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Sustainable Polyhydroxyalkanoates (PHA) Extraction Protocol Selection Using AHP-GRA

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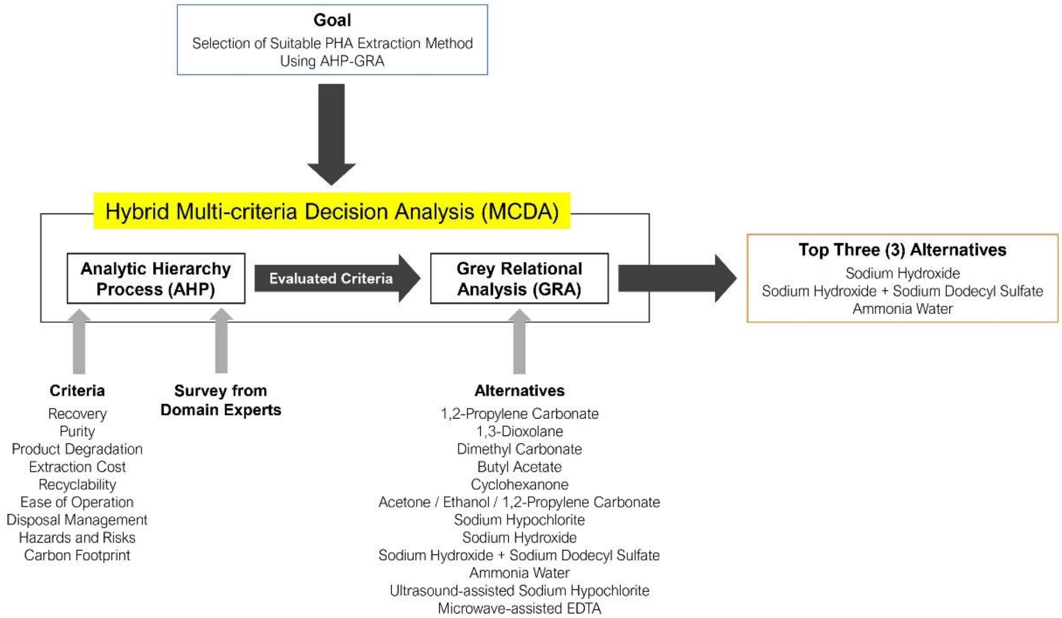
Graphical Abstract



Abstract

In this study, a sustainable protocol for PHA extraction was methodically selected using two (2) multi-criteria decision analysis (MCDA) tools, the analytic hierarchy process (AHP) and grey relational analysis (GRA). AHP was first used to evaluate the proposed criteria categorized into technical, economic, and environmental aspects using a collected survey of pairwise comparisons. Based on the results of AHP, it was identified that both environmental and economic aspects were given higher priorities. Among the criteria, hazards and risks has the highest overall importance, followed by extraction cost and purity. Using GRA, twelve (12) protocol alternatives categorized into solvent extraction and precipitation, non-PHA cell mass (NPCM) digestion, and assisted extraction methods were graded according to the criteria. Overall, the highest priority weights were given to NPCM digestion protocols including sodium hydroxide, sodium hydroxide + sodium dodecyl sulfate, and ammonia water. The reagents involved in these protocols are ecologically benign and cheaper compared to other solvents; hence, the higher grades in the environmental and economic aspect. Sensitivity analysis also proved that these protocols are excellent, particularly if extraction cost is given a higher priority. However, if hazards and risks and purity were given more importance, butyl acetate is preferable than sodium hydroxide. Further investigations such as the validation and optimization of protocols, together with feasibility studies and life cycle analyses may be integrated with the results of this study to comprehensively determine a sustainable PHA extraction protocol.

Keywords: analytic hierarchy process, extraction protocol, grey relational analysis, multi-criteria decision analysis, polyhydroxyalkanoates

Declarations

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Conflicts of interest

The authors declare that they have no competing interests.

Availability of data and material

Data may be made available upon request from the corresponding author.

Authors' contributions

All authors conceptualized and designed the work. JS Ventura and PJ Requiso contributed to the acquisition, analysis, and interpretation of data and results. PJ Requiso drafted the manuscript with consultation from the other authors. JS Ventura and PJ Requiso revised the manuscript for significant intellectual content. All authors approved the version of the manuscript for publication.

1. Introduction

To combat the problem against petroleum dependency and plastic pollution, bioplastics that are biodegradable, renewable, and biocompatible are receiving much attention as future alternatives to conventional plastics. Among the bioplastics, there has been a growing interest towards polyhydroxyalkanoates (PHA) that are synthesized by microorganisms as intracellular carbon and energy storage compounds. PHA synthesis increases under conditions of metabolic stress, often triggered by a limitation of nutrients required for cell growth such as nitrogen, phosphorus, or oxygen, associated with an availability of a suitable carbon substrate (Pérez-Rivero et al. 2019). These polyesters exhibit thermoplastic properties like polyethylene and polypropylene, which can be altered depending on their composition and molecular weight (Aramvash et al. 2015). This makes them appropriate for a wide variety of applications in packaging, agriculture, medicine, and pharmaceuticals.

The main drawback to the commercial production of PHA is its comparatively high cost compared to conventional plastics (Pagliano et al. 2021). Great efforts have already focused on optimizing the production of PHA using inexpensive substrates such as agricultural by-products and industrial wastes, as well as engineering of PHA-producing microbial strains (Du et al. 2012). However, developments in downstream processes for PHA recovery, which may represent up to 50% of the total production cost, have been rather slow (Pérez-Rivero et al. 2019). Extraction of intracellular PHA granules poses more difficulties than the separation of other industrial extracellular fermentation products. Extensive use of toxic non-recyclable materials and reagents, high energy consumption, and unwanted side reactions that can detrimentally affect polymer properties significantly increase the overall production cost (Samori et al. 2012). Industrially, these attributes somewhat antagonize sustainability and economic feasibility that the use of PHA promotes.

Selecting an efficient, economical, and environment friendly PHA extraction protocol is very important to reduce the cost of the polymer and minimize its impact. The protocol must be operationally fast and simple and can improve polymer yield and purity using minimal amounts of ecologically benign reagents. Nevertheless, choosing an appropriate protocol is quite complicated due to conflicting relations of technical, economic, social, and environmental attributes that must be taken into consideration and treated as an integrative whole. Besides, the criteria to be considered for a PHA extraction protocol selection has not been fully established. Researchers focus mostly on improving the feasibility of the process by optimizing the polymer recovery and purity, but other important factors that can affect protocol selection are often not evaluated. To make the selection process more strategic and comprehensive, a multicriteria decision analysis (MCDA) can be employed. MCDA methods differ from conventional decision techniques since they incorporate a decision-making that conforms with a synthetic numerical approach, leading to rankings and identification of trends (Szabo et al. 2021). They are used to formally solve actual problems by evaluating multiple conflicting criteria and identifying the "best" alternative from a pool of alternatives (Cobuloglu and Büyüktahtakin 2015; Nwokoagbara et al. 2015). Commonly, the techniques include the statement of the goal, identification of the alternatives, formulation of criteria and their respective indicators, weighing, and ranking of alternatives (Feiz and Ammenberg 2017).

