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# Preliminary integrated economic and environmental analysis of polyhydroxyalkanoates (PHAs) biosynthesis

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## Abstract

Being biodegradable and renewable, polyhydroxyalkanoates (PHAs), a green polymer, attract much attentions as potential alternative for conventional plastics due to increased concern towards environmental issue and resource depletion. However, PHAs not only have suffered some economic disadvantages on the market, and its environmental-friendliness has also been questioned as well. Therefore, there is a growing demand to improve both economic and environmental performances of PHAs production, especially at earlier stage of the process where there are plenty of opportunities and the modification cost is cheap. Therefore, a preliminary integrated assessment is introduced to provide a rapid evaluation for PHAs biosynthesis at R&D stage by coupling material cost analysis together with life-cycle assessment. Using fuzzy approach multi-objective optimization, crude glycerol is the most optimum substrate for biopolymer productions from *Cupriavidus necator*. The insight from sensitivity analysis has showed that the integrated assessment is sensitive to fluctuation in price and yield of substrate, while maintaining its robustness as similar result is obtained when using different multi-objective optimization tools. Providing some novel insights on PHAs biosynthesis like performance and site selection influencing factor, the integrated assessment can be used to facilitate screening for large-scale production of PHAs.

**Keywords:** *Cupriavidus necator*, Economic evaluation, Environmental impacts, Life-cycle assessment (LCA), Polyhydroxyalkanoates (PHAs), Waste reduction (WAR) algorithm

## Background

Intensively involved in our daily life, conventional plastics have a broad range of application, from machinery housings, disposable utensils, packaging, automobile, furniture to accessories. Motivated by the increasing concern towards environmental pollution and rapidly depleting petroleum reserve, industry, government and community are considering a biodegradable as well as economical replacement which made from renewable resources (Venkateswar Reddy et al. 2012).

Among all “green plastics”, polyhydroxyalkanoates (PHAs), a type of green polymer which is biodegradable, biocompatible, renewable (Hassan et al. 2013), and having high structural diversity (Steinbüchel and Valentin 1995) with broad range of applications have attract much attentions as potential alternative for conventional plastics. Accumulated under condition of excess carbon source with lacking of other nutrients (Magdouli et al. 2015; Dalal et al. 2013), PHAs serve as intracellular carbon and/or energy storage reserve in various microorganisms (Koller et al. 2011). PHAs can undergo rapid biodegradation (3–9 months) anaerobically and aerobically without the requirement of special environment conditions (Mumtaz et al. 2010; Keshavarz and Roy 2010).

However, there is an urgent need to improve economic performance of PHAs biosynthesis as its production cost

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is comparatively high compared with that of conventional plastics. There are some important elements that need to be considered when choosing the ideal microorganism and carbon source for industrial scale biosynthesis of PHAs, such as substrate cost, maximum polymer yield, growth rate, PHAs productivity, quality and quality of biopolymer accumulated, and downstream processing cost (Choi and Lee 1997, 1999). It is noteworthy that carbon source has been reported as the most influencing factor for PHAs price (Chanprateep 2010).

How green are green plastics? As the “green” substitute of petrochemical-based plastics, the overall process of PHAs biosynthesis must be environmental-friendly enough and causes less negative impacts on the environment (Belboom and Léonard 2016). Being a well-established and systematic concept that implements environmental sustainability (Kylili et al. 2016), life-cycle assessment (LCA) has been applied by several researchers (Kim and Dale 2005, 2008; GuriEFF and Lant 2007; Koller et al. 2013; Pawelzik et al. 2013; Harding et al. 2007; Khoo and Tan 2010; Akiyama and Tsuge 2003; Hottle et al. 2013; Heimersson et al. 2014; Álvarez-Chávez et al. 2012; Pietrini et al. 2007; Heyde 1998; Gerngross 1999) on “cradle-to-grave” or “cradle-to-gate” environmental analysis of PHAs biosynthesis, comparison with conventional plastics and other bioplastic, and from many different points of view. Most works demonstrates that PHAs are more environmental-friendly compared with conventional plastics, while some of the earlier works give different conclusions (Gerngross 1999; Heyde 1998).

