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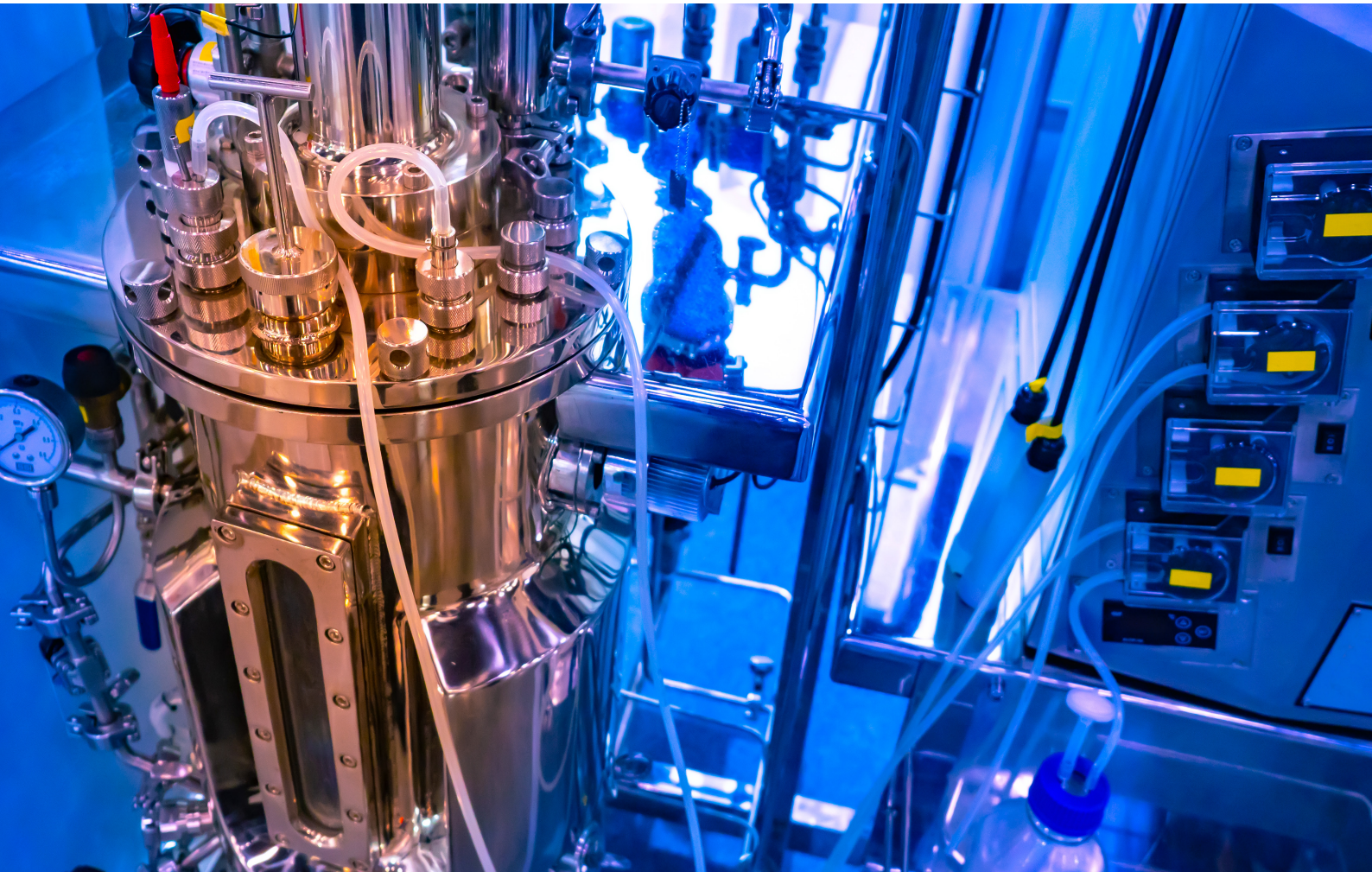
White Paper

Ready for Scale-Up?

