BIOCLEAN Report Summary

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Final Report Summary - BIOCLEAN (New BIOtechnologiCaL approaches for biodegrading and promoting the environmEntal biotrAnsformation of syNthetic polymeric materials)

Executive Summary:

About 31% of oil-deriving plastic waste is currently disposed in landfills in Europe, where it undergoes (photo)degradation with the production of small fragments & leachates, which enter the marine environment, where they can exert toxic effects. BIOCLEAN aimed at developing innovative, eco-efficient, pilot scale validated biotechnological solutions to safely degrade plastic wastes accumulated in terrestrial facilities thus preventing marine litter, and mitigating (micro)plastics effects in seas. Approach. Obtain novel, robust and efficient plastic-degrading microbes (i.e., bacteria and fungi, aerobic/anaerobic, pure cultures and consortia) from wasted plastics recovered from various terrestrial waste treating facilities and marine habitats (Aegean and Norwegian seas) and use them to develop i) novel biotechnological or physical/chemical-biotechnological integrated processes able to degrade plastic wastes, ii) bioaugmentation protocols to enhance biodegradation of (micro)plastics persisting in terrestrial waste treatment facilities and marine environments and iii) mitigation measures to achieve Good Environmental Status of the Aegean sea.

Results achieved + exploitation + dissemination

• A collection of 66 robust microbes able to partially degrade/transform films of plastics of polyethylene (PE) (7 cultures), including its different variants Low Density PE (LDPE) and Linear Low Density PE (LLDPE), polypropylene (PP) (2 cultures), polyvinyl chloride (PVC) (53 cultures) and polystyrene (PS) (4 cultures). The microbes belong to the partners who selected/isolated them. They can be patented, described in scientific international publications or used for developing tailored processes. Relevant for industry/environment.

• New, pilot-scale assessed, biological processes able to partially degrade PVC films or hybrid chemical/physical-biological processes able to degrade PS and PP plastics. They can be patented, and described in scientific international journals. Relevant for industry, environment and policy.

• Lab and pilot-scale bioaugmentation protocols for intensifying (micro)plastics biodegradation in soils, composting/anaerobic waste treatment facilities and marine environments. They often displayed limited activity but will be described in international publications. Relevant for industry, environment, and policy.

• Site-specific measures for mitigating plastic pollution and improving the environmental status of Aegean Sea. They can be described in international publications. Relevant for environment and policy.

• Dissemination to: i) the industry, environmental protection authorities and associations (via PlasticsEurope -full partner and leader of implementation/dissemination WP-, international initiatives and web-site); ii) the scientific community (via partners' participation in international conferences and workshops and international publications); Training and career development (training on plastic safe management and disposal to young scientists at ECOMONDO2014 exhibition -Italy, 2014- and at the DEDISA municipality -Crete 2015-); Communication with the general public (via partners and PlasticsEurope). Relevant for society, industry, environment and policy.

Further expected results i) The patenting/description in international scientific journals of the novel plastic degrading bacteria and fungi identified and tested; ii) validation of the pilot-scale biological process developed for the degradation of PVC film from the food packaging sector, of the integrated physical/chemical and biological treatment developed for PS and PP films and of the pilot-scale developed bioaugmentation protocols, and iii) finalization of protocols for mitigating plastic pollution and

improving the environmental status of Aegean Sea.
Impact. The knowledge developed by BIOCLEAN shall contribute to: i) better know the actual potential of plastic wastes occurring in the environment to undergo natural biodegradation and the major type of naturally-occurring microbes responsible for their biodegradation under a variety of terrestrial and marine habitats; ii) improve the monitoring tools and mitigation measures for better addressing marine litter in the Aegean Sea, and iii) clarify that the current environmental impact of the EU oil-based plastic sector can be significantly reduced only by assisting its gradual transition towards a market consisting of fully recyclable (bio)plastics and plastics fully biodegradable/compostable in terrestrial and marine environments.

Project Context and Objectives:
In 2014, there was a 7.9% increase in the worldwide plastic production compared to 2012. Specifically 311 million tonnes were produced, among these 59 million tons in Europe. About 64% of the total European plastic demand is concentrated in five countries, i.e. Germany, Italy, France, United Kingdom and Spain, and is mainly for polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC) and polystyrene (PS) (Plastics The Fact, 2015). These plastics are extensively used owing to their excellent mechanical/physical properties, low costs and for their chemical/biological inertness, stability and durability. Post-consumer waste plastics are currently recovered or disposed of in landfills. In particular, in 2014 in Europe, the plastic wastes amounted to 25.8 million tonnes that were recycled (29.7%), subjected to incineration for energy recovery (39.5%) or sent to landfill (30.8%). Although the amount of plastics wastes that is disposed of in landfill is decreasing (in 2010 it was 46% of the total plastic wastes), landfilling is still the first option in many EU countries (Plastics The Fact, 2015). Plastics wastes in landfill undergo photo oxidation, degradation and erosion that often give rise to small fragments. Plastics wastes and in particular such small fragments can sorb toxic compounds, e.g. persistent organic pollutants (POPs), which accumulated on their surfaces and can enter the food chain and cause toxic effects when fragments are ingested by marine animals (De Stephanis et al. 2013. Mar Pollut Bull, 69:206-214; Xia et al 2011. Chemosphere, 82:18-24; Roy et al. 2011. Environ Sci Technol, 45:4217-4227). Moreover, plasticizers and plastics additives as well as added monomers and oligomers can leach from plastics wastes into surface and/or ground water and thus the marine environment, where they can exert toxic effects (Gregory 2009. Phil Trans R Soc B, 364:2013-2025).
From a waste management perspective, strategies aiming to increase recycling, incineration and safe landfill disposal of such materials have been developed and practiced in several EU Countries; however, several limitations still persist. In particular, there is considerable regional variation in recycling rates and actually only a small proportion of plastic waste is recycled. Items made of a single polymer are easier and more efficient to recycle than composite items, films and mixed wastes (Thompson et al. 2009. Phil Trans R Soc B, 364:2153-2166) and finally, manual of mechanical sorting of plastics to be recycled lead to an increase of the recycled plastics costs. Incineration allows to recover some of the energy content of plastics, thus is better than landfilling, but is less energy efficient than plastic recycling (Thompson et al. 2009. Phil Trans R Soc B, 364:2153-2166). As a result, efficient recycling and incineration are only practiced in a few EU countries and in 11 countries more than 50% of 2014 plastics wastes was landfilled (Plastics The Fact, 2015). Thus, the large plastics production and their very poor (bio)degradability have led to the generation of huge pollution problems that could persist for centuries. To date, little is known about biodegradability of petroleum-deriving polymers/plastics and only very few reports are available in literature (Sivan. 2011. Curr Opin Biotechnol, 22:422–426; Shah et al. 2008. Biotech Adv, 26:246-265, Raddadi et al., 2014. CIESM Workshop Monograph n°46, pp. 49 - 54). Most of the reports available in literature are focused on biodegradable plastics, like poly(vinyl alcohol), aliphatic polyesters, polycaprolactone, polyamides, oligomeric ethylene, styrene, isoprene, butadiene, acrylonitrile, and acrylate.
Taking into account this scenario, BIOCLEAN project focused on evaluating the potential of biotechnological approaches for the safe disposal and possible valorization of plastic wastes. To achieve this goal, novel ad robust microbial cultures able to biodegrade the most prominent petroleum-based polymers/plastics produced in EU, i.e. PE, PP, PS and PVC, were isolated and thoroughly investigated and characterized. This to : i) develop with them new eco-efficient bio treatments for degrading and possibly valorizing (through tailored conversion towards useful products) wastes of such plastics and ii) develop bioaugmentation protocols for enhancing native biodegradation of plastic fragments persisting in composting plants, anaerobic digesters or occurring in marine ecosystems. This would in turn contribute to prevent and mitigate the environmental impacts of plastics.

