optimisation.

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1. Introduction

Global bio-based plastic production is approximately 1400 kt/a, accounting for nearly 0.5% of the current worldwide plastic production capacity (European Bioplastics, 2013; Plastics Europe, 2013). Hence, the development of competitive industrial processes which utilise renewable resources as feedstock for polymer production, can be a relevant aspect in the shift from a petrochemical-based chemical industry, towards a bio-based one.

In this context, the family of polyhydroxyalkanoates (PHAs) and its most common type, polyhydroxybutyrate (PHB), can be an interesting alternative to investigate. PHAs are biodegradable polyesters, naturally synthesized by bacterial fermentation, which can serve as polymer material (Endres and Siebert-Raths, 2011) or as raw material for the production of chemical building blocks (Koller et al., 2012). PHAs have attracted widespread interest as an alternative to conventional plastics due to their natural origin, biodegradability, and functionality. High production costs, which are estimated at 20–80% higher than for their petrochemical counterparts (Marketsandmarkets, 2013), remain a key bottleneck for their commercialisation. Three main factors contribute to this relative increase of the production costs of PHAs: (i) the energy used for the sterilisation of the fermentation equipment (Van Wegen

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et al., 1998), (ii) the PHA yield on the substrate, and (iii) the efficiency of the downstream processing (DSP) (Reddy et al., 2003). In terms of potential environmental impacts, it is not clear whether the production of PHAs using pure culture bacterial fermentation shows any clear advantage compared to fossil-based polymers. The substrates used in the pure culture bacterial fermentation, i.e. glucose, methanol or acetic acid, contribute significantly to the overall environmental impacts of the process, (Patel et al., 2005).

In order to address these issues on higher production costs and environmental impacts, a microbial community engineering process for PHA production has been proposed, as an alternative strategy (Salehizadeh and van Loosdrecht, 2004). Instead of the traditional pure culture bacterial fermentation, which consumes expensive feedstocks, the novel process is based on the selection of a microorganisms' populations from a variety present in the wastewater, which resulted in the provision of an enhanced PHA producing capacity (77% dry weight). Lower production costs and decreased related environmental impacts are expected as the process uses substrates such as industrial waste and non-aseptic process conditions (Salehizadeh and van Loosdrecht, 2004). A proof-of-concept has been achieved at laboratory and pilot scale using separately wastewater from a paper mill, and from the food industry (Jiang et al., 2012; Tamis et al., 2014). The first full scale demonstration plant of PHAs production has been initiated by Veolia (Press release, 06-14-2013).

In addition to an efficient fermentation process, the development of a competitive DSP facility to release the intracellular PHA is needed to further reduce manufacturing costs and environmental impacts. Based on studies with synthetic wastewater, Gurieff and Lant (2007) concluded that the main drawback of the mixed culture production of PHA from wastewater is the relatively large energy consumption within the DSP. However, their work is based on literature studies and only considers one DSP configuration. Several approaches have been described in the literature for recovery and purification of intracellular PHB after fermentation (Akiyama et al., 2003; Choi and Lee, 1997; Jacquel et al., 2008; Naranjo et al., 2013; Posada et al., 2011). The most common procedures involve the use of organic solvent, or treatment by chemical digestion. The use of solvent leads to improved purity and recovery yield. However, environmental impacts can increase if the solvent is not 100% recovered due to the surplus chemicals being released into the environment (de Koning and Witholt, 1997). From an environmental and an economic perspective, digestion with chemicals appears to be more attractive (Choi and Lee, 1997; Jacquel et al., 2008; Posada et al., 2011). Nevertheless, the thermal stability of the final product is lower, whilst in a solvent-based DSP the quality of the PHB is comparable to that of a commercial polymer (Jiang et al., 2015).

The aim of this research is twofold: (i) to design a system for the industrial production of PHB, with improved economic and environmental performance, and (ii) to identify specific processing steps that may be further improved prior to commercialisation. To aid in these objectives, data from laboratory and pilot plant scale, based on real effluents from industrial wastewater (Jiang et al., 2012; Tamis et al., 2014) is used. Whereby, one fermentation process and three DSP options are evaluated: two DSP routes are based on chemical treatment with surfactant combined with alkali or hypochlorite and the third one is based on solvent extraction with dichloromethane (DCM).

2. Methods

An ex-ante economic and environmental analysis of the full-scale production of PHB from wastewater is performed in this

study. The analysis is comprised of a conceptual process design, techno-economic evaluation and environmental Life Cycle Assessment (LCA).

2.1. Process design

The conceptual process design follows a hierarchical design strategy based on heuristics and experience, to develop the processing flowsheets. The processing units and operating conditions are selected from either data available at laboratory or pilot scale (Jiang et al., 2012, 2015; Tamis et al., 2014), or from literature review. Once the processing flowsheets are well defined, the mass and energy balances are obtained from conducting process modelling in ASPEN Plus software. The production capacity of PHB from wastewater deriving from a paper mill or the food industry is fixed at 1.5 kilotons per annum (kt/a) based on 6.8 kt COD (chemical oxygen demand)/a availability from wastewater and the yields reported in Section 2.1.1. The final product purity is 99.9 wt%.

2.1.1. Fermentation

PHB is produced in an aerobic conversion reaction by a microbial enrichment culture according to the schemes validated at laboratory and pilot plant scale (Jiang et al., 2012; Tamis et al., 2014). This microbial process consists of three sequential fermentation steps, as presented in Fig. 1a.

First, the organic material present in the wastewater is fermented into volatile fatty acids (VFA) in the acidification reactor (R-101) with a yield of 0.91 g COD/g COD (based on Jiang et al., 2012: $COD_{initial} = 26.3$ g/L, $COD_{end} = 24.0$ g/L, Table 1). Second, the resulting VFA rich solution is split into two streams. One stream is directed towards the selector (R-102), a sequencing batch reactor operating with a solids retention time of 1 day and a cycle duration of 0.5 days (Tamis et al., 2014). In the selector, the enrichment of PHA producing bacteria (X) takes place with a biomass yield on a substrate of 0.34 g X/g COD (Tamis et al., 2014). The maximal biomass concentration in the selector ($[X]_{max}$) is 0.5 kg/m³, based on a maximal biomass oxygen uptake rate (q_{O_2}) of 1 kg O₂/kg X h and a maximal oxygen transfer rate (OTR_{max}) of 0.5 kg O₂/m³ h ($[X]_{max} = OTR_{max}/q_{O_2}$). A dilution flow recycled from downstream (R-103) avoids surpassing the maximal biomass concentration.

