AGENCE NATIONALE DE LA RECHERCHE ANR	**Compte-rendu de fin de projet**	

Projet ANR- 16-CE34-0007-01

OXOMAR

Degradation, Biodegradation and toxicity of Oxo-biodegradable Plastics in the oceans

Programme PRC 2016

A IDENTIFICATION

Acronyme du projet	**OXOMAR**
Titre du projet	**Dégradation abiotique et biotique et toxicité des plastiques oxo-dégradables en mer**
Coordinateur du projet (société/organisme)	Ghiglione Jean-François, CNRS
Date de début du projet Date de fin du projet	**01/10/2016** **30/09/2019**
Labels et correspondants des pôles de compétitivité (pôle, nom et courriel du corresp.)	aucun
Site web du projet, le cas échéant	http://lomic.obs-banyuls.fr/fr/axe_4_ecotoxicologie_et_ingenierie_metabolique_microbienne/oxomar.html

Rédacteur de ce rapport	
Civilité, prénom, nom	Mr Ghiglione Jean-François
Téléphone	04 68 88 73 16
Courriel	ghiglione@obs-banyuls.fr
Date de rédaction	10/03/2021
Période faisant l'objet du rapport d'activité	01/10/2016 au 31/02/2021
Liste des partenaires présents à la fin du projet (société/organisme et responsable scientifique)	CNRS – LOMIC, J-F. Ghiglione CNRS – ICCF, A-M. Delort IFREMER – S. Stachowski CNEP – D. Fromageot SYMPHONY - M. Laurier

B RESUME CONSOLIDE PUBLIC

Ce résumé est destiné à être diffusé auprès d'un large public pour promouvoir les résultats du projet, il ne fera donc pas mention de résultats confidentiels et utilisera un vocabulaire adapté mais n'excluant pas les termes techniques. Il en sera fourni une version française et une version en anglais. Il est nécessaire de respecter les instructions ci-dessous.

B.1 RESUME CONSOLIDE PUBLIC EN FRANÇAIS

Contexte et objectif : Parmi les 300 000 tonnes de déchets plastiques restrouvés aujourd'hui à la surface des Océans, plus de la moitié sont composés de polyéthylène. La dégradation du polyéthylène (PE) par les microorganismes est très lente et même lorsqu'elle est couplée à la dégradation physico-chimique (abrasion, UV, vagues…), les durées de dégradation dépassent plusieurs décennies dans l'environnement marin. Une solution proposée par les industriels consiste à intégrer des additifs qui favorisent l'oxydation du PE pour le rendre plus accessible à la biodégradation: les oxo-biodégradables (OXO-bio). Le manque de connaissances sur ce type de produits a conduit à des mesures récentes d'interdiction de la commercialisation des oxo-dégradables (OXO) en Europe, les Oxo étant définit par la CEN TR15351 comme « associé à une dégradation associée à la coupure des macromolécules résultant de leur oxydation ».

Notre objectif était d'apporter des données scientifiques solides sur le devenir des OXO-bio en mer, les OXO-bio étant définit par le CEN TR15351 comme la « dégradation résultant de phénomènes liés à l'oxidation et à des phénomènes cellulaires ». La base du projet a reposé sur 3 axes pour évaluer (1) la dégradation abiotique, (2) la biodégradation et (3) la toxicité éventuelle des OXO lorsqu'ils sont rejettés accidentellement en mer.

Approches expérimentales : Ce projet ambitieux a reposé sur une approche pluridisciplinaire couplant la physique, la chimie et la biologie. Les tests ont été réalisés à partir de 5 films industriels à base de

polyéthylène basse densité (PE-BD) (film 1) additivé paér des mélanges de pro-oxydants à base de Cobalt (Co – film 2), manganèse (Mn – film 3), cobalt et manganèse (Co/Mn, film 4) et , manganèse et fer (Mn / Fe, film5) et en mélange avec un polymère biodégradable à base d'amidon (film 6).
Dans un premier temps, nous avons confronté les données de vieillissement des PE en milieu naturel par rapport à du vieillissement artificiel par l'action des ultraviolets (UV A et B) et de la température (incubateur SEPAP 12.24.H). Ce procédé permet d'accélérer artificiellement les processus de dégradation physico-chimique, qui prennent plusieurs décennies en milieu naturel.
Dans un second temps, nous avons testé la biodégradabilité des différents films OXO en utilisant soit une souche pure connue pour dégrader le PE (*Rhodococcus rhodochrous*), soit un inoculum bactérien complexe du milieu marin. La biodégradation a été caractérisée d'un point de vue :
-physique par microscopie à force atomique (AFM),
-chimique par Résonance Magnétique Nucléaire du proton (RMN 1H), Spectrométrie de Masse Haute Résolution couplée à de la Chromatographie Liquide (LC-HRMS) et Spectroscopie Infrarouge à Transformée de Fourier (FTIR)
-microbiologique par microscopie confocale et épifluoresence, cytométrie en flux, séquençage d'ADN haut débit, respirométrie (O_2 et CO_2)
-toxicologique par biotests réalisés à partir des lixiviats des différents OXO sur des embryons ou des larves de poissons (*Dicentrarchus labrax*), oursins (*Paracentrotus lividus*), huitres (*Crassotrea gigas*), ascidies (*Phallusia mammillata*), céphalochordés (*Branchiostoma lanceolatum*) et sur des microalgues (*Skeletonema marinoi*, *Chaetoceros calcitrans*, *Tetraselmis suecica*, *Emiliania huxleyi*).

Résultats majeurs :
Le vieillissement artificiel a montré un rôle crucial des mélanges pro-oxydants (Co, Mn et Fe) dans le devenir des films OXO dans l'environnement. L'efficacité de vieillissement était plus marquée sur les films contenant des pro-oxydants par rapport à l'OXO mélangé à un polymère biodégradable. Des signes évidents de biodégradabilité ont été observés pour l'OXO vieilli artificiellement dans des conditions équivalentes à une exposition naturelle d'un an au rayonnement solaire. La toxicité des lixiviats d'OXO a été considérée comme nulle à l'exception de l'utilisation de pro-oxydants à base de Co, soulignant ainsi l'importance de contrôler la composition des composés additifs pro-oxydants dans la formulation des OXO.

Production scientifique :
Le projet a permis de mettre en évidence la biodégradabilité des OXO à partir d'un vieillissement artificiel équivalent à une durée de vie d'environ 1 an. Les données recueillies serviront à rédiger une dizaine d'articles scientifiques dont 9 déjà publiés. Les résultats préliminaires ont été présentés lors de 13 conférences internationales et d'autres suivront au cours de la prochaine année.

Informations factuelles :
Le projet OXOMAR est un projet de recherche fondamentale coordonné par le CNRS LOMIC. Il associe les expertises des laboratoires publics CNRS-LOMIC et CNRS–ICCF, IFREMER-Nantes, public-privé et CNEP et un partenaire privé du Royaume-Uni SYMPHONY. Le projet a débuté en octobre 2016 et a terminé en mars 2022. Il a bénéficié d'une aide financière de l'ANR de 490 287 € pour un coût global de 1 990 616 €.

B.2 RESUME CONSOLIDE PUBLIC EN ANGLAIS

Context and objective: Of the 300,000 tonnes of plastic waste found today on the surface of the oceans, more than half are made of polyethylene.The degradation of polyethylene (PE) by microorganisms is very slow and even when it is coupled with physicochemical degradation (abrasion, UV, waves, etc.), degradation times exceed several decades in the marine environment. One solution proposed by manufacturers consists of integrating additives that promote the oxidation of PE to make it more accessible to biodegradation: oxo-biodegradable (OXO-bio). The lack of knowledge about this product has led to recent measures banning the marketing of oxo-degradable plastic (OXO) in Europe, OXO being defined by the CEN in TR15351 as “degradation identified as resulting from oxidative cleavage of macromolecules”. Our objective was to provide solid scientific data on the fate of OXO-bio at sea, OXO-biodegradation being defined by the CEN in TR15351 as “degradation resulting from oxidative and cell-mediated phenomena, either simultaneously or successively”. The basis of the project rested on 3 axes to assess (1) abiotic degradation, (2) biodegradation and (3) the possible toxicity of OXO-bio when thrown into the sea.

