NUCLEIC ACID INSIGHTS

DNA
ENGINEERING AND MANUFACTURING

SPOTLIGHT

COMMENTARY

Incorporating digitalization in the conceptual design, research and development of plasmid biomanufacturing

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The importance of large-scale production of plasmid DNA (pDNA) has increased steadily over the years due to the development of a growing number of direct and indirect applications. To meet the growing demand for pDNA, significant efforts must be made towards improving its manufacturing. In particular, the digitalization of pDNA manufacturing could enable faster process optimization, support data-driven decision-making, and contribute to waste reduction and more sustainable operations. In this commentary article, we further contend that the benefits of digitalization should be captured early on at the research and development stage of the manufacturing process. To support this vision, we present a conceptual framework for incorporating digitalization into pDNA process development, discuss technological enablers, explain how digital methods could overcome traditional limitations, and delve into implementation considerations.

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PLASMIDS AND THE DIGITALIZATION OF BIOMANUFACTURING

Plasmids are pervasive across the gene and cell therapy industry of today [1,2]. As biologicals, they are used to deliver genetic information to patient target cells or as vehicles to deliver the molecular components of gene editor systems. Moreover, plasmids serve as essential raw materials for the manufacturing of engineered cell products (e.g., CAR-T cells) or of other biologicals (e.g., viral vectors and mRNA). The ability to manufacture plasmids cost-effectively on a large scale is thus critical for many

biopharmaceutical companies and research institutions [3]. In other circumstances, for example, in the production of lentiviral vectors for cell therapies, the actual challenge may be to develop GMP-compliant scale-down models capable of producing pDNA in a cost-effective manner [4]. One approach to increase efficiency, throughput and scalability, conserve resources, and minimize environmental impact in pDNA manufacturing is to embrace digitalization [3].

A fundamental principle of digitalization is the mapping of the physical space in a digital object via a digital twin (DT) [5]. A DT is a continuously updated *in silico* representation of a real-world system or process that acts as an identical counterpart in the digital space. An essential feature of a fully functional DT is a two-way dataflow between the physical system and its digital counterpart [6]. Ultimately, the DT generates a dynamic or static profile of the process based on historical and near-real-time measurements across an array of dimensions [6]. DTs are valuable for system simulation, integration, testing, monitoring, maintenance and even training, and are an essential building block of model-based systems engineering. Furthermore, in conjunction with mathematical modeling, DTs are likely essential for the successful implementation of continuous biomanufacturing, as they enable real-time process control, predictive decision-making, and rapid optimization [7,8].