The third and last step is PHB accumulation, carried out in the fed-batch accumulation reactor (R-103), where the content of intracellular PHB is maximised to 70 wt% (Tamis et al., 2014). To avoid substrate inhibition (maximal allowed substrate concentration assumed is 0.25 kg COD/m³), the other sub-stream of the acidification reactor product stream is continuously dosed during the entire batch length. The total suspended solids (TSS) concentration in the outlet stream of the accumulation reactor is 2.7 kg TSS/m³. To concentrate the product stream, the solids are separated via settling during 30 min at the end of the cycle (based on laboratory and pilot plant experience). The clarified fraction is removed from the top of the settler and 75% of this stream is recycled for the purpose of dilution water to the selector. The settled product is sent to the buffer tank (T-101) and subsequently fed continuously to the DSP configuration for PHB extraction and purification.

2.1.2. Downstream processes (DSP)

Three DSP routes were evaluated for the recovery of intracellular PHB. The parameters of the cell disruption steps are based on both literature (Dong and Sun, 2000; Jacquel et al., 2008; Lee and Choi, 1998) and laboratory work carried out at Delft University and Eindhoven University in the Netherlands.

2.1.2.1. Case I: alkali-surfactant. The flow diagram of the DSP for Case I is shown Fig. 1b. The concentrated fermentation broth, containing 20 kg TSS/m³, with 70 wt% PHB, is preheated to 30 °C

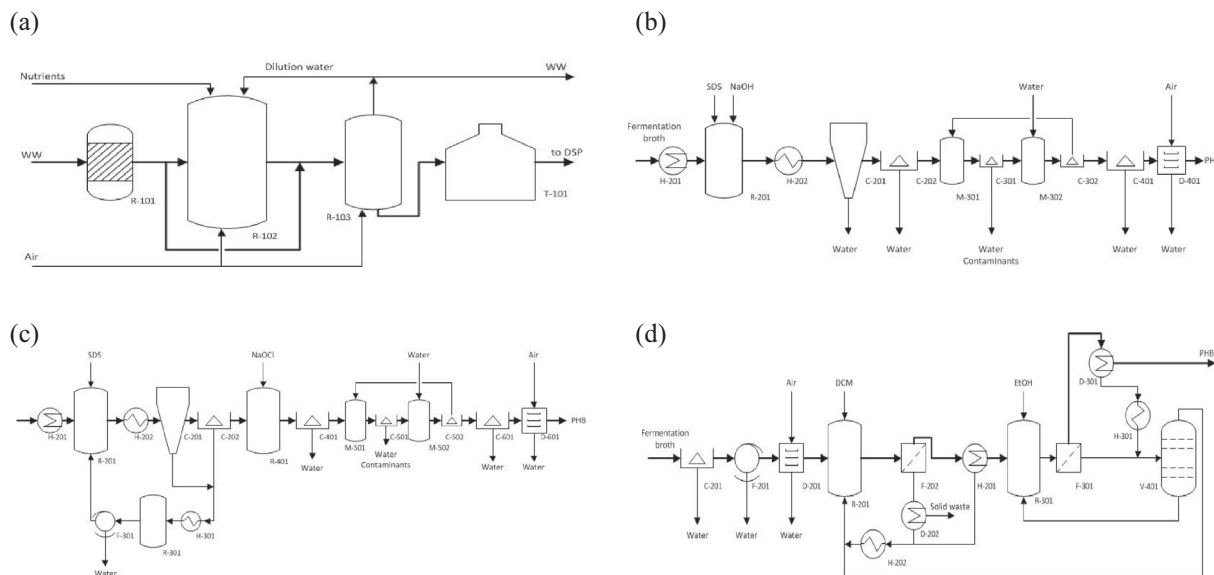


Fig. 1. Process flow diagrams. (a) WW fermentation to PHB. R-101: acidification fermenter, R-102: selection fermenter, R-103: accumulation fermenter, T-101: buffer tank. (b) Alkali-surfactant. H-201: preheater, R-201: alkali reactor, H-202: cooler, C-201: hydrocyclone, C-202: centrifuge, M-301: mixing tank, C-301: centrifuge, M-302: mixing tank, C-302: centrifuge, H-301: pre-cooler, R-301: SDS crystallisation reactor, F-301: filter, R-401: hypochlorite reactor, C-401: centrifuge, M-501: mixing tank, C-501: centrifuge, M-502: mixing tank, C-502: centrifuge, C-601: centrifuge, D-601: air dryer. (c) Surfactant-hypochlorite. H-201: preheater, R-201: surfactant reactor, H-202: cooler, C-201: hydrocyclone, C-202: centrifuge, H-301: pre-cooler, R-301: SDS crystallisation reactor, F-301: filter, R-401: hypochlorite reactor, C-401: centrifuge, M-501: mixing tank, C-501: centrifuge, M-502: mixing tank, C-502: centrifuge, C-601: centrifuge, D-601: air dryer. (d) DCM solvent route. C-201: centrifuge, F-201: filter, D-201: air dryer, R-201: DCM reactor, F-202: filter, D-202: solid phase dryer, H-201: DCM evaporator, H-202: DCM condenser, R-301: ethanol precipitation reactor, F-301: filter, D-301: dryer, H-301: condenser, V-401: vacuum distillation.

Table 1
Operational and biological parameters of wastewater fermentation towards PHB (Jiang et al., 2012; Tamis et al., 2014).

