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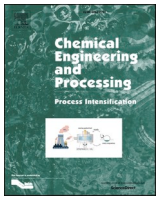

Bioprocess intensification: A route to efficient and sustainable biocatalytic transformations for the future

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ABSTRACT

With the current pressing need to rise to the ambition of net zero targets to mitigate carbon emissions and climate change impacts, sustainable processing has never been more critical. Bioprocessing has all the desirable attributes to respond to the sustainable processing challenge: use of cheap, renewable resources, nature-inspired, highly selective biocatalysts operating optimally under mild conditions and reduced energy consumption/carbon footprint. With bioprocessing productivity being far from ideal to meet the large-scale need for food, drugs, biofuels and bio-based chemicals, there has been tremendous interest of late in developing intensified bioprocesses, with significant advancement achieved in tailoring and utilising the technologies in the toolbox traditionally applied in chemical process intensification.

This review highlights the wide range of activities currently on-going in bioprocess intensification, focusing on upstream, bioreactor/fermentation and downstream separation steps. Great strides have been made in biocatalyst engineering and high density cell immobilisation for significant productivity enhancement, which, in conjunction with elegant process innovations such as novel bioreactor technologies and in-situ product separations, are enabling bioprocesses to become more competitive than ever before. The future prospects of bioprocess intensification are promising but there are still challenges that need to be overcome to fully exploit this technology.

1. Introduction

Few industrial examples of applying process intensification (PI) from the chemical and green chemistry industries [1–3] to intensify the rate, increase the yield, or significantly minimize power, footprint, and water use in biological and biosolar processes which utilize enzymes and live cells have been reported over the past decade. This is despite the overwhelming number of reports that synthetic biology can now rapidly generate novel biocatalysts, vaccines, antibodies, biological medicines, and engineered cells that will soon revolutionize our lives [4–8]. This apparent lack of emphasis on the efficiency, sustainability, cost effectiveness, and yield of biomanufacturing (Bioprocess Intensification, BPI) is primarily for three reasons: (1) biocatalysts are much slower (several orders of magnitude for cell culture and fermentation) and much less stable (for many cell-free enzymes) than the comparable rate and stability of chemical catalysts, (2) many bioprocesses are highly regulated (especially in the pharmaceutical and food industries), and (3) the scale of the products is market driven.

Many high value-added products, such as those in the pharmaceutical industry, produced by industrial biotechnological processes can remain profitable even with low efficiency batch manufacturing. The realisation of the potential for increased efficiency using truly continuous biomanufacturing is slowly developing for some bioproducts as their market penetration increases, but this is often frustrated by the lack of continuous product recovery and purification operations, particularly for complex labile molecules such as glycoproteins [9–11]. In contrast to high molecular weight glycoproteins, the continuous isolation of peptide-derived antibiotics such as beta lactams using the Podbielniak centrifugal extractor has been used since the 1940s and is an outstanding example of BPI [12]. Therefore, a new holistic approach to training

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engineers to design intensified bioprocesses (upstream and downstream) and new equipment for more efficient bioprocessing of live cells and continuous processes for the isolation of more complex molecules such as proteins, enzymes, vaccines, and peptides is critical.

In the last decade, our planet, our climate, and the future of sustaining our quality of life have changed. Due to population growth, changing climate, challenges for food supply, access to clean water, public health threats, inadequate sanitation, deteriorating air and water quality, the need has never been greater for engineering highly stable biocatalysts. Future biocatalysts must be as stable and efficient as chemical catalysts to process very large volumes of air, water, pollutants, capture and recycle greenhouse gases, recycle resources, and capture solar energy with minimal energy and water use. Unexpectedly during this past year, a new need surfaced - to rapidly develop, test in clinical trials, and scale-up the manufacture of hundreds of millions of doses of vaccines and distribute them for global vaccination to stop a novel Corona virus pandemic. None of these global challenges are driven by existing market economics. Addressing the above global needs using the selectivity of biological catalysts will need a very different engineering approach. New advanced materials or composite devices developed for other industries may need to be adapted for use in large-scale bioprocessing. The scale of the material to be processed using enzymes and live cells required to meet future global challenges will be far greater than those in existing bioprocesses that manufacture food components (amino acids, organic acids, flavours, fragrances, fine chemicals), alcohols, solvents, modify lipids, generate new antibiotics, pharmaceutical or chemical intermediates, enzymes (food grade, industrial, therapeutic), high-value human biological medicines (antibodies) or live cells for regenerative medicine. Only by intensifying the use of highly stable biological catalysts and developing continuous bioprocesses using novel approaches can these very significant global needs be addressed.

Alongside conventional process engineering approaches to intensify process, nature has endowed biocatalysts (no matter their format) with the ability to be altered through changes to the genetic code. Using the whole range of recombinant DNA technologies allows not only new-to-nature products to be made, but also at new-to-nature rates and with new-to-nature stabilities under the required industrial conditions. There is therefore much scope to open up the biotechnology field to new applications on wider scale, as will be highlighted in this article.

This review paper first introduces the concept of bioprocess intensification, making a clear distinction between the "intensification" and "optimisation" terminologies and emphasising the key approaches of novel equipment, process and material design that define bioprocess intensification. This is followed by a state-of-the-art review of the recent advances made in the intensification of upstream, biotransformation and downstream separation/purification stages of bioprocesses as well as in the integration or hybridisation of reaction-separation steps. Advances made in bioprocess modelling as supporting tools for bioprocess intensification are also highlighted. Finally, the future prospects for bioprocess intensification developments particularly in the context of the grand challenges currently facing humanity are presented, with a view to stimulating further research in these important areas.

2. Bioprocess intensification: targets and approaches

A conventional bioprocess typically comprises of 3 stages: upstream, biotransformation/bioreaction and downstream separation and purification steps, as illustrated in Fig. 1. Each of these stages offers several opportunities for intensifying a given bioprocess.

Bioprocess intensification has been defined as an increase in bioproduct output relative to cell concentration, time, reactor volume or cost [14]. One of the important questions however is what is regarded as 'intensification' as opposed to 'optimisation'. There are subtle differences between the two terms and often they are used interchangeably. Both 'intensification' and 'optimisation' refer to performance improvements leading to increases in productivity [15] but intensification is associated with more of a *step change* in technology resulting in not only a considerable increase in productivity but a significant improvement in other environmental and economic metrics such as energy consumption, carbon footprint, OPEX and CAPEX etc [2,16–18]. A step change involves a drastic change in equipment and/or process design e.g., moving from batch to continuous processing or integrating process steps such as reaction and separation or purification stages all in one multifunctional equipment [2,16,18]. Optimisation, on the other hand, is an *incremental improvement* in productivity or energy usage, which involves, for example, a straight-forward change in operating conditions in existing equipment; one simple example is adjusting the media composition in a chosen bioreactor to optimise cell density [14]. Optimisation can of course be superimposed upon the intensification step.

