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A life cycle assessment of poly-hydroxybutyrate extraction from microbial biomass using dimethyl carbonate

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5 **A life cycle assessment of poly-hydroxybutyrate extraction**
6 **from microbial biomass using dimethyl carbonate**
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Abstract

Poly-hydroxyalkanoates are an example of biodegradable and biocompatible polymers, produced from renewable raw materials. With respect to other bioplastics the market share of poly-hydroxyalkanoates is still limited because of their commercial costs. To develop more cost-effective processes, a multilevel approach is usually undertaken combining innovative, cheaper and more effective microbial cultivation with safe and cheap extraction and purification methodologies. This study assesses the potential life cycle environmental impacts related to a novel protocol poly-hydroxyalkanoates extraction based on dimethyl carbonate in comparison to the use of halogenated hydrocarbons (in particular 1,2 dichloroethane). Four scenarios are analysed for the dimethyl carbonate protocol considering: extraction from microbial slurry or from dried biomass, and recovery by solvent evaporation or polymer precipitation. The life cycle assessment demonstrates that the environmental performances of dimethyl carbonate-based protocols are far better than those of the most comparative process using the halogenated hydrocarbons. The scenario that foresees the extraction of dried biomass and recovers solvent by evaporation appears to be the most promising in terms of environmental sustainability performance.

Keywords: bio-based polymers; bioplastics; poly-hydroxyalkanoates; environmental impact; environmental performance; ecodesign.

1. Introduction

Plastic materials from fossil sources play 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 plastic 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 bio-based materials that have comparable properties and that are more readily degradable after being discarded has become an industrial, social and environmental priority. The current annual global production capacity of bioplastics, including both biodegradable and bio-based plastics, is about 2 Mt and a significant growth of the bioplastics market is expected in the next few years (European Bioplastic, 2015).

Among biopolymers, poly-hydroxyalkanoates (PHAs) have rapidly gained interest both in research and industry because they are greatly versatile, fully biodegradable and with properties similar to conventional plastics (Keshavarz and Roy, 2010; Vega-Castro et al., 2016; Dietrich et al., 2017). PHAs are linear polyesters produced by single (e.g. *Cupriavidus necator*) or mixed microbial consortia through aerobic fermentation of many carbon sources such as

1 polysaccharides or lipids, to store carbon and energy. At present, approximately 150 different
2 PHA monomers can be combined yielding materials with distinct physical properties as function
3 of the monomer composition including properties similar to those of polypropylene and
4 polyethylene, but with the advantage of being biodegradable, biocompatible and produced from
5 renewable raw materials (Steinbüchel and Lütke-Eversloh, 2003). Thanks to this peculiar
6 combination of suitable physical and mechanical properties and biodegradation abilities, PHAs
7 could be used in packaging but also in biomedical applications as biodegradable carriers for
8 drug release, disposable items, surgical pins, and wound dressings.

9
10 Poly-hydroxyalkanoates rank highly in terms of 'green design' but they can nonetheless exhibit
11 relatively large environmental impacts and, if all life cycle phases are taken into consideration,
12 the final environmental balance can be even worse than that of conventional polymers obtained
13 from petroleum (Tabone et al., 2010).

14
15 Several studies report that the dominant contributions to energy requirement (and,
16 consequently, environmental burden) in the production of PHAs are: i) the use of cultivated
17 feedstock, such as corn and sugar cane, to obtain substrates like as glucose, methanol or
18 acetic acid (Patel et al., 2005; Keshavarz and Roy, 2010), ii) the sterilization of fermentation
19 equipment (Van Wegen et al., 1998); iii) the recovery of the intracellular polymer (Keshavarz
20 and Roy, 2010; Rostkowski et al., 2012; Fernández-Dacosta et al. 2015). Persistent effort is
21 being made to find environmental friendly feedstock, like as waste vegetable oil, wastewater,
22 food scraps, waste oils, etc. (Heimersson et al., 2014; Anjum et al., 2016). In second point
23 regards, research is continuing on mixed microbial culture instead of the more expensive and
24 problematic pure culture (Hao et al., 2017; Montiel-Jarillo et al., 2017). As far as concerned the
25 last point, a more environmentally benign PHAs recovery method that is less energy intensive
26 and does not use harmful solvents appears one priority (Keshavarz and Roy, 2010; Rostkowski
27 et al., 2012). In general, the recovery of intracellular PHAs can be accomplished via two
28 processes: i) solvent extraction, or ii) digestion of the non-PHAs cellular matrix. The first process
29 is currently mainly based on the use of chlorinated solvents (e.g. chloroform), able to dissolve
30 the polymer in high quantity, and affording, at the same time, the highest levels of PHAs purity.
31 The second process is based on the dissolution of cellular membranes by using alkaline/acidic
32 solutions, sodium hypochlorite or surfactants, in order to release PHAs from cells. This process,
33 however, does not usually yield high levels of purity, can affect the mechanical properties of the
34 polymer and increase the recovery costs affecting wastewater treatment and re-use.

35
36 Samori et al. (2015) have designed a new protocol for the extraction of poly-hydroxybutyrate
37 (PHB) and various copolymers (e.g. poly-hydroxybutyrate-valerate, PHBHV) from single strains
38 and mixed microbial consortia with a high content of polymer (74 and 40% wt%, respectively)
39 using as solvent dimethyl carbonate (DMC). DMC is an acyclic alkyl carbonate and it has
40 become increasingly important in the chemical industry mainly because of its versatility as
41 reagent and solvent, and its relatively low toxicity for human health and for the environment
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1 (Delledonne et al., 2001). This study therefore aims to assess the potential environmental
2 impacts related to a production processes based on the protocol proposed by Samori et al.
3 (2015) in comparison to the extraction with halogenated. We apply an attributional Life Cycle
4 Assessment (LCA) adopting a 'gate-to-gate' perspective: we start from the microbial biomass
5 enriched in PHA and stop when the polymer is ready to be formed. Up to now, the DMC-based
6 protocol has been developed only on a laboratory scale thus an industrial-scale production has
7 been hypothesized. and this preliminary analysis of potential environmental impacts of a future
8 industrial-scale production can provide useful information to better orient the development and
9 scale-up activities.

16 2. Materials and Methods

18 2.1. Extraction processes using dimethyl carbonate

20 PHB is one of the most well-known and studied PHAs, therefore has been taken as the study
21 polymer. The method is based on the solubilisation of PHB with dimethyl carbonate (DMC). The
22 procedure can be applied directly to concentrated microbial slurries or to dry biomass, affording
23 very high polymer recovery (>92%) and excellent purity (>95%).

24 The direct extraction from microbial slurry requires a biomass concentration of 100 g L⁻¹. Such
25 concentration was achieved by centrifuging and concentrating a pure microbial culture after the
26 accumulation phase. The slurries were extracted with DMC for 1 h at 90 °C. After that, DMC
27 phase and the biomass slurry were centrifuged and separated, and the extracted polymer was
28 recovered after (i) evaporation of DMC or (ii) precipitation with ethanol (EtOH). The polymer
29 recovery was very high in both cases (>96%). The extraction from dried biomass requires a
30 biomass to solvent ratio of 2.5% (w/v). The biomass and the solvent were centrifuged and kept
31 at 90° C for 4 h. The residual biomass was then centrifuged and the polymer was recovered
32 after (i) evaporating the solvent or (ii) the addition of EtOH and precipitation. In this case, the
33 polymer recovery was above 92% in both recovery scenarios.

43 2.2. Extraction processes using halogenated hydrocarbons

44 Chlorinated solvents are the best performing organic solvents for solubilizing and recovering
45 PHAs from microbial cells, therefore, an extraction with halogenated hydrocarbons has been
46 chosen as comparison process. Among the patented processes, the US Patent 4324907
47 (Senior et al., 1982) has been selected for three reasons: 1) high PHA recovery (95%); 2) data
48 comprehensiveness; 3) similarity to DMC extraction process. **In the patented process, an
49 aqueous suspension of cells is spray-dried and then refluxed with 1,2-dichloroethane (DCE) at
50 83 °C for solubilizing PHB. Finally, PHB is precipitated by adding a methanol/water mixture and**

1 filtered. The polymer purity is very high (98%). Similar results are obtained using
2 dichloromethane or chloroform instead of 1,2-dichloroethane in the PHB extraction process.
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5 **2.3. Modelling and scale-up of the extraction processes**

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

32 <Figure 1>

33 **2.4. Application of environmental life cycle assessment**

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36 The goal of this study is to compare the environmental performance of the protocol proposed by
37 Samorì et al. (2015) for the extraction of PHB with DMC from microbial cells with the
38 environmental performance due to an alternative process using chlorinated solvents using LCA.
39 A 'gate-to-gate' approach is used, and only the extraction process has been considered since
40 the cultivation phase and the bioplastic product manufacture after the polymer extraction are
41 assumed to be equivalent for all considered extraction processes. The system boundaries of the
42 study include the following processes: 1) biomass preparation; 2) chemicals production; 3) PHB
43 extraction; 4) chemicals recovery; 5) air emissions abatement; 6) solid waste management.
44 Note that the microbial cells cultivation is not included in the system boundaries and that the
45 processes downstream from cultivation are often the most cost and energy consuming
46 (Fernández-Dacosta et al. 2015). The functional unit (FU) is defined as 1 kg of PHB ready for
47 the product's manufacturing.
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2.4.1. Scenarios description and assumptions

Two different ways for extracting PHB with DMC have been analysed: a) extraction from dried biomass (Dry) and b) extraction from microbial slurry (Slurry). For each of the two extraction ways, two sub-scenarios have been evaluated, which differ in the polymer recovery strategy: 1) after evaporation of the solvent (Evap); or 2) after the addition of EtOH and precipitation (Precip). 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 apparatus for mass transferring, electric consumption of catalytic oxidizer, water consumption of cooling pumps have been omitted (the first two because only low contributions are expected, the last one because water is supposed to be extracted and then returned to the water stream); 2) only the operational phase has been considered, excluding equipment construction, maintenance and dismantling; 3) all electricity comes from the Italian national grid power (2014 reference year); 4) the purge factor of air dryers has been fixed to 0.2% (according to Hischier et al. (2005) on diffusive and fugitive emissions to air from production plants); 5) an annual production of about 500 t/y of PHB has been considered (necessary to estimate the hourly air emission rates); 6) 100% pure PHB production; 7) DMC has been classified as a Class V of Annex I to Part Five of Italian Legislative Decree 152/2006 (Italian Parliament, 2006) following affinity rule (see explanation in section 2.4.4.); 8) DMC and 1,2-dichloroethene air emissions are below the materiality threshold (4000 g/h and 25 g/h, respectively); 8) NO_x emission factor for catalytic oxidizer has been considered equal to those occurring in thermal treatment processes of biodegradable waste; 9) emission factors for hazardous waste have been used to estimate the emission due to the catalytic oxidizer applied to extraction process through 1,2-dichloroethene; 10) the solid waste resulting from the DMC extraction is treated in a waste-to-energy plant for non-hazardous waste (see explanation in section 2.4.5.).