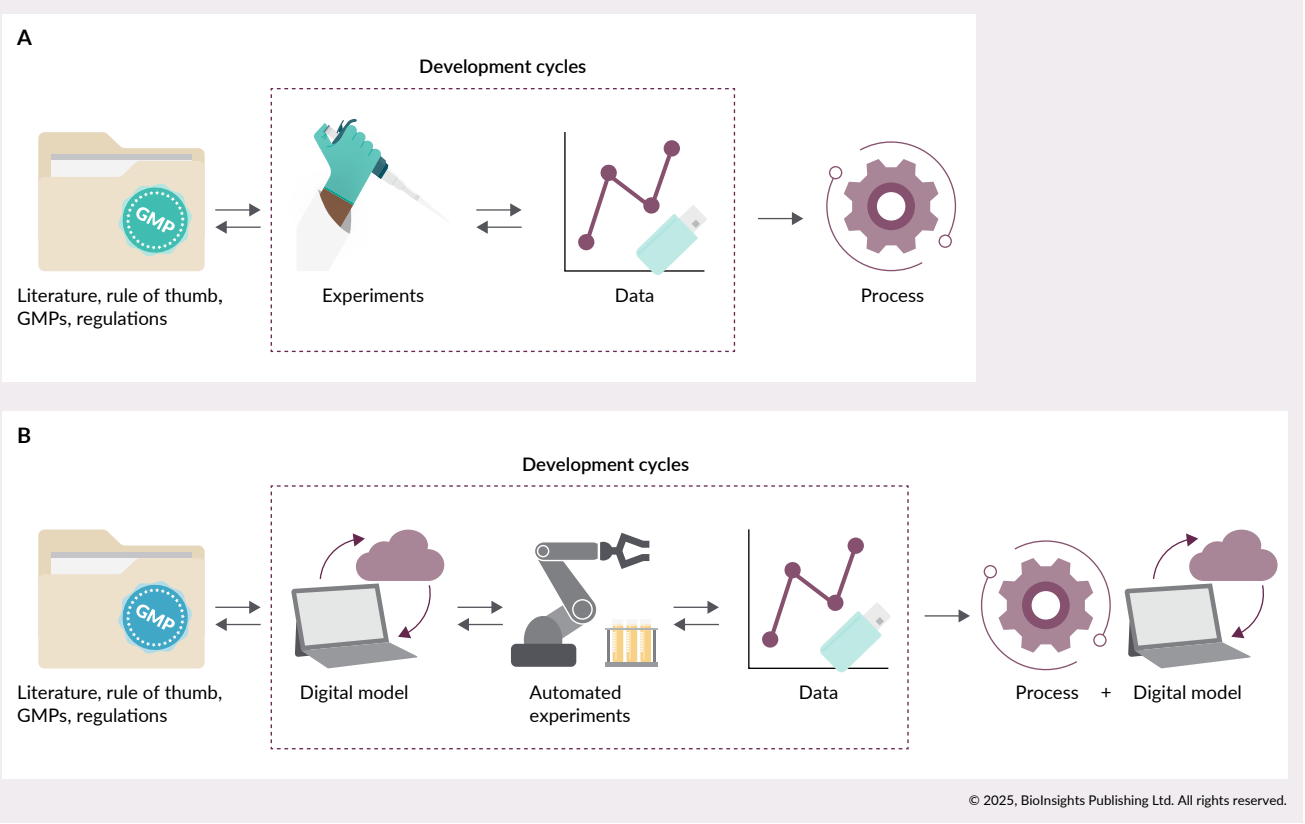
The creation of a DT of a biomanufacturing process has been advocated as one of the most compelling benefits of digitalization [6–9]. While as a first approach this will involve the digitalization of well-established manufacturing processes that are already in routine operation, several authors argue that the benefits of biomanufacturing should be captured early on at the research and development stage [10,11]. The development of a process compliant with Industry 4.0, which is characterized by the integration of digital technologies—such as the Internet of Things (IoT), artificial intelligence, data analytics, and automation—into manufacturing systems, inherently also demands the development of its DT [12]. The research question underlying this approach is therefore 'How to develop and incorporate a digitalization framework in the conceptual design, research and development of (pDNA) biomanufacturing processes?'. Such a framework is currently lacking, as most efforts are focused on digitizing established biomanufacturing processes.

THE LIMITATIONS OF TRADITIONAL PROCESS DEVELOPMENT

A real shift to a biomanufacturing scenario, where a physical process and its digital counterpart communicate, interact two ways, and operate in synchrony without interruption, requires digitalization to be embedded early in the biomanufacturing research and development stage [10]. This entails replacing the traditional process development pipeline, which follows a linear, step-by-step methodology known to be time consuming and laborious [10,11,13], with a digitally centered process development approach **(Figure 1)**. A process draft is usually designed based on the available literature, in-house experience, rules of thumb and GMPs. Key information to bear in mind pertains to final product specifications (e.g., pDNA topology, biological potency, impurity limits), some of which are established with guidance from regulations [14]. Examples of process-related impurities in pDNA manufacturing include host cell components (proteins, genomic DNA, RNA, endotoxins), residual reagents (solvents, salts, enzymes), and leachables from equipment, resins or filters. The final specifications will differ depending on the final application of the target pDNA [14]. For example, more stringent quality requirements regarding impurities will be in place if the pDNA is to be used in therapeutic applications, as opposed to cases

