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ECO-ENZ

Innovation Plan

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TABLE OF CONTENTS

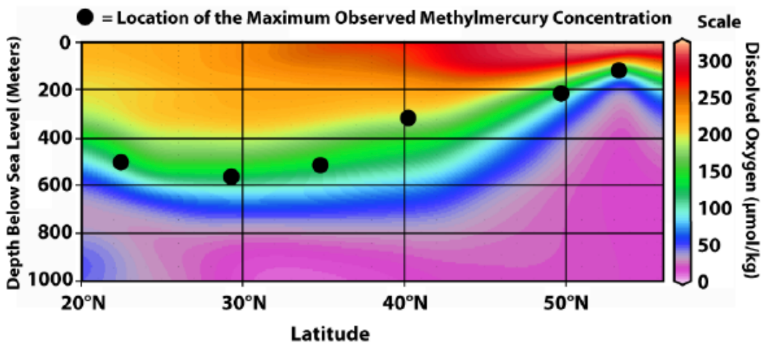
EXECUTIVE SUMMARY

Eco-Enz is a private company (non-profit) focused on creating a more productive planet for this generation, as well as generations to come. With every new innovation, Eco-Enz. keeps in mind its most basic goal: ridding the ocean of pollution. Eco-Enz, utilizing biotechnology, genetic engineering, environmental engineering, physics, and much more, aims to understand what each organism on Earth has to offer to make this planet thrive. Certain plants carry features that allow them to absorb certain toxic materials out of the environment. As a basic example, trees take in Carbon dioxide, something deadly to life on Earth if inhaled in large amounts, and turns it into Oxygen, something most organisms cannot live without. By understanding these efficient mechanisms, scientists within this company can create improved plants that can utilize these mechanisms, as well as their own stellar capabilities, and introduce them into various environments without affecting the wildlife.

PROBLEM

Ocean pollution has been an issue for many years, but more recently, the ocean is in more danger than ever. Entire ecosystems have become "dead zones" in which no life can thrive due to the excessive amounts of waste in the area like oil, septic sludge, dirt, and trash. Toxic chemicals from these kinds of pollution can cause more long-term issues like mutation and disease in marine life and life on land. One such toxic chemical is mercury. Scientists know that ⅔ (or 160 tons a year) of the elemental mercury deposited in the ocean comes from human activities such as the burning of fossil fuels. Elemental mercury is not considered very harmful to the marine and human life on earth, but still, if large amounts of it are exposed to humans, mercury poisoning can occur. This includes affecting the functions of the kidney, lungs, skin, and brains, along with many unsettling side effects. Although, an even more toxic form of mercury is created when the mercury reaches the ocean: methylmercury. The reason as to how methylmercury comes about is somewhat unknown but theories lead to the possible creation of the substance through chemical reactions on the sea floor/sea life in which sulfide and mercury ions combine and enter cell membranes of microbial cells to be methylated.

Low oxygen zones in the ocean can also create methylmercury (100 to 1,000 feet below the surface). Feces and dead plankton sink to the bottom of the ocean and absorb oxygen. Any bacteria relying on sulfate for respiration could create methylmercury. Methylmercury is passed up the food chain through a bioaccumulation process until it reaches the highest in the food chain in which the most methylmercury has accumulated. Coal is the biggest culprit in this pollution scene. High-sulfur coal ("Dirty" coal) tends to be high in mercury. When dirty coal is released into the atmosphere, a large amount of mercury and sulfur is created. From there, they can be washed back to Earth by rain or they can diffuse directly into bodies of water, making it the perfect combination for methylmercury in the ocean.



The location of the maximum methylmercury concentration at depth in the Pacific Ocean was the first evidence noted by the researchers pointing to the new methylation cycle. The graphic shows sampling depth on the left (in meters), and oxygen concentration on the right (in micromoles per kilogram of seawater [µmol/kg]) along a north-south latitudinal transect in the eastern North Pacific Ocean. The specific depth of maximal methylmercury concentration was consistently found at the ocean depth where the most rapid loss of oxygen was also observed. The process linking these two observations is microbial decomposition of "ocean rain", which is settling algae produced near the surface of the ocean. The decomposition process consumes oxygen from the water, but also leads to unintended methylmercury production. Graph created by David P. Krabbenhoft with data from Sunderland and others, 2009.

