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Polyvinyl chloride biodegradation by *Pseudomonas citronellolis* and *Bacillus flexus*

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Highlights

- *Pseudomonas citronellolis* and *Bacillus flexus* are PVC film biodegraders
- PVC biodegradation and fragmentation occurred after 45 days of incubation
- PVC number average molecular weight (M_n) decrement of about 10% by bacterial attack
- High (~19%) gravimetric weight loss observed for waste PVC degraded by *P. citronellolis*

Abstract

The accumulation of high amounts of petroleum-derived plastics in the environment has raised ecological and health concerns. The aim of this work was to study the biodegradative abilities of five bacterial strains, namely *Pseudomonas chlororaphis*, *Pseudomonas citronellolis*, *Bacillus subtilis*, *Bacillus flexus* and *Chelatococcus daeguensis*, towards polyethylene, polypropylene, polystyrene and polyvinyl chloride films under aerobic conditions. Preliminary screening resulted in

the selection of *P. citronellolis* and *B. flexus* as potential PVC film degraders. Both strains were able to form a biofilm on the plastic film surface and to cause some modifications to the FTIR spectra of biomass-free PVC films. The two strains were then used to set up a PVC film biodegradation assay in 2-liter flasks. After 45 days incubation, fragmentation of the film was observed, suggesting that PVC biodegradative activity took place. Gel permeation chromatography analysis showed a reduction in average molecular weight of 10% for PVC incubated with *P. citronellolis*, with PVC polymer chains apparently attacked. Based on these results, the *P. citronellolis* strain was selected for biodegradation assays of two waste PVC films, used either nonsterile or subjected to ethanol sterilization. Chemical analyses on the incubated films confirmed the biodegradation of waste PVC plastics as shown by a gravimetric weight loss of up to about 19% after 30 days incubation. In summary, this work reports the biodegradation of PVC films by *P. citronellolis* and *B. flexus*. Both strains were shown to act mainly against PVC additives, exhibiting a low biodegradation rate of PVC polymer.

Keywords: Waste plastic, Polyvinyl chloride biodegradation, *Pseudomonas citronellolis*, *Bacillus flexus*

Abbreviations: PE, polyethylene; PP, polypropylene; PS, polystyrene; PVC, polyvinyl chloride; HDPE, high density PE; LLDPE, linear low density PE; LDPE low density PE; MSM, mineral salt medium; ATR-FTIR, Attenuated Total Reflectance - Fourier-transform infrared spectroscopy, GPC, gel permeation chromatography; TGA, thermogravimetrical analysis; ESBO, epoxidized soybean oil; $M_{n,}$ number average molecular weight.

Introduction

In 2016, world and EU plastics production amounted to 335 and 60 million tons, respectively [1]. The main polymers used in plastic formulations are polyethylene (PE), polypropylene (PP),

polyvinyl chloride (PVC) and polystyrene (PS), accounting in total for about 65.8% of global plastic demand [1, 2]. About 40% of total European plastic demand is for packaging, with the most requested polymers for packaging being PE (≈14.5 million tons), PP (≈9.5 million tons), PVC (≈5 million tons), polyurethane (PU) and polyethylene terephthalate (PET) (≈3.5 million tons) and PS (≈2 million tons) [1]. Such high demand and production has increased the amount of waste plastics and raised issues related to its disposal and pollution [3, 4]. Despite increased recycling, 27.3% of total plastic waste was disposed of in landfill in 2016 [1]. Once there, plastics were reported to undergo partial degradation, mainly cracking and fracturing, producing small pieces called meso- and microplastics. Such smaller fragments may leach out from landfill and enter the marine environment, contributing to marine litter [3, 5, 6]. Plastics pollution in seas and oceans leads to further environmental and health concerns due to potential toxic effects on marine biota [3, 5-7].

There has been an increasing interest in the evaluation of conventional plastics (bio)degradability, in order to assess the fate of plastics accumulated in various environments [2-4] and to select potentially active microbes that could be applied as inocula to improve/speed up degradability in a composting process, for example as a waste management strategy.

This work assessed the abilities of five culture collection strains of the genera *Pseudomonas*, *Bacillus* and *Chelatococcus* to degrade PE, PP, PS and PVC plastic films under aerobic conditions. The most promising strain was used in further studies to obtain insights into the mechanism of biodegradation and to evaluate its ability to degrade the waste plastic, with or without an initial sterilization step. This was in the perspective of selecting bacterial strains that could be used as inocula to be applied to improve plastic degradability in aerobic waste management systems like the composting plants.

Material and Methods

Bacterial strains

In accord with literature research of bacterial plastic biodegradation, five culture collection strains were selected for plastic film biodegradation assays. The strains were from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Germany). They were *Pseudomonas chlororaphis* (DSM 50083), *Pseudomonas citronellolis* (DSM 50332), *Bacillus subtilis* subsp. *spizizenii* (DSM 15029), *Bacillus flexus* (DSM 1320) and *Chelatococcus daeguensis* (DSM 22069).

Plastic films

Four petroleum-derived plastic films were used, namely PE, PP, PS and PVC. PVC film contained about 30%$_{w/w}$ of additives/plasticizers, PS contained <0.5%$_{w/w}$ of mineral oil, high density PE (HDPE) about 0.5%$_{w/w}$ of 1-hexene, linear low density PE (LLDPE) about 4%$_{w/w}$ of butane, while low density PE (LDPE) and PP did not contain any additive components.

