WHITE PAPER

Revolutionizing Small Molecule API Production: Unveiling the impact of Fermentation as a Sustainable Solution

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While large molecules continue to advance in drug development pipelines, small molecule Active Pharmaceutical Ingredients (APIs) still retain their historical dominance among new drug applications (NDAs). However, the last few years have seen the lines blurring between traditional chemical synthesis of small molecule APIs and bio-fermentation of large molecules, as production of small molecule drug substances in recombinant hosts gains ground. This is due to recent technological developments which have led to significant growth in the suitability of fermentation for a myriad of applications. In this white paper, the impact of the evolution of synthetic biology and supporting technologies on modern fermentation practices will be discussed.

Advantages of fermentation

Synthetic biology is nothing new; for many years, it has been used in biopharmaceutical manufacturing. However, rising interest in fermentation stems from scientific advances that have increased its relevance, in addition to numerous advantages over chemical processes. Novel molecular structures can be created, and the flexibility of fermentation allows the use of microorganisms to create part or all of any desired molecule. In some cases, fermentation can be utilized to produce molecules that are not amenable to traditional synthetic organic chemistry. The technique has been used to make a wide variety of compounds, including pharmaceutical intermediates, food ingredients, agricultural products, and much more. Biocatalysts are now used to modify current commercial molecules as well.

In addition to being sustainable, fermentation avoids the handling and disposal of hazardous waste, and as such, this 'green' approach is safer for both manufacturers and the environment. Compared to cell culture,

microbial fermentation tends to be more facile and scalable as well. The raw materials are also inexpensive and widely available, while facilities are usually less costly to install.

Fermentation involves two classes of strains: natural classic producer strains (non-GMO) and engineered host strains (GMO; Genetically Modified Organisms). There are numerous microorganisms that are now widely used in biotechnology processes, crossing a variety of strains. A non-comprehensive list of examples includes:

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- *Streptomyces sp.* (the source of a majority of natural-origin antibiotics and immunosuppressants).
- *Escherichia coli* (GMO strains for the production of bioplastics, vitamins, large molecules, etc.).
- *Corynebacterium sp.* (for the production of amino acids).
- *Mycobacterium sp.* (best known for its pathogenic species which cause tuberculosis or leprosy, but some non-pathogenic species are useful for the bioconversion of phytosterols into steroids).
- *Paracoccus sp.* (bacteria known for its nitrate-reducing capabilities; some species produce carotenoids).

- *Lactobacillus sp*. (for the production of probiotics, yogurt, cheese, and milk derivatives).
- *Pseudomonas sp.* (for the production of oncology intermediates or bioremediation).
- *Bacillus sp.* (for agriculture applications or the production of enzymes).

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- *Aspergillus sp.* (a mold genus widely used in food and industrial applications).
- *Penicillium sp*. (a source of drugs such as penicillin and antifungal drugs such as griseofulvin).
- *Acremonium sp*. (a source of cephalosporin antibiotics).
- *Saccharomyces cerevisiae* (GMO strains for the production of antibiotics, vitamins, cannabinoids, large molecules, etc.).
- *Pichia pastoris* (GMO strains for the production of large molecules, etc.).
- *Tolypocladium inflatum* (a source of the immunosuppressant cyclosporine).
- *Claviceps purpurea* (a source of ergot alkaloids used to treat pain).
- *Blakeslea trispora* (used in the natural production of carotenoids, including beta-carotene and lycopene).

The advantages of fermentation over traditional processes have been bolstered by technological progress, which is the primary driving force behind this sustainable science's surge in applicability.

Technological advances

The latest developments in fermentation fall into two main categories: the use of synthetic biology and advances in analytical instrumentation. The evolution of improved tools (such as expression systems) and knowledge have led to increased yields and lower costs. Producer strains can now be formulated with better understanding and effectiveness, which enables new processes to be developed. Fine-tuning of the production is possible with synthetic biology, including directed evolution of biosynthetic pathways, which offers a multitude of advantages. The resulting higher yields, fewer impurities, abbreviated timeline, new molecules, and safer processes represent great progress in biosynthesis. Steroids, analgesics, opioids, cannabinoids, and anti-cancer drugs have clearly benefited from these advancements.

Innovations in analytical technology that support structure elucidation, high-throughput screening, and process control have had a profound impact on discovery, development, and manufacturing. Mass spectrometry (MS) and nuclear magnetic resonance (NMR) provide a clear picture of the process on a molecular level. This information facilitates prudent process design for improved outcomes. In addition, high-throughput platforms for the rapid screening of molecules can accelerate discovery and process development efforts. For process monitoring and control, analytical improvements provide more stable and efficient fermentation processes, which can now be monitored in detail, in real-time. This facilitates automated controls based on more than just the usual temperature, pH, and other critical process conditions; biosensors can also provide measurements of parameters such as cell mass, substrate, and metabolite concentration to a feedback loop.