FIGURE 1

Replacing the (A) traditional approach to biomanufacturing process development by a (B) digitally centered process development.



where it serves as a raw material for the manufacture of a viral vector. Today, several pDNA manufacturing platforms have been developed, which can be readily adopted for the production of various pDNA molecules. Nevertheless, the introduction of new methodologies or process modifications targeted at generating more efficient processes will still require several process development cycles, relying heavily on human operators performing lab-based experimentation at small scale (typically 100 mL–10 L cell culture). The disadvantages of this approach are well recognized and include:

- Time and cost inefficiencies
- Limited process understanding and data utilization
- Poor scalability
- Human error and variability
- Regulatory compliance challenges

A digitally centered process development approach, paired with automated experimentation, could contribute to delivering new methodologies that generate more efficient processes, ultimately mitigating some of these limitations [10,11].

ENVISIONING A DIGITALLY CENTERED PDNA PROCESS DEVELOPMENT

A digitally centered approach to process development relies heavily on

incorporating digitalization concepts and computational tools at the early stages of process conceptualization, design, and development. The technological enablers of this approach include mathematical modelling (mechanistic, hybrid, and data-driven models), computational fluid dynamics, machine learning and AI, generative AI, automation and smart sensors, high throughput (HT) experimentation, workflow management systems, and edge and cloud computing **(Table 1)**. This approach offers several benefits, including accelerating development, reducing consumables by avoiding uninformative experiments, requiring fewer experiments, lowering error rates, and enhancing process understanding. Furthermore, by the end of the process development stage, digital models will be readily available to support technology transfer, process scale-up, and subsequently routine operation and control.

Here we present our view on how a digitally centered alternative can be utilized to

TABLE 1

Scientific and technological tools for digitally centered plasmid DNA process development.

Enabler	Description
Mathematical modeling	Mathematical process models of diverse nature (mechanistic, surrogate, data-driven, hybrid) are set up to provide information about key properties, variables and performance parameters/indicators (e.g., yields) of the different sub-processes (e.g., cell culture, unit operations), the interactions between process parameters, and product quality attributes (e.g., purity); these models are a key component of a DT, providing deep insights into the current state of the process through simulation
CFD	Software tools for performing CFD dynamics simulations can play a crucial role in bioprocess scale-up by enabling the simulation and analysis of fluid flows within bioreactors; this facilitates the optimization of mixing, mass transfer, and overall reactor design, which are essential for efficient scale-up
Machine learning and AI	AI and machine learning contribute to smart automation and analytics through the identification of optimal process parameters, automation of complex tasks, prediction of potential issues; enabling the shift to predictive rather than reactive process control
Generative AI (large-language models)	If trained on large and adequate datasets of bioprocess parameters, LLMs can suggest improvements to increase efficiency and product quality, or assist in designing more effective experiments, potentially reducing the number of iterations required in bioprocess development
Automation and smart sensors	Bioprocessing workflows can be optimized, monitored and controlled in real-time by integrating advanced technologies such as sensors and IoT devices for data acquisition, and AI and machine learning for predictive modeling and decision-making; automated systems can handle tasks such as sampling, analysis, and equipment maintenance and contribute to enhance process efficiency, improve product quality, and reduce variability by minimizing human intervention
HT experimentation	HT experimentation using robotic platforms enable rapid, parallel execution of numerous experiments, significantly accelerating process optimization and development; these systems can dispense reagents, mix solutions, and transfer samples, minimizing human error and increasing experimental throughput; it is thus possible to explore a broader range of parameters and conditions simultaneously, leading to faster identification of optimal production conditions; if integrated with advanced data analytics and computational modeling, HT experimentation can enhance decision-making capabilities and reduce development timelines
Workflow management systems (WMS)	By implementing WMS, processes can become fully documented, traceable and reproducible, allowing for reuse of the generated data; WMS enhance interoperability, thus enabling better collaboration between scientists; they allow for the seamless choreographing of tasks, ensuring that complex workflows are executed efficiently and in the correct sequence; additionally, WMS facilitate structured storage for data and metadata, preserving essential context for future analyses; built-in error detection mechanisms help identify issues early, triggering automated error handling procedures to maintain workflow reliability and data integrity
Edge and cloud computing	Edge computing enables real time data processing and control of the biomanufacturing facilities, empowering quick adjustments; cloud computing provides scalable storage, big data analytics, and collaborative platforms for long term data analysis, process optimization and predictive modelling