Fermenter	Parameter	Value	Units
Acidification	Temperature	30	°C
	Effluent COD	26.3	kg/m ³
	SRT	1	day
	Conversion capacity	50	kg COD/m ³ d
	Yield	0.91	g COD/g COD COD
Selection	Temperature	30	°C
	SRT	1	day
	Cycle length	0.5	day
	OTR max	0.5	kg O ₂ /m ³ h
	Yield	0.34	g X/g COD
	q _{O2}	1	kg O ₂ /kg Xh
Accumulation	Temperature	30	°C
	Cycle length	0.5	day
	OTR max	0.5	kg O ₂ /m ³ h
	Yield	0.44	g PHA/g COD

(in H-201). The chemical digestion of the cell material is carried out in the alkali reactor (R-201), with sodium hydroxide (NaOH, 0.2 M) and sodium dodecyl sulphate (SDS, 0.2 wt/v%), and a residence time of 1 h (Jiang et al., 2015). 95% of the intracellular PHB is released (Jiang et al., 2015) and subsequently recovered in two consecutive solid-liquid separation units. 70% of the total wastewater content is assumed to be removed in a hydrocyclone (C-201) and 90% of the remaining wastewater is removed by centrifugation (C-202). Impurities, which correspond to about 9% of the solid phase, are removed by washing with counter current fresh water in mixing tanks and centrifuges (M-301, C-301, M-302, C-302). PHB is further concentrated by centrifugation (C-401) and evaporation of the residual humidity in a final air drying step (D-401). Air drying is favoured because it does not affect the thermal stability of PHB neither molecular weight during the melt processing, as temperatures below 65 °C are implemented (Chen et al., 2013). A 99.9 wt% purity of the final product is obtained.

2.1.2.2. Case II: surfactant-hypochlorite. Cell disruption by chemical digestion can also be achieved by using surfactant and hypochlorite (Lee and Choi, 1998). The non-PHB cell material (NPCM) in the fermented wastewater is solubilised by sodium dodecyl sulphate (SDS) in the surfactant reactor (R-201), as depicted in Fig. 1c. Treatment conditions are 3:1 SDS:NPCM mass ratio, 55 °C, and 15 min of residence time based on Jacquelin et al. (2008). 88% of the intracellular PHB (Jacquelin et al., 2008) is recovered in the SDS treatment and subsequently concentrated in a hydrocyclone (C-201), followed by a centrifuge (C-202). The liquid phase is subjected to a crystallisation process (R-301) at 9 °C to recover and recycle 80% of the SDS (Smith et al., 2001). Further treatment with sodium hypochlorite (NaOCl) in a second reactor for chemical digestion (R-401), during 10 min at 30 °C, recovers extra intracellular PHB in the solid phase. A mass ratio of 8:1 NaOCl:NPCM is used to recover 95% of the remaining PHB. The rest of the DSP is analogous to the alkali-surfactant route: PHB concentration by centrifugation (C-401), counter current water washing (M-501, C-501, M-502, C-502), centrifugation (C-601) and final air drying (D-601) allows the process to obtain a 99.9 wt% PHB.

2.1.2.3. Case III: dichloromethane solvent. PHAs are highly soluble in some halogenated solvents such as dichloromethane (22.8 kg PHB/m³ DCM, Jacquelin et al., 2007). The boiling point of DCM is 40 °C, which makes its recovery and recycle comparatively easy and results in potential energy and solvent costs savings. This also permits performing DSP at milder temperatures, thus, avoiding final product degradation.

Prior to an extraction step using DCM, and due to the limited solubility of PHB in water (7.6 10⁻⁶ kg PHB/m³ H₂O, Jacquelin et al., 2007), 100% water is removed from the fermentation product (as shown in Fig. 1d) by centrifugation (C-201), followed by filtration (F-201), and air drying (D-201). The intracellular PHB extraction is carried out in the solvent reactor (R-201) at 20 °C with an efficiency of 86%, as observed at laboratory conditions. Subsequently, the solid and liquid phases are separated via a filter (F-202). Only 50% of the DCM in the liquid phase is evaporated (H-201), and then condensed

(H-202) for recycling. This prevents high local supersaturation at the wall of the equipment and PHB film formation, which affects product quality. Following this, ethanol is fed to the precipitation reactor (R-301), with a mass ratio of 2 kg EtOH/kg DCM to precipitate 98% of the PHB (the solubility of PHB in the solvent mixture is 0.22 kg PHB/m³, [Jacquel et al., 2007](#)). The solid and liquid phases are then separated via the filter (F-301), the remaining solvent mixture in the solid phase is vaporised (D-301) to obtain a 99.9 wt% purity of PHB. In order to reduce operating costs and environmental concerns, DCM and ethanol are separated and recycled via distillation (V-401). High separation efficiency is required to reuse DCM and ethanol. Large amounts of energy are needed because DCM and ethanol form a homogeneous azeotrope ([Gmehling et al., 2014](#)). 99% purity of DCM and ethanol is reached over the top and bottoms, respectively by using 15 distillation stages at vacuum conditions, *i.e.* 0.5 bar.

2.2. Economic evaluation

The economic potential of the three process options was analysed by considering total capital investment, and material and energy consumption costs, which together account for the majority of the total production costs of PHB. Total capital expenditure was calculated using typical factors for delivered equipment costs ([Smith, 2005](#)). Delivered equipment costs were estimated from equipment manufacturers' indications and using correlations based on individual equipment's characteristic size ([Sinnott, 2005](#)). The calculated costs were updated to 2013 prices using the Chemical Engineering Plant Cost Index (CEPCI). Annual depreciation (AD) was calculated based on the total capital (f_1) and the start-up material costs (m_{SU}) with an interest rate (i) of 5% and a payback time (PB) of 20 years. See Eq. (1):

$$AD = (f_1 + m_{SU}) \cdot \frac{i(1+i)^{PB}}{(1+i)^{PB} - 1} \quad (1)$$

The total annual costs (TAC) were estimated using Eq. (2):

$$TAC = AD + U + m + L + M \quad (2)$$

Utilities (U) and materials (m) costs were based on consumption levels from the energy and mass balances and [Ulrich and Vasudevan \(2006\)](#). Maintenance costs (M) were assumed to account for 3% of the total fixed capital costs and the labour costs (L) were assumed to be 10% of the total annual costs (TAC) ([Peters et al., 2003](#)).