In particular, the main R&I objectives of the project were:

1) To select novel, robust and specialized microbial cultures able to significantly degrade PE, PP, PS and PVC plastic used as film through: a) enrichment and isolation of aerobic and anaerobic microbes from weathered plastic wastes obtained from marine habitats (Aegean sea and Northern sea) and terrestrial treatment facilities (i.e., European plastic storing landfills, composting facilities, anaerobic waste treatment plants) or contaminated industrial sites; b) screening of plastic-degrading bacteria and fungi available in European public and private collections;

2) To develop, optimize and assess physical (thermal, UV and gamma radiation) and chemical (O3) treatments for improving the biodegradability of selected plastics;

3) To develop pilot scale biotechnological processes and integrated physical-chemical pretreatment/ biotechnological processes, using the most effective PE, PP, PS and PVC plastic degrading microbial cultures obtained;

4) To develop tailored bioaugmentation strategies, based on the most promising plastic-degrading cultures obtained, for the intensification of biodegradation of plastic wastes and debris persisting in the Aegean Sea water and in composting/anaerobic digester facilities;

5) To develop monitoring protocols and assessment methods for plastics pollution in the marine environment of the Aegean Sea as a test case, and to develop a tentative program of measures to reduce plastics pollution in the same sea.

BIOCLEAN did not duplicate RTD activities planned in previous FP6 and FP7 projects funded in the area of environmental biotechnology and marine bioremediation. Conversely, it generated new and complementary knowledge in the sector by filling gaps currently existing in the bioremediation of petroleum-derived xenobiotic compounds, by supporting previous efforts made by EC in funding RTD projects. Further, none of the existing patents on biodegradation of synthetic plastics overlaps with the RTD activities planned in BIOCLEAN, which had much wider objectives in terms of: a) number, type and environmental and market relevance of plastics involved in the biodegradation studies, b) novelty, types and environmental relevance of microorganisms isolated/selected, and c) biodegradation tests employed and expected final outcomes and benefits for the environmental management of terrestrial solid waste disposals and the good environmental status of the aquatic environment with regard to marine litter.

Project Results:

The objectives of WP2 were the selection of microorganisms (characterized strains from culture collections and novel microbes isolated or enriched from waste plastics) and enzymes with the ability of degrading four target plastics/polymers, i.e. polyethylene (PE), polystyrene (PS), polypropylene (PP) and polyvinylchloride (PVC). In order to obtain sufficient material for the isolation of microorganisms, several sampling campaigns were carried out to obtain environmental plastic samples. Targeted regions have been the west coast of Norway in the North Sea and the Mediterranean Sea around Crete in order to collect marine samples, and one composting plant in Belgium, one landfill plant, one soil sample, one polymer production site (Styron) in Germany as well as anaerobic digestion plants in Belgium, the Netherlands, Germany, Italy and Spain. Moreover, new methodologies have been conceived and implemented for the enrichment, isolation and screening of polymer/plastic-degrading microorganisms from 63 different samples of plastic wastes collected from different environments. Screenings involved degradation tests of model compounds (recalcitrant organic compounds such as Azure B, compounds structurally related to plasticizers or additives, e.g. dimethylphthalate, BPA, butylparabene, or oligomers), growth monitoring of microbes in mineral media containing only plastics or polymers as carbon and energy sources and physico-chemical characterization of the plastic sources after prolonged contact with microbes (e.g. gravimetry, thermogravimetry analyses, size exclusion chromatography, etc.) as well as microscope analyses (SEM and AFM). The petroleum-deriving plastics/polymers used in the project were kindly provided by different Italian companies and were applied as powder or films with or without pretreatment. Microorganisms, i.e. bacteria (both anaerobic and aerobic) and fungi, but also enzymes produced by them, were thus selected as candidates for tailored polymer/plastic biodegradation screenings. Numerous candidate organisms were isolated from plastic wastes (hundreds of microbes obtained including aerobic and anaerobic bacteria, terrestrial and marine fungi, actinomycetes and anaerobic consortia from marine and terrestrial environments) in addition to those which were obtained from public or private collections (45 bacterial and 56 fungal strains). The polymer/plastic biodegradation activities were assessed by using various and complementary microbiological and chemical techniques. Microbial growth was measured by cell count, methane production (gas chromatography, GC-TCD) and consumption of electron acceptors, i.e. nitrates and sulfates (ion chromatography IC-CD), biofilm formation on plastic surfaces (Lowry method and SEM observations). The biodegradation of plastic films was evaluated by gravimetric weight loss, thermogravimetric analysis (TGA), attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR), differential scanning calorimetry (DSC), gel permeation chromatography (GPC), gas chromatography-mass spectroscopy (GC-MS), X-ray photoelectron spectroscopy (XPS), high performance size exclusion chromatography (HPSEC), high performance liquid chromatography-mass spectroscopy (HPLC-MS), 12CO2 and 14CO2 production analyses. After thorough screenings and evaluations, different microorganisms capable of pretreated and/or untreated target polymer/plastic degradation have been selected.

