RESEARCH

Open Access

CrossMark

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

Yoong Kit Leong[1], Pau Loke Show[1,2]*, Heng Chou Lin[3], Chih Kai Chang[3], Hwei-San Loh[4], John Chi-Wei Lan[3] and Tau Chuan Ling[5]

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

*Correspondence: PauLoke.Show@nottingham.edu.my;
showpauloke@gmail.com
[2] Food and Pharmaceutical Engineering Research Group, Faculty of Engineering, University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan, Malaysia
Full list of author information is available at the end of the article

Leong *et al. Bioresour. Bioprocess.* (2016) 3:41

Page 2 of 9

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.

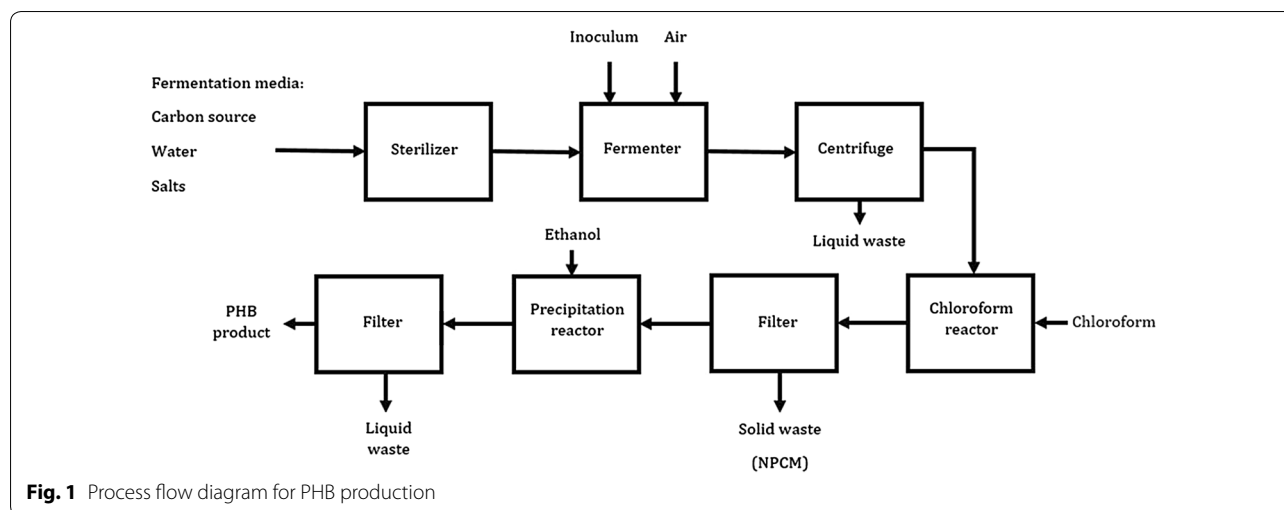## Methods

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_2HPO_4-$7H_2O$, 6.7 g/L; KH_2PO_4, 1.5 g/L; $(NH_4)_2SO_4$, 2.5 g/L; $MgSO_4$-$7H_2O$, 0.2 g/L; and $CaCl_2$, 10 mg/L) and 0.5 % v/v of trace mineral solution (Na_2EDTA, 6.0 g/L; $FeCl_3$-$6H_2O$, 0.29 g/L; H_3BO_3, 6.84 g/L; $MnCl_2$-$4H_2O$, 0.86 g/L; $ZnCl_2$, 0.06 g/L; $CoCl_2$-$6H_2O$, 0.026 g/L; and $CuSO_4$-$5H_2O$, 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_2 (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

Leong *et al. Bioresour. Bioprocess.* (2016) 3:41

Page 3 of 9

Fig. 1 Process flow diagram for PHB production

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:

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

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:

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

$$\text{PEI} = \sum_i^m \text{Impact}_i^{\text{total}} \tag{3}$$

$$\text{EB} = \sum \text{PEI introduced} - \sum \text{PEI deducted} \tag{4}$$

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

Leong *et al. Bioresour. Bioprocess.* (2016) 3:41

Page 4 of 9

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 λ_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:

$$\lambda_0 = \begin{cases} 0 & V_o \leq v_o^L \\ \frac{V_o - v_o^L}{v_o^U - v_o^L} & v_o^L \leq V_o \leq v_o^U \\ 1 & V_o \geq v_o^U \end{cases} \tag{5}$$