Literature have always reported environmental impacts in terms of carbon dioxide equivalent emissions (global warming potential) and/or energy consumptions (Akiyama and Tsuge 2003; Kim and Dale 2005, 2008; GuriEFF and Lant 2007). However, these are not comprehensive enough and hardly cover every aspect, which includes smog formation, ozone depletion, acid-rain formation, water pollution, and other pollutions (Harding et al. 2007). Developed based on sustainability concept of LCA, WAR algorithm is an environmental index that can covers a wide range of environmental concerns (Young et al. 2000). In addition, R&D stage is a crucial stage to integrate environmental concern inherently into the process design as there are plenty of opportunities and the modification cost is cheap (Koller et al. 2013). Therefore, focusing on R&D stage, this paper aimed to develop an integrated analysis of economic and environmental performances for PHAs by coupling material cost evaluation and WAR algorithm with the illustration of a case study to screen suitable carbon source for PHA biosynthesis.

## Methods

### Microorganism and culture conditions

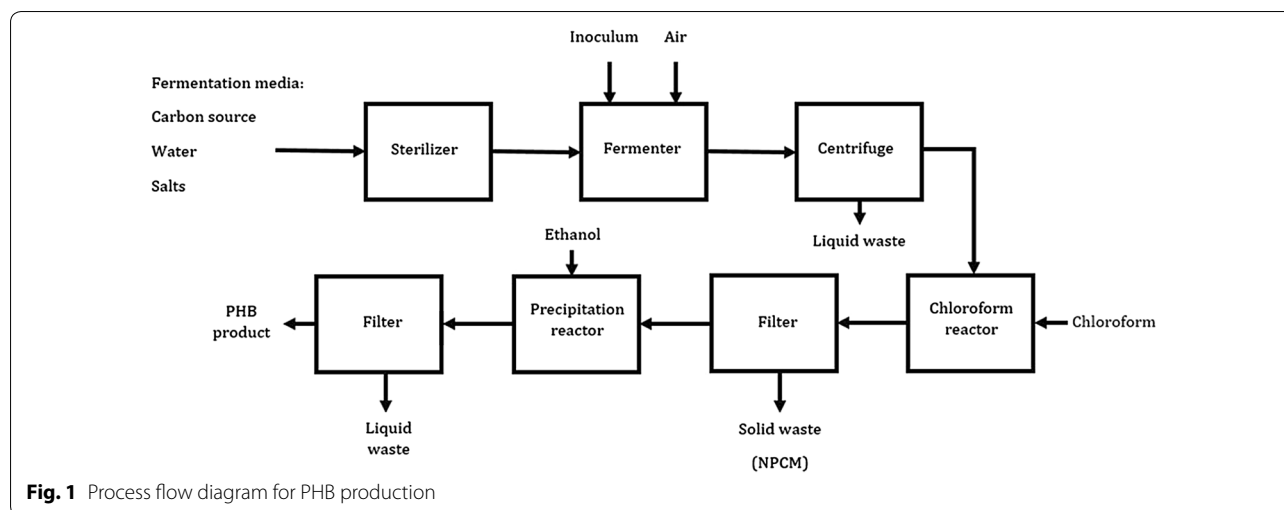
The bacterial strain used *Cupriavidus necator* H16 was maintained in nutrient plates at 4 °C. Cells were first cultured using agar plates at 30 °C for 24 h. Seeder cultures were prepared in 10 mL of nutrient broths (8 g/L) by inoculated with single colony from agar plates. The culture was then grown aerobically at 30 °C for 24 h. Subsequently, the inoculum (5 % v/v) was transferred to a 250-mL shake flask containing 100 mL operating volume of defined medium supplemented with glycerol of 30 g/L and yeast extract of 2 g/L. The inoculated fermentation medium was then incubated aerobically at 30 °C for 72 h. The defined medium was composed of Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O, 6.7 g/L; KH<sub>2</sub>PO<sub>4</sub>, 1.5 g/L; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2.5 g/L; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.2 g/L; and CaCl<sub>2</sub>, 10 mg/L) and 0.5 % v/v of trace mineral solution (Na<sub>2</sub>EDTA, 6.0 g/L; FeCl<sub>3</sub>·6H<sub>2</sub>O, 0.29 g/L; H<sub>3</sub>BO<sub>3</sub>, 6.84 g/L; MnCl<sub>2</sub>·4H<sub>2</sub>O, 0.86 g/L; ZnCl<sub>2</sub>, 0.06 g/L; CoCl<sub>2</sub>·6H<sub>2</sub>O, 0.026 g/L; and CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.002 g/L).

