Plastic Not Fantastic

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Background, Purpose and Hypothesis

Plastic bags are made from polyethylene (PE) which is a polymer consisting of long chains of the monomer ethylene. Plastic bags are very popular in our daily lives and have a harsh environmental impact on our ecosystems. Every year, approximately 500 billion plastic bags are used worldwide and billions of those are dumped into the oceans. Countless wildlife, including sea-lions, whales, birds and turtles ingest the plastic bags and die every year.

Plastic bags have very high durability, persisting in the environment for 20 to 1000 years before they decompose. The development of a biotechnological approach to PE degradation is the *main goal* of this project. The *hypothesis* is that if PE-degrading microorganisms do exist in the Nature, then it will be possible to isolate them and use them for degradation of plastic bags.

Procedure and Results

Part 1: Isolation and Testing of Polyethylene Degrading Microorganisms

1) Preparation of PE powder. A mixture made with 3g of cut PE film and 3g of NaCl was ground for 10 minutes using pestle and mortar. The mixture of ground PE film and NaCl was transferred into a cylinder containing distilled H_20. The floating layer of PE particles was collected on filter paper, washed three times with distilled H_20 and dried in an oven at ~60˚C overnight. Dried PE powder was then passed through a sieve mesh ($N^0$100).

2) Preparation of growth and enrichment medium. The rationale behind the enrichment procedure was to create strong selective conditions using powdered PE as the only source of carbon.

A growth medium consisting of 0.1% $(NH_4)_2SO_4$, 0.1% $NaNO_3$, 0.1% K_2HPO_4, 0.1% KCl, 0.02% $MgSO_4$, and 0.01 % yeast extract was prepared in 1L of tap H_20. The enrichment medium was created by adding 0.2g of PE powder to 100mL of growth medium.

3) Isolation of PE degrading microorganisms by enrichment procedure. A few soil samples were collected at a local landfill in Waterloo, Ontario, then mixed together and used as a source of

potential PE degrading microorganisms. 1g of the soil mix was added to the first enrichment flask, which was incubated at ~30°C for 4 weeks on shaker (200rpm). 10mL of broth was taken from the first enrichment flask, and re-inoculated into 100mL of fresh enrichment medium, then cultivated under the same conditions for 4 weeks. The same procedure was repeated a third time. The final enrichment culture (FEC) was filtered through filter paper to remove any remaining polyethylene powder.

4) Measurement of ability for PE degradation. PE degradation was performed in flasks containing 50mL of growth medium, 10mL of FEC, and weighed PE film strips. Flasks were incubated on a shaker for 6 weeks at 30^{0}C. Control flasks containing 10mL boiled FEC, to kill all microbes, 50mL growth medium and weighed PE film strips were incubated under same conditions.

PE degradation was determined by measuring the weight loss (%) of the PE film strips. After 6 weeks incubation, the average weight loss of PE film strips was 17.2% $\pm$ 1.45, while the PE film strips in control flasks demonstrated no weight loss. So the FEC is, in fact, able to degrade PE film strips. Results are presented in Table 1.

Table 1. PE degradation (%) by Final Enrichment Culture

Flask Number	Flask 1	Flask 2	Flask 3
PE Film Strip Weight Loss (%)	19.32	15.27	16.83

Part 2: Microbial Identification

1) Analysis of Final Enrichment Culture (FEC). FEC was subjected to serial dilutions and plating out on Luria-Bertani (LB) agar plates to observe morphology and amount of microorganisms in FEC. Four distinct colonial morphologies were observed, suggesting the existence of at least four different microbial strains in FEC.

2) Purification of microbial strains from FEC. Colonies of each microbial strain from FEC were picked up, resuspended in 0.2ml of 0.85% NaCl and streaked out on LB agar. This procedure was repeated until pure strains were obtained. Each strain was preserved on LB agar.

3) Measurement of ability for PE degradation by individual microbial strains from FEC.

Isolated microbial strains from FEC preserved on LB agar were individually tested for PE degrading ability. The results are demonstrated in Table 2, and showed that only strain №2 is capable of significant PE degradation. The numbers represent an average of three flasks that underwent the exact same treatment.

Table 2. PE degradation by individual microbial strains isolated from FEC

Strain Number	№1	№2	№3	№4
Average PE Film Strip Weight Loss (%)	4.8 ± 0.62	21.6 ± 2.57	1.2 ± 0.69	2.3 ± 1.03

4) Effect of combination of microbial strains from FEC on PE degradation. The degree of PE degradation was significantly increased when the strongest PE degrader, strain №2, was combined with the strain №1. Results are presented in Table 3. The numbers represent an average of three flasks that underwent the exact same treatment.