Several papers had already employed the MCDA approach in decision-making. In selecting a sustainable biomass crop for biofuel production, economic, environmental, and social aspects subdivided into 16 sub-criteria were assessed using the stochastic analytic hierarchy (AHP) methodology. Biomass crops considered were switchgrass, miscanthus, sugarcane, corn, and wheat (Cobuloglu and Büyüktahtakin 2015). AHP was also applied to find the most appropriate feedstock for biodiesel production in Vietnam among three possible options – namely jatropha oil, fish fat, and waste cooking oil. The waste cooking oil was considered the most preferred alternative (Khang et al. 2016). Using four (4) MCDA methods, namely Preference Ranking Organization Method for Enrichment Evaluation Graphical Analysis for Interactive Assistance (GAIA), Weighted Sum Method (WSM), Weighted Product Method (WPM), and Technique for Order Preference by Similarity to Ideal Solution (TOPSIS), coconut and soybean were regarded as the worst feedstock for biodiesel production. The criteria for selection involved the cost of production, physicochemical properties and structural composition of biodiesel, and sustainable land usage for crop production (Anwar 2021). Similarly, we also employed an MCDA hybrid consisting of AHP and Grey Relational Analysis (GRA) to select corn stover, sugarcane bagasse, and banana pseudostem as best agricultural feedstock substrates for PHA production. These raw materials were selected by prioritizing conversion efficiency, cellulose content, and processing cost (Requiso et al. 2018). In a similar study, a comprehensive TOPSIS evaluation of commonly used hydrogen bond acceptors and donors was done to assess the greenness of deep eutectic solvents (Bystrzanowska and Tobiszewski 2021). Upon ranking, results show that many solvents, synthesized by mixing sugars alcohols, alcohols, sugars, and amides are promising solvents and more preferred than imidazolium-based ionic liquids. To the best of our knowledge, this paper is the only existing study so far that utilizes MCDA in selecting a PHA extraction protocol.

PHA Extraction Strategies

In general, current PHA extraction strategies include series of operations such as separation of microbial cell biomass from the fermentation broth, biomass pretreatment, polymer extraction, and purification (Pérez-Rivero et al. 2019). Microbial cell biomass may be primarily separated from the broth by centrifugation, filtration, and sedimentation. Next, recovered cell biomass can be pretreated by heating, freezing, adding salts, grinding in liquid nitrogen, or treating with hot compressed water to increase the permeability of the bacterial cells and hasten the extraction process (Koller et al. 2013). The biomass, pretreated or not, is then subjected to main extraction to separate PHA from non-PHA cell materials (NPCM) surrounding the polymer. Finally, impurities such as hydrophobic lipids and proteins in the recovered polymer are removed through purification.

PHA extraction and purification methods can be categorized based on their basic approach and mechanism (Koller et al. 2013; Jiang et al. 2018). The first category is solvent extraction and precipitation, the still most widely used practice for PHA recovery. Solvents alter the permeability of the cell membrane and temporarily dissolve the polymer granules. Chloroform and dichloromethane are considered the best organic extraction solvents, but are dangerous both for humans and the environment, rendering them as undesirable for industrial applications (Samori et al. 2012). Greener non-halogenated solvents such as 1,2 propylene carbonate, 1,3-dioxolane, dimethyl carbonate, butyl acetate, and cyclohexanone were used to replace these compounds (Pérez-Rivero et al. 2019). After dissolution, PHA is recovered with a precipitating agent (antisolvent) such as acetone, water, ethanol, and methanol (Koller et al. 2013). These mild compounds polish the extract by solubilizing lipids that coat the polymer granules. Alternatively, PHA can be precipitated by lowering the temperature to a range where solubility of PHA in the solvent is not provided anymore. Although solvent and antisolvent systems often result in excellent polymer recoveries and purities, the required low precipitation and high extraction temperatures entails additional energy costs. Solvent extraction is operationally slow due to the nature of the solvents utilized. Large amounts of these solvents, usually reaching up to 20-fold mass of the PHA-rich biomass are also undesirably required, making the process too costly to be considered a feasible alternative (Koller et al. 2013).

The second approach for PHA purification aims at the digestion and dissolution of NPCM, leaving PHA as an insoluble solid (Burniol-Figols et al. 2019). A wide variety of compounds such as oxidizing agents, acids, bases, chelating agents, and surfactants are used to selectively digest NPCM. Examples of these are sodium hypochlorite, sodium hydroxide, ammonia, sulfuric acid, ethylenediaminetetraacetic acid (EDTA), and sodium dodecyl sulfate (SDS). The major advantage of NPCM digestion is the high polymer recoveries (> 90%) at reduced volumes of digestion agents (compared to the use of solvents) (Pérez-Rivero et al. 2019). Nevertheless, the ester bonds of PHA are very reactive to these agents, leading to hydrolysis and degradation. Due to the lower purity of the extracted polymer, an additional purification step is also often required.

To improve polymer recovery from NPCM digestion, it can be coupled with physical methods such as ultrasound (sonication) and microwave (Balakrishna Pillai et al. 2018; Martínez-Herrera at al. 2020). These tools help in speeding up the digestion process and increase the recovery and purity of the extracted polymer. Sonication generates extreme and rapid cyclic pressure changes in a fluid, generating microbubbles of gas and vapor. These bubbles implode and generate violent shock waves that propagate through the fluid (Ishak et al. 2015). The intense turbulence produced improves the mass transfer and dispersion of phases. Meanwhile, the specific electromagnetic effects of microwave radiation disintegrate microbial cells, aiding the release of accumulated polymer granules from the cytoplasm (Balakrishna Pillai et al. 2018).

This study determined a sustainable PHA extraction protocol from a set of alternatives by utilizing AHP-GRA, a hybrid MCDA approach. The hybrid MCDA provides a better decision-making tool because of the combination of both intangible (AHP) criteria and empirical (GRA) data in selecting and ranking the proposed protocols. The complex decision-making process was simplified by establishing a hierarchy involving goals, aspects, criteria, and alternatives. AHP was first used to clearly evaluate the proposed aspects and criteria through a collected survey of pairwise comparisons. At a given hierarchy level, elements were evaluated and given relative importance values. Then, a GRA scoring system was used to assess the proposed alternatives. A decision matrix was formulated by incorporating the scoring system with the set of criteria from AHP. Using the decision matrix, protocol alternatives were rated and ranked.

2. Materials and Methods

2.1 AHP

AHP was used to evaluate the proposed criteria in selecting the best PHA extraction protocol. The process was used in the study due to its simplicity, ease of use, and great flexibility (Ho 2008). It can handle multiple criteria and objectives in a complex decision-making process, which usually involves subjective variables (Saaty 1980). It simplifies decision-making by organizing a hierarchical framework with the target at the highest level, aspects and criteria on the middle, and alternatives remain at the last level, as summarized in the proposed selection process (Figure 1).

At a given hierarchy level, elements were compared in pairs for their relative importance. In case of the criterion of each aspect, the assessment of the pairwise comparison was made with respect to the former hierarchy level. The comparisons were made on an increasing importance from a scale of 1 to 9. Values 1, 3, 5, 7, and 9 indicated equal, moderately more, strongly more, very strongly, and extremely more importance, respectively, while the remaining intermediate judgment values 2, 4, 6, and 8 were intended to compromise value of importance (Saaty 1980; Pohekar and Ramachandran 2004).

The goal of the study was to determine a sustainable PHA extraction protocol. Nine (9) proposed criteria were categorized into three (3) main aspects, namely technical, economic, and environmental aspects. Technical aspect included recovery, purity, and product degradation. For the economic aspect, extraction cost, recyclability, and ease of operation were selected. Meanwhile, disposal management, hazards and risks, and carbon footprint were included in the environmental aspect. These sets of aspects and criteria were compared pairwise by four (4) domain experts.

The domain experts evaluated the pairwise sets of aspects and criteria based on a scale of importance. The comparisons generated a matrix indicating the relative value of aspects and criteria for the goal (Equation 1). Numerical ratings based on Saaty's scale were written on the upper triangular matrix while the inverse of these ratings can be seen on the lower triangular matrix (Saaty 1980). Correspondingly, the geometric mean scores of the pool of experts were inputted for the pairwise comparison.

$$A = \begin{bmatrix} a_{11} & a_{12} & \cdots & a_{1q} \\ a_{21} & a_{22} & \cdots & a_{2q} \\ \vdots & \vdots & \vdots & \vdots \\ a_{q1} & a_{q2} & \cdots & a_{qq} \end{bmatrix} = \begin{bmatrix} a_{11} & a_{12} & \cdots & a_{1q} \\ 1/a_{21} & a_{22} & \cdots & a_{2q} \\ \vdots & \vdots & \vdots & \vdots \\ 1/a_{q1} & 1/a_{q2} & \cdots & a_{qq} \end{bmatrix} \quad (1)$$

After consolidating the pairwise comparisons, the scores in the matrix were summed up to normalize the values. Normalization, which is needed to make all the indicators comparable on the same scale, involves dividing each element by the total score in the column and computing the average of each row to get the priority vector or weights. The priority weights within each aspect were multiplied with the weights obtained from the previous hierarchy level (aspects) to get the global weights of each criterion.