It is useful to define quantitative targets for the desired improvements in order to set an indicative benchmark for achieving bioprocess intensification. In our experience, a target of at least 50% and ideally >1-fold increase in productivity, titre, biomass concentration or any other relevant bioprocessing metric should be feasible, measured against an established base case in conventional bioprocessing system, where these parameters are already optimised. These metrics for bioprocessing are necessarily lower than in chemical process intensification (where

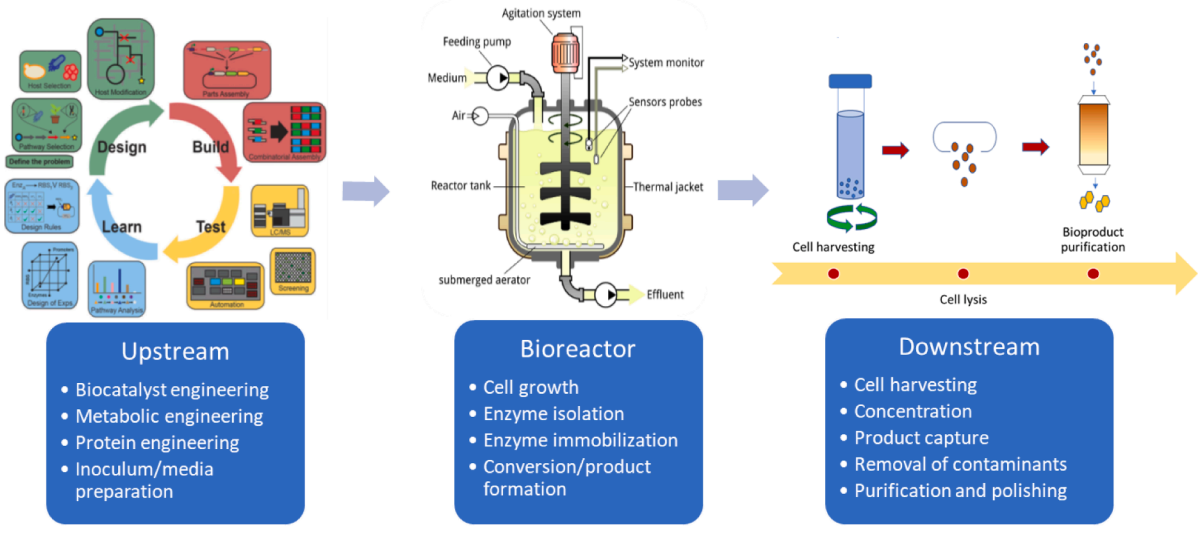

Fig. 1. Processing stages in a typical bioprocess (upstream and bioreactor images courtesy of Petzold et al. [13] and Y. Mrabet, respectively).

process enhancements, whether productivity, product characteristics etc. are expected to be several folds to qualify as intensification [19]) due to the handling of more sensitive biocatalysts which, as explained above, cannot operate under as wide as window of operating conditions as chemical catalysts can [20]. It is to be noted though, that whilst these quantitative measures of productivity and other bioprocess-related parameters are useful guidelines to enable a quick evaluation of the extent of processing improvements, there is much more to achieving bioprocess intensification. The development of greener, more environmentally friendly processes (e.g., by reducing water use, using less harmful solvent or replacing them with greener solvents, reduced energy consumption and carbon footprint) is also of great interest, as is the need for reducing the physical footprint of equipment to facilitate mobile, distributed or modular manufacturing. Therefore, smaller improvements in the stated metrics may still be regarded as intensification, particularly if the novel technologies or processing methods enable otherwise challenging biotransformations to be performed at large scale in an environmentally-friendly and sustainable manner and open up new possibilities in bioprocessing.

The implementation of BPI naturally follows from the generic PI principles developed for chemical processing and which involves the three often overlapping or integrated approaches of designing innovative equipment, methods and materials for more efficient, cleaner and inherently safer processing [21,22]. This is illustrated in Fig. 2 below. As has been elaborated on a number of times before, multiple benefits can be derived through this approach including smaller equipment and plant footprint, reduced operating and capital costs, faster processing, reduced waste and energy use and the ability to produce molecules with very tailored characteristics amongst others [1,219,23]. BPI by innovative equipment design can include technologies based on radically different concepts involving, for example, immobilised cell reactors, perfusion bioreactors, falling film reactors and so forth whilst innovative processing relate to multi-functionality or hybridisation concepts and use of alternative energy sources such as ultrasound, microwaves etc. for more efficient and targeted energy input.

It is important to highlight the importance of the 'material' approach of the general intensification strategy [,[21,22]] i.e., the 'biocatalyst engineering' in the context of bioprocessing which plays a crucial, synergistic role with the process engineering innovations in maximising the bioproductivity [24]. Bioprocesses typically involve whole cell microorganisms or enzymes which tend to work under tight conditions of substrate and/product concentrations for optimal performance. Outside these optimal process boundaries, cell growth in fermentation or enzyme activity in biocatalytically driven transformations may drop. By re-engineering the biocatalyst to make it more stable and more reactive at higher substrate and product concentrations, there is much scope for extending the operational boundaries in order to maximise productivity in tandem with the bioprocess improvement strategies. Material innovation therefore involves efficient biocatalyst design, support materials and novel approaches using biocomposites for example.

The most recent advancement in bioprocessing based on a selection of these individual approaches or ideally a combination of them will be highlighted in the state-of-the-art review of technologies.

3. State-of-the-art review of bioprocess intensification technologies

The state-of-the-art review is divided into sections according to upstream (biocatalyst engineering and novel immobilisation techniques), bioreactor (continuous processing) and downstream processing steps in a conventional bioprocess. This is followed by a review of intensification techniques for hybridisation and alternative energy input where the described applications complement the upstream, bioreactor and downstream processing. A review of bioprocess modelling progress achieved in all three stages of bioprocessing with specific references to intensification concludes this section.

3.1. Biocatalyst engineering

In bioprocesses, improvements through biocatalyst modification can result in step change enhancements in the resulting process metrics and thereby intensification. Several reviews have emphasized the particular and special role of biocatalyst modification using a range of recombinant techniques to enhance bioprocesses [24,25]. Such modifications are particularly powerful and can also result in secondary improvements in the process. A recent review of potential microbial modification techniques illustrates well the plethora of tools available [26]. A few examples divided into microbial and enzymatic processes are given here to further illustrate the point.

3.1.1. Microbial processes

In microbial processes a useful economic surrogate is to use the metrics of titer (g/L), rate (g/L/h) and yield (g/g). These three metrics reflect downstream, reactor and operating costs respectively and serve

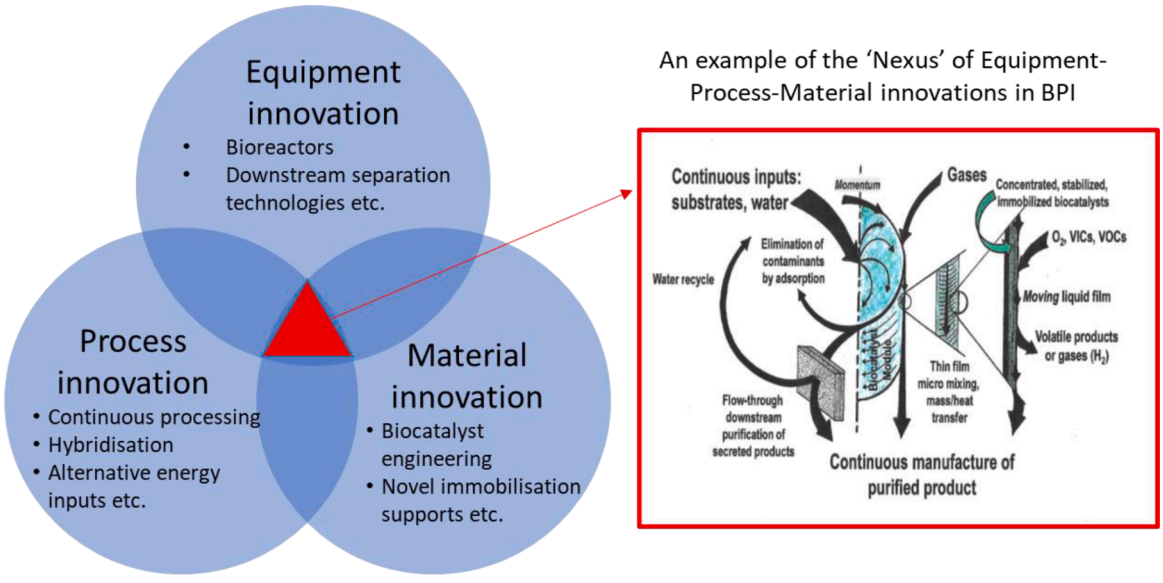

Fig. 2. Bioprocess intensification approaches.

as useful ways to benchmark intensified fermentation processes.

