CAPSTONE EXPERIENCE 2015

Genetically Modified Seed Inoculant

Agricultural & Biological ENGINEERING

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Objective

Increase the final iron content in plants, such as corn, by modifying Bacillus subtilis that will be used as a soil additive.

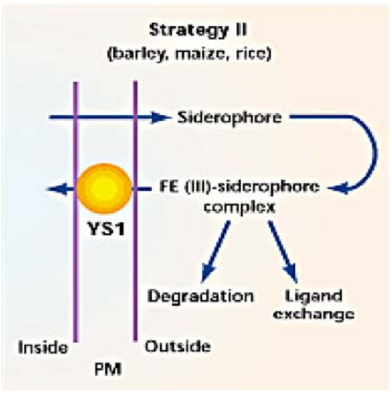
Design a process for industrial commercialization of genetically modified microbes to be applied to crops.

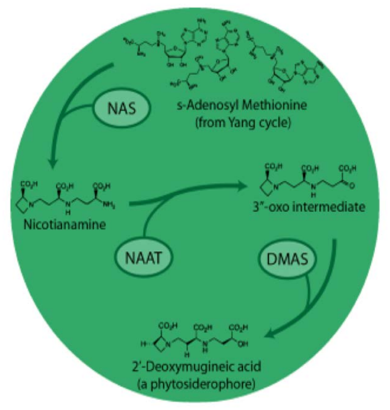
Problem Statement

The World Health Organization states that iron deficiency is one of the most common nutritional disorders in the world. Current approaches to increasing nutrient density in plants includes genetically modifying the plants, however this practice is highly controversial and has led to products failing to take hold in developing countries where it is needed. Our approach to solving this problem is to bypass genetically modifying the plant and instead modify bacteria found in soil to help increase the uptake of iron content in plants, a continuation of Purdue's 2014 iGem project. In our project, we hope to create a design that future companies may implement to modify multiple bacteria that can be optimized for various crops.

Previous Related Work

- Despite being one of the most abundant elements on Earth, is mostly found in its insoluble Fe(III) form.
- Most organisms can directly uptake only Fe(II).
- To facilitate the transport of insoluble Fe(III) into the cells, both plants produce chemicals called phytosiderophores, which bind to Fe(III) and transform it into a soluble compound that can be transported into the cells. (See Strategy 2 diagram below).





Microbe

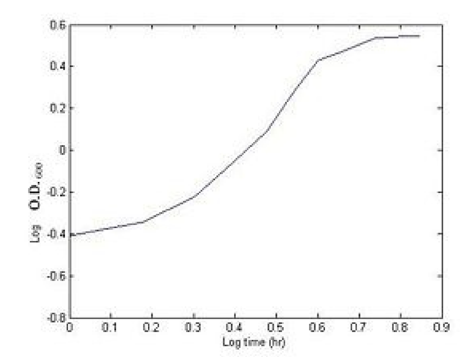
- Engineer Bacillus subtilis to produce the phytosiderophore used by corn, deoxymugineic acid.
- Uses the same metabolic pathway used by the plant: constructs will be synthesized from the sequences of these genes as found in corn.
- Codon optimization for a smoother transition from a eukaryotic plant cell to a prokaryotic bacterium.

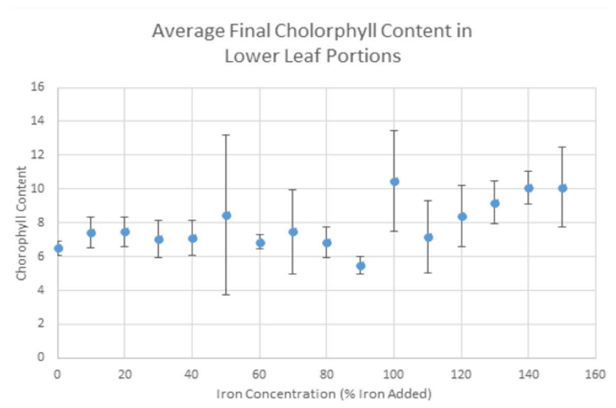
Data

Design:

