Review of Microbial Bioprocessing: Bioprospecting of microbial strains for development of products

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The review presents a full study and analysis of the microbial bioprocessing workflow-from microbial strain selection and development to fermentation, downstream processing, and product recovery. It highlights the extensive application of high throughput screenings, microbioreactor-platforms, and other process-intensification strategies to even out those great disparities that exist between research at the laboratory scale and commercial-scale manufacturing. The same respect is given to automated and data-driven control and media optimization, which further sets forth scalable, reproducible, and efficient production systems. Along these lines, the review aims at providing an integrative view of the present situation and future prospect of microbial bioprocessing through the analysis of concepts derived from modern research and industrial practices. It involves a discussion of technological innovations, practical considerations, and emerging trends that are redefining the role of microbes as sustainable biofactories for the vast assortment of industrial applications, including pharmaceutical, food and beverage, agriculture, cosmetics, and renewable energy.

Keywords: Microbial products · Strain Improvement · Downstream Processing · Product recovery

Introduction

Recent years have seen a tremendous advancement in microbial bioprocessing, especially with the combination of synthetic biology and systems metabolic engineering (Li et al., 2020). With the use of these techniques, it is possible to precisely alter the metabolic pathways of microbes like *Corynebacterium glutamicum* and *Escherichia coli*, increasing the yields of valuable products like chemicals, biofuels, and medications (Li et al., 2020). Microbial strain engineering has undergone a revolution because to the application of CRISPR/Cas9 technology, which enables precise genome editing to increase the tolerance and productivity of industrial strains (Zhang et al., 2021). Furthermore, bioprocess design is currently being aided by machine learning (ML) and artificial intelligence (AI), which speed up metabolic pathway prediction and optimization and lower the time and expense associated with developing new microbial strains (Lee et al., 2022).

Saccharomyces cerevisiae was designed to synthesize the precursor, enabling scalable manufacture of the antimalarial medication artemisinin, marking a notable milestone in microbial bioprocessing (Jiang and Pfeifer, 2021). The use of non-food biomass and the integration of circular bioeconomy concepts have emerged as key areas of focus for sustainability in bioprocessing, with the goal of minimizing environmental effect (Guo et al., 2021). Moreover, *Corynebacterium glutamicum* metabolic engineering has been used to produce amino acids on a large scale, which has led to more environmentally friendly industrial processes (Kind et al., 2020). The engineering of Escherichia coli to produce 1,4-butanediol, a substance usually obtained from petroleum-based sources, is another significant achievement (Yim et al., 2020). All of these developments are opening the door for the biotechnology sector to develop bioprocesses that are more productive, economical, and ecologically friendly (Guo et al., 2021; Yim et al., 2020).

From strain development to product development, the whole microbial bioprocessing pathway is covered in this overview. In order to increase productivity and robustness—two essential components for effective industrial processes—it starts with the genetic modification and selective breeding of microbial strains (Parekh, Vinci and Strobel, 2000). Bioprocess optimization is then covered in the review, with special attention to developments in high-throughput screening, microbioreactor systems, and miniaturized bioreactors for increasing strain productivity, as well as fermentation technologies including batch, fed-batch, and continuous cultures (Zeng et al., 2020; Neubauer et al., 2013; Hemmerich et al., 2018). In order to guarantee consistency and efficiency, it also discusses scaling up from laboratory to industrial production, with an emphasis on process parameter control and cutting-edge technologies like automated systems and data-driven modeling (Lim and Shin, 2013; Schäpper et al., 2009; Hegab, Elmekawy and Stakenborg, 2013).

Microbial bioprocessing is essential in both research and industry in order to produce useful products like enzymes, biofuels, and medicines, (Neubauer et al., 2013). Large-scale production efficiency is ensured by developing bioprocesses from microliter cultures to industrial sizes (Neubauer et al., 2013). Developments in fermentation techniques improve microbial strains, resulting in higher production and yield (Parekh, Vinci, and Strobel, 2000).

By quickly analyzing strains and maximizing output, high-throughput screening technology has transformed industrial biotechnology (Zeng et al., 2020). Technologies for high-throughput cultivation aid in bridging the gap between laboratory and industrial scales (Long et al., 2014). Fed-batch cultures increase yields by providing perfect nutrition management (Lim and Shin, 2013). By smaller-scale industrial settings being replicated, microbioreactors speed up the development of bioprocesses (Hemmerich et al., 2018). Recent advances in microfluidic microbioreactor technology improve control and automation, enhancing scalability (Hegab, Elmekawy and Stakenborg, 2013).

Microbial Strain Improvement

Genetic engineering techniques with CRISPR Cas 9

The generation of biofuels and other useful metabolites has been revolutionized by microbial strain enhancement through genetic engineering, specifically using CRISPR-Cas9 (Zhang et al., 2018). CRISPR enables precise alterations that can optimize metabolic pathways and improve microbial efficiency in the production of biofuels like ethanol and butanol by targeting particular genes (Dexter and Fu, 2009). For example, endogenous CRISPR-Cas systems have been used to design *Clostridium ljungdahlii* to generate butanol, resulting in yields that are noticeably greater than those obtained using conventional approaches (Köpke et al., 2010). Furthermore, *Escherichia coli* can now produce more fatty acids thanks to this technique, making it a strong microbial platform for the generation of biodiesel (Kim et al., 2019). By using modified strains of *Yarrowia lipolytica*, metabolic pathways have been optimized for the synthesis of valuable bioproducts such isoprenoids as well as for the generation of biofuel (Jia et al., 2019). Furthermore, multiplex editing of microbial genomes has been made easier by CRISPR-Cas9, allowing for simultaneous alterations across several genes. This has sped up strain growth and increased overall productivity (Cho et al., 2019). These developments show the promise of CRISPR-Cas9 in microbial biotechnology, opening the door to more effective and sustainable production methods (Zhang et al., 2018).

