A BEGINNER'S GUIDE TO

BIOPESTICIDE FORMULATIONS

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Introduction

A Beginner's Guide to Biopesticide Formulations is designed to provide an accessible and comprehensive overview of the various mediums and techniques used in formulating biopesticides. With the growing demand for sustainable agricultural practices, biopesticides have emerged as an eco-friendly alternative to conventional chemical pesticides. They utilize naturally occurring organisms or substances to control pests, minimizing environmental impact and reducing harm to non-target species, including humans.

This handbook is tailored for students, researchers, and practitioners who are new to the field of biopesticides. It offers insights into the different formulation types, such as wettable powders, emulsifiable concentrates, granules, and more, and delves deeper into its advantages and disadvantages. We also delve into key component distribution for biopesticide development, and a comprehensive comparison of all formulations.

Whether you are a budding scientist or an agricultural practitioner, this guide will help you navigate the world of biopesticides, empowering you to contribute to more sustainable and environmentally conscious pest management strategies.

Wettable powders (WP)

Wettable powders (WP) are used widely in agriculture for pesticide applications. They consist of a toxicant that is combined with inert carriers and wetting agents, which aid in effective water dispersion. Listed below are the main properties, components, pros, and cons we find in wettable powders.

Components of Wettable Powders

Component	Weight Percentage (%)	Description
Active Ingredient	10.0 - 80.0	The primary toxicant responsible for pest control [2].
Wetting Agent	1.0 - 2.0	Enhances the mixture's ability to disperse in water [2].
Dispersing Agent	2.0 - 5.0	Aids in maintaining suspension in the spray tank [2].
Antifoaming Agent	0.1 - 1.0	Reduces foam formation during mixing and application [2].
Filler/Carrier	Up to 100	Inert materials that help bulk up the formulation [2].

Common Wetting and Dispersing Agents

Trade Name	Chemistry	Property
LUCRAMUL® DAC 211	Condensated ketone sulfonate	Solid dispersing agent [2].
LUCRAMUL® DAC 220	Condensated naphthalene sulfonate	Solid dispersing agent [2].
LUCRAMUL® DAC 240	Alkyl naphthalene sulfonate	Solid dispersing agent [2].

Advantages of Wettable Powders

- **Lower Dermal Hazard:** Compared to liquid formulations, wettable powders are safer as they pose a lesser threat to skin exposure [5].
- **Reduced Phytotoxicity:** They are suitable for the treatment of sensitive crops and trees and do not pose a threat to treated surfaces [4].
- **Easy Storage and Handling:** They are stable and easier to transport than liquid formulations as they are in a dry state [5].
- **Versatile Application:** Suitable for various types of spray equipment where agitation is possible [2].

Disadvantages of Wettable Powders

- **Agitation Requirement:** Settling the wettable powder is an issue and could lead to complications hence agitation is a prerequisite [4].
- **Inhalation Risk:** The fine particles can pose an inhalation hazard during mixing, requiring careful handling [5].
- **Clogging Issues:** They may be abrasive to equipment and can clog nozzles and filters, affecting application efficiency [1].
- **Measurement Challenges:** They often require precise weighing for accurate dosing, which can be a cumbersome and time-intensive process [4].

Liquid Concentrates (LC)

Liquid Concentrates (LC) are formulations that include water-soluble toxicants. LC does not necessarily require emulsifying agents or organic solvents, unlike emulsifiable concentrates. Hence, liquid concentrates are more straightforward in their agricultural applications. Listed below are the main properties, components, pros, and cons we find in liquid concentrates.

Components of Liquid Concentrates

Component	Weight Percentage (%)	Description
Active Ingredient	10.0 - 100.0	The primary toxicant responsible for pest control [2].
Water	Variable	Acts as the solvent in which the active ingredient is dissolved [6].
Additives	1.0 - 5.0	May include stabilizers or preservatives to enhance shelf life and efficacy [4].

Common Additives

Trade Name	Function
Sodium alkylate	Acts as a defoamer
Sodium olefin sulfonate	Functions as a wetting agent

Advantages of Liquid Concentrates

- **Ease of Use:** They are not very difficult to maneuver, carry, and store, therefore relatively user-friendly for the applicator [2].
- **Solubility in Water:** It has the property of being a water-soluble compound. That is, it can dissolve directly in water without needing an emulsifier [6].

- **No Agitation Required:** Liquid Concentrates are Formulated such that agitation is not a must during application, unlike some other formulations, liquid concentrates do not require constant agitation during application [4].
- **Minimal Residue:** They are usually expected not to leave residues that would be observable on treated surfaces; this is an aesthetic advantage [2].

Disadvantages of Liquid Concentrates

- **Limited Availability:** Some active ingredients may not be available in liquid concentrate form [4].
- **Skin Absorption Risk:** Certain formulations can be easily absorbed through the skin, posing health risks to applicators [2].
- **Spill Cleanup Challenges:** Spills and splashes can be difficult to clean up and decontaminate effectively [2].
- **Container Residue:** It can be challenging to empty containers, leading to waste and potential environmental concerns [6].

Emulsifiable Concentrates (EC)

Emulsifiable Concentrates (EC) are formulations that contain a pesticide dissolved in a suitable organic solvent along with an emulsifying agent. These formulations are designed to be diluted with water and applied as sprays. ECs are known for leaving minimal visible residue on treated surfces, but they also carry a higher risk of causing injury to fruit and foliage compared to other formulations like wettable powders. The following includes a description of Emulsifiable concentrate formulations, including their components, pros, and cons.

Components of Emulsifiable Concentrates

Component	Weight Percentage (%)	Description
Active Ingredient	25.0 - 75.0	The primary toxicant responsible for pest control [1].
Organic Solvent	Variable	Dissolves the active ingredient and facilitates emulsification [4].
Emulsifying Agent	1.0 - 5.0	Allows the formulation to mix with water and form a stable emulsion [2].

Common Emulsifying Agents

Trade Name	Function
Atlox™ 4838B	Non-ionic emulsifier
Tween™ series	Non-ionic surfactants for stability

Advantages of Emulsifiable Concentrates

- **Ease of Handling:** They are relatively easy to handle, transport, and store, making them user-friendly for applicators [1].
- **Minimal Residue:** ECs typically leave little or no visible residues on treated surfaces, which is beneficial for aesthetic purposes [4].

- **Low Agitation Requirement:** They require minimal agitation during application compared to other formulations [2].
- **Versatile Application:** ECs can be used with various types of application equipment, including portable sprayers and mist blowers [2].

Disadvantages of Emulsifiable Concentrates

- **Phytotoxicity Risk:** They are more likely to cause injury to fruit and foliage compared to other formulations like wettable powders [1].
- **Skin Absorption Risk:** Certain solvents used in ECs can be easily absorbed through the skin, posing health risks to applicators [4].
- **Material Compatibility Issues:** Solvents can deteriorate rubber or plastic components in spray equipment [2].
- **Potential Corrosiveness:** Some ECs may be corrosive or flammable, requiring careful handling [1].

Suspension Concentrates (SC)

Suspension concentrates (SC) are liquid formulations that contain a high percentage of active ingredients suspended in a suitable solvent. This type of formulation is designed to dilute the components with water before application, which makes them more effective for pest control in agricultural use. Suspension concentrates allow for the efficient delivery of active ingredients while minimizing the volume needed for the application. Listed below are the main properties of Suspension concentrates including, components, pros, and cons.

Components of Suspension Concentrates

Component	Weight Percentage (%)	Description
Active Ingredient	400 to 800 g/L	The primary toxicant responsible for pest control [7].
Water	Variable	Acts as the solvent for dilution before application [8].
Additives	1.0 - 5.0	May include wetting agents, dispersants, and stabilizers [9].

Common Additives

Trade Name	Function
Wetting agents	Enhance dispersion and adherence
Dispersing agents	Help maintain suspension stability
Stabilizers	Improve shelf life and performance

Advantages of Suspension Concentrates

- **High Active Ingredient Content:** They contain a significant concentration of active ingredients, making them effective for pest control while requiring less product per application [7].
- **Ease of Use:** They can be easily diluted with water, facilitating straightforward application [8].
- **Reduced Packaging Volume:** The high concentration allows for smaller packaging sizes, reducing waste and storage space [9].

- **Versatile Application:** Suitable for various types of application equipment, including sprayers and mist blowers [7].

Disadvantages of Suspension Concentrates

- **Agitation Requirement:** They may require moderate agitation to ensure uniform distribution of active ingredients in the spray tank [9].
- **Potential for Clogging:** High concentrations of solids can lead to clogging in spray equipment if not properly managed [8].
- **Limited Compatibility:** Not all active ingredients are suitable for formulation as suspension concentrates; compatibility must be assessed [7].
- **Storage Stability Issues:** Long-term storage stability can be a concern if not formulated correctly, leading to sedimentation or separation [9].