Plastic film biodegradation assays

Screening for the selection of active bacteria

Experiments were set up in Mineral Salt Medium (MSM) having the following composition: K_2HPO_4 0.7 g/l; Na_2HPO_4 0.9 g/l; $NaNO_3$ 2.0 g/l; $MgSO_4 \cdot 7H_2O$ 0.4 g/l; $CaCl_2$ 0.1 g/l; trace element solution 2.0 ml/l; pH 6.7; trace element solution: $FeSO_4 \cdot 7H_2O$ 2.0 g/l; $MnSO_4 \cdot 4H_2O$ 1.5 g/l; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 0.6 g/l), supplemented with the appropriate carbon and energy source. The bacterial inocula were prepared by transferring single colonies from agar plates to 100-ml flasks containing 20 ml of MSM supplemented with 20 g/l glucose. The bacteria were grown to mid log phase (3-7 h incubation at 150 rpm and at 37°C for *C. daeguensis* or 30°C for the remaining strains) and the biomass was recovered by centrifugation (Thermo Scientific SL 16R; 6915 rcf, 15 min,

10°C). The bacterial cells were washed twice with sterile MSM and then inoculated, at a final concentration of 10^7-10^8 CFU/ml, into MSM supplemented with 2 g/l of plastic films previously cut into small pieces (3 cm^2), degreased and sterilized by immersion in 70%$_{v/v}$ ethanol (30 min, 150 rpm). A set of non-inoculated (abiotic) controls were also prepared. The biodegradation assays were performed in 100-ml flask containing 20 ml of MSM and the incubation was performed at 30° or 37°C and 150 rpm for 90 d. Additions of sterile water to the cultures were made from time to time to compensate for evaporation. Microbiological and plastic biodegradation analyses were performed as in the Analytical Methods section below.

PVC film biodegradation in 2L flask

From the results obtained in the screening experiment, PVC biodegradation assay was performed in 2L flasks containing 750 ml of MSM using *P. citronellolis* and *B. flexus*. Samplings were performed after 45 and 90 d incubation in order to evaluate bacterial growth, biofilm formation and film biodegradation (see Analytical Methods).

Assessment of waste PVC film biodegradation

In order to evaluate the ability of *P. citronellolis* to degrade previously used PVC film, an experiment using different waste PVC films was setup. Waste PVC films were recovered from a local market after their use as wrapping films for vegetables and fruit and their biodegradation was compared to that of virgin PVC film used in the previous experiments. The plastic films were cut into 3-cm^2 pieces and used as carbon and energy source either unsterilized or after ethanol sterilization (see above). Pre-cultured *P. citronellolis* was inoculated at a concentration of 10^9 CFU/ml in order to limit competition by other microorganisms in experiments where nonsterile PVC was used as substrate. The biodegradation assay was set up in a 250-ml flask containing 75 ml of MSM and the incubation was performed for 30 d. The incubation time was fixed at 30 d in order to retrieve data related to gravimetric weight loss, since longer incubation periods resulted in film

fragmentation, which prevented the gravimetrical weight loss evaluation. During the incubation period, sterile water was added to the cultures to compensate for evaporation.

Analytical methods

Evaluation of planktonic growth and biofilm formation on plastic films

Bacterial growth was monitored by the drop plate method [8] on solid MSM supplemented with 10 g/l glucose. At the end of the screening experiment, microbial adhesion and biofilm formation on plastic films incubated in the presence of the bacterial strains was evaluated using the qualitative crystal violet assay [9]. In the 2L flask PVC biodegradation experiment, the formation of dense biofilms on the plastic surface was quantified by determining the concentration of adherent proteins. PVC films were incubated overnight with 3 ml 6M urea at 4°C on an orbital shaker (150 rpm). Protein quantification according to Lowry method [10] was performed on the urea solutions, with 6M urea as reference solution/blank.

Assessment of plastic film biodegradation

Biomass-free plastic films were obtained by incubation in 6M urea (4°C, 150 rpm, overnight) and three washings with sterile distilled water. They were then subjected to the following analyses for the detection/assessment of biodegradation activity.

(a) Attenuated Total Reflectance - Fourier-Transform InfraRed Spectroscopy (ATR-FTIR). FTIR spectra of the dried washed plastic films were recorded over the wavelength range 450-4000 cm^{-1} using a Perkin Elmer FTIR (Waltham, USA) spectrometer operating in ATR mode. 32 scans were taken for each spectrum. Non-incubated films and films incubated into the abiotic controls were used as references.

(b) Gravimetric weight loss. The washed plastic films were dried under vacuum at room temperature to constant weight (at least 24 h) before weighing. The weight loss was calculated as a percentage of the original gravimetric weight.

(c) Gel Permeation Chromatography (GPC). The molecular weight of the PVC films was determined using gel permeation chromatography. GPC measurements were carried out on a HPLC Lab Flow 2000 equipped with Phenomenex Phenogel Mixed 5µ MXM/MXL columns and a Linear Instrument UVIS-200 detector operating at 254 nm. Tetrahydrofuran (THF) was used as mobile phase (1 ml/min) after calibration with polystyrene standards of known molecular mass. A sample concentration of 3 mg/ml was employed. Non-incubated PVC film and PVC films incubated under abiotic conditions were used as references.

(d) ThermoGravimetrical Analysis (TGA). Dried plastic samples of 5 to 10 mg were subjected to thermogravimetric analysis using a Perkin Elmer TGA7 thermal analyzer under a nitrogen atmosphere (gas flow: 40 ml/min). The thermograms were recorded from 40°C to 800°C at a heating rate of 10°C/min.