Trichoderma hamatum HF4 and Bacillus sp. were chosen as promising degraders of pretreated LLDPE for experiments in related WPs. Rhodococcus ruber strain C208 and a consortium consisting of five bacterial strains isolated at FHNW (Salinibacterium sp. strain BC6, Rhodococcus sp. strain BC7, Pseudomonas sp. strain BC8, Shewanella sp. strain BC9 and Lysinibacillus sp. strain BC27) were selected for further experiments due to their degradation potential towards PP. Bacillus flexus and Pseudomonas citronellolis were transferred to further WPs as they were able to cause considerable weight loss of virgin PVC films for food packaging. Penicillium variabile CCF3219 and Aspergillus terreus CCF3315, respectively, were also transferred to subsequent WPs as they were capable of considerably reducing the weight of pretreated PS. Marine fungal isolates and fungal strains from the UFZ collection were unable to cause a substantial degradation of the investigated plastic films in terms of mass losses, yet especially M-SR1-I1, 1-DS-2013-S2 and 1-DS-2013-S4 were able to degrade plasticizers.

Enzymes have been tested for their potential to modify the target polymers. Candidate enzymes including 5 different peroxidases [3 manganese peroxidases, MnP, from Bjerkandera adusta (BadMnP), Nematoloma frowardii and Stropharia rugosoannulata; 1 unspecific peroxidase, UPO, from Agrocybe aegerita (AaeAPO); and 1 dye-decolorizing peroxidase, DyP, from Auricularia auricular-judae] and one laccase (Lac, from Pycnoporus cinnabarinus) were selected for degradation of virgin PS, PVC and HDPE powder. Furthermore, 1 commercial glucose oxidase (AniGOX, from Aspergillus niger type VII) was tested for degradation/depolymerization of virgin PS powder, virgin and pretreated (UV and X-ray) PS, virgin PVC films and PVC film after removal of plasticizers. Finally, BadMnP, AaeAPO and AniGOX depolymerization activity was evaluated against linear polystyrene sulfonate (PSS) as soluble homologues of PS. The enzymatic depolymerization activity of BadMnP, AaeAPO and AniGOX was observed against PSS; the combination of the last two enzymes showed a breakdown of up to 70% of the PSS (at 2 g/L). FTIR, GPC and TGA analyses performed on pretreated PS films exposed to AniGOX displayed little changes of the polymer, while no degradation of virgin PS and PVC films and of pretreated PVC film was observed. No distinct degradation effects for virgin and pre-treated plastics were achieved by any of the tested enzymes and no release of significant amounts of fission products was observed. Hence, due to lack of effectiveness on target polymers in further tests, work on enzymes has been suspended in favor of whole cell degradation approaches.

The active microbes selected based on the previous screening activities were used to further study the plastics/polymers biodegradation extents and mechanisms (WP3). Specifically, mineralization of PS was proven for the first time using 14C-ozonated PS and P. variabile. Anaerobic consortia enriched from plastic waste obtained from both terrestrial and marine habitats showed virgin PVC film weight loss of up to 20 % after up to 18 months of incubation (abiotic controls up to 7%). Chemical analyses suggested that PVC polymer chains are attacked by some consortia; SEM observation showed extensive biofilm formation on PVC surfaces (Figure 2). Additives of virgin PVC films were shown to be the main components degraded by aerobic bacteria and fungi. Attack to low molecular weight chains of pretreated LLDPE was observed for the bacterial strain Bacillus sp. B17. Little biodegradation yields of PEs and PS (≈1.5% weight loss) by different fungal strains were detected. None of the tested enzymes resulted in substantial breakdown or conversion of plastics, only plastic-related model substrates are attacked by GOX-mediated Fenton systems or UPOs. Finally, no toxic evidences were found in cultural broth of bacteria incubated with PVC under aerobic conditions or fungi incubated with PVC and PS. Results of Ecotox (based on V. fischeri responses) displayed some toxic effects of the culture supernatants of the anaerobic consortia incubated with PVC but also in the related abiotic controls that could be due to the release of toxic compounds that probably were generated during the process of production of thick PVC films (obtained by pressing five PVC thin layers at P=17.44 bar, T=160 °C for 4-5 minutes) used, into aquatic systems after 18 months of incubation. Broths from 2 active fungal cultures incubated with PEs were found to be slightly toxic or mutagenic.

In the frame of the project, one workpackage was dedicated to the evaluation of the effect of different treatment methodologies on the biodegradability of the target plastics/polymers (WP4). Since the beginning of the project, different methodologies and processes have been built up to decreasethe hydrophobicity of the polymers/plastics, the polymer matrix molecular weight and the macrostructure (crystalline morphology) as prerequisites for improving plastic bioavailability and biodegradability. To be able to do that, oxidation processes were chosen and control on their kinetics was attempted. The chosen strategy is based upon two key concepts: single or cycled and/or coupled (various) oxidative stresses. Specifically, a single oxidative condition is firstly applied and in a second step, in a more innovatively manner, two oxidative stresses were applied consecutively in the case of cycling (for instance: gamma (or UV) irradiations + thermal treatment) or simultaneously in the case of coupling. Finally, the sustainable character of the developed processes and their ability to be scaled up (for WP5) were considered. All the various conditions of cycling and coupling (i.e., nature of oxidative stresses, conditions of exposition, etc.) have been tested on the different plastics. For all the target plastics X rays, Gamma and UV irradiations cycled or coupled with thermal treatments have been used. Hybrid cycling treatments (oxidation cycled with exposition to Rhodococcus rubber) were also performed onto PP, PS and ozonated PP, and PS. Finally, O_3 exposition, and the coupling of O_3 exposition with UV irradiation (air or water phase) have also been performed on all the target plastics. A great number of pre-treated samples were produced and analyzed from a chemical point of view (with infrared spectroscopy) and physical one (via melt viscoelasticity) to determine the best treatment for each target plastics as any different plastic material has its own specific degradation mechanisms. Biodegradation of untreated and treated plastics has been studied in liquid medium (respirometry), in compost and in soil. While liquid medium respirometry allows for the direct measurement of CO_2 emission, a new way of determination of molecular weight decrease in the case of soil and compost has been developed. This methodology is based upon the determination of the evolution of the melt Newtonian viscosity (η_0) which is directly linked to the weight average molecular weight Mw through a power law relationship: $\eta_0 = K.Mw$ The most important increase in biodegradability was found in the case of gamma-irradiated PVC and to a lesser extent with LLDPE, PP and PS. Anyway, no full biodegradation was achieved for any of the plastics tested. It was assumed that it is necessary to decrease the polymer average molecular weight below the critical molecular weight for chain entanglement (Mc). The disentanglement of macromolecular chain should be a key step to allow biodegradation and bioassimilation with CO_2 evolution; under experimental conditions performed, the range 1 to 10 critical molecular weight (Mc) was achieved with the treatment while it had probably necessary to go down one decade below (0.1 to 1 Mc). Finally, the coupling of UV exposition with ozone gas atmosphere in a fluid bed reactor has been selected as the most promising pretreatment for upscaling (WP5). This kind of Advanced Oxidation Process (AOP) is very versatile as it allows acting on the UV intensity, the ozone concentration, the time and temperature of treatment and it is also possible to prevent autoxidation (fluid bed thermal exchange). Even though, time constraints did not allow exploring all the capacities of the hybrid cycling chemical oxidation/biological exposition; however, it was scaled and tested. It is now possible to increase a lot the degradation level with the aim to reach the aforementioned molecular level (0.1 to 1 *Mc).