Dry Flowable (DF) and Water Dispersible Granules (WDG)

Water dispersible granules (WDG) are also considered as **Dry flowable (DF) formulations**. They differ from wettable powders because of their unique spherical granule structure. These formulations consist of active ingredients, such as tiny sphere-shaped clay particles, which allow them to flow easily from the product container. The following includes a description of DF and WDG formulations, including their components, pros and cons.

Components of Dry Flowable and Water Dispersible Granules

Component	Weight Percentage (%)	Description
Active Ingredient	5.0 - 50.0	The primary toxicant responsible for pest control [2].
Wetting Agent	1.0 - 5.0	Enhances dispersion and adherence when mixed with water [2].
Dispersing Agent	0.1 - 2.0	Helps maintain suspension stability in the spray mixture [7].
Filler/Carrier	Up to 100	Inert materials assist in the formulation's flowability [8].

Common Additives

Trade Name	Function
LUCRAMUL® DAC 211	Solid dispersing agent
LUCRAMUL® DAC 220	Solid dispersing agent
LUCRAMUL® DAC 240	Solid dispersing agent

Advantages of Dry Flowable and Water Dispersible Granules

- **Improved Flowability:** The spherical shape of the granules reduces packing and allows for easier handling and dispensing from containers [2].
- **Reduced Dust Formation:** Compared to traditional wettable powders, DF and WDG formulations produce less dust during handling, improving safety for applicators [7].

- **Ease of Use:** They can be easily mixed with water to create a spray mixture, facilitating straightforward application [8].
- **Stability:** These formulations generally exhibit good stability during storage, preventing clumping or caking [2].

Disadvantages of Dry Flowable and Water Dispersible Granules

- **Agitation Requirement:** They may require moderate agitation to ensure uniform distribution of active ingredients in the spray tank [8].
- **Potential for Clogging:** The granules can lead to clogging in spray equipment during application when it is not managed properly[7].
- **Limited Compatibility:** Not all active ingredients are suitable for formulation as DF or WDG; compatibility must be assessed before formulation [2].
- **Water Sensitivity:** Some formulations may be sensitive to moisture, which can affect their performance during storage or application [8].

Advantages of Water Dispersible Granules Over Wettable Powders:

- **Reduced Dust Production**: WDG formulations are designed to be non-dusty, which significantly reduces the inhalation hazards associated with handling and applying pesticides compared to WP formulations, which can produce high levels of dust during mixing and application [10,11].
- **High Active Ingredient Loading**: WDGs can accommodate a higher concentration of active ingredients, often up to 90%. This allows for more effective pest control with less product volume needed for application, making them more efficient than many wettable powders that typically contain 10% to 80% active ingredients [10,13].
- **Improved Flowability**: The spherical shape of WDG particles enhances their flowability, allowing for easier handling and dispensing from containers. This contrasts with WP formulations, which can pack together and become difficult to manage [10,12].
- **No Sedimentation Issues**: WDGs do not experience crystal growth or sedimentation when mixed with water, ensuring that the active ingredients remain suspended throughout the application process. In contrast, wettable powders can settle quickly unless constant agitation is maintained [10,13].
- **Easier Package Disposal**: The packaging for WDGs is often simpler and easier to dispose of compared to liquid formulations, making them more environmentally friendly in terms of waste management [10,11].
- **Enhanced Safety**: The non-dusty nature of WDGs reduces the risk of respiratory exposure for applicators during mixing and application, contributing to safer handling practices compared to WP formulations that generate significant dust [5,10].

- **Compatibility with Adjuvants**: While both formulations can include adjuvants, WDGs generally allow for better incorporation of wetting agents and dispersants during formulation, enhancing their performance in water [2,13].

Flowable (F) Formulations

Flowable (F) formulations are liquid or viscous concentrates containing suspendable pesticides in water. The formulations minimize the injury to fruits and foliage when compared to emulsifiable concentrates (EC), hence considered as safe as wettable powders. Flowable formulations provide more benefits in terms of ease of use and application efficiency. Listed below are the main properties of Flowable formulations including, components, pros, and cons.

Components of Flowable Formulations

Component	Weight Percentage (%)	Description
Active Ingredient	40% - 60%	The primary toxicant responsible for pest control [14].
Water	Variable	Acts as the solvent for dilution before application [15].
Suspending Agent	1% - 10%	Helps maintain the suspension of active ingredients [3].
Additives	1% - 5%	May include wetting agents and stabilizers [3].

Common Additives

Trade Name	Function
Alkali lignin	Acts as a suspending agent
Non-ionic surfactants	Enhance dispersion and adherence

Advantages of Flowable Formulations

- **Reduced Dust Formation**: Unlike wettable powders, flowable formulations do not produce dust during handling, improving safety for applicators [14].
- **Ease of Mixing**: They can be easily mixed with water to create a uniform suspension, facilitating straightforward application [15].
- **Stable Suspension**: Flowable formulations maintain their suspension without the need for constant agitation, allowing for easier transportation and application in the field [3].
- **Lower Phytotoxicity**: These formulations typically cause less injury to fruit and foliage compared to emulsifiable concentrates [14].

Disadvantages of Flowable Formulations

- **Potential for Clogging**: If not properly managed, suspended particles can lead to clogging in spray equipment during application [15].
- **Storage Stability Issues**: Long-term storage stability can be a concern if the formulation is not properly stabilized, potentially leading to sedimentation [3].
- **Compatibility Limitations**: Not all active ingredients are suitable for flowable formulations; compatibility must be assessed before formulation [14].

Soluble Powders (SP)

Soluble powders (SP) are designed to dissolve completely in water, allowing for effective application for agricultural use. These formulations are commonly used for a wide range of fertilizers and some pesticides. The ability to dissolve easily makes them convenient for mixing and application, providing flexibility for pest management methods. The following includes a description of Soluble powders, including their components, pros and cons.

Components of Soluble Powders

Component	Weight Percentage (%)	Description
Active Ingredient	10% - 90%	The primary toxicant or nutrient responsible for pest control or fertilization [7].
Fillers/Carriers	Variable	Inert materials that aid in the formulation and flowability [8].
Wetting Agents	1% - 5%	Enhance the solubility and dispersion of the active ingredient [15].

Common Additives

Trade Name	Function
Sodium alkyl sulfate	Acts as a wetting agent
Propylene glycol	Functions as a dispersant

Advantages of Soluble Powders

- **Complete Dissolution**: They dissolve completely in water, ensuring uniform distribution of the active ingredient in the spray mixture, which enhances efficacy [7].
- **Ease of Use**: Soluble powders are easy to mix with water, making them user-friendly for applicators [8].
- **Reduced Dust Formation**: Compared to wettable powders, soluble powders produce less dust during handling, improving safety for users [15].
- **Versatile Application**: Suitable for various types of application equipment, including backpack sprayers and large-scale agricultural machinery [7].

Disadvantages of Soluble Powders

- **Limited Compatibility**: Not all active ingredients can be formulated as soluble powders; compatibility must be assessed before formulation [8].
- **Potential for Crystallization**: If not properly managed, soluble powders can lead to crystallization in the spray tank if the concentration exceeds solubility limits [15].
- **Storage Stability Issues**: Long-term storage stability can be a concern if the formulation is not properly stabilized, leading to degradation or loss of efficacy over time [7].

Dust Formulations (D)

Dust formulations (D) are created by mixing a chemical toxicant with finely ground inert materials such as talc, clay, or dried plant materials. While they can provide effective pest control, presently, dusts are seldom used in orchards due to extreme drift hazards, which can lead to undesired exposure and environmental contamination. This overview provides a comprehensive explanation of dust formulations, including their components, advantages, disadvantages, and comparisons with other pesticide formulations.

Components of Dust Formulations

Component	Weight Percentage (%)	Description
Active Ingredient	0.5% - 10%	The primary toxicant responsible for pest control [16].
Inert Carrier	90% - 99.5%	Finely ground materials such as talc or clay that serve as a diluent [11].

Common Inert Carriers

Material	Function
Talc	Acts as a carrier and reduces clumping
Clay	Provides bulk and improves flowability
Chalk	Enhances coverage and adherence

Advantages of Dust Formulations

- **Ease of Use**: Dusts are ready-to-use formulations that do not require mixing with water, making them convenient for quick applications [17].
- **Excellent Coverage**: The fine particle size ensures thorough coverage of surfaces, which can be beneficial for targeting pests in otherwise difficult-to-reach areas [18].
- **High Specifity**: Dusts can be applied directly into cracks and crevices or other specific locations where pests are likely to reside, minimizing exposure to non-target organisms [19].

Disadvantages of Dust Formulations

- **Drift Hazards**: While fine particle size boosts coverage, dusts are prone to drift during application, which may lead to unintended exposure to non-target plants and animals [16].
- **Inhalation Risks**: The tiny particles may present inhalation hazards for users during handling and application [17].
- **Limited Residual Activity**: Dusts may not provide prolonged effects compared to other formulations like emulsifiable concentrates or wettable powders [18].
- **Environmental Concerns**: The potential for drift and non-target exposure raises concerns about the environmental impact of using dust formulations in sensitive areas like orchards [11].