Selection and screening of high-yield strains

Selection and screening of high-yield strains in microbial bioprocessing are essential for optimizing production efficiency in various industrial applications. High-throughput screening techniques, coupled with systems metabolic engineering, allow for the identification of microbial strains that exhibit superior production of desired compounds such as biofuels, amino acids, or pharmaceuticals (Lee and Kim, 2015). Researchers can engineer microorganisms such as *Corynebacterium glutamicum* and *Escherichia coli* to increase the production of valuable biochemicals like L-arginine, L-lysine, and 1,4-butanediol by using computational tools for pathway design and synthetic biology approaches (Kind et al., 2014; Park et al., 2014). Moreover, to increase strain tolerance to industrial circumstances such high substrate concentration or temperature, directed evolution and adaptive laboratory evolution are used (Cobb et al., 2015). Novel approaches using CRISPR-Cas9 have also advanced the selection and screening process by enabling precise and multiplex genome editing, leading to faster strain development (Tong et al., 2015). These techniques make microbial bioprocessing more economical and sustainable by enabling high-yield production while also guaranteeing the strain's resilience in industrial settings (Lee and Lee, 2005; Becker et al., 2011).

Table 1 Overview of microbial co-culture systems and their applications in bioprocessing, illustrating the strains, bioprocess, product, and co-culture partners

Strain	Bioprocess	Product	Co-culture partners
Escherichia coli	N-butanol production	N-butanol	*E. coli* glucose- and xylose-selective strains
Escherichia coli	Rosmarinic acid (RA) production	Rosmarinic acid	*E. coli* upstream and downstream strains
Escherichia coli	Muconic acid production	Muconic acid	Two *E. coli* strains
Escherichia coli Saccharomyces cerevisiae	Flavonoid (taxanes) production	Taxanes	*Escherichia coli Saccharomyces cerevisiae*
Clostridium acetobutylicum Bacillus cereus	Butanol production	Butanol	*Clostridium acetobutylicum Bacillus cereus*
Aspergillus niger Trichoderma reesei	Cellulose degradation and enzyme production	Cellulolytic enzymes	*Aspergillus niger Trichoderma reesei*
Synechococcus elongatus Escherichia coli	3-hydroxy propionic acid (3-HP) production	3-hydroxy propionic acid	*Synechococcus elongatus Escherichia coli*

Bioreactor Design and Operation

Types of bioreactors

Various types of bioreactors are used, including batch, fed-batch, and continuous systems, each offering specific advantages for different microbial processes. Fed-batch bioreactors, for example, allow controlled nutrient addition, which is crucial for maintaining optimal microbial growth conditions and improving the production of desired metabolites (Keil et al., 2019; Teworte et al., 2022). Microbioreactor systems and high-throughput screening technologies have made it possible to perform scalable fermentations and real-time process monitoring at the microscale, which has greatly accelerated the development of bioprocesses (Grünberger et al., 2019; Keil et al., 2020). (Jian et al., 2020; Wang et al., 2024). The integration of advanced process control and online monitoring with microbioreactors has bridged the gap between small-scale experimental setups and industrial-scale production, increasing the reliability and reproducibility of high-throughput screening (Keil et al., 2019).

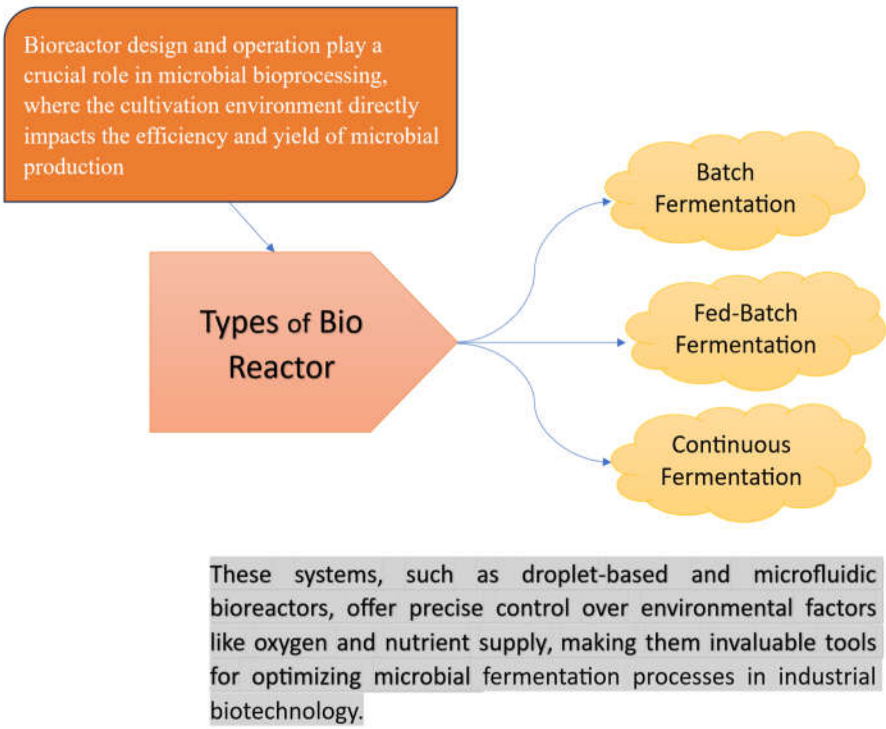

Figure 1: Types of bioreactors and their significance in microbial bioprocessing. This diagram highlights the importance of bioreactor design and operation in microbial bioprocessing, emphasizing the three main types: Batch Fermentation, Fed-Batch Fermentation, and Continuous Fermentation. Additionally, it mentions advanced systems like droplet-based and microfluidic bioreactors for optimizing microbial fermentation processes.

Scale up and optimization

Scale-up and optimization in microbial bioprocessing are essential to ensure that laboratory-scale processes maintain efficiency and quality when expanded to industrial scales. Maintaining appropriate oxygen transfer, nutrient distribution, and mixing while avoiding microbial stress from shear forces in bigger bioreactors are important difficulties during scale-up (Grünberger et al., 2022). The use of fed-batch and continuous bioreactors, which provide improved control over the input of nutrients and the accumulation of waste, is one way to address this problem (Teworte et al., 2022). When it comes to bioprocess optimization, computational models and real-time monitoring systems have grown in significance. They aid in the prediction and adjustment of crucial parameters like pH, temperature, and dissolved oxygen during scale-up (Jian et al., 2020; Wang et al., 2024). Furthermore, more efficient strain selection and process optimization at lower scales are made possible by sophisticated microscale bioreactors and high-throughput screening technologies, which also provide important data that support successful industrial applications (Grünberger et al., 2022; Funke et al., 2020). The industries can improve product production and consistency while cutting down on the time and expense needed for scale-up by incorporating these solutions (Keil et al., 2020; Bower et al., 2023).