Optimising bioreactor design and operation using computational fluid dynamics and digital twins

For biomanufacturing business models to become economically viable and stave off global environmental collapse, the production of nutrients, fibres, chemicals and fuels in bioreactors must be scaled up by many orders of magnitude without loss of process efficiency. But owing to the natural variability of living systems and different scaling laws and non-linearities at work, the complexity – and cost – of managing a bioreactor increases exponentially with size. The lack of suitable process technology for industrial-scale biomanufacturing therefore presents an existential risk.

Here, we explain why the time is right for the industry to embrace computational modelling as a tool to design large-scale bioprocesses and control technology in order to compete with, and eventually replace, traditional production processes based on fossil fuels and animals. We also showcase some of TTP's work in this area and demonstrate how we can help find better solutions to the scaling-up problem.



Introduction

Biomanufacturing promises sustainable food, textiles, chemicals and fuels without the use of animals or petroleum. Instead, these materials can now be made by exploiting the synthetic capabilities of engineered cells cultivated in bioreactors.

Designing and controlling economically viable in vitro biological processes is complicated. It typically requires much costly trial and error and a process referred to as "scale-up". During scale-up, the size of the bioreactor is increased in steps until an economically viable scale is reached.

For the small-scale manufacture of high-value products, designing and managing the bioprocess can be achieved through techniques established in the biopharma industry. Biosynthetic insulin, for example, has been produced by using E. coli containing human DNA for decades.

But if we now want to apply the biomanufacturing paradigm to high-volume, low-value goods, we need to scale up production by many orders of magnitude while maintaining high process efficiency. The scale-up challenge is a major obstacle in the way of a sustainable future.

Bioreactor design: the need to develop new solutions

To realise biomanufacturing at scale, radical innovation in reactor process design and control technology is urgently needed.

It is increasingly clear that commercially available bioreactors and fermenters from biopharma are unsuitable for manufacturing relatively low-value products at industrially relevant scales.

For example, Eat Just, who in 2020 became the first company to sell cultivated meat, acknowledge that they don't have sufficient capacity to solve the immediate challenge of scaling-up to Singapore's supermarket shelves, let alone to global markets.[1]

To manufacture products like synthetic meat, we need to re-configure existing technologies and invent novel approaches to meet a new set of requirements. To enable lower-cost capacity, design focus needs to shift away from purity, precision and containment towards high cell density, process intensification and volume scalability.[2]

Designing new large-scale synthetic bioprocesses requires significant engineering effort and investment (Figure 1). The current design process lacks sufficiently accurate computational tools and is therefore unable to predict the impact of design parameters on culture behaviour.

1. Asia's new food frontier: the rise of edible tech- Financial Times, subscription required
2. Scalability in industrial biomanufacturing: three objectives of better hardware design, TTP blog