Granule Formulations (G)

Granule formulations (G) are created by saturating an inert carrier with a pesticide, resulting in particles that typically range from 30 to 60 mesh in size. These formulations are used for managing soil-dwelling or water-dwelling pests and are considered to be a convenient method for targeted application. The following states the properties of Granule formulations including their description, additives (carriers), benefits and drawbacks.

Components of Granule Formulations

Component	Weight Percentage (%)	Description
Active Ingredient	1% - 15%	The primary toxicant responsible for pest control [20].
Inert Carrier	85% - 99%	Finely ground materials such as talc, clay, or organic matter that serve as a diluent [17].

Common Inert Carriers

Material	Function
Talc	Acts as a carrier and reduces clumping
Clay	Provides bulk and improves flowability
Corn cobs	Organic carrier for slow-release applications

Advantages of Granule Formulations

- **Ease of Application**: Granules can be applied directly to the soil or water without the need for dilution, making them convenient for quick applications [20].
- **Targeted Delivery**: They can effectively target soil-dwelling pests, such as nematodes and other insects, by delivering the active ingredient directly to the pest habitat [17].
- **Drift Risk Mitigation**: Compared to Dust formulations,Granules are less prone to drift during application, minimizing environmental contamination and non-target exposure [21].
- **Controlled Release**: Some granules are designed for slow release, allowing for long-lasting pest control effects over time [2].

Disadvantages of Granule Formulations

- **Moisture Activation Requirement**: Many granular pesticides require moisture from rainfall or irrigation to activate the active ingredient effectively [20].
- **Limited Mobility**: Once applied, granules may not move through the soil as effectively as liquid formulations, potentially limiting their effectiveness against mobile pests [21].
- **Storage Stability Issues**: Depending on the formulation, granules may be susceptible to clumping or degradation if not stored properly [2].

Controlled Release (CR) Formulations/ Microencapsulated Formulations (ME)

Controlled release (CR) formulations releases pesticides gradually via capsules or beads. With the help of this creative strategy, active chemicals can be delivered gradually and consistently, increasing pest control efficacy while reducing environmental impact. Applications needing extended efficacy and fewer application frequencies benefit greatly from CR formulations. The components, benefits, drawbacks, and comparisons with other pesticide formulations are all covered in length in this overview of controlled-release formulations.

Components of Controlled Release Formulations

Component	Weight Percentage (%)	Description
Active Ingredient	10% - 70%	The primary toxicant responsible for pest control [22].
Polymer Matrix	30% - 90%	Biodegradable polymers that encapsulate the active ingredient [9].
Additives	1% - 5%	May include stabilizers and surfactants to enhance performance [23].

Common Polymer Matrices

Material	Function
Polyurea	Provides a robust encapsulation layer
Urea-formaldehyde	Used for slow-release applications
Biodegradable polymers	Enhance environmental safety

Advantages of Controlled Release Formulations

- **Sustained Release**: These formulations provide a steady release of active ingredients over time, and maintain an effective concentration over a long period. [22].
- **Reduced Application Frequency**: The timed release mechanism reduces the need for frequent applications, This saves time and labour for agricultural workers.[9].

- **Minimized Environmental Impact**: By controlling the release rate, we can reduce the overall amount of pesticide entering the environment, thereby decreasing potential harm to non-target organisms [23].
- **Enhanced Efficacy**: CR technology allows for better targeting of pests by maintaining effective levels of active ingredients at the site of application [22].

Disadvantages of Controlled Release Formulations

- **Higher Production Costs**: The complexity involved in manufacturing CR formulations often results in higher costs compared to conventional pesticide formulations [9].
- **Potential Compatibility Issues**: Not all active ingredients are suitable for encapsulation; compatibility must be assessed before formulation [23].
- **Variable Release Rates**: The release rate can be influenced by environmental factors such as temperature and moisture, which may affect performance under different conditions [22].

Bait Formulations

Bait formulations consist of a poison combined with an attractive substance designed to lure pests. These formulations are commonly used in orchards for managing pests such as the cherry fruit fly, and they can be applied around tree trunks or in cover crops. Baits are generally considered less hazardous to the environment compared to many spray and dust formulations; however, there is a risk that birds and other animals may consume the bait and potentially suffer harmful effects if they ingest large quantities. The following includes a description of Bait formulations, including their components, pros and cons.

Components of Bait Formulations

Component	Weight Percentage (%)	Description
Active Ingredient	1% - 10%	The toxicant responsible for pest control [24].
Attractant	5% - 20%	A substance that lures the target pest [25].
Inert Carrier	70% - 94%	Materials such as sugars or starches that serve as a base for the bait [26].

Common Attractants

Attractant	Function
Ammonium carbonate	Attracts cherry fruit flies
Sugars	Enhances palatability for various pests

Advantages of Bait Formulations

- **Targeted Pest Control**: Baits help reduce pests hence decreasing the chances if the plant to be affected [24].
- **Reduced Environmental Hazard**: Compared to sprays and dust, baits typically pose a lower risk to the environment due to their localized application [25].
- **Ease of Application**: Baits can be used in various places like tree trunks or within cover crops easily, for effective pest control [26].
- **Less Frequent Applications**: The use of baits may allow for less frequent applications compared to traditional insecticides, which can save time and labor [16].

Disadvantages of Bait Formulations

- **Secondary Poisoning Risks**: Birds and other animals may consume the bait, leading to potential secondary poisoning if they ingest large amounts [24].
- **Limited Efficacy Against Certain Pests**: Not all pests may be attracted to bait formulations, limiting their effectiveness against a broad range of insects [25].
- **Environmental Conditions Impact Efficacy**: Factors such as rain or high humidity can affect the stability and attractiveness of bait formulations [26].

Oil Dispersions (OD)

Oil dispersions (OD) are formulations that contain active ingredients in an oil medium, which allows for effective dispersion in water during application.

Components of Oil Dispersions

Component	Weight Percentage (%)	Description
Active Ingredient	10.0 - 60.0	The primary toxicant responsible for pest control [27].
Oil Carrier	30.0 - 80.0	A liquid medium that helps in the dispersion of the active ingredient [21].
Emulsifier	1.0 - 5.0	A surfactant that stabilizes the emulsion [19].
Filler	Up to 100	Inert materials that bulk up the formulation [11].

Advantages of Oil Dispersions

- **Reduced Dermal Hazard:** Lower risk of skin exposure compared to some liquid formulations [19].
- **Effective Residual Control:** Provides longer-lasting effects on target pests due to slow release from the oil matrix [21].
- **Versatile Application:** Can be used with various types of spray equipment, especially where emulsification is required [11].

Disadvantages of Oil Dispersions

- **Higher Cost:** Generally more expensive than other formulations like wettable powders [19].
- **Potential Environmental Impact**: Oil-based formulations can have a higher risk of environmental contamination if not applied correctly [21].
- **Compatibility Issues:** May not mix well with all types of water or other pesticide formulations, requiring careful consideration during application [11].

Biodegradable Pellets (BP)

Biodegradable pellets (BP) are solid formulations designed to break down naturally over time, releasing active ingredients.

Components of Biodegradable Pellets

Component	Weight Percentage (%)	Description
Active Ingredient	1.0 - 30.0	The toxicant responsible for pest control [27].
Biodegradable Binder	10.0 - 50.0	Materials that hold the pellet together and decompose over time [19].
Filler	Up to 100	Inert materials that provide bulk and aid in application [11].

Advantages of Biodegradable Pellets

- **Environmental Safety:** It has a minimal environmental impact as it biodegrades easily and naturally [21].
- **Easy Application:** Offers easy application methods that avoid the mixing and dilution process [19].
- **Less Residue:** Accumulates less chemical residue on surfaces than liquid formulations [27].

Disadvantages of Biodegradable Pellets

- **Short Shelf Life:** May degrade before usage if utmost care is not taken while keeping them [21].
- **Moisture Sensitivity:** Very sensitive to humidity and moisture, which may degrade their potency [11].
- **Application Limitations:** This application is not suitable for all pest control situations especially where there must be an immediate solution [19]

Fumigants

Fumigants are volatile substances used to control pests through gas-phase action.

Components of Fumigants

Component	Weight Percentage (%)	Description
Active Ingredient	10.0 - 100.0	The primary toxicant that vaporizes to kill pests [27].
Carrier Gas	Variable	A gas or vapor that aids in dispersing the active ingredient into the target area [19].

Advantages of Fumigant

- **Efficient Pest Control:** Penetration into inaccessible areas may offer comprehensive pest control [21].
- **Rapid Action**: Usually acts very rapidly in killing the pest after exposure [11].
- **Widely Applied:** Applicable for all surroundings, whether applied to stored products or the soil [27].

