Probiotics in an Oral Dosage Form

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Intro

The purpose of this project is to create a sustainable, ethical, economical, high quality probiotic that can be taken in an oral dosage form. This will be done by isolating high concentrations of *Bacillus coagulans* from a fermentation broth via centrifugation, spray drying the bacteria, and encapsulating the bacteria into gel capsules. Objectives include obtaining and maintaining high viability through both production and first pass metabolism; minimizing environmental contaminants; and maximizing product shelf life by increasing tolerance to transportation, moisture, temperature fluctuations, and length of time to use.

Probiotics are a proven, preventative method of improving health; more sustainable that treating illness after contraction; and have been shown to prevent negative aspects associated with methods of treatment like antibiotics, with no adverse effects (Hickson, 2007). No health risks have been identified with the human use of probiotics (Martinez Cruz, et. al., 2012). Probiotics fit market trends of increasing popularity and demand for health foods in diverse forms. Scientific evidence for the benefits of live cells on human health continue to grow (Denkova, et. al., 2014). Additionally, in 2017, probiotic supplements were the best-selling ingredient in natural and specialty gourmet categories and by 2023 the global market is expected to exceed $64 billion (Prince, 2018).

Factors

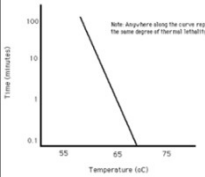
Despite the Western cultural obsession with killing "germs," the beneficial and necessary bacteria called by another name have sweetly endeared themselves to the population. Probiotics are commonly accepted as a preventative health measure and as a potential treatment for ailments ranging from discomfort to chronic illnesses. This cultural acceptance has created a rapidly growing market for probiotic food and dietary supplements. This is fortunate because effective, safe, and mass produced preventative health measures are essential to protecting the health of the global population as the stressors of global warming complicate the struggle to provide adequate water, nutrition, and sanitary conditions.

Several environmental considerations are central to the feasibility of probiotic mass production. First, water must be treated as a protected resource, and the consumption of water must be minimized in the production design. Secondly, the probiotic strain produced should be safe to release into the environment, since mass consumption may result in the proliferation of the organism outside of the human gastrointestinal tract. Finally, an ideal product would be stable over a wide range of temperatures and humidities in order to reduce energy consumption during distribution and storage.

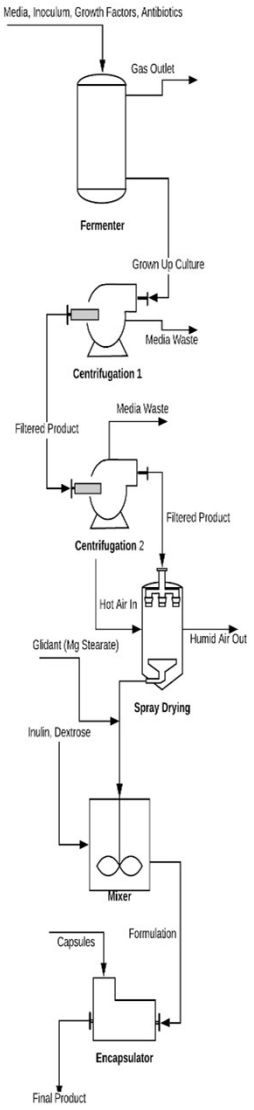
Quality

The main quality attributes of a probiotic is **cell viability, shelf-life, and proper dosing.**

In a plant setting, cell viability would be tested by plating the cells at multiple stages, such as at the end of fermentation, separation, and drying. Initial testing would provide estimates at the end of each operation and possible losses along the way. Most loss would be expected at the drying stage, as microorganisms are temperature sensitive.



Shelf-life would be a one-time or rare test to estimate the viability over time for our product. Most drugs are tested for effectiveness and when they reach 90% effectiveness, they are considered expired. Probiotics could be estimated the same way by considering expiration at 90% of original cell count.

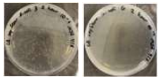


Alternatives/Experimentation

Fermentation

Bioreactors designed for high biomass yield were considered including bubble, air-lift, disposable, and impeller agitated reactors. Bubble reactors have low shear rates but also low mass and heat transfer rates due to limited mixing. Air-lift reactors similarly have low shear rates but have an improve heat transfer rate. Disposable reactors present an exciting new alternative with excellent sterility and control over internal conditions; however, small size and introduction of a new waste stream make them impractical. An impeller agitated system is chosen due to excellent mass and heat transfer rates.

Media design required the evaluation of various carbon and nitrogen sources. Media with soy meal, yeast extract, and ammonium sulfate was chosen for the fermentation.



Agar plates showing results of viability tests for 3 g/L K_2HPO_4 (left) and 6 g/L K_2HPO_4 (right)

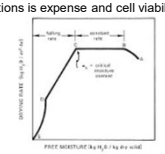
Separation

Filtration, sedimentation, and centrifugation are potential processes to separate the probiotic product from the fermentation media. Considering criteria such as cell viability and recovery, centrifugation was selected, as it is a method known to be effective in cell recovery (Russell, et. al., 2006). For industrial production, a continuous filter centrifuge is recommended. Different effective gravitational forces and durations of time can be tested to determine ideal conditions for viable separation. Greater force and longer time would lead to better recovery but would also be more likely to damage cells, thus a force and time must be selected to maximize both recovery and viability.

Agar plates showing results of viability tests for centrifugation for three minutes at 1k x g (left); three minutes at 10k x g (middle); and ten minutes at 10k x g (right)

Dehydration

The main goal of dehydration (drying) is to preserve the microorganisms to provide a shelf- stable product. The food and pharmaceutical industry uses many different ways to dry compounds. Most choices such as conveyor, drum, and tray drying would not be sufficient for our product. Two viable methods are freeze drying and spray drying. The balance between these two options is expense and cell viability.



