Biodegradability assessment of complex chemical mixtures using a carbon balance approach

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Abstract

The assessment of complex mixture biodegradability can be limited by technical issues and/or difficulties to rule on ready or inherent biodegradability. This work is composed of three different studies to introduce and improve Ultimately Transformed Organic Carbon (UTOC) as a quantification tool for biodegradation. The UTOC includes the inorganic carbon resulting from respiration and the carbon assimilated by microorganisms. The UTOC is correlated with dissolved organic carbon removal and can be a robust alternative for non-soluble substance evaluation. The UTOC was evaluated using a complex mixture of soluble and non-soluble substances for eventual verification with a UVCB (vegetal extract). The advantages of UTOC are clear; it is an appropriate method to quantify the initial raw material converted to an inert product by the action of microorganisms to determine the readily biodegradability of an unknown substance such as a vegetal extract.

Keywords:

Biodegradation

Ready biodegradability

UVCB substances

Non-soluble chemicals

Vegetal extract

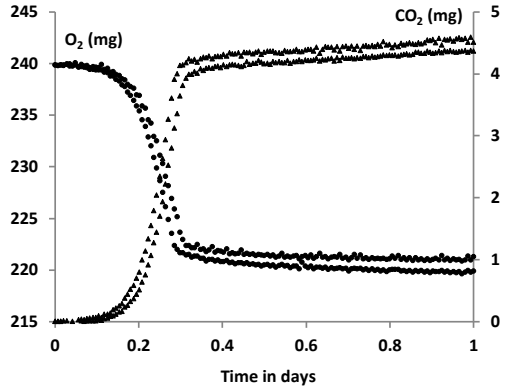
Supplementary-data-1.

Country	Regulation	A substance or chemical or chemical substance is…	A mixture is…	Considers UVCB
Japan	CSCL (Chemical Substance Control Law), 1973.	"... a chemical compound obtained by causing chemical reactions to elements or compounds (excluding a radioactive substance and the following substances): (i) Any specified poison prescribed in paragraph (3) of Article 2 of the Poisonous and Deleterious Substances Control Act (Act No. 303 of 1950) (ii) Any stimulant prescribed in paragraph (1) of Article 2 of the Stimulant Drug Control Act (Act No. 252 of 1951) and any raw material for stimulants prescribed in paragraph (5) of said Article (iii) Any narcotic prescribed in item (i) of Article 2 of the Narcotics and Psychotropics Control Act (Act No. 14 of 1953)."	Not defined	No
USA	TSCA (Toxic Substances Control Act), 1976.	"… any organic or inorganic substance of a particular molecular identity, including any combination of such substances occurring in whole or in part as a result of a chemical reaction or occurring in nature, and any chemical element or uncombined radical."	"… any combination of two or more chemical substances if the combination does not occur in nature and is not, in whole or in part, the result of a chemical reaction; except that "mixture" does include: (1) Any combination which occurs, in whole or in part, as a result of a chemical reaction if the combination could have been manufactured for commercial purposes without a chemical reaction at the time the chemical substances comprising the combination were combined and if, after the effective date of premanufacture notification requirements, none of the chemical substances comprising the combination is a new chemical substance, and (2) Hydrates of a chemical substance or hydrated ions formed by association of a chemical substance with water."	Yes
Australia	ICNA Act (Industrial Chemicals (Notification and Assessment) Act), 1989.	"… (a) a chemical element, including a chemical element contained in a mixture; or (b) a compound or complex of a chemical element, including such a compound or complex contained in a mixture; or (c) a UVCB substance; or (d) a naturally-occurring chemical; but does not include: (e) an article; or (f) a radioactive chemical; or (g) a mixture."	"… a physical combination of chemicals resulting from deliberate mixing of those chemicals or from a chemical reaction, but does not include a UVCB substance."	Yes
Korea	TCCA (Toxic Chemicals Control Act), 1991 AREC (Act on the Registration and Evaluation, etc. of Chemical Substances), 2013	"… elements, compounds and substances obtained by causing artificial reactions therewith and those obtained by extracting or refining substances existing in nature." "… an element, its compounds and substances obtained by artificial chemical reaction therewith, and substances obtained by chemical modification, extraction, purification of substances in their natural state."	Not defined "… a substance or solution composed of at least two substances."	No No
New Zealand	HSNO (Hazardous Substances and New Organisms) Act, 1996.	"… (a) any element, defined mixture of elements, compounds, or defined mixture of compounds, either naturally occurring or produced synthetically, or any mixtures thereof. (b) Any isotope, allotrope, isomer, congener, radical, or ion of an element or compound which has been declared by the Authority, by notice in the Gazette, to be a different substance from that element or compound. (c) Any mixtures or combinations of any of the above. (d) Any manufactured article containing, incorporating, or including any hazardous substance with explosive properties."	Not defined	No
Canada	CEPA (Canadian Environmental Protection Act), 1999.	"… any distinguishable kind of organic or inorganic matter, animate or inanimate, and includes (a) any matter that is capable of being dispersed in the environment or of being transformed in the environment into matter that is capable of being so dispersed or that is capable of causing such transformations in the environment; (b) any element or free radical; (c) any combination of elements of a particular molecular identity that occurs in nature or as a result of a chemical reaction; and (d) complex combinations of different molecules that originate in nature or are the result of chemical reactions but that could not practicably be formed by simply combining individual constituents"	"… a combination of substances that does not itself produce a substance that is different from the substances that were combined."	Yes
European Union	REACH (Registration, Evaluation, Authorization and Restriction of CHemicals), 2006.	"… a chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition."	"… a mixture or solution composed of two or more substances."	Yes