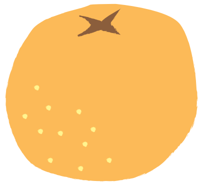
CUSTOMER SEGMENTS

The target market of this solution to manage the ocean's pollution is the United States Government, more specifically the Environmental Protection Agency (EPA). The reason why the EPA is the primary customer segment is that Eco-Enz is not focused on making a profit. Its main goal is to gain funding in order to implement its many agricultural innovations in various environments. There are numerous ways that the government works to protect America's many landscapes, especially the ocean and other bodies of water. Much of these services are contracted to third-party vendors who have specialized solutions to the pollutions issues the ocean incurs.

Some examples of third-party vendors include fisheries management, conservation and restoration of coastal lands and ocean habitats, wetlands, tidal marshes, and seagrasses, scientific research on species, plant life and reefs, environment data collection and testing, clean up of oil and chemical spills, and waste treatment and trash collection.

UNIQUE VALUE PROPOSITION

One proposition synthetic chemist Dr. Justin Chalker and his team at Flinders University, Australia created was to use oranges to purify water of mercury, eliminating environmental risks by using a cheap and eco-friendly alternative to other innovations. A polymer inside the oranges' makeup is used to fuel this idea. The plastic-like product absorbs mercury and other oils. This is a possible competition to Eco-Enz's proposition, but what makes Eco-Enz's idea unique is the fact that it identifies the root issue of mercury entering the ocean at all, rather than focusing on the aftermath of mercury entering the ocean. Most importantly, Eco-Enz's approach also provides a way to rid of the mercury naturally once absorbed, creating a solution to the decades-long struggle of safely disposing of mercury.



SOLUTION

In order to alleviate the effect of methylmercury on the ocean, Eco-Enz's goal is to prevent mercury deposits from entering the ocean at all, using the most eco-friendly and efficient ways possible. The root issue of the problem of ocean pollution lies in the pollution created by humans through industrial activities. Eco-Enz's solution is as follows: Utilize a plant that can purify soil and air near industrial areas within in the United States before runoff can reach large bodies of water. This plant is called Coprinus comatus, a kind of fungi. Coprinus comatus has been examined in North America to be used as a possible bio-extractor of mercury, as well as methylmercury, from polluted soils.

In a certain experiment published by Jerzy Falandysz, a researcher at the Laboratory of Environmental Chemistry and Ecotoxicology in Gdansk University, the contents of mercury in the caps and stipes of the C. comatus from soils correlated positively with the levels of contamination in the ground. Macrofungi are commonly known for their unique capability of absorbing various metallic elements and metalloids. Falandysz's goal was to examine if the C. comatus, which is a common plant in the northern hemisphere, had any potential to be a bio-extractor of Hg (mercury) in polluted grounds, as well as a bioindicator in citified soils. Falandysz collected samples from several sites from a town called Kartuzy in Northern Poland, using the fungi as an indicator of mercury in the soil. This land was surrounded by farmland and woodland with little industrial activity, restricted to small wood processing facilities and crop processing facilities, and limited tourist activity. Through a cutting and drying process of the caps and stipes, Falandysz found that the C. comatus was a sensitive bioindicator of urban soil pollution, essentially sequestering the Hg levels in the area (Appendix, Figure 1).