(e) Statistical analysis. All experiments were performed in duplicate. Data were statistically assessed using the analysis of variance (ANOVA) via MATLAB software (Version R2017a, The MathWorks Inc, Natick, USA). The significance of the data was determined by *Tukey* honestly significant different test. Statistically significant results were depicted by p values < 0.05.

Results and discussion

Preliminary screening for PE, PP, PS and PVC plastic films biodegradation

In a first experiment, the five bacterial strains were incubated with the different plastics in order to evaluate their biodegradative abilities against PE, PP, PS and PVC films. Bacterial growth in the presence of PE, PP, PS and PVC films showed a progressive decrease in planktonic cell count for all strains (data not shown). In order to assess if this was due to bacterial adhesion to the plastic film surfaces, the qualitative crystal violet assay was performed at the end of the experiment, i.e. after 3 months incubation. According to the results, interpreted following the method of [9], biofilm was found to be formed by only three strains on a PVC surface. Specifically, dense and weak biofilms were formed by *P. citronellolis /B. flexus* and *P. chlororaphis*, respectively (**Figure 1**a).

Plastic film biodegradation was evaluated using FTIR spectroscopy to reveal potential changes in the chemical structure of the plastics after incubation with the bacterial strains. Comparing the spectra of the non-incubated PEs (LDPE, LLDPE and HDPE), PP and PS films (Figure 1b) to those incubated with the bacteria and their corresponding abiotic controls, no differences were observed. These results, together with the previous finding of a decrease in planktonic count and absence of a biofilm on plastic surfaces, suggested that PEs, PP and PS were not attacked by the bacterial strains under the experimental conditions applied here. On the contrary, compared to non-incubated film, changes in the fingerprinting part of the spectra of PVC films incubated for 3 months in the presence of the bacterial strains and under abiotic conditions were observed at wavelengths ranging from 1500 cm^{-1} to 800 cm^{-1}, (Figure 1c). ATR-FTIR has been used to detect microbial degradation of different plastic materials as this technique could be useful to detect changes in surface chemistry [2, 11, 12]. They include the occurrence of new peaks attributed to new functional groups as well as the disappearance or the reduction of peaks attributed to surface functional groups. As an example, differences in peaks attributed to vinyl and double bonds have been found in partially degraded PS films after incubation with different marine communities, which were then considered as biodegraders [6]. According to the FTIR results, changes occurred on the surface of the PVC film in the presence of bacteria as well as under abiotic conditions. However, only *P. citronellolis* and *B. flexus* were shown to form a dense biofilm on the plastic film surface. As a dense biofilm has been considered the first step for biodegradation (of hydrophobic plastics) to occur [3, 6, 12, 16], only these strains were selected for further studies.

PVC film biodegradation assay

Based on the results of the preliminary screening, PVC biodegradation by *P. citronellolis* and *B. flexus* was further studied in 2L flasks containing 750 ml of MSM. Following a slight decrease to 10^7 CFU/ml observed after 20 d incubation, an increase in planktonic *P. citronellolis* cell count to 10^8 CFU/ml was recorded after 45 d and remained constant thereafter. From an initial *B. flexus* cell

count of 10^6 CFU/ml, a progressive increase up to 10^8 CFU/ml was recorded after 45 d, followed by a slight fall to 10^7 CFU/ml observed at the end of the experiment (**Figure 2**a). A high adherent protein concentration of 322.70±42.90 and 197.89±18.13 µg/mg of PVC film for *P. citronellolis* and *B. flexus*, respectively, was recorded after 45 d. At the end of the experiment, the concentrations of adherent protein fell to 37.78±0.81 and 113.22±2.32 µg/mg of PVC film incubated with *P. citronellolis* and *B. flexus*, respectively (Figure 2b). The reduction of adherent protein concentration between days 45 and 90 (Figure 2b) could be related to a partial biofilm detachment, as well as to differences in protein expression by bacteria growing in planktonic or biofilm mode. Indeed, when grown in a biofilm, bacterial cells, including *Pseudomonas* and *Bacillus* genera, were shown to adapt protein abundance and pattern transcription [13]. In particular, metabolic or housekeeping proteins (i.e. required for the maintenance of basic cellular functions and stably expressed under all conditions and during all developmental stages) are expressed in all biofilm stages, while adhesion and ribosomal proteins are overexpressed in earlier stages of biofilm formation. Hence, the high amount of protein detected after 45 d incubation could be related to their hyperexpression, which aided the formation of dense biofilm. After 90 d, higher amounts of adherent proteins were measured from the PVC incubated under abiotic conditions compared to that incubated with *P. citronellolis* (Figure 2b). This could be due to the presence of compounds other than proteins detectable by the Lowry assay, such as unsaturated fatty acids or molecules containing carbonyl groups [14] that may be present as PVC additives. Such compounds could leach out from PVC films during the incubation with urea prior to the Lowry assay and subsequently be detected. On the other hand, consumption of such leached compounds by bacteria resulted in a lower amount of superficial additives compared to those still present in PVC incubated in the abiotic control.

After 45 d, an almost intact PVC film was observed for the negative (abiotic) control while PVC brittle fracturing was present for PVC film incubated with either bacterial strain (**Figure 3**), making evaluation of the gravimetric weight loss impossible. From visual inspection, the degree of brittleness of PVC incubated with *P. citronellolis* was higher than that of films incubated with *B.*

flexus. This supports the higher amount of adherent protein and hence the denser biofilm formed by *P. citronellolis* on PVC film, suggesting a more extensive biodegradation activity of this strain towards PVC film.

