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# A life-cycle assessment of poly-hydroxybutyrate extraction from microbial biomass using dimethylcarbonate

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## Abstract

*Plastic materials have wide commercial applicability. However, they are made from non-renewable resources and are characterised by resistance to degradation. Poly-hydroxyalkanoates (PHAs) provides one example of a polymer biodegradable, biocompatible and produced from renewable raw materials. With respect to other bioplastics the share of PHAs in the market is very limited because of their commercial costs. To develop more cost effective processes for PHAs production, a multilevel approach is usually undertaken combining innovative, cheaper and more effective cultivation with safe and cheap extraction and purification methodologies. This study assesses the potential environmental impacts related to a production processes based on the novel protocol to extract PHAs comparing them to the impacts of extraction process based on the use of halogenated hydrocarbons.*

## 1. Introduction

Plastic materials from fossil sources have an important and pervasive role in our everyday life. However, the same characteristics of durability and resistance to degradation which account for their commercial and applicative success cause most of these synthetic polymers to withstand the ocean and terrestrial ecosystems for years to decades or longer, affecting organisms at multiple trophic levels (Ojeda, 2013). The replacement of petroleum-based non-biodegradable plastics with alternative biomaterials with comparable properties and that degrade after being discarded has become an industrial, social and environmental priority. The current global production capacity of bioplastics, including both biodegradable and bio-based plastics, is about 2 Mt and a significant growth of the bioplastic market is expected in the next few years (European Bioplastic, 2015).

Biopolymers rank highly in terms of green design but they can exhibit relatively large environmental impacts and, if all production steps are taken into consideration, the final environmental balance can be even worse than that of

conventional polymers obtained from petroleum (Tabone et al., 2010). Instead, polyhydroxyalkanoates (PHAs) produced from waste biomass have an excellent environmental ranking. PHAs are linear polyesters produced in nature by bacteria through aerobic fermentation of many carbon sources such as polysaccharides or lipids, to store carbon and energy. More than 150 different monomers can be combined within this family to give materials with properties similar to polypropylene and polyethylene but with the advantage of being completely biodegradable, biocompatible and produced from renewable raw materials. Thanks to this peculiar combination of suitable physical and mechanical properties and biodegradable abilities, PHAs could be used in packaging but also in biomedical applications as biodegradable carriers for drug release, disposable items, surgical pins, wound dressings. With respect to other bioplastics (e.g. PLA or starch-based polymers Mater-Bi®) already produced on a large manufacturing scale, the share of PHAs in the bioplastic market is very limited because PHAs are currently significantly more expensive than standard petrochemical plastics and even more costly than the most widespread commercially available bioplastics (Samorì et al., 2015).

To develop more cost effective processes for PHAs production, a multilevel approach is usually undertaken combining innovative, cheaper and more effective cultivation with safe and cheap extraction and purification methodologies allowing lower investments and operating costs for the global process, together with increasing the overall greenness of the PHA production. In general, the extraction of PHA can be accomplished through two strategies: i) solvent extraction, or ii) digestion of the non-PHAs cellular matrix. The first approach is mainly based on the use of chlorinated solvents (e.g. chloroform), able to dissolve the polymer in high quantity, affording, at the same time, the highest levels of PHAs purity. The second approach is based on the dissolution of cellular membranes by using alkaline/acidic solutions, sodium hypochlorite or surfactants, in order to release PHAs from cells. These methodologies do not usually give high level of purity, can affect the quality of the polymer and increase the recovery costs causing problems in wastewater treatment and re-use.

This study aims to assess the potential environmental impacts related to a production processes based on the novel protocol proposed by Samorì et al. (2015) to extract PHAs of single strain and mixed microbial consortia, comparing them to the impacts of extraction process described by Senior et al. (1982) and based on the use of halogenated hydrocarbons. Up to now, the novel protocols have been developed only on laboratory scale and this preliminary analysis of potential environmental impacts of industrial scale production can provide useful information to better orient the development and scale-up phases. The study applies an attributional Life Cycle Assessment (LCA) adopting a 'gate-to-gate' perspective.