Disadvantages of Fumigant

- **Higher Threat of Toxicity Hazard:** The health of humans and non-target organisms is at risk due to not following proper care [19].
- **Risk to Environment:** Aerial and soil contamination occurs when it is overused or applied improperly [21].
- **Regulatory Restrictions:** Most fumigants are kept under legal restrictions in terms of the practices and methods used to apply them [11].

Aerosols

Aerosols are pressurized formulations that release a fine mist or spray when activated.

Components of Aerosols

Component	Weight Percentage (%)	Description
Active Ingredient	1.0 - 20.0	The toxicant responsible for pest control [27].
Propellant	10.0 - 90.0	A gas that creates pressure within the container to expel the product [19].
Emulsifiers	1.0 - 5.0	Surfactants that stabilize the formulation and aid dispersion [11].

Advantages of Aerosols:

- **Easy Application:** It is easy to apply, and mixing is unnecessary - can be used to target specific areas [21].
- **Little Chance of Drifting:** Fine spray particles will not drift because of the small droplet sizes compared to other spray application methods; this improves application accuracy [19].
- **Rapid Knockdown:** Produces instantaneous kill of pests when contacted, thus fast knockdown due to fine size of particle and its uniform distribution [27].

Disadvantages of Aerosols:

- **Poor Coverage:** It might be impractical for extended coverage areas compared to other formulations such as spray or granule formulations [11].
- **Hazard by Inhalation:** Exposure through inhalation while application takes place requires safety precautions to users [21].

Comparison of all Formulations (based on Solubility):

Formulation Type	Active Ingredient (%)	Solubility	Agitation Required	Risk of Phytotoxicity
Wettable Powder (WP)	10% - 80%	Insoluble	Yes	Low
Liquid Concentrate (LC)	10% - 100%	Water-soluble	No	Low
Emulsifiable Concentrate (EC)	25% - 75%	Oil-soluble	Low	High
Suspension Concentrate (SC)	40% - 80%	Insoluble	Moderate	Low
Dry Flowable / WDG	5% - 50%	Insoluble	Moderate	Low
Flowable (F)	40% - 60%	Insoluble	Low	Low
Soluble Powder (SP)	10% - 90%	Water-soluble	No	Low
Dust (D)	0.5% - 10%	Insoluble	No	Moderate
Granules (G)	1% - 15%	Insoluble	No	Low
Oil Dispersions (OD)	10% - 50%	Oil-soluble	Yes	Moderate
Biodegradable Pellets (BP)	1% - 25%	Insoluble	No	Low
Fumigants	< 100% ,(nearly 100%)	Gas	No	High
Aerosols	1% - 10%	Insoluble in water, soluble in propellant	No	Moderate

Based on Release Mechanism:

Formulation Type	Active Ingredient (%)	Release Mechanism	Agitation Required	Risk of Phytotoxicity
Controlled Release (CR)	10% - 70%	Timed release	No	Low
Emulsifiable Concentrate (EC)	25% - 75%	Immediate release	Low	High
Wettable Powder (WP)	10% - 80%	Immediate release	Yes	Low
Granules (G)	1% - 15%	Immediate release	No	Low
Baits	1% - 10%	Timed release	No	Low

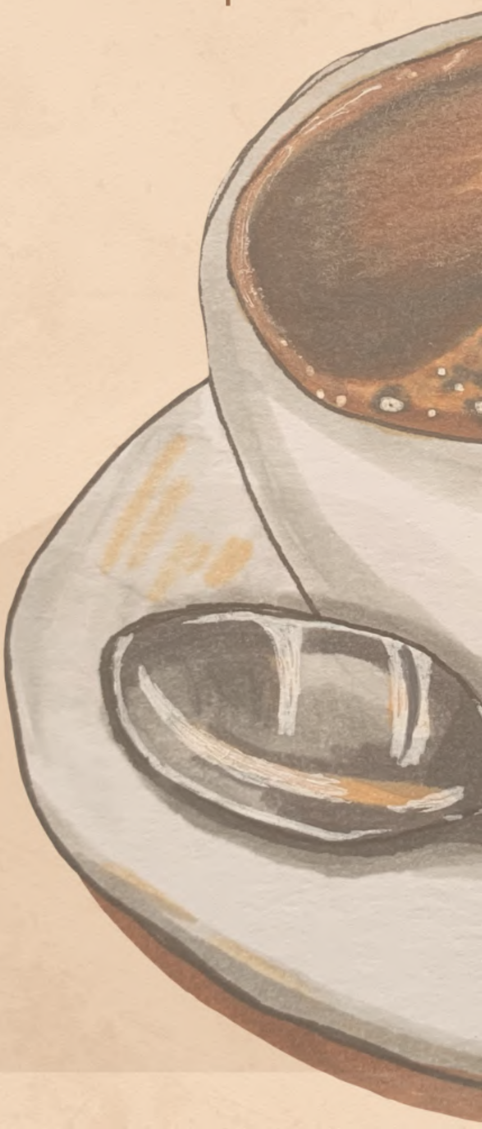
References:

1. *EP0962134A1 - A process for preparing a wettable powder formulation - Google Patents*. (1999, May 11). Google.com. https://patents.google.com/patent/EP0962134A1/en
2. *WP and WDG additives*. (2024). Levaco.com. https://agro-solutions.levaco.com/products/wp-and-wdg-additives.html
3. *EP0789510A1 - Wettable powder formulations - Google Patents*. (1995, November 2). Google.com. https://patents.google.com/patent/EP0789510A1/en
4. Rasoul Marzban, Jaber Babaei, Kalantari, M., & Saberi, F. (2021). PREPARATION OF WETTABLE POWDER FORMULATION OF BACILLUS THURINGIENSIS KD2. *Journal of Applied Biological Sciences*, *15*(3), 285–293. https://www.jabsonline.org/index.php/jabs/article/view/830
5. *Wettable powder (WP) | Croda Crop Care*. (n.d.). Www.crodacropcare.com. https://www.crodacropcare.com/en-gb/applications/wettable-powder
6. *Pesticide Formulation Demonstration*. (n.d.). Penn State Extension. https://extension.psu.edu/pesticide-formulation-demonstration
7. https://agraform.com/formulation-technologies/suspension-concentrate/
8. *Agricultural & Farming Chemicals | Suspension Concentrates | Azelis*. (n.d.). Www.azelis.com. https://www.azelis.com/en/markets/specialty-agrihorti/products/crop-protection/suspension-concentrates
9. *Capsule suspension (CS) | Croda Crop Care*. (n.d.). Www.crodacropcare.com. https://www.crodacropcare.com/en-gb/applications/capsule-suspension

10. *Water dispersible granule (WDG) | Croda Crop Care*. (2024). Crodacropcare.com. https://www.crodacropcare.com/en-gb/applications/water-dispersible-granule
11. *UNIT 3: Formulations*. (n.d.). Cms.ctahr.hawaii.edu. https://cms.ctahr.hawaii.edu/epp/Education/Study-Guide-Packets/APC-Core/APC-Unit3
12. *Agro Chemicals - Formulation*. (2024). Jeevanchemicals.com. http://www.jeevanchemicals.com/agro_formula2.html
13. *Water dispersible granules*. (2023). Azelis. https://www.azelis.com/en/markets/specialty-agrihorti/products/crop-protection/water-dispersible-granules
14. *US3992532A - Flowable pesticide formulations - Google Patents*. (1975, January 2). Google.com. https://patents.google.com/patent/US3992532A/en
15. *EP0052313A1 - Flowable pesticides - Google Patents*. (1981, November 6). Google.com. https://patents.google.com/patent/EP0052313A1/en
16. Nihal Kılıç. (2022). Efficacy of Dust and Wettable Powder Formulation of Diatomaceous Earth (Detech®) in the Control of Tyrophagus putrescentiae (Schrank) (Acari: Acaridae). *Insects*, *13*(10), 857–857. https://doi.org/10.3390/insects13100857
17. Bindu, S., & Akoijam. (2018). *Indian Journal of Hill Farming Insecticide Formulations*. *31*(2), 340–343. https://www.kiran.nic.in/pdf/IJHF/Vol_31_2/22%20Edited.pdf
18. Takeo Ohkouchi, & Tsuji, K. (2022). Basic Technology and Recent Trends in Agricultural Formulation and Application Technology. *Journal of Pesticide Science*, *47*(4), 155–171. https://doi.org/10.1584/jpestics.d22-055
19. *Pesticides and Formulation Technology Purdue extension*. (n.d.). https://www.extension.purdue.edu/extmedia/ppp/ppp-31.pdf