Supplementary-data-2. Definition of primary vs. ultimate biodegradation and readily vs. inherently biodegradable according to the OECD. Table adapted from ECETOC technical report n° 123 (2013).

Primary biodegradation (biotransformation)	**Alteration in the chemical structure of a substance, brought about by biological action, resulting in the loss of a specific property of that substance.**
Ultimate biodegradation (mineralization)	**The level of degradation achieved when the test compound is totally utilized by micro-organisms resulting in the production of carbon dioxide, water, mineral salts and new microbial cellular constituents (biomass).**
Readily biodegradable	**An arbitrary classification of chemicals that have passed certain specified screening tests for ultimate biodegradability; these tests are so stringent that it is assumed that such compounds will rapidly and completely biodegrade in aquatic environments under aerobic conditions.**
Inherently biodegradable	**A classification of chemicals for which there is unequivocal evidence of biodegradation (primary or ultimate) in any test of biodegradability.**

A



B

Sample		DOC	Min.	Biom.	Σ	R. (%)
NaB_1	T_{ini}	15,4	-	-	15,4	100
	T_{fin}	5,8	8,1	1,3	15,2	98
NaB_2	T_{ini}	14,9	-	-	14,9	100
	T_{fin}	5,5	6,3	2,3	14,1	94

Supplementary data Fig. 1 A: Quantity (mg) of oxygen consumption and carbon dioxide production in NaB duplicated bioreactors. Based on the similarities between duplicates and conjunctures regarding the oxygen consumption and carbon dioxide production, the respiration activity may have stopped after 0.4 day. The bioreactors were sampled after 1 day. The bioreactors containing 4-NP and DEG were stopped after 3 and 7 days, respectively. Note that for the mixture, the bioreactors were incubated for 28 days due to possible multiple kinetics phenomena due to the mixture. B: Quantity (mg) of initial (T_{ini}) and final (T_{fin}) DOC, Mineralized (Min.) carbon as carbon dioxide and converted to biomass (Biom.) carbon. Σ: sum, R: carbon recovery. Before inoculation, the DOC from the addition 30 $mgC.l^{-1}$ NaB to the culture media (0.514 l) should be 15.4 mg. The approximate values measured in the two assays were 15.4 and 14.9 mg, respectively. These quantities have been systematically weighted using the DOC naturally present in the mineral culture media (note that these quantities never exceeded 0.5 mg). Directly after bioreactor inoculation, the carbon from the microorganisms was measured for weighting of the final amount of carbon in this compartment. The inorganic carbon was measured in the bioreactor atmosphere (and considered negligible) and in the mineral culture media for weighting of the final values (note that these initial quantities never exceeded 0.5 mg). After 1 day, the DOC decreased from ≈ 15 to ≈ 5 mg and the mineralized gaseous and dissolved fractions equilibrated between the atmosphere (≈ 1.2 mg) and the culture media (≈ 6.9 and 5 mg for NaB1 and NaB2, respectively), for a sum of 8.1 (NaB1) and 6.3 mg (NaB2), as reported in the table in the "mineralization" column. If there is a difference in the measured inorganic fraction of carbon (NaB1 > NaB2), it appears to be balanced by an increased assimilation of carbon by biomass for the NaB2 assay (2.3 mg vs. 1.3 mg for NaB1). This leads to an equivalent quantity of organic carbon transformed during the biodegradation reaction. Considering biomass allows for adjustment of the differences that can exist with the sole measurement of mineralization and leads to an improved overview of UTOC during biodegradation. All values have been converted into percentages according to the initial quantity of organic carbon in each assay (15.4 and 14.9 mg). These data correspond to the bar graphs presented in Fig. 1A.