Titer: Co-production of acetone and ethanol using a modified strain of *E.coli*, incorporating additional enzymes gave a 35-fold improvement in product titer [27]. More recently data-driven approaches to guide metabolic engineering have also been employed to enhance product titer [28].

Productivity: Heat treatment of a psychrophilic organism which expresses heterologously the necessary genes for an interesting pathway, gave an improvement in cell permeability resulting in a 6-fold enhancement in itaconic acid productivity to 1.41 g/L/h [29]. Likewise transporter engineering through the deliberate insertion of genes expressing transporter proteins can be used to enhance rate, where the cell membrane otherwise acts as a limitation [30]. Finally, productivity can be increased via the use of a higher cell concentration in the fermentation, but this is at the expense of yield. To break this trade-off an alternative approach has been to separate the growth and conversion stages. Such decoupling of growth and conversion can result in significant improvements in productivity, and potentially yield [31–33].

Combined improvements: A superb example of improvements in all three metrics is presented by the process for the production of 1,4-butanediol in *E.coli*. Here detailed engineering of enzymes, pathways, metabolic network and the organism resulted in titer, rate and yield enhancements from 29 g/L, 0.6 g/L/h and 0.253 g/g to 99 g/L, 2.1 g/L/h and 0.351 g/g, respectively, with significant economic benefits [34].

Downstream processing: In the production of organic acids, 50–80% of the total processing costs lie downstream [35]. Succinic acid production using microbial species has been studied for many years. Although multiple product recovery schemes have been proposed, the major challenge downstream is the by-products [36]. In particular, the production of formic and lactic acids not only represent a loss of yield, but also result in a difficult separation, ultimately preventing crystallization. Using metabolic engineering a strain producing fewer by-products was recently developed [37]. Here only pyruvic acid and acetic acid (boiling points of 118 °C and 165 °C, respectively) are produced (in low amounts) enabling easy separation by distillation from succinic acid (boiling point 235 °C), allowing a simplified downstream processing and ultimately recovery via crystallization. Likewise, several *E.coli* strains have also been engineered to reduce other by-products in the biosynthesis of succinic [38] and other organic acid products [39].

The majority of metabolic engineering is focused on well-known and tested microorganisms such as *E. coli* and *S. cerevisiae*. Great success has been achieved in some cases, leading to commercialization. In reality, many other non-conventional platforms can also be used, opening still further opportunities [40] as well as cell-free and enzymatic processes.

3.1.2. Enzymatic processes

Enzymatic processes for the synthesis and production of chemical are gaining huge interest, in particular since the processes are without cellular constraints leading already to relatively intensified conversions compared to microbial fermentation. Recent reviews of this growing field in biotechnology include those by Sheldon and Woodley [41], Wu et al. [42], Romero et al. [43]. Still, improvements are required and metrics requiring improvement in enzymatic (biocatalytic) processes through protein engineering are focused on titer, activity and stability. Reaction yield is set by the stoichiometry of the reaction and the thermodynamic properties of the substrates and products. Other techniques exist to enhance these metrics [44]. Using protein engineering techniques, enormous increases in the metrics are possible. Protein engineering is a very powerful tool to alter enzyme properties [8,45,46]. Recent examples include pharmaceutical products such as the transaminase-based synthesis of Sitagliptin [47] and the enzymatic cascade for the production of Islatravir [48]. Here enormous improvements in rate of individual enzymes, under defined (and often special) conditions have enabled the implementation of entirely new synthetic schemes which are far simpler to operate and overcome cumbersome alternatives requiring multiple protection and de-protection steps. Stability can also be enhanced many fold through protein engineering methods, today guided by computational approaches [49].

3.2. Novel immobilisation techniques

Concentrating live cells or enzymes in thin nanoporous waterborne non-toxic polymer coatings on surfaces, on porous membranes, or on flexible biocomposite materials is a novel approach to increasing cell density and minimizing water to intensify bioprocesses. Biocomposites use thin (from cell monolayers to 50 μm thick multi-layers) coating deposition techniques such as waterborne coating, colloid convective assembly and dielectrophoresis, ink-jet bioprinting, aerosol delivery, and coating on woven or nonwoven fabrics.

Biocomposite materials (flexible or nonflexible) enable new bioreactor and photobioreactor designs that utilize less water and less power for mass transfer of O_2, CO_2, and nutrients, enhanced micro mixing. Coating architecture can facilitate uniform illumination of photosynthetic microbes, as well as intensify product separation and purification. This approach to BPI is analogous to the conversion of suspended chemical catalytic unit operations to surface catalysis flow methods. Thin biocoating immobilization techniques are limited to bioprocesses that assimilate or generate gases, degrade liquid wastes, or produce secreted products. Biocomposites may be particularly useful to intensify microalgae bioprocesses [50,51].

The critical aspects are coating thinness to reduce mass transfer resistance, strong wet adhesion, and coating nanoporosity – permanent coating pores that are smaller than the embedded biocatalysts. While there is much development of bioinks and bioprinting, most of the current developments in bioinks are hydrogels or reactive polymers for printing 3D structures as tissue scaffolds and tissue/organ engineering [52–54]. Bioprinting methods for process intensification differ significantly because the goal is to print highly reactive very thin (<10 μm thick) flexible highly porous biocatalyst layers and multilayer microstructures using rapid drying low viscosity adhesive latex emulsion ink-jet bioinks for intensifying biocatalysis [55,56]. How biocoatings and biocomposites can intensify bioprocess are summarized in Table 1 and in the conceptual schematic in Fig. 3 [50,51,57–63].

The concepts of very thin adhesive nano-porous coatings and printed microstructures of concentrated biocatalysts have only been demonstrated using laboratory model systems. However, these studies show the potential to significantly reduce water and energy use for very large-scale bioprocessing of wastewater, gaseous carbon emissions, improving indoor air quality using microbes, or intensifying microalgae and cyanobacteria bioprocesses [50,64–67].

Concentrated intact live cell biocatalysts in thin layers on surfaces can generate C—C bonds to recycle gaseous carbon wastes (gas-to-liquid bioconversions), continuously carrying out oxidations or reductions, secrete products (out of the coatings), secrete complex glycosylated proteins (antibodies, antigens), and are capable of efficiency harvesting solar energy to carry out photosynthesis (biomimetic leaves) similar to plant leaves [61–63]. However, generating coating microstructure and adhesion using latex binders that stabilize biocatalyst activity is challenging, particularly for whole cells that must remain viable, but not grow [57,58]. The microstructure and adhesive properties of biocoatings and bioprinted biocomposite materials are generated by a controlled drying process subjecting the entrapped biocatalysts to desiccation stress Some bacteria and yeasts are naturally desiccation tolerant, but most other cell types, particularly algae and mammalian cells are not and may be engineered in the future to withstand this stress [68,69]. Osmoprotective carbohydrate stabilizers can be added to coating formulations to protect cell viability during drying. Future desiccation tolerant engineered biocatalysts used in the environment will also require that they are permanently embedded in the polymer coatings and will not be released [51]. Very thin nanoporous top coatings may be used to minimize release of live biocatalysts.

Generation of biocoating nanoporosity architecture and sustaining

Table 1
Significant bioprocess intensification possible using biocoatings, bioprinting, or novel biocomposite materials may be achieved by engineering one or more features.