Places west and north of the center of the town had less contaminated samples of C. comatus, but less than the outskirts of two villages east of the town. At Lezno place, a place subject to intense road traffic, had an intermediate Hg content. Moreover, in older findings (Appendix, Figure 2), "... for specimens from the cinnabar (HgS) mining place in Germany with Hg in soil at 83-mg kg-1 dm, the content of Hg in two fruiting bodies was at 144-mg kg-1 dm, while for cinnabar mining place in Italy with Hg in soil at 210-mg kg-1 dm, the content of Hg in a single fruiting body was much less, i.e., at 23-mg kg-1 dm. Also high was Hg content in C. comatus emerged at the urbanized places in Finland, which showed up to 17-mg kg-1 dm." Figure 3 in the Appendix also shows the C. comatus' ability to absorb impressive amounts of mercury in relation to the soil content around it.

Another breakthrough scientists have achieved is the idea that enzymes can detoxify mercury compounds. MerB is an enzyme found in bacteria resistant to $HgCH_3^+$ toxicity, which has three cysteine residues in its active site that are known to be imperative for dividing the Hg-C bond. Although, the exact way this process is done, according to researchers, is somewhat a mystery. If this mystery were to be solved, the bioremediation of methylmercury may be possible. Another enzyme, MerA, reduces the resulting Hg^{2+} to less toxic elemental mercury. In order to combine these two powerful traits between the fungi and bacteria, genetic engineering must take place.

By inserting either MerB or MerA (Appendix, Figure 4) into the gene sequence of the C. comatus through bacterial plasmids, the fungi would have the ability to both sequester mercury in the soil around it and safely remove of the toxicity of the element. This can be done through viral transduction, transformation and transfection, Agrobacterium, or injection. Since C. comatus is an edible plant and the toxicity of the mushroom would be essentially zero with this process even after the chemical processes, then the ecosystems around the C. comatus would not be disrupted. The main location for these fungi should be near industrial plants and cities within the United States to safely remove mercury from the atmosphere and prevent it from entering large bodies of water.

START-UP COSTS FOR THE FIRST YEAR

Dedicated Infrastructure/Utility: $166,664
***University of Chicago Marine Biological Lab Rental**

MerA Sample: $930.75

C. comatus sample (100 kg): $6,992.99

Salaries for 25 Employees: $2,114,000

TOTAL: $2,288,587.74

The EPA grants $4 billion every year to various organization.

**Working with bacteria is much more cost effective than working with mammalian cell structures.*

CONCLUSION

Eco-Enz is a private company (non-profit) focused on creating a more productive planet for this generation, as well as generations to come. The preservation of the ocean was upheld by proposing the idea of genetically modified fungi, preventing any more mercury from getting into the ocean through industrial processes. These specific fungi, the Coprinus comatus, has the ability to absorb mercury from its environment. The enzymes MerB and MerA have the ability to reduce the toxicity level of mercury. By injecting one of these enzymes into the Coprinus comatus, this fungi will have the ability to absorb mercury and essentially rid the toxicity of the plant afterward, solving the two key issues of preventing mercury from getting in the ocean and finding a safe way to rid of mercury.

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APPENDIX

Place ID	Name of the place	Type of material Fungus	Soil
1	Kartuzy, Wzgórze Wolności/ Prokowska Str.	3 (13)[b]	3
2	Kartuzy, Sambora Str./3 Maja Str.	3 (13)	3
3	Kartuzy, Węglowa Str.	3 (15)	3
4	Kartuzy, Majkowskiego Str. (center of town)	3 (13)	3
5	Kartuzy, Majkowskiego Str. (northern edge)	3 (13)	3
6	Kczewo village	3 (15)	3
7	Pępowo village	3 (13)	3
8	Leźno village	3 (13)	3

Figure 1

Summary of data on total mercury concentrations in *C. comatus* and beneath soil, bioconcentration factor (BCF), and cap to stipe Hg content ration quotients ($Q_{C/S}$)