CFD: computational fluid dynamics. HT: high throughput. WMS: Workflow Management Systems.

aid, guide and accelerate the development and establishment of a pDNA manufacturing process. The overall goal is to develop an integrated model toolset that examines the entire biomanufacturing process, providing clarity on bottlenecks, highlighting optimization opportunities, and ultimately enhancing superior product quality and efficiency in laboratory operations. Specifically, we propose an approach that involves synergies between:

- Experimentation
- Digitalization
- HT model-assisted experimentation activities **(Figure 2)**

These intertwined collaborative research activities should cover the upstream and downstream processing stages of plasmid manufacturing.

Experimental setting up of a benchmark process

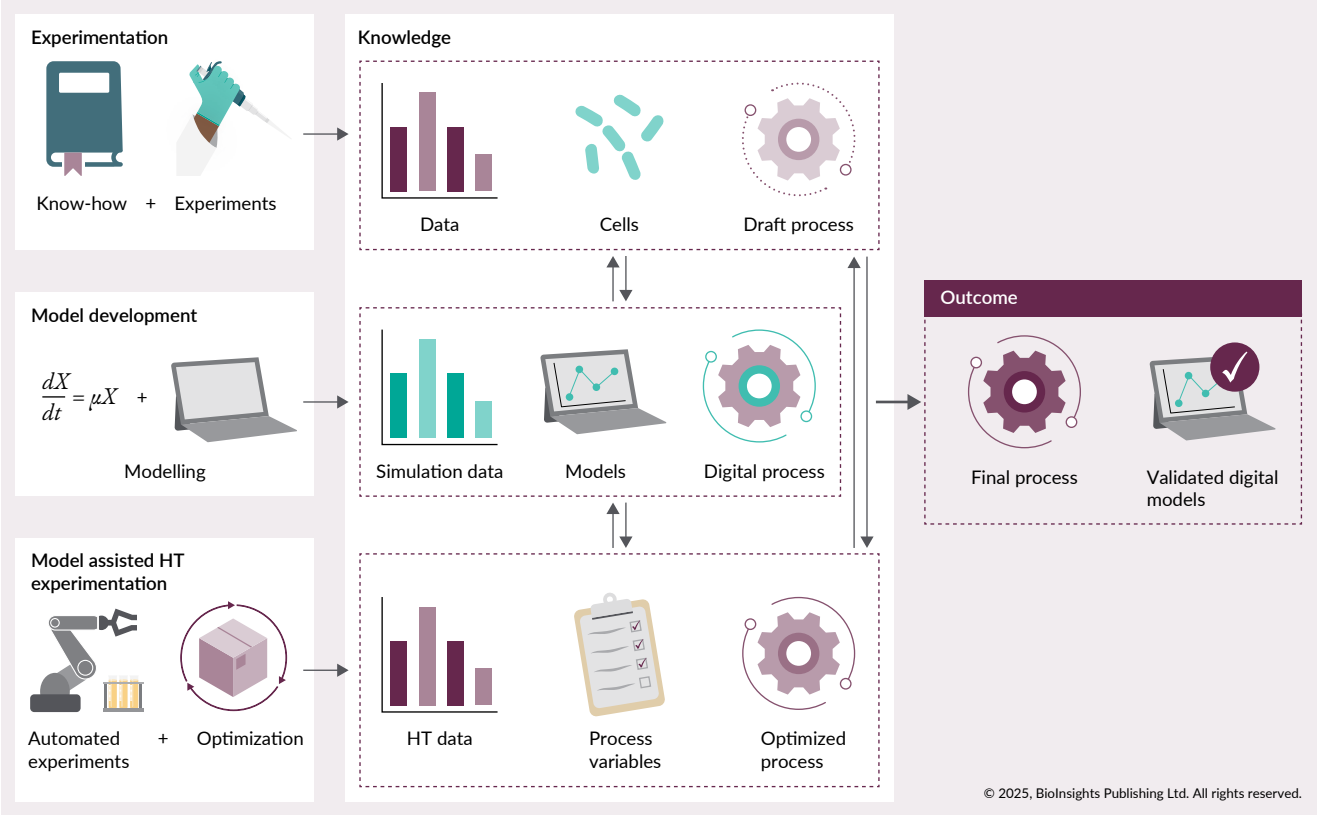
An experimental benchmark process is initially defined based on available knowledge and rules of thumb [15]. This heuristic approach involves the selection of a strain of the producer *Escherichia coli* with genotypes suitable for pDNA amplification, the preparation of banks of cells transformed with the target pDNA, and the set-up of key analytics (e.g., gel electrophoresis, HPLC, ELISA). Then, a working pDNA manufacturing process should be drafted and established at lab scale. This entails cultivating cells to amplify pDNA and then setting up a downstream processing train of operations to recover, isolate, and purify the pDNA. The goal is to quickly obtain initial datasets (e.g., time series data describing microbial cell culture and pDNA amplification, recovery yields of unit operations, etc.) that can be used to jump start and advance model development and guide HT experimentation for process optimization.