Furthermore, the wastewater generated in any factory has to undergo a wastewater treatment (WWT) process (which causes additional costs for the attached industries). It is here assumed that the PHB production process developed, produces water effluent at the same quality standards of the existing wastewater treatment process. Therefore, avoided wastewater treatment costs can be accounted as economic credits which are directly deducted from the total annual costs. A similar approach is followed for the environmental credits in the LCA. These economic credits were estimated based on the treatment cost per m³ of wastewater and the volumetric flow entering to the fermentation tank ([Ulrich and Vasudevan, 2006](#)). No additional WWT costs were considered in the PHB production process since the wastewater effluent was already treated during fermentation and thus suitable for being returned to the water cycle.

2.3. Life cycle assessment (LCA)

An LCA was performed according to ISO 14040 and ISO 14044 standards ([ISO, 2006a,b](#)). A cradle-to-gate approach was selected for the system boundaries, including wastewater fermentation

and PHB recovery, excluding the product use and end-of-life treatment. The functional unit selected was 1 kg of PHB production.

An assessment of mechanical properties and applicability of the final product is out of the scope of this research and thus are not discussed. Despite this omission, it is important to mention that the quality for thermoplastic application might not be achieved in Case I but reached in Case III ([Jiang et al., 2015](#)). Nevertheless, if PHB is considered as an intermediate for the production of chemical building blocks (*e.g.* methyl crotonate and methyl acrylate), its final quality is not considered a limiting factor. This value chain from wastewater to chemical building blocks via PHB will be addressed in a forthcoming paper.

The Life Cycle Inventory (LCI), including input and output flows of materials and energy, was derived from the mass and energy balances obtained from the process simulation. The focus of this study is on two impact categories: the global warming potential (GWP) and the non-renewable energy use (NREU). Individual impacts for materials and energy carriers were taken from the database EcoInvent v2.2. Combined with the inventory, the individual impacts when summed provided the overall impacts of each process option. Similarly as applied within the economic analysis, the environmental impacts that would have arisen from the avoided wastewater treatment (WWT) are considered as environmental credits. These environmental credits as well as the biogenic carbon embedded in the PHB are directly deducted from the total GWP and NREU following the guidelines of [Pawelzik et al., 2013](#); U.S. EPA, 2011.

2.4. Sensitivity analysis

The four most critical variables and assumptions of the process designs were analysed to determine their influence on the techno-economic and environmental performance, and to identify opportunities for further improvement: (i) the cell disruption efficiency obtained in laboratory was varied $\pm 10\%$, (ii) the SDS crystallisation step in Case II was removed, (iii) the distillation of methanol in Case III was carried out in two columns (instead of one), and (iv) 100% of DCM was vaporised after solvent extraction in Case III (instead of 50% DCM vaporisation).

3. Results and discussion

3.1. Process design

The considered DSP led to a final product purity of 99.9 wt% in the three cases investigated; however, the global PHB recovery yield was slightly different for each case, *i.e.* 73.5% for Case I, 75.8% for Case II and 82.2% for Case III.

The PHB released by chemical treatment (Cases I and II) was in solid phase and further purified in a 2 steps counter-current washing process, with the addition of water to remove the non-PHB cell material. This washing led to a PHB loss of about 10% of the released PHB. In Case III, PHB was extracted with DCM as solvent. The PHB in the liquid phase was recovered via precipitation with ethanol, therefore, no water-washing was required. Additionally, the overall recovery was enhanced in Case III, as the non-precipitated PHB was separated out at the bottoms stream of the distillation tower and recycled for further precipitation. No recycle loops, which could increase the recovery yield, were incorporated into Cases I and II.

3.2. Economic evaluation

The outcome of the economic evaluation of the fermentation and each DSP route is discussed in this section. The obtained

results are subsequently compared to the costs associated to the traditional sugar-based production process of PHAs and those of the production processes utilising petrochemical counterparts.

Based on the process conditions described in Section 2.1, and the models and assumptions presented in Section 2.2, the economic evaluation shows that Case I is the most cost-competitive alternative amongst the various options designed (see Table 2 and Fig. 2a). The total production costs, including economic credits, were: 1.40 €/kg PHB in Case I, 1.56 €/kg PHB in Case II and 1.95 €/kg PHB in Case III.

In all three options, the costs attached to DSP are larger than those for the fermentation stage, with contributions of 70% (Case I), 73% (Case II) and 79% (Case III) (Table 2). Utility costs represent a major share of the total production costs: 65% (Case I), 51% (Case II) and 74% (Case III). The largest share in Case III is due to the distillation column used to recover DCM and ethanol, leading to high duties in the reboiler and condenser.

Within the utilities (Table 2 and Fig. 2b), steam costs were 14% and 12% of the total production costs in Cases I and II, and 10% in Case III. The higher steam costs in Cases I and II were due to the lower PHB recovery. Cooling water costs were similar to those of steam: 13% (Case I), 12% (Case II) and 10% (Case III). Electricity consumption in Case I is observed as the most significant utility cost factor (21% of total costs) but its share is significantly lower in cases II and III (11% and 8%, respectively). The variance can be attributed to the larger volumetric flow of the centrifuges in Case I due to the more diluted process streams.

Case III was the least economically attractive option, mainly due to the high utilities consumption for hot water and hot-dry air. The air was applied to remove water from the fermentation product (95 wt%) before the extraction process with DCM since the water presence decreases the PHB solubility in DCM. Supply of hot-dry air accounted for 11% of the total costs. In Cases I and II, PHB was released in a solution, therefore, drying was not required.

Table 2
Economic evaluation results.

	Case I	Case II	Case III
<i>Total costs (€/kg PHB)</i>			
Annual depreciation	0.22	0.24	0.37
Utilities	1.30	1.08	1.82
Materials	0.33	0.65	0.06
Maintenance	0.01	0.01	0.01
Labour	0.14	0.16	0.19
Total (no WWT credits)	1.99	2.14	2.46
Inclusion of credits			
WWT (avoided)	−0.60	−0.58	−0.51
Total (with WWT credits)	1.40	1.56	1.95
Costs by processing sections			
Fermentation	0.59	0.57	0.51
DSP	1.40	1.57	1.95
<i>Utilities costs (€/kg PHB)</i>			
Electricity	0.41	0.24	0.19
Hot water	0.11	0.16	0.60
Steam	0.27	0.27	0.23
Air	0.21	0.09	0.30
Chilled water	–	0.01	0.13
CW	0.27	0.27	0.24
Natural gas	0.01	0.00	0.01
Solid waste	–	–	0.11
Water (wash)	0.00	0.04	–
Total (no WWT credits)	1.30	1.08	1.82
Inclusion of credits			
WWT (avoided)	−0.60	−0.58	−0.51
Total (with WWT credits)	0.70	0.51	1.31

Hot water dominated the operating costs of Case III with a share of 25% of the total costs.

