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Biodegradation of mixture of plastic films by tailored marine consortia

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ABSTRACT

This work sheds light on the physicochemical changes of naturally weathered polymer surfaces along with changes of polymer buoyancy due to biofilm formation and degradation processes. To support the degradation hypothesis, a microcosm experiment was conducted where a mixture of naturally weathered plastic pieces was incubated with an indigenous pelagic community. A series of analyses were employed in order to describe the alteration of the physicochemical characteristics of the polymer (FTIR, SEC and GPC, sinking velocity) as well as the biofilm community (NGS). At the end of phase II, the fraction of double bonds in the surface of microbially treated PE films increased while changes were also observed in the profile of the PS films. The molecular weight of PE pieces increased with incubation time reaching the molecular weight of the virgin pieces (230,000 g mol^{-1}) at month 5 but the buoyancy displayed no difference throughout the experimental period. The number-average molecular weight of PS pieces decreased (33% and 27% in INDG and BIOG treatment respectively), implying chain scission; accelerated (by more than 30%) sinking velocities compared to the initial weathered pieces were also measured for PS films with biofilm on their surface. The orders Rhodobacterales, Oceanospirillales and Burkholderiales dominated the distinct platisphere communities and the genera *Bacillus* and *Pseudonocardia* discriminate these assemblages from the planktonic counterpart. The functional analysis predicts overrepresentation of adhesive cells carrying xenobiotic and hydrocarbon degradation genes. Taking these into account, we can suggest that tailored marine consortia have the ability to thrive in the presence of mixtures of plastics and participate in their degradation.

1. Introduction

Plastics have widely replaced the natural products due to their intrinsic characteristics such as durability and low production cost. In 2016, the annual plastic production has reached 60 million tons in Europe [1]. The majority of them (60%) was used in packaging industry followed by the building and construction sector while only the 27.3% of the collected plastic waste ended up in landfills [1]. Marine plastic litter is considered a major challenge to be addressed, stemming from anthropogenic activities in terrestrial and marine ecosystems [2,3]. Plastic fragments are globally detected from equators to poles, from shallow to the deepest areas and from highly touristic beaches to remote locations [4–7]. They have been responsible for causing negative effects on all levels of organization in the marine environment, from single organisms to ecosystem function [8,9]. It has been demonstrated that floating microplastics in North Pacific accumulation zone may threaten the associated predators since their mass is 180 times on average higher than the surrounding biota and at least on chemical measured from every piece exceeds sediment threshold effect levels [10].

Annual river inputs have been estimated to be 1.15–2.41 million tons [11], however, the buoyant plastic mass ranges between 7000–35000 tons [12]. According to other model estimations, plastic mass increased up to 236,000 metric tons but still represents a low percentage of total plastic inputs [13]. There exists a knowledge gap concerning plastic pathways in the marine environment, while

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understanding the procedure is highly important to recognize and realize the possible risks and impacts. It is considered that once plastics enter the oceans they may undergo degradation due to the synergy of abiotic and biotic mechanisms [14]. Large plastic items are subjected to UV radiation, fluctuating temperatures and mechanical forces at ocean surface, which results in the alteration of their physicochemical properties and fragmentation to microplastics and further to nanoplastics [15,16]. Abiotic degradation precedes and stimulates biodegradation since carbonyl groups are generated on the surface along with the breakdown of the high molecular weight polymers to smaller ones [17]. Therefore, a wide range of organisms can settle on the weathered surface, using it as a substrate and as a carbon source [18–20]. Several bacterial genera have been identified as plastic colonizers, while at the same time diatoms, ciliates and bryozoan are some representatives of the diverse group of plastic associated taxa [21–23]. At the same time, a number of enzymes able to modify and/or degrade various polymers have been identified [24] while improve of their catalytic activity is investigated through enzyme engineering approaches [25,26].

Platisphere is considered a distinct ecological niche, where the inhabitants are equipped with specific metabolic characteristics. For example, genes related to xenobiotic degradation are enriched within the microbial assemblages [27,28]. Given that the bathypelagic community is strongly influenced by the sinking particles microbiome [29] questions are raised concerning the impact of these novel communities in the oceans. However, few studies have demonstrated the degradation potential of associated communities [14,30,31]. Since mineralization of polymer molecules is a complicated process, a network of species with functional complementarity may have higher chances to accomplish it. The present study aims to reveal the efficiency of an indigenous marine community (alone or bioaugmented) to colonize mixtures of plastic waste comprising of naturally weathered polyethylene (PE) and polystyrene (PS) films. The response of community in terms of structure and predicted metabolic activity was investigated together with the monitoring of its impact on the physicochemical properties of the plastic films.

2. Materials and methods

2.1. Sample collection

Naturally weathered PE and PS plastics were collected from two sandy beaches in Chania, Greece; Agios Onoufrios (coordinates: 35.549128, 24.061855) and Kalathas (coordinates: 35.554538, 24.085120). Pieces bearing clearly the polymers identification symbols scheme were only collected. Next, they were cleaned with water and soap and 70% ethanol solution was used for surface sterilization. Large plastic items were cut into smaller pieces with 1 cm^2 surface area, weighted and four pieces were attached in a fishing line with a specific sequence. Both polymer types were strung in the same line. Five fishing lines were put in one beaker. The pieces were identified according to their position and the number of the fishing line.

2.2. Experimental design

A microcosm experiment was performed in two phases in enriched filtered saline water (C:N:P ratio of 100:10:1). During the first phase, naturally weathered PE and PS films were exposed to a pelagic microbial community alone (characterized as “indigenous” (INDG)) or bioaugmented with strains able to grow with PE or PS as the sole carbon source (characterized as “bioaugmented” (BIOG)) [30,31]. At the end of phase I, the developed biofilm on the surface of the polymers was harvested and the whole experiment was repeated using new naturally weathered films (not exposed to microbial consortia during phase I) with the harvested biofilms as the inoculants. During phase II, one fishing line from every replicate was permanently removed every month for further analysis while every phase lasted for 5 months.