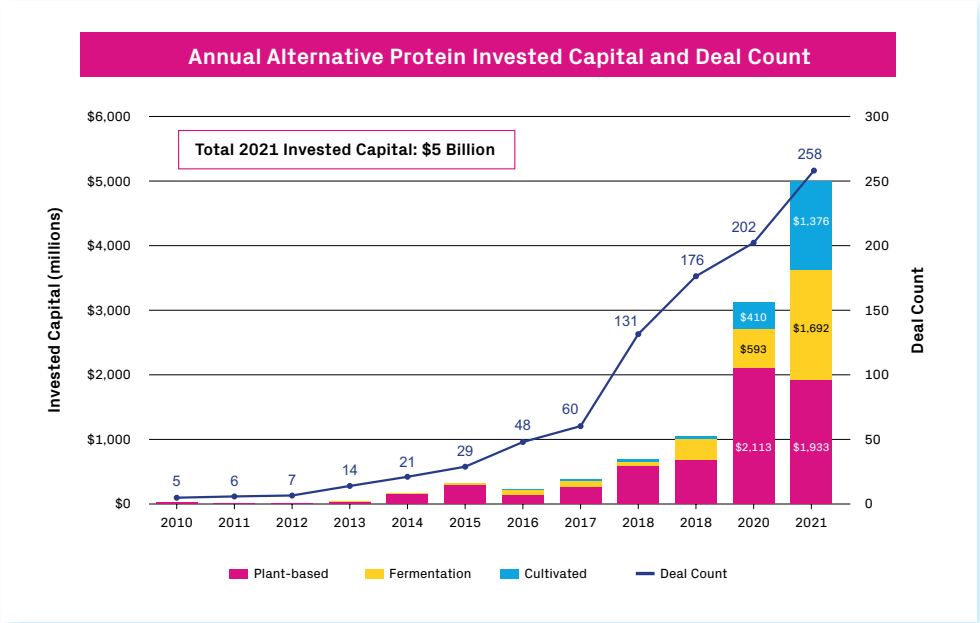


Figure 1 Investment in the alternative protein sector: Data compiled by The Good Food Institute (GFI) show that 2021 was a record year for investment in companies creating sustainable alternatives to conventional animal-based foods.[3] However, little of the capital raised is being put towards biomanufacturing infrastructure or bioprocess development innovations.[4,5] This is surprising given that an orders-of-magnitude increase in manufacturing capacity will be needed.

In the absence of predictive links between input and output, optimisation is achieved via extensive experiments, racking up large R&D costs and potentially missing global optimums lying further afield in the design space. The results are therefore small improvements on existing designs.

However, with the rise of greater computational resources, we see real opportunities to introduce computational tools to the bioreactor design process, as well as to the control systems used during eventual operation.

Modelling in vitro biology

A bioreactor is a controlled environment where cells can live and be productive. This environment consists of a suspending medium with many dissolved chemicals, nutrients mainly but also toxic metabolites. To sustain the culture, the medium is fed with gases and liquids while being mechanically stirred to facilitate the transfer of the nutrients and metabolites to and from the cells.

A healthy culture requires the concentrations of these chemicals to be within narrow tolerances, while the molecules take part in numerous biochemical reactions. When conditions are not precisely met, cell behaviour may change detrimentally, and entire batches, that have taken days or weeks to cultivate, may be lost.

3. Record $5 billion invested in alt proteins in 2021, surging 60 percent since 2020, Good Food Institute
4. It turns out we need to make (a huge amount of) stuff, Bio Endeavors
5. The "Coming Apocalypse:" Will Industrial Biotech Flourish or Flounder?, The Digest

The above considerations illustrate the complexity of in vitro bioprocesses. Consequently, much care must be taken to design reactor hardware and to tune operating conditions to allow the bioprocess to flourish in an economically viable manner.

Traditionally, bioreactor process design has mainly been driven by trial-and-error experimentation, which can consume vast amounts of time and money. In contrast to other areas of engineering, computational tools have not yet found widespread application in bioprocess design.

This is mainly due to the complexity of the computational problem, which involves the mixing of gases into turbulent suspensions of living cells, each a micro-bioreactor, transforming dazzling numbers of chemical species.

As computers become more powerful, however, these processes are becoming computationally tractable. Hence, we can expect that, in the coming decades, computer models will play a key role in designing and managing biomanufacturing processes at the scales needed for global impact.

By combining computational fluid dynamics (CFD) with metabolic computations, these models will provide a high-resolution digital representation of the complex interplay of turbulence and biochemistry. This information will provide two functions to the industry.

The first is as a tool to design reactor hardware, such as impellers, spargers, and feed tubes, to achieve the most efficient mass transfer of nutrients and toxics to and from the cells. Understanding the effect of, for example, flow conditions and nutrient dosing on cell proliferation and differentiation will likely accelerate design, prototyping and iteration.

The second function is to control reactor operation, i.e., for steering of the rates of the impellers, spargers, and feed tubes. Reactor control will involve running a real time simulation in parallel to the actual bioprocess (a digital twin).

In this scenario, sensor data is gathered from the bioprocess and sent to the simulation. From this data, the simulation reconstructs the detailed state of the process, computes the corresponding optimal control strategies, and sends the data back to the bioprocess. These strategies consist for instance of the timing and the dosing of the feed and harvest rates.

Computational fluid dynamics at TTP

At TTP, we are developing and using these types of CFD tools to design bioreactors and to explore the limits of their operation and optimisation.

In addition to well-known commercial software, we have developed proprietary CFD tools. The advantage of these in-house tools is that the software can be tailored around the problem at hand and is therefore computationally faster than the commercial equivalent. This allows us to simulate more detail at the same computational cost. Moreover, we can run the software on superclusters without paying parallel licence fees.