The carbon sources used are soybean oil, waste cooking oil, crude, and refined glycerol. *Cupriavidus necator* was cultivated for 72 h at 30 °C and pH 7 in 5-L bioreactor containing 3.5 L working volume of defined medium with 2-g/L yeast extract and optimized carbon source of 5 % wt/v for soybean oil and waste cooking oil, while 3 % wt/v for crude and refined glycerol. The stirring speed was controlled by dissolved O<sub>2</sub> (DO) concentration which maintained at 50 % and air flow rate at 1 vvm. The process flow diagram for production of PHB from *C. necator* is shown in Fig. 1.

### Biomass determination and PHA content analysis

Dry cell weight (DCW) was determined by weighing dry cells from 10 mL of culture broth as the mean of triplicate measurements. Cells were centrifuged and washed twice with deionized water before drying it overnight at 70 °C.

For the determination of PHB content, the GC method of Akaraonye et al. with slight modification was employed (Akaraonye et al. 2012). 2 mL of chloroform and 2 mL of acidified methanol which contained 1 % v/v of sulphuric acid were added to approximately 20-mg dried samples. After undergoes esterification at 100 °C for 15 h, 1 M sodium chloride was added to stop the reaction and the samples were cooled rapidly. The sample was then allowed to settle until separating into organic and aqueous phases. Then, 0.2 µL of organic phase (bottom phase) was injected into GC-2014 (Shimadzu, Japan) equipped with a ZB-5 column. The initial oven temperature is set at 80 °C and held for 1 min, then increased to 200 °C at a rate of 20 °C/min and held for 3 min at the same



temperature. The PHB content of the injected samples was determined by internal standard calibration using standard PHB (Sigma) with diphenyl ether: chloroform of ratio 1:9 as internal standard. The carrier gas used is nitrogen gas at constant pressure 80 kPa. The injection and detector temperature are both 250 °C with split flow 13 mL/min.

#### Integrated economic and environmental assessment

For all assessment, the system boundary only covered the “gate-to-gate” analysis of upstream PHA biosynthesis as the main objective of this work is to screen the suitable carbon source that satisfies both economic and environmental criteria. Cost and environmental impacts of utility are assumed to be the same for each carbon source due to the same fermentation condition.

#### Economic assessment of PHA biosynthesis

The economic performances of PHA biosynthesis are evaluated by calculating the raw material cost which dominates the major shares of PHA’s production cost. The material cost calculation is given as follows:

$$\text{Material cost} = \sum (\text{Cost of chemical components per kg} \times R^{\text{cp}}), \quad (1)$$

where cp is chemical components, and  $R$  is chemical components used per kg PHA produced (kg cp/kg PHA).

#### Environmental assessment of PHA biosynthesis

Based on the potential environmental impact (PEI) balance concept introduced by (Hilaly and Sikdar 1995; Young and Cabezas 1999) have introduced WAR algorithm that focuses on waste minimization across the process boundary. With an extensive chemicals database,

WAR algorithm covers four local toxicological impact categories which are human toxicity potential by ingestion (HTPI), terrestrial toxicity potential (TTP), human toxicity potential by either inhalation or dermal exposure (HTPE), and aquatic toxicity potential (ATP) and four global atmospheric impacts which are global warming potential (GWP), ozone depletion potential (ODP), photochemical oxidation or smog formation potential (POP), and acidification or acid-rain potential (AP). The equations used are as follows:

$$\text{Impact}_i^{\text{total}} = \sum_{\text{cp}}^n (\text{Impact}_i^{\text{cp}} \times R^{\text{cp}}) \quad (2)$$

$$\text{PEI} = \sum_i^m \text{Impact}_i^{\text{total}} \quad (3)$$

$$\text{EB} = \sum \text{PEI introduced} - \sum \text{PEI deducted} \quad (4)$$

where EB is environmental burdens.

PEI categories are combined into a single PEI index utilizing weighing factors which show the relative or site-specific considerations of user. Following the literature (Young et al. 2000), with no specific site in mind, the weighting factor for all categories in this case study will be given equivalent values of unity.