2.3. Weight reduction

The biofilm was removed from the attached plastic pieces and the pieces were further washed and dried at 50 °C for 3 days. The weight of all the flakes was measured using a balance with a 6-digit accuracy and the percentage of weight loss was calculated.

2.4. Analytical techniques

The functional groups on the surface of the polymer were detected with the attenuated total reflectance—Fourier transform infrared spectroscopy (FTIR) while the PS residual polymer was analyzed with gel-permeation chromatography (GPC) as previously described [31]. Size exclusion chromatography (SEC) was performed using SEC Agilent Technologies PL-GPC 220 for the analysis of the naturally weathered PE samples. It was calibrated with PS standards varying from M_p = 4,500 to M_p = 3,400,000 g mol^{-1} exhibiting low polydispersity (≤1.10). The eluent was o-dichlorobenzene (o-DCB) and all measurements were taken at 150 °C. The instrument is equipped with a precolumn PLgel Guard 10 µm 50 × 7.5 mm and three columns PL gel 10 µm MIXED-B 300 × 7.5 mm. The flow was chosen at 1 mL min^{-1} (the MIXED-B abbreviation corresponds to columns withstanding temperatures up to 220 °C according to the manufacturer).

2.5. Sinking velocity

The sinking velocity was determined using settling cylinders (50 cm height and 8 cm in diameter) filled with seawater (density 1027.1 kg m^{-3}) in a temperature controlled room (20 °C). Seawater was collected from Agios Onoufrios and filtered to remove the suspended particulate matter. Plastic films were washed with 2% (v/v) aqueous sodium dodecyl sulphate solution for 30 min followed by distilled water in order to remove the biofilm. In order to reveal the effect of the biofilm on the sinking velocity, the pieces were fixed with 2% (v/v) formaldehyde for 2 h under continuous shaking, following by successive washing in water and an ethanol gradient. The plastic films were cut into square pieces with 2 mm side and were rinsed in seawater overnight. The sinking speed of the pieces during the first 15 cm was considered as accelerated and was ignored. The settling time was measured along 5 cm sections while at least 6 pieces from every treatment were tested.

2.6. Community structure

The growth of the free and attached microbial populations was monthly estimated during phase II. Water and biofilm samples obtained by scratching polymers’ surface were serially diluted and cultured on plates with Standard I medium. The colonies were measured after 7 days incubation at 20 °C.

DNA was isolated from the biofilm developed on PE and PS pieces of the same replicate, pooled and eluted in Tris-EDTA (TE) buffer. DNA extraction was performed according to the CTAB protocol for the extraction of bacterial genomic DNA at the end of each phase. The concentration was measured with the Quantifluor dsDNA assay (Promega Corporation, USA) and adjusted to 4 nM before sequencing. Next generation sequencing of 16S rDNA genes amplified from DNA extractions were performed according to Illumina’s application note (part # 15044223, Illumina, San Diego, USA), using the primers: 515 F (5′ -GTG CCA GCM GCC GCG GTA A- 3′) and 806R (5′ -GGA CTA CHV GGG TWT CTA AT- 3′). PCR steps were performed as previously described [31]; the samples were loaded on an Illumina MiSeq and sequenced using a 600-cycles MiSeq Reagent Kit v3. The sequences were deposited in BioProject (PRJNA378706), the Submission ID is SUB2440072.

The functional genes were predicted based on the 16S rRNA gene sequencing data using a database of phylogenetically referenced genomes (PICRUSt, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) [32]. Metabolic pathways were

predicted from the Kyoto Encyclopedia of Genes and Genomes (KEGG) catalogue.

2.7. Data analysis

Next generation sequencing data analysis was performed on fastq files. Adaptors were trimmed with a threshold of 0.9 and the paired-end reads were joined using PANDAseq version 2.8 [33] and were further analyzed using QIIME package, version 1.9.1 [34]. The joined sequences were filtered and clustered *de novo* using the Greengenes database updated in May, 2013 (http://greengenes.lbl.gov) with a 97% identity threshold. Community similarity was represented by principal coordinate analysis (PCoA) using a normalized OTU table (cumulative sum scaling (CSS) normalization) [35]. A phylogenetic tree was constructed with OTUs displaying a mean relative abundance of ≥0.5%. This analysis was performed in R [36] using the phyloseq package [37]. The linear discriminant analysis (LDA) effect size (LEfSe) [38] was performed to identify the biomarker species between the two phases and between the planktonic and attached cells. The abundance of predicted functional genes was compared using STAMP software (Statistical Analysis of Metagenomic Profiles, v2.1.3.) [39].

3. Results and discussion

3.1. Impact of the communities on the chemical characteristics of plastic films

Abiotic oxidation is the prerequisite for biotic degradation of polymers, especially for polyolefins [40,41]. When plastics with a carbon-carbon backbone are exposed to abiotic conditions such as UV radiation, degradation initiates which further leads to increasing susceptibility of polymers to biodegradation [17]. During this experiment, naturally weathered PE and PS films were exposed to a marine community alone or bioaugmented in a simulated marine environment and the induced alterations on the characteristics of the plastic pieces were monitored.

Photooxidation acts primarily on the surface of the plastics; hence the surface of the weathered polymers may display variations in the topography, roughness and chemistry in comparison with the virgin ones. The degree of abiotic degradation depends on the environmental characteristics; it was demonstrative that the effect of UV radiation was more pronounced in plastic pieces exposed in the air environment in comparison with the seawater [42,43]. Several new bands assimilated to ester carbonyl, methyl and ester bonds were detected in beached PE films [44]. In this experiment, new absorption peaks were detected, with FTIR spectroscopy, in the surface of the weathered PE and PS films used as a substrate and as a carbon source (Figs. 1 & 2). For example, two peaks at 1600 cm^{-1} and 1580 cm^{-1} were observed in exposed PE films which correspond to the formation of C=C bond of the vinyl group. Hydroxyl groups (at 3300–3400 cm^{-1}) can be detected in the weathered PS films while changes were also observed in the peaks at 1000-1700 cm^{-1}.