Most effective physical/chemical pre-treatments developed in WP4 were scaled up and optimized in WP5. Scaling up of the AOP for polymer degradation through controlled oxidation, based upon the coupling of UV radiations together with an ozone enriched atmosphere, was performed through the development and construction of LUVOR reactor. The scaled up two-phase system comprised of a closed stainless steel reactor usable in both gas and liquid phases. Such design allowed for its additional usage in an external biodegradation cycle. Hence, after pretreatment, subsequent incubation of pretreated plastics with microbial biomass to achieve biodegradation was realized in the same system. The reactor was successfully applied to perform all steps necessary for pretreatment and biodegradation processes of PP by R. ruber and PS by P. variabile. For the pretreatment of PP, the LUVOR system proved to be especially useful, as UV irradiation in addition to ozonation drastically changed the properties of the substrate during treatment, based on infrared spectroscopy and melt viscoelasticity analyses. Protocols for the reproducible growth of strains selected in WP2/WP3 as efficient plastics degraders and for the inoculum production for pilot-scale biodegradation experiments carried out in other WPs were developed. These include detailed information on the growth conditions (carbon and nitrogen sources, exogenous electron acceptor, pH, inoculum, etc.) and the equipment needed as well as the methods to be applied for quantification of the amount of biomass produced. The list of developed protocols included liquid cultures of aerobic bacteria and fungi obtained from culture collections or isolated in the frame of the project from marine/terrestrial environment, and anaerobic consortia enriched from anaerobic digester or landfill-deriving plastic wastes as well as solid-phase fungal cultures.

Scaled up experiments investigating bioremediation of plastics under submerged conditions included the application of P. citronellolis for virgin PVC in 15-L stirred tank reactor, and of R. ruber C208 and P. variabile in the integrated physical/chemical (UV/O3) and biological treatments of PP and PS, respectively, in the LUVOR reactor. For virgin PVC films, no release of significant amount of Cl- was detected during biodegradation, but a gravimetric weight loss of 21±0.5% was observed at the end of the experiment (after 57 days). The PVC film incubated with P. citronellolis showed higher thermal stability compared to the non-incubated one, while the molecular weight of the incubated PVC did not change significantly. These findings suggest that PVC films was partially degraded, and that degradation mostly concerned the additives rather than the polymer chains. Gravimetric weight loss measurements, oxygen consumption and TGA analyses showed that the pretreatment of PP in the LUVOR system was effective and the subsequent biodegradation by R. ruber C208 provided results comparable to those obtained in small-scale experiments using this polymer/degrader combination. No significant biodegradation was observed in the case of PS subjected to the combined (UV/O3) and P. variabile treatments upscaled in the LUVOR reactor.
Moreover, degradation capacity of plastics-degrading organisms selected in previous WPs was investigated in soil, under solid-state fermentation (SSF) using 2 bacterial and 4 fungal strains and wheat straw as the substrate, and composting processes. The evaluation of plastic film biodegradation potential in artificial/inert soil (applying an artificial fungal consortium and 1 bacterial strain) and in vermiculite (as inert support to substitute mature compost) was carried out using P. citronellolis. Significant weight losses were obtained for virgin PE samples with Bacillus sp. (up to 7.4%) and for virgin PP with T. hamatum (up to 3.8%). Based on CO2 measurement, significant biodegradation was observed only for PVC film in inert soil (around 24%) inoculated with Gloeophyllum consortium and in the presence P. citronellolis in vermiculite (27.4±0.0).

Work Package 6 was dedicated to the development of bioaugmentation strategies for marine environment and for composting facilities applying the most effective plastic degrading microorganisms selected in the previous WPs, with an effort to enhance biodegradation of PE, PP, PS or PVC waste plastics occurring in polluted marine habitats or terrestrial treatment facilities (composting, landfill/natural soil and anaerobic digester).
In the frame of bioaugmentation under surface seawater conditions, a small platform was constructed and installed being able to carry stainless steel cages submerged in seawater that were close to the surface to have near maximum natural UV irradiation. Several designs were implemented but they were all beaten badly by the waves during the winter months (Nov 2013 to Feb 2014). The platform was finally successfully modified to stand severe weather conditions and carry at least 12 stainless steel cages. In addition, the initial design consisted in glass or fiberglass culture vessels placed on top of the platform with the objective to mimic seawater field conditions in terms of temperature, sunlight, wind excluding the dilution effect of the seawater currents. However, until the very end of the project, the biodegradation observed on virgin plastics was very small and it could be masked due to weathering by sunlight, i.e., loss of weight due to fragmentation to very small particles (creating the so called “invisible plastics”). Therefore, it was important to be able to discriminate weathering (leading to fragmentation) from biodegradation both leading to weight loss. Furthermore, because of continuous evaporation it would have been necessary to refill regularly with distilled water otherwise, the salinity would continuously increase to very high values. The above restrictions led to the conclusion that the best way to mimic seawater conditions but with no weathering due to UV light, was to bring the experiments “indoors” but use non-sterile seawater and supplement with distilled water any water loss that was observed during the 7-month bioaugmentation experiments.
Among the different categories of plastics, weathered PE and PS were selected in order to carry biodegradation experiments under near-field conditions (i.e., in non-sterile seawater and ambient temperatures) using bioaugmentation with special cultures from WP2. Tailored combinations of marine bacteria previously tested for their ability to degrade virgin polymers of PS and PE, i.e., three consortia composed of special marine bacterial cultures together with the autochthonous marine microbial community, were inoculated in microcosms in the presence of naturally weathered PS and PE. The final aim was to monitor any potential degradation of naturally weathered plastics caused by the microbial community. The experiments were conducted in microcosms simulating field seawater conditions excluding the dilution effect of the currents. Under such conditions, significant average weight losses were recorded for weathered PE (up to 16%) and PS (up to 15%) after seven months of incubation with different consortia, compared to a max of 4% for PE and 0% for PS of weight loss in the presence of only indigenous marine microbes (control treatments). Both plastic films showed a well-developed biofilm, those on PE were able to change the surface topography of the films, while those on PS created fissures and holes on PS surface. Thus,

BIOCLEAN consortia exhibited a high yield and rate of biodegradation of marine debris provided that the plastic pieces were sufficiently weathered (which also explains the observed variability among replicates).

