A Primer on the Process of Cannabinoids Derived Through Biosynthesis & Cellular Agriculture

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About Biosynthesis & SynBio

Biosynthesis is the stepwise process by which living things use enzymes to build or modify molecules into other molecules for the benefit of an organism. The path that these enzymes take, from starting materials to final product, is referred to as a biosynthetic pathway. Enzymes are remarkable biological machines and, in many cases, their ability to create or modify molecules is unmatched when compared to non-biological synthesis approaches. Scientists have learned to utilize enzymes to make useful molecules for many different purposes. This type of biotechnology is referred to as synthetic biology, or SynBio. Over the past several decades, the SynBio industry has created products including biofuels, rare oils, insulin, cosmetic and food ingredients, and vaccines – all molecules or proteins that are cost-prohibitive, complex to recover, or even impossible to produce. With the recent resurgence of the cannabis plant for medical and consumer applications, there is a growing demand for cannabinoids, the main active ingredients found in the cannabis plant. This demand specifically requires cannabinoid as isolates of high purity, free of contaminants, and at a significantly lower cost. In assessing the current methods to create and isolate cannabinoids, a SynBio approach was determined to be the most effective. To understand this conclusion, a discussion on cannabis cultivation, cannabinoid extraction and the challenges in the industry is detailed below.

The distinction between cannabis, marijuana and hemp

To begin we need to provide some clarity on names. Cannabinoids are a class of molecules that are produced by the plant species, *Cannabis sativa* L.[1]. Although other plant species have been observed to produce cannabinoids or cannabinoid-like compounds, *Cannabis sativa* is the plant of first discovery and produces the highest concentrations of these compounds. Cannabinoids that are found in the plant are more specifically referred to as phytocannabinoids (phyto, derived from the Latin word for plant) and the molecules produced in our bodies that have similar functions – but very different structures – are referred to as endocannabinoids. There are many different cannabinoids[2] found in the tissues of *Cannabis sativa* but the two most observed, and most abundant, are delta-9 tetrahydrocannabinol (Δ9-THC or just THC) and cannabidiol (CBD). THC and CBD are frequently referred to as the “major” cannabinoids, but this is only attributed to their concentration in the plant and not to their comparative importance among all the cannabinoids. Despite the presence of other “minor” cannabinoids, THC and CBD concentrations have been utilized as benchmark delineators of different varieties of the cannabis plant, often referred to incorrectly as strains[3], and different cannabis-derived products.

The cannabis industry categorizes plants using both biological definitions and legal/regulatory definitions, a source of much confusion in this constantly evolving industry. Biologically, a species is a group of organisms that share key traits and can produce offspring of the same type. These traits, and the rules by which to assess them, are agreed upon and modified by associated scientific bodies[4]. Within a species, there can be considerable variability in appearance. For example, a Chihuahua and a Great Dane look very different but are both part of the *Canis lupus* species, just as a Red Delicious apple and a Granny Smith apple look very different but are both part of the *Malus domestica* species**.** In the plant kingdom, if the variation is naturally occurring, it is referred to as a “variety” of the species. If the variation was created by human intervention, it is referred to as a cultivated variety or “cultivar”. Research on *Cannabis* evolution has revealed a convoluted history as to its origin and species definition. However, the current consensus is that the species is *Cannabis sativa L*., and the subspecies variation that we currently see in the cannabis industry—designations such as sativa, indica, ruderalis, and the extensive commercial lineages—are examples of either varieties or cultivars.

[1] the “L.” is an identifier for the person who classified the organism.

[2] Currently there are roughly 100 cannabinoids that have been isolated from the plant. For a thorough catalog and analysis see Berman, P., Futoran, K., Lewitus, G. M., Mukha, D., Benami, M., Shlomi, T., & Meiri, D. (2018). A new ESI-LC/MS approach for comprehensive metabolic profiling of phytocannabinoids in Cannabis. Scientific Reports, 8(1), 1–15. https://doi.org/10.1038/s41598-018-32651-4

[3] The term strain is used to describe subspecies of microorganisms, such as bacteria, that utilize asexual reproduction to propagate. Although cannabis plants can be cultivated by clonal propagation (asexually), this is an artificial approach. *Cannabis sativa* is a dioecious sexually reproducing organism (a single plant is either male or female) and follows the botanical sub-species delineations of variety or cultivar. Laboratory mouse “strains” are similarly mis-characterised.

[4] International Code of Nomenclature for algae, fungi, and plants (ICN), https://en.wikipedia.org/wiki/International_Code_of_Nomenclature_for_algae,_fungi,_and_plants

In parallel to a cannabis plant's botanical variety or cultivar distinction, there exists a legal/regulatory definition[5]. For example, in jurisdictions such as the United States and Canada:

- If a *Cannabis sativa* plant tissue or processed material contains THC levels equal to or less than 0.3% w/w[6], it is automatically classified as **hemp** and associated products as hemp-derived.
- If a *Cannabis sativa* plant tissue or processed material contains THC levels greater than 0.3% by weight, it is automatically classified as **marijuana** and associated products as marijuana-derived.