Engineered Feature	Functional Benefit to BPI
High cell concentration (non-growth conditions)	Adhesive polymers permanently concentrate live cells, combine different cell types, or enzymes (500 to 1,000-fold over suspension bioreactors) on surfaces in thin nano porous coatings. Enables continuous multi-step bioprocessing, elimination of biomass waste, eliminates separating biomass from secreted products.
Stability of reactivity	Non-growing biocatalysts stabilized for 1,000s of hours to achieve comparable half-lives with chemical catalysts. Dry stabilization (lyoprotection) enables storage/shipping without cold chain.
Ease of fabrication	Biocatalyst adhesive deposition on flexible materials enables linear roll-to-roll additive fabrication.
Light penetration, UV protection, reflectance, photoreactivity	Multi-layer biocomposite coatings enable engineering both light absorbing/scattering of polymer matrix and immobilized photoreactive cells for efficient light penetration, solar energy harvesting, UV protection for live cells, access to nutrients/water, photoreactivity.
Cell/enzyme reactivity	Upregulation of non-growth gene expression (1,000-fold) after immobilization demonstrated enhancement of cell specific reactivity. Increased live cell specific reactivity (5 to 10-fold) demonstrated in microalgae biocomposites (by direct cell-to-cell contact).
Process improvements: modular scale-out for reduced water, power consumption, footprint, more uniform product, eliminates seed stages.	Biocomposite material irrigated with thin liquid films enable increased mass transfer, micro mixing, secreted product concentration, efficiency of product recovery, potential for integration with downstream membrane product or contaminant adsorbers

porosity and adhesion following rehydration are critical to immobilized cell viability. Recently advances have been made in generating permanent coating nanoporosity and optimize light penetration by novel coating microstructure approaches [51,70]. The biology of coating-entrapped cells limited by nutrients, process conditions, or engineered by synthetic biology not to divide following immobilization is also challenging. Although the chemical and pharmaceutical intermediate industries have utilized "resting cells" as suspended biocatalysts for decades, the biology of non-growing nutrient-limited cells and how to sustain their activity and viability are still largely unknown [71].

Continued development of porous biocoatings, adhesive bioinks, colloidal biocoatings, and robust biocomposites will transform suspended cell bioreactors and photobioreactors into continuous flow-through intensified membrane or thin liquid film bioreactors (Fig. 4). These concepts could also be used in the future for secreted protein products integrated with product purification by contaminant capture membrane absorbers.

3.3. *Continuous bioprocessing*

Continuous bioprocessing is gaining more attention due to advantages such as constant nutrient conditions, removal of by-products/waste, an ability to generate high density cultures, no lag phase once the system operating and no end of culture viability issues. It is to be noted though that changes in cell metabolism are inevitable over long periods of time and the complexity involved in controlling the metabolism towards target product expression does not warrant operation beyond the 'useful' lifespan of the cells. Therefore, a typical timescale for a continuous biotransformation operating under steady-state at peak concentration and product yield is generally 30–90 days [10] after which a fresh culture is inoculated. Another driver for continuous manufacturing is to debottleneck the downstream processes. There are no issues such as emptying, cleaning and transfer which increase the risk of contamination or product degradation and is time consuming and labour intensive, resulting in a higher productivity for continuous processes. Continuous processing also allows for in line purification, advantageous as a key cost for bioprocesses is the downstream processing, as highlighted already earlier on. It is also often easier to scale up as the continuous units are generally smaller and suitable to numbering up approaches, ultimately allowing for cost effective, more space, time and resource efficient processes.

As the continuous processes are an integrated system, more advanced process control systems are needed, and if one unit is not working, the whole process is affected. Process analytical techniques are very important and there is future scope to further understand and predict the processes and optimise control and sampling. The approach taken must be systematic, combining process control and analysers, multivariate tools for design, data acquisitions and analysis as well as allowing for continuous improvements [72,73].

Continuous flow bioprocessing in microreactors (or microfluidics) has received much attention in recent years. There has also been a particularly strong focus on the development of perfusion processes in

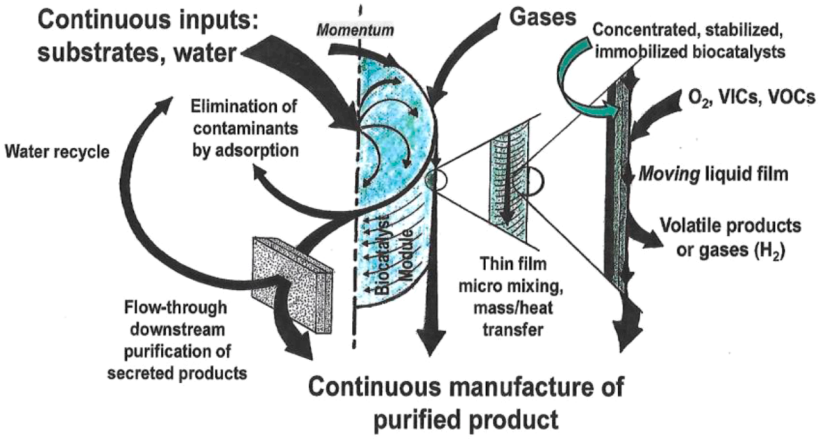

Fig. 3. Conceptual illustration of bioprocess intensification in integrated continuous flow reactor-biocatalyst design.

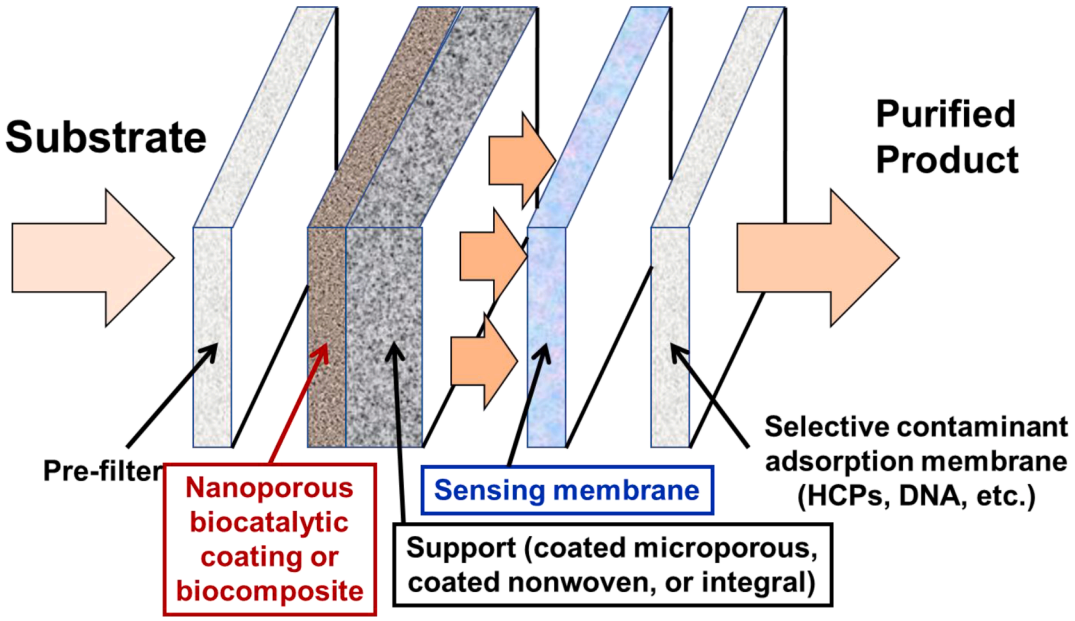

Fig. 4. A concept for a flow through biocomposite membrane bioreactor integrating product purification by contaminant capture membranes for bioprocess intensification of secreted protein products.

the pharmaceutical industry as an enabling technology for streamlined continuous biomanufacturing. Developments in both of these areas will be highlighted here.