$$\lambda_0 = \begin{cases} 0 & V_o \geq v_o^U \\ \frac{v_o^U - V_o}{v_o^U - v_o^L} & v_o^L \leq V_o \leq v_o^U \\ 1 & V_o \leq v_o^L \end{cases} \tag{6}$$

$$0 \leq \lambda_0 \leq 1, \tag{7}$$

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 λ_o have to be maximized for all λ_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 λ_o will be satisfied to at least the value of λ. Therefore, the overall objective now is to maximize λ as:

$$\lambda_0 \geq \lambda \tag{8}$$

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:

$$PEI\ weightage\ = 1 - Cost\ weightage \tag{9}$$

$$EB\ weightage\ = 1 - Cost\ weightage \tag{10}$$

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

Leong *et al. Bioresour. Bioprocess.* (2016) 3:41

Page 5 of 9

Table 1 Data of raw material cost and PEI

Chemicals[a]	Price (USD $/kg)[a]	HTPI (kg^{-1})[b]	TTP (kg^{-1})[b]	HTPE (kg^{-1})[b]	ATP (kg^{-1})[b]	GWP (kg^{-1})[b]	ODP (kg^{-1})[b]	PCOP (kg^{-1})[b]	AP (kg^{-1})[b]
Na_2HPO_4	0.25	0.045	0.045	0	0.002	0	0	0	0
KH_2PO_4	0.3	0.156	0.156	0	0.005	0	0	0	0
$(NH_4)_2SO_4$	0.1	0.177	0.177	0	0.025	0	0	0	0
$MgSO_4$	0.05	0.070	0.070	0	0	0	0	0	0
$CaCl_2$	0.08	0.191	0.191	0	0	0	0	0	0
Na_2EDTA	1.55	0.156	0.156	0	0.007	0	0	0	0
$FeCl_3$	0.2	0.835	0.835	0.082	0.011	0	0	0	0
H_3BO_3	0.4	0.141	0.141	0.119	0.028	0	0	0	0
$MnCl_2$	0.3	0.488	0.488	0.021	0.014	0	0	0	0
$ZnCl_2$	0.18	1.070	1.070	0.238	0.065	0	0	0	0
$CoCl_2$	0.8	0.490	0.490	0.595	0.033	0	0	0	0
$CuSO_4$	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[c]	0.2	0.098	0.098	0	0.792	0	0	0	0

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

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

[c] 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)[a]	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_2HPO_4 (kg)	23.45
KH_2PO_4 (kg)	5.25
$(NH_4)_2SO_4$ (kg)	8.75
$MgSO_4$ (kg)	0.7
$CaCl_2$ (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

[a] Energy requirement by fermenter is assumed as 0.5 kW/m^3 (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^{-1}, 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^{-1}, 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

Leong *et al. Bioresour. Bioprocess.* (2016) 3:41

Page 6 of 9

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^{-1})	0.192	0.533	0.061	1.000
TTP (kg PHA^{-1})	0.192	0.533	0.061	1.000
HTPE (kg PHA^{-1})	0.0002	0.0004	0.046	0.095
ATP (kg PHA^{-1})	1.184	3.590	0.003	0.003
GWP (kg PHA^{-1})	0.000	0.000	0.000	0.000
ODP (kg PHA^{-1})	0.000	0.000	0.000	0.000
POP (kg PHA^{-1})	0.000	0.000	0.959	1.958
AP (kg PHA^{-1})	0.000	0.000	0.000	0.000
Total PEI (kg PHA^{-1})	1.568	4.657	1.130	1.404
Environmental burdens (kg PHA^{-1})	+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); Gurieff 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 λ ($\lambda = 0.922$), which may due to its high yield and considerably cheap price among other substrates. Similarly, crude glycerol also achieves the highest λ ($\lambda = 0.494$) 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 λ_o	0	0.355	0.904	1.000
PEI λ_o	0.876	0	1.000	0.922
EB λ_o	0	1.000	0.450	0.494
(Cost + PEI) λ	0	0	0.904	0.922
(Cost + EB) λ	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^{-1}, and −1.320 to −1.466 kg PHA^{-1}, respectively, with the yield decrease by 10 %. Following that, for crude glycerol, the (Cost + PEI) λ decreases from 0.922 to 0.878, while (Cost + EB) λ 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^{-1}, and −1.062 to −0.966 kg PHA^{-1}, respectively, following 10 % rise in yield. On the basis of that, for refined glycerol, the (Cost + PEI) λ increases to 0.969 from 0.904, while the