Chemical material costs represented 16%, 31% and 2% of the total costs for the Cases I, II and III, respectively. The material costs in Case I were the half of the ones in Case II because of the relatively low price of NaOH (the cheapest of the utilised chemicals). In Case II, NaOCl and SDS were used. NaOCl has a comparable price to NaOH, but the price of SDS is three times higher. A similar amount of chemicals were used in Case I and II, but in Case III the make-up of solvents was minimal (1%) due to high recovery level of DCM and ethanol by distillation.

The total production costs endured from the processes assessed in this study were lower than the expected price of PHA production for a scaled-up industrial biotechnology based process (2–4 €/kg PHB, Jacquelin et al., 2008). Fig. 2c. compares the PHA production costs estimated to those values reported by previous studies and by commercial processes, considering different C-sources, microorganisms and DSP options.

Current industrial PHA production typically uses sugars, which can account for 23% of the total production costs (Lee and Choi, 1998). Wastewater as a feedstock for the mixed-culture bacterial fermentation is a key parameter that can explain the lower costs found in this study since sterilisation costs are negligible. Moreover, the costs of the avoided wastewater treatment were assigned as credits and therefore subtracted from the overall production costs. In Case I, the credits accounted for 30% of the total production costs, 27% in Case II and 21% in Case III. Economic credits are higher for Case I due to the higher volumetric flow of wastewater that is required to compensate its lower PHB recovery.

Although PHB production costs from wastewater are lower compared to sugar-based production routes, the production costs need to be reduced further to provide a competitive alternative to the petrochemical counterparts. Polyethylene terephthalate (PET), for example, has a current market price of approximately 1.3 €/kg (Packham, 2014). It should be noted that production process for PET has been commercially available for many years and has matured towards optimum conditions. There is therefore potential for increasing the efficiency of PHB production via wastewater fermentation, because the current design of the industrial process is still at a moderately early stage of development compared to fossil resources based production of PET. PET is selected as indicative benchmark because it is a polyester as are PHAs. Mechanical properties of the final product are not discussed in this study. When the application of PHAs is for thermoplastic material, isotactic polypropylene should be used for comparison.

3.3. Life cycle assessment

The overall GWP including; fermentation and DSP stages, the credits from avoided wastewater treatment, and biogenic carbon, was: 2.38 kg CO₂-eq/kg PHB for Case I, 2.06 kg CO₂-eq/kg PHB for Case II and 4.30 kg CO₂-eq/kg PHB for Case III. The NREU was 106, 109 and 158 MJ/kg PHB for Cases I, II and III, respectively (Table 3). Results of LCA are shown in Fig. 3. The environmental impacts of the fermentation step were relatively low compared to those induced by the DSP. The latter(s) accounted for 61% of total GWP in Case I, 60% in Case II and 76% in Case III. The share of total NREU arising from DSP was 70% in Case I, 72% in Case II and 82% in Case III.

The key factors driving the environmental impacts caused by the fermentation process were steam use for heating the fermenters, and growth nutrients. Steam use for fermentation accounted for 15% of the GWP in Cases I and II, and 9% in Case III. Steam accounted for 12% of the total NREU in cases I and II, and 6% in Case III. Urea and phosphate, the growth nutrients required for fermentation, represent 19% of the GWP in Cases I