Since the numeric grades were derived from personal or subjective judgments, some degree of inconsistency may be observed in the comparisons made by the surveyed experts (Khaira and Dwivedi 2018). To ensure that consistent judgments were made, consistency verification was incorporated in AHP. The procedure measured the degree of consistency among the pairwise comparisons in matrix (*A*) by calculating the consistency ratio (*CR*) (Equation 3) using the value of consistency index (*CI*) (Equation 2) and random index (*RI*).

$$CI = \frac{(\lambda_{max} - n)}{(n - 1)} \tag{2}$$

$$CR = \frac{CI}{RI} \tag{3}$$

The *CI* value was calculated using the maximum eigenvalue (λ_{max}) and order (*n*) of the comparison matrix. On the other hand, λ_{max} was calculated as the summation of the product of the priority vector and the column total of the pairwise comparison matrix of each corresponding criterion. The *CR* value determines the consistency of the subjective input in the matrix for pairwise comparisons. It was calculated as the ratio of *CI* and *RI*. Random *RI* values for various *n* has already been proposed by Saaty (1980) (Table 1). A *CR* of 10% or less implies that the comparisons are relatively consistent or acceptable. In contrast, a *CR* higher than 10% indicates an inconsistent data and the comparison matrix needs to be adjusted by an appropriate corrective measure or inconsistency identification.

Another reason why AHP was used in the study is that it can be integrated with other techniques, to consider not only both qualitative and quantitative factors, but also some real-world resource limitations. This approach makes a more realistic and promising decision than a stand-alone AHP (Ho 2008).

2.2 GRA

Results of AHP were integrated with GRA to determine the rank of each PHA protocol alternative. GRA has been proven to be useful for dealing with poor, incomplete, and uncertain information, and is suitable for studies with small samples (Sallehuddin et al. 2008). It can generate more reliable solutions efficiently when they are combined with the results of other MCDA methods (Deng 1982). Protocol alternatives with multiple attributes can be compared easily after the process by combining the entire range of performance attribute values for every alternative into a single value. With this, GRA scores of each alternative can be combined to the previously obtained AHP criteria weights. Using GRA, twelve (12) alternatives categorized into solvent extraction and precipitation, NPCM digestion, and assisted extraction methods were rated and ranked. The GRA scoring system used to evaluate the proposed alternatives for feedstock selection is shown in Table 2. To simplify calculations, scores ranging from 1 to 5 (with 5 as the highest score) were arranged by incorporating the positive and negative criteria that will most likely give advantages to the ranking of the protocol alternatives. For example, recovery and purity were considered as positive criteria while product degradation and extraction cost were treated as negative criteria. In effect, the scores were reversed for the negative criteria.

Initially, a decision matrix was formulated using the set of alternatives $(i = 1, 2, \dots, m)$ and criteria $((j = 1, 2, \dots, m)$. The i^{th} alternative was expressed as $Y_i = (y_{i1}, y_{i2}, y_{i3}, \dots, y_{ij}, \dots, y_{im})$, where y_{ij} is the performance value of the attribute j of alternative i. The Y_i could also be translated into a comparability sequence $X_i = (x_{i1}, x_{i2}, x_{i3}, \dots, x_{ij}, \dots, x_{im})$ by using one of the equations below (Equations 4, 5 or 6).

$$x_{ij} = \frac{y_{ij} - Min\{\, y_{ij}, i=1,2,\dots,m\}}{Max\{\, y_{ij}, i=1,2,\dots,m\} - Min\{\, y_{ij}, i=1,2,\dots,m\}} \qquad \text{for } i = 1, 2, \dots, m \qquad j = 1, 2, \dots, m \tag{4}$$

$$x_{ij} = \frac{Min\{\, y_{ij}, i=1,2,\dots,m\} - y_{ij}}{Max\{\, y_{ij}, i=1,2,\dots,m\} - Min\{\, y_{ij}, i=1,2,\dots,m\}} \qquad \text{for } i = 1, 2, \dots, m \qquad j = 1, 2, \dots, m \tag{5}$$

$$x_{ij} = 1 - \frac{\left| y_{ij} - y_j^* \right|}{Max\left\{ Max\{\, y_{ij}, i=1,2,\dots,m\} - y_{ij}^*, y_{ij}^* - Min\{\, y_{ij}, i=1,2,\dots,m\} \right\}} \quad \text{for } i = 1, 2, \dots, m \quad j = 1, 2, \dots, m \tag{6}$$

Equations 4, 5, and 6 are used for the larger-the-better, smaller-the-better, and closer-to-the-desired-value-y_j^*-the-better, respectively (Kuo et al. 2008). This study used only Equation 4 prior to the pre-arranged scoring system shown in Table 3.

The preference index was then normalized into $[0, 1]$ using the grey relational generating procedure (Equations 4, 5, or 6). An alternative with preference index closest to or equal to 1 is deemed the best; however, this does not usually exist (Kuo et al. 2008). Thus, reference sequence $X_0 = \left(x_{01}, x_{02}, x_{03}, \dots, x_{0j}, \dots, x_{0m}\right) = (1, 1, \dots, 1, \dots, 1)$ was made to find the comparability sequence close to the reference sequence.

Then, the grey relational coefficient was computed to determine the closeness of x_{ij} to x_{0j}. The closer the x_{ij} to x_{0j}, the larger the grey relational coefficient. In Equation 7, $\gamma\left(x_{0j}, x_{ij}\right)$ is the grey relational coefficient between x_{ij} and x_{0j}, and $\Delta_{ij} = \left|x_{0j} - x_{ij}\right|$, $\Delta_{min} = Min\ \left\{\Delta_{ij},\ \ i = 1, 2, \dots, n; j = 1, 2, \dots, m\right\}$, $\Delta_{max} = Max\ \left\{\Delta_{ij},\ \ i = 1, 2, \dots, n; j = 1, 2, \dots, m\right\}$, and ζ is the distinguishing coefficient. The ζ is in the range of 0 to 1 and could be set by the decision maker. In this study, the distinguishing coefficient was set at 0.5.

$$\gamma\left(x_{0j}, x_{ij}\right) = \frac{\Delta_{min} + \zeta\Delta_{max}}{\Delta_{ij} + \zeta\Delta_{max}} \tag{7}$$

Finally, the grey relational grade was obtained using Equation 8.

$$\Gamma(X_0, X_i) = \sum_{j=1}^{n} w_j\, \gamma(x_{0j}, x_{ij}) \tag{8}$$

The integrated grey relational grade between reference sequence (X_0) and comparative sequence (X_i) was computed from the summation of the product of the priority vector of each criteria (w_j, for $j = 1, 2, \dots, n$) multiplied by the corresponding grey relational coefficients between x_{0j} and x_{ij}. It usually denotes the level of correlation between the reference and comparability sequence. Thus, if the comparability sequence for an alternative has the highest grey relational grade with a reference sequence, (i.e., comparability sequence is almost similar to the reference sequence) the alternative would be the most preferred (Kuo et al. 2008).