Media Optimization

Media optimization plays a key role in maximizing the yield, efficiency, and cost-effective microbial growth and product formation. It involves refining the nutritional components of the culture medium to promote optimal microbial activity, ensuring the best possible outcomes in the production of biotechnological products, such as biofuels, pharmaceuticals, enzymes, and biofertilizers.

Nutrient Requirements

Since nutrients have a direct impact on microbial growth, metabolism, and total product production, they are an essential component in microbial bioprocessing. The three main nutrients—carbon, nitrogen, and trace elements—need to be carefully controlled in order to maximize microbial activity. Maintaining a high carbon-to-nitrogen ratio, for example, has been demonstrated to improve the formation of polysaccharides, and trace elements such as potassium, phosphorus, and calcium, depending on their amounts, can have a major impact on the process (Papagianni, 2017). Furthermore, when coupled with proper oxygen uptake and carbon source management, organic acids like pyruvic and succinic acids can promote the synthesis of xanthan gum (Papagianni, 2017; Sauer and Mattanovich, 2017). Additionally, some microbes—like Aspergillus niger, which is used to produce citric acid—are extremely sensitive to metal ion concentrations, necessitating tight control over trace elements in order to prevent product synthesis from being inhibited (Dhillon et al., 2020). Advances in genetic engineering and metabolic optimization have also allowed for better nutrient utilization in bioprocesses, improving yield and reducing production costs (Nikolaou et al., 2017; Sauer and Mattanovich, 2017). The ability to finely tune nutrient availability is thus essential for optimizing microbial growth and ensuring the scalability of industrial bioprocesses (Kubicek, 2018).

Table 2: Overview of various carbon and nitrogen sources used in microbial bioprocessing, including their roles and example microorganisms

Nutrient Type	Common Sources	Role in Microbial Bioprocessing	Example Microorganisms
Carbon	Glucose, sucrose, starch, hydrolysed starch	Energy source and cellular building blocks	*Saccharomyces cerevisiae*, *Escherchia coli*
Nitrogen	Ammonium chloride, casein, soybean hydrolysate	Protein synthesis and growth regulation	*Xanthomonas campestris*, *Corynebacterium glutamicum*
Carbon	Molasses, glycerol, ethanol, lactose	Alternative carbon sources for specific microbial processes	*Bacillus subtilis*, *Pseudomonas aeruginosa*
Nitrogen	Urea, ammonium sulfate, yeast extract	Supports growth and metabolite production	*Aspergillus niger*, *Clostridium acetobutylicum*

Cost-effective media formulations

Cost-effective media formulations are essential in microbial bioprocessing to reduce overall production costs, particularly when scaling up from laboratory to industrial levels (Kind et al., 2014; Lee et al., 2011). These formulations typically focus on using inexpensive, readily available carbon sources, and chemically defined minimal media to ensure reproducibility and ease of metabolic analysis while maintaining high yields of the target product (Zhuang and Herrgård, 2015; . et al., 2012). For bulk chemical production, the use of low-cost substrates, such as agricultural by-products or industrial waste, is often preferred (Croughan et al., 2015; Van Dien, 2013). Additionally, the minimization of byproducts during fermentation and the optimization of microbial growth conditions, such as pH and feeding strategies, contribute to enhancing cost-effectiveness (Paddon and Keasling, 2014; Choi et al., 2013). High cell density cultures and fed-batch processes are commonly employed to increase biomass and product concentrations, further improving the economic viability of microbial bioprocesses (Lee, 1996; Yadav et al., 2012).

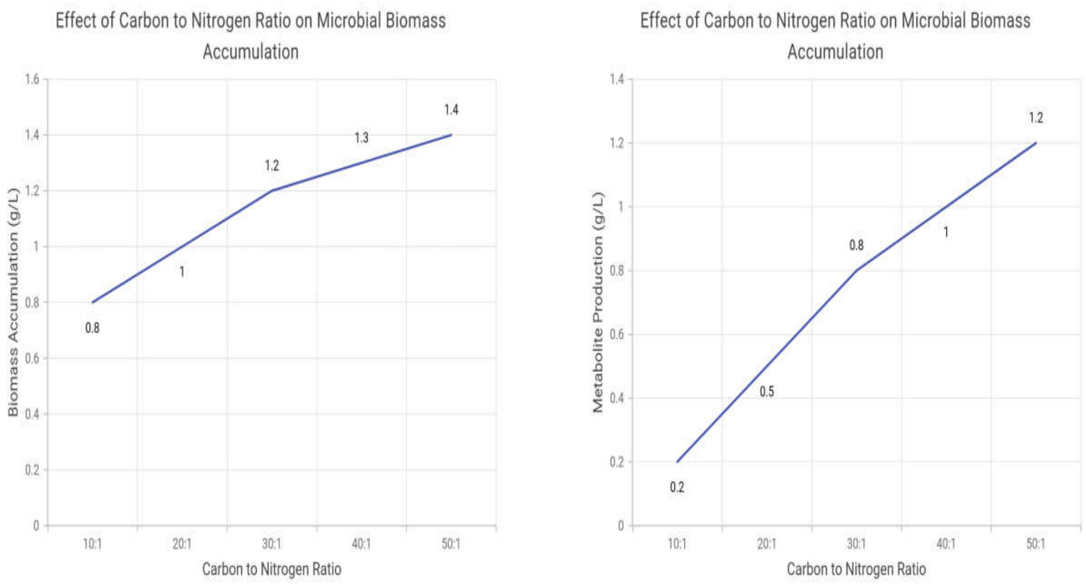

Figure 2: Effect of carbon to nitrogen ratio on microbial biomass accumulation and metabolite production: The graphs show a positive correlation between increasing carbon to nitrogen ratios and both microbial biomass accumulation and metabolite production

Fermentation Processes

Fermentation processes are fundamental in microbial bioprocessing, with batch, fed-batch, and continuous fermentations being the primary methods employed for product synthesis at different scales (Kim et al., 2017). Batch fermentation is widely used but often limited by nutrient depletion, which can lead to a decline in microbial productivity over time (Mans et al., 2018). To address this, fed-batch fermentation has become a prevalent choice in industrial applications, as it allows for the controlled addition of nutrients to maintain high productivity and prevent by-product formation (Keil et al., 2019). Continuous fermentation systems, such as chemostats, are ideal for achieving steady-state conditions, making them suitable for large-scale production (Grünberger et al., 2019). Recent advances in microbioreactor technologies, including automated and high-throughput platforms, have enhanced the ability to optimize fermentation conditions. These platforms enable precise control over parameters such as nutrient supply, pH, and dissolved oxygen, thereby improving the scalability and efficiency of fermentation processes (Totlani et al., 2023). Additionally, microfluidic and droplet-based microbioreactors offer promising solutions for nutrient-controlled microbial cultures, providing dynamic environments that closely mimic industrial-scale conditions (Kim et al., 2017; Bower et al., 2017).

