NC STATE

College of Engineering

Chemical and Biomolecular Engineering

Bio-upcycling of PET Plastic Waste

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Introduction

Plastic pollution is a growing problem as plastic can take 50 to 600 years to decompose.

Downcycling ← **Plastic Waste** → **Upcycling**

Existing recycling techniques; very expensive & less uses than virgin plastic.

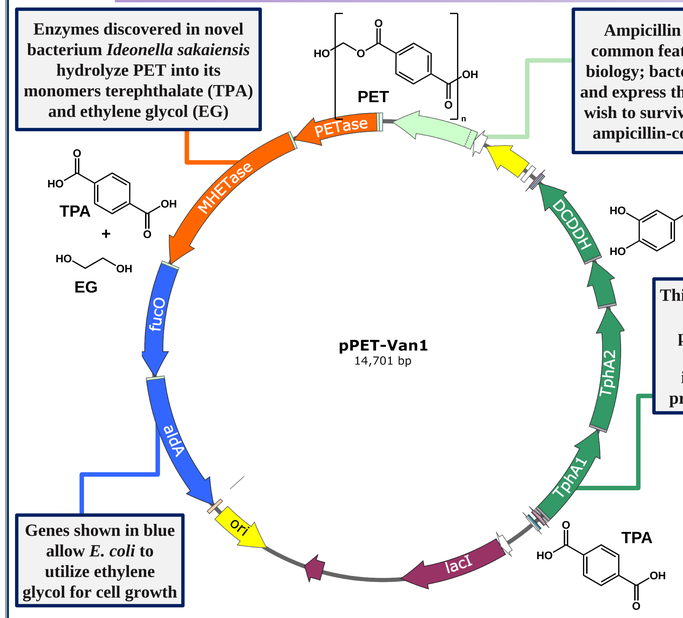
Biochemical methods of decomposition to produce valuable products.

The purpose of our project is to engineer microbes and develop a possible gene pathway to convert polyethylene terephthalate (PET) waste into a profitable product. Creating a valuable product provides an avenue for industry to reduce waste and help the environment.

Materials and Methods

Enzymes discovered in novel bacterium *Ideonella sakaiensis* hydrolyze PET into its monomers terephthalate (TPA) and ethylene glycol (EG)

Ampicillin resistance is a common feature of synthetic biology; bacteria must uptake and express this plasmid if they wish to survive when grown in ampicillin-containing media



This operon converts TPA to protocatechuate (PC), a key intermediate in product formation

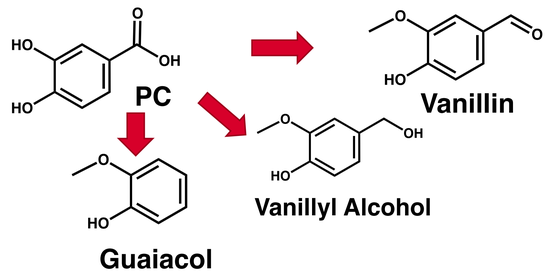
Genes shown in blue allow *E. coli* to utilize ethylene glycol for cell growth

- Novel enzymes from foreign sources can be expressed in bacteria such as *Escherichia coli* by inserting genes of interest into small circular pieces of DNA called "**plasmids**" or "**vectors**".
- National Center for Biotechnology Information (**NCBI**) is an online repository of genetic data that anyone can access.
- Building from previously published research, We used a program called **Snapgene** to design plasmids like the one shown above and simulate the steps needed to construct such a plasmid in a lab environment.
- **The plasmid shown above PET into Protocatechuate, a key intermediate in the synthesis of our final products, shown on the next panel.**

Results

Profitable Products Investigated

Vanillin	Vanillyl Alcohol	Guaiacol
Plant metabolite, anti-inflammatory, flavoring agent, antioxidant, and anticonvulsant.	**Plant metabolite, Anticonvulsive, suppresses seizures, anti-inflammatory, flavoring agent.**	**Antioxidant properties, expectorant, anti-skinning agent for paints, traditional dental pulp sedation**



pPET-Van1 is co-expressed with one of the plasmids shown to the right: pVan2, pVanOH, or pGuai to produce vanillin, vanillyl alcohol, or guaiacol from PET. Each fermentation scheme comes with distinct advantages and disadvantages