This designation causes much confusion in the industry due to the wide variability inherent to growing large numbers plants – or "crop production systems" in agriculture-speak. The degree of this variation increases as you move from indoor production to greenhouse production, and to outdoor field production. Cannabis plants produce cannabinoids to protect against pathogen infection, insect herbivory and UV damage from sunlight. Cannabinoid levels fluctuate with environmental conditions, and thus, a particular cannabis cultivar planted in the field can fluctuate between legal designation as hemp or marijuana. Similarly, as cannabis tissues are dried and processed, their relative cannabinoid concentrations can vary so producers must keep an eye on every stage to ensure the end product legally qualifies as hemp. Operators and cultivators are typically licensed according to legal or regulatory designations, and because of the variability in the crop system, THC concentrations can fluctuate in and out of legal compliance. These naturally variable cannabinoid concentrations continuously affect the global hemp CBD industry. In the 2019 United States hemp harvest season, more than half of the hemp fields planted had to be destroyed due to the levels of THC that crossed the 0.3% threshold[7]. This raises serious quality and supply chain concerns regarding the viability and sustainability of hemp as a production system for cannabinoids.

The cannabis sativa plant as a factory for cannabinoids

As a crop, plants are living factories that absorb light and carbon dioxide and convert it to sugar which the plant then converts into metabolites (e.g. glucose, starch, fiber, protein, nucleic acids). The majority of the metabolites produced are used for the primary functions of being a plant: building structures such as roots, stems, leaves and flowers as well as running the cellular machinery to produce energy to drive biologic processes. A small portion of these metabolites are considered secondary in function and are produced only when needed, such as for defense (e.g. toxic compounds that protect against predating insects) or to assist in reproduction (e.g. smells to attract pollinators).

Unlike the cultivation of individual plants, crop systems with thousands or millions of plants are difficult to grow successfully while ensuring they are free from both biological contaminants and chemical residues which affect the ability to pass quality controls. Effectively managing crop systems requires automation, external inputs and ongoing maintenance. This process is fraught with numerous risks including crop loss due to environmental changes, disease, predation, genetic variability, pathogen contamination and chemical contamination. By extension, these risks can cause significant fluctuations in the stability of the supply chain and the market value of the crop or crop derivatives. Today, many of these factors are mitigated through enhanced genetics and technology to reduce these risks. These risks are further reduced as cultivation moves from the field into greenhouses and further into indoor cultivation. However, control issues and the implementation of risk mitigation technologies often outweigh the cost-benefit of cultivation as compared to the market value of what the crop produces. This is why we don't see apple orchards in greenhouses.

Researchers are constantly improving crop plant species through traditional techniques including breeding and hybridization, and modern techniques such as mutagenesis and genetic engineering. Since agriculture was

[5] Legal regulatory definitions can differ by jurisdiction.
[6] Weight for weight, the proportion of a particular substance within a mixture, as measured by weight or mass. Note that current regulations require the anhydrous (with water) values.
[7] 2019 Hemp Benchmarks Report (https://www.hempbenchmarks.com/)

invented, these approaches have produced remarkable improvements to both crop yield and the mitigation of crop loss risks. Despite these improvements, the general architecture of the plant as a structural organized collection of cells that changes over time has fundamental limitations with diminishing returns. A plant species has a biologic and economic limit from the perspective of a factory that produces useful metabolites. To move beyond this limitation, a better factory is needed. To address this, scientists have developed approaches to move biosynthetic pathways from the plant to microorganisms such as algae and yeast. As these organisms are single cells, where each cell functions as an independent metabolite factory, they can be grown in large vessels with high efficiency, under sterile conditions, free from contaminants and over short periods of time. Although this approach has its own set of challenges, it removes all the risks in crop production systems discussed above. This type of biotechnology has been utilized for more than 40 years and is generally employed when the desired metabolite is present in the crop but cannot be economically recovered, the crop system is susceptible to risk, or a specific metabolite is required but the plant does not have the capacity to produce it in appreciable quantities.