Downstream Processing

Downstream processing in microbial bioprocessing is a pivotal step that affects both the yield and purity of the final product. It encompasses various stages, including separation, purification, and concentration of bioproducts, which are critical for ensuring the economic feasibility of the overall process (Nikolaou et al., 2010). Factors such as the rheology of the fermentation broth, product concentration, and the presence of contaminants greatly influence the selection of downstream methods like filtration, centrifugation, and chromatography (Sauer and Mattanovich, 2012; Totlani et al., 2023). With advancements in bioprocessing, membrane technologies, adsorptive techniques, and crystallization have become integral in improving product recovery and purity (Dhillon et al., 2019). Moreover, the rise of biobased chemical production, such as 1,3-propanediol, has emphasized the need for energy-efficient downstream processes, given that downstream costs can account for up to 50% of total production expenses (Banner et al., 2020). Genetic engineering innovations have led to microbial strains that minimize inhibitory byproducts, simplifying downstream purification (Gómez-Pastor et al., 2018; Kim et al., 2021). Additionally, integrated bioprocessing approaches, where upstream and downstream processes are combined, have been explored to enhance efficiency and reduce costs (Brar et al., 2021). These developments contribute to making microbial bioprocessing more sustainable and economically viable in industrial settings.

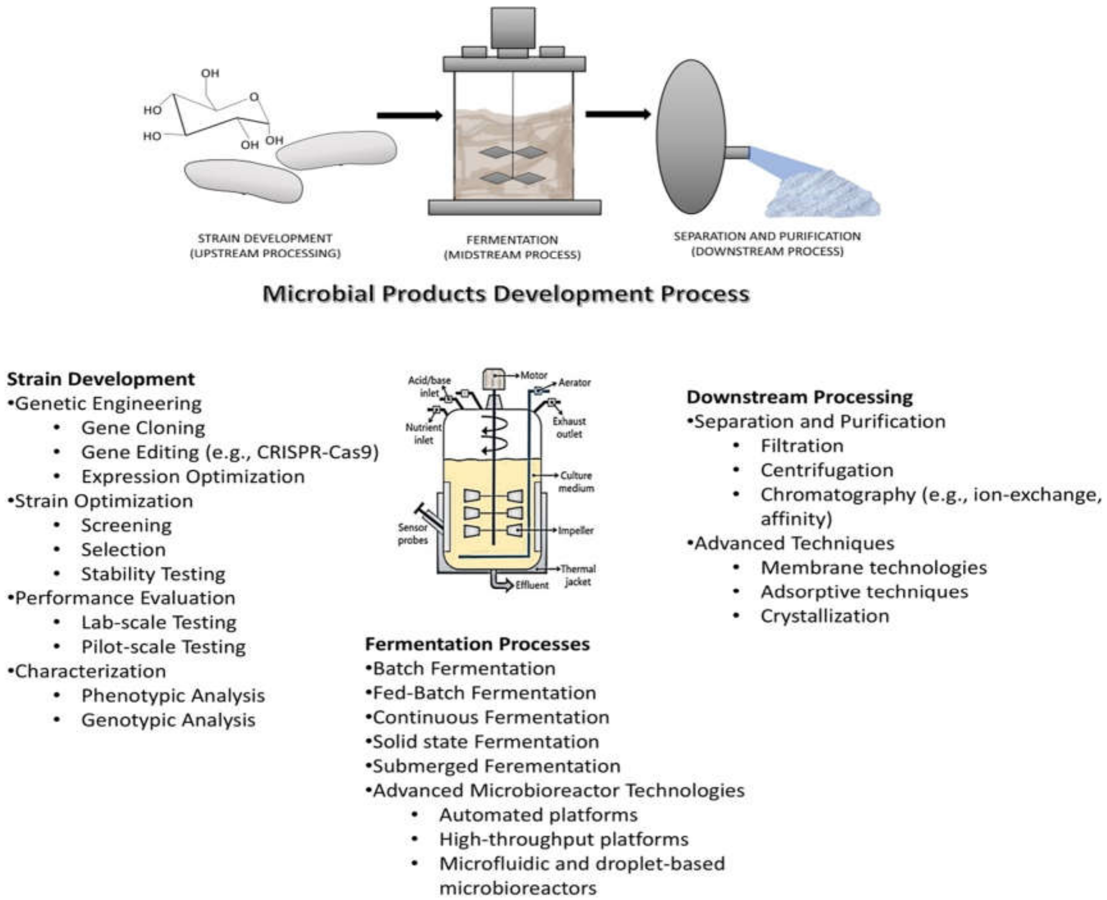

Figure 3 Microbial products development process illustrating strain development, fermentation processes, and downstream processing techniques

Product Recovery and Purification

Product recovery and purification in microbial bioprocessing involve techniques like chromatography and filtration to isolate desired biomolecules. Chromatography, including ion-exchange and affinity types, separates compounds based on molecular characteristics for high-purity outputs. Filtration methods, such as ultrafiltration and microfiltration, remove microbial cells and debris, enhancing product clarity (Toomer, 2020). Final formulation steps like lyophilization or adding stabilizers are crucial for product stability, especially in pharmaceuticals (Sagar et al., 2018). Solid-state fermentation (SSF) is a cost-effective, sustainable method for managing agro-industrial waste and improving product recovery (Kennes, 2018). Emerging downstream techniques, such as aqueous two-phase extraction (ATPE) and advanced filtration, enhance yield and reduce processing time, making microbial bioprocessing more efficient and scalable (Sadh et al., 2018; Thomas et al., 2013; Ghosh et al., 2016).

Applications of Microbial Products

Microbial bioprocessing has gained importance across industries due to its versatile applications. In the food industry, enzymes like amylases, cellulases, and proteases produced via microbial processes are key in breaking down complex biomolecules, enhancing food processing and biofuel production (Kennes, 2018). In the pharmaceutical sector, microbial bioprocessing aids in producing antibiotics, amino acids, and vitamins (Toomer, 2020). Solid-state fermentation (SSF) is particularly effective in generating bioactive compounds, such as phenolic antioxidants and antimicrobial agents, used in food preservation and health supplements (Sadh et al., 2018; Panda et al., 2018). SSF also supports zero-waste utilization by converting agro-industrial residues into valuable products, fostering a circular economy (Sheikha and Ray, 2022). Environmental applications include bioremediation and biofuel production from organic waste, addressing sustainability challenges (Baiano, 2014; Ghosh et al., 2016).