We can therefore run simulations with great detail, revealing for instance, the spatiotemporal structure of the turbulent eddies that are generated in a mixing tank (see Figure 2). These structures dictate the rate of mixing of chemical species as well as the hydrodynamic forces that the cells or organisms experience. These forces impact cell viability and therefore culture productivity, which is exemplified in the case study on the next page.

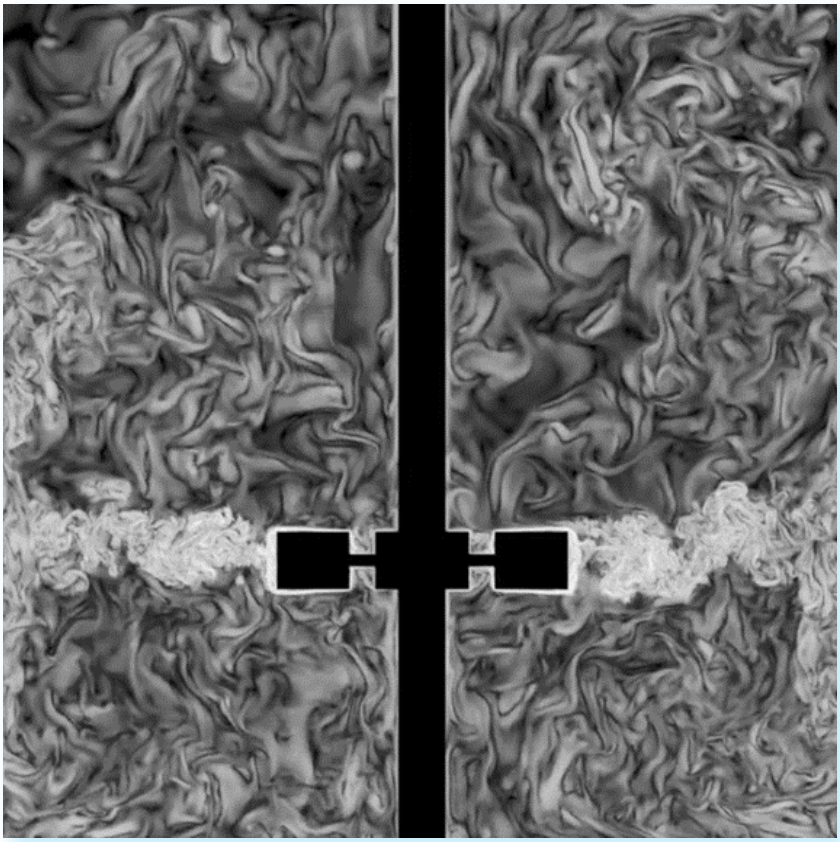


Figure 2 TTP uses in-house computational fluid dynamics (CFD) software to simulate the spatiotemporal structure of turbulent eddies in bioreactors. These eddies are responsible for the transfer of chemicals to and from the cells. The eddies also exert hydrodynamic forces on the cells, which may be detrimental.

Case Study: The effect of shear stress on cell culture performance in bioreactors

TTP has developed CFD modelling techniques to predict how bioreactor design can affect cell culture productivity.

Cells in bioreactors are sensitive to a wide range of influences which can, either singly or in combination, reduce cell culture productivity. Influences that may disturb cells include physical damage due to agitation, inadequate nutrient supply, unfavourable temperature or waste accumulation, population dynamics, diseases and foreign invasions.

In addition, biological studies show that cell productivity can be affected by prolonged low-level shear, but cells are also capable of recovering from, and may even be stimulated by, certain levels of mechanical stress.

Empirical approaches to bioreactor design fail to model and predict how the cumulative effects of mechanical stress and recovery affects cell productivity and yield. As reactor size increases, so does the power of the agitators required to ensure homogenous conditions. This raises the risk of mechanical stress and cell damage, thus forfeiting potential productivity gain from moving to a larger system.

Our CFD modelling techniques achieve this by first simulating the flows and shear histories experienced by hypothetical tracer cells inside different bioreactor designs and then using these shear history traces to drive a biological damage-and-recovery model to estimate cell performance in different reactor designs.