Table 3. PE degradation by combined action of microbial strains isolated from FEC

Strain Combination	№2 & №1	№2 & №3	№2 & №4
Average PE Film Strip Weight Loss (%)	32.2 ± 2.67	22.5 ± 1.41	21.2 ± 2.23

A reasonable explanation of this phenomenon is that strain №1 produces a biosurfactant- a surface active compound. Biosurfactants are compounds that emulsify (solubilize) PE oligomers. Emulsified oligomers are taken up and metabolized by microbial cells.

5) Identification of microbial strains. The microbial strains were characterized according to their phenotype, based on colony morphologies on LB agar. Bacterial cell morphology was observed under microscope after Gram staining. Further identification was done using the API 20NE test, according to the manufacturer's, bioMérieux, instructions.

Characterization of strain №2- Gram-negative cells, rod-shaped with rounded poles. On LB agar, it forms deep-yellow pigmented colonies around 0.2mm in diameter, circular, slightly convex and

opaque, smooth after 2 days of incubation at 30˚C. The cells did not grow in anaerobic conditions. The strain was negative for acid production from glucose. The catalase and oxidase-positive strain did assimilate acetate. Phenotypic characterization made it possible to assign strain №2 to the genus *Sphingomonas.*

Characterization of strain №1- Gram-negative, rod-shaped cells. After two days of incubation at 30˚C on LB agar the strain produced smooth white colonies that have a mucoid appearance. The strain was negative for acid production from glucose under aerobic conditions. It was able to grow on organic substrates like glucose, gluconate and malate and gave a positive result to the oxidase and catalase test. Phenotypic characteristic made it possible to assign strain №1 to the genus *Pseudomonas.*

Part 3: Optimization of PE Degradation by Combination of Strain №2 and №1

1) Effect of temperature of incubation on PE degradation. The results (see Table 4) showed that PE degradation significantly increased at 30^0C compared to 7^0C. The numbers represent an average of three flasks that underwent the exact same treatment.

Table 4. Effect of temperature on PE degradation by the mixed culture of strains №2 and №1

Incubation Temperature (^{0}C)	**~7^0C**	**~30^0C**	**~37^0C**
PE Film Strip Weight Loss (%)	2.8 ± 0.74	33.3 ± 2.22	36.5 ± 3.66

2) Effect of inoculum size on PE degradation. The experiment demonstrated that PE degradation increased with the increase of the inoculum size of the mixed culture. The increase stopped when volume of the cultures was more than 2mL. The numbers represent an average of three flasks that underwent the exact same treatment.

Table 5. Effect of inoculum size on PE degradation the mixed culture of strains №2 and №1

Amount of Strains №2 and №1 (mL)	**0**	**0.25**	**0.5**	**1.0**	**2.0**	**3.0**
PE Film Strip Weight Loss (%)	0.0 ± 0.0	4.4 ± 1.22	14.9 ± 1.96	26.4 ± 2.04	34.0 ± 3.17	33.5 ± 2.41

3) Effect of sodium acetate on PE degradation.

The rationale for this experiment was to investigate the influence of sodium acetate as an alternative source of carbon on PE degradation in order to increase PE degradation by the mixed culture of strains №2 and №1. The results showed (see Table 6) that low concentrations of sodium acetate (up to 0.1%) stimulate PE degradation. Higher concentrations of sodium acetate repressed PE degradation. The numbers represent an average of three flasks that underwent the exact same treatment.

Table 6. Effect of acetate on PE degradation by the mixed culture of strains №2 and №1

Concentration of Sodium Acetate (%)	**0**	**0.05**	**0.1**	**0.2**	**0.4**	**1.0**
PE Film Strip Weight Loss (%)	35.3 ± 3.88	39.7 ± 3.56	42.8 ± 3.23	40.3 ± 1.47	21.7 ± 2.43	6.1 ± 1.51

Conclusion

A microbial consortium capable of PE degradation was obtained as a result of the enrichment procedure. Two PE-degrading strains were isolated from the consortium and identified as bacteria belonging to the genus *Sphingomonas* and *Pseudomonas*. A high degree of PE degradation was achieved by combination of the two strains.

Plastic bags are usually buried in landfills or thrown into the oceans and surrounding ecosystems. The process of polyethylene degradation developed in this project can be used on an industrial scale for biodegradation of plastic bags. As a result, this would save the lives of millions of wildlife species and save space in landfills.

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