When the weathered pieces were exposed to microbial activity, the FTIR profile of the plastic surfaces was further altered. New bands at 1610 cm^{-1}, 1560 cm^{-1} and at 990 cm^{-1} appeared on the surface of the microbially treated PE films which correspond to C=C bond. In the BIOG treatment, a band at 1400 cm^{-1} was broadened while a new band was observed at 1380 cm^{-1} which is attributed to end methyl groups. Regarding PS films, it should be noted that the FTIR profile of the pieces previously exposed to INDG community for 6 months was similar to the profile of the virgin ones. A broadening band in the BIOG treated PS films was observed at 1600 cm^{-1} corresponding to conjugated carbon double bonds while only one broaden peak appeared between 1200 cm^{-1} and 920 cm^{-1}. Changes on the functional groups on the surface of the plastic films have been also demonstrated after their exposure to microorganisms [14,45]. For example, carbonyl groups and double bonds appeared on the surface of PE films when they were incubated with marine bacteria [46].

Increase in the fraction of double bonds of the naturally weathered PE pieces after their incubation with the marine consortia was observed. In detail, the vinyl bond index of the PE films increased after microbial exposure while all the other indices remained stable in comparison to the indices of the weathered pieces. Elevated vinyl functionalities in the polymer are produced due to Norrish type II reaction [47] and indicate chain scission. In general, microorganisms favor the formation of double bonds due to consuming of the carbonyl groups which leads to unsaturated chains, the breakdown of the plastic chain or biotic dehydrogenation [48].

Under oxidative conditions, polyolefins undergo primarily chain scission; thus polymers with decreased molecular weight (M_w) are generated [49,50]. At the same time, low molecular hydrocarbons such as hexadecane, octadecane and tricosane were detected in the chloroform extract of LDPE that has been previously exposed to UV-B radiation [51]. However, crosslinking may also occur due to abiotic factors [17,52], depending on the characteristics of the polymer itself such as morphology and crystallinity. As seen in Table 1, weathered PE films with reduced molecular weight were employed as a substrate and as a carbon source in the seawater microcosms. At both treatments, the molecular weight of PE pieces increased in correspondence with incubation time while the M_w of the treated films at month 5 is similar to the M_w of virgin PE pieces (230,000 g mol^{-1}). Biofilm populations may attack to the oxidized polymer; the highly oxidized oligomers with elevated molecular weight are consumed faster than the smaller molecules during biodegradation [53]. This process occurs primarily at the surface of the weathered polymers, since photo-oxidation takes place on the top 100 μm [54], resulting in thicker and smaller polymers which will be further degraded or not at a later stage.

A different impact of marine communities on PS films was detected. In detail, the M_w of the polymer pieces remained stable along exposure time while a decrease in the number average molecular weight (M_n) was observed, implying chain scission. The M_n of the weathered films was approximately 115,850 g mol^{-1} while the M_n of pieces exposed to BIOG and INDG community decreased to 83,744 g mol^{-1} and 77,201 g mol^{-1} respectively after 6 months. Interestingly, the M_n reduction was more pronounced in the films being subjected to INDG community in accordance with the higher weight decrease in this treatment.

Biofilm formation on the surface of the pieces is considered the first step to imply biodegradation and gravimetric measurements are considered the first hint to suggest it. Several studies have reported weight reduction due to the activity of marine microorganisms [46,55]. Both acclimated consortia seemed more efficient in decreasing the weight of PE films (Fig. 3). During phase II, the INDG consortia reduced by 7% the weight of naturally weathered PE flakes while only 0.5% weight reduction was noticed at the end of phase I. As regards the bioaugmented treatment, 4% and 1% weight reduction was observed by exploiting the acclimated and non-acclimated consortia respectively. Weight loss of naturally weathered PS pieces followed the same pattern in the INDG treatment. At the end of phase I, 0.2% weight decline was achieved while the acclimated autochthonous community reduce by 0.5% the PS mass already from the first month at phase II. During this phase, the weight of PS pieces progressively decreased along months and approximately 11% reduction was measured at month 5. The re-inoculation of BIOG biofilm community did not further enhance the weight decline of PS pieces since 2% reduction was observed at the end of both phases. A similar pattern has been noticed when polystyrene was the sole carbon source [31].

3.2. Sinking velocity

Weathered PS films had a sinking velocity of 0.008 m sec^{-1} when they were measured in the static seawater column. After six months of incubation with the acclimated BIOG and INDG marine communities,