Table 3 Microbial products, microorganisms, and their industrial applications

Microbial Product	Microorganism	Industry	Applications
Amylases	*Aspergillus spp.* *Bacillus spp.*	Food, Biofuel	Breakdown of starch into sugars, enhancing food processing and bioethanol production
Cellulases	*Trichoderma spp.* *Aspergillus spp.*	Food, Biofuel	Degradation of cellulose, aiding in food processing and biomass conversion for biofuel production

Proteases	*Bacillus spp. Aspergillus spp.*	Food	Protein hydrolysis in food processing for enhanced texture and flavor
Antibiotics	*Penicillium spp. Streptomyces spp.*	Pharmaceutical	Production of antimicrobial agents for treating bacterial infections (e.g., Penicillin)
Amino Acids	*Corynebacterium spp. Escherichia coli*	Pharmaceutical, Food	Used as nutritional supplements and in parenteral nutrition
Vitamins	*Propionibacterium spp.*	Pharmaceutical, Food	Production of vitamins for nutritional fortification and dietary supplements
Phenolic Antioxidants	*Aspergillus spp. Rhizopus spp.*	Food, Health Supplements	Used in food preservation and as nutraceuticals for their health benefits
Bioactive Compounds (from SSF)	*Fungi, Bacteria*	Food, Health	Production of antimicrobial agents and food preservatives through solid-state fermentation

Future Trends in Microbial Bioprocessing

The future of microbial bioprocessing is expected to emphasize sustainability, efficiency, and innovation. A significant trend is the growing use of agro-industrial waste as a substrate for microbial processes, supporting the circular economy by transforming waste products into high-value bioproducts such as enzymes, pigments, and biosurfactants (Astudillo et al., 2023). Solid-state fermentation (SSF) is increasingly recognized for its lower energy and water requirements, making it a more sustainable method for producing bioactive compounds (Ng et al., 2020; Sheikha & Ray, 2022). Advances in bioreactor design and scaling up fermentation processes are crucial for moving microbial bioprocesses from the laboratory to industrial scales, thereby enhancing production efficiency (Crater & Lievense, 2018). Additionally, integrating green extraction techniques and life cycle assessments is vital for ensuring the environmental sustainability of the entire production process (Xu et al., 2022). Microbial bioprocessing also holds significant potential in the bioenergy sector, where agricultural wastes are converted into biofuels, contributing to the global shift towards renewable energy sources (Ferreira et al., 2020; Valdez-Vazquez et al., 2010).

Conclusion

This review has provided a comprehensive exploration of microbial bioprocessing, with particular attention to the latest advancements and innovative methodologies that are driving the field forward. From the initial stages of microbial strain improvement and genetic engineering to large-scale fermentation and downstream processing, each aspect of the bioprocessing workflow has been examined in detail. Emerging technologies such as synthetic biology, CRISPR-based genome editing, and precision fermentation have been highlighted as transformative forces that are enhancing microbial productivity and process efficiency. By delving into the latest trends and synthesizing insights from a wide range of studies, this article offers a thorough understanding of the potential applications of microbial bioprocessing across multiple industries, including pharmaceuticals, agriculture, food, cosmetics, and biofuels. It also identifies future opportunities for innovation and the challenges that must be overcome to fully realize the potential of microbial bioprocessing in sustainable and industrial-scale production.

Reference

1. Anbarasan, P., et al., 2012. Integration of chemical catalysis with extractive fermentation to produce fuels. *Nature*, 491, pp.235–239. doi: 10.1038/nature11500.
2. Astudillo, Á., Rubilar, O., Briceño, G., Diez, M.C. and Schalchli, H., 2023. Advances in agroindustrial waste as a substrate for obtaining eco-friendly microbial products. *Sustainability*, 15(3467), pp.1-15. Available at: https://doi.org/10.3390/su15043467 [Accessed 12 September 2024].
3. Baiano, A., 2014. Recovery of biomolecules from food wastes—a review. *Molecules*, 19(9), pp.14821-14842. Available at: https://doi.org/10.3390/molecules19091482 [Accessed 12 September 2024].
4. Banner, T., Fosmer, A., Jessen, H., Marasco, E., Rush, B., Veldhouse, J. and De Souza, M., 2020. Microbial bioprocesses for industrial-scale chemical production. *Current Developments in Biotechnology and Bioengineering*. doi: 10.1016/B978-0-12-818199-6.00002-4.
5. Becker, J., Zelder, O., Häfner, S., Schröder, H. and Wittmann, C. (2011) 'From zero to hero—design-based systems metabolic engineering of *Corynebacterium glutamicum*

for L-lysine production’, *Metabolic Engineering*, 13, pp. 159–168. Available at: https://doi.org/10.1016/j.ymben.2010.10.001

6. Bower, D.M., Lee, K.S., Ram, R.J. and Prather, K.L.J. (2023) ‘Fed-batch microbioreactor platform for scale down and analysis of a plasmid DNA production process’, *Biotechnology and Bioengineering*, 109(8), pp. 1976–1986. Available at: https://doi.org/10.1002/bit.27778 (Accessed: 11 September 2024).
7. Brar, S.K., Verma, M., Dhillon, G.S. and Tyagi, R.D., 2021. Recent advances in downstream processing of bio-products from industrial wastewater. *Bioresource Technology*, 345, p.125898. doi: 10.1016/j.biortech.2021.125898.
8. Cho, C. et al. (2019) ‘Engineering clostridial aldehyde/alcohol dehydrogenase for selective butanol production’, *MBio*, 10(2), pp. e00067-19. Available at: https://doi.org/10.1128/mBio.00067-19 (Accessed: 11 September 2024).
9. Choi, S., et al., 2013. Production of 4-hydroxybutyric acid by metabolically engineered *Mannheimia succiniciproducens* and its conversion to γ-butyrolactone by acid treatment. *Metabolic Engineering*, 20, pp.73–83. doi: 10.1016/j.ymben.2013.03.003.
10. Cobb, R.E., Wang, Y. and Zhao, H. (2015) ‘High-efficiency multiplex genome editing of *Streptomyces* species using an engineered CRISPR/Cas system’, *ACS Synthetic Biology*, 4, pp. 723–728. Available at: https://doi.org/10.1021/sb500295h
11. Crater, J. and Lievense, J., 2018. Scale-up of industrial microbial processes. *FEMS Microbiology Letters*, 365(fny138). Available at: https://doi.org/10.1093/femsle/fny138 [Accessed 12 September 2024].
12. Croughan, M.S., Konstantinov, K.B. and Cooney, C., 2015. The future of industrial bioprocessing: batch or continuous? *Biotechnology and Bioengineering*, 112, pp.648–651. doi: 10.1002/bit.25673.
13. Dexter, J. and Fu, P. (2009) ‘Metabolic engineering of cyanobacteria for ethanol production’, *Energy and Environmental Science*, 2, pp. 857–864. Available at: https://doi.org/10.1039/b811141a (Accessed: 11 September 2024).
14. Dhillon, G.S., Brar, S.K., Verma, M. and Tyagi, R.D., 2019. Advances in downstream processing for bio-based chemical production. *Biotechnology Advances*, 37(6), p.107408. doi: 10.1016/j.biotechadv.2019.107408.