Leong *et al. Bioresour. Bioprocess.* (2016) 3:41

Page 7 of 9

Table 5 Summary of the sensitivity analysis using five different cases

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

Fig. 2 Multi-objective optimization for Cost + PEI (**a**) and EB (**b**) using weighting factor

(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.
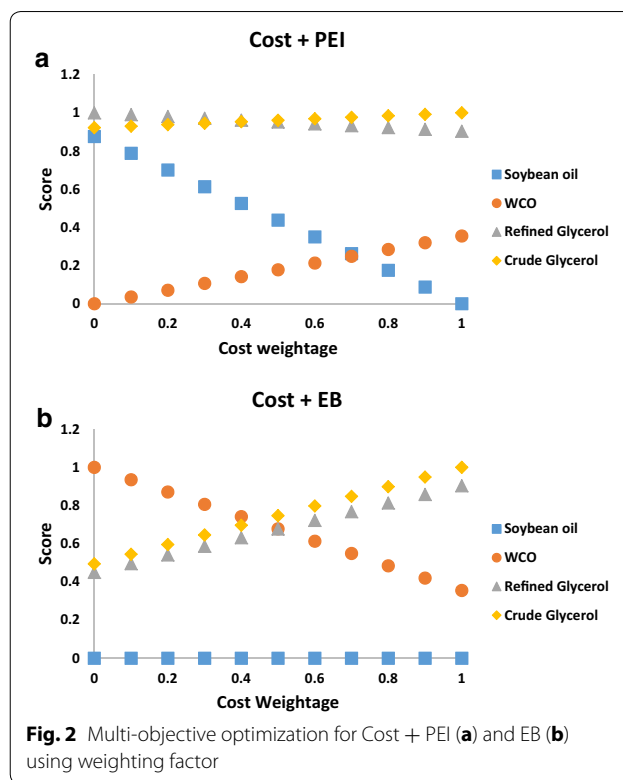
In case 3, as the crude glycerol price increases by 10 % (i.e., USD$ 0.22), the overall raw material cost of PHA from crude glycerol increases to 0.386 from 0.362 USD$/kg PHA. Following that, the (Cost + PEI) λ of refined glycerol increases from 0.904 to 0.921, and becomes quite close to that of crude 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.

Leong *et al. Bioresour. Bioprocess.* (2016) 3:41

Page 8 of 9

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.

Authors' information
YKL—Master in Chemical Engineering in University of Nottingham. Currently a Ph.D. student.

PLS—Associate professor in the Department of Chemical and Environmental Engineering, The University of Nottingham, Malaysia Campus. After completing his bachelor degree in Process and Food Engineering. He furthers his studied in Ph.D. in Bioprocess Engineering. His research focuses on bioprocess engineering from upstream to downstream processing in biotechnology and industrial microbiology as well as fermentation technology using green methods to produce sustainable chemistry.

HCL—Master in Chemical Engineering.

CKC—Master in Chemical Engineering, Ph.D. student.

HSL—A virologist and molecular biologist, graduated her bachelor's and Ph.D.'s degrees from Universiti Putra Malaysia (UPM). After her Ph.D. graduation, she worked as a post-doctoral research fellow in the Institute of Bioscience, UPM for one year, then, she joined the University of Nottingham Malaysia Campus (UNMC) in 2006 and has been working until present. She is now a Professor of the university, a Fellow of the Higher Education Academy, UK and has been lecturing in the molecular biology and biotechnology areas for more than nine years. Since October 2010, she has been appointed as the UNMC Director of Biotechnology Research Center which facilitates different areas of molecular biotechnology and plant tissue culture researches. She is the leader of Molecular Pharming and Bioproduction (MPharmBio) Research Group and one of the founding members of the Rainforest Plants Research Group at UNMC. Her research interests include application of molecular pharming approach in developing plant-based vaccine candidates for controlling significant human and poultry diseases and plant-made biopharmaceutical proteins as well as investigations on medicinal plant bioactives from primary rainforest (tropical) plant species and vitamin E isomers as new therapeutic agents.

JCWL—Ph.D., School of Chemical Engineering, University of Birmingham. After his Ph.D. graduation, she worked as a post-doctoral research fellow in Institute of BioAgricultural Sciences, Academia Sinica. Then, he worked a research fellow and lecturer, Department of Applied Chemistry & Chemical Engineering, Yamaguchi University, Japan. Following that, he also has been a research fellow, Biofuels Laboratory, Energy & Environment Laboratories, ITRI. Currently, he is an Assistant Professor in Department of Chemical Engineering & Materials Science, Yuan-Ze University.

