# Studies on Microbial Plastic Degradation in the Philippines

**VIRGINIA C. CUEVAS, Ph. D.**
**Professor Emeritus, UPLB,**
**Academician, NAST**

# OUTLINE OF PRESENTATION

I. Introduction

Philippines as no. 3 marine plastic polluter in the world

Partially degraded plastic debris, < 5 mm in diameter PE and PP pose the greatest danger to marine wildlife

Chemical composition of plastic

II. Researches on plastic degrading micro organisms in the Philippines

Potential fungal plastic degraders

Degradation of biodegradable plastics

Potential bacteria plastic degraders

III. Characteristics of plastic that make them recalcitrant to degradation

Need for a multidisciplinary approach to develop an efficient plastic microbial assisted degradation technology

IV. Call for exemption of S & T researches from Procurement Law or total revision of Procurement law

# 2010 – Philippines- listed as the no. 3 marine plastic polluter in the worl

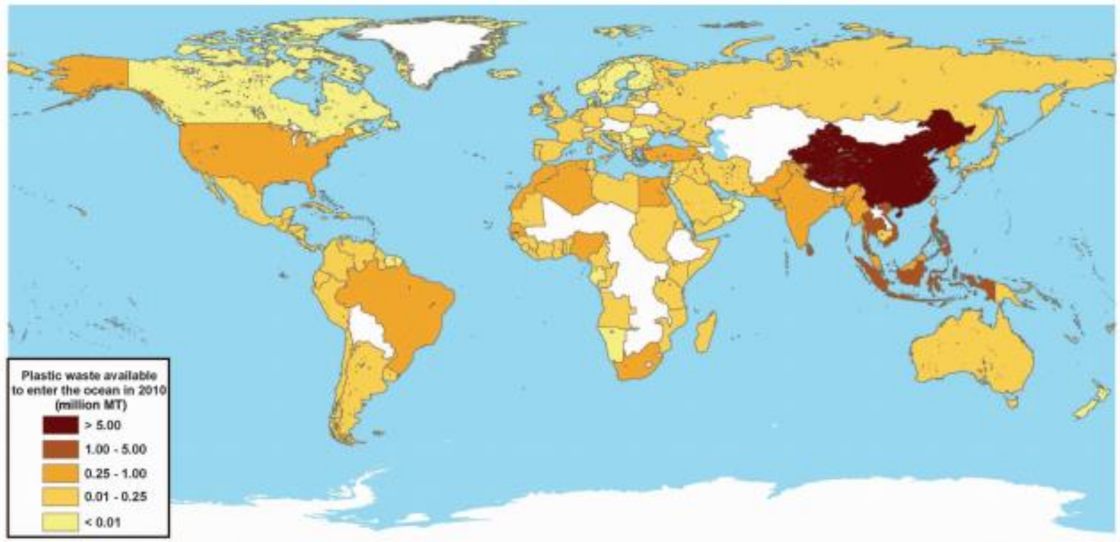



**Fig. 1. Global map with each country shaded according to the estimated mass of mismanaged plastic waste [millions of metric tons (MT)] generated in 2010 by populations living within 50 km of the coast.** We considered 192 countries. Countries not included in the study are shaded white.

**Table 1. Waste estimates for 2010 for the top 20 countries ranked by mass of mismanaged plastic waste (in units of millions of metric tons per year).** Econ classif., economic classification; HIC, high income; UMI, upper middle income; LMI, lower middle income; LI, low income (World Bank definitions based on 2010 Gross National Income). Mismanaged waste is the sum of inadequately managed waste plus 2% littering. Total mismanaged plastic waste is calculated for populations within 50 km of the coast in the 192 countries considered. pop., population; gen., generation; ppd, person per day; MMT, million metric tons.

| Rank | Country | Econ. classif. | Coastal pop. [millions] | Waste gen. rate [kg/ppd] | % plastic waste | % mismanaged waste | Mismanaged plastic waste [MMT/year] | % of total mismanaged plastic waste | Plastic marine debris [MMT/year] |
|---|---|---|---|---|---|---|---|---|---|
| 1 | China | UMI | 262.9 | 1.10 | 11 | 76 | 8.82 | 27.7 | 1.32–3.53 |
| 2 | Indonesia | LMI | 187.2 | 0.52 | 11 | 83 | 3.22 | 10.1 | 0.48–1.29 |
| 3 | Philippines | LMI | 83.4 | 0.5 | 15 | 83 | 1.88 | 5.9 | 0.28–0.75 |
| 4 | Vietnam | LMI | 55.9 | 0.79 | 13 | 88 | 1.83 | 5.8 | 0.28–0.73 |
| 5 | Sri Lanka | LMI | 14.6 | 5.1 | 7 | 84 | 1.59 | 5.0 | 0.24–0.64 |
| 6 | Thailand | UMI | 26.0 | 1.2 | 12 | 75 | 1.03 | 3.2 | 0.15–0.41 |
| 7 | Egypt | LMI | 21.8 | 1.37 | 13 | 69 | 0.97 | 3.0 | 0.15–0.39 |
| 8 | Malaysia | UMI | 22.9 | 1.52 | 13 | 57 | 0.94 | 2.9 | 0.14–0.37 |
| 9 | Nigeria | LMI | 27.5 | 0.79 | 13 | 83 | 0.85 | 2.7 | 0.13–0.34 |
| 10 | Bangladesh | LI | 70.9 | 0.43 | 8 | 89 | 0.79 | 2.5 | 0.12–0.31 |
| 11 | South Africa | UMI | 12.9 | 2.0 | 12 | 56 | 0.63 | 2.0 | 0.09–0.25 |
| 12 | India | LMI | 187.5 | 0.34 | 3 | 87 | 0.60 | 1.9 | 0.09–0.24 |
| 13 | Algeria | UMI | 16.6 | 1.2 | 12 | 60 | 0.52 | 1.6 | 0.08–0.21 |

Source –Jambeck, et. al. 2015. Plastic waste inputs from land into the ocean. Science magazine. 347: issue 6223.

# Plastic pollution coming from mismanaged plastic wastes

- not formally managed; includes disposal in dumps or open, uncontrolled landfills
- an estimated 4.8 to 12.7 million MT entered the ocean in 2010
- equivalent to 1.7 to 4.6% of the total plastic waste generated in those countries

- the top 20 countries' mismanaged plastic waste encompass 83% of the total in 2010

- top waste-producing countries having some of the largest coastal populations

# Mismanaged plastic wastes

- generated annually by populations living within 50 km of a coast worldwide
- enter the ocean via inland waterways, wastewater outflows, and transport by wind or tides
- mainly polyethylene and polypropylene
- partially degraded plastic debris, < 5 mm in diameter
- pose an even more serious impact on marine ecosystems by concentrating persistent organic pollutants;
- these particles enter the food chains through ingestion by marine wildlife -causes intestinal blockage in marine mammals, sea turtles and seabirds