## 2. Materials and Methods

### 2.1. Extraction processes through DMC

The protocol of Samorì et al. (2015) is designed for the extraction of polyhydroxybutyrate (PHB) and various copolymers (e.g. poly(hydroxybutyrate-valerate), PHBV) from single strain and mixed microbial consortia with a high content (from 50 to 70 wt%) of polymer. The method is based on the solubilisation of PHA with dimethyl carbonate (DMC). The procedure can be applied directly to concentrated microbial slurries or to dry biomass, affording very high polymer recovery (>85%) and excellent purity (>95%). DMC is an acyclic alkyl carbonate and it has become increasingly important in the chemical industry mainly because of its versatility as reagent and solvent, and its relatively low toxicity for human health and for the environment (Delledonne et al., 2001).

The direct extraction from microbial slurry requires a biomass concentration of 50 g L<sup>-1</sup>. Such concentration is achieved by centrifuging and concentrating the microbial culture after the accumulation phase. The slurries are extracted with DMC for 1 h at 90 °C. After that, the water phase and the residual biomass are centrifuged and separated, and the extracted polymer is recovered after (i) filtration and evaporation of the solvent or (ii) precipitation with ethanol (EtOH). The polymer recovery is 85%.

The extraction from dried biomass requires a biomass to solvent ratio of 2.5% (w/v). The biomass and the solvent are centrifuged and kept at 90° C for 4 h. The residual biomass is then centrifuged and the polymer is recovered after (i) filtering and evaporating the solvent or (ii) the addition of EtOH and precipitation. In this case, the polymer recovery is 88%.

### 2.2. . Extraction process through halogenated hydrocarbons

Chlorinated solvents are the best performing organic solvents for solubilizing and recovering PHAs from microbial cells. This evidence brings us to choose an extraction with halogenated hydrocarbons as comparison process. Among the patented processes, we have chosen the one detailed in the US Patent 4324907 (Senior et al., 1982) for three reasons: 1) high recovery (95%); 2) data comprehensiveness; 3) similarity to DMC extraction process. In the patented process, an aqueous suspension of cells is spray dried, refluxed with acetone at 56 °C to extract lipids and pigment and then deprived of the solvent by filtration. The residual cells are then refluxed with 1,2-dichloroethane at 83 °C for solubilizing polyhydroxybutyrate (PHB). Finally, the PHB is precipitated by adding a methanol/water mixture and filtered. The purity is very high (98%). Similar results are obtained using methanol instead of acetone as the lipid extraction solvent and dichloromethane or chloroform as the PHB extraction solvent.

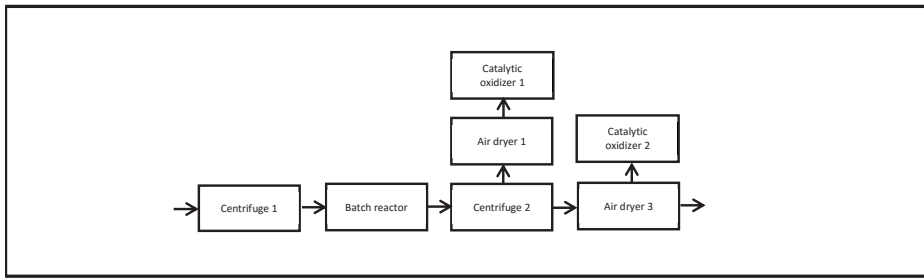
### 2.3. Modelling and scale-up of the extraction processes

Industrial scale production of PHAs with DMC is not yet established, resulting in lack of direct input/output data for the LCI. Therefore, the extraction processes at industrial scale have been simulated by a preliminary design of the envisaged process plant. This was based on the principia of good engineering practice in scale-up and process design (Sinnott, 1993; Bisio and Kabel, 1985; Zlokarnik, 2002). While it is recognized that the actual scale-up would require extensive research efforts, this simplified approach is considered adequate for the purpose of explorative LCA studies (Righi et al., 2011; Righi et al., 2016). The material and energy balances for the envisaged process flow diagram were quantified with the support of a Chemical Process Simulation (CPS) software (Aspen HYSYS® by Aspentech), and the main equipment units were preliminary sized on the basis of relevant scientific and technical references (Table 1). The extraction processes are composed by a series of equipment units: 1) centrifuges; 2) batch reaction vessels; 3) air dryers; 4) catalytic oxidizer; 5) pervaporation systems (only in the scenarios where the polymer is recovered with the addition of EtOH). The equipments are different in dimensions and arrangement in base on the different scenarios (see Fig. 1).