Modelling of upstream and downstream processing

Mathematical models are developed to represent, analyze, and predict the complex system surrounding pDNA manufacturing. This calls for selecting an appropriate model structure that aligns with the biological and physical nuances of the different operations in the manufacturing train. The models should be able to describe and predict the dynamics of cell growth and pDNA amplification. This requires the establishment of time course relations between variables such as the concentration of key nutrients (e.g., carbon source), biomass concentration, and pDNA titers [16,17]. Stoichiometric models of *E. coli* metabolism can also be useful in this context [18,19]. Models used to describe the isolation and purification of pDNA from the *E. coli* cells should predict the performance metrics of various operations (e.g., tangential flow filtration, precipitation, chromatography), especially in terms of yield and purification efficiency. Draft models for a particular operation are first tested using the corresponding initial data sets. Simulation results are then used to guide the design of additional experiments, such as model-based design of experiments [20–22]. The new sets of experimental data are further used to refine and validate the models. These experimental/modelling development cycles should be repeated until a satisfactory model is obtained. An illustration of this approach is provided by Muller *et al.* in the context of rAAV production [23]. Starting with shaker flask data, satisfactory process models were obtained after two to three iterative cycles combining high-throughput (HT) runs in a fully automated microbioreactor system with hybrid model refinement. Benchmarking this approach against a statistical Design

FIGURE 2

Synergies between (A) experimental setting up of a benchmark process, (B) digitalization, and (C) high-throughput model-assisted experimentation activities.



of Experiments method showed that the model-based experimental design consistently produced higher rAAV titers with fewer total experiments. Rigorous validation should be made by comparing model outputs against separate experimental datasets. The outcome is a set of robust models that reflect experimental observations, provide insights into the process and support subsequent process optimization.

High-throughput model-assisted experimentation activities

Optimization of pDNA manufacturing can then be performed by resorting to automated HT screening platforms [24–26], guided by the predictive models developed. Such platforms, which are being introduced into modern process development labs, can be used to screen process conditions and operating variables that maximize the performance of the unit operation being tested [27–29]. Experimental workflows can be integrated and automated in a laboratory environment by resorting to heterogeneous devices, including liquid handling stations, parallel cultivation systems, and mobile robots [30]. For example, advanced liquid handling stations with embedded parallel mini bioreactors can be used to run up to 48 parallel cell culture/pDNA amplification dynamic experiments in a process-wide design and optimization scheme [31,32]. The integration of a workflow management system [33] ensures the flexible yet reliable handling of complex HT experiments and FAIR data storage—findable, accessible,