3.3.1. Microfluidic systems for bioprocesses

Microfluidic systems process small amounts of liquids using channels at the microscale in bioprocessing, allowing for low volume and high surface area. Key advantages are the small size, rapidity, precise control of mixing, reagents, flowrates, addition of chemicals, flexibility of experimental set up, heterogeneity and an ability to perform multiple experiments under highly controlled conditions rapidly. The channels are fabricated from a range of materials such as silicone, glass, plastics and paper. They are well suited to biology due to their scales (typically of the order of 10–100 mm) being comparable to those of cells [74]. To address the operational challenges due to blockages by solids in such system, customised reactor design and operating conditions need to be considered [75].

Microfluidic channels have been successfully exploited for biocatalysed transformations, especially where integrated reaction-separation steps can be implemented [75]. There is a plethora of examples on continuous microflow technologies that have been developed at a rapid pace over the last decade. Tamborini et al. [76] and Žnidaršič-Plazl [77] provide well written reviews of such continuous flow bioprocesses, focusing on a variety of meso and microreactor configurations for application in enzymatic and whole cell biotransformations where several fold productivity enhancements and increased stability of biocatalysts are highlighted in a wide range of examples and opportunities for continuous biomanufacturing. De Santis et al. [78] highlight more recent updates in continuous flow biocatalysis since 2018. With application of continuous microreactors to bioprocesses being well known and well documented in the literature already, we have provided only a selection of the most recent investigations in microreactors and their intensification potentials [79–82] in Table 2. We would refer interested readers to the published reviews on this topic for more detailed information.

3.3.2. Perfusion processes

Perfusion technology which can be applied at both the seed and bioreactor stages is an alternative process intensification technique which has significant advantages over the traditional fed-batch reactor [83–86]. It operates by continuously removing media from the reactor whilst the cells are either retained or returned to the bioreactor allowing higher cell densities due to optimal nutrient conditions and the removal of toxic by-products. Key advantages are higher biomass yield, smaller batches, easier product purification, numbering-up and higher productivity whilst issues are fouling, plugging, higher media requirements and more complex operation [87] and how changes in scale-up affect the cells processes. Fraser and Endres [87] list a range of classic perfusion reactor systems which include membrane reactors, hollow fibre reactors and fixed and rotating bed reactors. Fig. 5 illustrates two typical set-ups of a perfusion reactor, an alternating tangential flow (ATF) and tangential flow filtration (TFF) [88]. There is little difference in the perfusion rate between the two systems, but the ATF exhibits smaller maximum stress values whilst there is significantly more hold up of product in the TFF. This is attributed to the different hydrodynamic patterns, where there is a constant backflush of the permeate in the ATF in comparison to the one directional flow for the TFF system.

Applications using perfusion bioreactors include tissue engineering, production of enzymes, animal cells etc [89]. Industrial examples have been reported by Boehringer who showed an increase in the cell specific productivity up to nearly 4x [84], Sanofi who were able to achieve 4 $gL^{-1}day^{-1}$for an IgG [83] and Talo et al. [90] who describe the non-straightforward process of the industrialization of a perfusion bioreactor, complying to GMP and taking an integrated approach where all disciplines worked collaboratively, ultimately producing a 12 disposable independent perfusion culture chambers generating various biological tissues. Research is moving from investigating methods to increase cell densities, to a larger focus on, for example, tissue engineering and its application in building cell structures and using advanced tools to understand the product quality [91]. For example, Tao et al. [92] highlighted the application of a wave reactor with a floating filter to produce high density cell banks with sufficient number of cells, replacing the traditional multi steps using shake flasks to generate sufficient cell culture and thereby reducing the manufacturing plant time to nine days. Some other approaches to increase the efficiency of the perfusion process include (i) design of the settlers where the Kompala research group have further designed their inclined settlers with new compact settlers with 6x more inclined settling areas, which achieved a four-fold increase in cell productivity [93] (ii) design of the bioreactors where Chouinard et al. [94] use a pulsative flow perfusion bioreactor to

Table 2
Overview of bioprocess intensification technologies, their intensification characteristics for selected examples and potential limitations of application.

Technology/ technique (listed alphabetically)	Biocatalyst	Examples of intensification characteristics and key observations in representative bioprocesses	References	General remarks (e.g., favourable application areas, technology/technique limitations in bioprocess applications)
Biocatalyst engineering	Live cells	• > 5 fold and up to 40 fold improvement in specific productivities and titer respectively in two stage (growth decoupled from metabolite formation) fermentation process • > 3x increase in overall productivity of succinic acid made possible by strain and metabolic engineering in fed-batch fermentation of *M. succiniciproducens* (3.49 g/L/h vs. 1.21 g/L/h compared to wild type organism); titer as high as 90.68 g/L enables easier downstream purification to >99.9% purity	[32] (and references within) [37]	Dependent on ability to switch between growth and production stages. Also dependent on viscosity, which in some cases may limit the production stage.
Biocatalyst immobilisation	Live cells (bacteria, cyanobacteria, microalgae, yeast)	• 5-10 fold enhancement in specific rate of carbon assimilation using very high concentrations of live cells in thin nanoporous adhesive coatings and biocomposite materials • 3x increase in ethanol concentration from 3D porous geometries printed from bioink material of live yeast cells compared to bulk material with 4x higher productivity (21 g ethanol/L h vs 5 g ethanol/L h); >2 orders of magnitude increase in cell density on porous scaffolds; improvement due to mass transfer enhancement in porous lattice structure.	[61,64] [56]	For biocoatings, thin layers of no more than 100 μm thickness are required to reduce mass transfer resistance and to maintain as uniform photoreactivity as possible in light-driven biocatalytic processes. Bioprinting can achieve very thin layers (<10 μm thickness), in multilayer microstructures for greater biocatalytic performance. Dessication stress during the biocoating/ bioprinting process may pose a problem for some cell types e.g., algae and mammalian cells, which may require engineering or addition of stabilizers for greater viability.
Continuous flow microreactor or microfludics	Enzyme	• Productivity enhanced by 315x with immobilised β-glucosidase in continuous glycosylation of the natural compound perillyl alcohol in a microbioreactor compared to a batch immobilized system; long term stability of the immobilised enzyme observed • 224-fold increase in productivity and 7-fold improvement in efficiency in a continuous flow microreactor compared with a batch reaction for lactose oxidation by galactose oxidase	[79] [80]	Microchannel blockages by solid particulates from reaction products or non-solubilised reagents may prevent uninterrupted operation [75,76].
	Whole cell biocatalyst	• Enhancement in gas-liquid mass transfer in whole cell biofilms in microchannels can increase productivity and long term stability of the biofilm if nutrient limitation is avoided (e.g., for styrene oxidation by *Pseudomonas taiwanensis* VLB120ΔC, increase in productivity from 11 to 46 g. L_{tube}^{-1} day^{-1} when supply of oxygen increased	[81,82]	In processes involving whole cells immobilised in a biofilm structure on the walls of a microchannel, it is important to carefully select operating conditions to prevent excessive film wash-out. Controlling the biofilm thickness is another an important consideration in such systems- excessive biomass formation may result in high pressure drops and in the worst case scenario even block the microchannel.
Electric fields	Enzyme	• Specific rate of enzymatic hydrolysis of high oleate sunflower oil in semi-batch reactor increased by up to ~4 fold when electric field strength increases from 10 kV to 30 kV • In a membrane-based microextractor, separation efficiency of electrically charged molecules doubled from 50% to 100% at short residence time under applied electric field,	[209] [211]	Joule heating effect of electric field application may thermally degrade enzymes. Controlling the magnitude of the external voltage would be critical here.
Falling film bioreactor (FFBR)	Potential for use with live cells (bacteria, cyanobacteria, algae)	• Prototype FFBR demonstrating efficient gas-to-liquid mass transfer in thin falling liquid film (high kLa values $>10^3$ h^{-1}); promising potential for continuous large-scale gas bioprocessing of gaseous carbon to chemicals using immobilised biocatalyst with benefits of reduced water and power input and product recovery costs (less dilute product stream)	[66]	
	Chlorella vulgaris culture	• Algal biomass concentrations up to 100 times higher than in a raceway-type PBR achieved under conditions of thin film flow (up to 1.5 mm thickness) of substrate over the immobilised cell structure; high productivity of 5.7 $kg{\cdot}m^{-3}{\cdot}day^{-1}$ (7.07 $kg{\cdot}m^{-3}{\cdot}day^{-1}$ in constant light); potential for reduced water use as an environmental benefit	[232]	Limitations in performance due to light attenuation through immobilised cell matrix.
	Enzyme	• Thin, wavy films with large specific surface area of $\sim10^5$ m^2/m^3 flowing over immobilised D-amino oxidase enabled high O_2 transfer rate to the enzyme surface and allowed re-use of enzyme 4×10^4 times vs. soluble enzyme	[257]	
Gas stripping	Whole cell microorganism	• In ABE fermentation for biobutanol production, significant productivity enhancements as high as 300% in fed-batch and 270% in continuous stirred	[134–136, 138]	Simple versatile technique which can be used in combination with other ISPR techniques e.g., pervaporation for enhanced product separation.