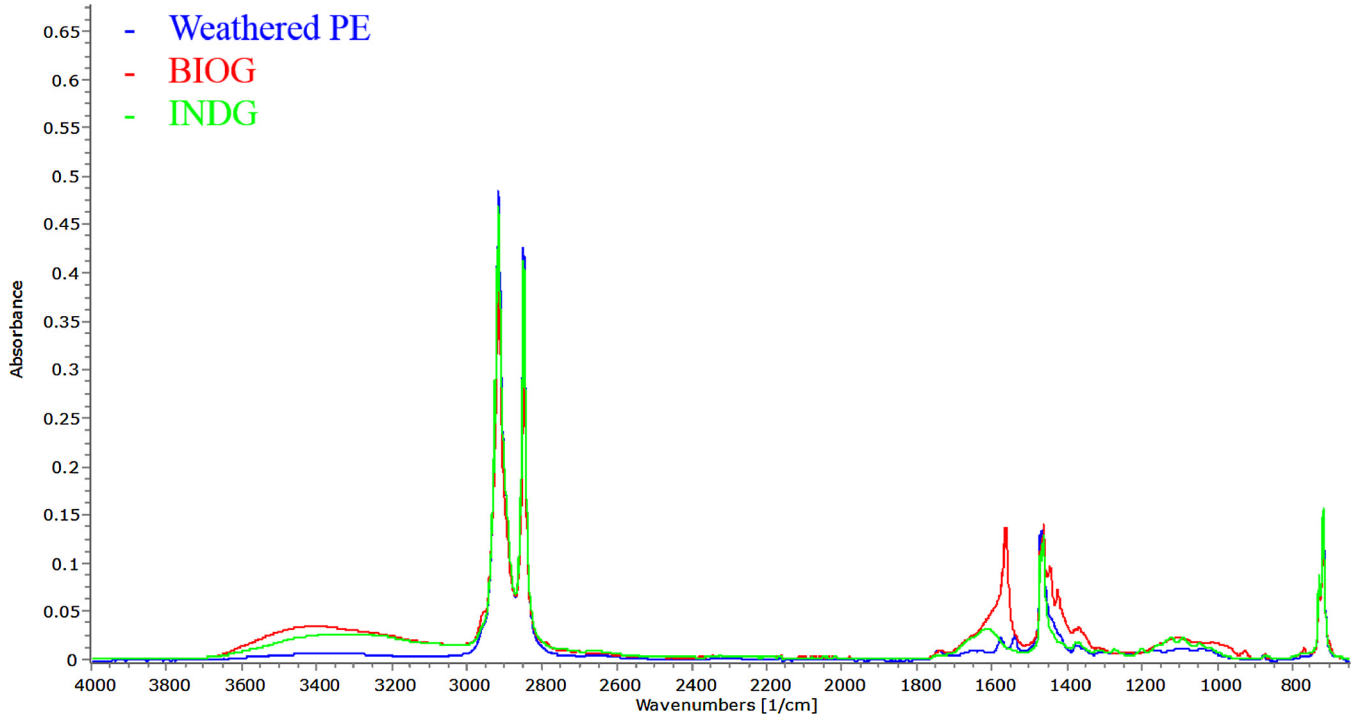

Fig. 1. FTIR spectra of the weathered PE pieces before and after their exposure to BIOG and IND marine consortia.

the sinking velocities of the pieces increased by 18% and 28% respectively (Fig. 4). Biofouling decreased the buoyancy of plastic pieces thus it enhanced their transport through the water column [56]. Accelerated sinking velocities were observed in pieces when incorporated into biofilm since 94% increase was noticed for pieces incorporated with the BIOG community and 31% increase was observed for pieces incorporated with the INDG community. Similar sinking velocities of PS particles along incubation time have been noticed in coastal waters [57]. The weathered PE films did not display negative sinking velocity despite their incubation with the marine communities and the formation of thick biofilm on the surface. It seems that fouling by macro-organisms could significantly alter the buoyancy of PE pieces from positive to negative [58]. According to this behavior, PE microplastics would remain in the pelagic zone for a long period of time since it is difficult for macro-foulers to attach to small particles, unless they are incorporated into marine snow and become negatively buoyant [59].

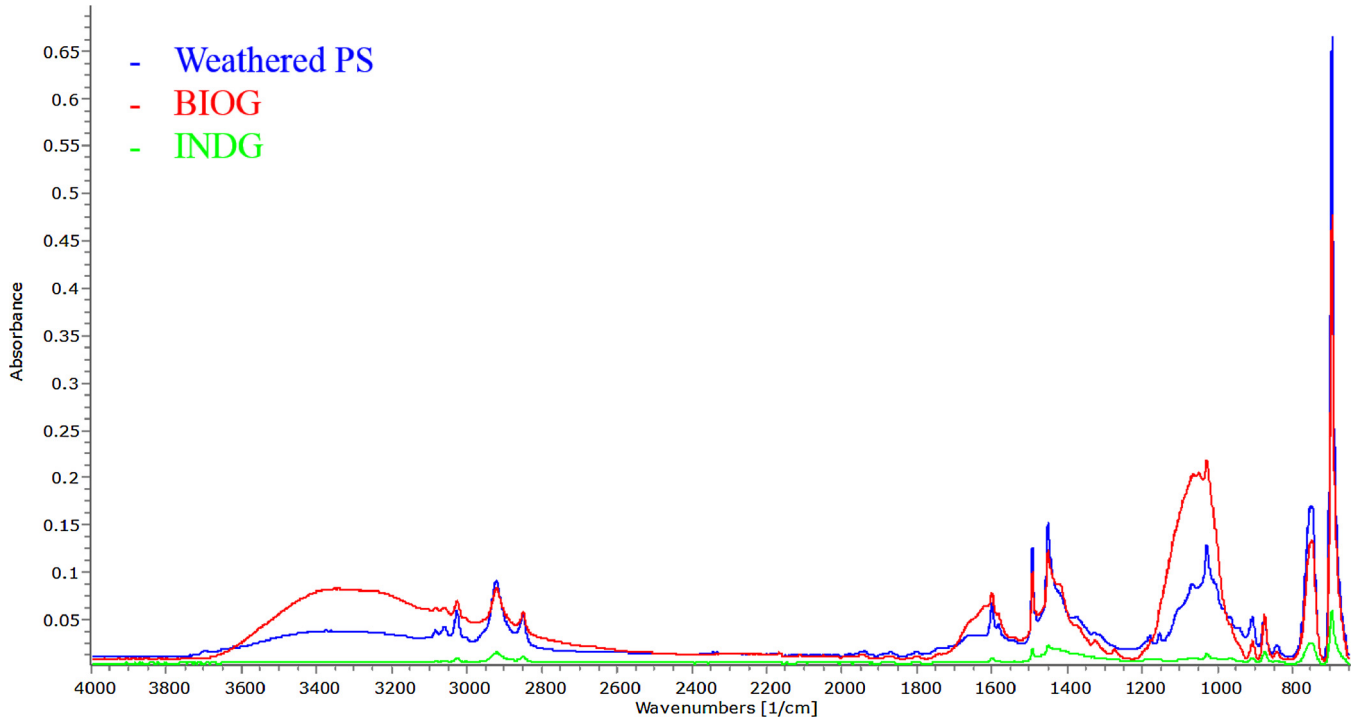

Fig. 2. FTIR spectra of the weathered PS pieces before and after their exposure to BIOG and IND marine consortia.

Table 1
Molecular weights and molecular weight distribution of the naturally weathered and microbially treated PE and PS pieces.