TCL—Ph.D., School of Chemical Engineering, University of Birmingham. Currently, he is a Professor in Institute of Biological Sciences, Faculty of Science, University of Malaya.

Author details
[1] Department of Chemical and Environmental Engineering, Faculty of Engineering, University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan, Malaysia. [2] Food and Pharmaceutical Engineering Research Group, Faculty of Engineering, University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan, Malaysia. [3] Biorefinery & Bioprocess Engineering Laboratory, Department of Chemical Engineering and Material Science, Yuan Ze University, No. 135 Yuan-Tung Road, Chungli, Taoyuan 320, Taiwan. [4] School of Biosciences, Faculty of Science, University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan, Malaysia. [5] Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.

Competing interests
The authors declare that they have no competing interests.

References
Akaraonye E, Moreno C, Knowles JC, Keshavarz T, Roy I (2012) Poly(3-hydroxy-butyrate) production by *Bacillus cereus* SPV using sugarcane molasses as the main carbon source. Biotechnol J 7:293–303

Akiyama M, Tsuge T, Doi Y (2003) Environmental life cycle comparison of polyhydroxyalkanoates produced from renewable carbon resources by bacterial fermentation. Polym Degrad Stab 80:183–194

Álvarez-Chávez CR, Edwards S, Moure-Eraso R, Geiser K (2012) Sustainability of bio-based plastics: general comparative analysis and recommendations for improvement. J Clean Prod 23:47–56

Belboom S, Léonard A (2016) Does biobased polymer achieve better environmental impacts than fossil polymer? Comparison of fossil HDPE and biobased HDPE produced from sugar beet and wheat. Biomass Bioenergy 85:159–167

Chanprateep S (2010) Current trends in biodegradable polyhydroxyalkanoates. J Biosci Bioeng 110:621–632

Leong *et al. Bioresour. Bioprocess.* (2016) 3:41

Page 9 of 9

Choi J-I, Lee YS (1997) Process analysis and economic evaluation for poly(3-hydroxybutyrate) production by fermentation. Bioprocess Eng 17:335–342

Choi J, Lee YS (1999) Factors affecting the economics of polyhydroxyalkanoate production by bacterial fermentation. Appl Microbiol Biotechnol 51:13–21

Choi D, Chipman DC, Bents SC, Brown RC (2010) A techno-economic analysis of polyhydroxyalkanoate and hydrogen production from syngas fermentation of gasified biomass. Appl Biochem Biotechnol 160:1032–1046

Dalal J, Sarma PM, Mandal AK, Lal B (2013) Response surface optimization of poly (3-hydroxyalkanoic acid) production using oleic acid as an alternative carbon source by *Pseudomonas aeruginosa*. Biomass Bioenergy 54:67–76

Gerngross TU (1999) Can biotechnology move us toward a sustainable society. Nat Biotechnol 17:541–544

Gurieff N, Lant P (2007) Comparative life cycle assessment and financial analysis of mixed culture polyhydroxyalkanoates production. Bioresour Technol 98:3393–3403

Harding K, Dennis J, Blottnitz H, Harrison S (2007) Environmental analysis of plastic production process: comparing petroleum based polypropylene and polyethylene with biologically-based poly-β-hydroxybutyric acid using life cycle analysis. J Biotechnol 130:57–66

Hassan MA, Yee L-N, Yee PL, Ariffin H, Raha AR, Shirai Y, Sudesh K (2013) Sustainable production of polyhydroxyalkanoates from renewable oil-palm biomass. Biomass Bioenergy 50:1–9

Heimersson S, Morgan-Sagastume F, Peters GM, Werker A, Svanstrom M (2014) Methodological issues in life cycle assessment of mixed-culture polyhydroxyalkanoate production utilising waste as feedstock. N Biotechnol 31:383–393

Heyde M (1998) Ecological consideration on the use and production of biosynthetic and synthetic biodegradable polymers. Polym Degrad Stab 59:3–6

Hilaly A, Sikdar S (1995) Pollution balance method and the demonstration of its application to minimizing waste in a biochemical process. Ind Eng Chem 34:2051–2059

Hottle TA, Bilec MM, Landis AE (2013) Sustainability assessments of bio-based polymers. Polym Degrad Stab 98:1898–1907