Different physical/chemical techniques, including TGA and GPC, were combined in order to verify if such degradation was towards the polymer backbone or if only the additives present in plastic formulation were degraded [6]. TGA showed a decreased thermal stability, mainly in the low temperature region, for the films incubated with both strains compared to the non-incubated film (**Figure 4**a). On the other hand, an increase in thermal stability was observed in the abiotic control, where the curve shifts towards that of the PVC powder, free if additives/plasticizers. The thermal stability of a plastic material is determined by the type, structure and length of the polymer chains as well as by the additives (type, structure and quantity) present in the plastic formulation. The PVC film used contained about 30%$_{w/w}$ additives, citrates, adipates, polyadipates, epoxidized soybean oil (ESBO), and Zn (data provided by the PVC production company, Gruppo Fabbri Vignola S.p.A., Italy). The presence of low molecular weight additives generally decreases the thermal stability of a plastic. ESBO is added to PVC formulation in amounts ranging from 1-2%$_{w/w}$ to 6%$_{w/w}$ in order to increase thermal stability of the plasticized films for food application [15]. The increased thermal stability of incubated PVC films may be related to the degradation of PVC additives, while its decrease could be due to the generation of functional groups along the main polymer chain and/or the production of low molecular weight PVC chains more prone to thermal degradation at low temperature [15, 16]. Nevertheless, an increase in thermal stability may not exclude polymer chain degradation, since the presence of a higher amount of ESBO compared to other additives has been reported to increase PVC film thermal stability [15]. Therefore, such an increase in thermal stability could be the result of scission of a few polymer chains and the degradation of additives other than ESBO. Hence, the increase in thermal stability due to the increase of ESBO content could be highly pronounced, making it harder to appreciate the decrease in thermal stability as a result of polymer chain scission. Taking into account this quite complex scenario, the observed increase in thermal

stability of the PVC film incubated under abiotic conditions could be due to the leaching of water-soluble additives to the liquid medium, while the lower thermal stability of the PVC films incubated under biotic conditions could be related to the formation of functional groups/shorter polymer chains accelerating the thermal degradation process, that indeed starts at lower temperature (**Figure 4**a). This effect is more pronounced for PVC films incubated with *P. citronellolis* than those subjected to *B. flexus* culture. Another interesting feature is the variation of the second step height, between 300 and 550°C, in the TGA curves. In particular, the neat (i.e. not incubated under biotic/abiotic conditions) PVC film (containing additives) shows the lowest step, while PVC powder (containing no additives) presented the most intense second step (Figure 4a). The abiotic control trace was very similar to that for PVC powder, suggesting that water-soluble additives were released into the medium culture. On the other hand, the films incubated with both bacteria showed an intermediate second step height, suggesting the corresponding films still contained additives and, consequently, the bacteria had attacked the polymer component as well as the additives.

An important polymer characteristic from which to assess polymer degradation is a reduction of the length of its backbone chains. Chain length affected all degradation types, i.e. physical, chemical and even biological. The higher the chain length, the less degradable is the polymer [2, 5]. GPC measurements showed a reduction in number average molecular weight ($\underline{M_n}$) from 100% to 90.87±4.54% and 93.48±4.67% after 90 d with *P. citronellolis* and *B. flexus*, respectively, compared to 102.90±5.15% of the abiotic control (Figure 4b). The reductions suggest that apparently both strains are able to attack the PVC polymer and cause some chains scission leading to the formation of smaller fragments, as well as the additive components. A decrease in M_n has been reported as proof of polymer chains degradation. Indeed, a superficial erosion could affect the shape of the molecular weight distribution curve, while changes in the mean molecular weight are due to degradation of the bulk polymer material [2, 6]. Thus, exocellular enzymes released into the culture medium could attack polymers at the ends of backbone chains as well as within the chains, resulting in a decreased polymer molecular weight [2].

Pseudomonas have been reported to degrade a wide range of recalcitrant compounds including plastics such as PE, PS and PP. Moreover, *P. putida* strain AJ was found to grow using vinyl chloride monomers as carbon source [17]. Hence, here we studied the ability of *P. citronellolis* to grow in the presence of PVC film as carbon and energy source and to partially degrade PVC by attacking both film additives and polymer chains. With regard to *Bacillus* organisms, this is the first reported PVC film biodegradation by these bacteria, although there is one report of their ability to degrade other petroleum-derived plastics such as UV-pretreated PP by *B. flexus* [18].

Evaluation of waste PVC film biodegradation

The *P. citronellolis* strain was selected for biodegradation assays of waste PVC films after their use as wrapping films for vegetables and fruit. The planktonic count showed the same trend under the different experimental conditions, i.e. in the presence of waste or virgin PVC films either ethanol sterilized or nonsterilized. The count was constant from the beginning of the experiment up to 20 d incubation (**Figure 5**), after which a decrease of 10-fold in CFU/ml count was found from d 20 to 30 (Figure 5). Only a few colonies differing from the inoculated strain were observed in cultures with non-sterilized PVC films from d 20 to 30. In the biotic control, i.e. *P. citronellolis* grown in MSM supplemented with 20 g/l of glucose instead of 2 g/l of PVC, no bacterial colonies were detected after 10 d incubation, suggesting cell death due to nutrient depletion. Hence, similar to virgin PVC films, waste PVC plastics were also able to support bacterial growth for a longer incubation period than MSM supplemented with glucose.