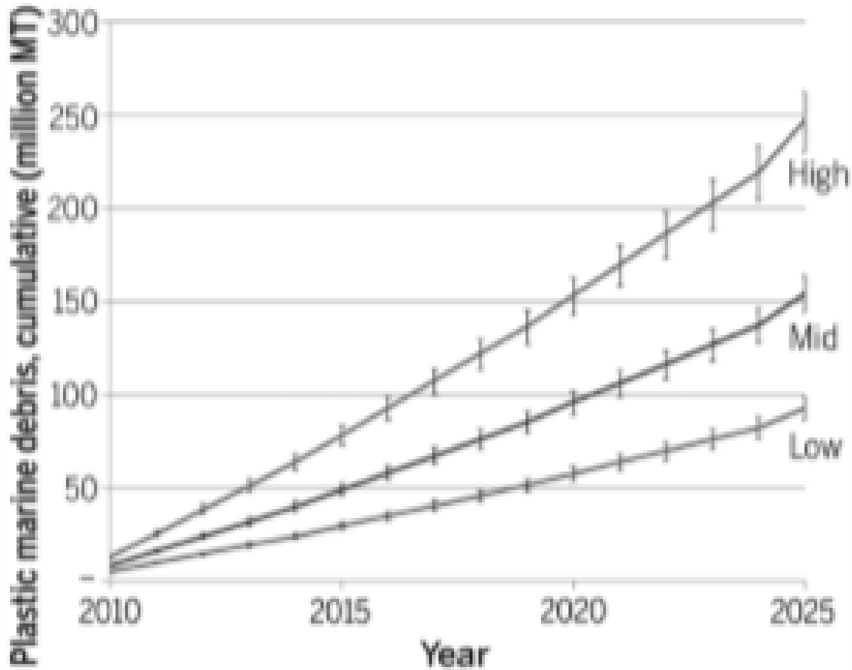



**Fig. 2. Estimated mass of mismanaged plastic waste (millions of metric tons) input to the ocean by populations living within 50 km of a coast in 192 countries, plotted as a cumulative sum from 2010 to 2025.** Estimates reflect assumed conversion rates of mismanaged plastic waste to marine debris (high, 40%; mid, 25%; low, 15%). Error bars were generated using mean and standard error from the predictive models for mismanaged waste fraction and percent plastic in the waste stream (*12*).

Source – Jambeck, et. al. 2015. Science magazine. 347: issue 6223.

# Chemical composition of plastic materials – fossil-based products

Wei and Zimmermann. 2017. Microbial Biotechnology .John Wiley & Sons Ltd and Society for Applied Microbiology

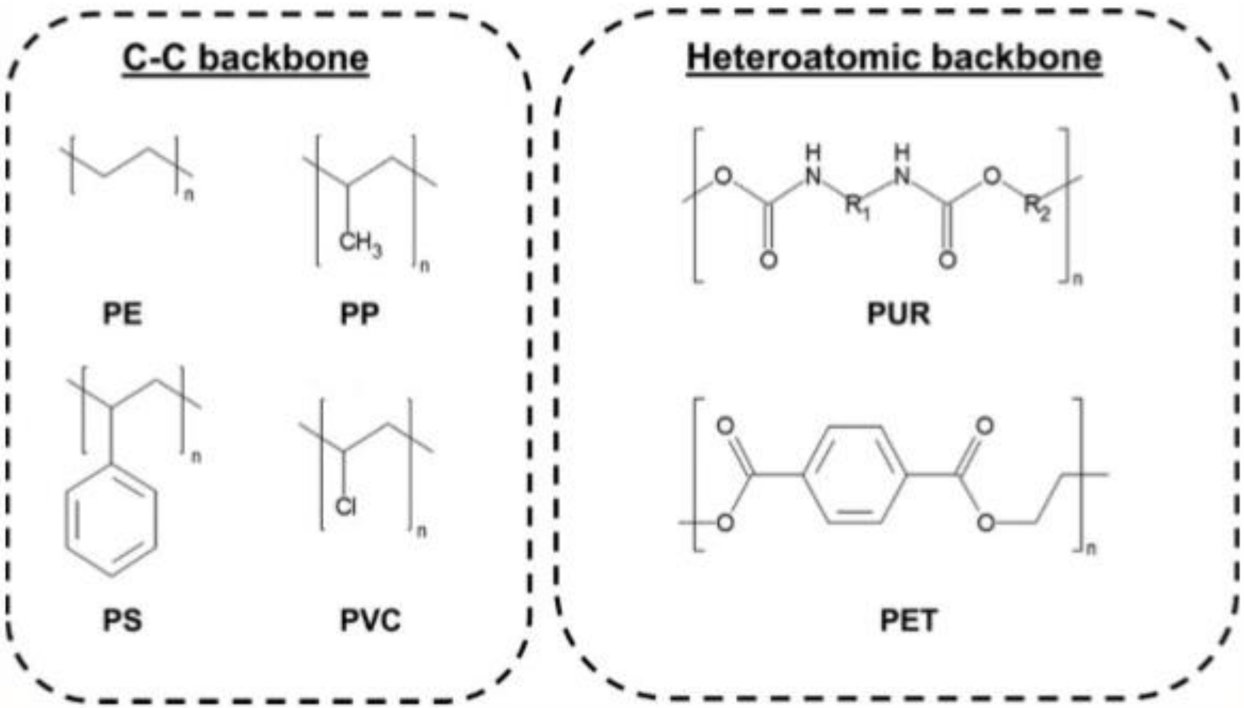



**Fig. 1.** Backbone structural formula of widely used petroleum-based plastics (PlasticsEurope, 2016).

polyethylene (PE), polystyrene (PS), polyurethane (PUR) and polyethylene terephthalate (PET)

# 1st study on potential fungal plastic degradation – mid 1990's

- Basic study showed - half life - thinnest, most transparent plastic bag – 14.5 years
- - will take 100 years to completely decompose
- leaf litter - half life-0.422 year; full decomposition 12 years
- Isolated sterile dark mycelia by 'baiting' method
- Caused 9 mg (15%) loss in wt of plastic film when grown in mineral medium with plastic strips and 0.5% glucose as sources of carbon, after 30 days incubation period; produced 40 mg dry wt of mycelia