Experimental approaches: This ambitious project was based on a multidisciplinary approach coupling physics, chemistry and biology. The tests were carried out using 5 industrial films based on low density polyethylene (LDPE) (film 1) additivated or not by pro-oxidants based on Cobalt (Co - film 2); Manganese (Mn - film 3); Co/Mn (film 4); and Mn/Fe (film 5); and mixed with a biobased blend-based polymer (film 6).

We first compared the data on the aging of LDPE in a natural environment, compared to artificial aging, by the action of ultraviolet (UV A and B) and temperature (SEPAP incubator 12.24.H). This process makes it possible to artificially accelerate the physico-chemical degradation processes, which would take several decades in the natural environment in the case of ordinary polymers.

Secondly, we tested the biodegradability of the various OXO-bio films using either a pure strain known to degrade PE (*Rhodococcus rhodochrous*), or a complex bacterial inoculum from the marine environment. Biodegradation has been characterized from the point of view of:
-physics: Atomic Force Microscopy (AFM),
-chemistry : nuclear magnetic resonance (1H NMR), High Resolution Mass Spectrometry coupled with Liquid Chromatography (LC-HRMS) and Fourier Transform Infrared Spectroscopy (FTIR)
-microbiology: confocal and epifluoresence microscopy, flow cytometry, high throughput DNA sequencing, respirometry (O2 and CO2)
-toxicology: biotests carried out from the leachates of the various OXO-bios on embryos or larvae of fish (*Dicentrarchus labrax*), sea urchins (*Paracentrotus lividus),* oysters (*Crassotrea gigas*), ascidians (*Phallusia mammillata*), cephalochordates (*Branchiostoma lanceolatum*) and on microalgae (*Skeletonema marinoi, Chaetoceros calcitrans, Tetraselmis suecica, Emiliania huxleyi*).

Major results: The artificial ageing has shown a crucial role of pro-oxidant additives (Co, Mn and Fe) in the fate of OXO-bio films in the environment. The aging efficiency was more marked on films containing pro-oxidants, compared to PE without pro-oxidants, or to OXO-bio mixed with a hydro-biodegradable polymer. Clear signs of biodegradability have been observed for artificially aged OXO-bio equivalent to natural sunlight exposure of 1 year.

The toxicity of the OXO-bio leachates was found to be nil, with the exception of the Co-based pro-oxidants, thus emphasizing the importance of controlling the composition of the pro-oxidant additives in the formulation of OXO-bios. In the case of Co-based pro-oxidants, toxicity is not however likely at the level at which this pro-oxidant would be used in practice. See below Fig 4, page 10.

Scientific production: The project made it possible to demonstrate the biodegradability of OXO-bios from artificial aging equivalent to a lifespan of approximately 1 year. The data collected will be used to write more than 10 scientific articles, including 9 already published. Results have already been presented at 13 international conferences and more will follow over the next years.

Factual information: The OXOMAR project is a fundamental research project coordinated by the CNRS LOMIC. It combines the expertise of the public laboratories CNRS-LOMIC, CNRS-ICCF and IFREMER-Nantes, public-private and CNEP and a private partner from the United Kingdom SYMPHONY ENVIRONMENTAL TECHNOLOGIES PLC – a company quoted on the London Stock Exchange. The project started in October 2016 and ended in March 2021. It received ANR aid of 490,287€ for a total cost of 1,990,616 €.

C MEMOIRE SCIENTIFIQUE

***Maximum 5 pages**. On donne ci-dessous des indications sur le contenu possible du mémoire. Ce mémoire peut être accompagné de rapports annexes plus détaillés.*

Le mémoire scientifique couvre la totalité de la durée du projet. Il doit présenter une synthèse auto-suffisante rappelant les objectifs, le travail réalisé et les résultats obtenus mis en perspective avec les attentes initiales et l'état de l'art. C'est un document d'un format semblable à celui des articles scientifiques ou des monographies. Il doit refléter le caractère collectif de l'effort fait par les partenaires au cours du projet. Le coordinateur prépare ce rapport sur la base des contributions de tous les partenaires. Une version préliminaire en est soumise à l'ANR pour la revue de fin de projet.

Un mémoire scientifique signalé comme confidentiel ne sera pas diffusé. Justifier brièvement la raison de la confidentialité demandée. Les mémoires non confidentiels seront susceptibles d'être diffusés par l'ANR, notamment via les archives ouvertes http://hal.archives-ouvertes.fr.

Mémoire scientifique confidentiel : NO

C.1 RESUME DU MEMOIRE

Ce résumé peut être repris du résumé consolidé public.

C.2 ENJEUX ET PROBLEMATIQUE, ETAT DE L'ART

Marine plastic litter is a global environmental problem (descriptor 10 of the EU Marine Strategy Framework Directive) since almost 10% of the 350 million tons of plastic produced worldwide gets accidentally or deliberately into the environment (Hammer et al. 2012, Galgani et al. 2013). The oceans are considered as the ultimate recipient of plastic litter. Oxo-biodegradable plastics become a complementary solution (together with better plastic collection and development of alternative products) to reverse the current trend of increasing pollution of the oceans by plastic litter, but their biodegradability at sea needs to be better understood. Plastics containing pro-oxidant additives called oxo-biodegradable (OXO-bio) have been introduced onto the market as material promising biodegradability, but their fate in marine waters was poorly investigated.

In the case of waste discarded in the environment, the pro-oxidants catalyze the normal processes of oxidation chemistry so that molecular mass reduction occurs in orders of magnitude faster than would otherwise happen (Jakubowicz et al. 2011). The oxidation (thermo and/or photo-oxidation induced by the pro-oxidants) of OXO-bio polymers results in the formation of short molecular chains whose molecular mass is under 5000 Daltons: these molecules bear oxygenated groups such as carboxylic acids, alcohols, and ketones (Albertsson et al. 1995, Roy et al 2013). It is well understood that the oxidation step transforms non oxygenated long chains (for example (CH2)n) - which cannot be accessed and assimilated by microorganisms - to short molecular chains, which can be integrated into the metabolism of microorganisms. Theoretically, the oxidation step transforms non oxygenated long chains (for example $(CH_2)_n$) - which cannot be accessed and assimilated by microorganisms - to short molecular chains, which can be integrated into the metabolism of microorganisms.

In addition to the American Standard ASTM D6954, an AFNOR Accord (AC T51-808 AFNOR) is a useful method to test for oxo-biodegradability. This Accord is based on ATP and ADP/ATP measurements to assess the oxo-biodegradability of polyolefin materials in the form of films by the bacterial strain *Rhodococcus rhodochrous* ATCC ® 29672 TM.

The European Union (Directive 2019/904 of 5 June 2019) has banned the use of "oxo-degradable" plastics because they perceived a lack of consistent evidence about biodegradation in the environment. The Directive foresees a ban on all products made of oxo-degradable plastic from July 2021.

The objective of OXOMAR was to provide solid scientific data on the fate of OXO-bios at sea, by evaluating the abiotic (task 1) and biotic (task 2) degradation of OXO-bio in natural conditions, as well as its potential toxicity for marine organisms (task 3).

C.3 Approche scientifique et technique

The OXOMAR project gathers together the largest manufacturer of OXO-bio masterbatches (Symphony Environmental Technologies Plc); together with a small or medium-sized enterprise specialized in valorization of academic researches on polymer (CNEP); and three academic units (ICCF, LOMIC, and IFREMER) specialized in chemistry of materials, marine microbiology, toxicology and ecotoxicology.

The base of the project is a combination of three complementary tasks to evaluate the abiotic degradation (TASK 1), the biodegradability (TASK 2) and the toxicity (TASK 3) of OXO-bio when discarded into the sea.