The strategy of reducing the incubation period from 90 to 30 d was successful and allowed the evaluation of gravimetric loss percentage, which showed statistically significant weight losses ($p<0.05$) of between 13.07±0.36% and 18.58±0.01% for PVC films incubated with *P. citronellolis*, compared to a maximum loss of 8.39±1.10% for the abiotic controls (Figure 6a). Waste PVC films showed statistically comparable or significant higher weight losses ($p<0.05$) compared to those of virgin PVC film (Figure 6a). These results confirmed the effectiveness of a PVC biodegradation

process by *P. citronellolis* even using PVC wastes. Moreover, the sterilization step was found to be unnecessary as non-sterilized PVC films showed even statistically higher weight losses ($p<0.05$) compared to sterilized films and *P. citronellolis* was not outcompeted by microbial contaminants. Specifically, waste PVC films showed a gravimetric weight loss of up to 18.58±0.01% compared to virgin PVC films, which exhibited up to 13.90±6.84%, while their corresponding abiotic controls lost 6.97±0.19% and 7.02±0.11% of gravimetric weight, respectively (Figure 6a).

In order to study if additives and/or polymer chains were degraded by the selected bacterium, TGA was performed on incubated films. Waste PVC films incubated for 30 d with *P. citronellolis* showed a higher thermal stability compared to the non-incubated films mainly in the second step region, i.e. between 300°C and 550°C (Figure 6b), confirming the ability of the organism to reduce the amount of additives in waste PVC. Virgin films incubated under the same conditions showed similar behavior (data not shown). These results appear to suggest that the attack on polymer chains is not particularly pronounced after 30 d biotic incubation.

Conclusions

Five culture collection bacterial strains were tested for their ability to degrade PE, PP, PS and PVC films under aerobic conditions. In preliminary screening, after 90 d incubation, potential biodegradation apparently occurred only in the case of PVC film in the presence of *P. citronellolis* and *B. flexus*. The PVC biodegradation process was further studied in 2L flasks in the presence of both strains. Results showed that the biodegradation activity was mainly directed towards PVC additives and to a lesser extent against the PVC polymer chains, as shown by the decrease in M_n. When grown in the presence of waste PVC films (from a food market), *P. citronellolis* was able to degrade partially the films after only 30 d incubation, even without sterilization of the waste PVC films. These results indicate the potential of *P. citronellolis* for the development of inocula to be

used in bioaugmentation trials to improve PVC degradation under aerobic conditions, as for example in the composting system.

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Figure Legends

a)

	PE	PP	PS	PVC
P. chlororaphis	-	-	-	+
P. citronellolis	-	-	-	++
B. subtilis	-	-	-	-
B. flexus	-	-	-	++
C. daeguensis	-	-	-	-

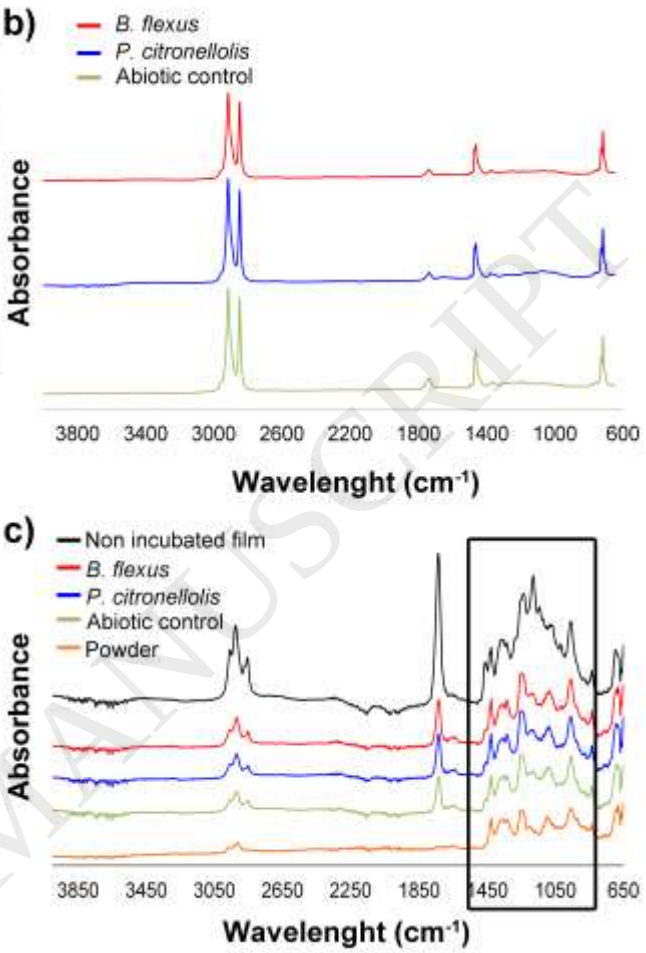

Figure 1. (a) Bacterial adhesion on plastic films; - no adhesion; + weak biofilm; ++ dense biofilm. (b, c) ATR-FTIR spectra of non-incubated powder/plastic films and of plastic films incubated with *P. citronellolis* and *B. flexus*: LDPE spectra as example of non biodegraded film (b) and PVC spectra (c); differences in spectra are highlighted in the boxed area. Data from experiments performed in duplicate.