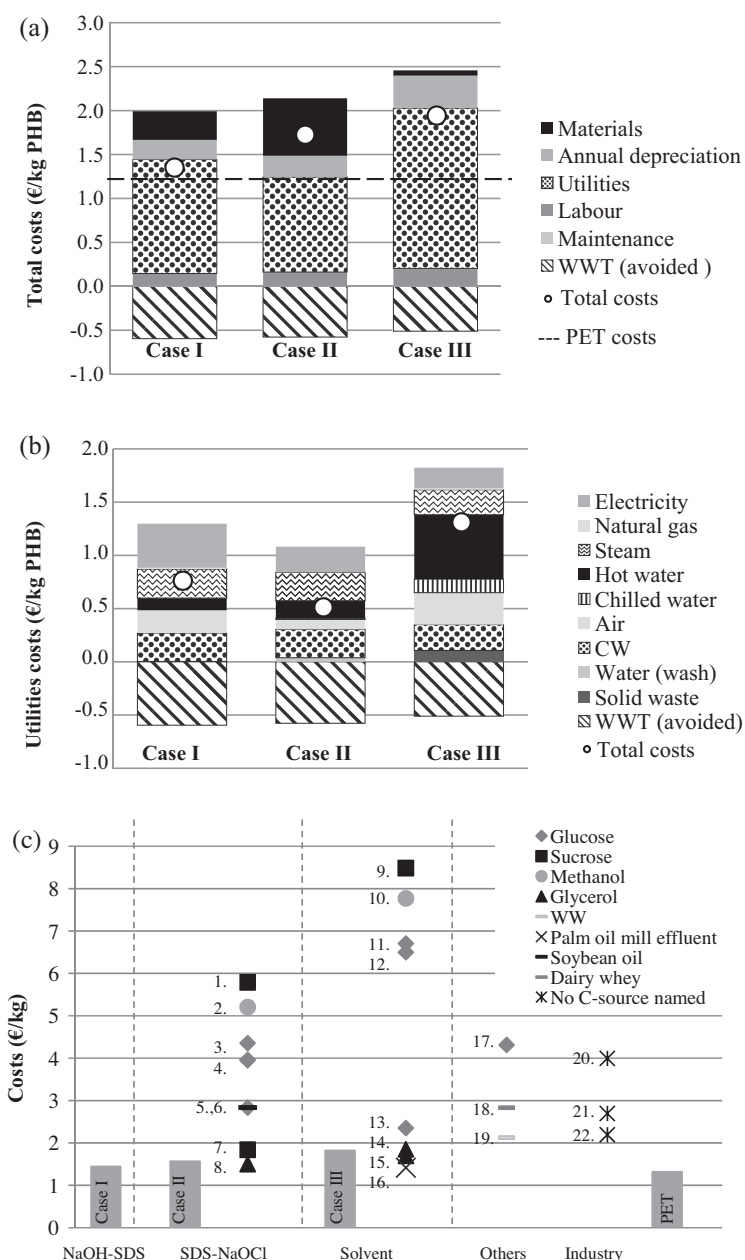


Fig. 2. Economic evaluation (fermentation + DSP). (a) Total production costs. (b) Utilities costs. (c) Comparison with literature, different substrate, microorganism and DSP. 1.2.3.4. Choi and Lee, 1997, *A. latus*, *M. organophilum*, *E. coli*, *A. eutrophus*. 5.6. Akiyama et al., 2003, *R. eutropha*. 7. Lee and Choi, 1998, *A. latus*. 8. Posada et al., 2011, *C. necator*. 9.10.11.12. Choi and Lee, 1997, *A. latus*, *M. organophilum*, *E. coli*, *A. eutrophus*. 13.14. Naranjo et al., 2013, *B. megaterium*. 15.16. Posada et al., 2011, *C. necator*. 17.18. van Wegen et al., 1998, *E. coli*. 19. Curieff and Lant, 2007. Mixed culture. 20.21.22. Jacquél et al., 2008, Biomer Biotechnology, Biogreen Mitsubishi, Metabolix. Process capacities range from 0.5 to 340 kt/a and are not harmonised in the figure.

and II, and 12% in Case III. In terms of NREU, the nutrients accounted for 15% of the overall NREU in Cases I and II, and 9% in Case III. For all cases, the electricity consumption during DSP (assumes as medium voltage, produced at grid in the Netherlands) delivered the highest contribution in the GWP. In Cases I and II, DSP shares were 40% and 33% of the total GWP, and 73% in Case III. The contribution from electricity usage to total NREU was 25% in Case I, 19% in Case II and 45% in Case III.

In Case I and II, the NREU relative to the natural gas was 29% and 27% of the total primary energy use. Case III proved the most energy intensive since the solvents recovery by distillation led to additional energy demand in the reboiler. Although the share of NREU attached to natural gas consumption in Case III was slightly higher in relative terms than for Cases I and II (37%), in absolute

terms it almost doubled the amount of primary energy use of Cases I and II (63 MJ/kg PHB in Case III, vs. 33 MJ/kg PHB in Cases I and II).

With respect to the chemicals used, NaOH and SDS when combined accounted for 18% of the total GWP and 16% of the NREU in Case I. Larger amounts of SDS were used in the chemical treatment in Case II, consequently their associated impacts were higher, i.e., 25% and 26% compared to the overall GWP and NREU, respectively. In Case III, the chemicals did not represent a significant environmental impact because they were recovered and recycled with very minor losses.

Results from the LCA indicate that the solvent treatment is the less preferred route for intracellular PHB release and purification. It has approximately 90% higher GWP and 50% higher NREU impacts

Table 3
Life cycle inventory and normalised impacts for Cases I, II and III including fermentation.

Process input	LCI (unit/kg PHB)			GWP (kg CO ₂ -eq/kg PHB)			NREU (MJ/kg PHB)		
	Case I	Case II	Case III	Case I	Case II	Case III	Case I	Case II	Case III
Nutrients (urea) (in kg)	0.25	0.24	0.21	0.79	0.77	0.68	16.37	15.87	14.03
Nutrients (phosphate) (in kg)	0.05	0.04	0.04	0.12	0.11	0.10	2.16	2.09	1.85
Steam (in kg)	3.11	3.02	2.67	0.70	0.60	0.60	12.30	11.92	10.54
Electricity fermentation (in kg)	1.40	1.36	1.20	0.27	0.23	0.23	4.14	4.01	3.55
Electricity DSP (in MJ)	10.15	7.75	25.86	1.94	1.48	4.94	30.07	22.96	76.59
Natural gas (in MJ)	32.78	30.83	60.24	0.14	0.13	0.26	34.42	32.37	63.25
Water (in kg)	235.91	420.24	1175.06	0.00	0.00	0.01	0.02	0.04	0.10
Solid waste (in kg)	-	-	0.66	-	-	-0.09	-	-	-1.41
NaOH (in kg)	0.74	-	-	0.88	-	-	17.70	-	-
SDS (in kg)	0.02	0.38	-	0.03	0.69	-	1.13	23.14	-
NaOCl (in kg)	-	0.51	-	-	0.43	-	-	8.31	-
DCM (in kg)	-	-	0.00	-	-	0.00	-	-	0.04
Ethanol (in kg)	-	-	0.00	-	-	0.00	-	-	0.01
WWT (avoided) (in m ³)	0.20	0.19	0.17	-0.45	-0.43	-0.38	-12.56	-12.17	-10.76
Biogenic carbon (in kg)	2.04	2.04	2.04	-2.04	-2.04	-2.04	-	-	-
Total				2.38	2.06	4.30	105.74	108.53	157.79