15. Dhillon, G.S., Brar, S.K., Verma, M. and Tyagi, R.D., 2020. Recent advances in citric acid bio-production and recovery. *Food and Bioprocess Technology*, 4(5), pp.505-529. doi: 10.1007/s11947-020-02667-0.
16. Ferreira, S., Buller, L., Maciel-Silva, F., et al., 2020. Waste management and bioenergy recovery from açaí processing in the Brazilian Amazonian region: A perspective for a circular economy. *Biofuels, Bioproducts and Biorefining*, 15, pp.37–46. Available at: https://doi.org/10.1002/bbb.2127 [Accessed 12 September 2024].
17. Funke, M. et al. (2020) 'Bioprocess control in microscale: Scalable fermentations in disposable and user-friendly microfluidic systems', *Microbial Cell Factories*, 9(1), pp. 1–13. Available at: https://doi.org/10.1186/s12934-020-01448-6 (Accessed: 11 September 2024).
18. Ghosh, P.R., Fawcett, D., Sharma, S.B. and Poinern, G.E.J., 2016. Progress towards sustainable utilisation and management of food wastes in the global economy. *International Journal of Food Science*, 2016(1), pp.1-20. Available at: https://doi.org/10.1155/2016/3563478 [Accessed 12 September 2024].
19. Gómez-Pastor, R., Pérez-Torrado, R., Garre, E. and Matallana, E., 2018. Recent advances in yeast biomass production. *Biotechnology Journal*, 13(4), p.1700141. doi: 10.1002/biot.201700141.
20. Grünberger, A. et al. (2019) 'A microfluidic co-cultivation platform to investigate microbial interactions at defined microenvironments', *Lab on a Chip*, 19(1), pp. 98–110. Available at: https://doi.org/10.1039/C8LC01185H (Accessed: 11 September 2024).
21. Grünberger, A., Paczia, N., Probst, C., Schendzielorz, G., Eggeling, L., Noack, S. and Kohlheyer, D., 2019. A disposable picolitre bioreactor for cultivation and investigation of industrially relevant bacteria on the single cell level. *Lab on A Chip*, 19(1), pp.98–110. doi: 10.1039/C8LC00927B.
22. Grünberger, A., Wiechert, W., Kohlheyer, D. and Noack, S. (2022) 'Advances in microbial single-cell analysis for industrial biotechnology', *Current Opinion in Biotechnology*, 74, pp. 26–33. Available at: https://doi.org/10.1016/j.copbio.2021.11.001 (Accessed: 11 September 2024).
23. Guo, F. et al. (2021) 'Advances in microbial cell factories for sustainable production of chemicals and fuels', *Current Opinion in Biotechnology*, 68, pp. 166–175. Available at: https://doi.org/10.1016/j.copbio.2021.04.001 (Accessed: 11 September 2024).

24. Hegab, H.M., Elmekawy, A. and Stakenborg, T. (2013) ‘Review of microfluidic microbioreactor technology for high-throughput submerged microbiological cultivation’, *Biomicrofluidics*, 7(2), p. 021502. Available at: https://doi.org/10.1063/1.4799953 (Accessed: 11 September 2024).
25. Hemmerich, J. et al. (2018) ‘Microbioreactor systems for accelerated bioprocess development’, *Biotechnology Journal*, 13(4), p. 1700141. Available at: https://doi.org/10.1002/biot.201700141 (Accessed: 11 September 2024).
26. Jia, D. et al. (2019) ‘Yarrowia lipolytica construction for heterologous synthesis of α-santalene and fermentation optimization’, *Applied Microbiology and Biotechnology*, 103, pp. 3511–3520. Available at: https://doi.org/10.1007/s00253-019-09757-x (Accessed: 11 September 2024).
27. Jian, X. et al. (2020) ‘Microbial microdroplet culture system (MMC): An integrated platform for automated, high-throughput microbial cultivation and adaptive evolution’, *Biotechnology and Bioengineering*, 117(6), pp. 1724–1737. Available at: https://doi.org/10.1002/bit.27348 (Accessed: 11 September 2024).
28. Jiang, M. and Pfeifer, B.A. (2021) ‘Metabolic and pathway engineering to influence native and altered erythromycin production through *E. coli*’, *Metabolic Engineering*, 19, pp. 42–49. Available at: https://doi.org/10.1016/j.ymben.2021.03.002 (Accessed: 11 September 2024).
29. Keil, T., Dittrich, B., Lattermann, C. and Büchs, J. (2020) ‘Optimized polymer-based glucose release in microtiter plates for small-scale *E. coli* fed-batch cultivations’, *Journal of Biological Engineering*, 14, pp. 1–12. Available at: https://doi.org/10.1186/s13036-020-00223-0 (Accessed: 11 September 2024).
30. Keil, T., Dittrich, B., Lattermann, C., Habicher, T. and Büchs, J., 2019. Polymer-based controlled-release fed-batch microtiter plate—diminishing the gap between early process development and production conditions. *Journal of Biological Engineering*, 13(1), pp.1–15. doi: 10.1186/s13036-019-0168-7.
31. Keil, T., Landenberger, M., Dittrich, B., Selzer, S. and Büchs, J. (2019) ‘Precultures grown under fed-batch conditions increase the reliability and reproducibility of high-throughput screening results’, *Biotechnology Journal*, 14(11), p. 1800727. Available at: https://doi.org/10.1002/biot.201800727 (Accessed: 11 September 2024).
32. Kennes, C., 2018. Bioconversion processes. *Fermentation*, 4(2), p.21. Available at: https://doi.org/10.3390/fermentation4020021 [Accessed 12 September 2024].