Information	Hg mg kg dm^{-1} Mushroom	Soil	BCF	Reference
Norway, background	1.7/0.85 (cap/stipe)			Allen and Steinnes 1978
Finland ($n = 2$)[#]	5.6 (1.5–9.8)			Laaksovirta and Lodenius 1979
Finland, rural ($n = 3$)	2.7 (2.5–2.9)			Kuusi et al. 1981
Finland, urban ($n = 37$)	4.7 (0.68–17)			Kuusi et al. 1981
Finland, urban ($n = 55$)	3.8 (1.4–10)			Lodenius et al. 1981
Finland, lead processing area ($n = 2$)	2.1 (0.6–3.5)			Liukkonen-Lilja et al. 1983
Finland ($n = 1$)	6.7			Kojo and Lodenius 1989
Germany ($n = 6$)	1.2 (0.4–2.2)			Seeger and Nützel 1976
Germany, Hg mining area ($n = 2$)	144	83	1.7	Fischer et al. 1995
Switzerland ($n = 1$)	2.8			Stijve and Roschnik 1974
Switzerland ($n = 4$)	3.3 (0.57–8.0)			Quinche and Dvorak 1975
Switzerland ($n = 7$)	3.1 (0.51–5.6)			Quinche 1976
Switzerland ($n = 17$)	2.5[a] (0.40–13)			Quinche 1992
Slovenia ($n = 1$)	2.1	0.22	9.5	Byrne et al. 1976
Croatia, eastern ($n = 1$)	1.4			Grgić et al. 1992
Spain, Lugo ($n = 10$)	2.6 ± 1.3 (H)		144	Melgar et al. 2009
	2.3 ± 1.2 (RFB)		115	
Italy, Mt. Amiata; HgS mine ($n = 1$)	23	210	0.11	Bargagli and Baldi 1984
Italy, Reggio Emilia	0.78 (0.42–1.1)[a]			Cocchi et al. 2006
China, Guangdong ($n = 7$)	0.04 (0.01–0.18)			Shen and Yu 2008

H hymenophore, *RFB* rest of fruit body

[a]number of fruit bodies, 95 % confidence interval

Figure 2

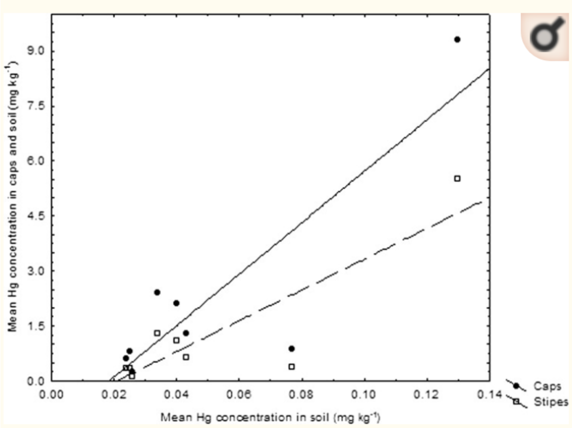

Fig. 2

Relationships between Hg content in the caps and stipes of *Coprinus comatus* and Hg in soil substratum (for caps: $y = -1.2933 + 70.1161*x$; $r = 0.87$; $p < 0.005$; $r^2 = 0.75$; $p = 0.0004$; for stipes: $y = -0.8774 + 41.9787*x$; $r = 0.87$; $p < 0.005$; $r^2 = 0.75$) for locations examined

Figure 3

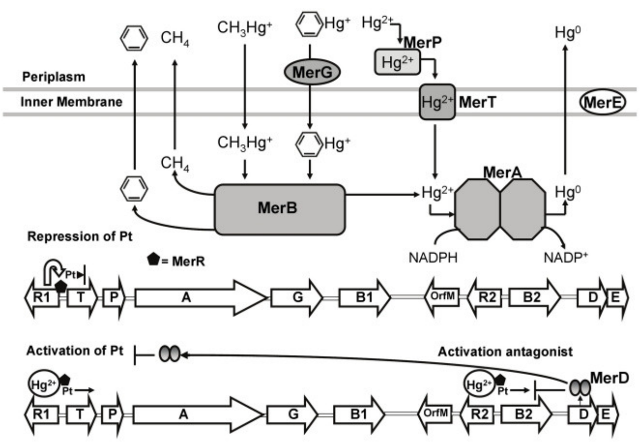

Figure 4