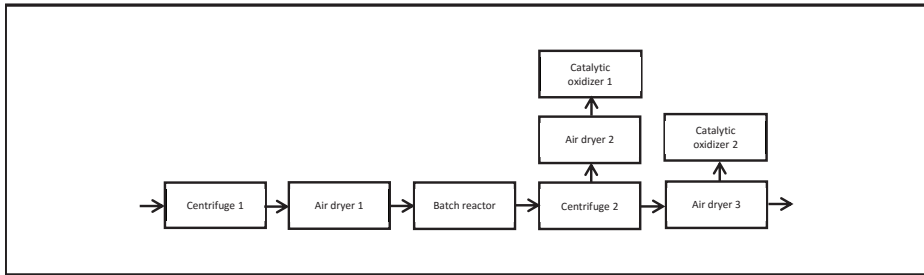
*Table 1: main parameters and data sources used to model the extraction processes*

<b>Equipment</b>	<b>Data</b>	<b>Data sources</b>
Centrifuges	Specific power	Perry et al., 1984
	Volumetric capacity	Perry et al., 1984
	Operating time	Harding et al., 2007
Batch reaction vessels	Specific power	Morfino, 2009
	Volume	Morfino, 2009
Air dryers	Energy consumption	Baker and McKenzie, 2005
	Purge flow	Aspen HYSYS®
	Heat loss	Aspen HYSYS®
Catalytic oxidizer	Emission factors	EEA, 2013
Pervaporation systems	General information	Kujawski et al, 2000
	General information	Neel, 1991