#### Multi-objective optimization

In this case study, there are two objectives, which are economic and environmental concerns that needed to be considered for alternative selection. As these objectives have the possibility to be conflicting in nature, this becomes a multi-objective optimization problem. Thus, there is a need for utilizing “fuzzy optimization

algorithm” as a decision-making tool on the trade-off between the different objectives of the process optimization. In fuzzy objective optimization, a degree of satisfaction  $\lambda_o$  has to be introduced to each objective which can be expressed as a linear function bounded by lower and upper limits of the target objective, as shown in Eqs. (5) and (6). Equation (5) is used for a target objective to be maximized, while Eq. (6) is used for a target objective to be minimized:

$$\lambda_o = \begin{cases} 0 & V_o \leq v_o^L \\ \frac{V_o - v_o^L}{v_o^U - v_o^L} & v_o^L \leq V_o \leq v_o^U \\ 1 & V_o \geq v_o^U \end{cases} \quad (5)$$

$$\lambda_o = \begin{cases} 0 & V_o \geq v_o^U \\ \frac{v_o^U - V_o}{v_o^U - v_o^L} & v_o^L \leq V_o \leq v_o^U \\ 1 & V_o \leq v_o^L \end{cases} \quad (6)$$

$$0 \leq \lambda_o \leq 1, \quad (7)$$

where  $V_o$  is the score of the carbon source (e.g., material cost for economic, and total PEI and EB for environmental),  $v_o^L$  and  $v_o^U$  are the lower and upper limits, respectively.

All  $\lambda_o$  have to be maximized for all  $\lambda_o$  to achieve high level of satisfaction. Developed by Zimmermann (1978), max–min operator tool can be utilized to maximize the least satisfied degree of satisfaction by guaranteeing that all  $\lambda_o$  will be satisfied to at least the value of  $\lambda$ . Therefore, the overall objective now is to maximize  $\lambda$  as:

$$\lambda_o \geq \lambda \quad (8)$$

#### Sensitivity analysis

Sensitivity analysis was conducted to determine the robustness and sensitivity of the integrated assessment. For this purpose, five different cases were considered where the most influencing factors were manipulated. In the first four cases, yield and price of both refined and crude glycerol were manipulated, while in the last case, the weighting factor was utilized for multi-objective optimization instead of fuzzy optimization algorithm using the following equations:

$$\text{PEI weightage} = 1 - \text{Cost weightage} \quad (9)$$

$$\text{EB weightage} = 1 - \text{Cost weightage} \quad (10)$$

## Results and discussion

### Basis for assessment of PHA biosynthesis

Showing the raw material cost at year 2015 in USD \$/kg and the PEI score for each chemical components in each categories, respectively, the foundation for the economic

and environment assessment of PHA production is presented in Table 1. The inoculum cells were deemed negligible to be considered as raw material cost due to rapid dividing rate of bacteria cells (Raynolds et al. 2000). The PHA biosynthesis from *C. necator* H16 using four different substrates, soybean oil, waste cooking oil (WCO), refined, and crude glycerol, which yielded 20.73, 11.05, 31.07, and 25.01 g/L of PHAs, respectively, has been studied. Following that, the relative masses of carbon sources of soybean oil, WCO, refined, and crude glycerol are 2.412, 4.524, 0.966, and 1.2 kg/kg PHA. The material and energy flows used in the study are provided in Table 2.

### Economic assessment of PHA biosynthesis

The results for both economic and environmental assessment for PHA biosynthesis are shown in Table 3. From Table 3, it is clearly shown that crude glycerol (0.362 USD\$/kg PHA) is the most economic competitive substrate, followed by refined glycerol (0.484 USD\$/kg PHA) as they gave a better biopolymer yield compared to others. The raw material cost calculated is comparable with the literature value of 0.118 and 0.149 USD\$/kg for crude and refined glycerol, respectively (Posada et al. 2011). As the by-product in biodiesel synthesis, glycerol's price reduces drastically following the rapid growth of biodiesel industry and has huge potential as carbon source for PHA biosynthesis (Posada et al. 2011). Being the unwanted waste from food industry, WCO also demonstrates its value as substrate for PHA production (1.180 USD\$/kg PHA). Agreeing with the literature (Akaraonye et al. 2012), further analysis has shown that carbon source has dominated the share of raw material cost which contribute at least 66.3 % of the total cost. Internal rate of return (IRR) and total annual cost (TAC) have always been used by the literature (Gurieff and Lant 2007; Van Wegen et al. 1998; Choi et al. 2010) to evaluate economic performance of PHAs biosynthesis process. However, these assessment techniques are not suitable for rapid screening at early stage as tedious and rigorous calculations are required.