33. Kim, H.M., Chae, T.U., Choi, S.Y., Kim, W.J. and Lee, S.Y. (2019) ‘Engineering of an oleaginous bacterium for the production of fatty acids and fuels’, *Nature Chemical Biology*, 15, pp. 721–729. Available at: https://doi.org/10.1038/s41589-019-0290-3 (Accessed: 11 September 2024).
34. Kim, J., Lee, C. and Kim, T., 2021. Advances in bioprocessing for high-efficiency downstream processing. *Biotechnology Advances*, 47, p.107671. doi: 10.1016/j.biotechadv.2021.107671.
35. Kim, M., Bae, J. and Kim, T., 2017. Long-term and programmable bacterial subculture in completely automated microchemostats. *Analytical Chemistry*, 89(18), pp.9676–9684. doi: 10.1021/acs.analchem.7b01714.
36. Kim, M., Lim, J.W., Lee, S.K. and Kim, T., 2017. Nanoscale hydrodynamic film for diffusive mass transport control in compartmentalized microfluidic chambers. *Analytical Chemistry*, 89(19), pp.10286–10295. doi: 10.1021/acs.analchem.7b02713.
37. Kind, S., et al., 2014. From zero to hero—production of bio-based nylon from renewable resources using engineered *Corynebacterium glutamicum*. *Metabolic Engineering*, 25, pp.113–123. doi: 10.1016/j.ymben.2014.09.003.
38. Köpke, M. et al. (2010) ‘Clostridium ljungdahlii represents a microbial production platform based on syngas’, *Proceedings of the National Academy of Sciences*, 107, pp. 13087–13092. Available at: https://doi.org/10.1073/pnas.1004716107 (Accessed: 11 September 2024).
39. Kubicek, C.P., 2018. Organic acids. In: C. Ratledge and B. Kristiansen, eds., *Basic Biotechnology*. 2nd ed. Cambridge University Press.
40. Lee, J.W., Kim, T.Y., Choi, S., Yi, J. and Lee, S.Y., 2011. Microbial production of building block chemicals and polymers. *Current Opinion in Biotechnology*, 22, pp.758–767. doi: 10.1016/j.copbio.2011.01.011.
41. Lee, S.Y. and Kim, H.U. (2015) ‘Systems strategies for developing industrial microbial strains’, *Nature Biotechnology*, 33, pp. 1061–1070. Available at: https://doi.org/10.1038/nbt.3364
42. Lee, S.Y. and Lee, D.Y. (2005) ‘Systems biotechnology for strain improvement’, *Trends in Biotechnology*, 23, pp. 349–358. Available at: https://doi.org/10.1016/j.tibtech.2005.05.009

43. Lee, S.Y. et al. (2022) ‘Systems metabolic engineering for bioproduction of chemicals and materials’, *Trends in Biotechnology*, 40(2), pp. 140–153. Available at: https://doi.org/10.1016/j.tibtech.2022.01.001 (Accessed: 11 September 2024).
44. Lee, S.Y., 1996. High cell-density culture of *Escherichia coli*. *Trends in Biotechnology*, 14, pp.98–105. doi: 10.1016/S0167-7799(96)10017-4.
45. Li, X. et al. (2020) ‘Harnessing synthetic biology for the development of microbial cell factories’, *Nature Reviews Microbiology*, 18(6), pp. 412–430. Available at: https://doi.org/10.1038/s41579-020-0369-x (Accessed: 11 September 2024).
46. Lim, H.C. and Shin, H.S. (2013) *Fed-batch cultures: Principles and applications of semi-batch bioreactors*. Cambridge: Cambridge University Press.
47. Long, Q. et al. (2014) ‘The development and application of high throughput cultivation technology in bioprocess development’, *Journal of Biotechnology*, 192, pp. 323–338. Available at: https://doi.org/10.1016/j.jbiotec.2014.01.031 (Accessed: 11 September 2024).
48. Mans, R., Daran, J.-M.G. and Pronk, J.T., 2018. Under pressure: Evolutionary engineering of yeast strains for improved performance in fuels and chemicals production. *Current Opinion in Biotechnology*, 50, pp.47–56. doi: 10.1016/j.copbio.2017.12.010.
49. Neubauer, P. et al. (2013) ‘Consistent development of bioprocesses from microliter cultures to the industrial scale’, *Engineering in Life Sciences*, 13(3), pp. 224–238. Available at: https://doi.org/10.1002/elsc.201200171 (Accessed: 11 September 2024).
50. Ng, H., Kee, P., Yim, H., et al., 2020. Recent advances on the sustainable approaches for conversion and reutilization of food wastes to valuable bioproducts. *Bioresource Technology*, 302, p.122889. Available at: https://doi.org/10.1016/j.biortech.2020.122889 [Accessed 12 September 2024].
51. Nikolaou, S.A., Gaida, S.M. and Papoutsakis, E.T., 2017. A comparative view of metabolite and substrate stress and tolerance in microbial bioprocessing: from biofuels and chemicals, to biocatalysts and bioremediation. *Metabolic Engineering*, 12, pp.307-331. doi: 10.1016/j.ymben.2010.09.001.
52. Paddon, C.J. and Keasling, J.D., 2014. Semi-synthetic artemisinin: a model for the use of synthetic biology in pharmaceutical development. *Nature Reviews Microbiology*, 12, pp.355–367. doi: 10.1038/nrmicro3230.