Type of plastic	Month	Treatment	M_n	M_w	I_p
PE	0	Weathered PE	18000	42000	2.33
	3	BIOG	25000	60000	2.40
	3	INDG	24000	56000	2.33
	5	BIOG	85000	188000	2.21
	5	INDG	113000	285000	2.52
PS	0	Weathered PS	115850	165653	1.44
	3	BIOG	97781	180392	1.84
	3	INDG	87326	163764	1.88
	5	BIOG	83,744	164403	2.11
	5	INDG	77,201	169400	2.19

3.3. Biofilm communities composition

Biofouling is a crucial step in plastic degradation process; thus the ability of the pelagic microbiota to colonize the plastic surfaces was monitored during phase II (Fig. 8). It seems that PE and PS films harbor high bacterial population throughout the experimental period. The BIOG community seems more efficient in adhering to the weathered pieces and developing a biofilm community since its abundance remained above 10^7 cells cm^{-2}. Decreased INDG biofilm cell densities were enumerated until month 3 (approximately 10^5 cells cm^{-2}), afterwards, the population increased at month 4 (10^{10} cells cm^{-2}) and decreased again at month 5 (10^8 cells cm^{-2}). Interestingly, this pattern is in line with the weight decrease of PE and PS pieces. Planktonic assemblages exhibited different growth patterns across phase II. The highest cell abundance was observed at month 1 in the INDG treatment while the cell densities ranged from 10^6 cells ml^{-1} until 10^8 cells ml^{-1} the following months. Concerning BIOG treatment, the free cells population reached its maximum at month 2 and then the population progressively decreased until month 5. Although culture dependent methods underestimate the actual microbial population, high cell densities were recorded. Similar bacterial counts were measured from marine plastic pieces by using the epifluorescence microscopy [60].

The initial pelagic and biofilm communities were also described in terms of community composition while PE and PS associated communities were pooled for downstream analysis. When the same pelagic community was incubated with either PE or PS weathered pieces in microcosm experiments, no significant differences were noticed in biofilm community composition [30,31]. It seems that environmental parameters and polymer characteristics as a substrate significantly discriminate the plastic marine debris associated communities from the planktonic counterpart [27,60]. The colonization process is separated

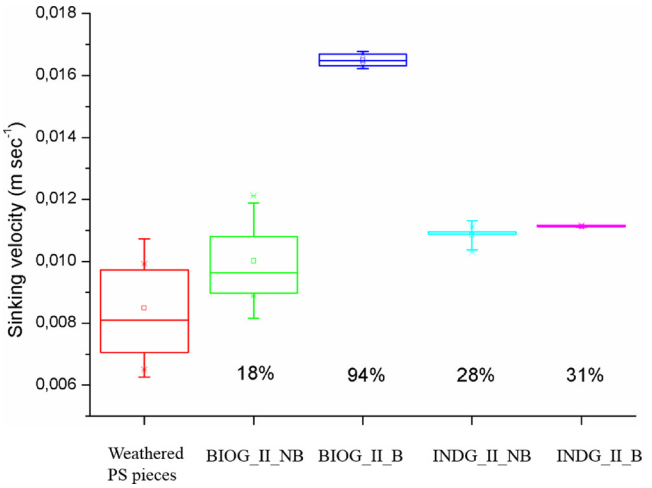

Fig. 4. Sinking velocities of PS films previously subjected to microbial communities for 5 months; numbers in the figure correspond to the increase in the median sinking velocity compared to weathered films.

into three stages, in where attachment is followed by the selection and further by the domination of secondary consumers [61]. In this experiment, the indigenous and bioaugmented biofilm communities were compared in order to reveal the effect of acclimatization and bioaugmentation in community composition. It seems that the plastic associated marine communities significantly differed (PERMANOVA: $p < 0.05$) with respect to stage of acclimatization (Fig. 5A). Two distinct groups were formed; one involved the biofilm communities at the end of phase II and the other involved the communities of phase I. Divergence occurred in the plastic associated communities during phase II since the evolution of species network may be governed by ecological interactions and spatial assembly together with the metabolic complementation [62]. At the same time, inoculation with the potential plastic degraders did not significantly influence community composition (PERMANOVA: $p > 0.05$).

Plastics pieces harbors unique microbial assemblages that are distinct from the organic particle attached counterparts [60]. The phylum Proteobacteria dominated the plastic associated communities while the Alpha- and Gammaproteobacteria exhibited higher abundances in the acclimated biofilm communities. These classes dominated the plastic litter associated communities in the Pacific and Atlantic areas [63]. Members of the order Bacillales, Caulobacterales and Rhizobiales were highly enriched in the acclimated biofilm communities while the abundance of the orders Oceanospirillalles and Alteromonadales did

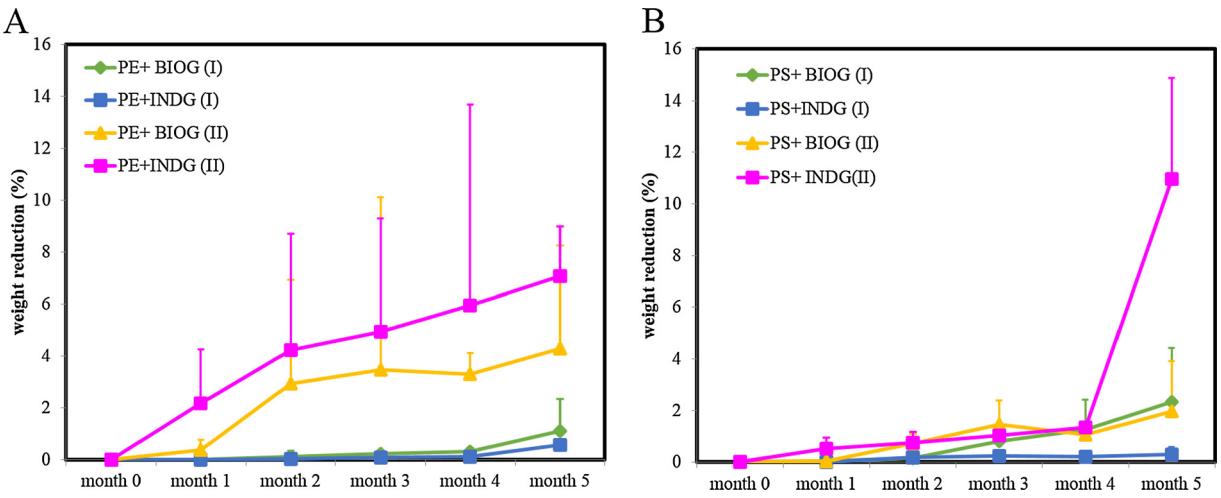

Fig. 3. Weight reduction of PE (A) and PS (B) films along exposure time during phase I (I) and phase II (II). Bars indicate standard deviation.