**Cuevas, VC and RL Manaligod. 1997. Phillip J Sc (126(2):117-130.**

# Plastic degradation in forest environment

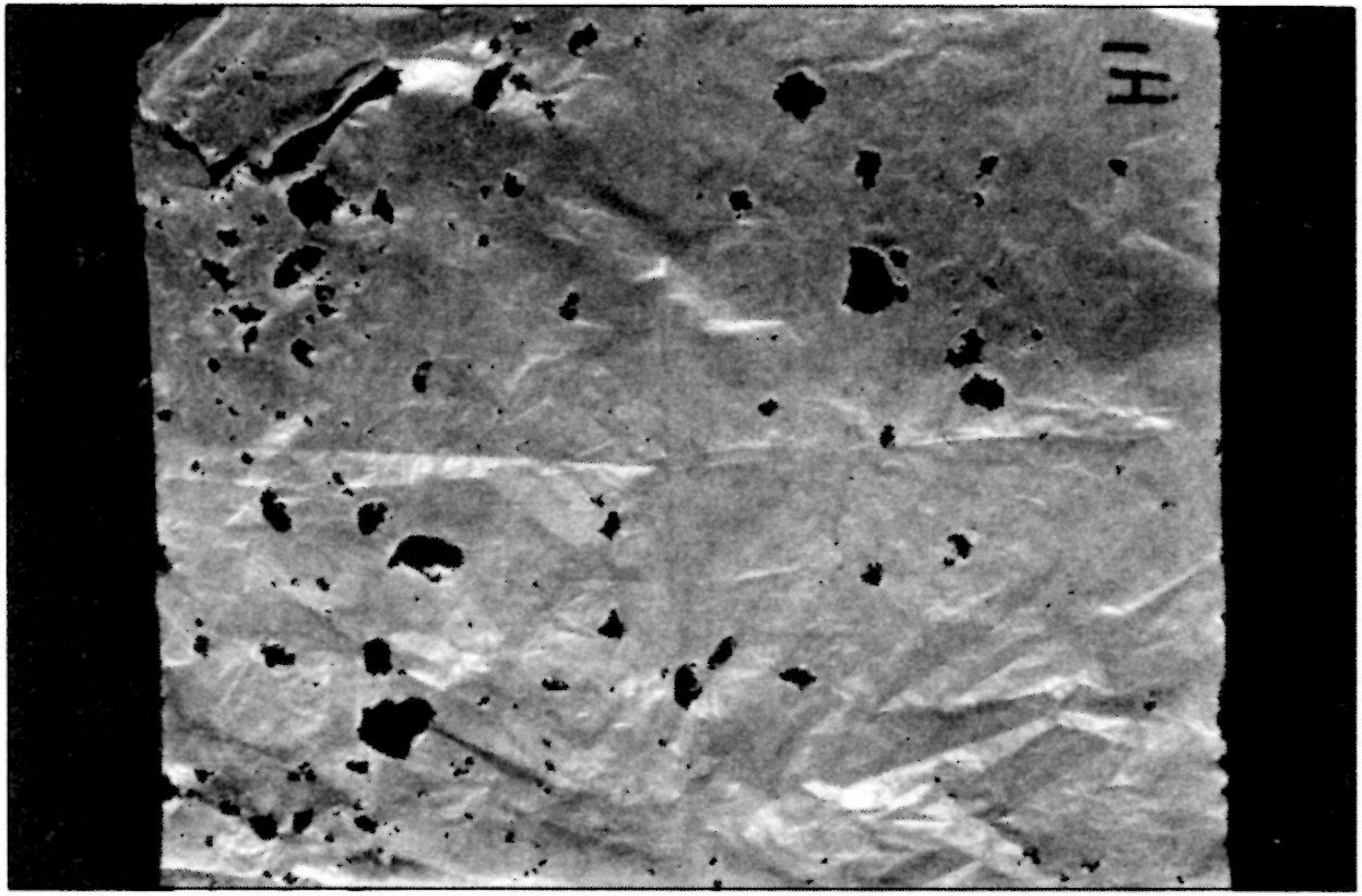



Figure 6. Plastic sheet (Type II) buried for 4 months in soil and litter.

Cuevas, VC and RL Manaligod. 1997. Phillip J Sc (126(2):117-130.

# Further study on plastic degrading ability of sterile dark mycelia

- Misidentified as *Xylaria* sp.
- An active cellulose and lignin degrader
- Had profuse growth in mineral medium at optimum pH and temp under lab condition with plastic strips and 0.5% glucose
- produced mucilaginous sheath that enabled adhesion of mycelia on plastic strips
- SEM showed tearing, striations and burrowing of hypha on plastic material

**Clutario, MTP and Cuevas, VC. 2001. Phillip J Sc 130(2):89-95.**

# Plastic degradation by isolate sterile dark mycelia

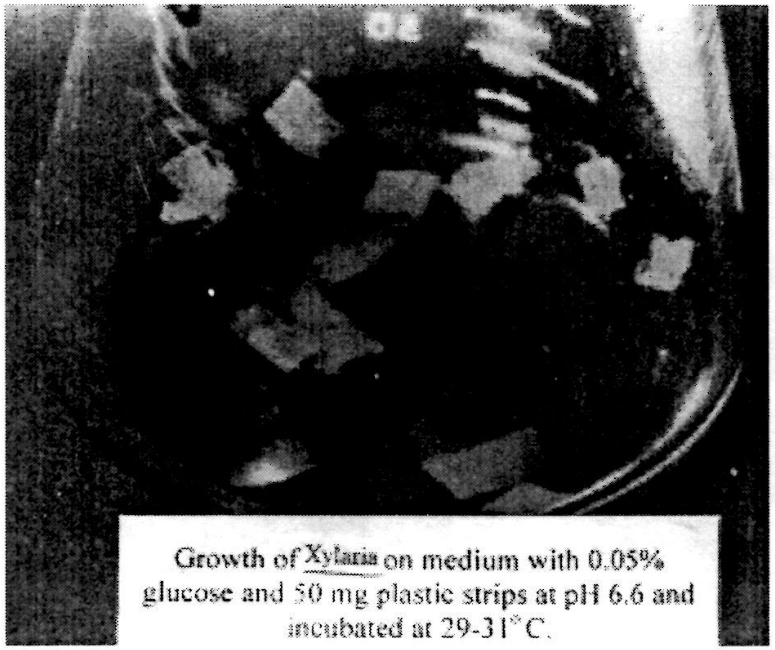



**Figure 2a**. Growth of *Xylaria* sp. in mineral medium with 0.5% glucose and plastic strips; pH of medium – 6.6 and incubation temperature at 29°C – 31°C for 50 days.

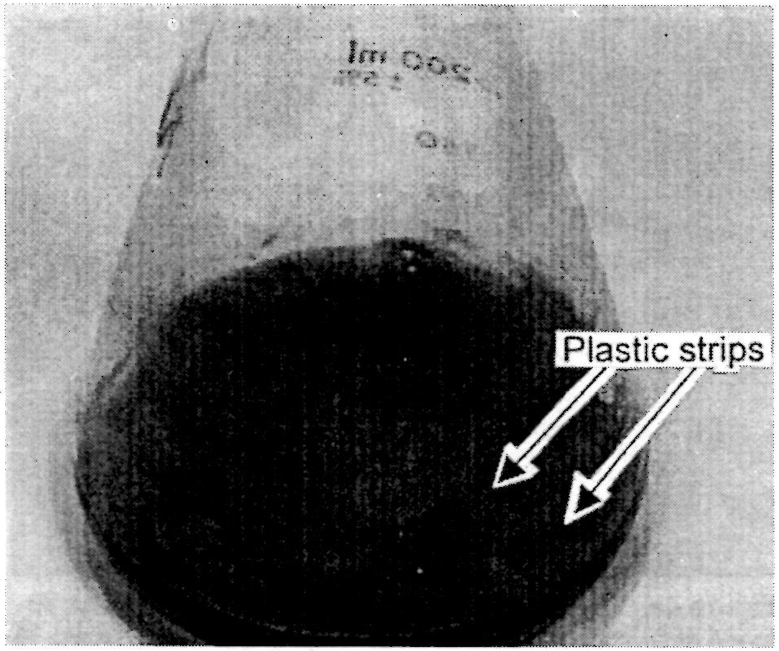



**Figure 2b**. Growth of *Xylaria* sp. in mineral medium with 0.5% glucose and plastic strips; pH of medium – 5.0 and incubation temperature at 25°C – 28°C for 50 days.

Clutario, MTP and Cuevas, VC. 2001. Phillip J Sc 130(2):89-95.