20. Goss, G., Taylor, D., & Kallay, W. (2009). Granular Pesticide Formulations. *ASTM International EBooks*, 114–110. https://doi.org/10.1520/stp16036s
21. *Pesticide Formulations - Kentucky Pesticide Safety Education*. (n.d.). Www.uky.edu. https://www.uky.edu/Ag/Entomology/PSEP/3formulations.html
22. Roy, A., Singh, S., Bajpai, J., & Bajpai, A. (2014). Controlled pesticide release from biodegradable polymers. Open Chemistry, 12(4), 453–469. https://doi.org/10.2478/s11532-013-0405-2
23. *CN103340197A - Slow/controlled-release microcapsule pesticide and preparation method thereof - Google Patents*. (2013, July 24). Google.com. https://patents.google.com/patent/CN103340197A/en
24. Utah State University. (2020). *Western Cherry Fruit Fly*. Usu.edu. https://extension.usu.edu/planthealth/research/western-cherry-fruit-fly
25. *Western Cherry Fruit Fly | WSU Tree Fruit | Washington State University*. (n.d.). https://treefruit.wsu.edu/crop-protection/opm/western-cherry-fruit-fly/
26. Charlotte. (2022, December 30). *Cherry fruit fly: identification & treatment - Plantura*. Plantura. https://plantura.garden/uk/fruits/cherry-tree/cherry-fruit-fly
27. *School IPM*. (n.d.). Schoolipm.ifas.ufl.edu. https://schoolipm.ifas.ufl.edu/newtechp5.htm
28. *PI231/PI231: Pesticide Formulations*. (n.d.-b). Edis.ifas.ufl.edu. https://edis.ifas.ufl.edu/publication/PI231
29. *Formulation*. (2024). Growsafe.co.nz. https://www.growsafe.co.nz/StandardManual/Agrichemicals/Formulation/StandardManual/Agri/Formulation.aspx?hkey=f19b0293-7e1d-46fb-93d0-5fdfbde9d682

Promoter Design for Dummies



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GLOSSARY

1. **RNA Polymerase:** An enzyme that produces RNA with a template DNA from the process of transcription.
2. **Sigma Factor:** A bacterial protein that initiates the binding of RNA polymerase to a promoter.
3. **Transcription Start Site:** Promoter region where transcription begins. Usually referred to as +1.
4. **Consensus regions:** Sequences of DNA that show the highest probability of performing a certain function across many organisms.
5. **Open complex:** The Transcription bubble structure (opening of the double stranded DNA) formed prior to Transcription
6. **Upstream:** Direction away from the coding region moving further.
7. **Downstream:** Direction that the gene is read.

The advancement of synthetic and systems biology calls attention away from gene engineering to complex circuits with many interrelated parts. Understanding the working of a promoter system is essential as they control gene expression. This handbook will equip you with the foundation to engineer promoters.

INTRODUCTION

The rapid advancement of synthetic and systems biology is shifting the focus from traditional gene engineering towards the intricate design of complex genetic circuits with multiple interconnected components. At the heart of this lies the promoter system—a key element in regulating gene expression. Understanding how promoter-systems function is vital, as they serve as the “Master Controllers” of a gene.

A promoter is located upstream of a coding gene and essentially functions as an ON/OFF Switch triggered due to the presence or absence of a molecule or a compound. The promoter could be as small as a few tens of base pairs and could extend to hundreds of base pairs in length with sites for the binding of various factors and polymerases responsible for the coding of the gene downstream.

RNA Polymerase

Transcription occurs due to the enzyme RNA Polymerase. Its structure is conserved amongst prokaryotes and eukaryotes, but the difference arises in the various sub-functions, which are dependent on the transcription factors that bind to this enzyme. Here we will be constraining ourselves to prokaryotic transcription, which occurs in the active site of RNA polymerase [1].

The RNA polymerase core enzyme comprises five subunits- 2 alpha, one beta, one beta’ and omega. A sixth subunit called the sigma factor which helps in identification of the promoter region, binds to the core enzyme to form the holoenzyme, which then helps to assemble onto the core promoter region [2]. The sigma factor also helps in melting the double stranded dna to form the open complex (transcription bubble) [3].

Core Enzyme Subunits

The alpha subunit of RNA polymerase acts as a scaffold to assemble the larger beta and beta' subunits, with each alpha subunit pairing with one of these larger subunits. It consists of two domains, the alpha-CTD (C-terminal domain) and alpha-NTD (N-terminal domain), connected by a flexible linker. The alpha-CTD is involved in transcription activation by interacting with transcription factors and upstream promoter elements, while the alpha-NTD plays a role in gene regulation through interactions with transcription factors. The beta and beta' subunits bind the sigma factor and nucleotide triphosphates interacting with dna, facilitates RNA synthesis with respect to template DNA. The omega subunit helps stabilize the RNA polymerase by assisting with proper folding [4].

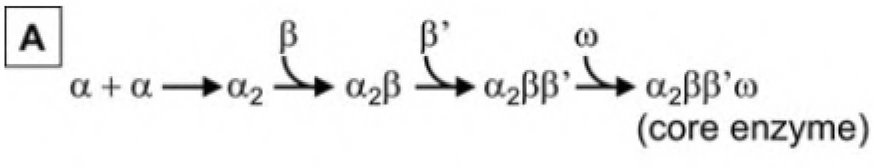


Fig.1 Formation of the RNA polymerase core enzyme [10]

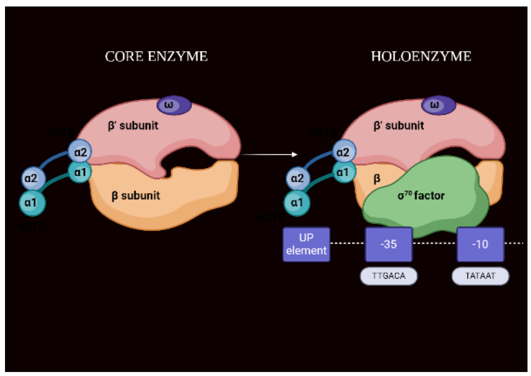


Fig.5 E. coli RNA Polymerase structure

Sigma Factor

Sigma factors are multi domain proteins that bind with the RNA polymerase core enzyme to form the holoenzyme. The sigma factors contains 4 domains : $\boldsymbol{\sigma_{1.1}, \sigma_2, \sigma_3, \sigma_4}$

Domain	Function
Domain 1.1	Found in primary sigma factors; ensures sigma binds to the promoter only when complexed with RNA polymerase to prevent non-specific interactions.

TABLE 1: DOMAINS OF THE SIGMA FACTOR [12]

Domain 2	Divided into 2.1, 2.2, 2.3, 2.4; region 2.4 binds to the −10 element (Pribnow box); region 2.3 stabilizes the open complex during transcription initiation.
Domain 3	Interacts with core RNA polymerase, stabilizing sigma factor binding and facilitating holoenzyme complex formation for transcription initiation.
Domain 4	Contains region 4.2; binds to the −35 element of the promoter, establishing specific DNA contacts for accurate RNA polymerase positioning at the transcription start.

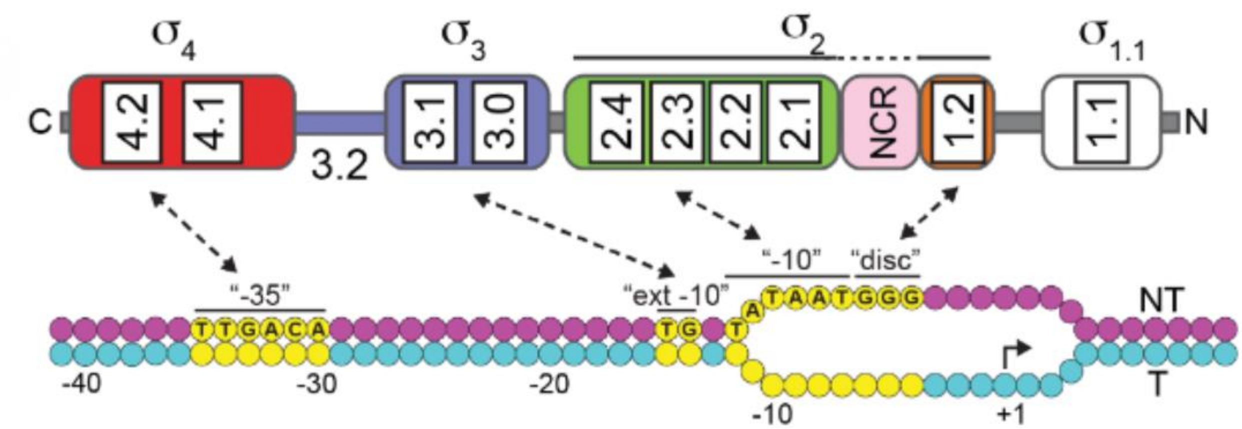


Fig.2: interaction of the different domains of sigma factor [5]

Bacillus subtilis has 10 sigma factors (so far identified), each expressed at a different phase in the bacterial life cycle and performing a variety of functions. Sigma A is the most prevalent, expressed throughout the bacterial life cycle.