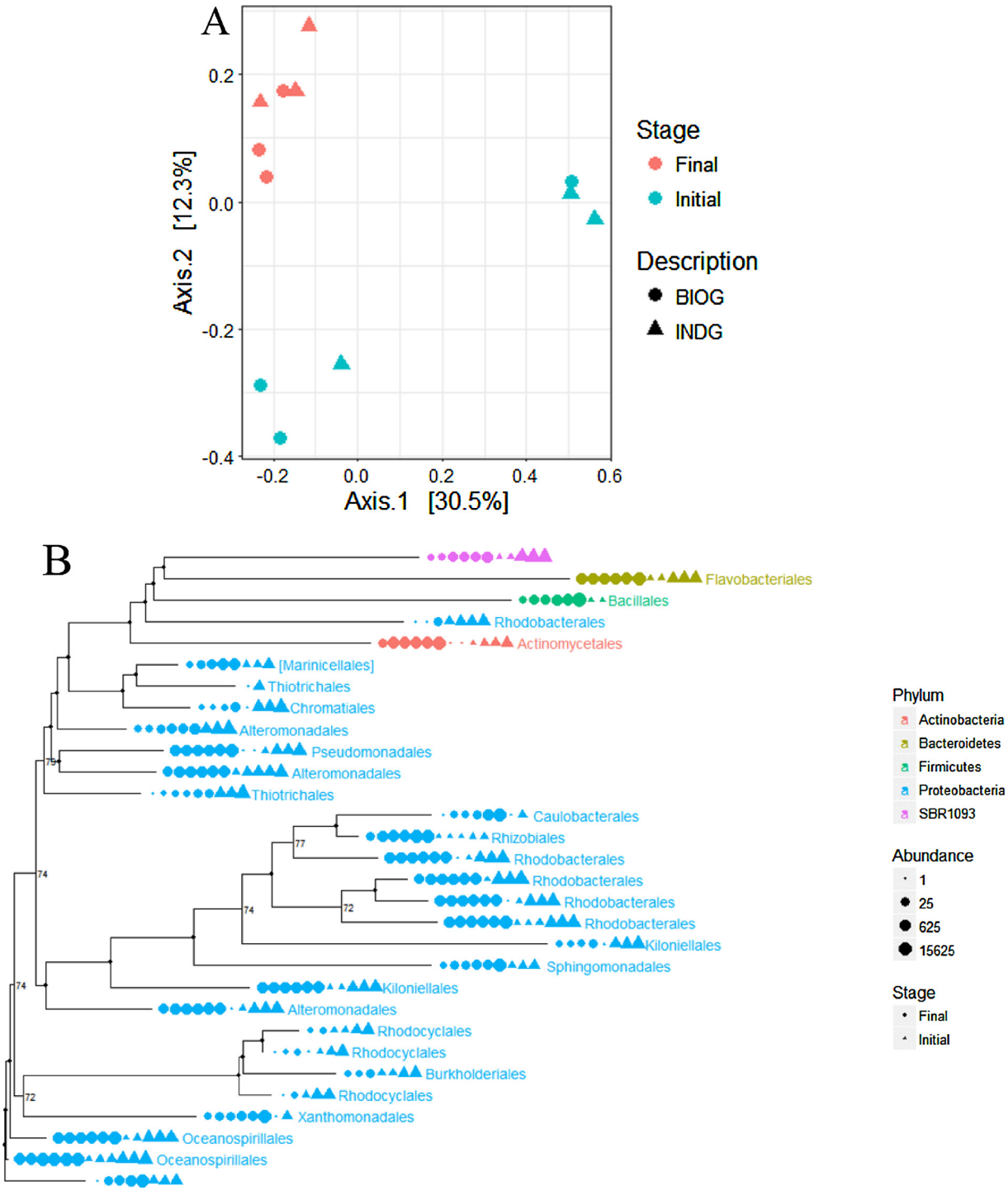

Fig. 5. PCoA plot of the biofilm communities at phase I and II (A) and phylogenetic tree of the most abundant orders (≥0.5%) at phase I and II.

not display significant differences between the two phases (Fig. 5B). In general, the orders Rhodobacterales, Oceanospirillales and Burkholderiales are the most abundant within the platisphere communities. In accordance, Rhodobacterales have been characterized as keystone inhabitants of plastic pieces [27,64] and Burkholderiales displayed high abundance in the bacterial communities growing on plastic substrates [63].

It may be hypothesized that the majority of biofilm inhabitants belong to the primary colonizers and secondary consumers; within this diverse community there are species that could play a significant role in plastic degradation pathway. The genera *Alcanivorax* and *Ochrobactrum* exhibited the higher abundances within the acclimated biofilm communities since they accounted for more than 40% of relative abundance. These two genera can be detected in the pelagic community but they were enriched within the plastic associated assemblages. *Alcanivorax* belongs to the group of obligate hydrocarbonoclastic bacteria while *A. borkumensis* cells are hydrophobic when they are cultured in the presence of hydrocarbons; thus they exhibit increased ability to adhere to oil/water interfaces and form biofilm [65]. The genera *Bacillus* and *Pseudonocardia* were among those that discriminate acclimated biofilm communities from the planktonic counterpart (Fig. 6). Interestingly, both genera have been characterized as fossil based