interoperable, and reusable (FAIR) [34]. Here, model-based tools can enhance information gain and process robustness by enabling, for example, real-time process monitoring, the selection of the most relevant sampling times, and the optimization of process control. Many downstream processing unit operations used in pDNA manufacturing such as precipitation [35,36] and chromatography [37,38], can also be optimized using HT platforms [28,29]. One important aspect to mention is that HT experimentation critically depends on HT analytics to rapidly evaluate multiple conditions [39]. For example, an evaluation of 48 parallel cell culture/pDNA amplification experiments run on a mini bioreactor platform would undoubtedly involve assessing pDNA titers and topology. Since most scale-down reactor systems only incorporate DO and pH measurements, this would require collecting samples, performing miniprep isolation, and running agarose or capillary electrophoresis analysis in parallel, which is not trivial to implement [40]. Further challenges include the small volumes of scale-down reactors, which restrict the sampling frequency and volume, as well as the large number of samples generated. Reality shows that, unfortunately, analytical capacity often lags behind experimental throughput, creating a significant bottleneck [39]. This mismatch between experimental throughput and analytical capacity can slow down decision-making and delay process optimization, particularly in complex biological systems. Notwithstanding the analytical challenge, the large amounts of heterogeneous experimental data generated by HT platforms contain valuable information that can be explored using a wide variety of machine learning (ML) approaches [41–43]. Examples of data-driven methods that may be useful for optimization purposes include artificial neural networks [44], Bayesian optimization [45–47], deep reinforcement learning [48], and others [49].

Key benefits of HT experimentation include accelerated development, and the ability to perform a higher number of experiments while keeping the number of needed consumables low due to the smaller volumes. Applying model-based methods to design experiments with optimal information gain ensures that only the minimum number of experiments is performed [50,51]. The new data generated can be used to refine and validate the models that have been developed. The goal of these activities is to determine the optimal conditions for pDNA manufacturing and to develop a reliable digital model of the process. On the other hand, one should be aware that miniaturized systems may not replicate large-scale pDNA manufacturing (e.g., bioreactor dynamics and substrate heterogeneities/gradients [52]), and that analytical and data handling limitations can hinder the translation of results. Furthermore, the complexity of integrating automated platforms and the resources required to ensure regulatory compliance cannot be overstated.

Model integration and process validation

Ideally, models describing both upstream and downstream processing sections should be merged into a singular, unified model. This integration is still perceived as a bottleneck, often because upstream and downstream process models have focused on describing different sets of variables. Once integration is achieved, the consolidated model should be rigorously validated against lab-scale datasets (e.g., at the 1–2 L lab scale), ensuring it reflects real system dynamics, and that it is robust and reliable [53].

TRANSLATION INSIGHT

Embracing digitalization concepts and tools at the early stages of process conceptualization, design, and development

can accelerate development, reduce consumables and error rates, increase the number of informative experiments, and ultimately improve process understanding. In the context of plasmid manufacturing, this digitally centered approach to process development requires synergies and interconnection of:

- Experimentation
- Digitalization
- HT model-assisted experimentation activities

However, the field is in its infancy, with several areas requiring further study or pilot testing.

For once, many of the digitalization tools at our disposal **(Table 1)** are still underexplored in the context of process development. For example, there is clearly room for the development of LLMs tailored to the conceptual development of processes for the biomanufacturing of a particular class of bioproducts (e.g. nucleic acids, pDNA, mRNA), leveraging existing literature data and pre-existing knowledge (e.g., company data, expertise). Such dedicated LLMs could be invaluable, for example, in the initial drafting of a manufacturing process. The use of CFD in the context of process scale-up can also be considered sub-optimal due to its high computational cost, reliance on simplifications that may not fully capture complex interactions, and challenges in accurately predicting scale-dependent phenomena (e.g. turbulence, mixing, and heat transfer). Another important area that requires investment is the development of more advanced and refined mathematical models capable of accurately representing complex biological systems, for example, microbial cell culture and pDNA amplification. The importance of mathematical models in conjunction with the adoption of digitalization will be especially relevant in the context of continuous manufacturing, which is an industry trend likely to change the way plasmids are manufactured in the future [54–56].