Computational advances have furthered the research and development of biosynthetic processes as well. Bioinformatics, molecular modeling, and artificial intelligence (AI) are indispensable for '-omics' analyses, including genomics, metabolomics, and transcriptomics. They provide critical information regarding modifications of a microorganism's genome to support the tailored construction of an improved strain.

The future of fermentation

The future of biopharmaceutical fermentation is bright. Rapid advances in molecular biology and engineering tools will deliver far-reaching impacts over the next several years. Continued exponential growth in molecular biology techniques will greatly improve not only bio/pharmaceuticals, but also food, agriculture, enzymes for biocatalysis, and more.

Competitive fermentation processes will allow the design of microorganisms producing different families of therapeutic molecules which are currently obtained from animal or vegetal sources. This will decrease costs, improve quality, and increase their worldwide availability.

The longer-term outlook predicts the use of microorganisms to produce high-demand products such as nutritional compounds, new antimicrobials, biomaterials and much more, using sustainable technologies that offer significant benefits over traditional chemical synthesis.

Curia as a fermentation partner

Curia's unique combination of expertise and state-of-the-art facilities delivers a distinct advantage to our partners. The experts at Curia provide customized solutions from development to manufacturing with a proven track record of quality and regulatory compliance.

CASE STUDIES

There is a multitude of projects that highlight Curia's distinguished capabilities. For example, our experts applied their proficiency in synthetic biology to replace the traditional extraction of cannabinoids from hemp, which yields an impure mixture. In this application, the genetic modification of *Saccharomyces cerevisiae* was utilized to produce a single, pure cannabinoid which was manufactured at gram to kg levels. Another project involved developing and scaling up a robust process to make antibiotics from actinomycetes with a customer who had performed the discovery work with a grant from NIH. Curia has also generated tons of steroids via the bioconversion of phytosterols from oil or pine. Various bacteria and fungi were screened for their ability to modify the phytosterol molecule, which is the backbone for several kinds of steroids. The chosen microorganisms were then used to make steroid intermediates which were purified before their use in the chemical synthesis of the desired products, such as dexamethasone (proved efficient during Covid pandemic, saving thousands of lives), hydrocortisone, testosterone, etc. Many more case studies can be provided to demonstrate the rapid delivery of working quantities of novel natural products, accelerated product delivery, substantially improved robustness, cost-advantaged processes, and new IP.

As an integral part of our varied services, Curia has significant experience developing processes for efficient and scalable isolation and purification of diverse natural products, secondary metabolites, and other trace chemicals from complex biological mixtures. These 'Source Mixtures' include a diverse set of fermentation broths generated from a broad array of microbial strains. Curia has multiple chemists and process engineers who have performed hundreds of isolations of natural products at practical scales, as well as other secondary metabolites over the past twenty years. They understand the unique characteristics of biological source extracts, including efficient strategies to address biopolymers and other impurities of biological origin that impact effective isolations of natural product compounds.

The differentiating capabilities of Curia in the fermentation field include both upstream process (USP) and downstream process (DSP) expertise. Our primary USP technologies include cell bank cGMP construction and storage, strain improvement and screening, fermentation development (bacteria, yeast, fungi) and scale-up, bioconversions, synthetic biology, gene cloning and expression, and microbiology. For DSP, we are adept at process development and scale-up, including filtration, centrifugation, TFF (microfiltration/ultrafiltration/nanofiltration), purification with resins (adsorption/ionic), chromatographic purification (2-500 L scale) and crystallization.

Curia leads the industry with our ability to convert part of a chemical process to a microbial methodology, then integrate the biotechnology and chemical synthesis to make one, complete process. With our wide range of fermentation capacities (from sub-mL to 50,000 L), going from lab scale to pilot and industrial scale is yet another of our specialties. With large scale fermentation processes, it can be difficult to keep process variables and mixing under precise control. However, Curia's experts design and optimize processes at the benchtop with the challenges of scale up in mind, so that increasing the scale goes smoothly and accurately.

Curia's experts are highly skilled in working with natural classic producer strains (non-GMO) and engineered strains (GMO; mainly *E. coli, S. cerevisiae* and *Pichia pastoris*). GMO can be used to produce food ingredients, APIs and their intermediates, agricultural products, and much more. Finally, recombinant protein and enzyme expression and purification is one of Curia's more experienced disciplines, including strain construction, fermentation development and manufacturing.