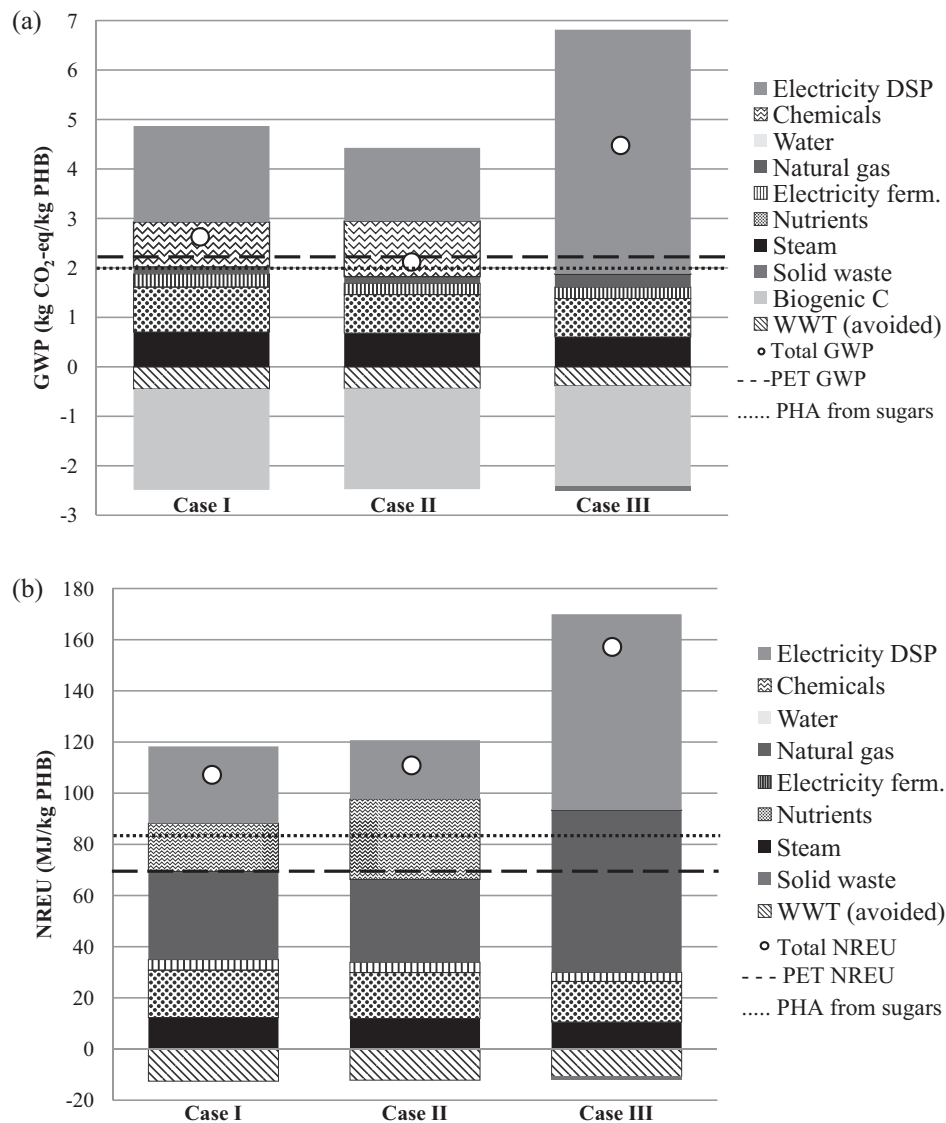


Fig. 3. Life cycle assessment of the three case studies (fermentation + DSP). (a) Global warming potential and (b) non-renewable energy usage. PET info from [Liebich and Gieglich, 2010](#), PHA from sugars information from [Patel et al., 2005](#).

than Cases I and II. In Cases I and II, the GWP was in line with that reported for sugar-based PHA production (2.0 kg CO₂-eq/kg PHA, Patel et al., 2005) and analogous fossil based plastics (2.15 kg CO₂-eq/kg PET, Liebich and Giegrich, 2010); however this is not the case for Case III. For the DSP that involves solvent, the GWP obtained is double that of sugar-based PHA or PET. The NREU for Cases I and II were comparable to sugar based PHA (81 MJ/kg PHA, Patel et al., 2005) and PET production (69 MJ/kg PET, Liebich and Giegrich, 2010). Cases I and II had approximately 30% and 55% respectively increased NREU per kg of PHB produced, compared to 1 kg of sugar-based PHA and 1 kg of PET production. The NREU results following the solvent route were 95% higher than the sugar-based PHA, and 130% higher than the NREU required for PET production.

3.4. Sensitivity analysis

3.4.1. Cell disruption efficiency

As shown in Table 4, sensitivity analysis was performed on the base design for each of the three cases. The observed efficiency of the cell disruption when taken from laboratory data was varied $\pm 10\%$ with respect to the base design. The costs (€ per kg PHB) and the environmental impacts (GWP and NREU per kg PHB) increased with reduced recovery due to a lower overall PHB yield. At a higher efficiency, more PHB was recovered, thus, the costs and environmental impacts decreased. The solvent route (Case III) was the least affected by the changes. This can be explained by the most influential factors (*i.e.* energy use in the distillation for solvents recovery) not varying under conditions of lower cell disruption efficiency.

3.4.2. SDS crystallization (Case II)

In Case II, the surfactant was recovered and recycled via crystallisation. A filter was also included for the separation of the crystallised surfactant from the liquid phase. The process was carried out at 9 °C, requiring the use of chilled water in a pre-cooler. As an alternative, the crystallisation step can be removed, and all of the SDS would enter the process as fresh material. The capital and utilities costs would then be reduced by 13% and 4%, respectively; but material costs would increase by 22%. Thus, the drawbacks of using extra material outweigh the benefits in terms of investment and utilities savings. The total production costs would then be 3.07 €/kg PHB, doubling the base design costs.

The GWP and NREU, both with and without SDS recovery are compared in Fig. 4a and b. Although the electricity consumption dropped because no chilled water was required when the crystallisation step was bypassed, the overall environmental impacts increased. Without crystallisation, the larger amount of make-up material would result in a 90% larger GWP and 60% higher NREU compared to the base design.

3.4.3. Distillation (Case III)

The utilities costs in Case III were 74% of the total production costs, of which 40% is directly linked to the energy demand for the distillation process. Instead of one column operating at 0.5 bar of absolute pressure, two sequential columns were considered within the sensitivity analysis. The first separation is performed at atmospheric pressure and the second at vacuum conditions (0.5 bar) to obtain the desired purity in the product streams. The process option with two columns did not show any benefits compared to a single vacuum column. The separation efficiency was lower, therefore, additional DCM was needed. The material costs rose from 0.06 €/kg PHB to 0.51 €/kg PHB and the annual depreciation increased by 4% due to the extra equipment. The total production costs increased 27%, from 1.95 €/kg PHB to 2.48 €/kg PHB.