(*continued on next page*)

Table 2 (*continued*)

Technology/ technique (listed alphabetically)	Biocatalyst	Examples of intensification characteristics and key observations in representative bioprocesses	References	General remarks (e.g., favourable application areas, technology/technique limitations in bioprocess applications)
		tank fermenters and energy savings of up to 50% in a two-stage gas stripping process		Limited to removal of volatile products such as alcohols.
Liquid-liquid extraction (LLE)	Whole cell microorganism Enzymes	• Up to 30% yield enhancement for p-coumaric acid (p-CA), a hydrophobic product obtained from an engineered *S. cerevisiae* strain and extracted with oleyl alcohol, whilst its degradation is significantly reduced by up to 3-fold • 5-fold increase in bio-based carboxylic acids concentration from 10g/L to 50 g/L in an optimised LLE-membrane ISPR process coupled with distillation giving a 3-fold reduction in carbon footprint at the higher titer; compared to a non ISPR process, the carbon footprint reduction is 560-fold. • In ω-transaminase-catalysed reaction, product concentration (26.5 g L^{-1}), product purity up to 70% g/g and product recovery of ~80% achieved in a 2 stage in-situ LLE extraction process, much higher than can be obtained with conventional batch or fed-batch.	[124] [129] [258]	Solvent/ionic liquid toxicity to microorgansims requires careful selection of biocompatible solvent, especially if the system is designed for direct contacting. Perstraction, involving a physical barrier such as a liquid or solid membrane, may be considered instead if toxicity is likely to be a major drawback. Solvent may also interact with immobilisation structures e.g., polymers [123] and limit their effectiveness to support the biocatalyst.
Membrane bioreactor (MBR)	Enzyme	• In protein hydrolysis to peptides in enzymatic membrane reactor, productivity more than doubled and yield increased by 7 fold when comparing continuous MBR with batch reactor with no membrane or with off-line membrane • Similar enhancements observed in other enzymatic transformations due to reduced product inhibition by its rapid removal in continuous MBR system	[165, 259–261]	Membrane fouling can limit long term continuous operation. Conventional techniques for fouling mitigation (e.g., ultrasound) may not be directly transferable due to the sensitivity of biocatalyst employed; a case-by-case approach is needed. In membrane immobilised enzyme, activity may be affected; effective enzyme immobilisation strategies are needed to solve this limitation.
Microwave	Enzyme	• In immobilised lipase-catalysed synthesis of n-butyl palmitate, conversion of 97.1% obtained after 25 minutes at 60°C in a 20 ml batch vessel (no power input specified), representing a 12x reduction in reaction time compared to conventional heating • Lipase-catalysed esterification of lauric acid, reaction times of less than 6 minutes were needed for high conversions of ~80% at 20 W power input	[224] [223]	Too high temperatures from microwave application may degrade biocatalysts; this can be controlled by reduced exposure time and by immobilisation structures.
Oscillatory baffled reactor (OBR)	Whole cell microorganism	• Up to 24% improvement in bioethanol production compared to STR in a SSF process using cassava as biomass, α-amylase and *Saccharomyces cerevisiae;* up to 63% reduction in shear compared to STR; uniform suspension of cells	[186]	Gentle mixing for shear sensitive biocatalyst. Too high solid loading (suspended cells, immobilised enzymes or substrates) may limit uniformity in suspension and therefore productivity.
	Enzyme	• Enzymatic hydrolysis of α-cellulose biomass at 15% loading in batch mode showed a modest conversion increase of ~7% in OBR vs STR but at a much reduced power density (7 % that of the STR)	[187]	
	Microalgae cultivation	• 95% increase in the average maximum growth rate compared to T-flask cultures due to better mixing and CO_2 availability to microalgae in OBR	[188]	
Perfusion	Live cells	• Several industrial examples by Boehringer, Sanofi, Bristol-Myers Squibb etc. demonstrate significant increases in cell specific productivity and titer by several folds in large scale processing; cell densities as high as 100 million cells/mL possible; improvements in downstream processing also highlighted as a result of higher productivity.	[10,83,84, 86]	Allows continuous bioprocessing in practice, although the perceived operational complexities and control challenges can limit wider industrial uptake of the technology [10]
Perstraction (with or w/o SLM, SILM)	Whole cells microorganism	• Using SILM for ethanol recovery from 2wt% ethanol in water, high ethanol flux of > 2.2 kg/m^2•h (vs. ~10^{-4} – 10^{-1} kg/m^2•h in pervaporation) and selectivity of > 320 (vs. ~20 in liquid-liquid extraction). • Based on a composite membrane coated with a thin film of 2-ethyl-1-hexanol as an extractant, high purity (>99.5%) biobutanol recovered from fermentation broth using less than 25% of the typical energy usage of other recovery techniques such as gas stripping and LLE; n-BuOH flux of one order of magnitude higher than state-of-the-art perstraction systems	[161] [158]	Useful when using toxic solvent as it prevents direct contact between microorgisms and solvent via a solid or liquid membrane. Problems of permeability limits with solid membrane; can be addressed by using liquid membranes. Fouling on solid membrane surface may prevent long term continuous processing.
Reactive distillation	Enzyme	• Reduced product inhibition and greater conversion in transesterification with immobilized *Candida antarctica* lipase enzyme beads; ability to achieve normally difficult separation of chiral molecules and product	[170,173]	Generally limited window of temperature-pressure conditions available to ensure optimal activity of enzymes in reaction stage.
Spinning disc bioreactor	Microalgae biocomposite	• 73% enhancement in CO_2 biofixation compared to a suspended cell photobioreactor; biocomposite matrix enables high density of living photoactive cells (8.8 ×	[50]	Potential for application in large scale processing based on continuous thin film flow by varying disc size and operational conditions; also allows more tunable thin films compared to gravity-induced

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Table 2 (*continued*)