- Fermenter: Glacier Tanks, 3.73 kW/hr.
- Piping: 11.24 m.
- Pumps: 39.99 kW.
- Microfilter: Δ19kPa.
- Granulator/Dryer: Glatt GPCG30/60 Fluid Bed Processor volume determined to be 22L.
- Packaging: Cryovac Onpack 2045 D vertical form fill seal machine; package size 510 mm by 242 mm by variable height and 5L.
- Marel Multihead Weigher, TSB-100 Tray Former, Markem-Imaje Code Dater.

Experimental:

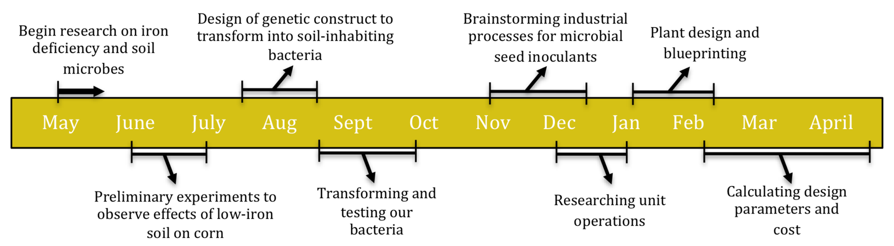


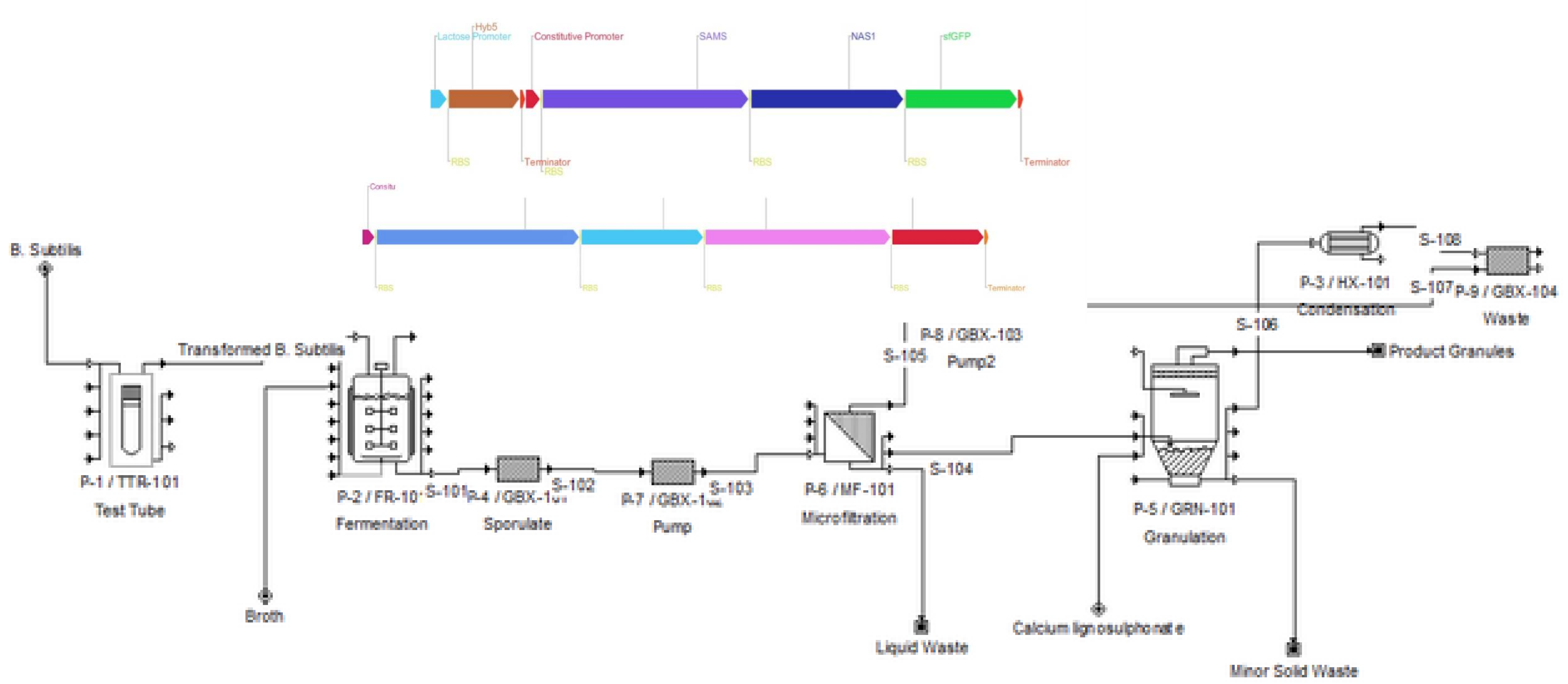


Future Process Design Studies

Constraints:

- Production time relies heavily on fermentation time.
- Time of sporulation.
- Maximum population.
- Water and oxygen barrier packaging.
- Cost of equipment.



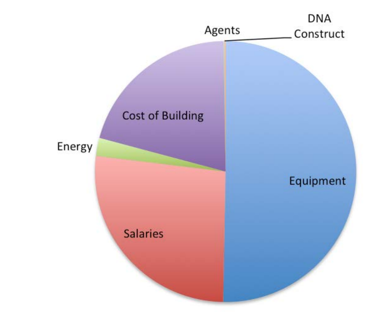


Budget

The pie chart depicts how our money will be spent to build and run our plant.

Estimated Costs:

- Equipment = $484518.18
- Building = $198000
- Total Workers' Yearly Salaries = $256496
- Yearly Energy Costs = $21600
- Corn Steep Liquor and Calcium Lignosulfonate= $850
- Custom DNA Construct = $1897



Global/Societal Impact

Iron deficiency and anemia

- 2 billion people – over 30% of the world's population – are anemic, many due to iron deficiency

Our Product's Influence on corn:

- Corn currently contains 25% daily value of iron.
- Our product should increase iron content in corn by about 5%.
- Current day cost of corn: 378 cents per bushel.
- Farmers could sell corn at increased price of $0.49 per iron percent increase achieved.
- Traditional methods of using iron chelates would cost $1000 per acre, so lots of saved money!

Regulations around the world