Bioaugmentation experiments were performed also under terrestrial environments. Specifically, the ability of 4 fungal and 2 bacterial strains to grow and degrade virgin PEs, PP, PS and PVC as well as (ɣ+T) treated LLDPE films under composting conditions was studied. The addition of microbes obtained from terrestrial environments resulted in biodegradation yields above 9% weight loss vs. PVC films under controlled composting conditions where, however, none or very low weight losses (max 2.0±2.9%) were recorded for virgin and pretreated PEs, PP and PS films. These findings confirm the low influence of inoculation using selected cultures on plastics biodegradation under real composting conditions. Also, bioaugmentation strategies focusing on PVC film in mature compost (with P. citronellolis), soil (using a consortium of different Gloeophyllum strains or R. ruber) and under anaerobic conditions (with consortia of methanogenic archaea and nitrate reducing bacteria) were investigated. PVC films can be degraded in mature compost, soil or anaerobic digester sludge conditions given the biodegradability of the additives. The addition of PVC film biodegrading bacteria resulted in significant increases of the rate of biodegradation of PVC films under different real conditions (eg, anaerobic nitrate-reducing consortium, under anaerobic digestion conditions, and with P. citronellolis in compost aerobic conditions).

WP7 was dedicated to demonstrate and validate the effectiveness of the bioaugmentation protocols developed in WP6 for the biodegradation of plastic debris and wastes i) in seawater with local currents, in the Northwestern coastline of Crete Aegean Sea under site conditions and ii) in a large scale composting facility in DEDISA. The evaluation of bioaugmentation under field conditions and the monitoring of the degradation of plastics in the marine environment were performed carrying out field experiments in the Aegean Sea (in Eastern Mediterranean Sea) near the northwest corner of the TUC campus. In order to examine whether biodegradation takes place after significant weathering in seawater, the weathering process was studied first using plastic bags and hard plastic materials made of PE, PP, PS or PVC that were placed in submerged cages near the water surface for one year. Weathering (fragmentation) was also studied under lab conditions (in aquariums and on sand) mimicking the real conditions. Based on the experimental data collected, a mathematical model for the rate of fragmentation was developed. Furthermore, a microscopy-based image analysis protocol was developed to enable the estimation of the "invisible plastics" produced due to weathering. Analysis of the results proved that, despite the fact that Aegean Sea is an oligotrophic environment, biofouling, i.e., adhesion of living organisms to plastic surfaces, occurred very quickly. Marginal physical and chemical modifications of weathered plastics, especially those that did not contain any UV stabilizer, were measured based on tensile and GPC analysis under both, seawater (field) and onshore (beach sand)conditions. Experimental findings suggest that fragmentation of plastic bags depends on the degree of weathering on beach sand (best quantified by luminance exposure) and on mild mechanical stress exerted on the weathered plastics. If the mechanical forces are not included, the weathered plastic may look as if it is intact, however, with the slightest mechanical force it crumples into many fragments.

Attempts to validate the bioaugmentation strategy in DEDISA composting facility were carried out by evaluating the ability of terrestrial strains (selected in previous WP) to degrade PP, HDPE and LDPE plastics, commonly found in compost material. Mesocosms consisting in 100 kg of maturing compost in the presence of A. terreus and T. hamatum were set. Abiotic controls were also performed. After 175 days of mesocosm incubation under conditions simulating the composting process in DEDISA's facility, i.e. water addition, mixing for aeration and temperature monitoring; no significant biodegradation of the plastics occurred according to gravimetric weight loss percentage criterion.

The Life Cycle Assessment (LCA) of the developed bioremediation processes was performed in order to assess their environmental impact and sustainability (WP8). The Eco-indicator 99 methodology was applied for the calculation and presentation of the results. The development of bioremediation processes exploiting the microorganisms selected in the project was limited to the two kinds of plastics that were significantly biodegraded by the isolated and tested cultures, namely PP and PVC. Experiments with the other plastics, PE and PS, did not lead to significant biodegradation and therefore they were not taken into account for this LCA study. Based on the data inventory gathered for LCA and data provided by partners, an economic assessment was carried out. In general, the economic evaluation followed the trend of the environmental assessment. The PVC biodegradation by P. citronellolis and PP pre-treatment and bioremediation with R. ruber are the most expensive treatments and far too expensive to compete with conventional plastic waste treatment methods. The bioremediation based on industrial composting and soil bioremediation resulted in relatively low score. It is important to keep in mind that these processes were not subjected to a scale-up. The data obtained from lab scale experiments may differ significantly from that ones obtained from up-scaled processes (especially in terms of energy consumption). As such, this information should only be used as an indication providing guidance for further research and optimization. Finally, the microbiological risk assessment of the developed processes/strategies was carried out. It focused on hazard identification, hazard characterization, exposure assessment and risk characterization in accordance with the guidelines of Food and Agriculture Organization of the United Nations (FAO), World Health Organization (WHO), U.S. Department of Agriculture/Food Safety and Inspection Service (USDA/FSIS) and U.S. Environmental Protection Agency (EPA). The risk assessment performed led to the conclusion that R. ruber, P. citronellolis and P. variabile microorganisms used for the bioremediation processes of PP and PVC are not known to cause diseases in healthy adult humans and are of only minimal potential hazard to humans and the environment (CDC). Therefore, standard open laboratory benches may be used to handle these strains e.g. for inoculum production. Their further use for bioremediation purposes does not require a special containment or a confined environment. None of the applied microorganisms were genetically manipulated. Therefore, the overall microbial risk associated with the developed biotechnological processes in large-scale bioreactors or for bioaugmentation of compost matrix can be considered to be very low.

In the frame of the Bioclean project, WP9 was dedicated to the development of tools supporting policy of the Marine Strategy Framework Directive (MSFD) related to plastics debris. Specifically, the objectives were: i) to provide support to policy-makers by developing monitoring protocols and assessment methods for plastics pollution in the marine environment focusing on the Aegean Sea as a test case; ii) to develop a program of measures to prevent plastics and microplastics entering the marine environment and iii) to promote synergistic activities with other research initiatives on micro-plastics.