Technology/ technique (listed alphabetically)	Biocatalyst	Examples of intensification characteristics and key observations in representative bioprocesses	References	General remarks (e.g., favourable application areas, technology/technique limitations in bioprocess applications)
		10^{12} cell loading/m^2) to absorb CO_2 over 15 hours of operation		falling film reactor. Strong adhesion of the biocomposite structure to the disc required; mechanical strength of the biocomposite structure also important for the cells to be retained in the biocomposite matrix during rotation of the disc. Thin, porous biocomposite layer is required to ensure high viability of cells throughout the structure and to reduce mass transfer limitations.
	Enzyme	• Hydrolysis rate in the spinning cloth reactor up to 75% higher than in a conventional batch stirred tank with immobilized lipase enzymes. • ~5x faster reaction with spinning cloths at 450 rpm than in batch configuration in enzymatic resolution of 1-phenylethanol process; 35% enhancement in productivity compared to batch processing using a stack of 3 cloths	[178,179]	Enzymes significantly detached from the cloth at a critical average shear of 9,500 s^{-1}. The spinning speed of the reactor should operate below this critical shear (~400 rpm) [178]
Taylor-Couette reactor	Enzyme	• Efficient suspension of shear-sensitive enzymes at even at low rotation rates • In hydrolysis of starch by α-amylase, use of a ribbed cylinder reduced axial dispersion in the wavy Taylor vortex region, to enable better mixing without compromising on the plug flow capability of the device.	[183,184]	Low and controlled shear beneficial for shear-sensitive solids such as immobilised enzymes and mammalian cells cultivation [182]. Too high rotation rates can lead to axial flow by-passing the vortices and reducing effectiveness of contact between enzyme particles and substrate [183].
	Microalgae	• 100% increase in dry biomass concentration from 6g/L to 12 g/L with increase in rotation of inner cylinder from 0 to 600 rpm, after 100 hours of batch operation at 10% CO_2 concentration in gas flow at 0.05 vvm • 60% increase in biomass productivity from 3 g/L/day vs 4.8 g/L/day under same conditions	[185]	
Ultrasound	Enzyme (immobilised)	• Increase in conversion of furfuryl alcohol from 48% to 99% in the ultrasonicated system vs. the non-sonicated set-up. • 67% energy saving in ultrasound-assisted transformation to 99% conversion vs non-sonicated system	[196]	Degradation of free enzyme by ultrasound is more readily observed whilst immobilisation provides greater protection and stability of the enzyme [193,194]. Ultrasound may compromise enzyme activity under excessive exposure times (>1 hour typically) and at high power due to high temperatures attained in the sonicated medium [194].
	Bacteria/fungal cells	• Up to 83% increase in specific growth rate and 36% increase in cell membrane permeability in low intensity US-assisted fermentation vs non sonicated. • 2.5x increase in kinetics of fermentation and 2 × rise in xylose uptake and utilization by the cells in fermentation of xylose from sugarcane bagasse using free cells of *Candida tropicalis* to produce xylitol.	[200,201]	Optimal performance with whole cells generally achieved at moderate frequency (20-40 kHz), low amplitude and mild power input. Beyond these limits, viability of cells may be compromised, especially with larger bacterial cells and bacilli-shaped cells [199] .

ABE: acetone-butanol-ethanol; LLE: liquid-liquid extraction; SLM: supported liquid membrane; SILM: supported ionic liquid membrane; STR: stirred tank reactor; SSF: solid-state fermentation; US: ultrasound.

produce a 3D cell culture and found viability and a uniform distribution of cells across the whole gel, whilst cells were mainly found on the top in the static traditional incubator. They attribute this to the ability to continuously supply nutrients and oxygen whilst removing metabolic wastes (iii) filter/membrane design to mitigate fouling/clogging during long term operation of filters/membranes where the research focus is typically on the choice of the membranes and/or the operating strategies [95–98].

3.4. Advances in downstream processing

Efforts in developing intensified downstream separation and purification steps in bioprocessing have been ongoing for a long time, with the need to develop integrated continuous upstream and downstream stages being recognised to be of utmost importance for the continuous operation philosophy to be put into practice in bioprocessing [99]. Large improvements in, for example, cell culture titers has resulted in further pressure and cost of the downstream processes [100] and future improvements in downstream processing is required to debottleneck it [101] as well as significantly reduce costs, which, for industrial scale manufacture of biopharmaceuticals, currently accounts for between 45 and 92% of the total cost [102]. Key steps can be divided in cell separation & products extraction, purification and formulation [103]. Well researched technologies include continuous chromatography [101, 104], clarification technologies [105] and viral inactivation [106] and tools include microbial engineering [103] and specialist microorganisms [107]. Further downstream processing developments have been reviewed for specific products such as fuels/commodity chemicals [108] and itaconic acid [109]. A selection of the key technologies is highlighted below.

3.4.1. Chromatography

Chromatography has shown potential to capture and purifying a range of biologics, including recombinant enzymes and monoclonal antibodies (mAb). Key design parameters are type of chromatography format (resin columns, membrane, monolith) resin stability, binding conditions, size and the biochemical properties of the target protein [101,104]. There is considerable interest in continuous chromatography and its integration with other production processes. An industrial example highlighted by Sanofi has emphasised the importance of

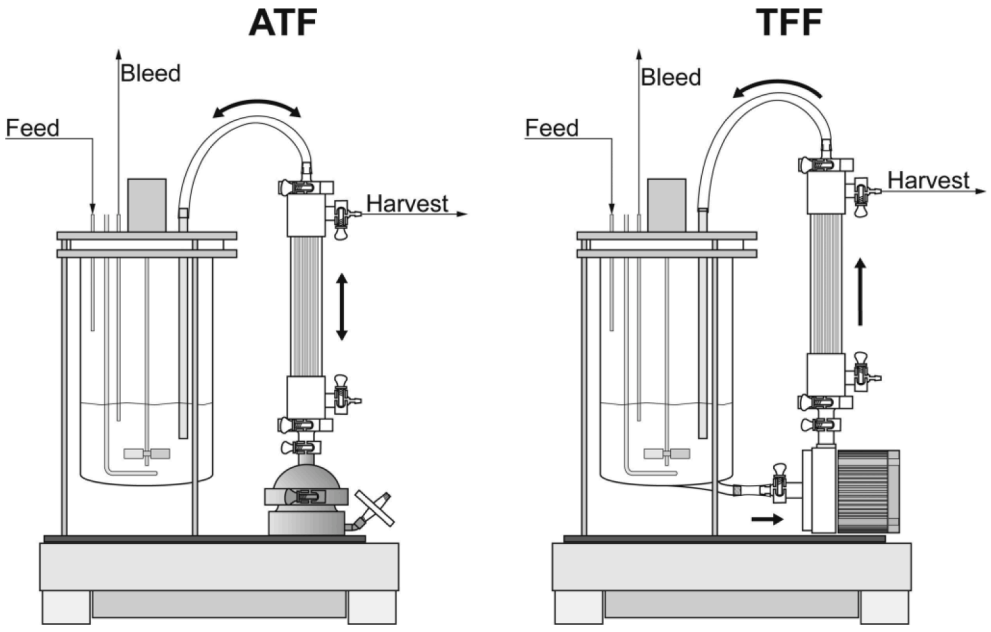

Fig. 5. Illustrations of typical perfusion bioreactors [88].

integrating processes in the design of a periodic counter-current chromatography for recombinant enzymes and monoclonal antibody. The small column integrated with the upstream process results in fast cycling, high throughput using less resins and buffers, highlighting that both the mechanical and the microbial robustness are key aspects for the system to operate continuously for months without breakdown [104]. Future research opportunities include the development of green organic solvents, reducing the waste and developing faster and more efficient separations [110] as well as addressing process modelling and control challenges [101].

3.4.2. *Viral inactivation systems*

Johnson et al. [111] review challenges in viral inactivation, including testing used and how can this also be applied for increased productivity, providing strategies and technologies and potential modifications to existing processes. Traditionally, viral inactivation occurs in large holding tanks for relatively long durations whilst continuous processing in incubation chambers with narrow residence time distributions can overcome these challenges. Specific examples include a continuous viral inactivation chamber for which a range of designs (coil, serpentine, tubular) have been compared by Orozco et al. [106]. They devised a small scale unit that is modular and scalable with small residence time distributions, thus improving efficiency and small pressure drops, showing the potential for commercially available continuous systems. Gillespie et al. [112] also studied similar chambers with inactivation within minutes, with the best design being 'packets of fluids' as that generated the most narrow residence time distribution. Filtration is well established in industry, with most application being batch processes. David et al. [113] investigated continuous filtration which was found to achieve the desired inactivation, although further research is recommended to understand the mechanisms long term.