<Table 2>

2.4.2 Data collection and elaboration

Primary data have been used for the processes taking place in laboratory, 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 Version 2 database (Frischknecht et al., 2005). All main background processes used in this study are shown in Table 3. Detailed Life Cycle Inventories of the five scenarios are presented in Annexes A-E.

1 With regards to life cycle impact assessment (LCIA), the following impact categories have been
2 included: climate change (GWP), ozone depletion (OD), human toxicity cancer (HTc) and non-
3 cancer (HTnc), freshwater ecotoxicity (FE), acidification (AC), photochemical ozone formation
4 (POF), freshwater eutrophication (EuF), marine eutrophication (EuM), terrestrial eutrophication
5 (EuT), particulate matter formation (PM), ionizing radiation (IR), resource depletion (RD) and
6 water depletion (WD). The methods recommended in the ILCD Handbook (EC-JRC, 2011,
7 2012) have been applied.
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13 <Table 3>

14 15 16 **2.4.3. Dimethyl carbonate toxicity and ecotoxicity characterization**

17 Since DMC characterization factors (CFs) of human toxicity and freshwater aquatic ecosystem
18 toxicity were not available, they have been calculated following the approach proposed in the
19 scientific consensus model USEtox (www.usetox.org) that is endorsed by the UNEP/SETAC
20 Life Cycle Initiative and recommended by the ILCD Handbook for characterizing human toxicity
21 and ecotoxicity in LCA (Westh et al., 2015). The CF calculation requires several types of input
22 data: physicochemical substance properties, bioconcentration data and toxicological and
23 ecotoxicological information (Fantke et al., 2017). Physicochemical and bioconcentration data of
24 DMC were mainly derived from EPI Suite™ (US EPA, 2016), while (eco)toxicological data were
25 derived from ECHA's database on registered substances (European Chemicals Agency, 2017).
26 Ecotoxicological and toxicological data used in this study are reported in Table 4. Regarding
27 human toxicity, the ED₅₀ values have been extrapolated from NOEC or NOEL based on a
28 generic conversion factor of 9 (Huijbregts et al., 2005). Since experimental data are from
29 subchronic and acute tests, a subchronic-to-chronic extrapolation factor of 2 and an acute-to-
30 chronic extrapolation factor of 5 have been used to extrapolate to chronic ED₅₀ (Fantke et al.,
31 2017). For ecotoxicity, chronic EC₅₀ values have been calculated from NOEC using the
32 extrapolation factors suggested by Payet (2004) and, when necessary, applying a generic
33 chronic-to-acute ratio of 2 (Müller et al., 2017). All newly developed input data used in the
34 USEtox model are reported in Table 5.
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46 <Table 4>

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49 50 51 **2.4.5. Characterization of solid waste from extraction processes**

52 At first, elemental analysis was carried out on a C, H, and N Elemental Analyser. Next, organic
53 compounds measurements were performed by gas chromatography coupled to mass
54 spectrometry (GC/MS): the concentrations of dimethyl carbonate and 42 hazardous compounds
55 (toxic, carcinogenic, mutagenic, flammable, etc.) were determined (see Annex F). Then, a
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1 colorimetric method was applied for the determination of aldehyde concentrations (expressed
2 as formaldehyde). Afterward, 19 metals (Be, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Ag, Cd, Sb,
3 Ba, Tl, Pb, Hg, Sn) were evaluated by inductively coupled plasma mass spectrometry (ICP-MS).
4 Finally, ion chromatography was applied for determination of chlorine content (fundamental test
5 in solid biomass for power sector). As last step, Mahler bomb calorimeter was used for
6 determining higher heating value (HHV) of the solid waste and subsequently its lower heating
7 value (LHV).
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10 11 12 13 **3. Results and discussion**

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15 The first part of the study results describes the outcome of analyses performed and models
16 applied to obtain all data necessary to carry out the LCA. The second part of the study results
17 reports the evaluation by means of LCA.
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20 21 **3.1. Toxicity for DMC and solid waste**

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23 Using the extraction process through halogenated hydrocarbons presents a considerable
24 disadvantage, namely the use of highly toxic solvents. It is noteworthy that IARC has classified
25 dichloromethane as probable carcinogen to humans (Group 2A) and 1,2-dichloroethane and
26 chloroform as possibly carcinogens (Group 2B). On the contrary, dimethyl carbonate - according
27 to ACGIH, IARC, NTP - has not been classified as carcinogen. Consequently, it is fundamental
28 that the comparison between DMC-based protocol and the extraction through halogenated
29 hydrocarbons considers also the (eco)toxicity effects of the extraction solvents. Results of
30 human toxicity and freshwater aquatic ecotoxicity characterization of DMC calculated with
31 USEtox are reported in Table 6. The corresponding characterization factors of 1,2-
32 dichloroethane are shown in the same table and are up to 2 orders of magnitude higher than for
33 DMC. Dichloromethane or chloroform, both used in extraction processes of PHB, show
34 characterization results very similar to 1,2-dichloroethane. As it is possible to observe, the
35 toxicity CFs of DMC are comparatively low ranging from 2.5×10^{-10} to 2.7×10^{-6} cases/kg emitted
36 and from 6.7×10^{-10} to 7.4×10^{-6} DALY/kg emitted, respectively, for different emission
37 compartments. Also the freshwater aquatic ecotoxicity CFs are low ranging from 0.2 to 6.7
38 PDF·m³·day/kg emitted. As for ecotoxicity, it is noteworthy that freshwater aquatic ecotoxicity
39 CFs have been obtained by so-called 'limit tests'. A 'limit test' is an acute toxicity test in which, if
40 no ill-effects occur at a pre-selected maximum dose, no further testing at greater exposure
41 levels is required (Duffus, 2009). Therefore, a conservative approach has been applied
42 considering no observed effect concentrations (NOEC) to extrapolate to concentrations
43 resulting in 50% effect in the exposed freshwater ecosystem species (EC₅₀).
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47 As additional comparison to give an impression of the order of magnitude of presented CFs, the
48 human toxicity CF of formaldehyde (carcinogen to humans Group 1) for the emission to
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1 household indoor air is 4.55×10^{-3} cases/kg emitted, while the corresponding CF of DMC is
2 2.72×10^{-6} cases/kg emitted and the aquatic ecotoxicity CF of DDT (persistent organic pollutant,
3 worldwide banned) for the emission to continental freshwater is 2.78×10^5 PAF·m³·day/kg
4 emitted, while the corresponding CF of DMC is 10.8 PAF·m³·day/kg emitted. These findings and
5 considerations agree with a number of authors that referred to DMC as a substance benign to
6 human health (Lissel et al., 1989; Ono, 1997; Tundo and Selva, 2002) and eco-friendly (Tundo
7 et al., 2000; Vasapollo et al., 2003; Miao et al., 2008).

8 While EU legislations concerning air quality is rather homogeneous, based on the setting of
9 limits for the concentration of certain pollutants into the air, the EU rules regarding industrial
10 emissions into the atmosphere is more heterogeneous. In Italy this issue is regulated by the
11 Legislative Decree 152/2006 (Italian Parliament, 2006). National emission limits have been set
12 by the Decree for different substances based on their hazard properties. The Decree organizes
13 the organic substances in five classes in order of decreasing toxicity. Not all organic substances
14 are listed, but it is possible to define the class of a substances referring to substances occurring
15 in the list and with similar toxic properties ('affinity rule'). Dimethyl carbonate is not listed by the
16 Decree but through the 'affinity rule' it can be classified in Class V. For this class, the emission
17 limit is set to 600 mg/Nm³ that corresponds to 4000 g/h. These values have been used for LCA
18 modelling.

19 The solid waste resulting from PHB extraction process was analysed to attempt a first
20 classification of it as 'hazardous' or 'not hazardous' waste. These analyses were necessary to
21 hypothesize its end-of-life. In fact, all 'hazardous' waste must be disposed in accordance with
22 regulations more stringent than for 'not hazardous' waste. Main results of chemical analyses
23 carried out on solid waste coming from the extraction process of PHB from microbial cells are
24 presented in Table 7. All hazardous organic compounds show results lower than the detection
25 limits (data not reported). According to these results and in first approximation, it is possible to
26 categorize this waste as 'not hazardous'. The content lower than 1% of halogenated organic
27 substances, expressed as chlorine, suggests that the waste could be sent to incineration plants
28 respecting the restriction that the gas resulting from the incineration is raised to a temperature
29 of at least 850 °C for at least two seconds (as established by Directive 2010/75/EU, European
30 Commission, 2010). The incineration way is reinforced by the low heating value (LHV) of the
31 waste (14,400 J/g) that is well comparable with other waste usually sent to waste-to-energy
32 plants, like textile (11,789 J/g), leather and rubber (14,265 J/g), and wood (9,310 J/g) (World
33 Bank, 1999). Another interesting end-of-life option for the extraction waste could be the
34 composting, suggested by the high carbon content (47.6%) and the low concentration of metals
35 considered hazardous for such employment (Legislative Decrees, 217/06, Italian Parliament,
36 2006; and D.G.R. 1528/2006, Regional Council of Abruzzo Region, 2006). Composting of
37 biomass remaining after PHB recovery was a solution proposed also by Nonato et al. (2001).