# Plastic degradation by isolate sterile dark mycelia

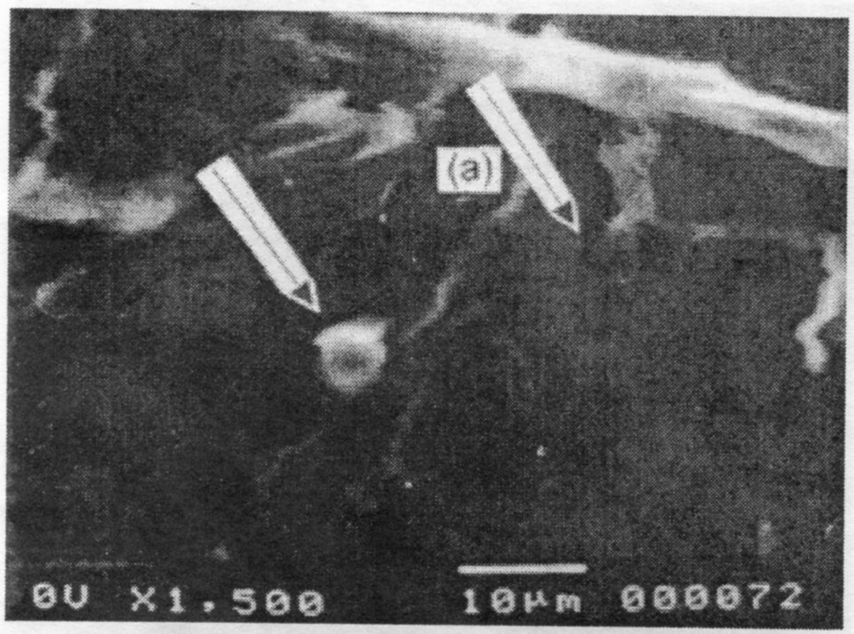



**Figure 4a**. Tearing of plastic surface as shown in SEM. Arrow A shows a hypha in actual process of burrowing. Arrow B shows a hypha rising from a torn surface.

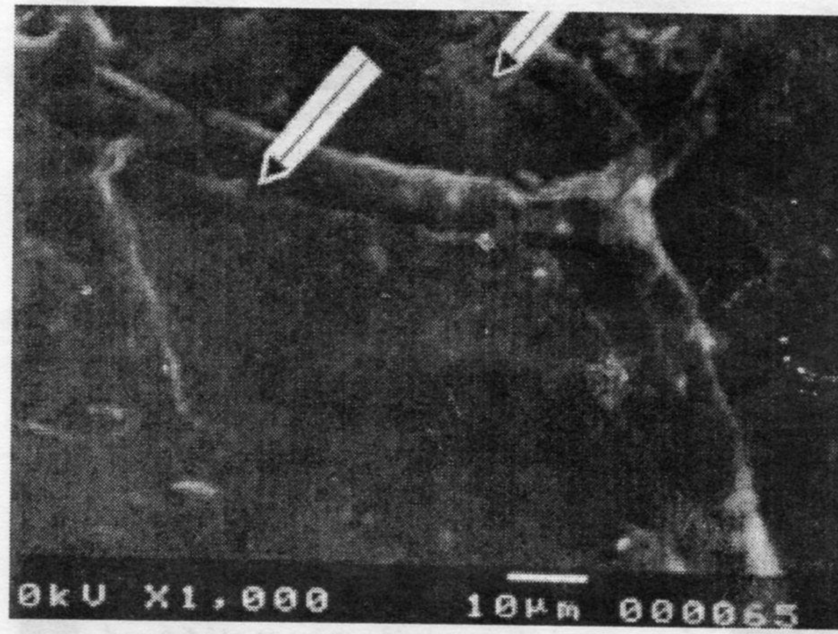



**Figure 4b**. Evidence of degradation revealed by SEM.. Striations (pointed by arrows) on the component of plastic surrounding the hypha.

After 50 days incubation period

**Clutario, MTP and Cuevas, VC. 2001. Phillip J Sc 130(2):89-95.**

# Plastic degradation by isolate sterile dark mycelia

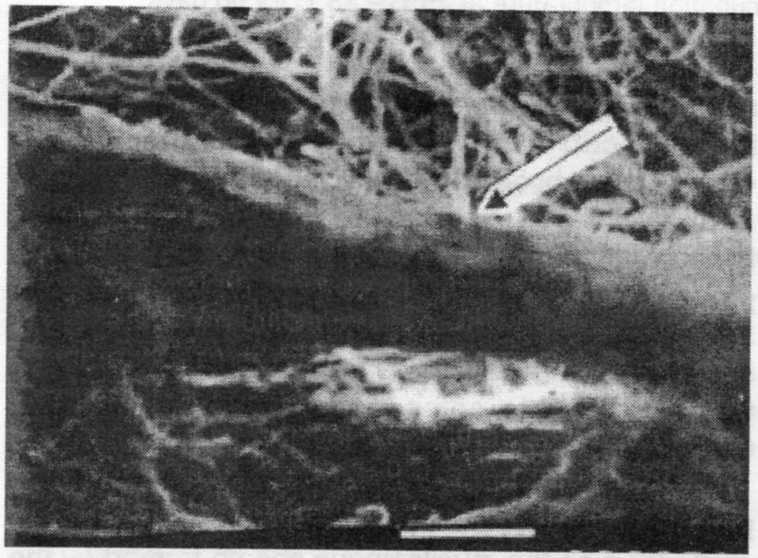


**Figure 4c**. Undersurface of the plastic strip as seen in SEM. Arrow points an actively burrowing hypha on the edge of the material.

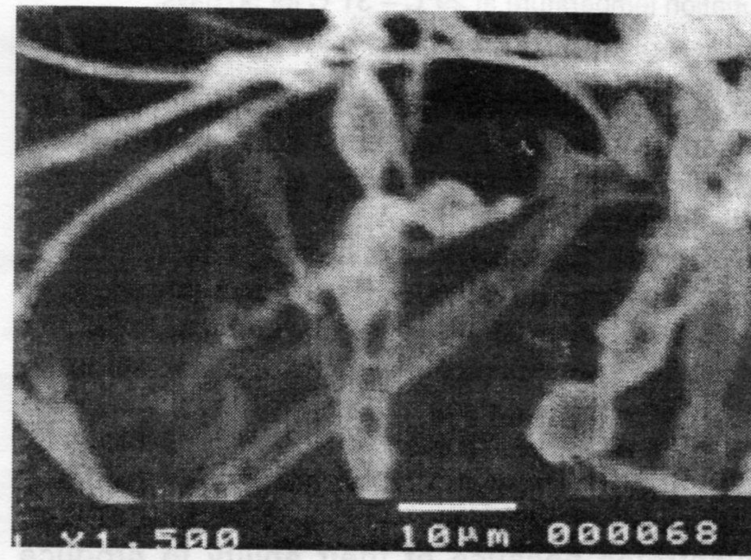



**Figure 4d**. Formation of capsular material outside the hypha.

Clutario, MTP and Cuevas, VC. 2001. Phillip J Sc 130(2):89-95.

# Other fungal potential plastic degraders

Collected efficient lignin degraders from stressed environment – soil covered with mine tailings undergoing ecological succession
- Identified the isolates by molecular method using analysis of sequence of internal transcribed spacer region between 18s and 28s ribonuclear RNA

*Lasiodiplodia theobromae, (sterile dark mycelia), Fusarium verticilloides, Penicilium janthenilium Paecilomyces puntonii*

Urzo MLR, Cuevas VC and Opulencia RB. 2017. Phillip. Agric. Sc. 100 Special Issue. 572-580.