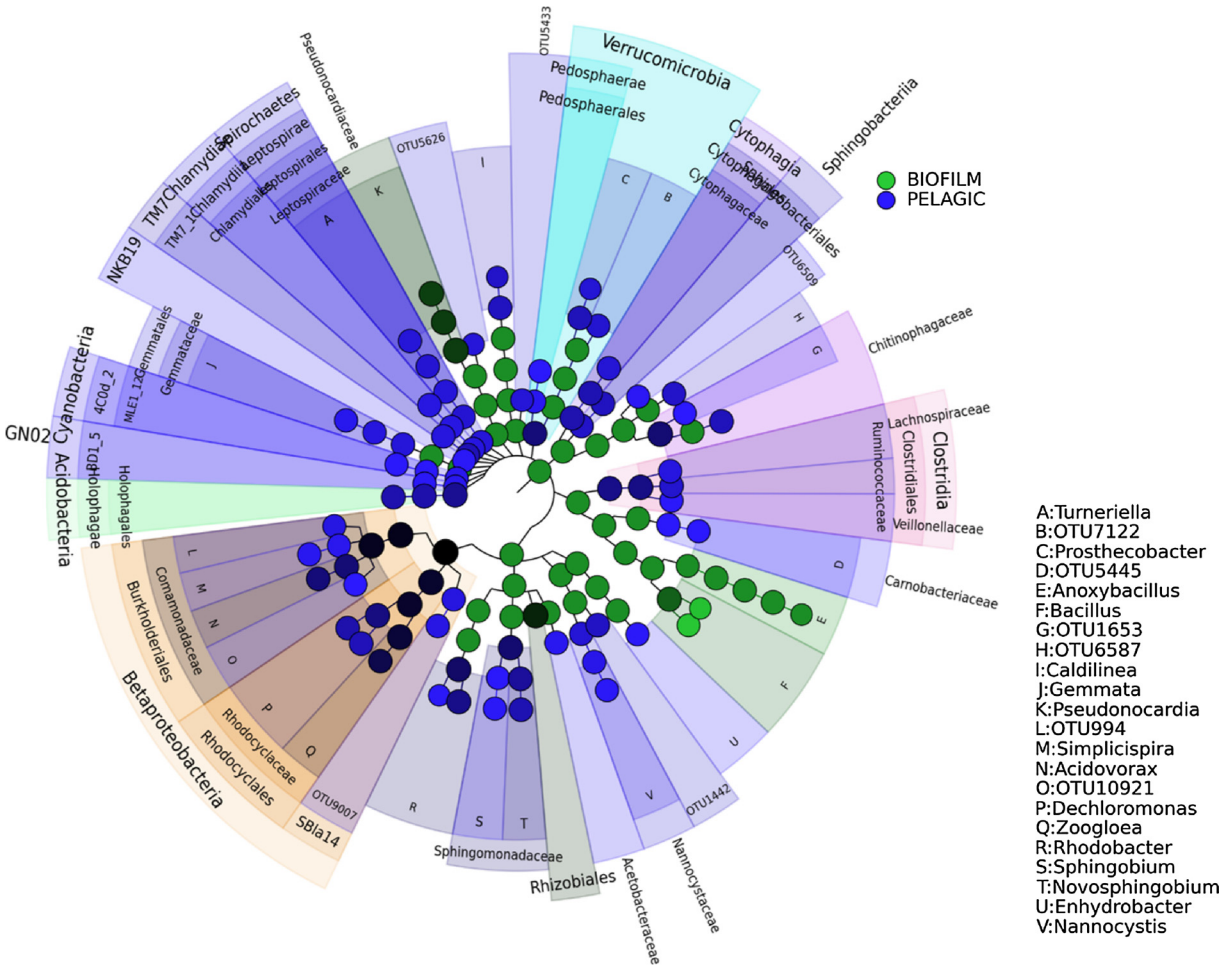

Fig. 6. Pelagic and biofilm (at phase II) biomarkers identified by LefSe analysis.

polymer or biopolymer degraders [66,67].

3.4. Functional prediction of the biofilm communities

In an oligotrophic environment, cells with high load of metabolic enzymes are selected since efficient respiration is favored under low resource availability [68]. At these conditions, the need of a network of specialized bacteria with neutral interactions becomes more important over niche complementarity [69]. The recalcitrant resources drives towards more specific metabolic pathways; thus the cells are equipped with a limited number of efficient enzymes. A decrease in the number of enzyme families was observed in the predicted metabolic profile of the acclimated biofilm communities in comparison with the planktonic ones and is in line with this hypothesis (Fig. 7). Moreover, the adhered communities exhibited significantly lower bacterial chemotaxis and motility in their predicted functional profile. In cases where the substratum provides the nutrients, adhesiveness is an important factor that determines cell proliferation along biofilm growth [70]. The more adhesive genotypes could reside on the desired surface and overgrow thus displacing the antagonists.

An increase in the predicted metabolic activity concerning fatty acid metabolism and biosynthesis of unsaturated fatty acids was also noticed in the acclimated communities (data not shown). Microorganisms can assimilate the oxidized oligomers; once these compounds are inside the cells, they are recognized as fatty acids analogues and β-oxidation initiates, leading to mineralization [53]. In alkane biodegradation pathway, alkane, which is a similar compound to PE, undergoes successive oxidations until its conversion to fatty acid in order to be incorporated in the cell metabolism [71]. Interestingly, KEGG terms related to “xenobiotics degradation”, “Bisphenol degradation”, “chloroalkane and chloroalkene degradation”, “ehtylbenzene degradation”, “naphthalene degradation”, “polycyclic aromatic hydrocarbon degradation” and “styrene degradation” were overrepresented in acclimated biofilm assemblages in comparison to the planktonic counterparts as well as the biofilm communities at phase I (Fig. 7). These results are in accordance with other studies investigating the predicted functional profile of plastic marine debris associated communities [27,28,60]. Conclusions based only on the predicted metabolic profile of the communities are risky and too general. Although, they pave the way for more targeted research in the field of gene expression and thus the discovery of the key catalytic enzymes participating in the biodegradation pathway of polymers.