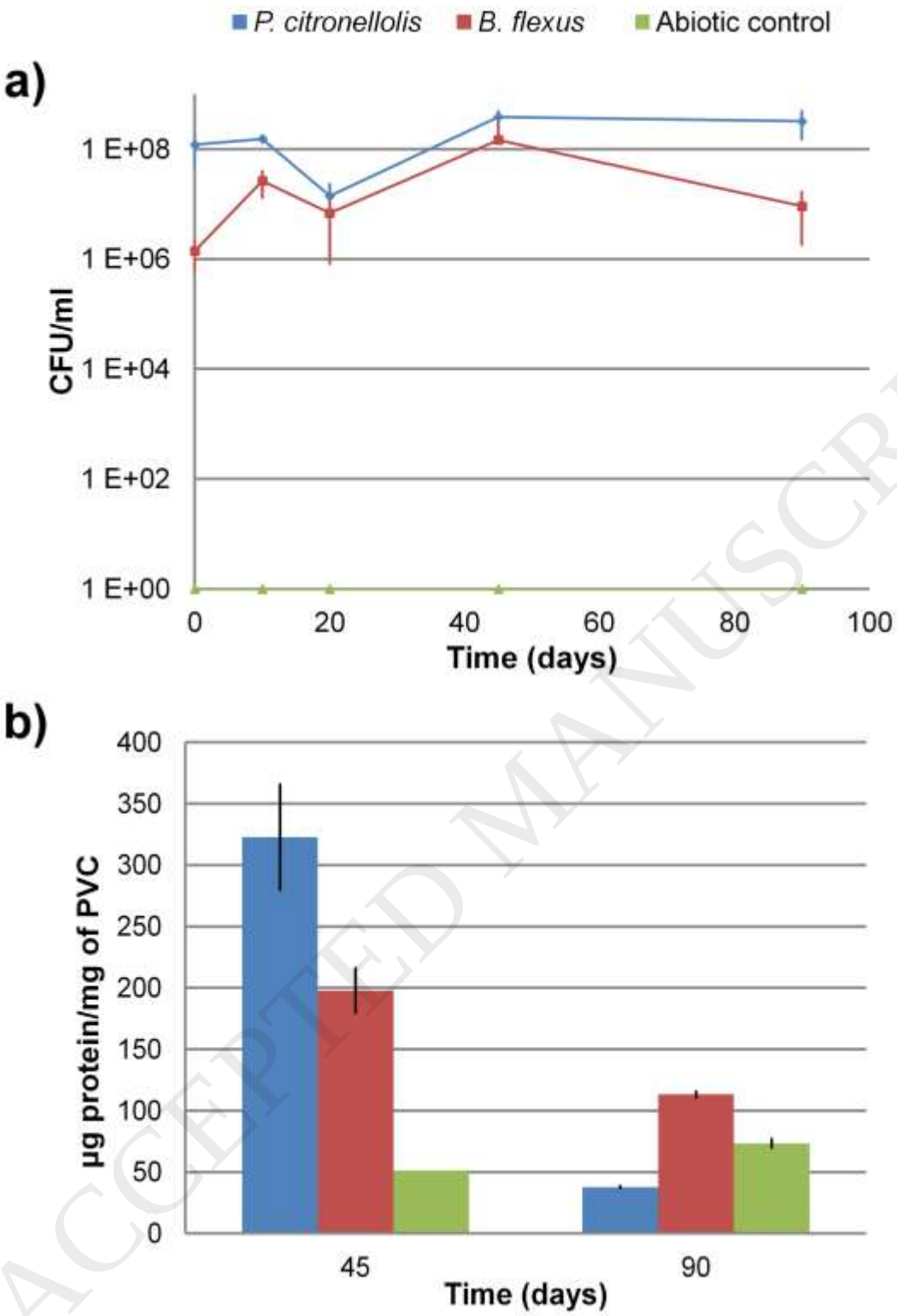

Figure 2. Planktonic cell count (a) and adhered proteins (b) on PVC films incubated for up to 90 days in the presence of *P. citronellolis* and *B. flexus.* All results are presented as the average ± SD of data from experiments performed in duplicate.

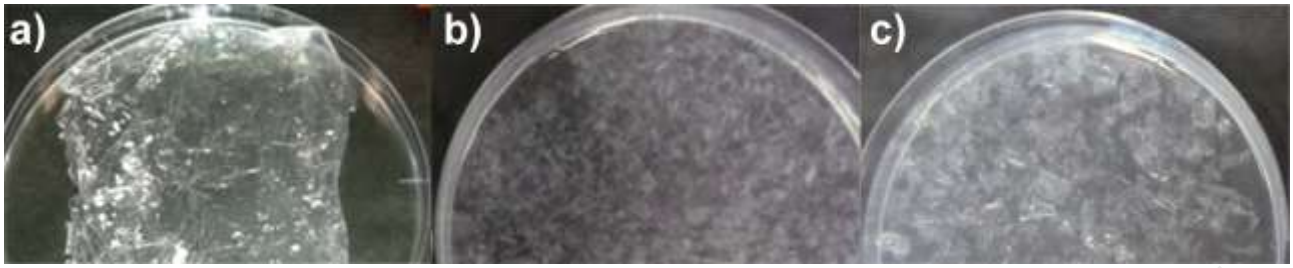

Figure 3. PVC films incubated in the abiotic control (a) and in the presence of *P. citronellolis* (b) or *B. flexus* (c) for 70 days. Data from experiments performed in duplicate.