3. Results and Discussion

3.1 Selection of PHA Extraction Protocol

To develop a method for selecting a suitable and sustainable extraction protocol for intracellular PHA, it is important to evaluate a protocol in terms of its efficiency, economic feasibility, and environmental effects. The first main aspect for evaluation, named as technical aspect, measures the overall effectiveness of the protocol indicated by recovery, purity, and possible degradation of the polymer extract. These properties are highly reliant on the nature of the extraction procedure, particularly the characteristics of the solvents and digestion agents (Koller et al. 2013). Table 3 compares the surveyed protocol alternatives in terms of extraction mechanism, operating conditions, recovery, and purity. Recovery accounts for the extracted amount of crude polymer with respect to the amount of cell biomass loaded to the extraction process. An extraction protocol must have an average recovery of 90% of higher for it to be considered feasible. However, incomplete separation of NPCM from the extracted polymer may result in unwanted impurities, undesirably increasing the apparent PHA recovery. Hence, recovery must always be evaluated with purity, which is quantified as the mass fraction content of pure PHA in the crude polymer extract. An ideal protocol is similarly characterized by an average purity of 90% of higher. Lastly, product degradation pertains to the decomposition of long chains of PHA into smaller molecular counterparts. It is usually measured either by average molar mass or polydispersity index of the extracted polymer. A minimal to negligible PHA degradation at any extraction temperature is deemed desirable for an extraction process.

Economic aspect assesses the economic potential of an extraction protocol in terms of extraction cost, recyclability of the solvents and reagents involved, and the ease of conducting the procedure. Extraction cost, the sum of material cost and processing cost, accounts for the total of the expenses that can be incurred in recovering the polymer from the cell biomass. Material cost is highly dependent on the extraction solvents, antisolvents, digestion agents, and purification reagents. Additionally, processing cost very much depends on the energy requirements of the extraction operations and processes. A low extraction cost is an ideal attribute of a feasible and sustainable protocol. Organic solvents and precipitation agents are more expensive compared to digestion agents and purification solvents (Samori et al. 2012). Also, the overall energy requirements for solvent extraction and precipitation are higher due to extreme temperatures and longer incubation periods required by the processes. The recyclability of the reagents varies with the mechanism of PHA extraction. Solvents and antisolvent systems, as well as purification agents are often recyclable and can be separated by unit operations such as distillation. The convenience of separation mainly depends on the number of solvents and precipitating agents used to extract the polymer. Digestion agents, in contrast, are often deemed nonrecyclable and treated as wastewater after the process. On the other hand, the ease of operation criteria deals with the integral extraction operations and the corresponding operating conditions, as well as the required materials and equipment. Operationally fast and simple extraction processes are preferrable since they are more convenient to perform. Ideal protocols must not involve biomass pretreatment prior to extraction, extreme extraction conditions, and highly sophisticated equipment that entail added costs.

Finally, environmental aspect gauges the impacts of an extraction protocol to the environment, addressing concerns regarding the sustainability of PHA production. The criteria included in this aspect are disposal management, hazards and risks, and carbon footprint. Disposal management examines the relative amounts, composition, and properties of reagents used to recover PHA. Hazards and risks pertain to the probability of a a person being harmed or experiencing adverse health effects when exposed to the process, especially to the extraction agents and other toxic by-products. Ideally, the protocol must involve the use of non-explosive, non-toxic, non-carcinogenic, biodegradable, and non-flammable chemicals to minimize the chance of danger that may occur during extraction. Carbon footprint is the estimated amount of carbon dioxide and other greenhouse gases emitted during PHA recovery. To simplify the assessment, carbon footprint was indirectly measured by counting the number of reagents utilized in the process.

Pairwise comparisons for the main aspects and criteria are summarized in Tables 4 to 7. AHP results indicate that both environmental and economic aspects are crucial in the selection a sustainable PHA extraction protocol (Table 8). This is indicated by the closeness of their weights (0.377 and 0.342, respectively). Technical aspect came in at third priority at a score of 0.281. Among the aspects, hazards and risks (0.194) received the highest overall importance. This emphasizes the importance of using safe and ecologically benign extraction reagents. Extraction cost (0.179) and purity (0.114) were also highly ranked at second and third, respectively, stressing the necessity for an efficient and cost-effective extraction process. Meanwhile, ease of operation (0.063) and disposal management (0.090) were given the lowest scores.

The final ranking of the protocols was then conducted using GRA. Initially, a decision matrix was made using a scoring system (Table 3) to rate the protocols. Based on literature surveys, the scores of the different feedstocks in relation to the proposed criteria are presented in Table 9. Scores were normalized and processed to obtain the grey relational coefficients and grey relational grades.

GRA coefficients and grades of the proposed protocol alternatives under the technical aspect are shown in Table 10. In terms of recovery, 1,2-propylene carbonate, butyl acetate, and cyclohexanone – with recovery values of at least 95% were equally given the highest score of 0.096. Additionally, the latter two protocols, together with 1,3-dioxolane, sodium hypochlorite, and sodium hydroxide + sodium dodecyl sulfate equally received the highest score for the purity criteria (0.114). For product degradation, all solvent extraction and precipitation protocols, except 1,2-propylene carbonate received the highest grade (0.071). Ammonia water and microwave-assisted EDTA protocols were also given the same grade. NPCM digestion protocols are known to cause PHA hydrolysis and degradation; hence, the low scores for the criterion. Overall, the top three scores were given to butyl acetate (0.281), cyclohexanone (0.281), and 1,3-dioxolane protocols (0.249).

Table 11 shows the grey relational grades of the protocol alternatives for the economic aspect. The highest scores (0.179) were given to most of the NPCM digestion and assisted protocols because digestion agents are relatively cheaper compared to organic solvents and antisolvents. Dimethyl carbonate protocol scored the highest for recyclability since the solvent and ethanol, the corresponding precipitating agent, can be easily separated by unit operations such as distillation. Solvent extraction and precipitation protocols were graded the lowest in the ease of operation criteria, mainly due to relatively higher temperature and longer incubation period requirement for extraction, and the corresponding special equipment needed for the process. In contrast, sodium hypochlorite, sodium hydroxide, and sodium hydroxide + sodium dodecyl sulfate protocols received the highest scores (0.063) for the criterion due to their lower temperature requirement (around 30°C) and shorter reaction time (1 h), making them simpler and more convenient to perform. With this, the three (3) protocols also dominated the pool of alternatives under the economic aspect.

Dimethyl carbonate, ammonia water, and microwave-assisted EDTA protocols obtained the highest scores (0.090) for the disposal management criterion under the environmental aspect (Table 12). For hazards and risks, dimethyl carbonate, butyl acetate, and sodium hydroxide scored the highest at 0.194. For the carbon footprint criterion, most of the NPCM digestion protocols, together with 1,3-dioxolane and dimethyl carbonate protocols ranked the highest with a score of 0.093. In summary, dimethyl carbonate and sodium hydroxide, which came separately from two different protocol categories, were graded the highest (0.377) for the environmental aspect. Both protocols involve reagents that are relatively safe for human health and for the environment, easily disposable, non-irritating at low concentrations, and most importantly, bear no mutagenic and carcinogenic effects either by contact or inhalation (Samori et al. 2012).

Overall scores for the protocol alternatives are listed in Table 13. The highest priority weights were given to sodium hydroxide (0.797), sodium hydroxide + sodium dodecyl sulfate (0.783), and ammonia water (0.765). These NPCM digestion protocols scored lower in the technical aspect, but since the remaining two aspects were highly prioritized, they ranked the highest after combining the scores. The top three protocols require relatively safe and environment friendly reagents, which gave them the advantage in the environmental aspect. Also, since these reagents are relatively cheaper compared to the solvents used in other alternatives, they were given the highest scores in the economic aspect.

3.2 Sensitivity Analysis

A sensitivity analysis was made to estimate probable modifications and shifts on the scores of the protocol alternatives for PHA extraction. It was done by adjusting the selected top three criteria (hazards and risks, extraction cost, and purity) weights from 0 to 1 with an interval of 0.1. Based on the results of the analysis, the top three protocols, namely sodium hydroxide, sodium hydroxide + sodium dodecyl sulfate, and ammonia water are very excellent, particularly if extraction cost is given a higher priority (Figure 2b). However, the ranking of protocol alternatives was very sensitive to changes in the priority weights of the remaining top two criteria. If hazards and risks were given more importance, dimethyl carbonate and butyl acetate protocols are preferable than sodium hydroxide (Figure 2a).