-Task 1: The goal of this task was to evaluate the abiotic degradation of OXO-bio in marine waters. Six formulations of plastics were used in this project: (film 1) Low density polyethylene (LDPE) control; (film 2) LDPE + 1% Additive A (Cobalt Prodegradant, Calcium Carbonate, Phenolic Stabiliser); (film 3) LDPE + 1% Additive B (Manganese Prodegradant, Calcium Carbonate, Phenolic Stabiliser); (film 4) LDPE + 1% Additive C (Cobalt & Manganese Prodegradant, Calcium Carbonate, Phenolic Stabiliser); (film 5) LDPE + 1% Additive D (Manganese Prodegradant & Iron photosensitiser, Calcium Carbonate, Phenolic Stabiliser); and (film 6) (OXO-HYDRO) = LDPE + LDPE/Biobased Blend (stabilized) + Cobalt prodegradant (50% total biobased content).

Artificial ageing of the 6 polymers were performed in photo-thermic chambers to reach 4 ageing states with different oxidation states. The samples were exposed in photo-thermal ageing units (SEPAP 12.24H and SEPAP 12.24) during 0-400 hours to accelerate their ageing. The chemical nature of the oxidation compounds formed after accelerated photoaging and photooxidation was identified by infrared characterization in films based on LDPE OXO-bio and in OXO-HYDRO film (in particular hydroxyl, lactonic and carboxylic acid groups). The follow up of the formation of critical photoproducts (carboxylic acids) was carried out until photofragmentation reached oxidation extent □abs(1715 cm^{-1})= x/100, where x is the thickness of the film in microns. The ageing in seawater carried out in SEPAP 12.24H was also used for toxicological tests.

-Task 2: The goal of this task was to evaluate the biodegradation of OXO-bio in marine waters. This task has been divided in two parts by (i) following several months of OXO-bio colonization by marine microorganisms under natural conditions and (ii) by evaluating the biodegradability of OXO under natural conditions as compared to a cultivated microorganism with known PE-biodegradation abilities (*Rhodococcus rhodochrous* ATCC ® 29672 TM).

OXO-bio colonization has been studied during 2-month periods and at different seasons to reach a level of colonization similar to colonization encountered on plastics sampled in natural seawater (~10^6 cells.mm^2). We provide new data on the temporal changes of the bacterial abundance (atomic force microscopy, fluorescence microscopy, flow cytometry), bacterial activities (exoenzymes activities such as lipase, aminopeptidase, β-glucosidase) and heterotrophic activity, bacterial diversity (MiSeq metabarcoding on 16S rRNA genes). The specificity of the 'plastisphere' by comparison to seawater bacteria was also described by comparing the results to micoorgansims living in the seawater at free-living (fraction <3 μm) or particle-attached (fraction >3μm) states.

OXO-biodegradation was studied following two complementary approaches. The first approach was based on the transfer of natural communities growing on OXO-bio (see above – colonization phase) under new minimum medium with OXO-bio as sole carbon source and energy. Biomass production (measured by epifluorescence microscopy or flow cytometry) together with oxygen consumption

(Presens FiBox 4 recorder) and bacterial heterotrophic activity (^{3}H leucine incorporation) were showing evidence of biodegradation.

Tests of incorporation of stable isotope ^{13}C-labeled OXO-bio were also performed in order to estimate the rate of OXO-biodegradation under natural conditions and estimation of the ratio of bacterial biomass vs. CO_2 production, by EA-IRMS (Elementary Analysis-Isotopic Ratio Mass Spectrometry) and GC/MS (Mass Spectrometry coupled with Gas Chromatography), as well as the description of the bacterial communities involved in the biodegradation (DNA-stable isotope probing coupled with MiSeq metabarcoding on 16S rRNA genes). The second approach was based on culture methods using the bacterial strain *Rhodococcus rhodochrous* ATCC ® 29672 TM. An analytical chemistry approach allowed the analysis of isotopomers (^{12}C /^{13}C composition of molecules) to provide metabolism markers of the labeled polymers. In particular, protocols had to be set up to analyze the ^{13}C enrichment (i) of CO_2 from microbial metabolism by GC-MS, (ii) of microbial biomass by GC-MS–Pyrolysis and (iii) of intracellular microbial metabolites.

-Task 3: The goal of this task was to evaluate the toxicity of OXO-bio in marine waters. Given that organisms from various trophic levels, habitats and feeding behaviors may exhibit different sensitivities to chemical contaminants, it is of major importance to use several model organisms to draw any firm conclusions on the potential toxicity of OXO-bio in the marine environment.

The impacts were assessed in organisms representing several trophic levels of marine ecosystems. Phytoplankton cultures (*Skeletonema marinoi, Chaetoceros calcitrans, Tetraselmis suecica, Emiliania huxleyi*) were tested as pelagic microalgae representing primary producers, a first trophic level possibly consumed by some of the primary consumers tested in the Oxomar project.

Urchin (*Paracentrotus lividus*) and oyster (*Crassotrea gigas*) are both benthic organisms at their adult life stage, grazer for the former and filter-feeding (namely on phytoplankton) for the latter, with planktonic early life stages. Both organisms are models used in ecotoxicology, as the sensitivity of their early life stages to chemical contamination is well known. The cephalochordate amphioxus (*Branchiostoma lanceolatum)* and the tunicate ascidian (*Phallusia mammillata*) are also benthic as adults and planktonic as larvae, both being filter feeders consuming phytoplankton. Less studied in ecotoxicology, these two organisms belonging to chordates, closer to vertebrates, could provide valuable additional information about contaminant impacts on the first marine trophic levels.

A biotest was also carried out on a model of vertebrate, the European sea bass (*Dicentrarchus labrax*), a marine Teleost, a carnivorous organism at all stages of its life cycle including the larval stage, used here for testing the leachates.Toxicological tests were done on leachates from non-aged and artificially aged OXO-bio films tested at 1, 1/10 and 1/100 dilutions. Dose-response experiments were also perfomed to evaluate the toxicity of TME (Trace Metal Elements) used as additives to formulate the OXO-bio films.

C.4 RESULTATS OBTENUS

Several novel factors are pointed out here with respect to the complementary tasks of the OXOMAR project:

1- Oxidation kinetics of the six formulations of plastics under artificial ageing. The kinetics of formation of critical oxidation products (carboxylic acids) were determined in all the 6 films either by 100 h of irradiation in SEPAP 12.24., or 300 h of thermooxidation at 60 ° C or 300 h of thermooxidation after pre-photooxidation for 100 h in SEPAP 12.24. These conditions were chosen because they correspond to a service life of approximately 1 year under natural light. The purpose of these tests was to verify the pro-oxidant role of the masterbatches introduced into the films. Several batches of industrial film have been tested to optimize the formulations of these films by making them highly oxidizable to meet the criteria defined in the AFNOR T51-808 Accord. It was shown that the oxidation kinetics of the non-additive OXO-HYDRO film was much slower than that of the OXO-bio films.

The rate of formed carboxylic acid compounds (known as the critical product) was the criterion which made it possible to link the oxidation state of a PE to the loss of its mechanical properties (fragmentation for example).

In a second step, and in order to study the chemical modifications made to the photooxidation of films immersed in water, the formation kinetics of so-called critical oxidation compounds were studied in a SEPAP 12.24.H chamber. These studies made it possible to study the role and effectiveness of prooxidant masterbatches in aqueous media. As shown in Figure 1, it appeared that the masterbatches accelerated the photooxidation of films exposed in water (compare films 2, 3, 4 to film 1, left figure). This result is interesting because it appears that the masterbatches do not migrate out of the films when the films are immersed in water. After thermooxidation at 60 ° C in seawater of pre-photooxidized films in SEPAP 12.24.H, the prooxidizing additives make it possible to reach high oxidation levels at 3x / 100 according to the AFNOR ACT51-808 Accord (x = thickness of the film in microns - figure on the right)

After establishing the oxidation kinetics, several samples of 1-2 g of films oxidized at a given oxidation level (2x / 100 and 3x / 100), in samples of seawater maintained at 60 ° C and having contained films at a given oxidation level were sent to the project partners involved in WP 2 and 3.