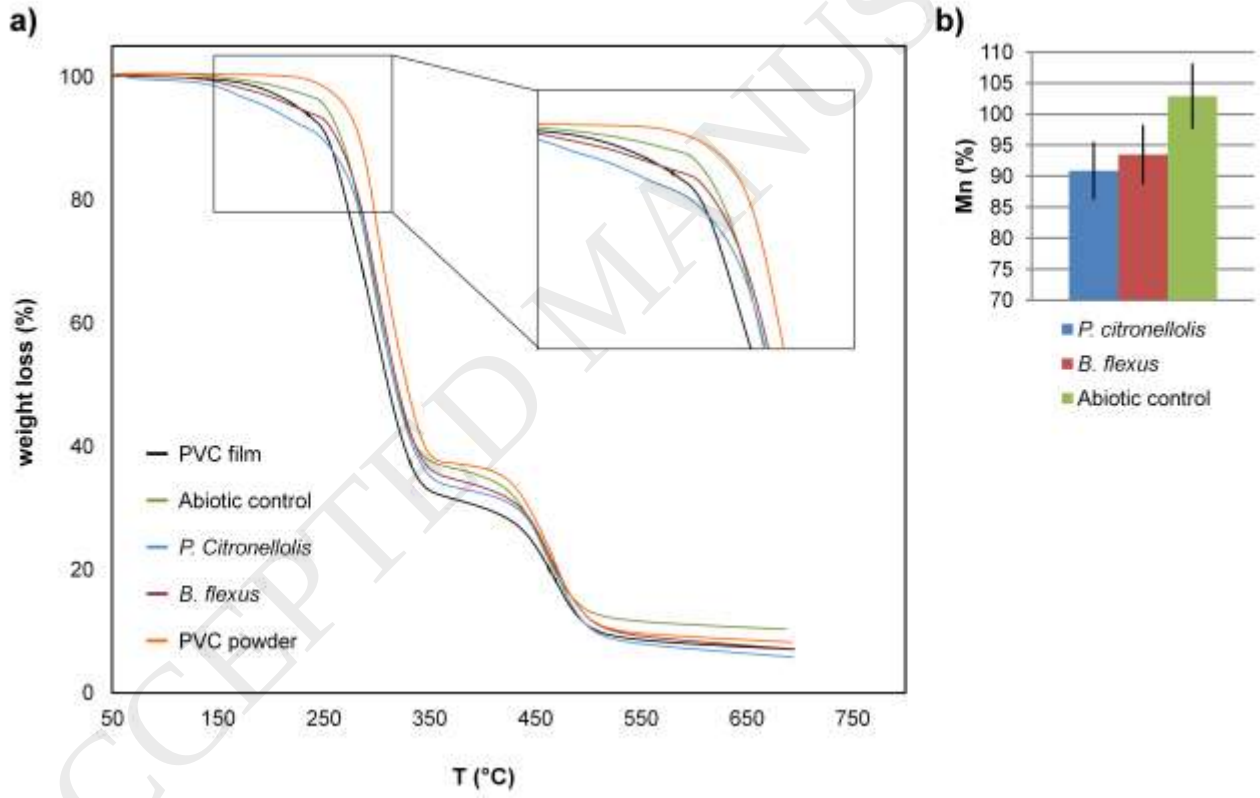

Figure 4. Thermogravimetric curves of PVC powder and film incubated under abiotic conditions or in the presence of *P. citronellolis* and *B. flexus* (a). GPC results of PVC incubated in the abiotic control or in the presence of *P. citronellolis* and *B. flexus* (b). All results are presented as the average ± SD of data from experiments performed in duplicate.