## Urzo MLR, Cuevas VC and Opulencia RB. 2017. Phillip. Agric. Sc. 100 Special Issue. 572-580.

Substrate – polyester polyurethane (PUR) in amorphous, colloidal form - raw materials for making foams, mattresses

- Isolates grown in mineral medium with PUR as source of C & N

- compared growth in potato dextrose broth and mineral medium with PUR as source of C and NH4SO4 as source of N

Degrading ability assessed by

- by liquid clearance assay

- dry mycelial production in PUR medium and Potato dextrose broth (PDB)

# Liquid clearance assay

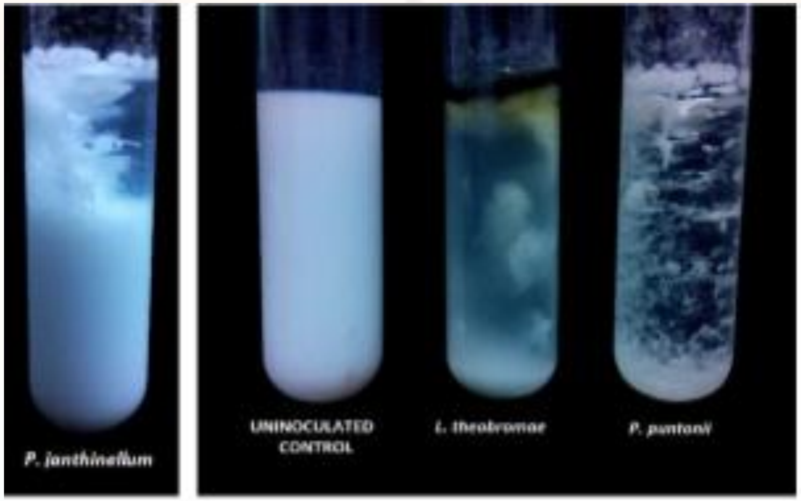



**Fig. 3.** Liquid Clearance Assay. Left: MM/L$_{PUL}$ inoculated with *P. janthinellum* showing clearance after 6 days of incubation; Right:

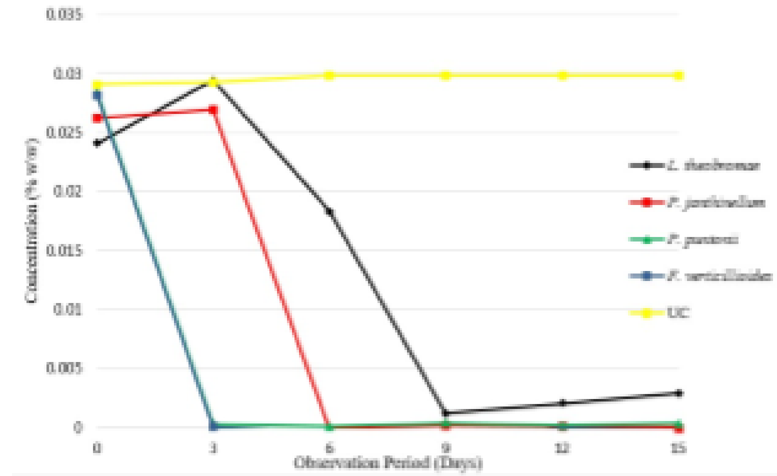



**Fig. 4.** Concentration of PU in MM/L$_{PUL}$ during 15 day incubation period.

PUR – more susceptible to microbial attack than PE

Urzo MLR, Cuevas VC and Opulencia RB. 2017. Phillip. Agric. Sc. 100 Special Issue. 572-580.

# Growth in mineral medium with PUR as source of C and N

Urzo MLR, Cuevas VC and Opulencia RB. 2017. Phillip. Agric. Sc. 100 Special Issue. 572-580.

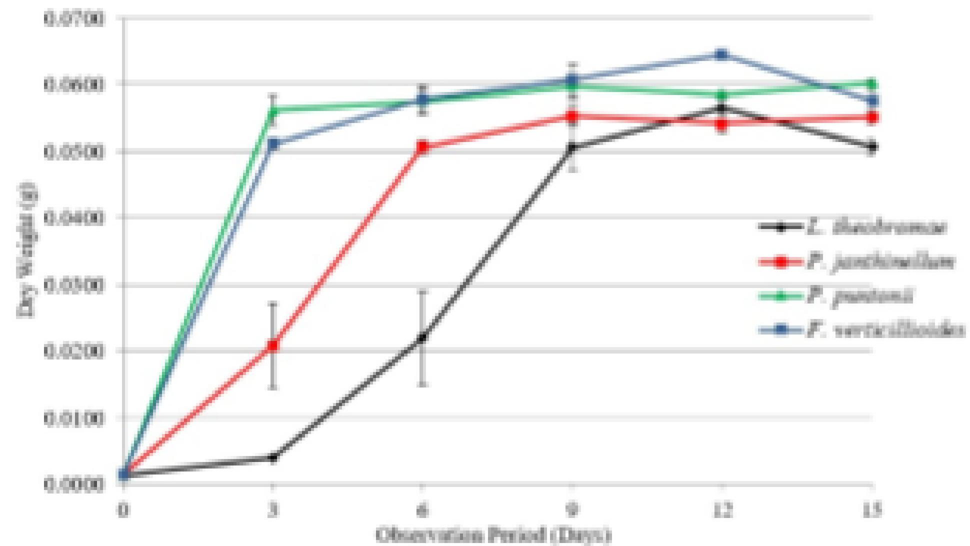



**Fig. 5.** Growth curves of top four PU degraders grown in $MM/L_{PUL}$.

**Table 1.** Computed P value from the Pairwise T test comparison of biomass grown in PDB and mineral medium. Data used was from mean dry weight of fungal biomass of each isolate grown in $MM/L_{PUL}$ and PDB.

| Isolate | P value |
|---|---|
| FI0101 | 0.883 |
| FI0602 | 0.549 |
| FI1503 | 0.377 |
| FI1803 | 0.105 |

# Microbial degradation of biodegradable plastics

**Substrates used**:

commercial biodegradable plastic -3 types of oxobiodegradable plastic (OBD);

Control - non-biodegradable ordinary plastic bag – low density polyethylene - LDPE

Test organisms – obtained from BIOTECH UPLB Culture collection

- *Cellulomonas flavigena; Arthrobacter luteus* – cellulose degraders
- *Phanerochaete chrysosporium* – lignin degrader
- Natural soil microflora

**Method of evaluation**

- Soil burial for 90 days
- Grown in synthetic medium with plastic strips as sole C source under lab condition – 90 days

} wt loss plastic strip / SEM observation

Gutierrez, et al. 2015. Asian J. Applied Sciences. 3(1):75-81.

# Results

Wt loss of biodegradable plastic under lab condition by two cellulose degrader bacteria

– dependent on the chemical composition of plasticicer used

- less than 1% wt loss was observed starting from 30 days exposure to the two test organisms

- Soil burial test – little higher (1.5%) than lab condition; biodegradable plastic – slightly higher wt loss than LDPE

Gutierrez, et al. 2015. Asian J. Applied Sciences. 3(1):75-81.