Take, for instance, a cylindrical reactor design with two impellors where the main variable is the angle of the impellor blades, i.e., a pair of radial Rushton impellors or a pair of axial impellors in the same tank. The paths followed by cells in these reactors are revealed by tracer particles, whose exposure to shear and other variables can be mapped out and analysed (Figure 3).

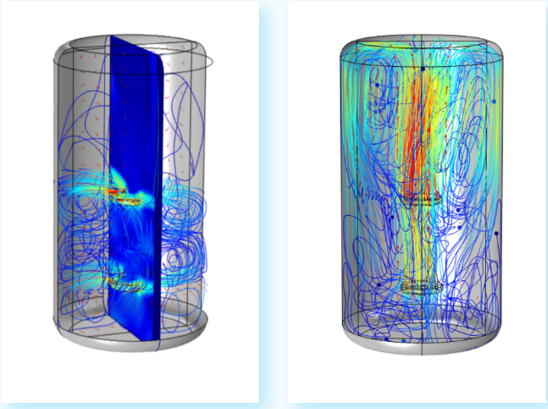


Figure 3 Simulations of the flow regimes and paths followed by tracer cells in identical bioreactors except for agitator angle.

Our models allow us to quantify differences between these systems in terms of peak shear values, the frequency of their occurrences and the durations of shear stress experienced by tracer cells. This reveals markedly different shear histories in the two bioreactors (Figure 4).

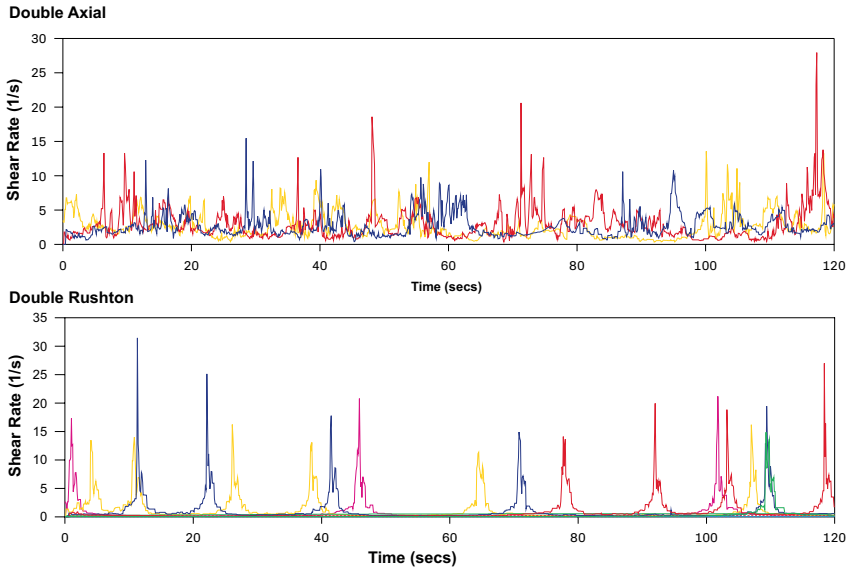


Figure 4 Tracer cells experience markedly different shear rate histories due to the flow regimes in the two reactor designs.

Figure 5 shows the results of combining this data with a shear damage-recovery model. In the example, cells in the radial Rushton impeller-driven reactor recover from periodic shear stress, thanks to intervening periods in environments of relative calm, while cells in the double axial impeller reactor suffer escalating shear stress.

We are thus able to use these modelling techniques as a design tool by comparing the specific estimates of cell viability and productivity across different bioreactor designs.

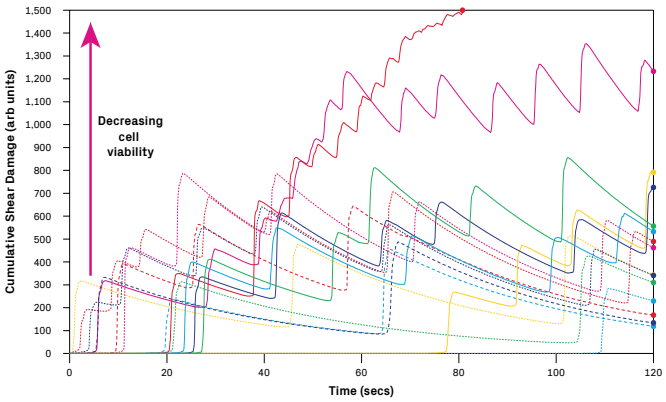


Figure 5 The cumulative impact of the shear rate histories on cell viability (and productivity) of the tracer cells from Figure 4.