In order to assess the current environmental status of Aegean Sea, preliminary investigation on the identification and characterization of major terrestrial sources (of marine litter) around the Aegean Sea started in 2013. Search for polluted areas along the Cretan coastline, including those where there is no human intervention (as control cites), was also conducted. The nine (9) most heavily polluted with marine litter/debris sandy beaches along the north and north-west coastline of the island of Crete (e.g., Cavo Sidero, Dionyssos, Pachia Ammos, Heraklio, Rethymno, Almyrida, Stavros, Balos and Falassarna) were then chosen as sampling sites in order to study, also, the presence of persistent organic pollutants (POPs, i.e. polycyclic aromatic hydrocarbons –PAHs- and polychlorinated biphenyls -PCBs) on plastic debris. Three sampling campaigns were conducted and plastic fragments < 5 mm and beached plastic pellets (3000 plastics fragments and 3000 plastics pellets) of all types (various colors, new, weathered, etc.) were found and collected. Counting the number of fragments and pellets and weighting were done in the laboratory, in order to estimate their densities (abundance) and load in the sea area in front of the selected sampling sites. Seasonal variations of these densities were examined by means of repeated sampling every month in the same areas. Accordingly, pyrogenic and/or petrogenic origin of PAHs was determined and interestingly a seasonal fluctuation in type and concentration of PAHs into plastic debris was observed. Contamination by PCBs varied from 23 to 256 ng/g plastic debris, the highest concentration was found in a bay beach close to an urban area with a commercial port and a Naval base, which confirm PCBs tendency to remain close to their source area.

Micro- and macroplastics pollution was evaluated for different seasons in 4 beaches in the northern coast of Crete. Microplastics (about 2000 items including fragments and pellets) were sorted, categorized according to certain physical characteristics such as size, shape and type (transparency, granules, fibers...), counted and weighed. It was found that mean densities (abundance) and weights were generally lower in winter than in summer, while no unique tendency in debris stratification in the beach (shoreline, middle part and away from the shoreline) was observed. No pellets were detected in the water column, while fragments abundance of microplastic differed within sampling sites and a homogeneous distribution of the size categories (mm) examined was observed. It was deduced that microplastics are generated on beaches and then they disperse into the sea, probably due to rough weather conditions and wave action during wintertime. It was estimated that landfills, urban areas and beaches and rivers accounted respectively for 6300; 2.4x106 and about 545 tons/year of plastics influxes to the Aegean sea, quantities higher than those from marine sources (fishing gear, passenger and cargo vessels). Finally, from the analysis of mass balance of plastics it was found that the continuous accumulation of plastics in the marine environment keeps on increasing at the rate between 51272 and 123472 tons/y.

Based on these findings, moving towards a Good Environmental Status of the Aegean Sea, requires the immediate implementation of defined mitigation measures/strategies. These include regular clean ups of beaches with plastic debris, incentives for trawlers to bring collected plastic debris to the shore, phase out plastic micro-granules in cosmetics and health care products, and promote sustainable handling procedures for plastics. It is also very significant to raise awareness of the public particularly to the inhabitants of the many islands in the Aegean Sea.

Synergy and integration with other scientific groups involved in Marine Litter was also taken into account. The BIOCLEAN project invited other marine litter EU projects, namely CLEANSEA - "Towards a Clean Litter-Free European Marine Environment through Scientific Evidence Innovative Tools and Good Governance" (www.cleansea-project.eu; coordinator Dr. Heather Leslie) and MARLISCO – "Marine Litter in European Seas - Social Awareness and Co-Responsibility" (www.marlisco.eu; coordinator Ms. Doriana Calilli) and DeFishGear – "Derelict Fishing Gear Project in the Adriatic Sea" (http://www.defishgear.net/; coordinator Dr. Andrej Kržan) to disseminate/discuss during scientific and technical events like Ecomondo (Rimini, IT). Discussions have been focused on the organization of a common project meeting and exchange of scientific information. A brochure of 18 projects in the Mediterranean, Black and Adriatic Seas has been prepared and distributed widely by UNEP/Barcelona Convention supporting an Ecosystem Approach.

Moreover, one of the identified key initiatives was GESAMP's Working Group 40 (WG40). WG40 was working between 2012 and 2014 on the publication of Report 90 in which three elements also tackled in project BIOCLEAN were of value for the GESAMP team: i) Are biological mechanisms able to degrade plastics and to which extent?, ii) how does physical effects like UV radiation, ozone and heat impact plastics as this might support evidence how plastics behave in the environment? and iii) how does plastics accumulate on beaches, surface and deeper sediments? The BIOCLEAN was represented by PLASTEU who attended as official observer various working group sessions of GESAMP WG 40 (Paris 02.2012, London 09.2013, Busan 07.2014 and Ecuador 10.2015) and used those opportunities to present certain interim results (e.g. presentation shown during the half term meeting in Ostrava (PL, 06.2014) were partially shown at the GESAMP meeting in Busan KR) and used the knowledge gained by BIOCLEAN to comment on the draft versions of the GESAMP Report 90.

Potential Impact:

The major results obtained in the frame of BIOCLEAN project can be summarized as follows:

• A collection of 65 robust microbes able to partially degrade/transform films of plastics of polyethylene (PE) (7 cultures), including its different variants Low Density PE (LDPE) and Linear Low Density PE (LLDPE), polypropylene (PP) (2 cultures), polyvinyl chloride (PVC) (53 cultures) and polystyrene (PS) (4 cultures). The microbes are belonging to the partners who selected/isolated them. They can be patented, described in scientific international publications, used for developing tailored processes. These results are relevant under industrial point of view and for the protection of the environment.

• New, pilot-scale assessed biological or hybrid chemical/physical-biological processes able to partially degrade PVC films and, to less extent, PE and PP plastic films. They can be patented, and described in scientific international journals. These results are relevant under industrial point of view and for the protection of the environment.

• Lab and pilot-scale bioaugmentation protocols for intensifying (micro)plastics biodegradation in soils, composting/anaerobic waste treatment facilities and marine environments. They often displayed limited activity but will be described in international publications.

• Site-specific measures for mitigating plastic pollution and improving the environmental status of Aegean Sea. They can be described in international publications. These results are relevant for the protection of the environment and the development of local policies.