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2 <Table 6>

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5 6 7 **3.2. Overall environmental performance results**

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9 Environmental performance results of PHB production processes based on DMC protocol
10 considering two alternative ways of extraction (from microbial slurry or from dried biomass) and
11 taking into account two different ways of polymer recovery (solvent evaporation or polymer
12 precipitation) are reported in Table 8. The four scenarios are compared to the reference
13 scenario 'DCE' (extraction with 1,2-dichloroethane). The relative contributions to each impact
14 category from the main steps involved within each scenarios are illustrated in Fig. 2 to 4. Each
15 PHB production process is subdivided into six steps: 1) biomass preparation (which includes
16 drying in scenarios 'Dry-Evap' and 'Dry-precip'), 2) chemicals production, 3) PHB extraction, 4)
17 chemicals recovery, 5) catalytic oxidation for air emission abatement, 6) solid waste treatment.

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30 Table 8 shows that all four scenarios using DMC show better environmental performances than
31 the process employing 1,2-dichloroethane for all considered impact categories. GHG emissions
32 due to 'DCE scenario' are about 5 to 15 times higher than scenarios representing the extraction
33 via DMC. Acidification, ozone formation precursors, marine and terrestrial eutrophication,
34 particulate matter formation and water depletion due to process by DCE are about 4 to 20 times
35 higher than those of the DMC protocol. The difference between 'DCE' and 'DMC' scenarios for
36 resources depletion shows 40 times difference. The emissions of (eco)toxic substances of 'DCE
37 scenario' are up to 2 orders of magnitude higher than the other scenarios. Finally, ionizing
38 radiation, ozone-depletion gas emissions and freshwater eutrophication related to process
39 through DCE is up to 350 times higher than 'DMC scenarios'. Note that the biggest differences
40 are always between 'DCE' and 'Dry-Evap' scenarios.

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47 When comparing the four scenarios related to DMC protocol, we observe that extraction applied
48 to dry biomass is always preferable to the one from slurry, and that recovery by solvent
49 evaporation is always preferable to polymer precipitation with EtOH. Therefore, the scenario
50 which evaluates extraction of the biopolymer from dried biomass and recovery by precipitation
51 (Dry-Evap scenario) seems to be the most promising, in spite of the fact that recovery obtained
52 through extraction from dried biomass is lower (92%) than obtained with the extraction from
53 slurry (96%). Such a result is dependent on three main factors: 1) the moderate solubilization,

1 and consequent loss, of DMC in the slurry; 2) the high energy requirement of pervaporation
2 systems (in the scenarios "Precip"); 3) the adding of EtOH which increases the mass that has to
3 be dried (in the scenarios "Precip").
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5 For the contribution analysis, the impact categories have been arranged in two groups. The first
6 group includes GWP, AC, EuT, EuM, POF, PM, HTnc, RD and WD. The second group
7 comprises EuF, FE, HTc, IR, and OD. As we observe from Fig. 2 and 3, the first group shows
8 several dominant processes for each impact category. Frequently, PHB extraction is a dominant
9 process for 'evaporation' scenarios (scenarios 1 and 2); chemicals production and recovery
10 (sometimes also catalytic oxidation) are the dominant processes in 'precipitation' scenarios (3
11 and 4); chemicals production is dominant in 'slurry' scenarios (1 and 3); and biomass
12 preparation is dominant in 'dry' scenarios (2 and 4). The distinct dominating factors provide a
13 quite differentiated picture in the contribution analysis. 'Slurry-evap' is dominated by chemicals
14 production and PHB extraction; in 'Dry-evap', biomass preparation and PHB extraction play the
15 main role; 'Slurry-precip' shows chemicals production, chemicals recovery and catalytic
16 oxidation as the main processes (the last one is important only in POF); 'Dry-precip' is
17 dominated by biomass preparation, chemicals production, chemicals recovery, and catalytic
18 oxidation (the last one is important only in POF). As indicated by Fig. 4, the second group is
19 entirely dominated by the chemical production in all four scenarios.
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22 From these results, we identified three main processes: chemical production, PHB extraction
23 and chemicals recovery. In particular, the most important contributor to chemicals production
24 processes is DMC that is used in abundant amount especially in scenarios 'Slurry-evap' and
25 'Slurry-precip' (see Annexes A and C). As shown, DMC consumption can be strongly reduced
26 using dry-biomass instead of microbial slurry. Concluding, a reduction in DMC consumption and
27 an increase of its recovery rate should therefore be among the major goals in the future scale-
28 up and optimization of DMC based processes.
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30 Regarding PHB extraction and chemicals recovery, these processes are dominated,
31 respectively, by DMC evaporation through air dryers (see Annexes A and B) and pervaporation
32 (see Annexes C and D), both high energy-requiring processes. It is possible that a recovery of
33 heat from material flows to be cooled by means of a heat exchanger would lead to a significant
34 thermal energy saving. Lastly, in order to reduce the energy consumption due to the DMC
35 recovery, an alternative to pervaporation could be analysed. All these recommendations should
36 be considered for the scaling up of the processes to industrial-scale. It should be in fact
37 reminded that the preliminary scale-up carried out in current study was only oriented to the
38 comparison of the basic information available for alternative process schemes: the process
39 optimization to be carried out during a more detailed scale-up is actually expected to further
40 increase the performances of the industrial process (Sinnott, 1993; Bisio and Kabel, 1985).
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43 From the point of view of the role of DMC in the environmental sustainability of the process, it is
44 important to highlight that the chemical pathway of DMC production (in this case-study via the
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oxidative carbonylation process; Righi et al., 2016) plays a fundamental role for the obtained results: the 'greener' the solvent, the less environmentally impacting the extraction.

To compare the results of our study to those of other scientific works is challenging. One reason is that to the best of our knowledge no LCA studies focusing only on PHAs extraction phase have been published. Moreover, although several scientific studies have been published about LCA method applied to PHAs production, they are usually not comparable. Among them, three reviews (Hottle et al., 2013; Yates and Barlow, 2013; Narodslawsky et al., 2015) analyse and compare the results obtained by a total of fifteen studies. All studies report the global warming potential (GWP), while only a limited number of studies has also evaluated other environmental impacts (Harding et al., 2007; Khoo et al., 2010; Kendall, 2012). Global warming potential ranges from -4 kg CO₂eq. (Kurdikar et al., 2000) to 11.9 kg CO₂eq. (Kendall, 2012) based on different system boundaries, feedstock, source of energy, etc. Completely different is the value reported by Rostkowski et al. (2012) with 942 kg CO₂eq, mainly due to biopolymer recovery. The values of GWP provided by the present study (3.9-11 kg CO₂eq) lie within the range of values reported in other studies. Since our values refer only to polymer extraction, our results would agree to the above range only if the PHAs extraction were one of the main contributors to the total impact. This hypothesis agrees to previous scientific works who observed that recovery is the dominant process (Rostkowski et al., 2012) or one of the dominant processes (Kendall et al., 2012; Fernández-Dacosta et al. 2015) contributing to the total environmental impacts of PHB production.

<Table 8>

4. Conclusions

LCA was applied to a novel procedure based on DMC for the extraction of PHB from bacterial biomass. Extraction of the polymer from microbial slurry or from dried biomass and recovery by solvent evaporation or polymer precipitation were assessed, obtaining four different scenarios. LCA results demonstrate that the environmental performances of DMC protocol are far better than those of the most common processes using halogenated hydrocarbons. Among the four scenarios tested the one that evaluates extraction from dried biomass and PHB recovery by precipitation is always the most promising. These findings encourage the research towards the application on pilot scale of DMC with the aim to verify the first results and to study its possible economic and technical implications at the industrial production scale. The main recommendations for the scaling up drawn from this study are: i) to prefer processes using dry biomass instead of concentrated microbial slurries since in the latter the loss of DMC is higher; 2) to enhance the efficiency of polymer recovery and the DMC recovery efficiency after the polymer extraction in order to reduce the DMC consumption; 3) to consider a heat exchanger

1 for thermal energy saving, and 4) to consider an alternative to the pervaporation for chemicals
2 recovering since this technology is very energy-requiring.
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7
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1 **Table 1 Main parameters and data sources used to model the extraction processes.**

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Equipment	Data	Data sources
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

1 **Table 2 Main features of the five scenarios analysed in our study.**

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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

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Table 3 Main background processes used in this study.

Process name	Data source	Geographic location
Electricity grid mix	PE International [§]	IT
Process steam from natural gas 95%	PE International [§]	IT
Ethylene dichloride, at plant	Ecoinvent	RER
Ethanol from ethylene, at plant	Ecoinvent	RER
Methanol, at plant	Ecoinvent	GLO
Water (desalinated, deionized)	PE International [§]	DE
Dimethyl carbonate, at plant	Righi et al, 2016	DE
Truck, Euro 0-6 mix, 20-26 t gross weight / 17.3 t payload capacity	PE International [§]	GLO
Diesel production mix, at refinery	PE International [§]	EU-27
Waste incineration of biodegradable waste fraction in municipal solid waste (MSW), ELCD/CEWEP	PE International [§]	EU-27

[§] PE International is now Thinkstep

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Table 4 Toxicological and ecotoxicological data of DMC used to calculate characterization factors for human toxicity and freshwater aquatic ecosystem toxicity. All data are from the ECHA registered substances database (European Chemicals Agency, 2017).