# Results :

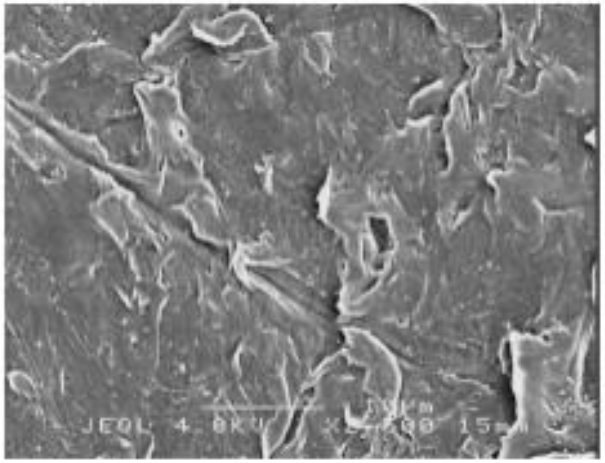


**Figure 10.** Flakes detached on the surface of LDPE incubated with *P. chrysosporium* as seen through the SEM.

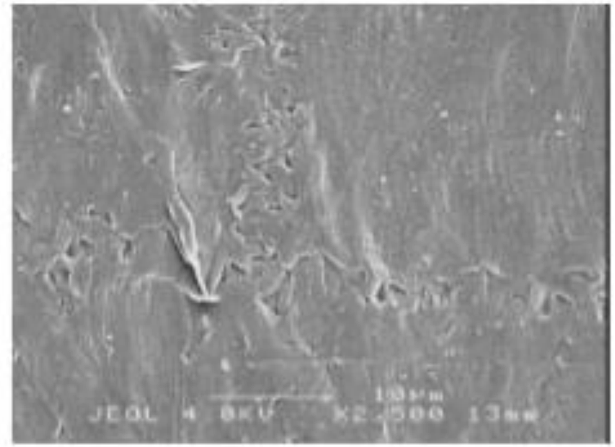


Figure 11. Flakes detached on the surface of OBP incubated with *P. chrysosporium* as seen through the SEM.

LDPE- had higher wt loss (9%) than OBD (4%)with *Phanerochaete chrysosporium*, under lab condition

Gutierrez, et al. 2015. Asian J. Applied Sciences. 3(1):75-81.

# Potential bacterial plastic degraders from Payatas dump site. 2015

Substrates used: polyethylene glycol (PEG) and low density polyethylene (LDPE

Methodology: substrate enrichment of soil samples and leachate from Payatas dumpsite, incubated for one week; diluents plated in mineral medium with PEG sole source of C,
-isolates taken from colonies with clearing zone

Identification of isolates based on results on cultural, morphology and biochemical tests

Evaluation of plastic degrading ability:
- $CO_2$ evolution from mineral culture medium with LDPE strips as sole source of C and inoculated with isolates with continues shaking incubated for 1 week
- observation of LDPE strips in SEM

Bolo et al. 2015. Phil J Health Res. & Devt. 19)1:50-59.

*Kocuria kristinae, Dermacoccus nishiomiyaensis, Pseudomonas stutzeri* and *Actinobacter haemolyticus*

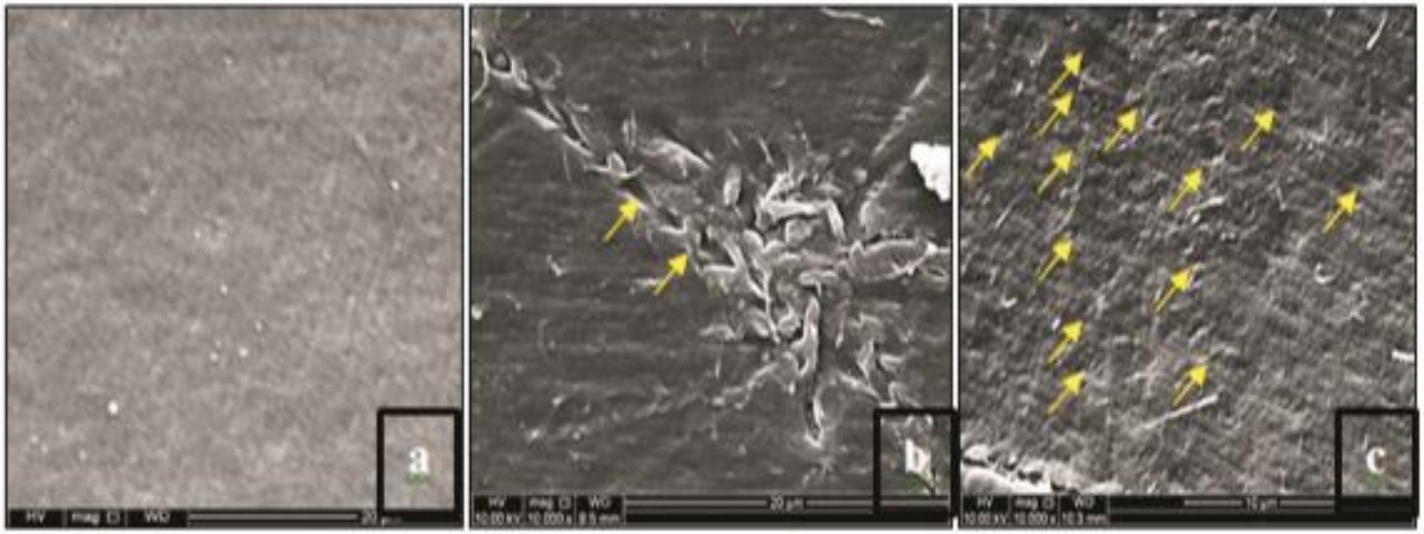



**Figure 5**. ***(a)*** *An SEM photomicrograph of the control group showing a smooth surface, without any dents;* ***(b)*** *Photomicrograph of the low density polyethylene (LDPE) film showing dents (yellow arrows) as evidence for biodegradation by Kocuria kristinae;* ***(c)*** *Photomicrograph of low density polyethylene (LDPE) film showing wears in the form of etching (yellow arrows) as evidence for biodegradation by Dermacoccus nishinomiyaensis.*

Bolo et al. 2015. Phil J Health Res. & Devt. 19)1:50-59.

# HDPE bacterial plastic degraders from root nodules of *Phaseolus vulgaris*

Substrate: high density polyethylene (HPDE) plastic bag

Test organisms: isolated from root nodules of *Phaseolus vulgaris* by plating homogenized root nodules samples in mineral medium with HPDE powder as sole source of C

Evaluation of plastic degrading ability

- Loss in dry wt of HDPE strips when incubated with isolates grown in mineral medium for 90 days
- SEM Observation of HPDE strips
- Fourier transform infrared spectroscopy (FTIR) analysis