The LCA results for both process options using either one or two distillation columns are shown in Fig. 4c and d. Electricity impacts decreased in the case with two columns due to less chilled water being required in the condenser of the vacuum column, but the impacts caused by DCM increased because more fresh material was used. The NREU in the two columns setup dropped by 15% with respect to the base design, but the GWP was 12% higher.

3.4.4. DCM evaporation (Case III)

In the base design, only 50% of the DCM was vaporised after extraction to avoid reaching supersaturation in the wall of the equipment and lower product quality, due to a less controlled crystallisation. On the assumption that optimised large-scale equipment is used and all DCM is evaporated, the DSP was simplified because no precipitation with ethanol or distillation was necessary. On the contrary, extra energy and larger heat exchangers were considered due to the absolute amount of DCM passing through vaporisation, condensation and recycling stages, which is doubled. The product purity and recovery yield decreased to 97.6 wt% and 81.9%, respectively. Capital and utilities costs dropped by 7% and 26%, resulting in 21% lower total production costs (1.54 €/kg PHB).

Fig. 4c and d compare LCA results of three alternative designs for Case III: (i) 50% DCM evaporation and one distillation column for ethanol-DCM recovery (base case), (ii) 50% DCM evaporation and two distillation columns, and (iii) all DCM vaporised. Since distillation is not used in this third case, chilled water and large amounts of natural gas are not necessary. Furthermore, the GWP decreased to negative values (−0.10 kg CO₂-eq/kg PHB) due to the credits from the avoided wastewater treatment and the biogenic carbon embedded in the PHB product. Additionally, the NREU values were halved (83 MJ/kg PHB).

Evaporation of all DCM simplifies the DSP significantly, leading to the most promising alternative from both an economic and environmental perspective. However, this high-evaporation level concept should experimentally be proven and its influence on the final product quality should be determined.

Table 4

Results of the sensitivity analysis for the disruption efficiency.

Parameter	Case I: NaOH-SDS			Case II: SDS-NaOCl			Case III: DCM		
	Base case	−10%	+10%	Base case	−10%	+10%	Base case	−10%	+10%
Disruption efficiency (%)	95.0	85.5	100.0 ^a	88–95 ^b	79.2–85.5 ^b	96.8–100 ^{a,b}	86.0	77.6	94.9
Overall recovery (%)	73.5	66.2	77.4	75.8	74.2	76.0	82.2	74.4	89.2
Depreciation (€)	0.22	0.25	0.21	0.24	0.25	0.24	0.37	0.37	0.37
Utilities (€)	0.70	0.76	0.68	0.51	0.51	0.51	1.31	1.35	1.27
Total costs (€)	1.40	1.53	1.34	1.56	1.74	1.42	1.95	2.00	1.90
GWP (kg CO ₂ -eq)	2.38	2.88	2.11	2.06	2.28	1.58	4.30	5.00	4.26
NREU (MJ)	106	118	101	108	114	99	158	174	156

^a Cell disruption efficiency was only increased 5% respect to the base case because it was already 95%.

^b Two values are given for the cell disruption efficiency corresponding to SDS treatment and NaOCl steps respectively.

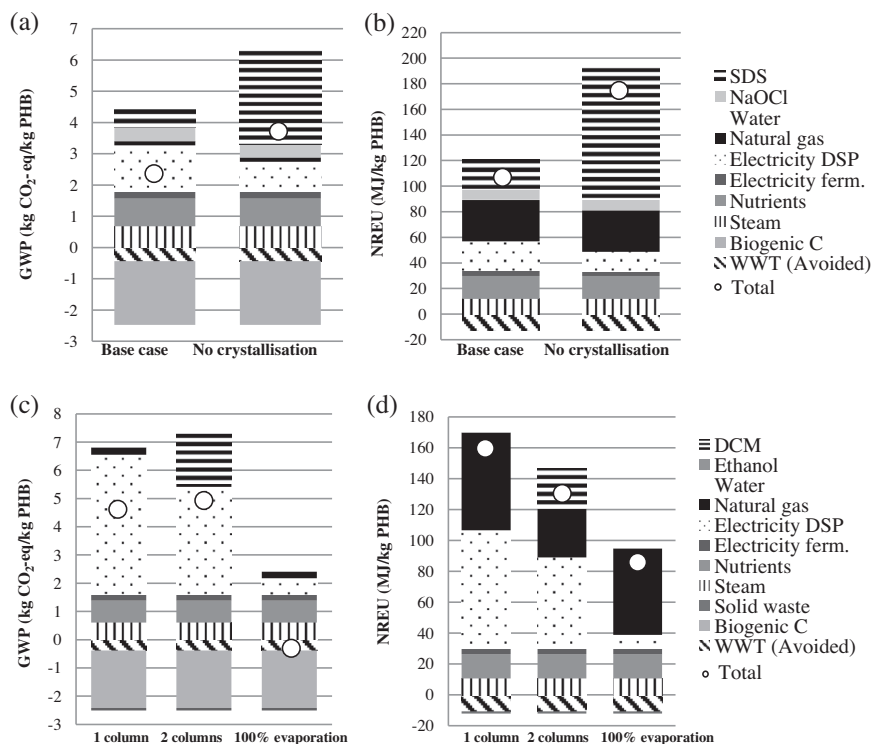


Fig. 4. Sensitivity analysis of LCA (fermentation + DSP). Case II with SDS recovery (base case) and without SDS recovery via crystallisation: (a) GWP and (b) NREU comparison. Case III with 50% DCM evaporation and one distillation column for ethanol-DCM recovery (base case), 50% DCM evaporation and two distillation columns and 100% DCM vaporised: (c) GWP and (d) NREU comparison.

4. Conclusions

Results of this study indicate that PHB production from wastewater could become an interesting alternative to the expensive sugar-based PHAs production. The process benefits from the use of wastewater as feedstock. An integrated approach including process design, economic and environmental assessments identifies alkali treatment for cell disruption as the most promising route. When compared to the alkali route, treatment with surfactant-hypochlorite needs one additional chemical digestion step. A downstream process using solvent includes a distillation step for the solvent recovery, increasing the final product costs and environmental impacts.

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