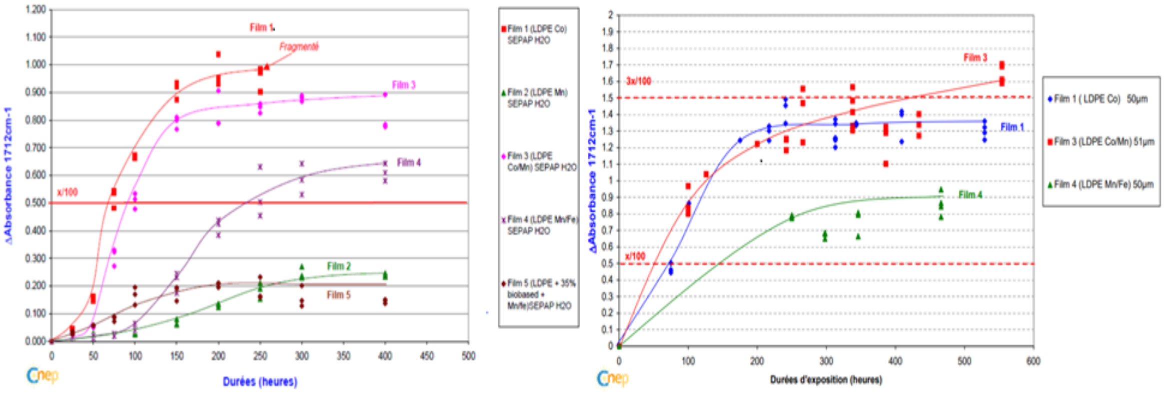


Figure 1: Formation of carboxylic acid groups after photo-oxidation in SEPAP 12.24.H (left) and after thermooxidation of the photo-oxidized films (right).
See also related publications by Eyheraguibel et al. (2018) Chemosphere.

2- Characterization of the plastisphere. Long term colonization of OXO-bio films by marine bacteria was followed at different seasons and in comparison with other biodegradable films. Artificially pre-aged OXO-bio (AA-OXO of different composition and with different ageing conditions – see above) or not OXO together with conventional low density PE and other polymers (PS, PHBV, PCL, PLA) were incubated for more than 6 weeks (covering spring-summer and summer-autumn periods) in an aquarium with natural circulation of seawater from the Banyuls Bay (NW Mediterranean Sea). Our experimental setup allowed us to characterize the successive phases of primo-colonization, growing, and maturation of the biofilms. We highlighted different trends between polymer types with distinct surface properties and composition, the biodegradable AA-OXO, PCL and PHBV presenting higher colonization by active and specific bacteria compared to non-biodegradable polymers (PE, PS, PLA and non-aged OXO-bio). Succession of bacterial population occurred during the three colonization phases, with hydrocarbonoclastic bacteria being highly abundant on all plastic types. OTUs belonging to Flavobacteriaceae, Gammaproteobacteria, Cellvibrionaceae and Myxococlaes were particularly dominant in artificially pre-aged OXO-bio ($AA_{144}OXO$), which may be particularly involved in OXO-biodegradation in natural seawater.

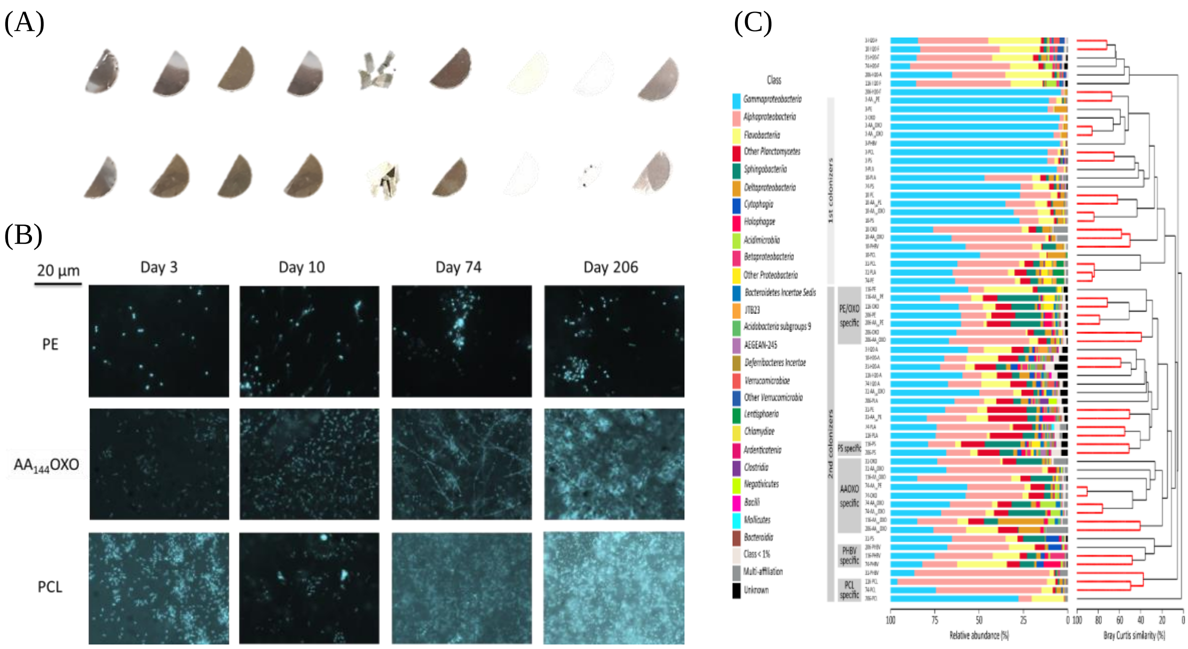
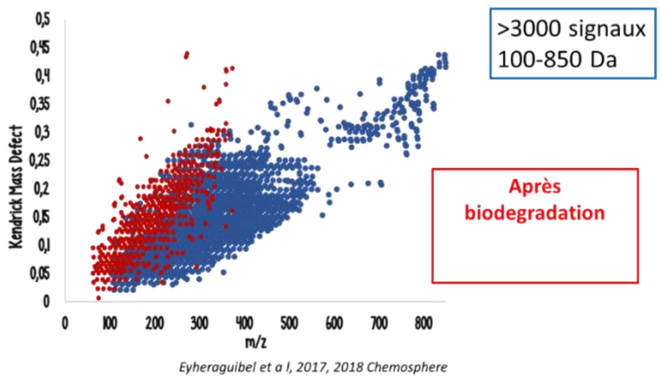


Figure 2. Microbial colonization of non pre-aged polyethylene (PE); oxobiodegradable (OXO-bio) polystyrene (PS); polycaprolactone (PCL); polylactic acid (PLA); as compared to artificially aged (48 to 144 days) PE (AA_{144}-PE) and OXO-bio (AA_{48}-OXO, AA_{144}-OXO). (A) Physical evolution of different polymer types after 3 and 206 days of immersion in seawater, (B) Epifluorescence micrographs of DAPI-stained polymers surfaces (PE, AA144OXO and PCL) showing differences in bacterial colonization and formation of a biofilm with EPS after 3, 10, 74 and 206 days of immersion in seawater and (C) Relative class abundances associated with the bacterial community structure of bacteria attached on plastic polymers, particle-attached (H2O-A) and free-living (H2O-F) in seawater during 7 months of immersion in seawater.
See also related publications by Dussud et al. (2018a) Frontiers in microbiology, Dussud et al. (2018) Environmental Pollution and Odobel et al. (2021) Frontiers in microbiology.

3- Biodegradation of OXO-bio polymers and development of new tools to prove its biodegradation. A first series of experiments consisted in developing an analytical approach combining ^{1}H NMR and LC-HRMS in order to be able to evaluate the biodegradation of oligomers resulting from the abiotic transformation (photo- and / or thermo-oxidation) of PE (OXO-bio). This approach has been successfully applied to the *Rhodococcus rhodochrous* strain (Fig. 3) and may serve as a basis in the future for a rapid screening of strains isolated from marine environments.