**Baculi RQ, JB Melegrito, RDS Sabalen, and MR Caranto. 2017. Philip Agric Sc. 100 (Special Issue). S21- S31.**

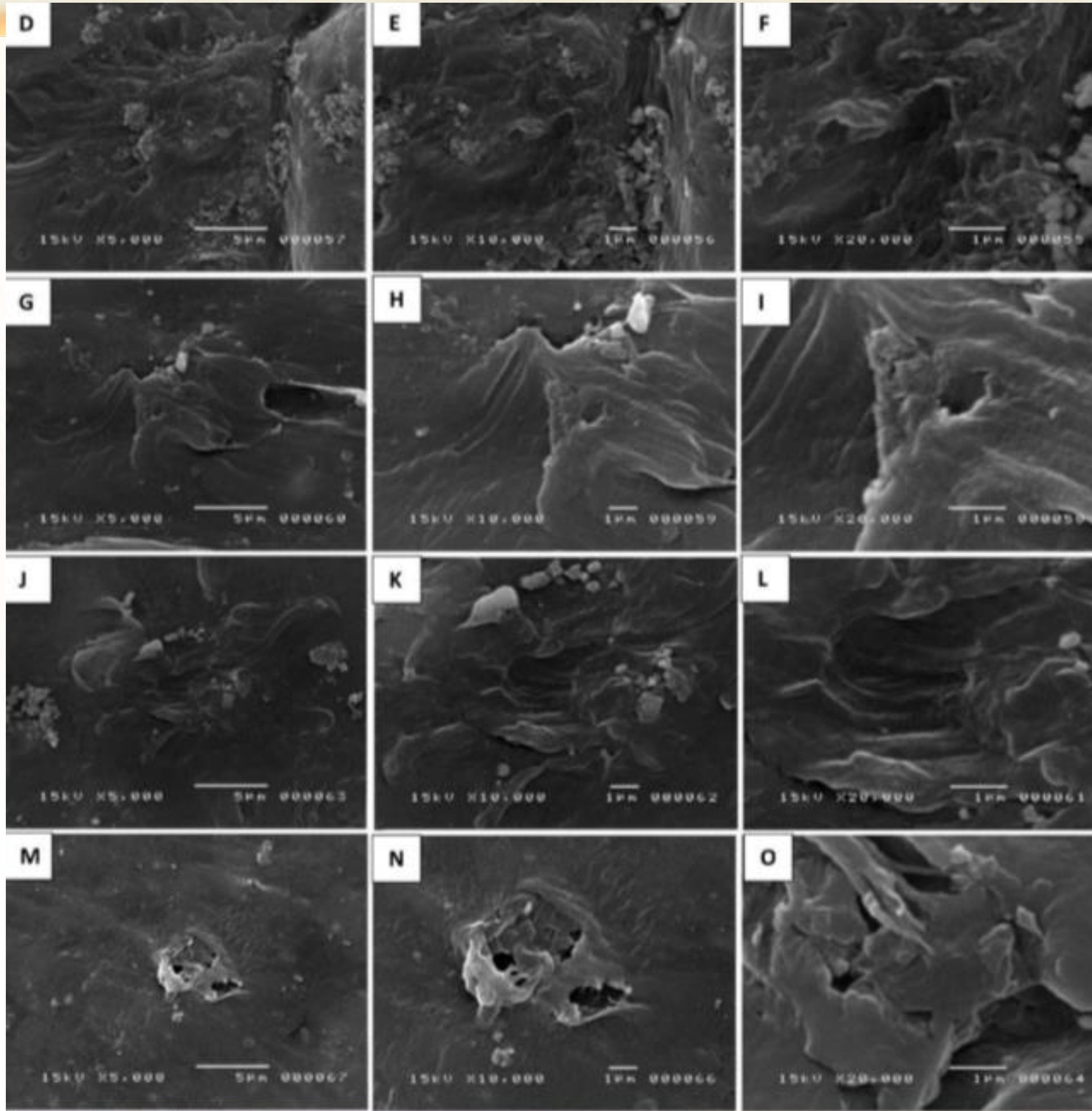
## Baculi RQ, JB Melegrito, RDS Sabalen, and MR Caranto. 2017. Philip Agric Sc. 100 (Special Issue). S21- S31.

Identification of isolates by molecular method – analysis of the 16S rRNA gene sequence

*Pseudomonas flourescens* and *Serratia marcescens* caused 2% and 8.6% loss of dry wt of HPDE strips respectively after 90 days incubation

FTIR analysis showed degradation happened at the carbonyl residues

SEM pictures of HDPE strips after 90 days incubation - 5000x, 10,000x and 20,000 x



## Potential plastic bacterial degraders from serpentine (alkaline) spring

Substrate: low density polyethylene (LDPE) plastic bag

Test organisms: isolates from hyperalkaline spring water by enrichment culture technique using LDPE powder as sole source of C in mineral medium

Evaluation of plastic degrading ability:

- Loss in dry wt of LDPE strips when incubated with isolates grown in mineral medium for 90 days
- SEM Observation of LPDE strips
- Fourier transform infrared spectroscopy (FTIR) analysis

Dela Torre DYZ, LA Delos Santos, MLC Reyes and RQ Baculi. 2018. Philip Sc Letters. 11 (Supplement): 1-12.

## Dela Torre DYZ, LA Delos Santos, MLC Reyes and RQ Baculi. 2018. Philip Sc Letters. 11 (Supplement): 1-12.

*Bacillus kruzlwichiae, Bacillus pseudofirmus, Prolinoborus fasciculus* and *Bacillus sp.* identified by molecular method

- Reduced weight of residual polymer up to 9.9%, 8.3%, 5.1%, and 6.3%, respectively
- isolates have the capability to adhere to the plastic surface

FTIR analysis showed changes in

- keto carbonyl index, ester carbonyl bond index, internal double bond index, and vinyl bond index supporting the depolymerization activity of the isolates

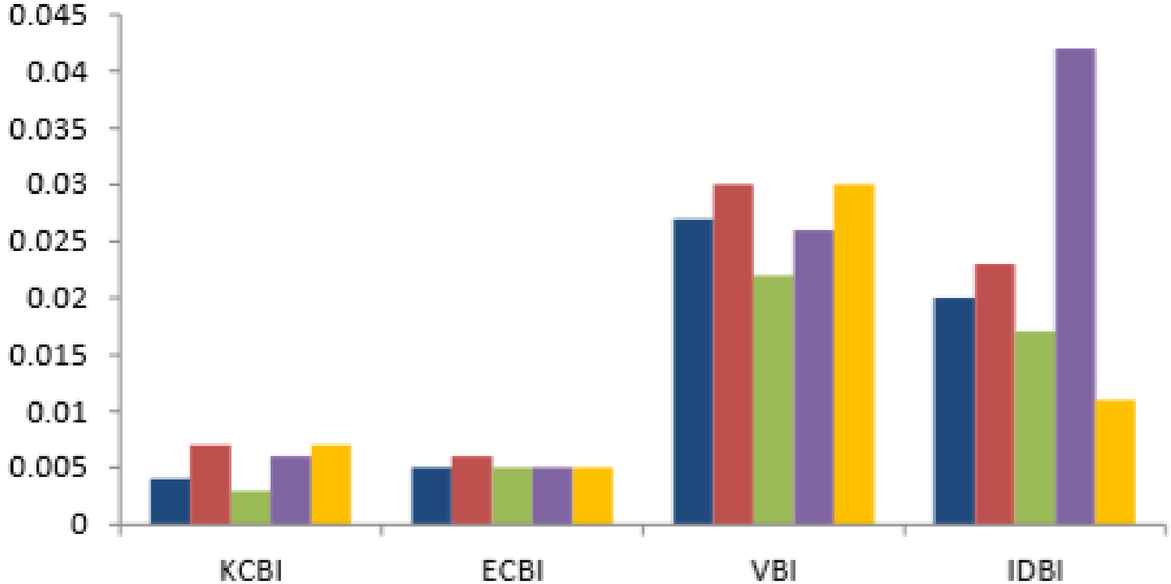



**Figure 5: FTIR indices of LDPE films exposed to the isolates for 90 days.** (KCBI-keto carbonyl index; ECBI-ester carbonyl index; VBI-vinyl bond index; IDBI-internal double bond index) [(Control ( );Bacillus krulwichiae PB1( );Bacillus pseudofirmus PB5( );Prolinoborus fasciculus PB8 ( ); Bacillus sp. PB14 ( )].

Dela Torre DYZ, LA Delos Santos, MLC Reyes and RQ Baculi. 2018. Philip Sc Letters. 11 (Supplement): 1-12.