TOXICOLOGICAL DATA					
Route	Animal	Test type	End-point	Value	Chronic ED ₅₀
Inhalation	Rat	Acute	NOEC	>5.36 mg/L	9.65 mg/L
Ingestion	Rat	Subchronic	NOEL	>500 mg/kg bw/day	2250 mg/kg bw/day
ECOTOXICOLOGICAL DATA					
Trophic level	Specie	Test type	End-point	Value	Chronic EC ₅₀
Fish	<i>Danio rerio</i>	ST (96 h)	NOEC	100 mg/L	165 mg/L
Invertebrates	<i>Daphnia magna</i>	LT (21 d)	NOEC	25 mg/L	120 mg/L
Algae	<i>Selenastrum capricornutum</i>	LT (72 h)	NOEC	100 mg/L	480 mg/L

Table 5 DMC data used in the USEtox model, version 2.02.

PARAMETERS	Unit	Value	References [notes]
CAS number	-	616-38-6	-
Physical and chemical data			
Molar mass	g/mol	9.0 10 ¹	-
pKa chemical class	-	Neutral	-
Partitioning coefficient between n-octanol and water	L/L	1.7	US EPA, 2016 [EPI Suite™]
Partitioning coefficient between organic carbon and water	L/kg	2.9	US EPA, 2016 [EPI Suite™]
Henry's law constant (at 25°C)	Pa·m ³ /mol	8.7	US EPA, 2016 [EPI Suite™]
Vapor pressure (at 25°C)	Pa	7.6 10 ³	European Chemicals Agency, 2017
Solubility (at 25°C)	mg/L	1.15 10 ⁵	European Chemicals Agency, 2017
Rate constant degradation and bioaccumulation			
Rate constant degradation in air	1/s	2.3 10 ⁻⁷	Based on US EPA, 2016 [EPI Suite™]
Rate constant degradation in water	1/s	5.4 10 ⁻⁷	Based on US EPA, 2016 [EPI Suite™]
Rate constant degradation in sediment	1/s	2.7 10 ⁻⁷	Based on US EPA, 2016 [EPI Suite™]
Rate constant degradation in soil	1/s	5.9 10 ⁻⁸	Based on US EPA, 2016 [EPI Suite™]
Bioaccumulation factor in fish	L/kg _{fish}	3.2	Based on US EPA, 2016 [EPI Suite™]
Toxicological and ecotoxicological data			
Average of the log of the species-specific geometric means of concentrations affecting 50% of the exposed species population for a defined endpoint	mg/L	2.3	Calculated [from ecotoxicological data, Table 4]
Human-equivalent lifetime dose per person that causes a non-cancer disease probability of 50% via inhalation	kg/lifetime	7.8 10 ²	Calculated [from acute inhalation LC50, Table 4]
Human-equivalent lifetime dose per person that causes a non-cancer disease probability of 50% via ingestion	kg/lifetime	9.8 10 ²	Calculated [from subchronic ingestion NOEL, Table 4]

Table 6 Human toxicity and freshwater aquatic ecotoxicity characterization factors of dimethyl carbonate (DMC) and 1,2-dichloroethane (DCE).

MIDPOINT LEVEL CHARACTERIZATION FACTORS				
Type of emission	Human toxicity [cases/kg emitted]		Freshwater aq. ecotoxicity [PAF·m ³ ·day/kg emitted]	
	DMC	DCE	DMC	DCE
to household indoor air	2.72 10 ⁻⁶	6.05 10 ⁻⁵	3.79 10 ⁻¹	1.22 10 ⁻¹
to industrial indoor air	9.24 10 ⁻⁸	2.13 10 ⁻⁶	3.79 10 ⁻¹	1.22 10 ⁻¹
to urban air	1.94 10 ⁻⁸	5.14 10 ⁻⁷	3.79 10 ⁻¹	1.22 10 ⁻¹
to continental rural air	3.93 10 ⁻⁹	1.70 10 ⁻⁷	3.79 10 ⁻¹	1.22 10 ⁻¹
to continental freshwater	8.85 10 ⁻⁹	1.57 10 ⁻⁶	1.35 10 ¹	1.51 10 ¹
to continental sea water	2.46 10 ⁻¹⁰	3.98 10 ⁻⁸	2.28 10 ⁻²	2.33 10 ⁻²
to continental natural soil	3.89 10 ⁻⁹	2.33 10 ⁻⁷	3.60 10 ⁰	1.17 10 ⁰
to continental agricultural soil	1.59 10 ⁻⁸	1.58 10 ⁻⁶	3.60 10 ⁰	1.17 10 ⁰
DAMAGE LEVEL CHARACTERIZATION FACTORS				
Type of emission	Human toxicity [DALY/kg emitted]		Freshwater aq. ecotoxicity [PDF·m ³ ·day/kg emitted]	
	DMC	DCE	DMC	DCE
to household indoor air	7.35 10 ⁻⁶	7.56 10 ⁻⁴	1.90 10 ⁻¹	6.08 10 ⁻²
to industrial indoor air	2.50 10 ⁻⁷	2.67 10 ⁻⁵	1.90 10 ⁻¹	6.08 10 ⁻²
to urban air	5.25 10 ⁻⁸	6.43 10 ⁻⁶	1.90 10 ⁻¹	6.08 10 ⁻²
to continental rural air	1.06 10 ⁻⁸	2.13 10 ⁻⁶	1.90 10 ⁻¹	6.08 10 ⁻²
to continental freshwater	2.39 10 ⁻⁸	1.96 10 ⁻⁵	6.73 10 ⁰	7.55 10 ⁰
to continental sea water	6.65 10 ⁻¹⁰	4.97 10 ⁻⁷	1.14 10 ⁻²	1.16 10 ⁻²
to continental natural soil	1.05 10 ⁻⁸	2.91 10 ⁻⁶	1.80 10 ⁰	5.86 10 ⁻¹
to continental agricultural soil	4.29 10 ⁻⁸	1.98 10 ⁻⁵	1.80 10 ⁰	5.86 10 ⁻¹

Table 7. Chemical analyses on solid waste from PHB extraction from microbial cells.

PARAMETERS	Unit	Value
C	%	47.6
H	%	9.6
N	%	5.2
S	%	0.1
Cl	%	0.03
DMC	mg/kg	0.4
Be	mg/kg	<1
Al	mg/kg	180.9
V	mg/kg	1.67
Cr	mg/kg	5.3
Mn	mg/kg	144.4
Fe	mg/kg	1092.8
Co	mg/kg	36.5
Ni	mg/kg	2.12
Cu	mg/kg	95.5
Zn	mg/kg	496
As	mg/kg	<1
Ag	mg/kg	4.1
Cd	mg/kg	<0,5
Sb	mg/kg	<1
Ba	mg/kg	41.6
Tl	mg/kg	<1
Pb	mg/kg	5.2
Hg	mg/kg	<0,2
Sn	mg/kg	1.32
HHV	J/g	16,455
LHV	J/g	14,400

Table 8 LCIA scores related to ‘new extraction process’ scenarios: extraction from microbial slurry + evaporation of the solvent (Slurry-Evap), extraction from dried biomass + evaporation of the solvent (Dry-Evap), extraction from microbial slurry + addition of EtOH and precipitation (Slurry-Precip), extraction from dried biomass + addition of EtOH and precipitation (Dry-Precip). Reference scenario is extraction with 1,2-dichloroethane (DCE). FU is 1 kg of PHB ready for the product’s manufacturing.

Impact category	Unit	Slurry-Evap	Dry-Evap	Slurry-Precip	Dry-Precip	DCE
GWP	kg CO ₂ eq.	7.0 10 ⁰	3.9 10 ⁰	1.1 10 ¹	7.9 10 ⁰	6.3 10 ¹
OD	kg CFC-11eq.	2.5 10 ⁻⁷	6.4 10 ⁻⁹	2.9 10 ⁻⁷	4.8 10 ⁻⁸	1.6 10 ⁻⁶
HTc	CTUh	2.6 10 ⁻⁸	2.0 10 ⁻⁹	3.8 10 ⁻⁸	1.5 10 ⁻⁸	1.7 10 ⁻⁷
HTnc	CTUh	8.5 10 ⁻⁸	2.5 10 ⁻⁸	1.2 10 ⁻⁷	6.6 10 ⁻⁸	9.8 10 ⁻⁷
FE	CTUe	1.8 10 ⁰	8.4 10 ⁻²	2.8 10 ⁰	1.1 10 ⁰	1.5 10 ¹
AC	Mole of H ⁺ eq.	9.7 10 ⁻³	4.9 10 ⁻³	1.6 10 ⁻²	1.1 10 ⁻²	9.2 10 ⁻²
POF	kg NMVOC	1.0 10 ⁻²	4.5 10 ⁻³	2.4 10 ⁻²	1.6 10 ⁻²	8.6 10 ⁻²
EuF	kg P eq.	1.4 10 ⁻⁴	4.7 10 ⁻⁶	2.6 10 ⁻⁴	1.3 10 ⁻⁴	1.1 10 ⁻³
EuM	kg N eq.	1.3 10 ⁻⁴	4.7 10 ⁻⁵	1.9 10 ⁻⁴	1.1 10 ⁻⁴	9.8 10 ⁻⁴
EuT	Mole of N eq.	2.8 10 ⁻²	1.5 10 ⁻²	4.3 10 ⁻²	3.0 10 ⁻²	2.5 10 ⁻¹
PM	kg PM _{2.5} eq.	3.9 10 ⁻⁴	1.9 10 ⁻⁴	6.6 10 ⁻⁴	4.7 10 ⁻⁴	3.9 10 ⁻³
IR	kBq U235eq.	5.1 10 ¹	1.3 10 ⁰	6.4 10 ¹	1.6 10 ¹	4.5 10 ²
RD	kg Sb eq.	1.6 10 ⁰	1.0 10 ⁰	3.1 10 ⁰	2.6 10 ⁰	2.0 10 ¹
WD	m ³ eq.	1.0 10 ⁻⁵	1.6 10 ⁻⁶	1.4 10 ⁻⁵	6.0 10 ⁻⁶	6.4 10 ⁻⁵