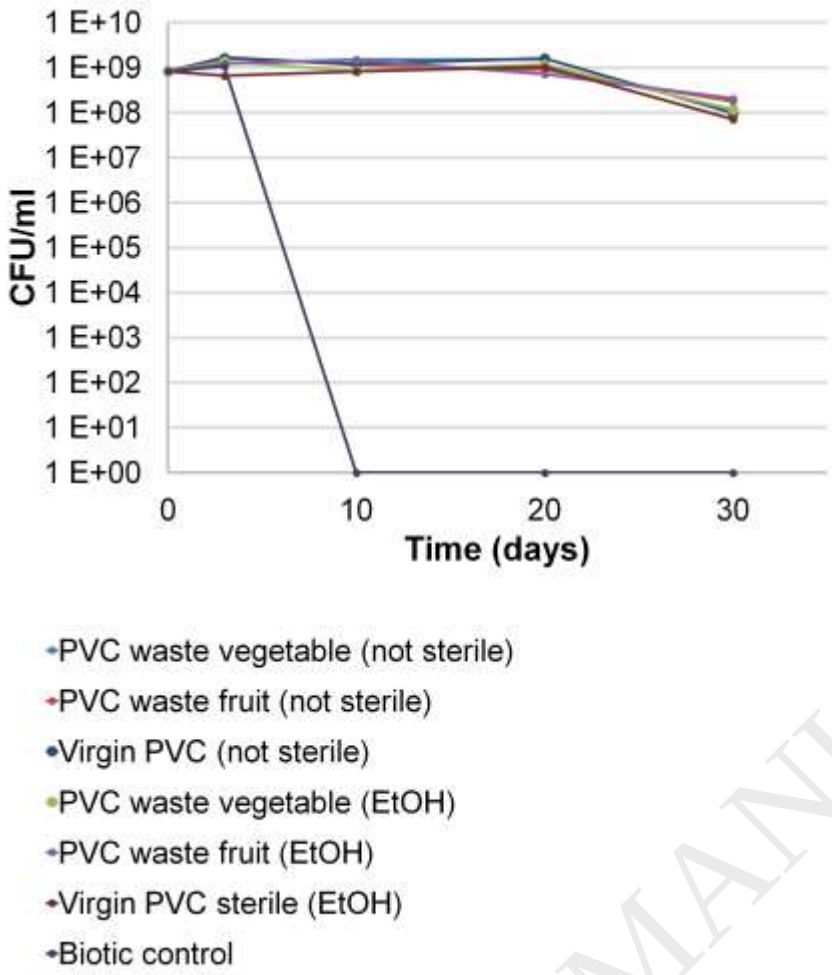

Figure 5. Planktonic cell count of *P. citronellolis* incubated with neat or waste PVC films subjected or not to sterilization as well as the biotic control. All results are presented as the average ± SD of data from experiments performed in duplicates.

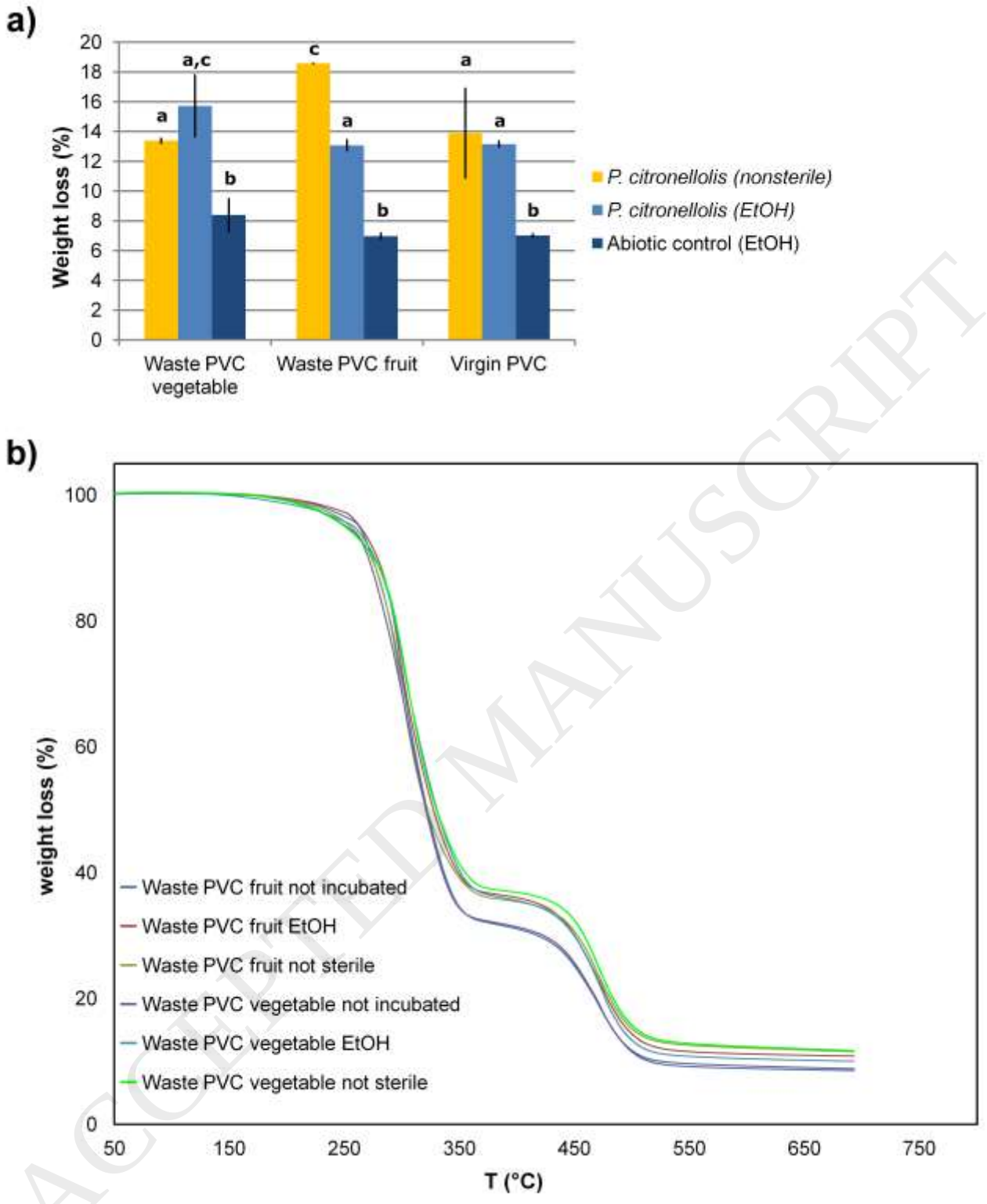

Figure 6. Gravimetric weight loss of virgin and waste PVC films subjected to different sterilization methods and incubated with *P. citronellolis* or in the abiotic control (ANOVA: $p<0.0001$; $F=23.07$; $df=8$). Results of post-hoc analysis of each PVC type and treatment were presented as letters: different letters correspond to statistically different weight loss results ($p<0.05$) (a).

Thermogravimetric curves of waste PVC films subjected or not to a sterilization step and incubated with *P. citronellolis* (b). Nonincubated waste PVC film as well as PVC powder were used to compare TGA results.