We have also used this approach to map out and analyse the fluctuating nutrient micro-environments as well as temperature and gas concentrations experienced by tracer cells, thus inferring single-cell stress and other micro-response variables from macroscopic reactor design elements.

These results can be fed back into the bioreactor design process in order to achieve environmental exposure profiles more appropriate to cell requirements. Our CFD models thus provide a route to rapid development, optimised scale-up and efficient exploitation of valuable cell cultures.

Fluctuations matter

In large reactors, mixing becomes increasingly inefficient resulting in variations in chemical concentrations, which can have detrimental effects on biological conversion efficiencies. Owing to the non-linear nature of the biochemical conversions, the bioprocess will behave differently when exposed to varying nutrient concentrations as compared to a process operating at the equivalent average conditions.

Small (say litre) scale reactors can be easily designed and controlled to provide the required mixing and transfer of nutrients to the cells. However, mass transfer becomes problematic at larger scales (say cubic meter), where mixing of fluids at the reactor scale takes a substantial amount of time when compared to biochemical reaction rates.

This means that reactors cannot be modelled as being perfectly mixed, but instead, the effects of spatiotemporal variations need to be explicitly incorporated in a numerical simulation, which calls for detailed CFD models.

At TTP, we aim to quantify the effect of fluctuations on the biochemistry, by combining CFD models with biochemistry models. As exemplified in our case study above, we do this by calculating the physicochemical effects on individual cells, which are tracked along turbulent flow paths, and which chemically interact with the dissolved chemicals. Our modelling also includes the non-Newtonian nature of the culture broth due to the presence, for instance, of filamentous or adhesive cells.

By contrasting computational results to large-scale experimental data from partners and literature we can make model adjustments that improve the corresponding agreement. This may involve, for instance, adjusting the computational rules for the breakup and the coalescence of the gas bubbles, which depend in an intricate way on the non-Newtonian fluid properties and on the hydrophobic and hydrophilic nature of the cells and the secreted products.

Scaling up

For high-value products, such as vaccines, hardware costs are not the limiting factor, and scaling up can be achieved by scaling out, i.e., by running many small-scale reactors in parallel.

For low-value products, however, scaling out is too costly, and scaling up the production volume requires larger reactor sizes, thereby reducing the amount of costly stainless steel and other hardware per culture volume.

Different mass transfer processes obey different scaling laws, which results in scale-dependent process efficiencies. For instance, the time to disperse nutrients from the source over the entire tank depends linearly on the tank size. The consumption rate of the nutrients by the cells, on the other hand, does not explicitly depend on the size. As a result, nutrient concentration profiles are scale dependent, being homogeneous in small reactors, while showing large variations in large reactors.

By combining mathematical methods with CFD, we can relate process conditions to the spatiotemporal species distributions. In Figure 6, we show oxygen transport simulations from the single litre to the mega-litre scale. These simulations are a cheap way to provide design rules for efficient mixing, which depend critically on the hardware configuration employed.

Optimising hardware using trial-and-error experimentation is very costly and time consuming since hardware modifications may introduce unforeseen side effects, like creating dead-zones, increased fouling, or detrimental shear stresses. In this context, CFD is an essential tool to predict these effects and guide hardware selection before committing to real-world experimentation.