4. Conclusions

Engineering the functional potential of natural microbial assemblages that colonize plastic surfaces is a key issue in plastic waste management and in mitigation of plastic debris in the marine environment. The present work provides insights on the degradation pathway of weathered plastics in the marine environment by exploring the ability of indigenous pelagic community alone or bioaugmented to alter the physicochemical characteristics of PE and PS pieces. Besides a significant weight loss, elevated vinyl functionalities in the surface of PE pieces and a broadening band in the BIOG treated PS films were observed after their exposure to microorganisms. The molecular weight of PE pieces increased along incubation time while PS pieces with

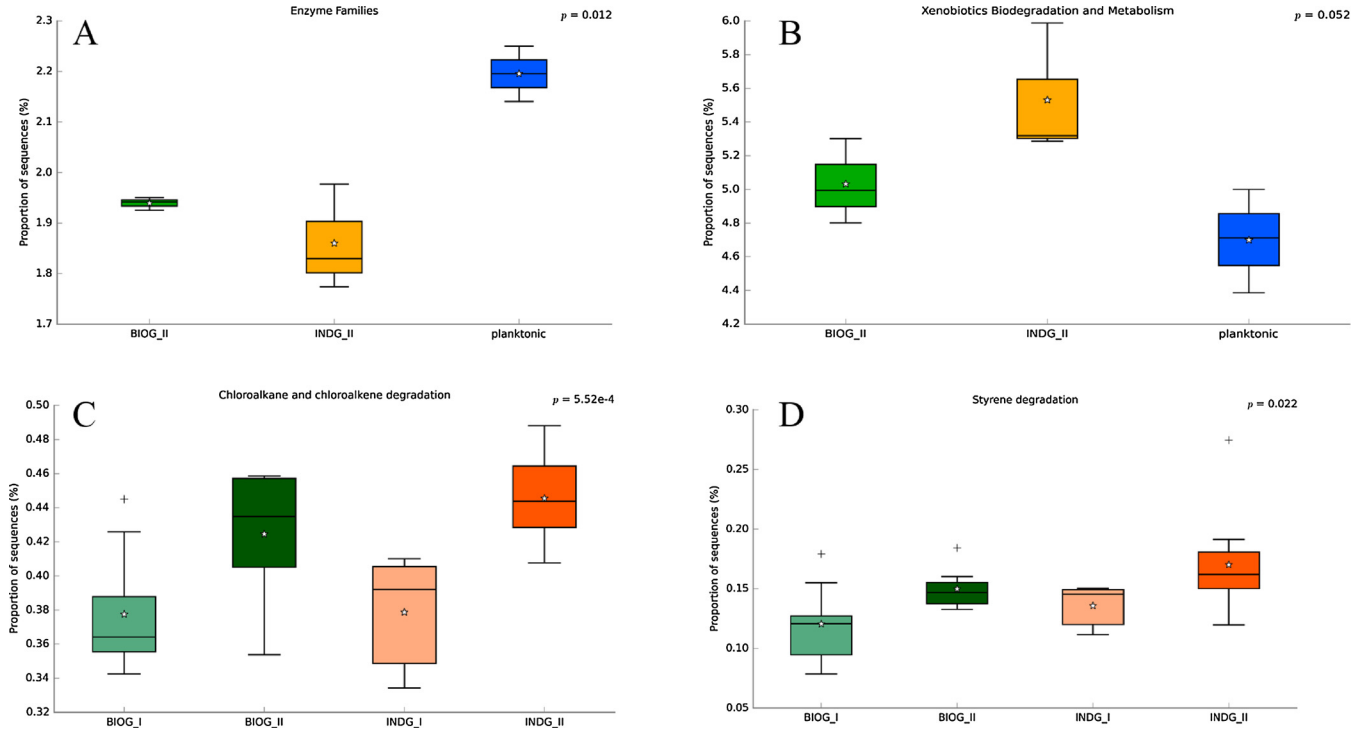

Fig. 7. Presentation of KEGG pathways related to enzyme families (A) and xenobiotics biodegradation and metabolism (B) among the acclimated biofilm communities and the pelagic counterpart and KEGG pathways related to chloroalkane and chloroalkene degradation (C) and styrene degradation among the biofilm communities at phase I and II.

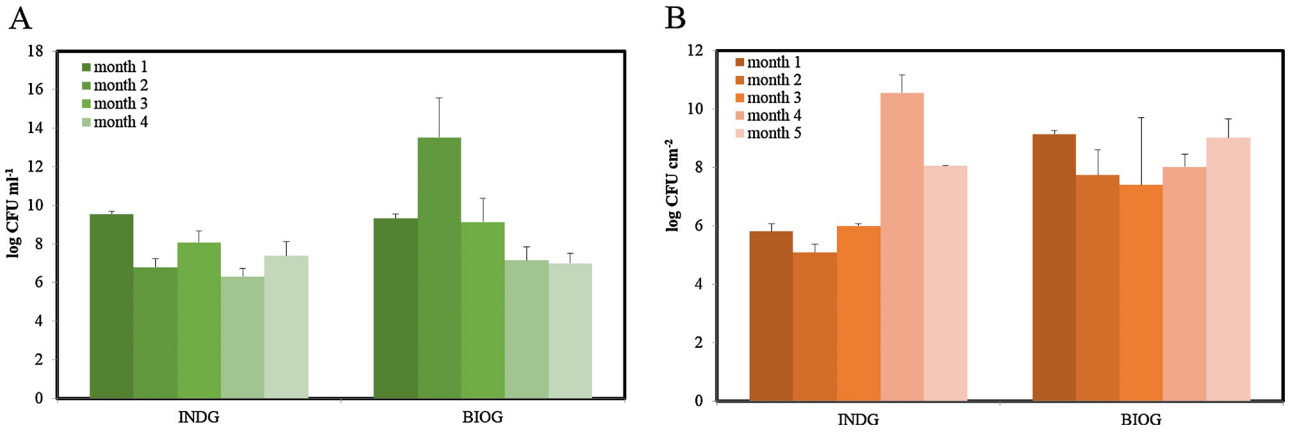

Fig. 8. Abundances of (A) free cells in the seawater medium and (B) abundances of the biofilm cells on the plastic pieces during phase II. Bars indicate standard deviation.

decreased M_n and accelerated sinking velocity were observed at the end of the experimental period. At the same time, plastics harbor distinct communities in terms of community composition and functional profile in comparison with the planktonic counterpart. Closing the gap between the hypothetic and realistic employment of microbial networks for plastic degradation could contribute to the development of mitigation measures and sustainable policies.

Acknowledgments

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