Likewise, prioritizing the importance of purity gives a preference to butyl acetate and 1,3-dioxolane (Figure 2c).

Sodium hydroxide + sodium dodecyl sulfate is an exceptional protocol candidate for PHA extraction. The protocol is always included in the top three alternatives even if the priority weights of the top three criteria were adjusted. Although sodium hydroxide scored high in both economic and environmental aspect due to the low cost and practically safe nature of the digestion agent, it can be one of the least preferred alternatives because of its low grades in the technical aspect. Moreover, butyl acetate also bears a potential for PHA extraction, but its use is mainly limited by its cost, recyclability, and ease of conducting the protocol. These variations in the ranking of alternatives show that selecting a PHA extraction is very much affected by the priorities and importance assigned to the criteria used in evaluating the extraction alternatives.

4. Conclusions

The study identified the best extraction protocol for sustainable PHA production by surveying a pool of experts. Responses were evaluated using MCDA methods. AHP was used to evaluate the criteria used in the selection process. The results of the study showed that economic and environmental aspects were given higher importance over the technical aspect of the protocol. Furthermore, the hazards and risks criterion received the highest overall weight among all the criteria, followed by extraction cost and purity of the extracted polymer. Based on the responses of the experts, the level of toxicity and the probable harm that the reagents would cause to man and environment, as well as the cost of reagents and energy required to carry out the extraction process were the main considerations for selection.

Meanwhile, GRA was combined with AHP results to assess the suitability of proposed protocol alternatives. Combined MCDA results showed that NPCM digestion protocols, namely sodium hydroxide, sodium hydroxide + sodium dodecyl sulfate, and ammonia water were the most suitable protocols for PHA extraction. This is mainly due to their favorable economic and environmental attributes. Sodium hydroxide + sodium dodecyl sulfate is an exceptional protocol candidate for sustainable PHA extraction, withstanding the adjustments made in the top three criteria in the sensitivity analysis. Butyl acetate also bears a potential for PHA extraction, but its use is mainly limited by its cost, recyclability, as well as its high temperature and longer reaction time requirement. Further studies such as the validation and optimization of protocols, together with economic and life cycle analyses may be integrated with the results of this study to comprehensively determine a suitable PHA extraction and recovery protocol.

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Tables and Figures

Tables

Table 1. Proposed random index (Saaty 1980).

n	1	2	3	4	5	6	7	8	9
RI	0.00	0.00	0.58	0.90	1.12	1.24	1.32	1.41	1.45

603 **Table 2**. Scoring system used to evaluate PHA extraction protocol alternatives.

Criteria	Score 1.0	2.0	3.0	4.0	5.0
Recovery	< 80%	80 to 85%	85 to 90%	90 to 95%	> 95%
Purity	< 80%	80 to 85%	85 to 90%	90 to 95%	> 95%
Product degradation	Degradation at low temperatures and low solvent concentrations	Degradation at low temperatures and high solvent concentrations	Moderate or controlled degradation	Minimal degradation only at very high temperatures	No degradation at any temperature
Extraction cost	Very high	High	Moderate	Low	Very low
Recyclability	Solvent used in cell disruption	Four (4) or more solvents separable by unit operations (distillation)	Three (3) solvents separable by unit operations (distillation) where solvents are used for other purposes	Binary solvent system separable by unit operations (distillation)	Solvents not combined with other solvents
Ease of operation	High temperature extraction, with hot filtration setup	High temperature extraction, with long extraction or precipitation time	Extraction requiring cell biomass pretreatment	No cell biomass pretreatment, but requires special equipment	Simple and direct operations at low temperatures
Disposal management	With three (3) or more solvents	Binary solvent systems	With two (2) solvents separately used in extraction	Solvents corrosive only at very high concentrations	With only one (1) solvent Solvents relatively safe for ordinary disposal at very low concentrations
Hazards and risks	Solvents with very high toxicity, carcinogenic, and mutagenicity	Carcinogenic and mutagenic solvents	Corrosive and environment pollutant at very high concentrations	With two (2) or more solvents that are flammable and irritant	With only one (1) solvent that is flammable and irritant

Carbon footprint	With four (4) or more volatile solvents	With three (3) volatile solvents	With two (2) volatile solvents	With one (1) volatile solvent	No volatile solvent

605 **Table 3**. PHA extraction and recovery protocol alternatives.

Protocol alternative	Mechanism	Special chemicals or equipment used	Extraction conditions	Recovery, %	Purity, %	Reference
A1	Solvent extraction and precipitation	1,2 propylene carbonate, acetone, 0.45 µm polypropylene filter, flask equipped with condenser and magnetic stirring	11.5 g wet biomass per 150 mL 1,2-propylene carbonate, 130°C, 30 min, precipitated with acetone	95	84	Fiorese et al. 2009
A2	Solvent extraction and precipitation	1,3-dioxolane, water	5% w/v biomass (dry basis) per 2 mL 1,3-dioxolane, 80°C, 6 h, precipitated with water	92.7	97.9	Yabueng et al. 2018
A3	Solvent extraction and precipitation	Dimethyl carbonate, ethanol, 0.45 µm polypropylene filter	2.5% w/v biomass (dry basis) (50 mg) cells per 2 mL dimethyl carbonate, 90°C, 1 h, precipitated with ethanol	88	95	Samori et al. 2012
A4	Solvent extraction and precipitation	Butyl acetate, acetone, thermostatic water bath with stirring	1 g wet biomass per 100 mL butyl acetate, 103°C, 30 min, precipitated with acetone	96	98	Aramvash et al. 2015
A5	Solvent extraction and precipitation	Cyclohexanone, acetone, methanol, hot filtration setup	Degreasing: Dried biomass (20:1 volume to mass ratio) in acetone, overnight at room temperature with magnetic stirring Extraction: 1 g degreased biomass in 50 mL cyclohexanone, 120°C, 30 min, vigorous stirring, precipitated with methanol	95	97.2	Jiang et al. 2015

A6	Solvent extraction and precipitation	Acetone, ethanol, 1,2 propylene carbonate, hexane, hot filtration setup	Pretreatment: 100 g wet biomass sonicated at 70% amplitude, 10 min Extraction: 500 mg sonicated biomass (61% moisture) per 5 mL of solvent mixture (1:1:1 v/v/v), 120°C, 1 h, precipitated by 2.5 mL hexane for 48 h	92	93	Fei et al. 2016
A7	NPCM digestion and dissolution	Sodium hypochlorite, acetone	Cell pellet from 10 mL of culture broth per 10 mL of sodium hypochlorite solution, 37°C, 1 h, PHA pellet washed with acetone	Not stated	95	Berger et al. 1989
A8	NPCM digestion and dissolution	Sodium hydroxide	40 g/L (dry basis), 10 mL 0.1 N sodium hydroxide, 30°C, 1 h	90.8	88.4	Lee et al. 1995
A9	NPCM digestion and dissolution	Sodium hydroxide, sodium dodecyl sulfate	20 g/L (dry basis), 10 mL 0.2 M NaOH + 0.02% w/v sodium dodecyl sulfate (volume not mentioned), 30°C, 1 h, 200 rpm	91	99.1	Jiang et al. 2015
A10	NPCM digestion and dissolution	Liquid ammonia, ultrasound bath, heating block	Pretreatment: 100 mg dry biomass suspended in 5 mL water, sonicated at 100% amplitude, pulse of 0.5 for 10 min, then dried again Extraction: 100 mg dry pretreated biomass per 4 mL 0.2 M ammonia, 115°C, 30 min	92	89	Burniol-Figols et al. 2019
A11	Ultrasonic-assisted NPCM digestion	5% v/v sodium hypochlorite, ultrasound bath (up to 20°C)	Wet biomass treated with 5% v/v sodium hypochlorite, sonicated at 42 kHz frequency, 20°C, 30 min	Not stated	Not stated	Martínez-Herrera et al. 2020

A12	Microwave-assisted NPCM digestion	Ethylenediaminetetraacetic acid (EDTA), ethanol, microwave oven	Cell biomass from 250 mL culture suspended in 10mM EDTA, 1 h, room temperature, with intermittent shaking, microwaved (2450 MHz) at maximum power of 700 W, 10 min, washed with ethanol	93.75	97.21	Balakrishna Pillai et al. 2018

606

Table 4. Pairwise comparison matrix of technical, economic, and environmental aspects.