### Environmental assessment of PHA biosynthesis

During environmental assessment for different process alternatives, WAR algorithm considers the “worst-case scenario” which is the accidental release and disperse of all chemicals into the environment, and achieves dangerous levels of concentration, causing short- or long-term impacts to human, terrestrial, aquatic, and global atmospheric. Therefore, from Table 3, the production of PHA from refined glycerol gives the least environmental impact (Total PEI = 1.130), where WCO scores the worst (Total PEI = 4.657) as total loss of containment

**Table 1 Data of raw material cost and PEI**

Chemicals <sup>a</sup>	Price (USD \$/kg) <sup>a</sup>	HTPI (kg <sup>-1</sup> ) <sup>b</sup>	TTP (kg <sup>-1</sup> ) <sup>b</sup>	HTPE (kg <sup>-1</sup> ) <sup>b</sup>	ATP (kg <sup>-1</sup> ) <sup>b</sup>	GWP (kg <sup>-1</sup> ) <sup>b</sup>	ODP (kg <sup>-1</sup> ) <sup>b</sup>	PCOP (kg <sup>-1</sup> ) <sup>b</sup>	AP (kg <sup>-1</sup> ) <sup>b</sup>
Na <sub>2</sub> HPO <sub>4</sub>	0.25	0.045	0.045	0	0.002	0	0	0	0
KH <sub>2</sub> PO <sub>4</sub>	0.3	0.156	0.156	0	0.005	0	0	0	0
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.1	0.177	0.177	0	0.025	0	0	0	0
MgSO <sub>4</sub>	0.05	0.070	0.070	0	0	0	0	0	0
CaCl <sub>2</sub>	0.08	0.191	0.191	0	0	0	0	0	0
Na <sub>2</sub> EDTA	1.55	0.156	0.156	0	0.007	0	0	0	0
FeCl <sub>3</sub>	0.2	0.835	0.835	0.082	0.011	0	0	0	0
H <sub>3</sub> BO <sub>3</sub>	0.4	0.141	0.141	0.119	0.028	0	0	0	0
MnCl <sub>2</sub>	0.3	0.488	0.488	0.021	0.014	0	0	0	0
ZnCl <sub>2</sub>	0.18	1.070	1.070	0.238	0.065	0	0	0	0
CoCl <sub>2</sub>	0.8	0.490	0.490	0.595	0.033	0	0	0	0
CuSO <sub>4</sub>	1.15	1.250	1.250	0.095	1.380	0	0	0	0
Yeast extract	0.3	0	0	0	0	0	0	0	0
Soybean oil	0.615	0.059	0.059	0	0.489	0	0	0	0
Refined glycerol	0.4	0.030	0.030	0.048	0	0	0	0.993	0
Crude glycerol	0.2	0.030	0.030	0.048	0	0	0	0.993	0
Waste cooking oil <sup>c</sup>	0.2	0.098	0.098	0	0.792	0	0	0	0

<sup>a</sup> All the chemical prices were obtained from Alibaba.com (Steinbüchel and Valentin 1995), with exception of soybean oil (Van Wegen et al. 1998)

<sup>b</sup> The PEI score can be obtained from the database of WAR algorithm graphical user interface (Environmental Protection Agency, U.S.)

<sup>c</sup> PEI for waste cooking oil are of palmitic acid as most of the waste cooking oil are palm oil-based

**Table 2 Material and energy values for PHAs production**

Feed	
Electricity (MJ) <sup>a</sup>	648
Air (kg)	19.4
Water (kg)	3.5
Carbon source	
Crude glycerol (kg)	105
Pure glycerol (kg)	105
Soybean oil (kg)	175
Waste cooking oil (kg)	175
Salts	
Na <sub>2</sub> HPO <sub>4</sub> (kg)	23.45
KH <sub>2</sub> PO <sub>4</sub> (kg)	5.25
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> (kg)	8.75
MgSO <sub>4</sub> (kg)	0.7
CaCl <sub>2</sub> (kg)	0.035
Trace mineral salts (kg)	0.25
PHB products	
Crude glycerol (kg)	87.54
Pure glycerol (kg)	108.75
Soybean oil (kg)	72.56
Waste cooking oil (kg)	38.68
Solid waste (biomass)	
Crude glycerol (kg)	40.29
Pure glycerol (kg)	38.01
Soybean oil (kg)	56.88
Waste cooking oil (kg)	83.44

<sup>a</sup> Energy requirement by fermenter is assumed as 0.5 kW/m<sup>3</sup> (Harding et al. 2007)

for this process will give the most negative impact on the environment.