The cultivation of *Cannabis sativa* as a crop system, uses the plant to produce cannabinoids, or to produce other products including seed and fiber. Some cannabis products use fresh or dried plant material, but other products that are rapidly growing in the "cannabis" market require the cannabinoids to be extracted from the plant material which introduces additional complexity, risks and associated costs. This crop, like other crop productions systems, also has its limitations. Existing varieties and cultivars are highly susceptible to yield variability, microbial and chemical contamination and crop-loss. In recent years, these risks have been improved by technology, agronomy and the implementation of cannabis-specific greenhouse and indoor cultivation methods. However, the ability to improve the plant's production capabilities is finite and has diminishing economic returns. Due to the value of cannabinoids and the rarity of the minor cannabinoids in the plant species, moving the biosynthetic pathway (specifically, the genes that encode the enzymes that make cannabinoids) from the plant factory into one of the well-known microorganism factories (e.g. algae, yeast, E.coli) is not only proving to be successful and economically-viable, but is eliminating much of the risks of the crop production system and supporting the emergence of the cannabinoid biosynthesis industry, or more commonly referred to as cannabinoid cellular agriculture.

The biosynthesis of cannabinoids through cellular agriculture

The large-scale production of cannabinoids using single-cell microorganisms is accomplished through a process called cellular agriculture (Figure below). It's similar to traditional agriculture but instead of large fields of individual plants, large vessels of individual cells are grown and harvested. Cellular agriculture does not involve the *Cannabis sativa* plant and, on their own, these microorganisms do not produce cannabinoids. Thus, cellular agriculture is a two-stage process: 1) the biosynthetic pathway that produces cannabinoids in the cannabis plant is recreated in the microorganism, and 2) the microorganism is grown to commercial scale in large aseptic fermentation vessels, then processed and purified.

Recreating the biosynthetic pathway was accomplished through research that identified all the enzymes and the associated genes that encode those enzymes in the *Cannabis sativa* plant, then using molecular tools to install them in the genome of the host microorganism. Because these enzymes operate in the same way in the host microorganism as in the plant, the resulting cannabinoids from both systems are identical. In contrast to plants that can make their own sugar using photosynthesis, the newly created microorganism needs to be fed sugar to produce the cannabinoids. This process is done in large steel fermentation vessels, similar to those used in beer production[8]. The cannabinoids are then separated from the microorganism and purified. The life cycle of microorganisms in fermenters (7-10 days) is far shorter than that of the cannabis plant (90-200 days), enabling dramatically increased yield while using a fraction of the material inputs. This increased capacity greatly reduces the relative cost and environmental impact as compared to traditional crop production. The

[8] Beer is produced using the microorganism *Saccharomyces cerevisiae* (yeast) to produce alcohol from sugar.

microorganism factory offers unparalleled production efficiency through the following channels:

- Increased production of cannabinoids per area of land.
- More consistent production rates.
- Greater production reliability without risk of crop-loss due to environment changes, disease, predation, pathogen contamination, and chemical contamination.
- Creation of major, minor and ultra-rare cannabinoids through an equivalent process.
- High purity cannabinoid isolates that are devoid of unwanted cannabinoids, chemicals and microbiological contamination.
- Significant reduction of the cost to produce pure cannabinoid isolate.

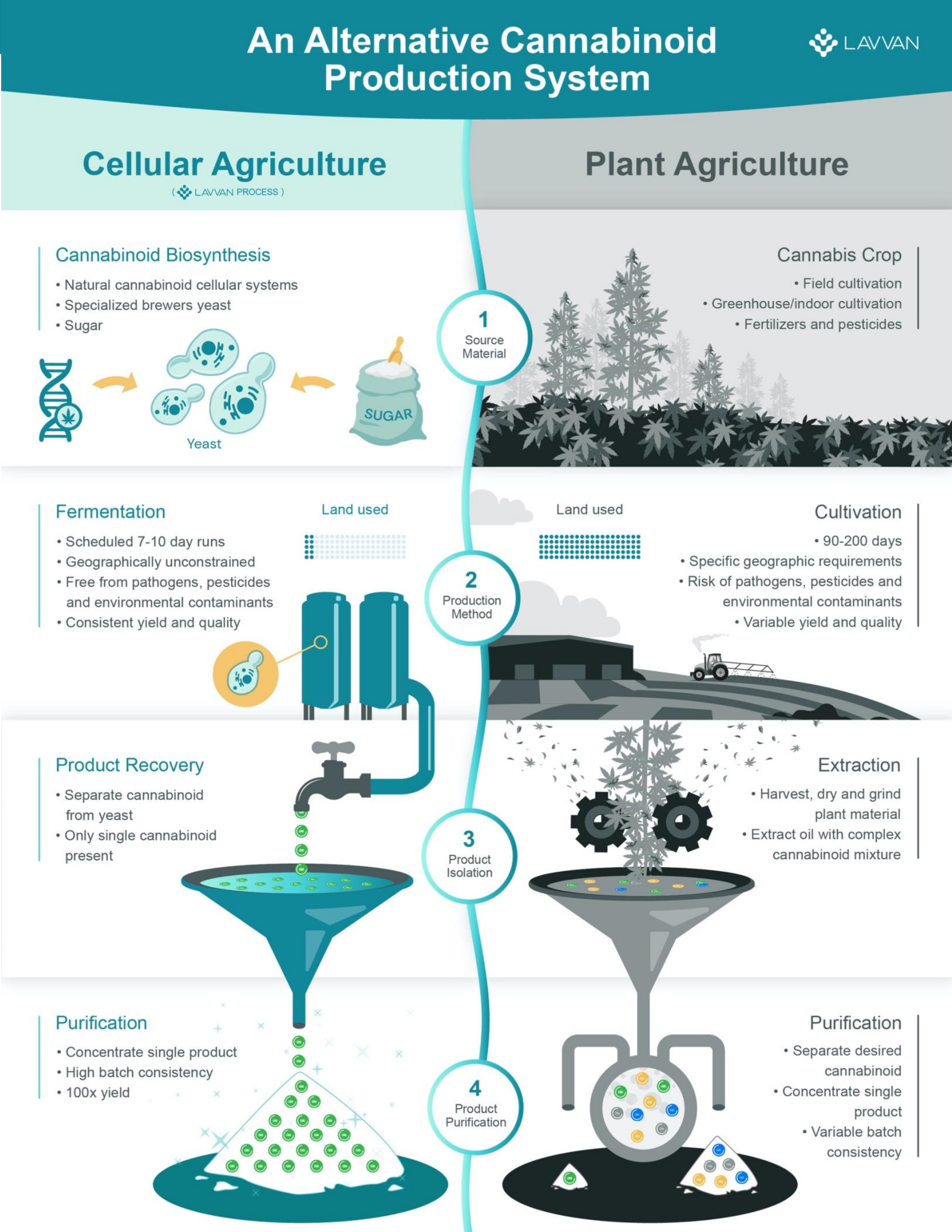
Designation of Cannabinoids Derived Through Biosynthesis