Aspect	TA	EC	EV
Technical Aspect (TA)	1.00	0.60	1.00
Economic Aspect (EC)	1.65	1.00	0.67
Environmental Aspect (EV)	1.00	1.50	1.00
Total	3.65	3.10	2.67

Note: $\lambda_{max} = 3.09$, $CI = 0.05$, $CR = 0.08$

Table 5. Pairwise comparison matrix of the criteria under the technical aspect.

Criterion	RE	PR	PD
Recovery (RE)	1.00	0.90	1.24
Purity (PU)	1.11	1.00	1.73
Product Degradation (PD)	0.80	0.58	1.00
Total	2.91	2.48	3.98

Note: $\lambda_{max} = 3.01$, $CI = 0.00$, $CR = 0.01$

Table 6. Pairwise comparison matrix of the criteria under the economic aspect.

Criterion	EC	RC	EO
Extraction Cost (EC)	1.00	1.50	3.44
Recyclability (RC)	0.67	1.00	1.32
Ease of Operation (EO)	0.29	0.76	1.00
Total	1.96	3.26	5.76

Note: $\lambda_{max} = 3.04$, $CI = 0.02$, $CR = 0.04$

Table 7. Pairwise comparison matrix of the criteria under the environmental aspect.

Criterion	DM	HR	CF
Disposal Management (DM)	1.00	0.39	1.16
Hazards and Risks (HR)	2.59	1.00	1.78
Carbon Footprint (CF)	0.86	0.56	1.00
Total	4.45	1.95	3.94

Note: $\lambda_{max} = 3.04$, $CI = 0.02$, $CR = 0.03$

Table 8. Weights of the aspects and criteria for PHA extraction protocol selection.

Aspect	Weight	Criteria	Overall Weight
Technical Aspect	0.281	Recovery	0.096
		Purity	0.114
		Product Degradation	0.071
Economic Aspect	0.342	Extraction Cost	0.179
		Recyclability	0.100
		Ease of Operation	0.063
Environmental Aspect	0.377	Disposal Management	0.090
		Hazards and Risks	0.194
		Carbon Footprint	0.093

Table 9. Decision matrix for the selection of PHA extraction protocol.

Protocol alternative	Criteria								
	RE	PU	PD	EC	RC	EO	DM	HR	CF
1,2-propylene carbonate	5.00	2.00	4.00	2.00	4.00	1.00	2.00	4.00	4.00
1,3-dioxolane	4.00	5.00	5.00	3.00	4.00	2.00	2.00	2.00	5.00
Dimethyl carbonate	2.00	4.00	5.00	2.00	5.00	2.00	5.00	5.00	5.00
Butyl acetate	5.00	5.00	5.00	1.00	4.00	1.00	2.00	5.00	4.00
Cyclohexanone	5.00	5.00	5.00	1.00	3.00	1.00	2.00	1.00	3.00
Acetone / ethanol / 1,2-propylene carbonate	4.00	4.00	5.00	1.00	2.00	1.00	1.00	1.00	1.00
Sodium hypochlorite	3.00	5.00	2.00	3.00	1.00	5.00	3.00	3.00	4.00
Sodium hydroxide	4.00	3.00	1.00	5.00	1.00	5.00	5.00	5.00	5.00
Sodium hydroxide + sodium dodecyl sulfate	4.00	5.00	4.00	5.00	1.00	5.00	4.00	4.00	5.00
Ammonia water	4.00	3.00	5.00	5.00	3.00	3.00	5.00	4.00	5.00
Ultrasound-assisted sodium hypochlorite	4.00	4.00	3.00	4.00	1.00	4.00	4.00	3.00	5.00
Microwave-assisted EDTA	1.00	4.00	5.00	4.00	1.00	4.00	5.00	2.00	5.00

Note: RE – recovery; PU – purity; PD – product degradation; EC – extraction cost; RC – recyclability; EO – ease of operation; DM – disposal management; HR – hazards and risks; CF – carbon footprint

Table 10. Weights of the proposed PHA extraction protocols for the technical aspect.

Protocol alternative	Criteria			Total grade
	Recovery	Purity	Product Degradation	
1,2-propylene carbonate	0.096	0.046	0.047	0.189
1,3-dioxolane	0.064	0.114	0.071	0.249
Dimethyl carbonate	0.038	0.076	0.071	0.186
Butyl acetate	0.096	0.114	0.071	0.281
Cyclohexanone	0.096	0.114	0.071	0.281
Acetone / ethanol / 1,2-propylene carbonate	0.064	0.076	0.071	0.211
Sodium hypochlorite	0.048	0.114	0.028	0.191
Sodium hydroxide	0.064	0.057	0.024	0.145
Sodium hydroxide + sodium dodecyl sulfate	0.064	0.114	0.047	0.225
Ammonia water	0.064	0.057	0.071	0.192

Ultrasound-assisted sodium hypochlorite	0.064	0.076	0.036	0.176
Microwave-assisted EDTA	0.032	0.076	0.071	0.179

Table 11. Weights of the proposed PHA extraction protocols for the economic aspect.

Protocol alternative	Criteria			Total grade
	Extraction Cost	Recyclability	Ease of Operation	
1,2-propylene carbonate	0.071	0.067	0.021	0.159
1,3-dioxolane	0.089	0.067	0.025	0.181
Dimethyl carbonate	0.071	0.100	0.025	0.197
Butyl acetate	0.060	0.067	0.021	0.147
Cyclohexanone	0.060	0.050	0.021	0.131
Acetone / ethanol / 1,2-propylene carbonate	0.060	0.040	0.021	0.121
Sodium hypochlorite	0.089	0.033	0.063	0.186
Sodium hydroxide	0.179	0.033	0.063	0.275
Sodium hydroxide + sodium dodecyl sulfate	0.179	0.033	0.063	0.275
Ammonia water	0.179	0.050	0.032	0.260
Ultrasound-assisted sodium hypochlorite	0.119	0.033	0.042	0.195
Microwave-assisted EDTA	0.119	0.033	0.042	0.195

Table 12. Weights of the proposed PHA extraction protocols for the environmental aspect.

Protocol alternative	Criteria			Total grade
	Disposal Management	Hazards and Risks	Carbon Footprint	
1,2-propylene carbonate	0.036	0.130	0.062	0.227
1,3-dioxolane	0.036	0.078	0.093	0.206
Dimethyl carbonate	0.090	0.194	0.093	0.377
Butyl acetate	0.036	0.194	0.062	0.292
Cyclohexanone	0.036	0.065	0.046	0.147
Acetone / ethanol / 1,2-propylene carbonate	0.030	0.065	0.031	0.126
Sodium hypochlorite	0.045	0.097	0.062	0.204

Sodium hydroxide	0.090	0.194	0.093	0.377
Sodium hydroxide + sodium dodecyl sulfate	0.060	0.130	0.093	0.282
Ammonia water	0.090	0.130	0.093	0.312
Ultrasound-assisted sodium hypochlorite	0.060	0.097	0.093	0.250
Microwave-assisted EDTA	0.090	0.078	0.093	0.260

Table 13. Overall grades of the proposed PHA extraction protocols.