Detailed environmental analysis showed that biosynthesis of PHA does not cause much burden on global atmospheric except when utilizing glycerol as carbon source. Release of crude and refined glycerol to the surroundings will cause the formation of photochemical smog with POP of 0.959- and 1.404-kg PHA<sup>-1</sup>, respectively. Other than that, comparing between different carbon sources, accidental release of soybean oil and WCO into water body will cause water pollution and endangers aquatic life with ATP of 1.184- and 3.590-kg PHA<sup>-1</sup>, respectively. Thus, these concerns needed to be considered when choosing PHA production plant site.

On the other hand, the process of turning unwanted byproducts into value-added products helps in reduction of environmental impacts by eliminating the release of waste to the environment from the point of view of overall sustainability. Therefore, utilizing glycerol and WCO as substrate for PHA biosynthesis causes deduction in environmental burden, as shown in Table 3. Hence, the environmental burdens for PHA synthesized from WCO, refined, and crude glycerol have the value of -4.275, -1.062, and -1.320, respectively, while environmental burden for soybean oil has the same value as that of Total PEI.

Compared to CML 2 baseline 2000 V2.03 and TRACI model used by Harding and his coworkers (2007) and

**Table 3 Economic and environmental evaluation between different carbon sources for *C. necator***

Carbon sources	Soybean oil	WCO	Refined glycerol	Crude glycerol
Material cost (USD\$/kg PHA)	1.631	1.180	0.484	0.362
HTPI (kg PHA <sup>-1</sup> )	0.192	0.533	0.061	1.000
TTP (kg PHA <sup>-1</sup> )	0.192	0.533	0.061	1.000
HTPE (kg PHA <sup>-1</sup> )	0.0002	0.0004	0.046	0.095
ATP (kg PHA <sup>-1</sup> )	1.184	3.590	0.003	0.003
GWP (kg PHA <sup>-1</sup> )	0.000	0.000	0.000	0.000
ODP (kg PHA <sup>-1</sup> )	0.000	0.000	0.000	0.000
POP (kg PHA <sup>-1</sup> )	0.000	0.000	0.959	1.958
AP (kg PHA <sup>-1</sup> )	0.000	0.000	0.000	0.000
Total PEI (kg PHA <sup>-1</sup> )	1.568	4.657	1.130	1.404
Environmental burdens (kg PHA <sup>-1</sup> )	+1.568	-4.275	-1.062	-1.320

Kim and Dale (2008), respectively, the chemical database of both the environmental assessment techniques is not comprehensive enough to cover the whole range of chemicals consumed in biosynthesis of PHAs from *C. necator*. On the other hand, non-renewable energy use (NREU) or fossil energy consumption and global warming potential (GWP) or greenhouse gasses (GHG) emissions used by Akiyama and his colleagues (2003); Guriieff and Lant (2007); Yu and Chen (2008) as well as Miller and his coworkers (2013) requires massive amount of information to obtain the impact value for every single component. Although these strategies may not be suitable in rapid screening for choice of bacteria and carbon source, they are able to provide detailed insights on cradle-to-gate environmental performance of PHAs and can be further improved if the assessment could cover a wider range of environmental impacts.

#### Multi-objective optimization using fuzzy approach

Following the two different environmental perspectives, there are two different scenarios for multi-objective optimization. First scenario aims to minimize both raw material cost and PEI by considering the “worst-case scenario”, while the second scenario focusing on overall sustainability which minimizes the raw material cost as well as environmental burden. The result of multi-objective optimization using fuzzy approach is shown in Table 4. In the first scenario, crude glycerol obtains the highest  $\lambda$  ( $\lambda = 0.922$ ), which may due to its high yield and considerably cheap price among other substrates. Similarly, crude glycerol also achieves the highest  $\lambda$  ( $\lambda = 0.494$ ) for the second scenario, due to its economic advantages and sustainability using it as carbon source for PHA biosynthesis. This has concluded that the crude glycerol is the most optimal carbon source for PHA biosynthesis.

**Table 4 Results of multi-objective optimization for two different scenarios**

Carbon source	Soybean oil	WCO	Refined glycerol	Crude glycerol
Cost $\lambda_o$	0	0.355	0.904	1.000
PEI $\lambda_o$	0.876	0	1.000	0.922
EB $\lambda_o$	0	1.000	0.450	0.494
(Cost + PEI) $\lambda$	0	0	0.904	0.922
(Cost + EB) $\lambda$	0	0.355	0.450	0.494

#### Sensitivity analysis

Aiming to investigate the consequences of “what if?” scenarios, a sensitivity analysis was conducted by considering the main parameters that influence both economic and environmental performance of PHA production by different carbon sources. Five different cases were considered as the parameters have huge influence on the results of multi-objective optimization and might have an influence on the result when the value of the parameters fluctuates. The results of five cases are shown in Table 5a, b, c and d and Fig. 2a, b.