For Industrial Fermentation and Biocatalysis, Curia has developed processes to create:

- APIs for the pharmaceutical field, including antibacterials (mainly produced by *Streptomyces* species), immunosuppressants (from *Streptomyces* and fungi), and enzymes (mainly produced by *E. coli* and *Pichia*).
- Food ingredients, including carotenoids produced by fungi and bacteria, and enzymes (mainly produced by *E. coli* and *Pichia*).
- Microorganisms for agriculture, mainly bacteria.

The table below illustrates Curia's breadth of expertise in industrial fermentations.

EXPERTISE ON INDUSTRIAL FERMENTATION

Food ingredients	Microorganism
Beta-carotene	*Blakeslea trispora*
Lycopene	*Blakeslea trispora*
Astaxanthin	*Phaffia rhodozyma*
Zeaxanthin	*Paracoccus carotinifaciens*
Canthaxanthin	*Paracoccus carotinifaciens*
Phytoene	*Paracoccus carotinifaciens*
Chymosin	*Pichia pastoris*
Renin	*Mucor miehei*
Beta-galactosidase	*Escherichia coli*
Enzymes	*Escherichia coli*

APIs	Microorganism
Penicillin	*Penicillium chrysogenum*
Cephalosporin C	*Acremonium chrysogenum*
Clavulanic acid	*Streptomyces clavuligerus*
Penicillin acylase	*Escherichia coli*
Pneumocandin	*Glarea lozoyensis*
Safranin B	*Pseudomonas sp.*
Pimaricin	*Streptomyces natalensis*
Tacrolimus	*Streptomyces sp.*
Sirolimus	*Streptomyces hygroscopicus*
Ascomycin	*Streptomyces hygroscopicus*
Steroids	*Mycobacterium sp.*
Daptomycin	*Streptomyces roseosporus*
Pristinamycin	*Streptomyces pristinaespiralis*

Benefit from modern facilities and exceptional skills

Curia has proven successful in rapidly scaling and improving effective fermentation processes for a wide range of strains (bacteria, fungi, yeasts) and numerous compounds (steroids, antibiotics, cannabinoids, oncology intermediates, microorganisms for agriculture, enzymes, etc.). Fermentation scale-up and process development efforts target very aggressive pharmaceutical research timeframes and/or other priority criteria most relevant to a given client and project goals.

Diverse and extensive expertise in fermentation optimization and scale-up, process chemistry, as well as natural product biosynthesis, allows Curia to reliably deliver new natural products and follow-on support. The comprehensive integration of proven expertise, technology, and support resources for fermentation development, scale-up, semisynthetic chemistry and downstream processing includes:

- Proven experience and resources for scaling fermentations from sub-milliliter screening systems to small fermenters (5-L, 20-L, 40-L, 600-L) and scale-up to a range of production fermenters (from 5 m^3 to 50 m^3).
- Deep experience, with broad variety of common engineered hosts (including *E. coli, Pichia, Saccharomyces,* etc.) as well as native producers, including marine and terrestrial actinomycetes, fungi, eubacteria, etc.
- Extensive analytical monitoring instrumentation on all fermenters. 24-hour fermentation monitoring and control with versatile feedback control options using state-of-the-art control systems.
- High throughput analytical systems (HPLC, UPLC, LC-MS/MS) for rapid and detailed process evaluation.
- Extensive practical experience using statistical Design of Experiments (DoE) for detailed and comprehensive evaluation of physiology and process variables.
- Extensive collection of >190,000 strains for screening alternative producers.
- Full process, integrating fermentation and chemical synthesis.
- Strong collaborative relationship with Curia's world-class chemistry development, natural products chemistry, analytical, QC, and biochemistry teams.
- Extensive experience in developing processes from liter scale to 50,000-L scale and building efficient working relationships with manufacturing partners.
- Highly successful technology transfer procedures.

Curia's partners around the world have benefitted from our strength in developing and scaling up fermentation processes. The flexible business model makes Curia easy to work with, as projects are customized to meet a client's needs in any stage of their project.

Contact Curia to discuss your **Discovery, Development, Analytical Services, API Manufacturing**, or **Drug Product needs.**

ABOUT CURIA

Curia is a Contract Development and Manufacturing Organization with over 30 years of experience, an integrated network of 29 global sites and over 3,500 employees partnering with customers to make treatments broadly accessible to patients. Our biologics and small molecule offering spans discovery through commercialization, with integrated regulatory and analytical capabilities. Our scientific and process experts and state-of-the-art facilities deliver best-in-class experience across drug substance and drug product manufacturing. From curiosity to cure, we deliver every step to accelerate and sustain life-changing therapeutics.

Learn more at curiaglobal.com

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