Protocol alternative	Technical aspect	Economic aspect	Environmental aspect	Overall grade
1,2-propylene carbonate	0.189	0.159	0.227	0.575
1,3-dioxolane	0.249	0.181	0.206	0.637
Dimethyl carbonate	0.186	0.197	0.377	0.759
Butyl acetate	0.281	0.147	0.292	0.720
Cyclohexanone	0.281	0.131	0.147	0.559
Acetone / ethanol / 1,2-propylene carbonate	0.211	0.121	0.126	0.457
Sodium hypochlorite	0.191	0.186	0.204	0.580
Sodium hydroxide	0.145	0.275	0.377	0.797
Sodium hydroxide + sodium dodecyl sulfate	0.225	0.275	0.282	0.783
Ammonia water	0.192	0.260	0.312	0.765
Ultrasound-assisted sodium hypochlorite	0.176	0.195	0.250	0.620
Microwave-assisted EDTA	0.179	0.195	0.260	0.634

Figures

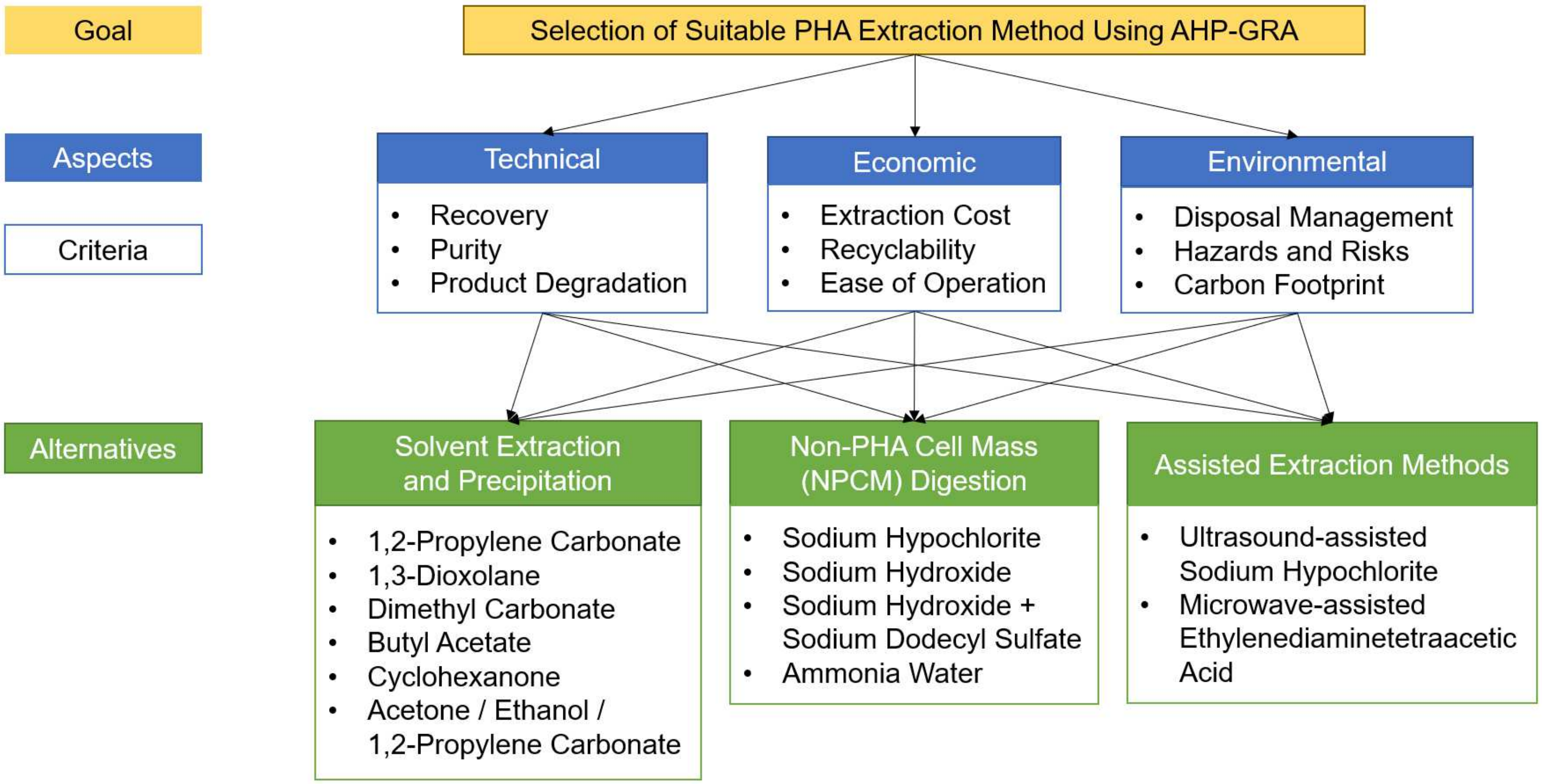

Fig 1 Multi-criteria decision hierarchy for the selection of PHA extraction and recovery protocol

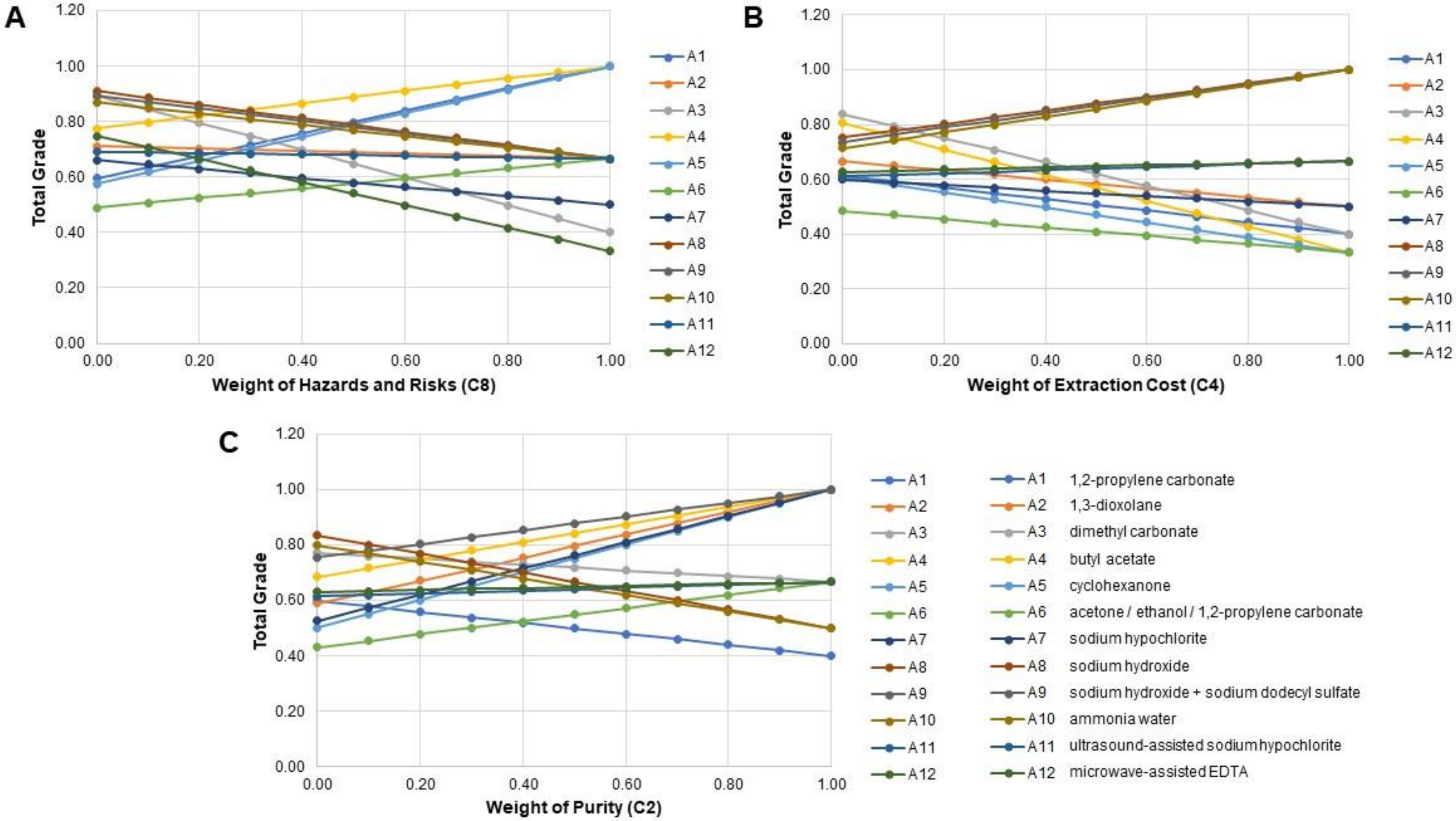

Fig 2 Sensitivity analysis of the priority weights of protocol alternatives for PHA extraction and recovery at various criteria weight intervals of (A) hazards and risks, (B) extraction cost, and (C) purity