Annex A

Scenario 1 (Slurry-Evap)

Step	Process	Input/Output	Flow	Quantity	U.M.	Note
Biomass preparation	Centrifuge 1	Input	Pure microbial culture	$1.4 \cdot 10^2$	kg	from cultivation phase
			Electricity	$3.7 \cdot 10^1$	MJ	from electricity grid mix
		Output	Concentrated wet biomass	$1.6 \cdot 10^1$	kg	to batch reactor
Water	$1.3 \cdot 10^2$		kg	reusable for a successive cultivation		
Chemicals production			DMC new	2.0	kg	from production plant
PHB extraction	Batch reactor	Input	Concentrated wet biomass	$1.6 \cdot 10^1$	kg	from centrifuge 1
			DMC recovered	$2.8 \cdot 10^1$	kg	from condenser 1 and condenser 2
			Electricity	$1.6 \cdot 10^1$	MJ	from electricity grid mix
			Steam	7.8	MJ	from natural gas
		Output	Biomass-DMC mixture	$4.6 \cdot 10^1$	kg	to centrifuge 2
PHB extraction	Centrifuge 2	Input	Biomass-DMC mixture	$4.6 \cdot 10^1$	kg	from batch reactor
			Electricity	$1.1 \cdot 10^1$	MJ	from electricity grid mix
		Output	PHB-DMC solution	$2.9 \cdot 10^1$	kg	to air dryer 2
			Residual biomass-DMC mixture	$8.3 \cdot 10^1$	kg	to air dryer 1
Chemicals recovery	Air dryer 1	Input	Water	$1.6 \cdot 10^1$	kg	reusable for a successive cultivation
			Residual biomass-DMC mixture	$8.3 \cdot 10^1$	kg	from centrifuge 2
			Electricity	$2.2 \cdot 10^2$	MJ	from electricity grid mix
		Output	Steam	$2.0 \cdot 10^1$	MJ	from natural gas
Chemicals recovery	Condenser 1	Input	Residual biomass	$4.3 \cdot 10^1$	kg	to waste inceneration
			DMC	$4.0 \cdot 10^1$	kg	to condenser 1
		Output	DMC recovered	$4.0 \cdot 10^1$	kg	to batch reactor
PHB extraction	Air dryer 2	Input	DMC purge	$6.3 \cdot 10^{-4}$	kg	to catalytic oxydizer
			PHB-DMC solution	$2.9 \cdot 10^1$	kg	from centrifuge 2
			Electricity	1.6	MJ	from electricity grid mix
		Output	Steam	$1.4 \cdot 10^1$	MJ	from natural gas
			PHB	1.0	kg	ready for the product's manufacturing
Chemicals recovery	Condenser 2	Input	DMC	$2.8 \cdot 10^1$	kg	from air dryer 2
			Electricity	$6.8 \cdot 10^1$	MJ	from electricity grid mix
		Output	DMC recovered	$2.8 \cdot 10^1$	kg	to batch reactor
			DMC purge	$4.5 \cdot 10^{-2}$	kg	to catalytic oxydizer
Catalytic oxidizer	Catalytic oxidizer	Input	DMC purge	$4.5 \cdot 10^{-2}$	kg	from condenser 1 and condenser 2
			Output	DMC emission	$3.9 \cdot 10^{-4}$	kg
		Output	CO ₂	$6.6 \cdot 10^{-2}$	kg	emission to air
			Water vapour	$2.7 \cdot 10^{-2}$	kg	emission to air
			NO _x	$2.8 \cdot 10^{-5}$	kg	emission to air

Annex B

Scenario 2 (Dry-Evap)

Step	Process	Input/Output	Flow	Quantity	U.M.	Note	
Biomass preparation	Centrifuge 1	Input	Pure microbial culture	$1.5 \cdot 10^2$	kg	from cultivation phase	
			Electricity	$3.8 \cdot 10^1$	MJ	from electricity grid mix	
		Output	Concentrated wet biomass	8.9	kg	to batch reactor	
			Water	$1.4 \cdot 10^2$	kg	reusable for a successive cultivation	
Biomass preparation	Air dryer 1	Input	Concentrated wet biomass	8.9	kg	from centrifuge 1	
			Electricity	1.9	MJ	from electricity grid mix	
			Steam	$1.7 \cdot 10^1$	MJ	from natural gas	
		Output	Dried biomass	1.5	kg	to batch reactor	
Water vapour	7.5		kg	emission to air			
Chemicals production	Batch reactor	Input	DMC new	$5.1 \cdot 10^2$	kg	from production plant	
PHB extraction			Dried biomass	1.5	kg	from air dryer 1	
			DMC recovered	$3.2 \cdot 10^1$	kg	from condenser 1 and condenser 2	
			Electricity	$1.1 \cdot 10^1$	MJ	from electricity grid mix	
		Steam	4.0	MJ	from natural gas		
PHB extraction		Centrifuge 2	Output	Biomass-DMC mixture	$3.3 \cdot 10^1$	kg	to centrifuge 2
				Biomass-DMC mixture	$3.3 \cdot 10^1$	kg	from batch reactor
			Input	Electricity	$7.9 \cdot 10^2$	MJ	from electricity grid mix
	PHB-DMC solution			$3.2 \cdot 10^1$	kg	to air dryer 3	
Chemicals recovery	Air dryer 2	Input	Residual biomass-DMC mixture	$9.5 \cdot 10^1$	kg	to air dryer 2	
			Electricity	$2.6 \cdot 10^2$	MJ	from electricity grid mix	
			Steam	$2.3 \cdot 10^1$	MJ	from natural gas	
		Output	Residual biomass	$4.9 \cdot 10^1$	kg	to waste incineration	
DMC	$4.6 \cdot 10^1$		kg	to condenser 1			
Chemicals recovery	Condenser 1	Input	DMC	$4.6 \cdot 10^1$	kg	from air dryer 2	
			Electricity	$1.1 \cdot 10^2$	MJ	from electricity grid mix	
		Output	DMC recovered	$4.6 \cdot 10^1$	kg	to batch reactor	
			DMC purge	$7.3 \cdot 10^4$	kg	to catalytic oxidizer	
PHB extraction	Air dryer 3	Input	PHB-DMC solution	$3.2 \cdot 10^1$	kg	from centrifuge 2	
			Electricity	1.8	MJ	from electricity grid mix	
			Steam	$1.6 \cdot 10^1$	MJ	from natural gas	
		Output	PHB	1.0	kg	ready for the product's manufacturing	
DMC	$3.1 \cdot 10^1$		kg	to condenser 2			
Chemicals recovery	Condenser 2	Input	DMC	$3.1 \cdot 10^1$	kg	from air dryer 3	
			Electricity	$7.5 \cdot 10^1$	MJ	from electricity grid mix	
		Output	DMC recovered	$3.1 \cdot 10^1$	kg	to batch reactor	
			DMC purge	$5.0 \cdot 10^2$	kg	to catalytic oxidizer	
Catalytic oxidizer	Catalytic oxidizer	Input	DMC purge	$5.1 \cdot 10^2$	kg	from condenser 1 and condenser 2	
			Output	DMC emission	$2.7 \cdot 10^4$	kg	emission to air
		CO ₂		$7.4 \cdot 10^2$	kg	emission to air	
		Water vapour	$3.0 \cdot 10^2$	kg	emission to air		
NO _x	$3.2 \cdot 10^5$	kg	emission to air				

Annex C

Scenario 3 (Slurry-Precip)