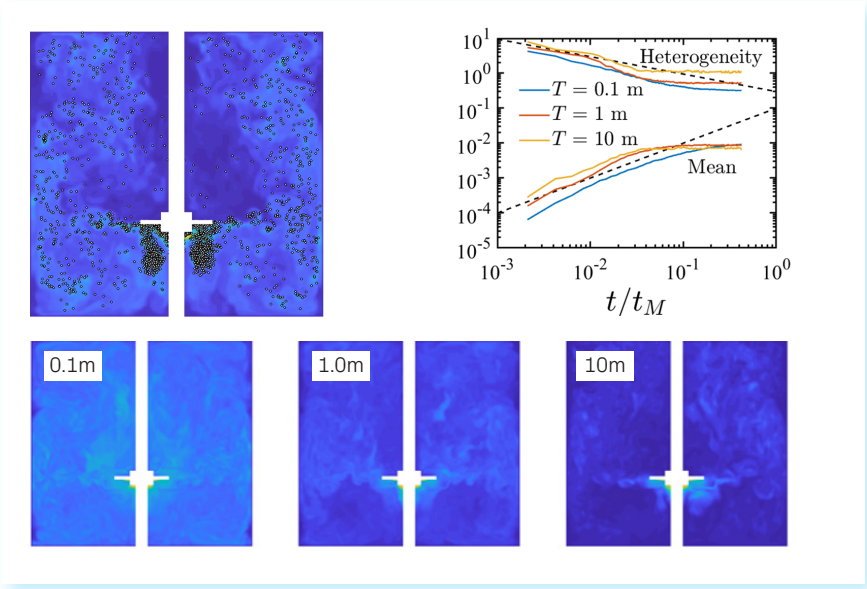


Figure 6 (Top left). Bubble positions and oxygen concentration in vertical plane intersecting the turbine axis (Top right) Mean and variation (heterogeneity) of dissolved oxygen concentration as functions of time for different tank sizes T=0.1 m,1 m and 10 m. (Bottom) instantaneous dissolved oxygen concentration for different tank sizes.

Guiding operation with digital twin technology

Running a successful bioprocess relies both on optimised hardware as well as on advanced control software.

Despite all efforts to the contrary, the complexity and unpredictability of cells growing in a reactor imply that each reactor batch will be subtly different, and conditions need constant monitoring and adjusting by highly experienced biotechnicians. These manual operations, however, are too labour intensive to be practical for the large-scale demands of the future bioeconomy. Instead, these demands require automated monitoring and control algorithms, based on a digital representation of the bioreactor, referred to as a digital twin (DT) .

A DT embodies a real-time simulation that is being driven by measurement data, and that is used to predict and control the reactor process. The aim of the DT is to provide as much information as possible on the spatiotemporal distributions of the biochemical species, and other physiochemical quantities. The DT achieves this by real-time fitting of the simulation to the measurement data, thereby reconstructing additional information that is not directly contained in the data.

Like the CFD simulations described above, the core of the DT consists of the transport equations for mass, momentum, and energy. However, since the DT runs in real-time, the computational complexity of the DT model should be reduced as compared to the full-blown CFD model. Consequently, the DT transport equations contain empirical correlations for various mass transfer and mixing processes. These correlations can be found in the literature or by conducting experiments or CFD simulations. In addition, the DT may refine these correlations, based on the continuous comparison between simulation and measurement data.

The complexity of computational bioprocessing cannot be overstated, and DTs have not yet been employed in industrial bioprocessing. The reasons for this include the cost of developing such complex modelling systems, the lack of multi-disciplinary expert knowledge, the lack of computational resources at line, and the aversion of the industry to change. Notable exceptions, however, include companies developing novel cell-and-gene therapies.[6]

For high-volumes of low-value biomanufacturing applications, DTs may well prove essential to reach commercial viability. Once implemented, the DT may provide the ability to dynamically control conditions by predicting, for instance, optimum strategies to feed the nutrients and harvest the cells at the most efficient time in the growth cycle. These automated solutions could unlock savings - valuable when working to the small margins, typical in food, textile, chemical and biofuel production.

6. Digital Twins Help Bioreactors to Produce Personalized, Cell-Based Therapies, Ansys

Come and talk to us!