The global jurisdictional regulation of cannabis and cannabinoids is continuously evolving. The biosynthesis of cannabinoids from microorganisms can produce both major, minor, rare, as well uniquely modified, cannabinoids. Depending on the jurisdiction in which a producer is operating, some or all of these cannabinoids may or may not be considered controlled substances. As the final cannabinoid molecules from both production processes are identical, regulations are applicable across the board. If a jurisdiction considers THC a controlled substance, generally, regulation will be enforced regardless of the production source. When it comes to providing authorization to produce the cannabinoid, some jurisdictions rely on definitions around parameters of the source material used. In the United States, licensed hemp cultivators are authorized to cultivate cannabis plants with less than 0.3% THC[9]. This currently leaves the process of biosynthesis in an unregulated space – but an issue where policy resolutions are actively being discussed. However, in Canada and parts of the European Union, cannabinoid regulations have already established equivalent definitions and regulatory approval for plant cultivation and cellular agriculture.

About LAVVAN

At the forefront of cannabinoid cellular agriculture, LAVVAN utilizes yeast fermentation technology to produce high-quality, reliably sourced, natural cannabinoid ingredients. LAVVAN provides cannabinoids with unparalleled purity, consistency, potency and sustainability at a scale capable of serving a range of industries including health, beauty, food and beverage, and pharmaceuticals. LAVVAN's cannabinoids are identical to those found in nature and are produced in a cGMP facility in accordance with the most stringent standards including being devoid of pesticides, mold, bacteria, and other contaminants often found in traditional cannabis agriculture. In addition to providing high purity cannabinoid ingredients, LAVVAN leverages its cannabinoid formulation expertise to support its industry partners in integrating cannabinoids into various end products and distinct applications.

9 FDA Regulation of Cannabis and Cannabis-Derived Products, Including Cannabidiol (CBD) (https://www.fda.gov/news-events/public-health-focus/fda-regulation-cannabis-and-cannabis-derived-products-including-cannabidiol-cbd)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LAVVAN
Cellular Agriculture
(LAVVAN PROCESS)
Plant Agriculture
Cannabinoid Biosynthesis
• Natural cannabinoid cellular systems
• Specialized brewers yeast
• Sugar
Yeast
SUGAR
1
Source Material
Cannabis Crop
• Field cultivation
• Greenhouse/indoor cultivation
• Fertilizers and pesticides
Fermentation
• Scheduled 7-10 day runs
• Geographically unconstrained
• Free from pathogens, pesticides and environmental contaminants
• Consistent yield and quality
Land used
2
Production Method
Land used
Cultivation
• 90-200 days
• Specific geographic requirements
• Risk of pathogens, pesticides and environmental contaminants
• Variable yield and quality
Product Recovery
• Separate cannabinoid from yeast
• Only single cannabinoid present
3
Product Isolation
Extraction
• Harvest, dry and grind plant material
• Extract oil with complex cannabinoid mixture
Purification
• Concentrate single product
• High batch consistency
• 100x yield
4
Product Purification
Purification
• Separate desired cannabinoid
• Concentrate single product
• Variable batch consistency