Sigma factor (alternative designation)	Gene(s)	Function	Promoter sequence[b]			Reference
			−35	Spacer (bp)	−10	
Vegetative-cell factors						
σ^A (σ^{43}, σ^{55})	*sigA*, *rpoD*	Housekeeping/early sporulation	TTGACA	17	TATAAT	210
σ^B (σ^{37})	*sigB*	General stress response	RGGXTTRA	14	GGGTAT	24
σ^C (σ^{32})	Unknown	Postexponential gene expression	AAATC	15	TAXTGYTTZTA	145
σ^D (σ^{28})	*sigD*, *flaB*	Chemotaxis/autolysin/flagellar gene expression	TAAA	15	GCCGATAT	119
σ^H (σ^{30})	*sigH*, *spoOH*	Postexponential gene expression; competence and early sporulation genes	RWAGGAXXT	14	HGAAT	237
σ^L	*sigL*	Degradative enzyme gene expression	TGGCAC	5	TTGCANNN	59
Sporulation-specific factors						
σ^E (σ^{29})	*sigE*, *spoIIGB*	Early mother cell gene expression	ZHATAXX	14	CATACAHT	252
σ^F ($\sigma^{spoIIAC}$)	*sigF*, *spoIIAC*	Early forespore gene expression	GCATR	15	GGHRARHTX	291
σ^G	*sigG*, *spoIIIG*	Late forespore gene expression	GHATR	18	CATXHTA	217
σ^K (σ^{27})	*sigK*, *spoIVCB:spoIIIC*	Late mother cell gene expression	AC	17	CATANNNTA	338

[a] The designations for the sigma proteins and their structural genes as well as likely functions of their regulons are listed. References for each item can be found in the text. The probable consensus sequences for the holoenzyme forms are aligned at their −10 positions (underlined). The spacer region represents the number of bases between the upstreammost −10 region base that is given and the downstreammost base of the −35 region. The reference for each consensus sequence is listed to its right.

[b] H, A or C; N, A, G, C, or T; R, A or G; W, A, G, or C; X, A or T; Y, C or T; Z, T or G.

Fig.3. Bacillus subtills sigma factors [6]

PROMOTER

Parts of a Promoter

The length of a promoter is divided into various segments depending on the functions and the compounds they bind to.

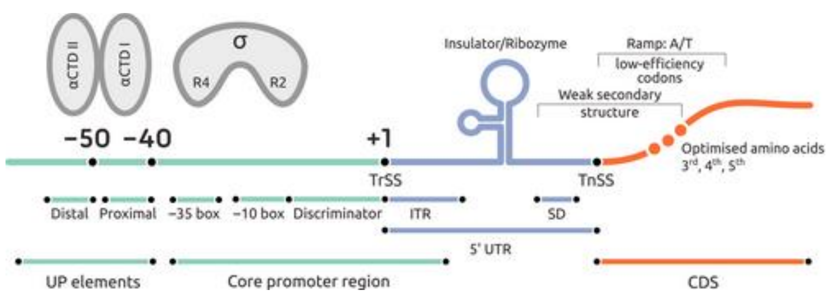


Fig.4 Parts of a Promoter [7]

Core Promoter

The promoter's core is roughly from the upstream end of the RBS to 40 base pairs upstream of the transcription start site. The –35 and –10 regions are identified by sigma subunits of the RNA polymerase, the mutations in which can vary the expression levels for a promoter.

The region of these sigma factor binding sites are crucial and the replacement with consensus sequences has shown a significant improvement in binding affinity and expression levels [8].

The discriminator region is a high GC containing sequence that is responsible for controlling promoter expression as GC bonds are harder to break. The region interacts with the alpha subunit of the RNA polymerase and tends to be heavily conserved and is responsible for the open complex formation [9].

Promoters are divided into strong, moderated and weak, depending on their transcription rates. These transcription rates vary as they rely on the escape of the RNAP at the end of transcription, which depends on the stability of the open complex. The higher the stability, the longer the escape time and weaker the promoter [10].

Upstream Elements

Elements in the upstream of the core region, from the –40 to the –60 region of the promoters, comprise of its upstream elements. These sequences are identified by the alpha subunits of the promoter contributing to the initial binding of the RNA polymerase. The Upstream elements are split into two parts namely the proximal, positioned at the –40 region and the distal, at the –50 region. The proximal region has been associated with the Catabolic Activator Protein (CAP) to unzip the DNA [11].

These regions are primarily responsible for the transcription rates in *E. coli* as the RNA polymerase of *E. coli* presents with extra subunits called alpha-C Terminal Domains (alpha-CTD), absent in *Bacillus subtills,* that bind strongly to these regions, but they still function as recognition sites in Bacillus [12]. RNA polymerase binding efficiency can be increased by enhancing the consistency of UP region with the consensus sequence of the same [13].

5’ Untranslated Region

The 5’ Untranslated Region of a promoter contains the Ribosome Binding Site in the form of a Shine-Dalgarno sequence that guides and aligns the Ribosome with the start codon to being transcription [10].

Types of Promoters

Promoters that get activated in the presence of a trigger molecule and begins to code for the downstream gene is referred to as an *Inducible promoter*. One such promoter is a Lead induced promoter (BBa_I721001).

Promoters that are consistently active through a cell life cycle requiring no triggers to be activated is a *Constitutive promoter* such as P43 (BBa_K1628006)

Promoters that get activated due to absence of a trigger molecule, that stays bound to the promoter until removed, is a *Repressible promoter* such as PbacA (BBa_K4912002)

Promoter Replacement

Promoter replacement is a foundational technique in synthetic biology, offering a direct route to overexpressing a gene of interest. In most cases, it's a straightforward process, but sometimes we must take a more innovative approach. This is where the design of composite parts comes into play—specially tailored promoter constructs that synchronize gene expression with different phases of production or specific triggers.

A Composite part is a combination of one or more pre-existing Bio parts combined to achieve a specific goal, in the context of a composite promoter, an additive or multiplicative effect on the transcription of the downstream gene.

Although wet lab testing is necessary to validate the functioning of composite part, there could be some literature backup on whether two parts when combined would achieve an expected result.

When considering a composite promoter, their compatibility is essential. Studies show that Sigma A-activated promoters showed synergistic activity with Sigma B-dependent promoters when Sigma A-dependent promoters are placed upstream of the coding sequence. However, the exact mechanism is still unclear[1].

The combination of two different promoters in tandem makes the composite part a dual promoter.

Dual Promoter

Dual promoter composite parts have been designed and tested for various systems including bacteria, yeast and mammalian cells to improve yield. The promoters in tandem need not necessarily be in the same direction to be considered dual promoters in which case it's called a bidirectional dual promoter.

Dual promoter systems have been documented for bacillus subtilis chassis as subtilis is a common industrial bacterium and has shown promising results for biomanufacturing.

Below is a list of documented dual promoter parts for *Bacillus subtilis*.

Strain	Plasmid	Promoter	Regulation	Induction	Product	Increase in Production Level
PL1801*spoIIE*	pDG268ΔNeo	P_{amyQ} (consensus) + P_{cry3A}	Constitutive + Constitutive	–	Subtilisin	* 5-fold (P_{amyQ} or $P_{cry3A'}$) *1.5-fold (P_{cry3A}) * 3-fold (P_{amyQ} + P_{cry3A} stab)
ϕ105MU331	pSGA2 pSGP2	Phage promoter + P_{amy} or P_{pac}	Inducible + Constitutive	Temperature shift from 37 °C to 50 °C	α-Amylase Penicillin acylase	* 133% α-amylase (phage promoter) * 113% penicillin acylase (phage promoter)
LKS87	pHA-TSαGT pHB-TSαGT	P_{HpaII} + P_{amyR2} or P_{blma}	Constitutive + Constitutive	–	Thermostable 4-α-glucanotransferase	* P_{HpaII} + P_{amyR2}: 11-fold (P_{HpaII}) * P_{HpaII} + P_{blma}: 12-fold (P_{HpaII})
DB104	pSM108 pSM109	P_{aprN} + P_{aprN} or P_{aprN_M10} + P_{aprN_M10}	Constitutive + Constitutive	–	Nattokinase	* P_{aprN} + P_{aprN}: 94% (P_{aprN}) * P_{aprN_M10} + P_{aprN_M10}: 95% (P_{aprN_M10})
168	pMA5	P_{HpaII} + P_{sacB}	Constitutive + Inducible	2% (*w*/*v*) sucrose	Endoglucanase	* 20-fold (P_{HpaII}) * No significant increase (P_{sacB})
1A747	pGJ103 (pShuttleF)	Putative promoter + P_{luxS}	Constitutive + Constitutive	–	β-Galactosidase	* Putative promoter + P_{luxS}: 8-fold (P43) * Hybrid promoter: 1.6-fold (putative promoter + P_{luxS})
168	pRB373-GFP pRB373-HSA	P_{ykzA}, P_{gsiB} or P_{ydaD} + P_{secA} or P_{yxiE}	Constitutive or Inducible + Constitutive	–	Green fluorescent protein Human serum albumin	* 2–3-fold GFP and HSA ($P_{\sigma A}$; namely P_{secA} or P_{yxiE})
WB600	pBSG24-MCS	P_{HpaII}+ P_{gsiB}	Constitutive + Inducible	–	Aminopeptidase	* 2.3-fold (P_{HpaII}) * 2.2-fold (P_{gsiB}) * 19-fold (P43)
BSG1682	pBSG16-pBSG29	P_{srfA}+ P_{HpaII} or P_{gsiB}	Inducible + Constitutive or Inducible	–	Green fluorescent protein Aminopeptidase Nattokinase	* P_{srfA} + P_{gsiB}: 12% GFP (P_{srfA}) * P_{srfA} + P_{HpaII}: 0.5-fold (P_{srfA}) Higher expression of both AP and NK (P_{srfA} or P_{HpaII})
CTCC M 2016536	pHYCGTd4 pHYPULd4 pHYαCGTd4	P_{HpaII}+ $P_{amyQ'}$	Constitutive + Constitutive	–	α- or β-Cyclodextrin glycosyltransferase Pullulanase	* 1.3-fold β-CGTase ($P_{amyQ'}$) * 1.5-fold pullulanase($P_{amyQ'}$) * 2.9-fold α-CGTase ($P_{amyQ'}$)