Step	Process	Input/Output	Flow	Quantity	U.M.	Note
Biomass preparation	Centrifuge 1	Input	Pure microbial culture	$1.4 \cdot 10^2$	kg	from cultivation phase
			Electricity	$3.7 \cdot 10^{-1}$	MJ	from electricity grid mix
		Output	Concentrated wet biomass	$1.6 \cdot 10^1$	kg	to batch reactor
Water	$1.3 \cdot 10^2$		kg	reusable for a successive cultivation		
Chemicals production	Batch reactor 1	Input	DMC new	2.3	kg	from production plant
PHB extraction			Concentrated wet biomass	$1.6 \cdot 10^1$	kg	from centrifuge 1
			DMC recovered	$2.8 \cdot 10^1$	kg	from condenser 1 and pervaporation
			Electricity	$1.6 \cdot 10^{-1}$	MJ	from electricity grid mix
		Steam	7.8	MJ	from natural gas	
		Output	Biomass-DMC mixture	$4.6 \cdot 10^1$	kg	to centrifuge 2
PHB extraction	Centrifuge 2	Input	Biomass-DMC mixture	$4.6 \cdot 10^1$	kg	from batch reactor 1
			Electricity	$1.1 \cdot 10^{-1}$	MJ	from electricity grid mix
		Output	PHB-DMC solution	$2.9 \cdot 10^1$	kg	to batch reactor 2
			Residual biomass-DMC mixture	$8.3 \cdot 10^{-1}$	kg	to air dryer 1
Chemicals recovery	Air dryer 1	Input	Water	$1.6 \cdot 10^1$	kg	reusable for a successive cultivation
			Residual biomass-DMC mixture	$8.3 \cdot 10^{-1}$	kg	from centrifuge 2
			Electricity	$2.2 \cdot 10^{-2}$	MJ	from electricity grid mix
		Output	Steam	$2.0 \cdot 10^{-1}$	MJ	from natural gas
Residual biomass	$4.3 \cdot 10^{-1}$		kg	to waste incineration		
Chemicals recovery	Condenser 1	Input	DMC	$4.0 \cdot 10^{-1}$	kg	to condenser 1
			DMC recovered	$4.0 \cdot 10^{-1}$	kg	from air dryer 1
		Output	DMC purge	$6.3 \cdot 10^{-4}$	kg	to catalytic oxidizer
Chemicals production	Batch reactor 2	Input	EtOH new	$2.2 \cdot 10^{-1}$	kg	from production plant
			PHB-DMC solution	$2.9 \cdot 10^1$	kg	from centrifuge 2
			EtOH recovered	$2.2 \cdot 10^1$	kg	from pervaporation
			Electricity	$2.0 \cdot 10^{-1}$	MJ	from electricity grid mix
		Output	PHB-DMC-EtOH mixture	$5.1 \cdot 10^1$	kg	to centrifuge 3
PHB extraction	Centrifuge 3	Input	PHB-DMC-EtOH mixture	$5.1 \cdot 10^1$	kg	from batch reactor 2
			Electricity	$1.4 \cdot 10^{-1}$	MJ	from electricity grid mix
		Output	PHB-DMC-EtOH concentrated mixture	2.0	kg	to air dryer 2
			DMC-EtOH solution	$4.9 \cdot 10^1$	kg	to pervaporation
PHB extraction	Air dryer 2	Input	PHB-DMC-EtOH concentrated mixture	2.0	kg	from centrifuge 3
			Electricity	$9.8 \cdot 10^{-2}$	MJ	from electricity grid mix
			Steam	$8.8 \cdot 10^{-1}$	MJ	from natural gas
		Output	PHB	1.0	kg	ready for the product's manufacturing
DMC-EtOH solution	1.0		kg	to condenser 2		
Chemicals recovery	Condenser 2	Input	DMC-EtOH solution	1.0	kg	from air dryer 2
			Electricity	$4.2 \cdot 10^{-2}$	MJ	from electricity grid mix
		Output	DMC-EtOH solution recovered	1.0	kg	to pervaporation
			DMC purge	$2.2 \cdot 10^{-3}$	kg	to catalytic oxidizer
Chemicals recovery	Pervaporation	Input	EtOH purge	$1.7 \cdot 10^{-3}$	kg	to catalytic oxidizer
			DMC-EtOH solution	$5.0 \cdot 10^1$	kg	from centrifuge 3 and condenser 2
			Electricity	$1.0 \cdot 10^1$	MJ	from electricity grid mix
		Output	Steam	$2.7 \cdot 10^1$	MJ	from natural gas
			DMC recovered	$2.8 \cdot 10^1$	kg	to batch reactor 1
			EtOH recovered	$2.2 \cdot 10^1$	kg	to batch reactor 2
			DMC purge	$2.8 \cdot 10^{-1}$	kg	to catalytic oxidizer
Catalytic oxidizer	Catalytic oxidizer	Input	EtOH purge	$2.2 \cdot 10^{-1}$	kg	to catalytic oxidizer
			DMC purge	$2.8 \cdot 10^{-1}$	kg	from condensers 1-2 and pervaporation
		Output	DMC emission	$1.6 \cdot 10^{-2}$	kg	emission to air
			EtOH emission	$1.2 \cdot 10^{-2}$	kg	emission to air
			CO ₂	$8.0 \cdot 10^{-1}$	kg	emission to air
Catalytic oxidizer	Catalytic oxidizer	Output	Water vapour	$4.1 \cdot 10^{-1}$	kg	emission to air
			NO _x	$3.0 \cdot 10^{-4}$	kg	emission to air

Annex D

Scenario 4 (Dry-Precip)

Step	Process	Input/Output	Flow	Quantity	U.M.	Note
Biomass preparation	Centrifuge 1	Input	Pure microbial culture	$1.5 \cdot 10^2$	kg	from cultivation phase
			Electricity	$3.8 \cdot 10^{-1}$	MJ	from electricity grid mix
		Output	Concentrated wet biomass	8.9	kg	to batch reactor
Water	$1.4 \cdot 10^2$		kg	reusable for a successive cultivation		
Biomass preparation	Air dryer 1	Input	Concentrated wet biomass	8.9	kg	from centrifuge 1
			Electricity	1.9	MJ	from electricity grid mix
			Steam	$1.7 \cdot 10^1$	MJ	from natural gas
		Output	Dried biomass	1.5	kg	to batch reactor
Water vapour	7.5		kg	emission to air		
Chemicals production			DMC new	$3.2 \cdot 10^{-1}$	kg	from production plant
PHB extraction	Batch reactor 1	Input	Dried biomass	1.5	kg	from air dryer 1
			DMC recovered	$3.2 \cdot 10^1$	kg	from condenser 1 and pervaporation
			Electricity	$1.1 \cdot 10^{-1}$	MJ	from electricity grid mix
			Steam	4.0	MJ	from natural gas
		Output	Biomass-DMC mixture	$3.3 \cdot 10^1$	kg	to centrifuge 2
PHB extraction	Centrifuge 2	Input	Biomass-DMC mixture	$3.3 \cdot 10^1$	kg	from batch reactor 1
			Electricity	$7.9 \cdot 10^{-2}$	MJ	from electricity grid mix
		Output	PHB-DMC solution	$3.2 \cdot 10^1$	kg	to reactor batch 2
Residual biomass-DMC mixture	$9.5 \cdot 10^{-1}$		kg	to air dryer 2		
Chemicals recovery	Air dryer 2	Input	Residual biomass-DMC mixture	$9.5 \cdot 10^{-1}$	kg	from centrifuge 2
			Electricity	$2.6 \cdot 10^{-2}$	MJ	from electricity grid mix
			Steam	$2.3 \cdot 10^{-1}$	MJ	from natural gas
		Output	Residual biomass	$4.9 \cdot 10^{-1}$	kg	to waste incineration
DMC	$4.6 \cdot 10^{-1}$		kg	to condenser 1		
Chemicals recovery	Condenser 1	Input	DMC	$4.6 \cdot 10^{-1}$	kg	from air dryer 2
			Electricity	$1.1 \cdot 10^{-2}$	MJ	from electricity grid mix
		Output	DMC recovered	$4.6 \cdot 10^{-1}$	kg	to batch reactor 1
DMC purge	$7.3 \cdot 10^{-4}$		kg	to catalytic oxidizer		
Chemicals production			EtOH new	$2.3 \cdot 10^{-1}$	kg	from production plant
PHB extraction	Batch reactor 2	Input	PHB-DMC solution	$3.2 \cdot 10^1$	kg	from centrifuge 2
			EtOH recovered	$2.3 \cdot 10^1$	kg	from pervaporation
			Electricity	$2.2 \cdot 10^{-1}$	MJ	from electricity grid mix
		Output	PHB-DMC-EtOH mixture	$5.6 \cdot 10^1$	kg	to centrifuge 3
PHB extraction	Centrifuge 3	Input	PHB-DMC-EtOH mixture	$5.6 \cdot 10^1$	kg	from batch reactor 2
			Electricity	$1.5 \cdot 10^{-1}$	MJ	from electricity grid mix
		Output	PHB-DMC-EtOH concentrated mixture	2.0	kg	to air dryer 3
DMC+EtOH solution	$5.4 \cdot 10^1$		kg	to pervaporation		
PHB extraction	Air dryer 3	Input	PHB-DMC-EtOH concentrated mixture	2.0	kg	from centrifuge 3
			Electricity	$9.7 \cdot 10^{-2}$	MJ	from electricity grid mix
			Steam	$8.6 \cdot 10^{-1}$	MJ	from natural gas
		Output	PHB	1.0	kg	ready for the product's manufacturing
DMC-EtOH solution	1.0		kg	to condenser 2		
Chemicals recovery	Condenser 2	Input	DMC-EtOH solution	1.0	kg	from air dryer 3
			Electricity	1.0	MJ	from electricity grid mix
			DMC-EtOH solution recovered	1.0	kg	to pervaporation
		Output	DMC purge	$2.2 \cdot 10^{-3}$	kg	to catalytic oxidizer
EtOH purge	$1.6 \cdot 10^{-3}$		kg	to catalytic oxidizer		
Chemicals recovery	Pervaporation	Input	DMC-EtOH solution	$5.5 \cdot 10^1$	kg	from centrifuge 3 and condenser 2
			Electricity	$1.1 \cdot 10^1$	MJ	from electricity grid mix
			Steam	$2.8 \cdot 10^1$	MJ	from natural gas
		Output	DMC recovered	$3.1 \cdot 10^1$	kg	to batch reactor 1
			EtOH recovered	$2.3 \cdot 10^1$	kg	to batch reactor 2
			DMC purge	$3.1 \cdot 10^{-1}$	kg	to catalytic oxidizer
Catalytic oxidizer	Catalytic oxidizer	Input	EtOH purge	$2.3 \cdot 10^{-1}$	kg	to catalytic oxidizer
			DMC purge	$3.2 \cdot 10^{-1}$	kg	from condensers 1-2 and pervaporation
			EtOH purge	$2.3 \cdot 10^{-1}$	kg	from condenser 2 and pervaporation
			DMC emission	$1.1 \cdot 10^{-2}$	kg	emission to air
		Output	EtOH emission	$8.1 \cdot 10^{-3}$	kg	emission to air
			CO ₂	$8.8 \cdot 10^{-1}$	kg	emission to air
			Water vapour	$4.5 \cdot 10^{-1}$	kg	emission to air
			NO _x	$3.3 \cdot 10^{-4}$	kg	emission to air

Annex E

Scenario 5 (DCE)