<u>Figure 3</u>: A study by LC-HRMS made it possible to identify more than 3000 signals corresponding to the different oligomers of mass 110 to 850 Da, generated during the oxidation of the OXO-bio polymer (in blue). After incubation with the Rhodococcus rhodochrous bacterium, most of these oligomers have been consumed (in red), it can be noted that the remaining oligomers have much lower masses (<350 Da). This approach is particularly original (striking fact).
See also related publications by Eyheraguibel et al. 2017 and Harrison et al. 2018.

4-Nil to moderate toxicity on OXO-bio leachate. The combination of toxicity tests based on different organisms allowed us to show different sensibilities in response to OXO-bio leachates exposure. Nil or low toxicity was observed for the films, except for the OXO-bio containing Cobalt-based additive (Figure 4). It can be argued that the ageing process of the film may lead to cobalt discharge in the surrounding media. Interestingly, when Co was tested as a single compound on some of the model organisms (microalgae, oyster, amphioxus and sea-urchin), toxicity effects occurred only at mg/L concentrations, which is unlikely to occur in the marine environment and especially in relation to the presence of OXO-bio leachates. The question is still pending for the possible cocktail effect with other unidentified compounds that were responsible for the toxicity of the leachates from Co-based OXO-bio.

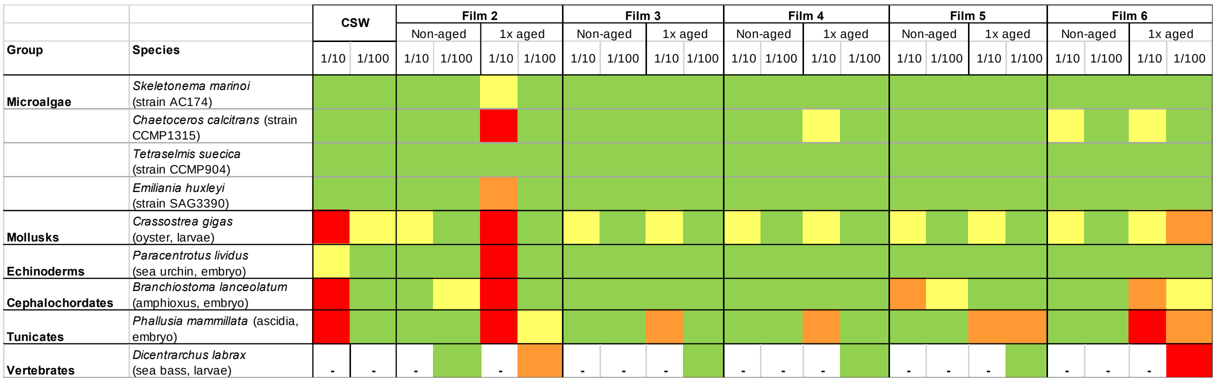


Figure 4: Toxicological tests on Microalgae, Mollusks, Echonderms, Cephalochordates, Tunicates and Vertebrates. Color code represent the significant effects (growth inhibition, abnormal development or mortality) per intensity for each model organism tested (green: microalgae growth inhibition <5%, vertebrate mortality <40%, normal larvae for others >80%; yellow: microalgae growth inhibition 5<x<25%, vertebrate mortality 50%<x<80%, normal larvae for others 50%<x<80%; orange: microalgae growth inhibition 25<x<50%, vertebrate mortality 40%<x<60%, normal larvae for others 20%<x<50%; red: microalgae growth inhibition <20%, vertebrate mortality >80%, normal larvae for others <20% ("-" means that no test could be run).

Exploitation des résultats

A combination of results from the different tasks have been already published in 8 peer-review journals (with impact factors >4). Most of the papers included authors from laboratories involved in the project, showing the complementarity of the teams involved in the project. Data have also been presented in 13 national and international conferences.

C.5 Discussion

Discussion sur le degré de réalisation des objectifs initiaux, les verrous restant à franchir, les ruptures, les élargissements possibles, les perspectives ouvertes par le projet, l'impact scientifique, industriel ou sociétal des résultats.

Most of the initial objectives have been achieved. The success of the project has been mainly due to the abililty of the team to work together in different fields of expertise.

One aspect of the project has not been finalized during the time of the project, which involved the use of stable isotope ^{13}C-labeled OXO-bio (see page 7 above). The production of ^{13}C OXO-bio was delayed for one year, first by the toxicity of cobalt, and also because it turned out to be more expensive than expected to obtain enough extruded plastic material. This part of the project, financed by the industrial partner SYMPHONY outside the framework of ANR financial support, experienced an unforeseen delay. Moreover, we realized that the ^{13}C OXO-bio differed in its characteristics as compared to the initial ^{12}C OXO-bio (5 times higher molecular weight than the initial OXO-bio), thus necessiting different pre-ageing conditions that needed to be optimized. We finally demonstrated the presence shifts in the positions of the absorption bands in ^{12}C and ^{13}C OXO-bio. Tests of biodegradation of artificially aged ^{13}C-OXO-bio are still running at the present time, and evidence of biodegradation has been confirmed.

Our results have several consequences in the market of oxo-biodegradable polymers. The perspective for development of biodegradable plastics in general depends on the regulatory context and the governmental will of each country. The market of OXO-bio is in a favorable position particularly in Africa, Asia, Middle East, and Latin America. In the European Union, the placing on the market of products made with oxo-degradable plastic is governed by EU's Directive 2019/904 of 5 June 2019 on the Reduction of the Impact of Certain Plastic Products on the Environment. The Directive foresees a ban on all products made of oxo-degradable plastic from July 2021.

However, in relation to oxo-biodegradable plastic our study shows evidence of biodegradation of OXO-bio after artificial ageing, which correspond to a service life of approximately one year under natural light. However, our study could not comment on its complete degradation in marine environment after periods classically used in actual norms (more than 60% degradation for ISO 18830 & ISO 19679 or 70% ASTM D6691-09 after 24 months).

Finally, the toxicity tests performed on a large panel of marine organisms allowed us to prove nil toxicity of some formulations of OXO-bio, but toxicity of OXO-bio made with Co-based additive was clearly observed at the level tested.

C.6 Conclusions

We have obtained congruent results from our multidisciplinary approach that clearly shows that Oxo-biodegradable plastics biodegrade in seawater and do so with a significantly higher efficiency than conventional plastics. The oxidation level obtained due to the d2w prodegradant catalyst was found to be of crucial importance in the degradation process. Out of the six-formulations tested, the Mn/Fe pro-oxidant was the most efficient, with no toxic effects under our experimental conditions. Biodegradability was demonstrated either by using the culture bacteria Rhodococcus rhodochrous or by a complex natural marine community of microorganisms.

The project ended with significant advances in the understanding of the biodegradation and toxicity of oxo-biodegradable plastics in the marine environment:

-we confirm that accelerated artificial aging (UV, temperature) which was perfectly mastered in this project, is a tool of choice which is particularly well suited to the study of the fate of OXO-bios in the marine environment (task 1). Accelerated artificial ageing does not invalidate the results.

-we highlight the kinetics of the succession of phases of biofilm colonization (primary colonization, growth, maturation), the characteristics of which will allow us a better understanding of the mechanisms of OXO-biodegradation (task 2).

-we developed an original method coupling ^{1}H NMR and LC-MS to analyze the biodegradation of oligomers from OXO-bios (task 2).

-we showed biodegradation of artificially aged OXO-bio (after a minimum of 300h of thermooxidation following photooxidation for 100 h in SEPAP 12.24), equivalent to natural sunlight exposure of 1 year.

-while some results show the potential toxicity of OXO-bio formulations based on cobalt for the various marine organisms tested, other formulations with similar properties show no toxicity. This result will allow OXO-bio plastics suppliers to direct their production towards formulations that are proven to be safe for the marine environment (task 3).