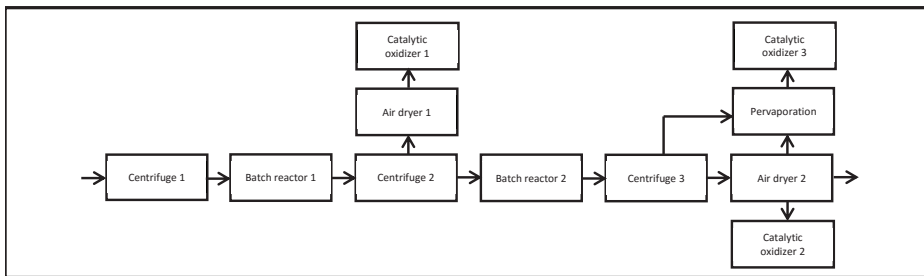
Scenario 1



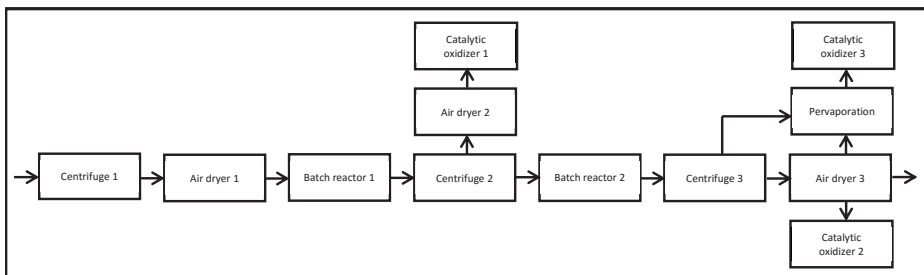
Scenario 2



Scenario 3



Scenario 4



Scenario 5

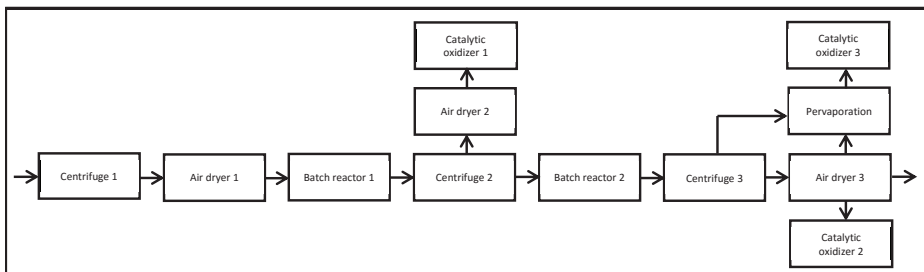


Figure 1: main equipment unit arrangement of each scenario

## 2.4. LCA method

The goal of this study is to evaluate the environmental performances of the novel protocol proposed by Samorì et al. (2015) for the extraction of PHAs with DMC from microbial cells and compare them with the environmental impacts due to an alternative process using chlorinated solvents. A 'gate-to-gate' approach is used, and only the extraction process has been considered since the cultivation phase is supposed the same for all the extraction processes. The system boundaries of the study include the following processes: 1) chemicals production; 2) thermal and electric energy production; 3) extraction process and PHB production; 4) waste management. Note that the microbial cells cultivation is not included in the system boundaries. The functional unit is defined as 1 kg of PHB ready for the bioplastic product manufacturing.

Two different ways for extracting PHAs with DMC have been analysed: a) extraction from dried biomass and b) extraction from microbial slurry. For each of the two extraction ways, two sub-scenarios have been evaluated, which differ in the polymer recovery strategy: 1) after filtration and evaporation of the solvent; or 2) after the addition of EtOH and precipitation. Each of the four resulting scenarios has been compared to the '1,2-dichloroethane scenario' which supplies the same quantity of PHB applying the patent of Senior et al. (1982). Table 2 shows the main features of each scenario.

The following cut-off rules and assumptions have been adopted: 1) electric consumption of pumps carrying the biomass to the reactor, water consumption of cooling pumps, electric consumption of catalytic oxidizer; 2) only the operational phase has been considered, excluding equipment construction, maintenance and dismantling; 3) all electricity comes from the Italian national grid power; 4) the purge factor of air dryers has been fixed to 0.1; 5) an annual production of about 500 t/y of PHB has been considered (assumption necessary to estimate the hourly air emissions); 6) 100% pure PHB production; 7) DMC has been classified as a Class V of Annex I to Part Five of Legislative Decree 152/2006 (classification of air pollutants based on health hazard); 8) DMC and 1,2-dichloroethene air emissions are below the materiality threshold (4000 g/h and 25 g/h, respectively); 9) NO<sub>x</sub> emission factor for catalytic oxidizer has been considered equal to those one occurring in thermal treatment processes of biodegradable waste; 10) emission factors of hazardous waste have been used to estimate the emission due to the catalytic oxidizer applied to extraction process through 1,2-dichloroethene; 11) the solid waste resulting from the DMC extraction is treated in a waste-to-energy plant for non-hazardous waste (see explanation below).

Table 2: main features of the five scenarios analyzed in the paper

Code	Solvent	Biomass state	Separation
1 Slurry-Evap	DMC	microbial slurry	DMC evaporation
2 Dry-Evap	DMC	dry biomass	DMC evaporation
3 Slurry-Precip	DMC	microbial slurry	precipitation with EtOH
4 Dry-Precip	DMC	dry biomass	precipitation with EtOH
5 DCE	1,2-dichloroethane	dry biomass	Precipitation with MeOH/water

Primary data have been used for the processes taking place in our laboratories, databases have been used for background processes and estimates have been used for emissions or processes not taking place in the current plant, such as catalytic scrubbing. LCA was conducted utilizing GaBi 6 software. The databases used for obtaining background data were Gabi Professional Database (Thinkstep, 2015) and Ecoinvent Database (Ecoinvent Centre, 2012). All main background processes used in this study are shown in Table 3.

With regards to life cycle impact assessment (LCIA), the following impact categories has been chosen: climate change (GWP 100), photochemical ozone formation (POP) and ecotoxicity (freshwater) (FETP). The methods recommended by ILCD Handbook (EC-JRC, 2011, 2012) have been used.

Since freshwater characterization factor (CF) of DMC is not available on GaBi 6 software, it has been calculated following the approach of USETox model (www.usetox.org). The calculations require two types of data: physical and chemical properties, bioconcentration data and ecotoxicological information. All physical and chemical data derived from EPI Suite™ (US EPA, 2016) while the ecotoxicological data derived from ECHA database (European Chemicals Agency, 2010).