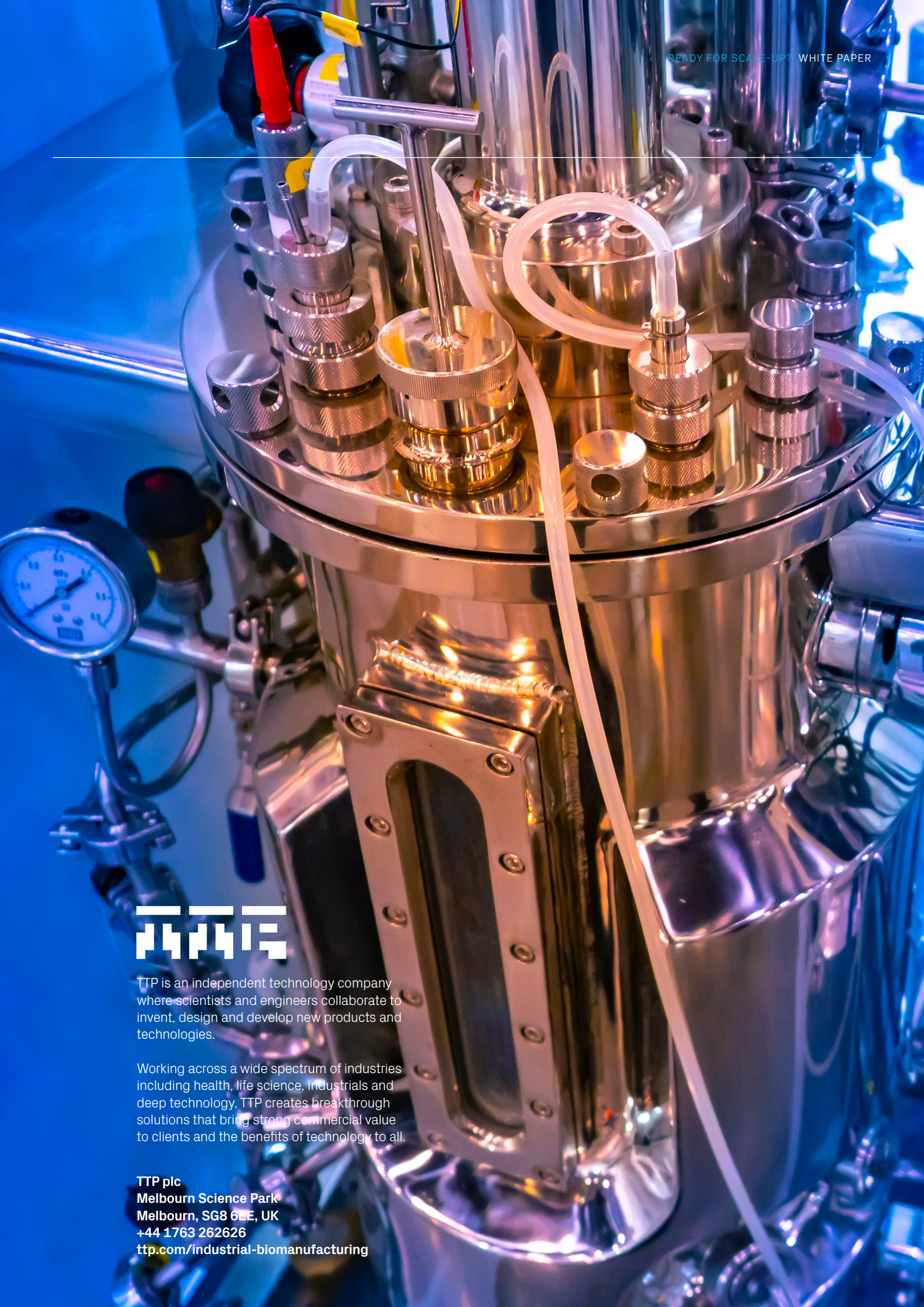
Biomanufacturing at TTP includes bioprocess, process technology, advanced manufacturing, biosensing and advanced material deposition, combined with expertise in material science, cell and molecular biology, machine learning and artificial intelligence.

We combine these technologies and our know-how to solve large-scale engineering challenges to enable more sustainable manufacturing business models and improved resource efficiency.

Whether you are the developer of a breakthrough cell culture or fermentation process, an equipment manufacturer looking to create a new offering for this market, or a business determined to create Intellectual Property in this area, TTP offers a 30-year track record in developing novel and successful technologies for industries from bioprocess to cell therapy.

Please reach out to learn how our experts can help.

Find us at ttp.com/industrial-biomanufacturing or contact us at enquiries@ttp.com



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Working across a wide spectrum of industries including health, life science, industrials and deep technology, TTP creates breakthrough solutions that bring strong commercial value to clients and the benefits of technology to all.

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