C.7 REFERENCES

1. Eyheraguibel B, Leremboure M, Traikia M, Sancelme M, Bonhomme S, Fromageot D, Lemaire J, Lacoste J, Delort A.M. (2018). Environmental scenarii for the degradation of oxo-polymers. Chemosphere. 198 182-190. DOI:10.1016/j.chemosphere.2018.01.1530. (IF=4.2)
2. Eyheraguibel B, M. Traikia, S. Fontanella, M. Sancelme, S. Bonhomme, D. Fromageot, J. Lemaire, G. Laurenson, J. Lacoste, A-M. Delort (2017). Characterization of oxidized oligomers from polyethylene films by mass spectrometry and NMR spectroscopy before and after biodegradation by a *Rhodococcus rhodochrous* strain. Chemosphere.184, 366-374.DOI: 10.1016/j.chemosphere.2017.05.137. (IF=4.2)
3. Harrison JP, Boardman C, O'Callaghan K, Delort AM and Song J (2018) Biodegradability standards for carrier bags and plastic films in aquatic environments: a critical review. R. Soc. open sci. 5: 171792.
4. Dussud C, Hudec C, George M, Fabre P, Higgs P, Bruzaud S, Eyheraguibel B, Meistertzheim AL, Jacquin J, Cheng J, Callac N, Odobel C, Rabouille S, Ghiglione JF (2018). Colonization of non-biodegradable and biodegradable plastics by marine microorganisms. Frontiers in microbiology (IF 4.52) 9:1571.
5. Dussud C, Meistertzheim AL, Conan P, Pujo-Pay M, George M, Fabre P, Coudane J, Higgs P, Elineau A, Pedrotti ML, Gorsky G, Ghiglione JF (2018) Evidence of niche partitioning among bacteria living on plastics, organic particles and surrounding seawaters. Environmental Pollution (IF 5.09) 236: 807-816.
6. Odobel C, Dussud C, Conan P, Pujo-Pay M, Meistertzheim AL, Eyheraguibel B, Delort AM, Ter Halle A, Bruzaud S, Barbe V, Ghiglione JF. Long-term colonization (7 months) of non-biodegradable and biodegradable microplastics by marine bacteria. Frontiers in microbiology (IF 4.52), submitted

D LISTE DES LIVRABLES

Date de livraison	**N°**	**Titre**	**Nature** (rapport, logiciel, prototype, données, ...)	**Partenaires** (souligner le responsable)	**Commentaires**
1	0.1	Quick off meeting (start)		All	Presentation of the project and tasks at Banyuls Observatory (Nov. 2016)
2	0.2	Website		<u>LOMIC</u> and All	Presentation of te website and possibility of internal sharing of files and documens
12	0.3	Group meeting	data	<u>LOMIC</u> and All	At Montpellier University (Nov. 2017)
20	0.4	Group meeting	data	<u>LOMIC</u> and All	At Banyuls Observatory (June. 2018)
29	0.5	Group meeting	data	<u>LOMIC</u> and All	At Banyuls Observatory (March 2019)
42	0.6	Group meeting	data	<u>LOMIC</u> and All	Visioconference (June 2020)

Date de livraison	N°	Titre	Nature (rapport, logiciel, prototype, données, …)	Partenaires (souligner le responsable)	Commentaires
51	0.7	Conclusion meeting meeting	data	LOMIC and All	Visioconference (Jan 2021)
51	0.8	Intermediate report	report	LOMIC and All	Report (July 2018)
53	0.9	Final report	report	LOMIC and All	Report (March 2021)
19	1.1	Production of OXO-bio	Materials	SYMPHONY	Done
24	1.2	Artificial ageing of OXO-bio for biodegradation and toxicity tests	Data and materials	CNEP, SYMPHONY	Done
29	2.1	Description of the colonization on OXO-bio 'plastisphere'	Data	LOMIC	Done
52	2.2	Biodegradability of OXO-bio at sea	Data	LOMIC, ICCF	Done
37	2.3	Production of 13C-OXO-bio and ageing	Materials	SYMPHONY, CNEP	Done
32	3.1	Ingestion tests	Data	LOMIC	Done
32	3.2	Tests on pro-oxidant leaching	Data	IFREMER, LOMIC	Done

E IMPACT DU PROJET

Ce rapport rassemble des éléments nécessaires au bilan du projet et plus globalement permettant d'apprécier l'impact du programme à différents niveaux.

E.1 INDICATEURS D'IMPACT

Nombre de publications et de communications (à détailler en E.2)

Comptabiliser séparément les actions monopartenaires, impliquant un seul partenaire, et les actions multipartenaires résultant d'un travail en commun.
***Attention** : éviter une inflation artificielle des publications, mentionner uniquement celles qui résultent directement du projet (postérieures à son démarrage, et qui citent le soutien de l'ANR et la référence du projet).*

		Publications multipartenaires	Publications monopartenaires
International	Revues à comité de lecture	1. Eyheraguibel B, Leremboure M, Traikia M, Sancelme M, Bonhomme S, Fromageot D, Lemaire J, Lacoste J, Delort A.M. (2018). Environmental scenarii for the degradation of oxo-polymers. Chemosphere. 198 182-190. DOI:10.1016/j.chemosphere.2018.01.1530. (IF=4.2) 2. Eyheraguibel B, M. Traikia, S. Fontanella, M. Sancelme, S. Bonhomme, D. Fromageot, J. Lemaire, G. Laurenson, J. Lacoste, A-M. Delort (2017). Characterization of oxidized oligomers from polyethylene films by mass spectrometry and NMR spectroscopy before and after biodegradation by a *Rhodococcus rhodochrous* strain. Chemosphere.184, 366-374.DOI:	6. Harrison JP, Boardman C, O'Callaghan K, Delort AM and Song J (2018) Biodegradability standards for carrier bags and plastic films in aquatic environments: a critical review. R. Soc. open sci. 5: 171792. 7. Ghiglione JF and Laudet V. (2020) Fish larvae: a polluted *Terra Incognita* unveils. *Current Biology* (*IF 9.19*) 30: 112–133. 8. Jacquin J, Cheng J, Odobel C, Pandin C, Conan P, Pujo-Pay M, Barbe V, Meistertzheim AL, Ghiglione JF (2019) Microbial ecotoxicology of marine plastic debris: a review on colonization and biodegradation by the 'plastisphere' *Frontiers in microbiology (IF 4.52)* 10:865.

		10.1016/j.chemosphere.2017.05.137. (IF=4.2) 3. Dussud C, Hudec C, George M, Fabre P, Higgs P, Bruzaud S, Eyheraguibel B, Delort A.M. Meistertzheim AL, Jacquin J, Cheng J, Callac N, Odobel C, Rabouille S, Ghiglione JF (2018). Colonization of non-biodegradable and biodegradable plastics by marine microorganisms. Frontiers in microbiology (IF 4.52) 9:1571. 4. Dussud C, Meistertzheim AL, Conan P, Pujo-Pay M, George M, Fabre P, Coudane J, Higgs P, Elineau A, Pedrotti ML, Gorsky G, Ghiglione JF (2018) Evidence of niche partitioning among bacteria living on plastics, organic particles and surrounding seawaters. Environmental Pollution (IF 5.09) 236: 807-816. 5. Odobel C, Dussud C, Conan P, Pujo-Pay M, Meistertzheim AL, Eyheraguibel B, Delort AM, Ter Halle A, Bruzaud S, Barbe V, Ghiglione JF. Long-term colonization (7 months) of non-biodegradable and biodegradable microplastics by marine bacteria. Frontiers in microbiology (IF 4.52), submitted	
	Ouvrages ou chapitres d'ouvrage		
	Communications (conférence)	1. Ghiglione JF, J Cheng , B Eyheraguibel , A. Ter Halle, S Bruzaud, AL Meistertzheim. (2020) Marine biodegradability of biobased and petroleum-based polymers as substitutes of conventional microbeads. Micro2020 Conference, Lanzarote, Spain.	1. Meistertzheim AL, Dussud C, George M, Fabre P, Pedrotti ML, Gorsky G, Ghiglione JF. Influence of plastic litters on marine microbial life. Oral presentation. ECOTOXICOMIC - First international conference on microbial ecotoxicology (Lyon, France, 21-24 Nov 2017). 2. Dussud C, Meistertzheim AL, George M, Fabre P, Pedrotti ML, Grosky G, Ghiglione JF. Impact of microplastics on marine microbial life. International Conference on Microplastic Pollution in the Mediterranean Sea in Capri (µMED). ORAL presentation. (Capri, Italie - 26-29 Sept 2017) 3. Dussud C, Hudec C, Elineau A, Coudane J, Ghiglione JF. Bacterial communities living on plastic litters in the Mediterranean sea. ASLO ORAL presentation. Aquatic Sciences Meeting (Honolulu, *Hawaii*, 26/02 au 03/03 2017)
France	**Revues à comité de lecture**		
	Ouvrages ou chapitres d'ouvrage		1. Galgani F, Bruzaud S, Duflos G, Fabre P, Gastaldi E, Ghiglione JF, Grimaud R, George M, Huvet A, Lagarde F, Paul-Pont I, ter Halle A (2020) Pollution des océans par les plastiques et les microplastiques. *Techniques de l'ingénieur.*
	Communications (conférence)		1. A.-M. Delort, E. Gastaldi (2020) Les différentes facettes de la biodégradabilité des plastiques. Colloque Biodégradabilité des Plastiques : du fondamental aux enjeux industriels, Biocitech (5 février 2020, Romainville.) 2. A.-M. Delort (2020) Nouveaux outils analytiques pour l'évaluation de la biodégradabilité des polymères . Webinaire Innovations : les membres