Encapsulation

When considering the dosage form of our product, we had to decide between giving our product as a compressed tablet, or a capsule. While tablets are easier to manufacture, offer more dose-per-volume, they subject the formulation to extreme pressures (>10 MPa) (*Sinka et. al.,2009*). This could kill our bacteria, thus the form of encapsulation was chosen. In addition, a capsule dosage form gives more freedom for choosing the makeup of the formulation.

Encapsulation also comes with the added benefit of increasing consumption options for consumers. Many users prefer to incorporate their probiotic into their daily meals. A capsule allows one to open and supplement any food with the dose.

Design/Optimization

Fermentation

Fermentation control factors include temperature, pH, aeration rate, agitation rate, media composition and concentration. Together, these factors control specific growth rate, concentration of saturation, and homogeneity of the solution. In order to produce the most probiotic in a reasonable amount of time, specific growth rate is prioritized followed by concentration for saturation. Homogeneity is least prioritized because the high specific growth rate should outweigh losses due to poorly mixed pockets of the reactor.

Optimal growth rate conditions for temperature, pH, and aeration rate have been evolutionarily selected for and mimic the conditions of the human gastrointestinal tract at 37 ℃ and 7 pH. Since the fermentation is designed to produce biomass, an excess of the carbon and nitrogen sources can be present in the media to increase the growth rate. However, the ease of separation and ability to recover unused media determine the limit of adding excess ingredients.

Separation

The two main variables considered in optimizing the centrifugation process were effective gravitational force and duration of centrifugation. Criteria of evaluation included quality, economic, and environmental factors.

For all three factors, high recovery is desired, as greater quantities of probiotic are desired in the product to be effective; it is economically favorable to recover more of the product; and recovering product prevents product from exiting in the waste stream and those resources being wasted.

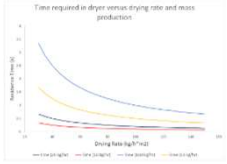
High cell viability is also desired, as damaged cells will result in ineffective product.

Greater force and time will result in higher recovery, while lower force and time will result in higher viability as well as lower cost. Thus, optimization of the centrifugation process is done by balancing the force and time to maximize recovery, viability, and cost.

Dehydration

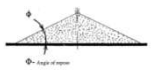
The main variables for spray drying are gas flow rate, air temperature, and air humidity.Increasing gas flow rate and air temperature would increase drying rate. Our design choice is to use a gas flow rate of 50,000 kg/hr and a final moisture content of 5 kg water/ kg dry.

Optimization is done by balancing the cost of blowing more gas and heating of the overall gas enough to reach the final desired moisture and provide desired cell viability by using other accessory systems.

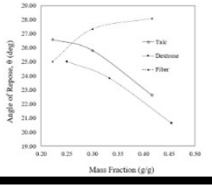


Encapsulation

Consideration was given to optimizing to formulation of our product. Powder flowability is hugely important when it comes to the process flow and economics of our design. Experiments were conducted to determine the angle of repose for various formulation blends. Angle of repose is a key metric for determining a powders flowability.



We independently changed the mass fraction of various excipients to get an idea of how their relative ratios affected the angle of repose for our product



Economics

Fermentation

The majority of raw materials cost is incurred during fermentation.

Cost rate	Soy Meal	Yeast Extract	$(NH_4)_2SO_4$	KH_2PO_4	$CaCl_2$	$MgSO_4$
Cost/hr	25	24	2	823	0.50	1.15
Cost/Year	187,355	172,965	13,583	6,053,789	3,751	8,435
			Total Product Cost/yr		6,439,878	

The purchased equipment cost for the fermenter is $120,000.

Metric	Percent	Cost ($)
Equipment	100	120,000
Installation	39	46,800
WC	75	90,000
TCI	503	603,600

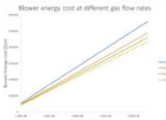
Separation

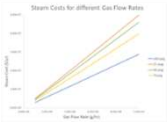
The cost of an industrial centrifuge suited for the purpose of this process is estimated to be around $55,000. Following the ratios for estimating capital investment for a solid-fluid processing plant given in *Plant Design and Economics for Chemical Engineers*: the total capital investment (TCI) can be estimated as 503% of the purchased equipment cost; equipment installation as 39%; and working capital (WC) as 75%. From this the following calculations can be made via multiplying the percent by the equipment cost:

Metric	Percent	Cost ($)
Equipment	100	55,000
Installation	39	21,450
WC	75	41,250
TCI	503	276,650

Dehydration

Costs for the spray drying section include the spray dryer itself and other costs from utilities, such as the blower and heat exchanger. A regeneration system can be used to reduce some heating costs.





Metric	Percent	Cost ($)
Equipment	100	180,000
Installation	39	70,200
WC	75	135,000
TCI	503	905,400

Encapsulation

The main costs in the encapsulation process will be: **Equipment, Installation, Piping, Electric Costs, and Materials.**

Equipment	Cost ($)
Vacuum Conveyor	38,000
Capsule Filler	32,000
V-Mixer	40,000

Cost rate	Talc	Dextrose	Inulin
Cost/hr	27	35	106
Cost/Year	200,239	257,608	762,374
Total Product Cost/yr		1,220,222	

We can estimate the costs for equipment installation as 25% the cost of equipment. Piping and electrical were each estimated as 10% the total cost of equipment. This lets us calculate the Total Capital investment from encapsulation and get a number on Working Capital.

Metric	Cost ($)
Equipment	110,000
Installation	27,500

Metric	Cost ($)
Piping	11,000
Electrical	11,000

Metric	Cost ($)
TCI	186,083
WC	18,608

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