The solid waste resulting from the extraction of PHB from the biomass was analysed and it resulted a non-hazardous waste since no cancerogenic or mutagenic or hazardous substances have been detected (data not published). Its lower heating value (LHV) results 16500 J/g, comparable to LHV of wood and paper, therefore the treatment in a waste-to-energy plant has been hypothesized.

Table 3: main background processes used in this study

Process name	Data source	Geographic location
Electricity grid mix	PE International <sup>§</sup>	IT
Process steam from natural gas 95%	PE International <sup>§</sup>	IT
Trichloromethane, at plant	Ecoinvent	RER
Dichloromethane, at plant	Ecoinvent	RER
Ethylene dichloride, at plant	Ecoinvent	RER
Ethanol from ethylene, at plant	Ecoinvent	RER
Methanol, at plant	Ecoinvent	GLO
Acetone	PE International <sup>§</sup>	IT
Water (desalinated, deionized)	PE International <sup>§</sup>	DE
DMC, at plant	Righi et al, 2016	DE
Waste incineration of biodegradable waste fraction in municipal solid waste (MSW), ELCD/CEWEP	PE International <sup>§</sup>	EU-27

<sup>§</sup> PE International is now Thinkstep



### 3. Results and discussion

Environmental performances of production processes based on the novel protocol considering two alternative ways of extraction of PHB (from microbial slurry and from dried biomass) and taking into account two different ways of polymer recovery are presented in Fig. 2. The four scenarios are compared to the reference scenario 'DCE'. The relative contributions to environmental performance by life cycle stage for each scenario are also shown in the Figure.

All extraction processes through DMC show environmental performances better than 'DCE' scenario for all three impact categories. GHG emissions due to extract process through DCE are about six times higher than scenarios representing the extraction via DMC. Similarly, the contribution to ecotoxicity of 'DCE' scenario is from 2 to 8 times higher than those ones of novel protocol and photochemical ozone formation score of reference scenario is from 2 to 3 higher than novel protocol scenarios. Nonetheless, it is important to highlight that the chemical pathway of DMC production (in this case-study via oxy-carbonylation process) has a fundamental role for the obtained results.

To the best of our knowledge, this is the second report to give a set of LCA results for PHB production, the first one was by Harding et al. (2007). Both works report GHG emissions expressed as CO<sub>2</sub>-equivalent and the values per FU are well comparable (about 1 kg versus 2 kg, respectively) considering that Harding et al. (2007) included also the cultivation phase. The other impact categories are not comparable since the characterization methods used are different.

As far as a comparison among the four scenarios related to the novel protocol is concerned, it is possible to observe that extraction applied to dry biomass is always preferable to the one from slurry. Such a result is dependent on two main factors: 1) the recovery obtained through extraction from dried biomass is higher (88%) than the one obtained with the extraction from slurry (85%); 2) the extraction from dry biomass presents lower loss and, therefore consume, of solvent.

As far as polymer recovery method is concerned, there are no clear indications of which type is preferable from life cycle analysis. GHGs and photochemical ozone formation emissions are lower evaporating the solvent than precipitating the polymer with EtOH. On the contrary, precipitation appears more suitable from the ecotoxicological point of view than evaporation.

The contribution analysis for climate change impacts (Figure 2a) illustrates that catalytic oxidizer and PHB extraction are the dominant processes for both the 1 and 2 'evaporation' scenarios, while in 'precipitation' scenarios (3 and 4) catalytic oxidizer, chemical recovery and PHB extraction are the dominant stages. As clearly indicated by Figure 2b, the ecotoxicity is completely dominated by the chemical production in all five scenarios.

Relative contributions of life cycle stages to photochemical ozone formation are visible from Figure 2c. Scenarios 1 and 2 are dominated by the chemical production while PHB extraction and catalytic oxidizer play a minor - but appreciable - role. Scenarios 3 and 4 show a more complex situation where there are three main contributors: chemical production; chemical recovery and catalytic oxidizer.