			d'Adebiotech ont la parole. (24 Novembre 2020) 3. Société Française de Microbiologie (30 septembre 2019, Cité des Sciences et de L'industrie PARIS) Ghiglione JF. Etat des connaissances sur l'écotoxicologie microbienne appliquée à la pollution plastique. Conférencier invité / conférence plénière 4. B.Eyheraguibel (2018). Biodégradation des plastiques dans l'environnement- 15ème rencontre des microbiologistes du pole Clermontois (26 avril- Clermont Ferrand). Conférencier invité. 5. B.Eyheraguibel (2018). Impact des conditions biotiques et abiotiques sur la biodégradation de polyéthylènes oxobiodégra-dables. Workshop Polymères et Océan (15-18 janvier, Montpellier). Conférencier invité. 6. Ghiglione JF. Colonisation et biodégradation bactérienne des microplastiques en mer. Conférencier invité.____Société Française de Microbiologie (9 - 11 octobre 2017, Cité des Sciences et de L'industrie PARIS) 7. B.Eyheraguibel (2017). De nouveaux outils pour étudier la biodégradation des polymères oxobiodégradables. Congrès National de la Société Française de Microbiologie. (9 - 11 octobre 2017, Cité des Sciences et de l'Industrie – Paris) 8. B. Eyheraguibel :2018 De la fragmentation à la biodégradation des plastiques. (IMRCP. Unibersité Paul Sabatier. 9. B.Eyheraguibel 2019 Les outils pour étudier la biodégradation des plastiques dans l'environnement. Workshop CEA Plastiques et environnement. 10. B.Eyheraguibel 31 Janvier 2020 Les plastiques dans l'environnement. Conférence-débat de l'association Montcel durable 11. B.Eyheraguibel 10 Octobre 2020 Biodégradation des matériaux polymères- Lycee Paul Constans, Montluçon
Actions de diffusion	**Articles vulgarisation**		1. 2020 Le Monde (24 Juillet 2020) La pollution par le plastique pourrait être réduite de 80 % en adoptant des mesures ambitieuses - JF Ghiglione 2. 2020 Le Figaro (29/01/2020) 2050- Déchets: peut-on éviter le pire? - JF Ghiglione 3. 2019 Science et vie junior (numéro #363 – Novembre 2019) "5 solutions pour les océans" - JF Ghiglione 4. 2019 - Environnement magazine (16 Octobre 2019) Plastique en mer, les solutions sont à terre - JF Ghiglione 5. 2019 Agropolis Origine, impact et solutions à la pollution des microplastiques en Méditerranée (N°24, Fév. 2019) - JF Ghiglione 6. 2018 20 minutes (13 Juin) : Nettoyer les Océans : la mer à boire - JF Ghiglione 7. 2018 Point de vue (8 Juin) Journée mondiale de l'Océan. Pollution plastique : la solution ne viendra pas du nettoyage de l'Océan - JF Ghiglione

	Conférences vulgarisation		1. Conférence grand public Semaine de la Mer - Région Occitanie / Pyrénées (Grau du Roi 29 Novembre 2019) Pollution en Méditerranée : comment agir ? - JF Ghiglione 2. 2018 Collège de France -– conférence invitée (20 Novembre 2018) Pollution des Océans par les plastiques : à quel point les « Sciences de l'Environnement » peuvent-elles contribuer à inverser la donne ? - JF Ghiglione 3. 2018. Conférence publique – Fondation Rovaltain (26 Avril 2018) Plastiques d'aujourd'hui et de demain, un enjeu environnemental & économique. - JF Ghiglione
	Autres : Documentaires		1. Télévision W9 28/01/2021. « 2050, Déchets: peut-on encore éviter le pire » 1h40 - JF Ghiglione 2. TV Chaîne ARTE - Reportage 52' - Océans, le mystère plastique – Inspiration scientifique - JF Ghiglione 3. Radio France Inter ; Emission « La Terre au carré » (25/08/2020) la pollution plastique : comment en venir à bout ? - JF Ghiglione 4. TV France 2 01/8/2020– Emission 13h15 le samedi – 30 minutes - «Docteur Plastiques» - JF Ghiglione 5. France 2 20h Journal Télévisé (23 Novembre) - JF Ghiglione

Autres valorisations scientifiques (à détailler en E.3)

Ce tableau dénombre et liste les brevets nationaux et internationaux, licences, et autres éléments de propriété intellectuelle consécutifs au projet, du savoir faire, des retombées diverses en précisant les partenariats éventuels. Voir en particulier celles annoncées dans l'annexe technique).

	Nombre, années et commentaires (valorisations avérées ou probables)
Brevets internationaux obtenus	none
Brevet internationaux en cours d'obtention	none
Brevets nationaux obtenus	none
Brevet nationaux en cours d'obtention	none
Licences d'exploitation (obtention / cession)	none
Créations d'entreprises ou essaimage	none
Nouveaux projets collaboratifs	none
Colloques scientifiques	13 congress (4 international, 9 national)
Autres (préciser)	3 public conferences and 5 audio-visual interviews

E.2 LISTE DES PUBLICATIONS ET COMMUNICATIONS

*Répertorier les publications résultant des travaux effectués dans le cadre du projet. On suivra les catégories du premier tableau de la section **Error! Reference source not found.** en suivant les normes éditoriales habituelles. En ce qui concerne les conférences, on spécifiera les conférences invitées.*

Revues à comité de lecture

1. Eyheraguibel B, Leremboure M, Traikia M, Sancelme M, Bonhomme S, Fromageot D, Lemaire J, Lacoste J, Delort A.M. (2018). Environmental scenarii for the degradation of oxo-polymers. Chemosphere. 198 182-190. DOI:10.1016/j.chemosphere.2018.01.1530. (IF=4.2)
2. Eyheraguibel B, M. Traikia, S. Fontanella, M. Sancelme, S. Bonhomme, D. Fromageot, J. Lemaire, G. Laurenson, J. Lacoste, A-M. Delort (2017). Characterization of oxidized oligomers from polyethylene films by mass spectrometry and NMR spectroscopy before and after biodegradation by a *Rhodococcus rhodochrous* strain. Chemosphere.184, 366-374.DOI: 10.1016/j.chemosphere.2017.05.137. (IF=4.2)
3. Dussud C, Hudec C, George M, Fabre P, Higgs P, Bruzaud S, Eyheraguibel B, Delort A.M. Meistertzheim AL, Jacquin J, Cheng J, Callac N, Odobel C, Rabouille S, Ghiglione JF (2018). Colonization of non-biodegradable and biodegradable plastics by marine microorganisms. Frontiers in microbiology (IF 4.52) 9:1571.
4. Dussud C, Meistertzheim AL, Conan P, Pujo-Pay M, George M, Fabre P, Coudane J, Higgs P, Elineau A, Pedrotti ML, Gorsky G, Ghiglione JF (2018) Evidence of niche partitioning among bacteria living on plastics, organic particles and surrounding seawaters. Environmental Pollution (IF 5.09) 236: 807-816.
5. Odobel C, Dussud C, Conan P, Pujo-Pay M, Meistertzheim AL, Eyheraguibel B, Delort AM, Ter Halle A, Bruzaud S, Barbe V, Ghiglione JF. Long-term colonization (7 months) of non-biodegradable and biodegradable microplastics by marine bacteria. Frontiers in microbiology (IF 4.52), submitted
6. Ghiglione JF and Laudet V. (2020) Fish larvae: a polluted *Terra Incognita* unveils. *Current Biology* (*IF 9.19)* 30: 112–133.
7. Harrison JP, Boardman C, O'Callaghan K, Delort AM and Song J (2018) Biodegradability standards for carrier bags and plastic films in aquatic environments: a critical review. R. Soc. open sci. 5: 171792.
8. Jacquin J, Cheng J, Odobel C, Pandin C, Conan P, Pujo-Pay M, Barbe V, Meistertzheim AL, Ghiglione JF (2019) Microbial ecotoxicology of marine plastic debris: a review on colonization and biodegradation by the 'plastisphere' *Frontiers in microbiology (IF 4.52)* 10:865.