Additionally, the full technical integration of the digitalization tools available **(Table 1)** in the context of process development is still a bottleneck. Clearly, we need to improve our ability to manage the loop of hypothesis formulation, model-based experimental design, high-throughput experimentation, data evaluation, model adaptation, conclusion, and new hypothesis generation, which still requires considerable human intervention. Although we are far from creating a 'Robot Process Development Scientist' designed to autonomously automate process development, akin to the Robot Scientist discussed by King *et al.* [57], the potential for digitalization to contribute to the generation of process knowledge is huge. The necessity to upgrade technological infrastructure for real-time data integration in process development laboratories is also imperative. Examples include the integration of HT experimentation and advanced analytics capabilities, the implementation of integrated Laboratory Information Management Systems (LIMS) or Electronic Lab Notebooks (ELN) [58], the replacement of legacy laboratory instruments with digitally enabled, IoT-compatible sensors and Process Analytical Technology tools (e.g., Raman, NIR, FTIR, and *in situ* microscopy) [59], and the installation of systems to ensure data integrity, traceability, and regulatory compliance in digital environments [60].

The implementation of digitalization in biomanufacturing—both in process development and operation—further requires a fundamental shift in how data are acquired and managed, aligning with the FAIR principles to ensure seamless integration, traceability, and utility across digital systems [61,62]. For example, this requires transforming heterogeneous data

formats (e.g., PDFs, Excel sheets) into structured, machine-readable formats (e.g., XML, JSON) to enable real-time synchronization between physical systems and their digital counterparts. Furthermore, the thorough tracking and recording of all tasks performed throughout experimentation at both experimental and computational levels is critical to ensure data reproducibility [33]. Another important aspect of digitalization is data safety, also known as cybersecurity, which involves managing data in a responsible manner to minimize the risk of a data breach. However, users are often not sufficiently aware of such safety aspects [63].

One significant challenge in embracing digitally centered process development is resistance to change among stakeholders. This can be addressed by demonstrating clear return on investment, ensuring data security, and fostering cross-disciplinary collaboration to build trust in digital innovations. This resistance may be exacerbated further by the lack of user knowledge—many potential users simply do not know how to use digital tools effectively or where to begin—as well as by the lack of tools specifically tailored for bioengineering. Clearly, a skilled workforce with competencies that differ from those of the past must be trained to understand the importance and value of digitalization tools, to utilize the new methodologies and associated devices in the laboratory, and to handle complex data outputs. This requires universities and research institutes to develop world-class educational programs in digital biomanufacturing, which are currently not widely available.

Although quantitative data on the digitalization of pDNA manufacturing is still scarce, it is reasonable to anticipate benefits comparable to those reported in other biomanufacturing domains where AI and advanced analytics have been integrated—such as improvements of throughput upstream (15–30%) and downstream (up to 60%) and significant improvements in resource efficiency and process robustness [64]. The digital shift in pDNA production is thus expected to enhance efficiency, sustainability, and decision-making in a similar manner.

Moving forward, academia can play a crucial role in exploring innovative digitalization approaches for early-stage biomanufacturing research, while industry should focus on pilot-testing digital tools in process development to assess their practical applications. Policymakers, on the other hand, must work to develop clear guidelines and regulatory frameworks that support the adoption of digitalization in biomanufacturing, ensuring both compliance and technological advancement. In conclusion, incorporating digitalization into manufacturing development is a strategic move towards efficiency and sustainability; however, its full potential depends on further research, industry validation, and supportive regulatory frameworks to ensure seamless integration and long-term impact.

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AUTHORSHIP & CONFLICT OF INTEREST

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