Step	Process	Input/Output	Flow	Quantity	U.M.	Note
Biomass preparation	Centrifuge 1	Input	Pure microbial culture	1.5 10 ²	kg	from cultivation phase
			Electricity	3.9 10 ⁻¹	MJ	from electricity grid mix
		Output	Concentrated wet biomass	9.1	kg	to batch reactor
Water	1.4 10 ²		kg	reusable for a successive cultivation		
Biomass preparation	Air dryer 1	Input	Concentrated wet biomass	9.1	kg	from centrifuge 1
			Electricity	1.9	MJ	from electricity grid mix
		Output	Steam	1.7 10 ¹	MJ	from natural gas
			Dried biomass	1.5	kg	to batch reactor
Chemicals production	Batch reactor 1	Input	Water vapour	7.6	kg	emission to air
			DCE new	9.5 10 ⁻¹	kg	from production plant
			Dried biomass	1.5	kg	from air dryer 1
			DCE recovered	9.4 10 ¹	kg	from condenser 1 and pervaporation
			Electricity	7.0 10 ²	MJ	from electricity grid mix
PHB extraction	Centrifuge 2	Output	Steam	7.3	MJ	from natural gas
			Biomass-DCE mixture	9.7 10 ¹	kg	to centrifuge 2
PHB extraction	Centrifuge 2	Input	Biomass-DCE mixture	9.7 10 ¹	kg	from batch reactor 1
			Electricity	2.0 10 ⁻¹	MJ	from electricity grid mix
		Output	PHB-DCE solution	9.5 10 ¹	kg	to reactor batch 2
Residual biomass-DCE mixture	1.0		kg	to air dryer 2		
Chemicals recovery	Air dryer 2	Input	Residual biomass-DCE mixture	1.0	kg	from centrifuge 2
			Electricity	2.5 10 ⁻²	MJ	from electricity grid mix
		Output	Steam	2.2 10 ⁻¹	MJ	from natural gas
			Residual biomass	5.2 10 ⁻¹	kg	to waste incineration
Chemicals recovery	Condenser 1	Input	DCE	5.1 10 ⁻¹	kg	to condenser 1
			Electricity	1.1 10 ⁻²	MJ	from electricity grid mix
		Output	DCE recovered	5.1 10 ⁻¹	kg	to batch reactor 1
DCE purge	2.5 10 ⁻³		kg	to catalytic oxidizer		
Chemicals production	Batch reactor 2	Input	Methanol-water solution new	3.1	kg	from production plant
			PHB-DCE solution	9.5 10 ¹	kg	from centrifuge 2
			Methanol-water solution recovered	3.1 10 ²	kg	from pervaporation
			Electricity	1.6	MJ	from electricity grid mix
PHB extraction	Centrifuge 3	Output	PHB-DCE-methanol-water mixture	4.1 10 ²	kg	to centrifuge 3
			PHB-DCE-methanol-water mixture	4.1 10 ²	kg	from batch reactor 2
PHB extraction	Air dryer 3	Input	Electricity	1.2	MJ	from electricity grid mix
			PHB-DCE-methanol-water concentrated mixture	2.0	kg	to air dryer 3
		Output	DCE-methanol-water solution	4.1 10 ²	kg	to pervaporation
PHB-DCE-methanol-water concentrated mixture	2.0		kg	from centrifuge 3		
Chemicals recovery	Condenser 2	Input	Electricity	2.4 10 ⁻¹	MJ	from electricity grid mix
			Steam	2.2	MJ	from natural gas
		Output	PHB	1.0	kg	ready for the product's manufacturing
			DCE-methanol-water solution	1.0	kg	to condenser 2
Chemicals recovery	Pervaporation	Input	DCE-methanol-water solution	1.0	kg	from air dryer 3
			Electricity	1.0 10 ⁻¹	MJ	from electricity grid mix
		Output	DCE-methanol-water solution recovered	9.9 10 ⁻¹	kg	to pervaporation
			DCE purge	1.7 10 ⁻³	kg	to catalytic oxidizer
			Methanol-water purge	5.7 10 ⁻³	kg	to catalytic oxidizer
Chemicals recovery	Catalytic oxidizer	Input	DCE-methanol-water solution	4.1 10 ²	kg	from centrifuge 3 and condenser 2
			Electricity	1.1 10 ²	MJ	from electricity grid mix
			Steam	5.0 10 ²	MJ	from natural gas
		Output	1,2-dichloroethane (DCE)	9.4 10 ¹	kg	to batch reactor 1
			Methanol-water solution	3.1 10 ²	kg	to batch reactor 2
			DCE purge	9.4 10 ⁻¹	kg	to catalytic oxidizer
Catalytic oxidizer	Catalytic oxidizer	Output	Methanol-water purge	3.1	kg	to catalytic oxidizer
			DCE purge	9.5 10 ⁻¹	kg	from condensers 1-2 and pervaporation
			Methanol-water purge	3.1	kg	from condenser 2 and pervaporation
			DCE emission	4.7 10 ⁻³	kg	emission to air
			Metanolo emission	1.2 10 ⁻²	kg	emission to air
			CO ₂	3.3	kg	emission to air
			Water vapour	3.4	kg	emission to air
NO _x	1.5 10 ⁻³	kg	emission to air			
Other emissions to air	N.R.	kg	emission to air (from EEA, 2013)			

N.R. = not reported

Annex F

ID	CHEMICALS	CAS NUMBER	ID	CHEMICALS	CAS NUMBER
1	1,1-dichloroethene	75-35-4	22	tetrachloroethylene	127-18-4
2	chloromethane	74-87-3	23	chlorobenzene	108-90-7
3	vinyl chloride	75-01-4	24	ethylbenzene	100-41-4
4	dichloromethane	75-09-2	25	meta- and para-xylene	N/A ^s
5	1,2-dichloroethene trans	156-60-5	26	bromoform	75-25-2
6	<i>tert</i> -butyl methyl ether	1634-04-4	27	styrene	100-42-5
7	1,1-dichloroethane	75-34-3	28	1,1,2,2-tetrachloroethane	79-34-5
8	1,2-dichloroethene cis	156-59-2	29	<i>o</i> -xylene	95-47-6
9	chloroform	67-66-3	30	1,2,3-trichloropropane	96-18-4
10	<i>tert</i> -butyl ethyl ether	637-92-3	31	isopropylbenzene	98-82-8
11	1,2-dichloroethane	107-06-2	32	2-chlorotoluene	95-49-8
12	1,1,1-trichloroethane	71-55-6	33	4-chlorotoluene	106-43-4
13	tetrachloromethane	56-23-5	34	1,3-dichlorobenzene	541-73-1
14	benzene	71-43-2	35	1,4-dichlorobenzene	106-46-7
15	1,2-dichloropropane	78-87-5	36	1,2-dichlorobenzene	95-50-1
16	1,1,2-trichloroethylene	79-01-6	37	nitrobenzene	98-95-3
17	bromodichloromethane	75-27-4	38	naphthalene	91-20-3
18	1,1,2-trichloroethane	79-00-5	39	1,2,4-trichlorobenzene	120-82-1
19	toluene	108-88-3	40	Hexachloro-1,3-butadiene	87-68-3
20	dibromochloromethane	124-48-1	41	2-methylnaphthalene	91-57-6
21	1,2-dibromoethane	106-93-4	42	1-methylnaphthalene	90-12-0

^s not applicable

1 **Figure captions**

2 Fig. 1: Process block diagram of each production scenario.

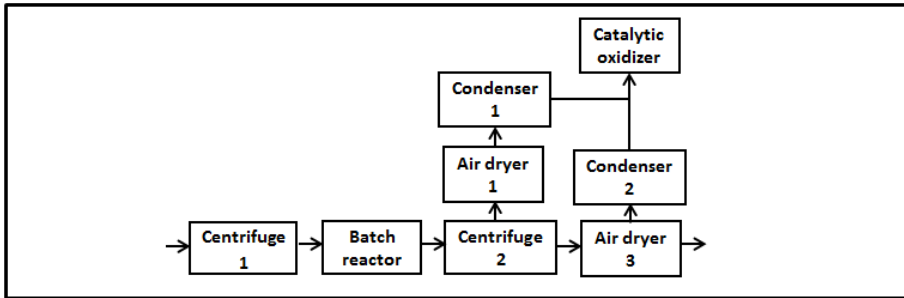
3
4
5
6 Fig. 2: Process contributions to GWP, AC, EuT, EuM and POF of each considered step: biomass
7 preparation, chemical production, PHB extraction, chemicals recovery, catalytic oxidation and
8 solid waste treatment (FU: 1 kg of PHB).

9
10
11
12 Fig. 3: Process contributions to PM, HTnc, RD and WD of each considered step: biomass
13 preparation, chemical production, PHB extraction, chemicals recovery, catalytic oxidation and
14 solid waste treatment (FU: 1 kg of PHB).

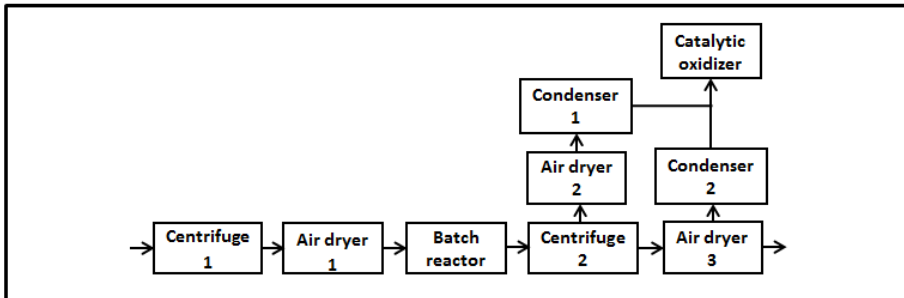
15
16
17
18 Fig. 4: Process contributions to EuF, FE, HTc, IR and OD of each considered step: biomass
19 preparation, chemical production, PHB extraction, chemicals recovery, catalytic oxidation and
20 solid waste treatment (FU: 1 kg of PHB).