- United States: GMO guidelines focus on plants, classified as soil additives.
- Europe: GMOs banned without rigorous field testing.
- Africa: Lack of established regulations.
- India: Moratorium on usage of GM crops.

Sustainability

- Unlimited shelf life when in spore state.
- Consumers need to add our product to the soil for five years; after five years the additive will remain and replenishing does not need to occur.
- Product is using Fe^{3+} that is already in abundance in the soil, therefore it will not deplete the soil of its nutrients.

Alternative Solutions

	Microbe	Fermenter	Heater	Microfilter	Granulator /Dryer	Condenser	Vertical Form Fill Seal Machine	Tray Former Box Machine
Criteria	Increase iron content in plants	Produce 10^7 cfu/mL in 500 L fermenter	Sporulate bacteria into a more stable form	Separate spores from fermentation broth and debris.	Coat spores with a water soluble chemical to increase durability	Condense vapor from heater and recycle.	Processing products at the correct weight into packages to be sealed	Needs to box the packages with products
Problem	Regulations/safety	Time	Yield, energy	Fouling			Cost of equipment, sanitation	Cost of equipment, additional boxes required
Alternative	GMO crops, soil additives or fertilizer	Airlift, fluidized bed, packed bed fermenters	UV shock, acid shock, broth	Using no microfilter and only drying, centrifuge	Wet versus dry granulation, extruder	Heat exchanger, rotary evaporator, reusing as a form of heat	Horizontal form fill seal machine	Case Erectors

Technical Advisor:
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