According to the information available on existing patents and previously funded European projects in the sector of plastics bioremediation, BIOCLEAN is the first European RTD project to provide concrete evidence and experimental results. In particular BIOCLEAN is:

• the first broad and systematic program dedicated to the enrichment, isolation and characterization of specialized consortia and pure cultures of bacteria and fungi capable of degrading PE, PP, PS, and PVC polymers and plastics from a variety of on site aged plastic wastes collected from European contaminated marine areas, landfills, and waste composting and anaerobic digesting facilities ;

• the first attempt to evaluate the effectiveness of a variety of biooxidative (lignin- modifying), unspecific macromolecule-

depolymerising and hydrolytic enzymes towards PE, PP, PS and PVC polymers and plastics;
• the first attempt to develop, assess, compare and integrate physical methods (thermal, UV, gamma radiation) and ozonation as pretreatment to improve the biodegradability of PE, PP, PS and PVC polymers and plastics at laboratory and pilot scale.
• the first project in which a wide and systematic study on the biodegradation of PE, PP, PS and PVC polymers and plastics under laboratory, pilot scale and field conditions is proposed and assessed under environmental and economical points of view. It is also the first project in which the possibility of valorizing PVC plastics via conversion into valuable chemicals and building blocks for new green polymers preparation was investigated;
• the first attempt to test bioaugmentation strategies for intensifying the natural biodegradation of microplastic and debris in surface water (in this case of the Aegean Sea), and in contaminated matrices coming from composting and anaerobic digesting facilities from different European Countries;
• the first attempt to quantify fragmentation rates of weathered plastics in the water column and onshore. Fragmentation rates were found to be related to cumulative luminance exposure, presence of oxygen during exposure and application of mild mechanical forces.
• the first attempt to develop mitigation measures for the Aegean Sea based on validated monitoring protocols for polymeric pellets and microplastics debris found onshore (surface & buried in the sand) and in the water column and on fragmentation rates of beached plastics.
The knowledge developed by BIOCLEAN should contribute to: i) better understand and evaluate the amenability of plastic wastes occurring in the environment to undergo natural biodegradation in different environments (terrestrial and marine) and identify the major type of naturally-occurring microbes responsible for their biodegradation in the different terrestrial and marine habitats studied; ii) improve the monitoring tools and mitigation measures to better address the problem of marine litter in the Aegean Sea, and iii) better clarify if the current environmental impact of the EU oil-based plastic sector can be effectively mitigated solely by assisting its gradual transition towards a market consisting of fully recyclable (bio)plastics or biodegradable plastics (in the terrestrial and marine environments).
Consequently, the results generated by the BIOCLEAN project provide new knowledge and depicts future innovation in the management of conventional plastics waste and the reduction of marine litter. Furthermore, the proposed site-specific measures for mitigating plastic pollution of the Aegean Sea will contribute to the implementation of EU policies and regulatory frameworks (i.e., Marine Strategy Framework Directive (MSFD) with respect to plastics) in this area and in other EU plastic-polluted marine basins.
In a nutshell, BIOCLEAN proposed solutions towards achieving a Good Environmental Status of EU sea regions with regard to marine litter as required by the MSFD. Good environmental status of the marine environment means to significantly reduce “Harm”, which can be divided into three general categories: i) Ecological in relation to mortality or sub-lethal effects on plants, and animals through entanglements, capture and entanglement from ghost nets, physical damage and ingestion including uptake of microparticles (mainly microplastics) and release of sorbed chemicals; ii) Social, which is related to the acceptance of aesthetic pollution and public safety; and iii) Economic which is related to different contexts, such as additional costs in terms of touristic activities, damage to vessels, economic losses concerning fishing gear facilities and further fishery activities such as the emerging aquaculture sector as well as cleaning coasts.

A special effort has been devoted to ensure that BIOCLEAN-produced knowledge could be easily uptaken, already during the project progression, directly, by the industrial partners and, indirectly, by the members of the Stakeholder Advisory Board (SAB). The key role played by PlasticsEurope in the exploitation of the results of the BIOCLEAN project have markedly contributed to this objective.

Finally, BIOCLEAN contributes to at least some of the societal challenges that EU faces by:
i. providing information on the actual potential of biological processes and strategies for mitigating the marine and terrestrial environmental impact associated with the growing amount of plastic wastes produced and accumulated yearly in EU, where they are considered highly recalcitrant and strongly impacting the marine ecosystems.
ii. favouring innovation and technology transfer, with a particular attention to SMEs, through the involvement of 7 SMEs active in complementary areas, by considering and mutualizing their complementary expertise and interests, thus contributing to the

development of innovative and integrated solutions and tailored strategies for complementary and diversified markets. Therefore, BIOCLEAN brought one of the first stones for the improvement of the quality of life through health, environmental and economic benefits to the local communities.

Dissemination and exploitation of project results

Dissemination and exploitation activities have been carefully planned by BIOCLEAN consortium since the beginning and continuously monitored and implemented along with the project execution.

In order to achieve effective dissemination of results, the following strategies were adopted:

1. Website creation and updating. The website was the main face of BIOCLEAN, enabling anyone to find out about the project, request more information. It was established in the framework of PasticsEurope's website which also managed it in close cooperation with the coordinator of the Project. All products, unless differently specified by Consortium Agreement, were freely available. The website in addition provided a password protected area allowing all project partners to regularly contribute to it with information on progress of the project. This function of "consortium partners to share information amongst each other" was additionally supported by a BASECAMP project area which allowed all members to upload and share presentations and reports.
The project website was and is a platform for external communication, fed with background information about the project.
i. Information about the consortium partners, detailed explanations of the scope of the project and the different working packages.
ii. Information on the training activities and active participation of consortium partners at conferences - prepared within the dissemination WP (WP10)

2. Electronic newsletter. PlasticsEurope provided several articles and interviews in its industry newsletter called NEWSLINK and by that informed the plastics producing companies in Europe about latest developments of project BIOCLEAN. In addition, PlasticsEurope established a BIOCLEAN Microblog (http://www.scoop.it/t/ bioclean) informing public not only about the progress of project BIOCLEAN but also on innovations and legislative, socio-economic, environmental and economic information addressing the sustainable management of plastic wastes, biodegradable plastics and the protection of marine environment from plastics. This information included topics like legislation and technological developments, content from leading relevant journals, including abstracts of highly relevant papers and international conferences and industrial symposiums related to the specific objectives of BIOCLEAN work plan and project-related communications, including newsletters. This channel was available to everyone with the additional ability to receive ad hoc news to mail via RSS feed and disseminated the results to the stakeholders. No relevant research data, nor confidential data were diffused with this tool.

3. Press releases. Specific press releases were prepared and sent out to a list of relevant scientific journalists interested in the thematic area before and after core events, workshops, meetings to which BIOCLEAN partners took part, training session organized, to inform about the events, promote the project activities and results with the most dynamic approach (2 articles were published including one on European Biotechnology and one on YourIS.com). Further articles were placed on Plastics the Mag an online e-magazine informing about latest innovation in the plastics world. This e-magazine is dedicated to the general public but also read but the plastics value chain in Europe.