Keshavarz T, Roy I (2010) Polyhydroxyalkanoates: bioplastics with a green agenda. Curr Opin Microbiol 13:321–326

Khoo HH, Tan RBH (2010) Environmental impacts of conventional plastic and bio-based carrier bags. Int J Life Cycle Assess 15:338–345

Kim S, Dale B (2005) Lifecycle assessment study of biopolymer (polyhydroxyalkanoates) derived from no-tilled corn. Int J Life Cycle Assess 10:200–210

Kim S, Dale B (2008) Energy and greenhouse gas profiles of polyhydroxybutyrates derived from corn grain: a life cycle perspective. Environ Sci Technol 42:7690–7695

Koller M, Hesse P, Salerno A, Reiterer A, Braunegg G (2011) A viable antibiotic strategy against microbial contamination in biotechnological production of polyhydroxyalkanoates from surplus whey. Biomass Bioenergy 35:748–753

Koller M, Sandholzer D, Salerno A, Braunegg G, Narodoslawsky M (2013) Biopolymer from industrial residues: life cycle assessment of poly(hydroxyalkanoates) from whey. Resour Conserv Recycl 73:64–71

Kylili A, Christoforou E, Fokaides PA (2016) Environmental evaluation of biomass pelleting using life cycle assessment. Biomass Bioenergy 84:107–117

Magdouli S, Brar SK, Blais JF, Tyagi RD (2015) How to direct the fatty acid biosynthesis towards polyhydroxyalkanoates production? Biomass Bioenergy 74:268–279

Miller SA, Billington SL, Lepech MD (2013) Improvement in environmental performance of poly(β-hydroxybutyrate)-co-(β-hydroxyvalerate) composites through process modifications. J Clean Prod 40:190–198

Mumtaz T, Yahaya NA, Abd-Aziz S, Abdul Rahman NA, Yee PL, Shirai Y, Hassan MA (2010) Turning waste to wealth-biodegradable plastics polyhydroxyalkanoates from palm oil mill effluent—a Malaysian perspective. J Clean Prod 18:1393–1402

Pawelzik P, Carus M, Hotchkiss J, Narayan R, Selke S, Wellisch M, Weiss M, Wicke B, Patel MK (2013) Critical aspects in the life cycle assessment (LCA) of bio-based materials—reviewing methodologies and deriving recommendations. Resour Conserv Recycl 73:211–228

Pietrini M, Roes L, Patel MK, Chiellini E (2007) Comparative life cycle studies on poly(3-hydroxybutyrate)-based composites on potential replacement for conventional petrochemical plastics. Biomacromolecules 8:2210–2218

Posada JA, Naranjo JM, López JA, Higuita JC, Cardona CA (2011) Design and analysis of poly-3-hydroxybutyrate production processes from crude glycerol. Process Biochem 46:310–317

Raynolds M, Fraser R, Checkel D (2000) The relative mass-energy-economic (RMEE) method for system boundary selection Part 1: a means to systematically and quantitatively select LCA boundaries. Int J Life Cycle Assess 5:37–46

Steinbüchel A, Valentin HE (1995) Diversity of bacterial polyhydroxyalkanoic acids. FEMS Microbiol Lett 128:219–228

Van Wegen RJ, Ling Y, Middelberg APJ (1998) Industrial production of polyhydroxyalkanoates using *Escherichia coli*: an economic analysis. Chem Eng Res Des 76:417–426

Venkateswar Reddy M, Nikhil GN, Venkata Mohan S, Swamy YV, Sarma PN (2012) Pseudomonas otitidis as a potential biocatalyst for polyhydroxyalkanoates (PHA) synthesis using synthetic wastewater and acidogenic effluents. Bioresour Technol 123:471–479

Young D, Cabezas H (1999) Designing sustainable processes with simulation: the waste reduction (WAR) algorithm. Comput Chem Eng 23:1477–1491

Young D, Scharp R, Cabezas H (2000) The waste reduction (WAR) algorithm: environmental impacts, energy consumption, and engineering economics. Waste Manag 20:605–615

Yu J, Chen LXL (2008) The greenhouse gas emissions and fossil energy requirement of bioplastics from cradle to gate of a biomass refinery. Environ Sci Technol 42:6961–6966

Zimmermann HJ (1978) Fuzzy programming and linear programming with several objective function. Fuzzy Sets Syst 1:45–55