Fig. 1

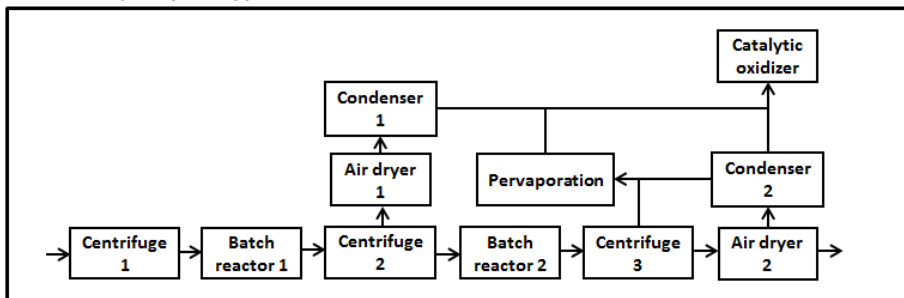
Scenario 1 (Slurry-Evap)



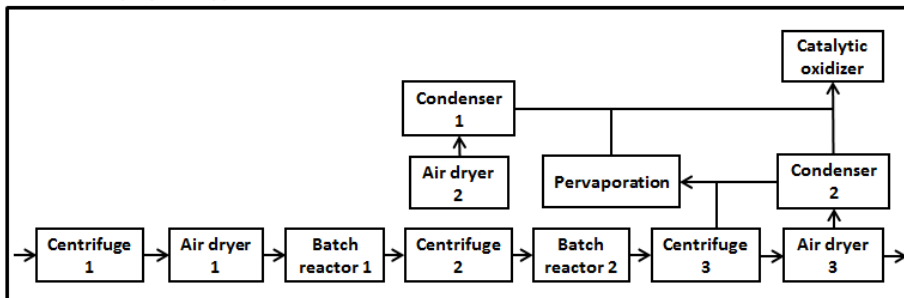
Scenario 2 (Dry-Evap)



Scenario 3 (Slurry-Precip)



Scenario 4 (Dry-Precip)



Scenario 5 (DCE)

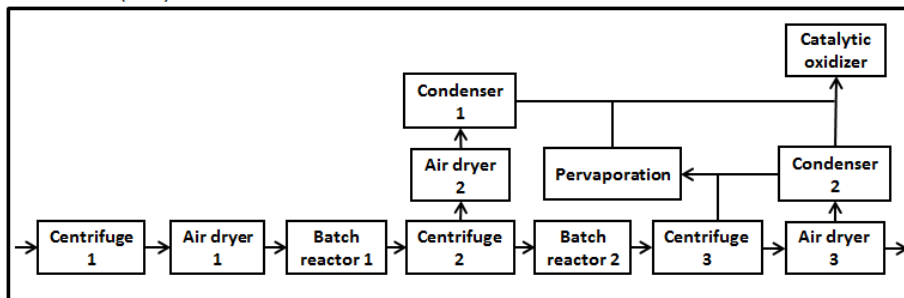


Fig. 2

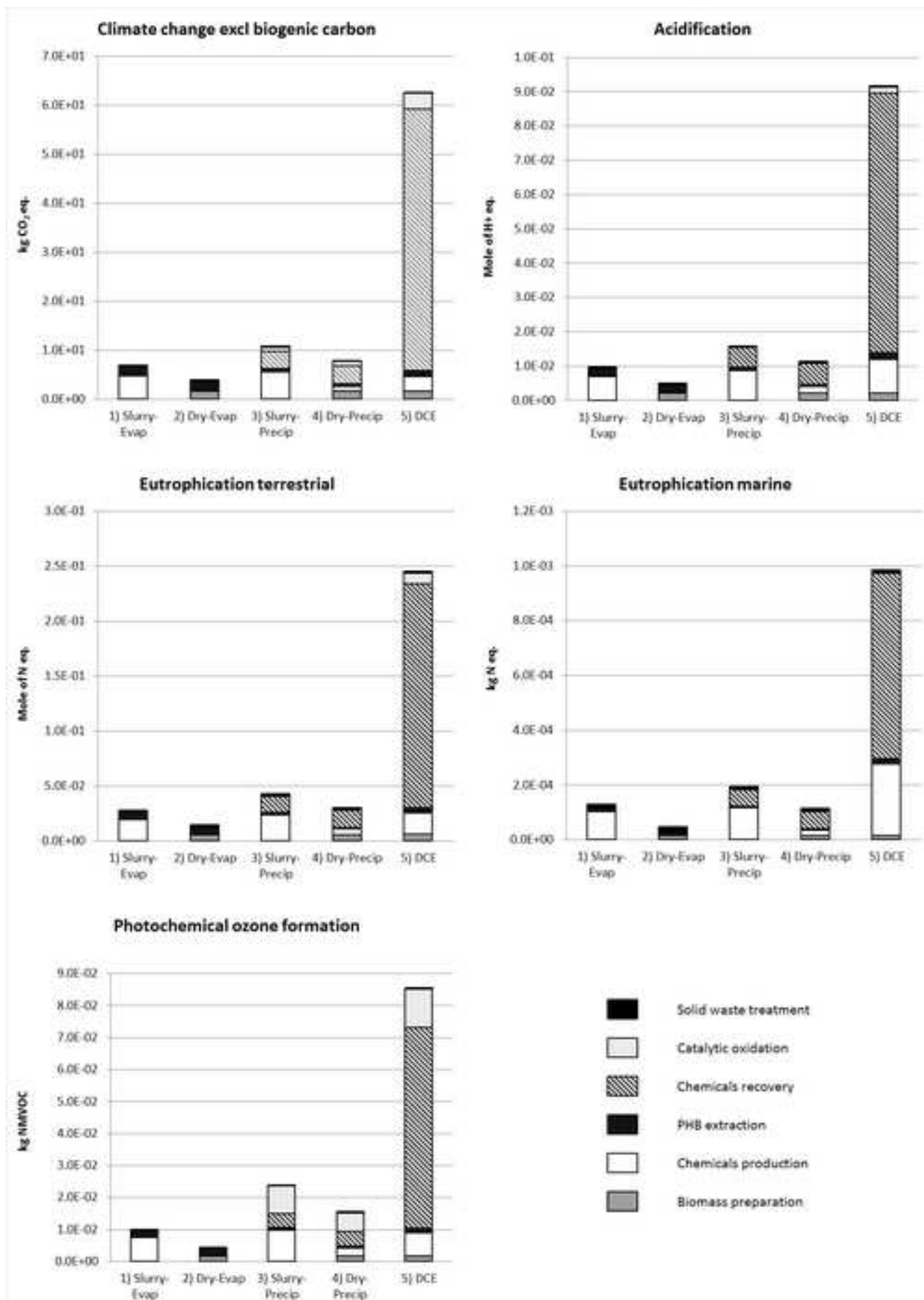


Fig. 3

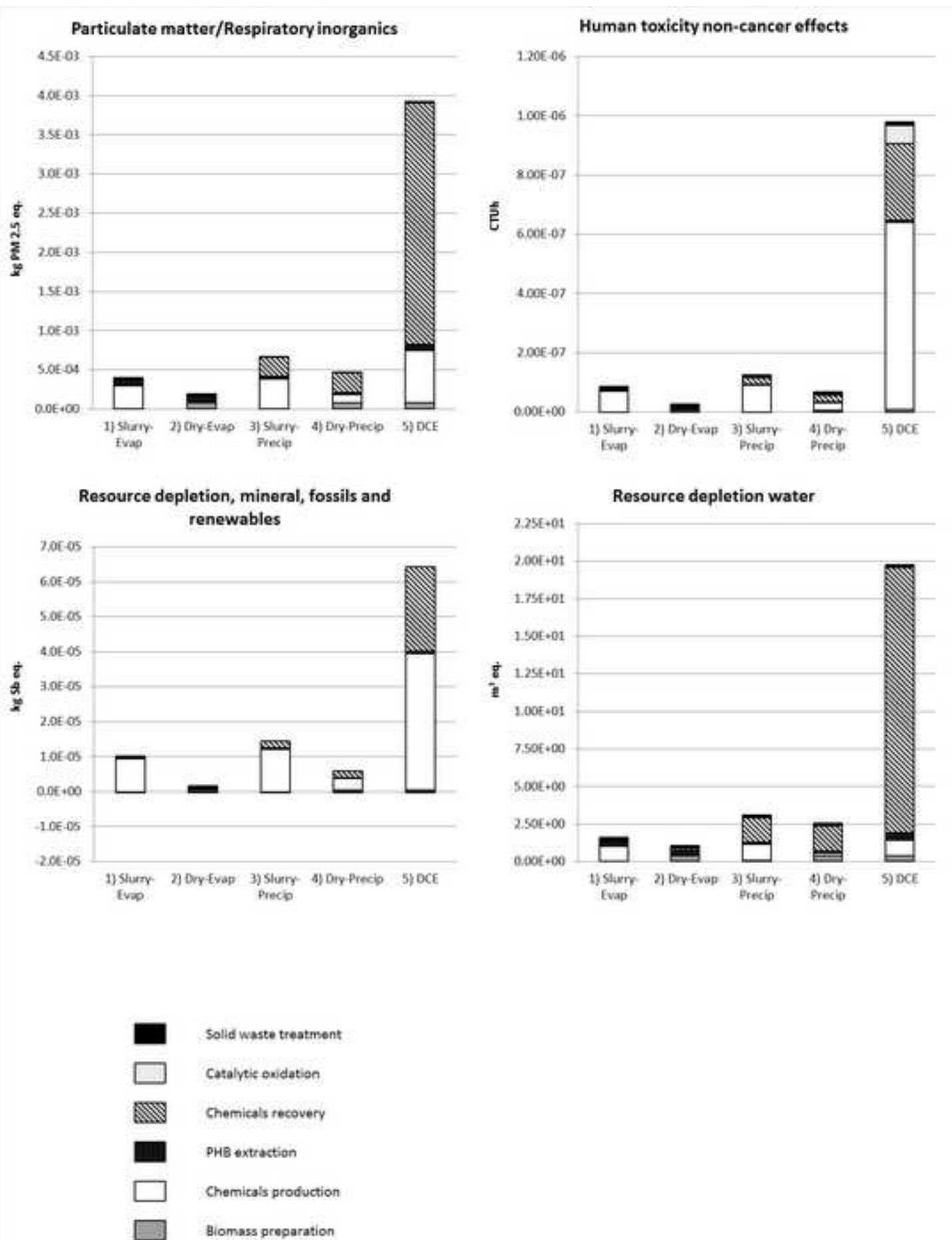


Fig. 4