Chapitres d'ouvrages

9. Galgani F, Bruzaud S, Duflos G, Fabre P, Gastaldi E, Ghiglione JF, Grimaud R, George M, Huvet A, Lagarde F, Paul-Pont I, ter Halle A (2020) Pollution des océans par les plastiques et les microplastiques. *Techniques de l'ingénieur.*

Conférences invitées :

1. Ghiglione JF. Colonisation et biodégradation bactérienne des microplastiques en mer. Conférencier invité. Société Française de Microbiologie (9 - 11 octobre 2017, Cité des Sciences et de L'industrie PARIS).
2. Ghiglione JF. ECOTOXICOMIC - First international conference on microbial ecotoxicology (Lyon, France, 21-24 Nov 2017). Influence of plastic litters on marine microbial life.
2. B. Eyheraguibel (2018). Biodégradation des plastiques dans l'environnement- 15ème rencontre des microbiologistes du pole Clermontois (26 avril- Clermont Ferrand).
3. B. Eyheraguibel (2018). Impact des conditions biotiques et abiotiques sur la biodégradation de polyéthylènes oxobiodégradables. Workshop Polymères et Océan (15-18 janvier, Montpellier).
4. Ghiglione JF Société Française de Microbiologie (30 septembre 2019, Cité des Sciences et de L'industrie PARIS). Etat des connaissances sur l'écotoxicologie microbienne appliquée à la pollution plastique.
5. A.-M. Delort, E. Gastaldi (2020) Les différentes facettes de la biodégradabilité des plastiques. Colloque Biodégradabilité des Plastiques : du fondamental aux enjeux industriels, Biocitech (5 février 2020, Romainville.)
6. A.-M. Delort (2020) Nouveaux outils analytiques pour l'évaluation de la biodégradabilité des polymères . Webinaire Innovations : les membres d'Adebiotech ont la parole. (24 Novembre 2020)

Autres conférences scientifiques:

7. B.Eyheraguibel (2017). De nouveaux outils pour étudier la biodégradation des polymères oxobiodégradables. Congrès National de la Société Française de Microbiologie. (9 - 11 octobre 2017, Cité des Sciences et de l'Industrie – Paris)
8. Meistertzheim AL, Dussud C, George M, Fabre P, Pedrotti ML, Gorsky G, Ghiglione JF. Influence of plastic litters on marine microbial life. Oral presentation. ECOTOXICOMIC - First international conference on microbial ecotoxicology (Lyon, France, 21-24 Nov 2017).
9. Dussud C, Meistertzheim AL, George M, Fabre P, Pedrotti ML, Grosky G, Ghiglione JF. Impact of microplastics on marine microbial life. International Conference on Microplastic Pollution in the Mediterranean Sea in Capri (μMED). ORAL presentation. (Capri, Italie - 26-29 Sept 2017)
10. Dussud C, Hudec C, Elineau A, Coudane J, Ghiglione JF. Bacterial communities living on plastic litters in the Mediterranean sea. ASLO ORAL presentation. Aquatic Sciences Meeting (Honolulu, *Hawaii*, 26/02 au 03/03 2017)
11. B.Eyheraguibel 2019 Les outils pour étudier la biodégradation des plastiques dans l'environnement. Workshop CEA Plastiques et environnement.
12. B.Eyheraguibel 31 Janvier 2020 Les plastiques dans l'environnement. Conférence-débat de l'association Montcel durable
13. B.Eyheraguibel 10 Octobre 2020 Biodégradation des matériaux polymères- Lycee Paul Constans, Montluçon

E.3 LISTE DES ELEMENTS DE VALORISATION

None

E.4 BILAN ET SUIVI DES PERSONNELS RECRUTES EN CDD (HORS STAGIAIRES)

Ce tableau dresse le bilan du projet en termes de recrutement de personnels non permanents sur CDD ou assimilé. Renseigner une ligne par personne embauchée sur le projet quand l'embauche a été financée partiellement ou en totalité par l'aide de l'ANR et quand la contribution au projet a été d'une durée au moins égale à 3 mois, tous contrats confondus, l'aide de l'ANR pouvant ne représenter qu'une partie de la rémunération de la personne sur la durée de sa participation au projet.
Les stagiaires bénéficiant d'une convention de stage avec un établissement d'enseignement ne doivent pas être mentionnés.

Les données recueillies pourront faire l'objet d'une demande de mise à jour par l'ANR jusqu'à 5 ans après la fin du projet.

Identification				Avant le recrutement sur le projet			Recrutement sur le projet				Après le projet				
Nom et prénom	Sexe H/F	Adresse email (1)	Date des dernières nouvelles	Dernier diplôme obtenu au moment du recrutement	Lieu d'études (France, UE, hors UE)	Expérience prof. Antérieure, y compris post-docs (ans)	Partenaire ayant embauché la personne	Poste dans le projet (2)	Durée missions (mois) (3)	Date de fin de mission sur le projet	Devenir professionnel (4)	Type d'employeur (5)	Type d'emploi (6)	Lien au projet ANR (7)	Valorisation expérience (8)
EYHERA GUIBEL Boris	H	boris.eyheraguibel@uca.fr	01/03/2021	Doctorat en Sciences	France	Post-doc	ICCF	Post-doctorat	13 mois	31/12/2019	CDD	Université Clermont-Ferrand	Chercheur	Non	Oui
PANDIN Caroline	F	caroline.pandin@gmail.com	01/12/2020	Doctorat	France	Thèse	LOMIC	Post-doctorat	12 mois	31/12/2019	CDD	CNRS	Chercheur	Non	Oui
DURAN Clelia	F	duran.clelia1@gmail.com	01/12/2020	Master 2	France	Master 2	LOMIC	Ingénieure d'étude	6 mois	14/08/2020	CDD	Université de Cambridge	Thèse	Non	Oui

Aide pour le remplissage

(1) Adresse email : indiquer une adresse email la plus pérenne possible
(2) Poste dans le projet : post-doc, doctorant, ingénieur ou niveau ingénieur, technicien, vacataire, autre (préciser)
(3) Durée missions : indiquer en mois la durée totale des missions (y compris celles non financées par l'ANR) effectuées sur le projet
(4) Devenir professionnel : CDI, CDD, chef d'entreprise, encore sur le projet, post-doc France, post-doc étranger, étudiant, recherche d'emploi, sans nouvelles
(5) Type d'employeur : enseignement et recherche publique, EPIC de recherche, grande entreprise, PME/TPE, création d'entreprise, autre public, autre privé, libéral, autre (préciser)
(6) Type d'emploi : ingénieur, chercheur, enseignant-chercheur, cadre, technicien, autre (préciser)
(7) Lien au projet ANR : préciser si l'employeur est ou non un partenaire du projet
(8) Valorisation expérience : préciser si le poste occupé valorise l'expérience acquise pendant le projet.