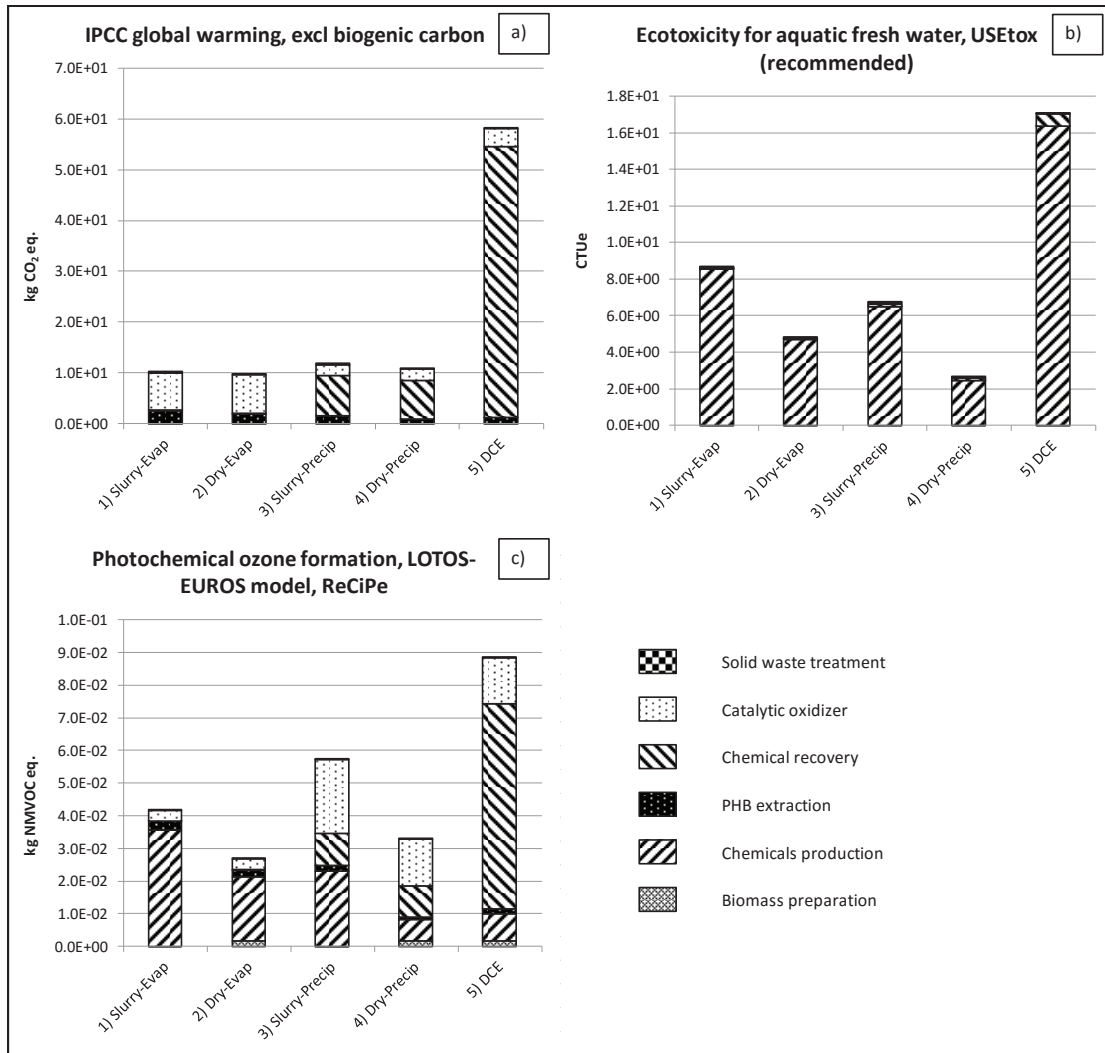


Figure 2: environmental performances of production processes based on the novel protocol considering two alternative ways of extraction of PHB (from microbial slurry and from dried biomass) and taking into account two different ways of polymer recovery (DMC evaporation and precipitation with EtOH)

#### 4. Conclusions

The extraction of polyhydroxybutyrate through the DMC is more beneficial in a gate-to-gate life cycle assessment study than extraction process based on the use of halogenated hydrocarbons. Impacts in all other categories are lower for PHB from novel protocol than the one obtained using halogenated.

These findings encourage the research towards the application on pilot scale of the new protocol with the aim to verify the first results and study its possible economic and technical implications at the industrial production. The dominant contributions to the environmental burden in the production of PHB through DMC are the production of chemicals and the catalytic oxidizing of airborne effluents.

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