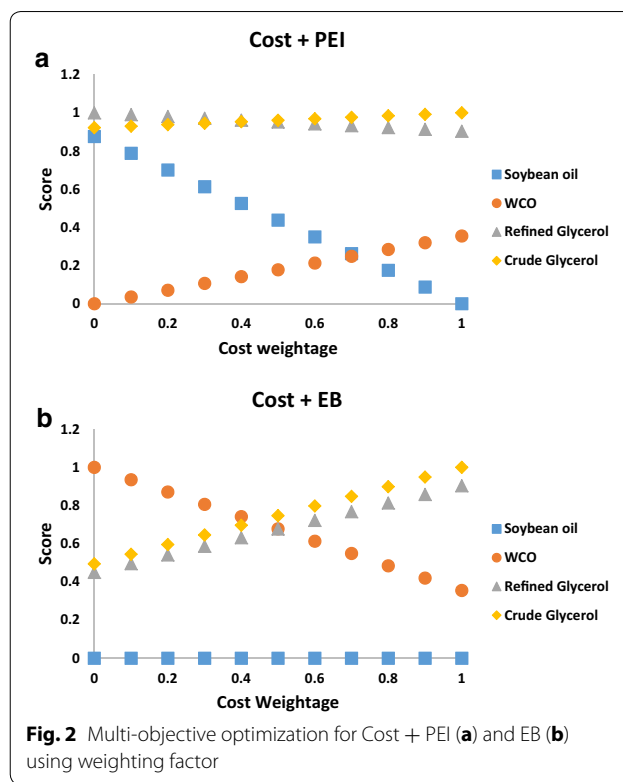
In case 1, the raw material cost, PEI, and EB of PHA production from crude glycerol increase from 0.362 to 0.538 USD\$/kg PHA, 1.404 to 1.560 kg PHA<sup>-1</sup>, and -1.320 to -1.466 kg PHA<sup>-1</sup>, respectively, with the yield decrease by 10 %. Following that, for crude glycerol, the (Cost + PEI)  $\lambda$  decreases from 0.922 to 0.878, while (Cost + EB)  $\lambda$  increases slightly from 0.494 to 0.519. Thus, refined glycerol has become the best carbon source from the point of view “worst-case scenario”.

In case 2, the raw material cost, PEI, and EB of PHA synthesized from refined glycerol decrease from 0.484 to 0.440 USD\$/kg PHA, 1.130 to 1.027 kg PHA<sup>-1</sup>, and -1.062 to -0.966 kg PHA<sup>-1</sup>, respectively, following 10 % rise in yield. On the basis of that, for refined glycerol, the (Cost + PEI)  $\lambda$  increases to 0.969 from 0.904, while the

**Table 5 Summary of the sensitivity analysis using five different cases**

Carbon source	Soybean oil	WCO	Refined glycerol	Crude glycerol
<b>a. Case 1</b>				
Cost (USD\$/kg PHA)	1.631	1.180	0.484	0.402
PEI (kg PHA <sup>-1</sup> )	1.568	4.657	1.130	1.560
EB (kg PHA <sup>-1</sup> )	1.568	-4.275	-1.062	-1.466
Cost λ <sub>o</sub>	0	0.367	0.933	1
PEI λ <sub>o</sub>	0.876	0	1	0.878
EB λ <sub>o</sub>	0	1	0.450	0.519
(Cost + PEI) λ	0	0	0.933	0.878
(Cost + EB) λ	0	0.367	0.450	0.519
<b>b. Case 2</b>				
Cost (USD\$/kg PHA)	1.631	1.180	0.440	0.362
PEI (kg PHA <sup>-1</sup> )	1.568	4.657	1.027	1.560
EB (kg PHA <sup>-1</sup> )	1.568	-4.275	-0.966	-1.466
Cost λ <sub>o</sub>	0	0.367	0.969	1
PEI λ <sub>o</sub>	0.851	0	1	0.853
EB λ <sub>o</sub>	0	1	0.434	0.519
(Cost + PEI) λ	0	0	0.969	0.853
(Cost + EB) λ	0	0.367	0.434	0.519
<b>c. Case 3</b>				
Cost (USD\$/kg PHA)	1.631	1.180	0.484	0.386
PEI (kg PHA <sup>-1</sup> )	1.568	4.657	1.130	1.404
EB (kg PHA <sup>-1</sup> )	1.568	-4.275	-1.062	-1.32
Cost λ <sub>o</sub>	0	0.367	0.921	1
PEI λ <sub>o</sub>	0.876	0	1	0.922
EB λ <sub>o</sub>	0	1	0.450	0.494
(Cost + PEI) λ	0	0	0.921	0.922
(Cost + EB) λ	0	0.367	0.450	0.494
<b>d. Case 4</b>				
Cost (USD\$/kg PHA)	1.631	1.180	0.446	0.362
PEI (kg PHA <sup>-1</sup> )	1.568	4.657	1.130	1.404
EB (kg PHA <sup>-1</sup> )	1.568	-4.275	-1.062	-1.320
Cost λ <sub>o</sub>	0	0.355	0.934	1
PEI λ <sub>o</sub>	0.876	0	1	0.922
EB λ <sub>o</sub>	0	1	0.450	0.494
(Cost + PEI) λ	0	0	0.934	0.922
(Cost + EB) λ	0	0.355	0.450	0.494