Fig.6 Studies in the literature related to double promoter expression system design for B. subtilis

The use of two constitutive promoters arranged in tandem has shown promising results. For example, combination of the *cry3A* promoter from *Bacillus thuringiensis* and the *amyQ* promoter from *Bacillus amyloliquefaciens* significantly enhanced subtilisin production. Similarly, the combination of the *amyR2* or *blma* promoters with the strong *PHpaII* promoters led to a remarkable increase in 4-α-glucotransferase production by up to 12-fold [5].

Promoters in-sillico can be tested by docking to their respective sigma factors to measure Gibbs free energy to test the binding affinities but will have to be backed up by wet lab studies.

NOTES

from Dry Lab Team MIT-MAHE -2024

When using the PDB structures of sigma factor for binding studies, make sure that the conformational change for the sigma factor has been accounted for.

Use pyMOL or CHIMERA softwares to visualise,animate and align your PDB files.

PDBviewer webserver tool can also be used to visualise the pdb structures.

CHECKLIST

Our checklist of questions to answer while designing your own promoter!

- ☐ Is the promoter inducible, constitutive or repressible?
- ☐ Does the promoter have the Pribnow box(-10region) and –35 region.
- ☐ What is the transcription factors binding sites present on the promoter?
- ☐ Does the promoter have an alpha-CTD binding region.
- ☐ Is the spacer region between the -35 and -10 regions equivalent to the spacer length for the specific sigma factor (refer *Fig.3)?*
- ☐ What is the promoter strength (e.g., weak, medium, or strong) and how does it affect transcription levels?
- ☐ Is the promoter compatible with the host organism or expression system being used?
- ☐ Are there any upstream activation sequences (UAS) or enhancers that modulate promoter activity?
- ☐ Does the promoter have any regulatory elements, such as operators, for feedback control or fine-tuning gene expression?

☐ References

[1] T. Phanaksri, P. Luxananil, S. Panyim, and W. Tirasophon, “Synergism of regulatory elements in σB- and σA-dependent promoters enhances recombinant protein expression in Bacillus subtilis,” *J Biosci Bioeng*, vol. 120, no. 4, pp. 470–475, Oct. 2015, doi: 10.1016/J.JBIOSC.2015.02.008.

[2] S. de A. e Silva and S. Echeverrigaray, “Bacterial Promoter Features Description and Their Application on E. coli in silico Prediction and Recognition Approaches,” in *Bioinformatics*, InTech, 2012. doi: 10.5772/48149.

[3] M. C. Davis, C. A. Kesthely, E. A. Franklin, and S. R. MacLellan, “The essential activities of the bacterial sigma factor,” *Can J Microbiol*, vol. 63, no. 2, pp. 89–99, Feb. 2017, doi: 10.1139/cjm-2016-0576.

[4] Y. Rao *et al.*, “A novel toolbox for precise regulation of gene expression and metabolic engineering in Bacillus licheniformis,” *Metab Eng*, vol. 78, pp. 159–170, Jul. 2023, doi: 10.1016/J.YMBEN.2023.06.004.

[5] S. Öztürk, B. G. Ergün, and P. Çalık, “Double promoter expression systems for recombinant protein production by industrial microorganisms,” *Appl Microbiol Biotechnol*, vol. 101, no. 20, pp. 7459–7475, Oct. 2017, doi: 10.1007/S00253-017-8487-Y.

[6] W. G. Haldenwang, “The sigma factors of Bacillus subtilis,” *Microbiol Rev*, vol. 59, no. 1, pp. 1–30, Mar. 1995, doi: 10.1128/MR.59.1.1-30.1995.

[7] L. Tietze and R. Lale, “Importance of the 5' regulatory region to bacterial synthetic biology applications,” *Microb Biotechnol*, vol. 14, no. 6, p. 2291, Nov. 2021, doi: 10.1111/1751-7915.13868.

[8] Y. Rao *et al.*, “Construction and application of a dual promoter system for efficient protein production and metabolic pathway enhancement in Bacillus licheniformis,” *J Biotechnol*, vol. 312, pp. 1–10, Mar. 2020, doi: 10.1016/J.JBIOTEC.2020.02.015.

[9] S. P. Haugen, W. Ross, M. Manrique, and R. L. Gourse, “Fine structure of the promoter–σ region 1.2 interaction,” *Proceedings of the National Academy of Sciences*, vol. 105, no. 9, pp. 3292–3297, Mar. 2008, doi: 10.1073/pnas.0709513105.

[10] L. Tietze and R. Lale, “Importance of the 5' regulatory region to bacterial synthetic biology applications,” *Microb Biotechnol*, vol. 14, no. 6, pp. 2291–2315, Nov. 2021, doi: 10.1111/1751-7915.13868.

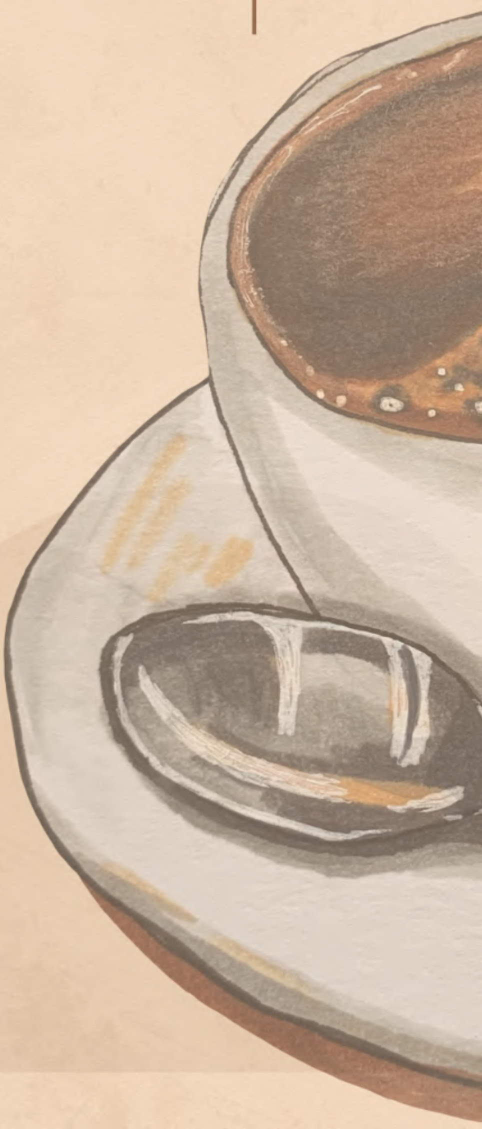
[11] S. T. Estrem *et al.*, “Bacterial promoter architecture: subsite structure of UP elements and interactions with the carboxy-terminal domain of the RNA polymerase subunit,” 1999. [Online]. Available: www.genesdev.org

[12] I. Artsimovitch, V. Svetlov, L. Anthony, R. R. Burgess, and R. Landick, “RNA Polymerases from *Bacillus subtilis* and *Escherichia coli* Differ in Recognition of Regulatory Signals In Vitro,” *J Bacteriol*, vol. 182, no. 21, pp. 6027–6035, Nov. 2000, doi: 10.1128/JB.182.21.6027-6035.2000.

[13] Y. Rao *et al.*, “A novel toolbox for precise regulation of gene expression and metabolic engineering in Bacillus licheniformis,” *Metab Eng*, vol. 78, pp. 159–170, Jul. 2023, doi: 10.1016/j.ymben.2023.06.004.