	Vanillin production with pVan2	Vanillyl alcohol production with pVanOH	Guaiacol production with pGuai
Pros	• most profitable product of the three	• Reduction to alcohol could alleviate vanillin toxicity	• Doesn't require co-expression with a 3rd plasmid • Decarboxylation w/ vdcBCD doesn't require energy or cofactors
Cons	• Requires co-expression w/ a 3rd plasmid for CAR activation • Reactive aldehyde group is toxic to E. coli	• Requires co-expression w/ a 3rd plasmid for CAR activation • lowest predicted product titre	• least expensive product

This plasmid, pVan2, encodes genes carboxylic acid reductase (CAR), and catechol O-methyltransferse (COMT), that convert protocatechuate to the final product, vanillin.

pVanOH can be constructed from pVan2 with one additional cloning step and produces vanillyl alcohol which is less toxic to *E. coli* than vanillin.

pGuai converts protocatechuate to guiacol via COMT and a non oxidative vanillic acid decarboxylase (vdcBCD)

Flux Balance Analysis

Flux balance analysis was conducted using the Python version of the COBRA package and the GLPK solver. *E. coli* MG1655 core model (BIGG ID: iJO1366) was modified to include one of the three pathways while supplying the cell with unlimited extracellular PET. The model was set to maximize production of the desired product.

Maximum Theoretical Yields of Product Molecules	
Product	Yield (mmol /g DCW-h)
Vanillin	10.0
Vanillyl Alcohol	9.2
Guaiacol	11.3

Limitations:

- Non-native metabolites only appear in reactions for which they are explicitly defined; pathway intermediates may be metabolized in unpredicted ways
- Product formation is controlled under inducible promoters; FBA cannot account for this

Economic Analysis

We considered a steady-state lab scale process with all equipment and utilities already provided, including a 1L bioreactor for E.coli process.

Below is a list of the related costs for this reactive system:

- Lysogeny broth (LB) media in bulk, $2.73 for 0.5L LB powder.
- PET source, either recycled or raw. As of January 2023, the national average price of post-consumer PET beverage bottles & jars was averaging 12.02 cents per pound. 75g of PET needed costs around $1.95 and the stead state cost is around $4.68.
- Vanillin, natural, ≥97%, FCC, FG"; the sale price is $267 for 100g.
- Vanillyl alcohol, ≥98%, FG"; the sale price is $115 for 100g.
- Guaiacol, natural, ≥99%, FG"; the sale price is $98.40 for 1000g.

Techno-economic analysis was conducted to determine the output prices. The following was calculated using FBA analysis and molar masses.

Techno-economic Analysis of Products					
Product	Yield (mol /g DCW-h)	Molar Mass (g/mol)	Yield (g product/ g DCW-h)	Product Sale Price ($/g product)	Sale Price ($/ g DCW-h)
Vanillin	0.0100	152.15	1.5215	2.67	4.06
Vanillyl Alcohol	0.0092	154.165	1.4183	1.15	1.63
Guaiacol	0.0113	124.14	1.4028	0.0984	0.13

Techno-economic analysis was conducted to determine the output prices.

- **Vanillin had the greatest sale price ($/g DCW-h) and marketability with a projected growth of 308 million from 2020 to 2025.** Additionally, the sales are projected to grow from 37,286 tons in 2018 to 59,458 from in 2024.
- Vanillyl alcohol is projected to increase ~ 5% globally by next year.
- Guaiacol has a steady growth with a projected growth from 310 million to 346 million in 2029.

Conclusions

- We have drafted several genome sequences for 3 target molecules for the biochemical conversion of PET into valuable products.
- Our techno-economic analysis determined that vanillin had the greatest sale price ($/g DCW-h) and marketability

Acknowledgements

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References

Scan QR Code for complete list of references.
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