4. Participation to international meetings. Partners have been invited as speakers in 04 conferences related to innovation in the sector of plastics, that of environmental microbiology and biotechnology as well as marine ecosystem protection and heath (1st Annual Conference of International Society for Biomedical Polymers and Polymeric Materials, Washington DC, USA; EurOcean 2014, Rome, Italy; CIESM 2014, Tirana, Albania; Mediterranean Conference on Combating Marine Litter in the Adriatic MacroRegion 2014, Athens, Greece). Furthermore, 35 oral presentations and 25 posters were presented in European and international conferences by partners. These activities were extremely relevant to raise awareness about the project

research and resulting outcomes and to give and assure a high visibility and networking possibilities.
Four special sessions dedicated to the BIOCLEAN project were organized including one within the 3rd International PLASTICE Conference Future of Bioplastics (Warsaw, 2013), one at the ECOMONDO fair (Rimini, Italy-2014) and two at the 6th European Bioremediation Conference (Chania, Crete-2015).

5. Publications. Currently, 05 papers of scientific relevance (including peer reviewed papers and extended abstracts of international congresses) have been published and more than 10 will be submitted to the most opportune scientific journals, by the partners, in order to disseminate results to the scientific community and increase global expertise and sharing the generated knowledge.

6. Trainings organisation. Trainings were critical in engaging the targeted audience in the discussions of BIOCLEAN key-concepts. They were organized in DEDISA municipality plant, where pilot and field experiments were carried out, in conjugation with project meetings, by the hosting partners under the supervision of PLASTEU. The technical specification of such sessions, their scheduling and content were disseminated through the project web site. Training activities addressed to transfer and apply the foreground generated in terms of protocols, technologies and assessment analysis tools and methods, identified at lab scale and exploitable at industrial and filed scale, were also organized for the young scientists and engineers joining the Project. In particular, in the frame of ECOMONDO 2014 fair a workshop for BIOCLEAN young scientists was organized.

7. Stakeholder information. An Advisory Board (SAB) composed, among other, by outstanding (European, and extra European) scientists and stakeholders with complementary skills and technical/scientific expertise was established. SAB members were informed on achievements of the Consortium during the SAB meeting and had the opportunity to discuss the results and provide inputs to the ongoing/further RTD activities. Both the Stakeholder event and the SAB meeting were organized during the ECOMONDO 2014 fair. Other prominent stakeholders were reached through ECOMONDO 2015 fair, Bioeconomy Investment Summit 2015, (Brussels, Belgium), the website and 3 newsletters of PLASTEU and of initiatives in which PLASTEU is involved in, such as GESAMP.

8. Final conference. UniBo in cooperation with Plasteu, TUC and DEDISA organized the BIOCLEAN conference within EBC-VI (06th European Bioremediation conference) for BIOCLEAN dissemination. The EBC-VI took place in Chania, Crete from June 29th to July 2nd, 2015; is the 6th edition of a series of quite successful conferences all hosted in Chania, series that remarkably contributed all along the last 15 years, to an effective implementation of environmental biotechnology principles and practices in Europe. The decision to organize the BIOCLEAN scientific conference, specifically two special sessions open to the public, within EBC-VI is justified by the fact that the event was considered extremely relevant to raise awareness about the project research and resulting outcomes and to give and assure a high visibility and networking possibilities. The conference was organized by the Technical University of Crete in cooperation with the University of Bologna. It was supported by the Environmental Biotechnology section of the European Federation of Biotechnology and by the Asian Federation of Biotechnology (marine biotechnology section) as well as by Wiley and Elsevier. It was sponsored by EPE, Hellenic Petroleum, INTEGEO and ECOMONDO. The International Scientific Committee of EBC-IV included 24 skilled scientists from different institutions above the world including Germany, China, Austria, Italy, Jordan, Greece, Belgium, USA, Morocco, Tunisia, Switzerland, Saudi Arabia, Czech Republic and Egypt.

9. Guidelines for Policy Makers and Authorities. Guidelines, regarding the results from the project and its implications towards waste management and mitigation of environmental pollution through plastics were developed in form of a brochure and subsequently transferred to EU policy makers (e.g. BioEconomy Summit 2015) and to industry during the global meeting of national plastics associations in Yuyao 09.2015.

10. Guidelines for general Audience. Guidelines of practical use and describing the project results of interest for the citizens were delivered to EU commission, National Authorities and competent bodies, to be further disseminated by the

National/European channels ordinarily in charge of the communication and education activities. Like described in 9) the developed brochure is published in open areas of the website to be of immediate and direct use from the citizenship. This material will increase the general knowledge of the EU citizens in methods, solution and tools which can be implemented and adopted for reducing plastics accumulation in landfills and the plastic occurrence and impacts in marine ecosystems.

The project produced foreground that will be protected through patents in the near future. The IPR protection of the novel microbes and biological processes/technologies for the valorisation and/or biodegradation of plastics is essentially ambitioned. Specifically, the foregrounds suitable for IPR protection include: i) the novel/robust plastic-degrading pure or mixed cultures of bacteria and fungi; ii) the novel or improved physical and/or chemical treatment of PP and PS polymers and plastics for improving their biodegradability; iii) the design and development of the LUVOR reactor for integrated physical, chemical and biological treatment of plastic and the pilot-scale slurry bioremediation processes for the biodegradation of virgin or pre-treated plastic films and iv) the acclimated bacterial consortia with PS and PE degrading capabilities (resulting from specialized cultures added to indigenous marine microbes with PS and PE as sole carbon sources), including their potential synergistic effects for PE degradation.
Moreover, the project produced foreground that is suitable for dissemination such as: i) protocols for the enrichment and isolation of plastic degrading aerobic and anaerobic microbes, bacteria and fungi, from plastic wastes; ii) integrated analytical methodology to determine rates, extents and final products of the biodegradation of target plastics by the selected microbes; iii) efficiency of biodegradation of weathered plastics under surface seawater conditions; iv) extent of the biodegradation of plastics under terrestrial conditions (compost, soil anaerobic digester); v) mathematical models for fragmentation rates and development of novel protocols for evaluating the level of fragmentation and weathering of plastics offshore and onshore; vi) fate and efficiency of biodegradative activities of BIOCLEAN strains used in bioaugmentation in DEDISA composting plant; vii) environmental and economical assessment of the technologies and bioaugmentation strategies developed; viii) development and validation of monitoring protocols and assessment methods for marine pollution by plastics debris (including polymeric pellets and microplastics); ix) development of mitigation measures for plastics and microplastics towards achieving Good Environmental Status of the Aegean Sea.

List of Websites:
http://www.biocleanproject.eu

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