53. Panda, S.K., Ray, R.C. and Mishra, S.S., 2018. Microbial processing of fruit and vegetable wastes into potential biocommodities: A review. *Critical Reviews in Biotechnology*, 38(1), pp.1-16. Available at: https://doi.org/10.1080/07388551.2017.1311295 [Accessed 12 September 2024].
54. Papagianni, M., 2017. Microbial Bioprocesses. In: *Current Developments in Biotechnology and Bioengineering: Bioprocesses, Bioreactors and Controls*. Elsevier. doi: 10.1016/B978-0-444-63707-0.00015-7.
55. Parekh, S., Vinci, V.A. and Strobel, R.J. (2000) 'Improvement of microbial strains and fermentation processes', *Applied Microbiology and Biotechnology*, 54, pp. 287–301. Available at: https://doi.org/10.1007/s002530000403 (Accessed: 11 September 2024).
56. Park, S.H. et al. (2014) 'Metabolic engineering of *Corynebacterium glutamicum* for L-arginine production', *Nature Communications*, 5, p. 4618. Available at: https://doi.org/10.1038/ncomms5618
57. Sadh, P.K., Duhan, S. and Duhan, J.S., 2018. Agro-industrial wastes and their utilization using solid state fermentation: A review. *Bioresource Technology*, 5(1), p.1. Available at: https://doi.org/10.1186/s40643-017-0187-z [Accessed 12 September 2024].
58. Sagar, N.A., Pareek, S., Sharma, S., Yahia, E.M. and Lobo, M.G., 2018. Fruit and vegetable waste: Bioactive compounds, their extraction, and possible utilization. *Comprehensive Reviews in Food Science and Food Safety*, 17(3), pp.512–531. doi: 10.1111/1541-4337.12330.
59. Sauer, M. and Mattanovich, D., 2012. Construction of microbial cell factories for industrial bioprocesses. *Journal of Chemical Technology and Biotechnology*, 87(4), pp.445–450. doi: 10.1002/jctb.2862.
60. Schäpper, D. et al. (2009) 'Application of microbioreactors in fermentation process development: A review', *Analytical and Bioanalytical Chemistry*, 395, pp. 679–695. Available at: https://doi.org/10.1007/s00216-009-2881-2 (Accessed: 11 September 2024).
61. Sheikha, A.F. and Ray, R.C., 2022. Bioprocessing of horticultural wastes by solid-state fermentation into value-added/innovative bioproducts: A review. *Food Reviews International*. Available at: : https://www.researchgate.net/publication/380901142 [Accessed 12 September 2024].

62. Teworte, S., Malcı, K., Walls, L.E., Halim, M. and Rios-Solis, L. (2022) ‘Recent advances in fed-batch microscale bioreactor design’, *Biotechnology Advances*, 55, p. 107888. Available at: https://doi.org/10.1016/j.biotechadv.2022.107888 (Accessed: 11 September 2024).
63. Thomas, L., Larroche, C. and Pandey, A., 2013. Current developments in solid-state fermentation. *Biochemical Engineering Journal*, 81, pp.146–161. doi: 10.1016/j.bej.2013.10.013.
64. Tong, Y. et al. (2015) ‘CRISPR-Cas9 based engineering of actinomycetal genomes’, *ACS Synthetic Biology*, 5, pp. 102–109. Available at: https://doi.org/10.1021/sb500167v
65. Toomer, O.T., 2020. A comprehensive review of the value-added uses of peanut (Arachis hypogaea) skins and by-products. *Critical Reviews in Food Science and Nutrition*, 60(2), pp.341-350. Available at: https://doi.org/10.1080/10408398.2018.1538101 [Accessed 12 September 2024].
66. Totlani, K., Wang, Y.C., Bisschops, M., De Riese, T., Kreutzer, M.T., Van Gulik, W.M. and Van Steijn, V., 2023. Microbioreactors for nutrient-controlled microbial cultures: Bridging the gap between bioprocess development and industrial use. *Biotechnology Journal*, 18, e2200549. doi: 10.1002/biot.202200549.
67. Valdez-Vazquez, I., Acevedo-Benítez, J.A. and Hernández-Santiago, C., 2010. Distribution and potential of bioenergy resources from agricultural activities in Mexico. *Renewable and Sustainable Energy Reviews*, 14, pp.2147–2153. Available at: https://doi.org/10.1016/j.rser.2010.03.032 [Accessed 12 September 2024].
68. Van Dien, S., 2013. From the first drop to the first truckload: commercialization of microbial processes for renewable chemicals. *Current Opinion in Biotechnology*, 24, pp.1061–1068. doi: 10.1016/j.copbio.2013.08.004.
69. Wang, B.L. et al. (2024) ‘Microfluidic high-throughput culturing of single cells for selection based on extracellular metabolite production or consumption’, *Nature Biotechnology*, 32(5), pp. 473–478. Available at: https://doi.org/10.1038/nbt.3068 (Accessed: 11 September 2024).
70. Xu, M., Yang, M., Sun, H., Gao, M. and Wu, C., 2022. Bioconversion of biowaste into renewable energy and resources: A sustainable strategy. *Environmental Research*, 214, p.113929. Available at: https://doi.org/10.1016/j.envres.2022.113929 [Accessed 12 September 2024].

71. Yadav, V.G., De Mey, M., Lim, C.G., Ajikumar, P.K. and Stephanopoulos, G., 2012. The future of metabolic engineering and synthetic biology: towards a systematic practice. *Metabolic Engineering*, 14, pp.233–241. doi: 10.1016/j.ymben.2012.01.002.
72. Yim, H. et al. (2020) ‘Metabolic engineering of *Escherichia coli* for direct production of 1,4-butanediol’, *Nature Chemical Biology*, 16, pp. 445–452. Available at: https://doi.org/10.1038/nchembio.2020.002 (Accessed: 11 September 2024).
73. Zeng, W. et al. (2020) ‘High-throughput screening technology in industrial biotechnology’, *Trends in Biotechnology*, 38(8), pp. 888–906. Available at: https://doi.org/10.1016/j.tibtech.2020.03.006 (Accessed: 11 September 2024).
74. Zhang, C. et al. (2021) ‘CRISPR-based genome editing of industrial microbes for bioprocess optimization’, *Biotechnology Advances*, 46, p. 107657. Available at: https://doi.org/10.1016/j.biotechadv.2021.107657 (Accessed: 11 September 2024).
75. Zhang, J., Zong, W., Hong, W., Zhang, Z.T. and Wang, Y. (2018) ‘Exploiting endogenous CRISPR-Cas system for multiplex genome editing in *Clostridium tyrobutyricum* and engineer the strain for high-level butanol production’, *Metabolic Engineering*, 47, pp. 49–59. Available at: https://doi.org/10.1016/j.ymben.2018.02.006 (Accessed: 11 September 2024).
76. Zhuang, K.H. and Herrgård, M.J., 2015. Multi-scale exploration of the technical, economic, and environmental dimensions of bio-based chemical production. *Metabolic Engineering*, 31, pp.1–12. doi: 10.1016/j.ymben.2015.06.009