Figures

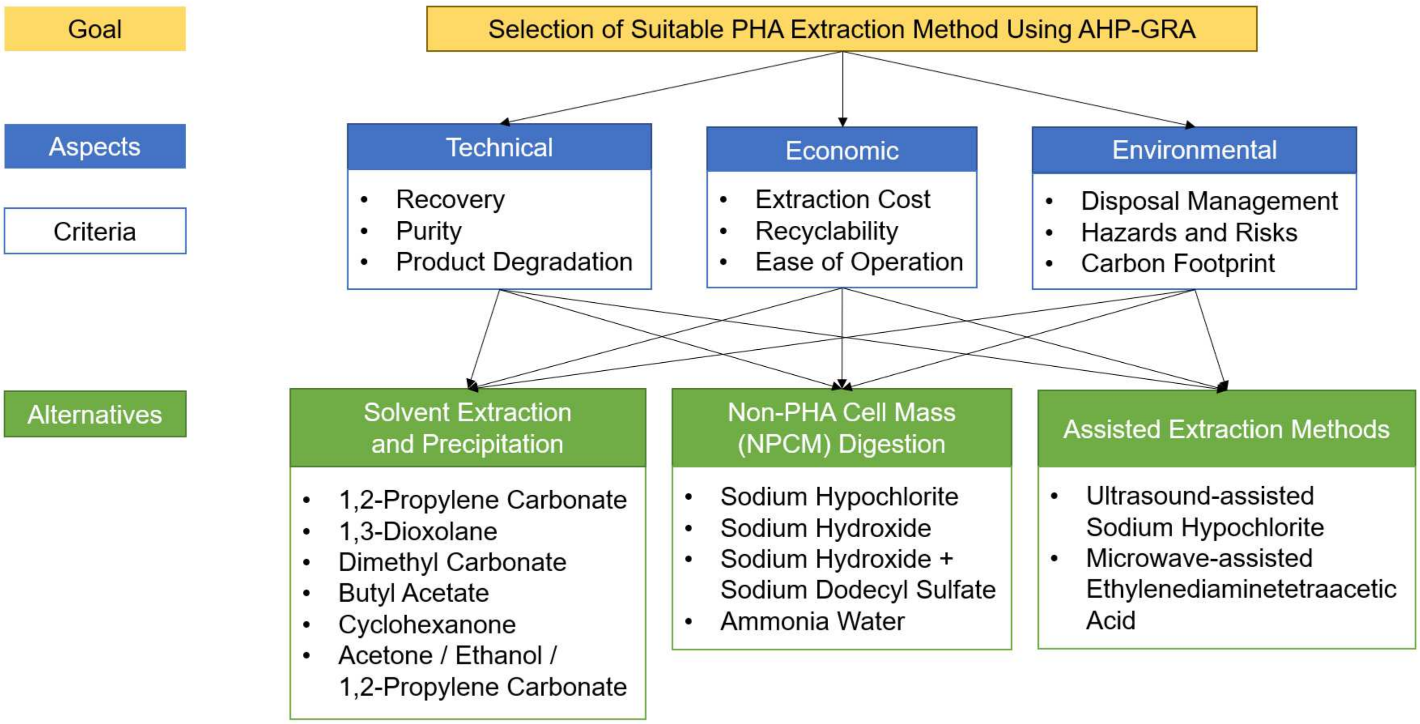

Figure 1

Multi-criteria decision hierarchy for the selection of PHA extraction and recovery protocol

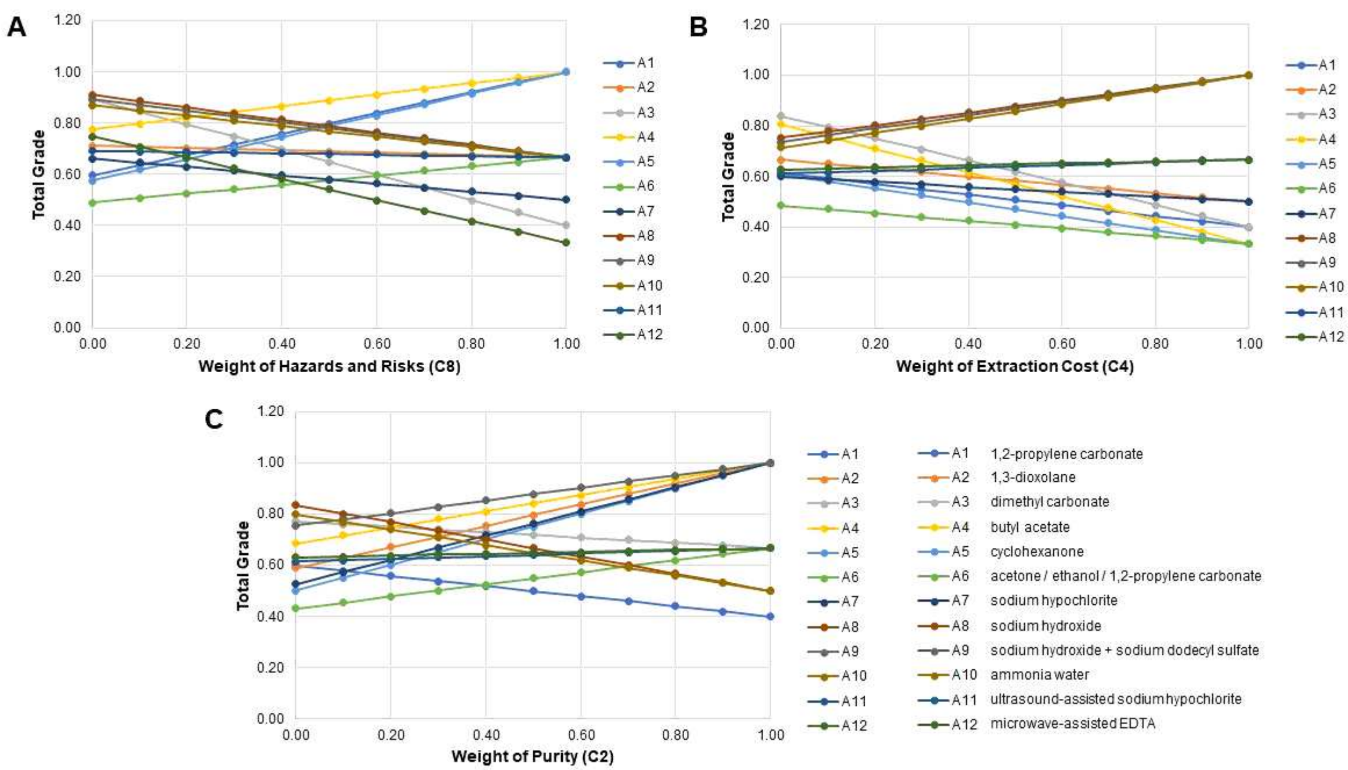

Figure 2

Sensitivity analysis of the priority weights of protocol alternatives for PHA extraction and recovery at various criteria weight intervals of (A) hazards and risks, (B) extraction cost, and (C) purity

Supplementary Files

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