- not all of the bioplastics derived from renewable resources are readily biodegradable

- have lower durability and lack of compatibility with existing equipment and end-of-life management systems

- their scale of production and use are still very limited

**Important factors restricting plastic biodegradability:**

a) hydrophobicity,
b) degree of crystallinity - complex three-dimensional structure,
c) surface topography and
d) molecular size- high molecular weight

# Problems associated with microbial plastic degradation

**Characteristics of plastic materials**

❖ **smooth surface topography and** low specific surface area

- degradation by enzymes - a surface erosion process
- difficulty for microorganisms to adhere to the surface of plastic film
- restricts the formation of a biofilm

❖ **high molecular weight, complex three-dimensional structure**

- lack of exposed surfaces available for biological attack

❖ **Hydrophobicity**

- enzymes attacking polymers are hydrolases

# Enzymes involved in polymer degradation

- ❖ enzymes involved in the metabolism of plant lignin

- lignin degrading microorganisms

Lignin - heterogeneous cross-linked phenolic polymer with oxidizable C-C bonds - enzymes laccases, manganese peroxidase, lignin peroxidase

- PE more complex than lignin; requires higher redox potential due to homogenous C-C backbone ,
- an efficient degradation of PE by these lignolytic enzymes not to be expected

- ❖ key enzymes capable of attacking the polymer backbones such as cutinases

are also able to hydrolyze the ester bonds in PET and PUR

PET and PUR – more biodegradable than PE, PS, PP AND PVC

- PET and PUR are polymers with hydrolysable chemical bonds in their backbone
- Latter types have to be oxidized first prior to their further depolymerization

- UV irradiation, oxygen, temperature, and chemical oxidants can be used as pre- treatments for oxidation

**Other pre treatments necessary**

PET materials must be reduced to particle sizes between 0.25 and 0.5 mm

- faster degradation by a bacterial polyester hydrolase due to increased accessibility of surface area for the enzyme action

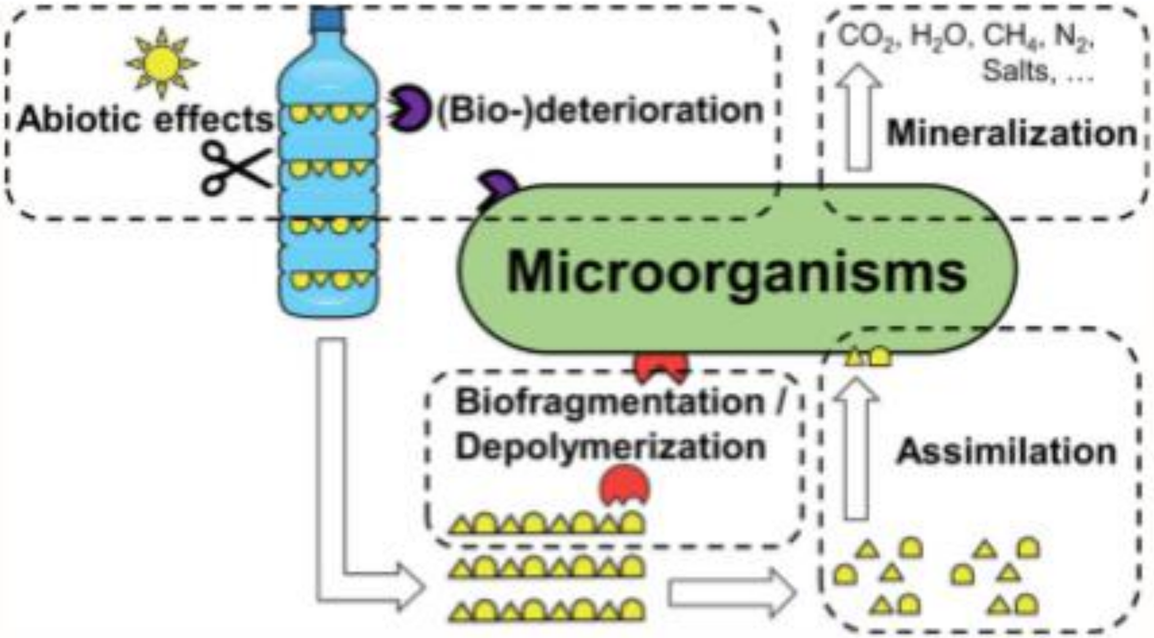



Fig. 2. Schematic illustration of plastic biodegradation (Lucas *et al*., 2008).



Source: Wei and Zimmermann. 2017. Microbial Biotechnology published by John Wiley & Sons Ltd and Society for Applied Microbiology.

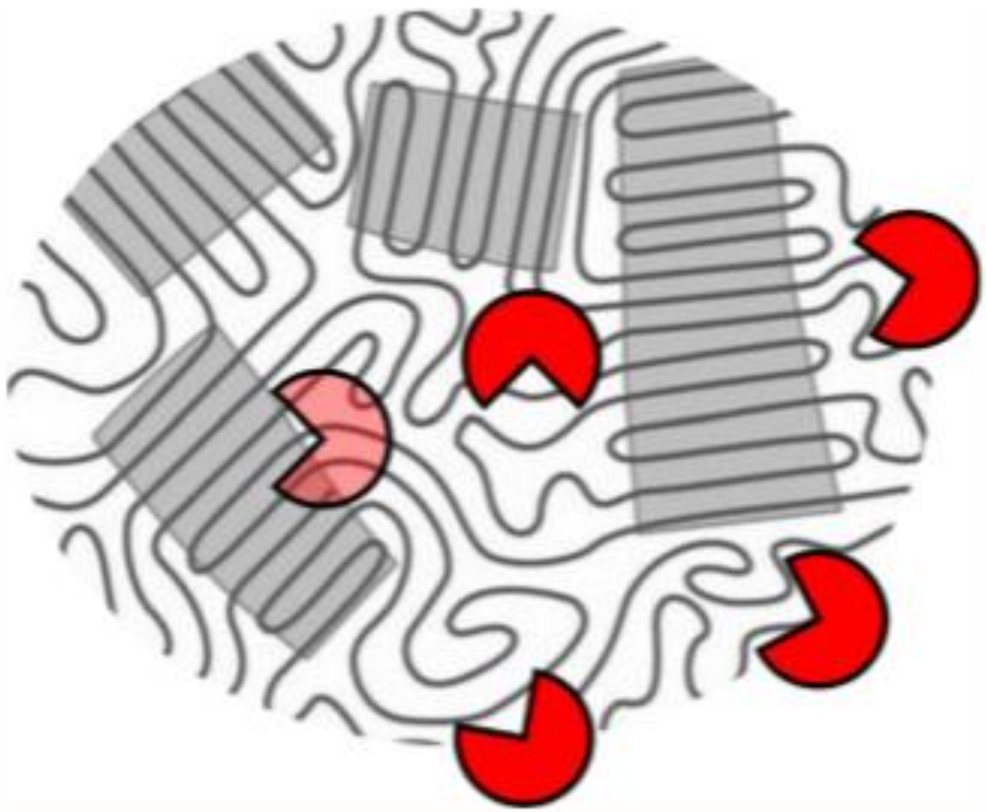


**Fig. 4.** Schematic illustration of a semi-crystalline polymer containing both amorphous and crystalline regions (grey areas). The amorphous parts are more susceptible to enzymatic attacks.

Source: Wei and Zimmermann. 2017. Microbial Biotechnology published by John Wiley & Sons Ltd and Society for Applied Microbiology.

# Different studies conducted in UP campuses show

a good number of potential microbial plastic degraders (bacteria and fungi) have been isolated and characterized

- much work needs to be done to utilize these microorganisms to develop the technology that can be used to safely dispose plastic wastes.

- 2017 review article has emphasized pre treatments processes are needed before efficient biocatalytic plastic recycling processes can be attained/

There is a need for a multidisciplinary approach – microbiologists, engineers, biochemists are needed to work together - to develop such technology for an environment-friendly plastic waste disposal system

- **1st – PROCUREMENT LAW MUST BE REVISED**. It is not a deterrent for corruption. Good, meaningful research will never prosper with this law.

# THANK YOU!