[14] S. Öztürk, B. G. Ergün, and P. Çalık, “Double promoter expression systems for recombinant protein production by industrial microorganisms,” *Appl Microbiol Biotechnol*, vol. 101, no. 20, pp. 7459–7475, Oct. 2017, doi: 10.1007/s00253-017-8487-y.

A Handy Guide to Stakeholder Identification



A Handy Guide to Stakeholder Identification

Stakeholders are an integral part of any project, specifically in the case of iGEM where projects themselves are born out of stakeholder engagement efforts. We at iGEM MIT-MAHE (2024) take great pleasure in guiding future iGEM teams to identify their stakeholders so that their human practices efforts can be the best it can be!

So, if you are reading this in the future, Greetings & good luck on your iGEM journey!

Stakeholders are defined in a plethora of ways but for iGEM purposes we shall define stakeholders as "all parties relevant to a project who gain to benefit from the project in a variety of ways." The stakeholders can change the entire course of the project or add on to it in a small way but they're all stakeholders nonetheless.

Now that we know what stakeholders are, it is time to classify stakeholders into various categories and what each of them mean.

STAKEHOLDER CLASSFICATION

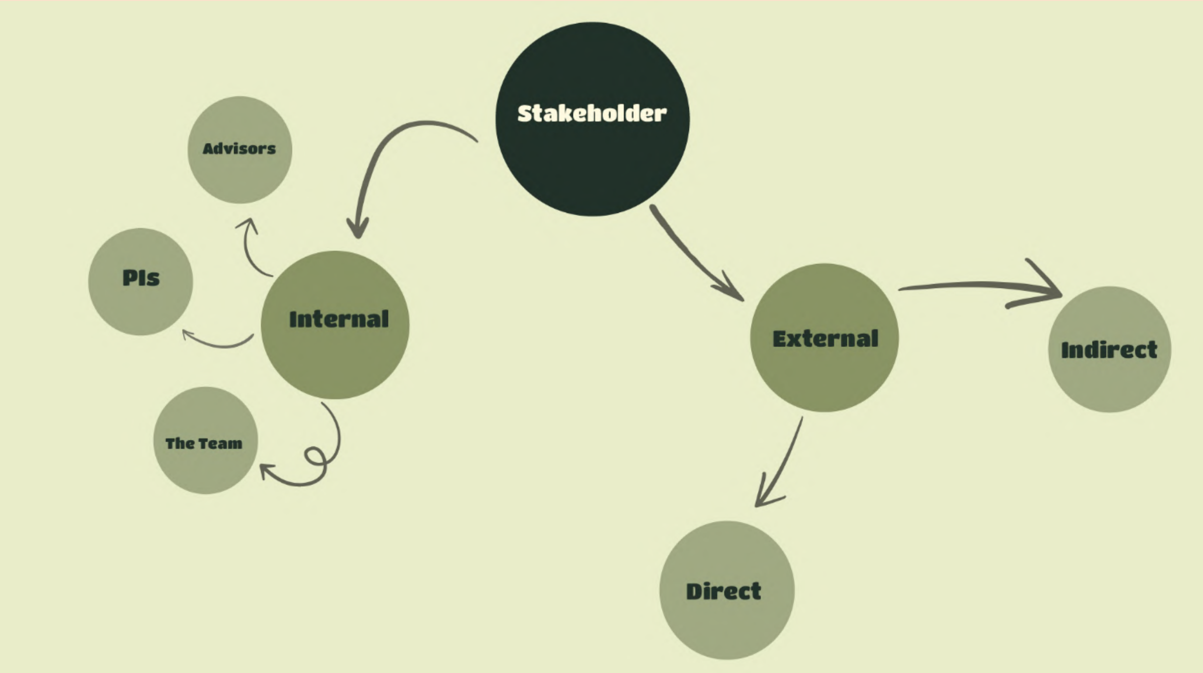


Figure 1: Classification of Stakeholders by iGEM MIT-MAHE 2024

Internal Stakeholder: Internal stakeholders are members working actively on the project under the guidance and along with all of their advisors and mentor(s). These stakeholders quite arguably are the most important stakeholders as they actively influence the decision-making process of the project. It is essential that there is a well-defined hierarchy and open mindedness amongst the team to ensure a smooth workflow especially during time crunches.

External Stakeholders: All external entities who are involved in any degree count as external stakeholders. These individuals/organizations may be collaborators, external advisors, sponsors, individuals and organizations that provide monetary/in-kind support.

External stakeholders may be further expanded as direct and indirect stakeholders for further stakeholder classification.

Direct Stakeholders: Direct Stakeholders are closely involved in the production, design and working in relation to the project's goals. These can include industrialists or researchers who closely work with the key elements of the project. For example, a project related to climate change would have climate change activists and the government's pollution control boards as the direct stakeholders.

Indirect Stakeholders: These types of stakeholders are usually those who are affected by the changes in the project but not on the same scale as that of direct stakeholders. More often than not, the indirect stakeholders are affected by the work performed by the direct stakeholders who in turn are affected by the change in the project or the problem statement. In most cases, consumers are indirect stakeholders as they're affected by the changes in the raw materials which are utilized by the direct stakeholders for engineering the final product reaching the consumers.

STAKEHOLDER IDENTIFICATION

Understanding the various types of stakeholders is the first step to identifying any team or project's stakeholders. When you want to identify your stakeholder, remind yourself to ask these question words:

1. WHO is my project addressing?
2. WHAT is my project addressing?
3. WHEN is my project going to be implemented?
4. HOW is my project relevant and HOW is it being implemented?
5. WHY is this project important for the society?
6. DOES my project have any ethical implications or impact?

The above list of questions is never-ending and can be endlessly done to identify more stakeholders or help you connect with other stakeholders from one to another. It is important to note that a lot of projects arise simply out of asking these questions.

Stakeholders can shape a project by providing ideas, changing the course of the project or even by contributing a few words of advice.

Indirect stakeholders although sounding unimportant prove to be really crucial as they tend to be the end goal of any project or product. Furthermore, indirect stakeholders can also be classified further by demographic.

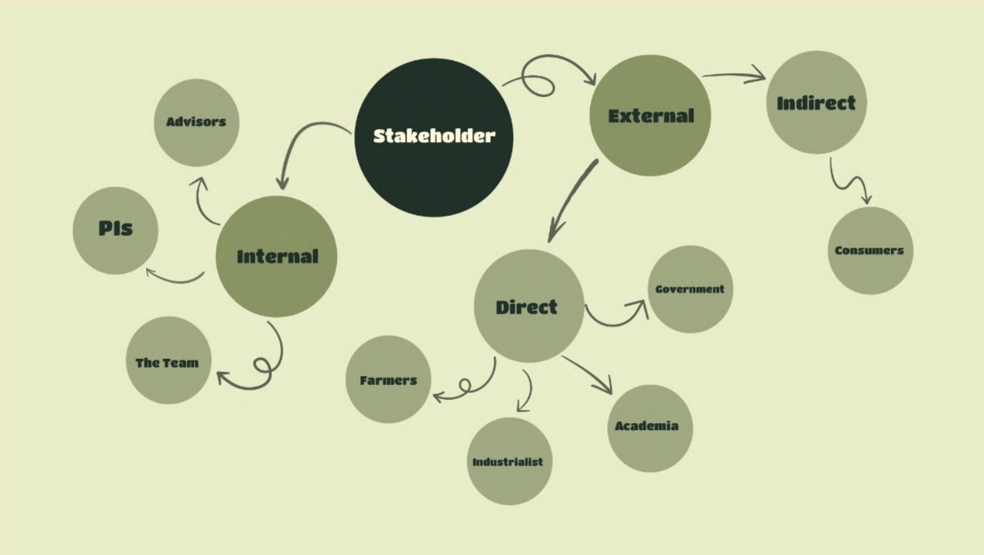


Figure 2: Stakeholder identification map for CAAPI (iGEM MIT-MAHE 2024)

A stakeholder map for any project can be mapped like the above given example and the farther a particular stakeholder strays away from the center, the closer they are to being an indirect stakeholder (more the arrows, closer to indirect stakeholder).

A PROJECT IS NOTHING WITHOUT ITS STAKEHOLDERS

When a project lacks stakeholders, it faces significant challenges that can impede its progress and long-term viability. Stakeholders are crucial in providing essential support, guiding the project's direction, and ensuring its relevance in the broader context. Without their involvement, a project may struggle to secure the resources it needs—be it financial backing, expertise, or access to critical tools.

The absence of external stakeholders often leads to a lack of clear objectives, as there is no external input to refine goals or provide the necessary feedback for continuous improvement. This can result in a project that drifts without purpose, failing to align with market needs or industry standards. Furthermore, without stakeholders to hold the team accountable, timelines and deliverables may become ambiguous, leading to inefficiencies and missed opportunities. Ultimately, stakeholders bring credibility, resources, and focus to a project, and their absence can leave it vulnerable to stagnation, irrelevance, or even failure.

BUT!

Worry not for you have our handy guide to stakeholder identification!