(Cost + EB) λ decreases from 0.450 to 0.434. The same as the previous case, the multi-objective optimization gives the same result where refined glycerol became a better substrate than crude glycerol.



In case 3, as the crude glycerol price increases by 10% (i.e., USD\$ 0.22), the overall raw material cost of PHA from crude glycerol increases to 0.386 from 0.362 USD\$/kg PHA. Following that, the (Cost + PEI) λ of refined glycerol increases from 0.904 to 0.921, and becomes quite close to that of crude glycerol (0.922). Therefore, it can be predicted that the refined glycerol will replace crude glycerol as the best carbon source as the price of crude glycerol further increase.

In case 4, the total raw material cost of PHA from refined glycerol decreases from 0.484 to 0.446 USD\$/kg PHA following the reduction of 10% in refined glycerol price (i.e., USD\$ 0.36). With that, the (Cost + PEI) λ of refined glycerol increases to 0.934 from 0.904, making the refined glycerol a more desired substrate for PHA biosynthesis. Therefore, it can be concluded that the integrated assessment is sensitive to the price and PHA yield of the substrate.

In case 5, weighting factor is utilized for multi-objective optimization instead of fuzzy approach. From the “worst-case scenario” viewpoint, WCO and crude glycerol have the similar increasing trend as the weightage of cost increases, while soybean oil and refined glycerol present a different trend. Crude and refined glycerol maintains a high score of at least 0.9 throughout the range of weightage with crude glycerol take over the most optimal substrate after cost weightage of 0.5.

On the other hand, based on the concept of overall sustainability, WCO's score falling as the cost weightage increases, while soybean oil's score remains 0 throughout the range of weightage. Both crude and refined glycerol have increasing score following the rise of cost weightage, with crude glycerol's score constantly on top of refined glycerol. Agreeing with fuzzy approach, crude glycerol is the most optimum carbon source for both scenarios, followed by refined glycerol.

### Concluding remarks

The integrated economic and environmental assessment has successfully compared and analyzed PHA production from different carbon sources. It can be concluded that crude glycerol is the most optimum carbon source for PHAs biosynthesis from the viewpoint of both economic and environmental. Insight from sensitivity analysis has showed that this integrated assessment is sensitive to yield and price of the substrate, while giving similar results when using different multi-objective optimization tools. This integrated economic and environmental assessment has successfully proved to aid in providing a rapid preliminary analysis of carbon sources for PHAs biosynthesis.

### Abbreviations

AP: acidification or acid-rain potential; ATP: aquatic toxicity potential; DCW: dry cell weight; GWP: global warming potential; HTPe: human toxicity potential by either inhalation or dermal exposure; HTPi: human toxicity potential by ingestion; LCA: life-cycle assessment; ODP: ozone depletion potential; PEI: potential environmental impact; PHA: polyhydroxyalkanoates; POP: photochemical oxidation or smog formation potential; TMS: trace mineral solution; TTP: terrestrial toxicity potential; WCO: waste cooking oil.

### Authors' contributions

YKL carried out the economic and environment analysis as well as drafted the manuscript. PLS helped to draft the manuscript and proofread it. HCL and CKC carried out the experiments and provide the experimental data. HSL provide technical guidance on statistical analysis. JCWL conceived of the study and participated in its design. TCL participated in the design of the studies. All authors read and approved the final manuscript.

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### Competing interests

The authors declare that they have no competing interests.

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