3.4.3. *Microbial engineering*

Microbial engineering, in contrast to the above techniques, involves the direct upstream manipulation of the microorganisms to facilitate downstream processing. Approaches include making the cells easier to disrupt to improve cell lysis [114] or simplifying the separation by changing the cell shapes (i.e., morphology engineering) [115]. Wang et al. [103] have reviewed the major microbial engineering methods showing how it can become part of a toolbox to reduce complexity and cost in microbial processing.

3.5. *Advances in multifunctional/hybrid biotechnologies*

Integration of process steps is one of the most studied strategies for achieving bioprocess intensification. The most common integration involves reaction and product separation-often referred to in biotechnology as *in-situ* product recovery (ISPR)-which has resulted in bioprocessing technology developments in extractive fermentations, membrane bioreactors, reactive distillation, amongst others. Many of these (bio) reactive separation systems have been extensively reviewed recently for enzymatic processes [116] and for whole-cell fermentations [117–119]. This so-called hybridisation of functionality offers many process advantages in the context of bioprocessing. In the case of equilibrium-driven processes, increased substrate conversion and targeted product yield are obtained by forward shifting of reaction equilibrium to products as the product removal is enhanced. Very often, the process drivers are to maintain high biocatalyst activity by rapidly removing products with inhibitory or toxic properties- one well-known example of this being the inhibitory effects of ethanol in *Saccharomyces cerevisiae* fermentation- and/or to prevent degradation of products particularly sensitive to operating conditions such as pH, temperature etc [117]. There are also potential economic advantages for integrating process steps in this way, such as reduction in capital and operating costs and physical footprint for space saving on a plant. It is recognised, however, that combining process steps may result in more complex systems requiring more advanced control strategies [120]. A selection of the most prominent reaction-separation hybridisation concepts for both whole cell and enzymatic transformations is highlighted here.

3.5.1. *Extractive fermentation*

Extractive fermentation has received much attention over the last few decades as a technique for improving productivity in fermentation processes. It works on the principles of combining a continuous product extraction step with the fermentation process and is commonly carried out as an ISPR technique. Four different ISPR configurations are possible, depending on the contact with fermentation medium (direct vs indirect) and location of the product separation process (internal vs external) among which the internal direct configuration is the most studied and involves less complexity [120]. Various strategies of

extractive fermentation have been developed dependent upon the characteristics of the desired product being recovered (e.g. its volatility, hydrophobicity, polarity amongst others) [120,121]. Key ISPR technologies of interest, particularly related to biofuel production by microbial fermentations, include liquid-liquid extraction, gas stripping, pervaporation, membrane permeation, adsorption, etc [118,122]. The recently reported advances of some of the most applied techniques are highlighted here.

Liquid-liquid extraction. Liquid-liquid extraction (LLE) in biotechnology is by no means a new technology; it has been widely employed as a downstream purification technique at commercial scale in Podbielniak centrifugal extractors for penicillin recovery in the pharmaceutical industry [9], which are the one of earliest examples of bioprocess intensification technology. However, the more recent developments in LLE have been concerned with its incorporation *in-situ* within fermentation systems using conventional organic solvents [121,123,124] or more novel solvents e.g. ionic liquids [125,126]. Solvent toxicity to microorganisms is an important consideration that has limited the range of solvents that can be applied to a given fermentation process [127]. The fact that different types of microorgansims exhibit a range of toxicity behaviour vis-à-vis a certain class of solvent [123] means that solvent screening on a case-by-case basis is an important step in applying LLE to biocatalytic systems. Provided biocompatibility between the microorganisms and the solvent can be achieved, the in-situ LLE extraction technique can result in considerable improvements in product yield [121,122,124]. Even greater processing benefits for LLE as an ISPR technique in whole cell biocatalysis have been demonstrated in continuous flow reactors. In one such recently reported work [128], a microreactor equipped with a membrane dispersion system resulted in 70% reduction in reaction time to reach >99% chiral diaryl alcohol product yield compared to a stirred tank reactor due to the enhanced mass transfer across the small transient droplets of 30 μm average diameter. Often the energy requirement to recover the solvent extractant by distillation is deemed to be detrimental to the economic and environmental viability of the LLE ISPR technology. However, an LLE-based membrane process coupled with distillation to recover purified bio-based carboxylic acids at high product concentrations has shown that, compared with a non-ISPR based process, a 560-fold reduction in carbon footprint is possible [129]. With as high a titer as possible, LLE ISPR combined with distillation may become energetically and environmentally competitive compared to other ISPR extraction techniques.

Gas stripping. Gas stripping is another well-known ISPR strategy commonly applied to acetone-butanol-ethanol (ABE) and ethanol fermentation processes and is based on using a gas stream to carry volatile products such as alcohols out of the fermentation broth [130–133]. It is a simple, versatile technique which, for instance, in the hugely important ABE fermentations for biobutanol production, has demonstrated significant productivity enhancements as high as 300% in fed-batch and 270% in continuous stirred tank fermenters [134–136] and energy savings of up to 50% in a two-stage gas stripping process [137]. Gas stripping combined with other technologies such as pervaporation [138] can yield even higher titres than the individual ISPR technologies (up to 550 g/L for biobutanol for has been reported [122].

Pervaporation. Pervaporation makes use of selective membranes to separate desired components from a mixture. Its operation is based on creating a concentration gradient between the liquid feed mixture on one side of the membrane and the vapour phase of the separated molecules on the permeate side of the membrane [139]. With the continuous removal of the vapour on the permeate side, formed under vacuum conditions, a concentration gradient is set up across the membrane to drive the selective separation of molecules from the liquid to the vapour phase. Pervaporation has been intensively studied as an ISPR technique in ethanol and ABE fermentations [140–143], with productivity enhancements >200% and >120% using this integrated technology being reported for batch and continuous ABE fermentations respectively [134,144,145]. The development of high performing (in terms of selectivity and flux), economically competitive organophilic membranes for such sustainable processing applications is the subject of much interest amongst the research community [146–148], with the focus being on composite membranes [149,150] and supported liquid and ionic liquid membranes [151,152]. There is also a need to develop more fouling resistant pervaporation membranes for long term application in continuous fermentation systems [153].

Perstraction. Perstraction technology is another membrane-based technology that works in conjunction with LLE. Instead of direct contact between the organic extractant and the fermentation broth in cases where toxicity of the organic phase may pose a problem, a solid or liquid membrane acts as barrier between the two phases [154]. Significant productivity enhancements have been observed using the perstraction technology based on free circulation of the liquid extractant on the permeate side especially in continuous fermentation processes [136,155–157]. Most recently, a spray-coated thin film of extractant on a membrane surface has been reported to give n-BuOH flux of one order of magnitude higher than state-of-the-art perstraction systems and up to 10x reduction in energy use for recovering high purity n-BuOH compared to energy intensive recovery techniques such as LLE extraction and gas stripping [158]. The use of supported liquid membranes (SLMs), where a hydrophobic liquid is confined within a supporting structure by capillary forces [159] widens the scope for employing more toxic but high performing liquid extractants that would otherwise not be tolerated by the microbial cells. SLMs can also offer even greater opportunity for higher permeability than solid membranes by enhancing the diffusion in the liquid phase [159]. SLM has been demonstrated to be effective in a number of in-situ extractive processes in whole cell biotechnological applications such as the biocatalytic synthesis of a chiral amine, (S)-α-methylbenzylamine (MBA), by ω-transaminases in E. coli [160]. Continuous extraction of the amine product enabled the conversion to be practically doubled to 98% compared to when no extraction was implemented, with a high MBA concentration of 55 g/l reached at the end the process. Further improvements are possible using ionic liquids as the supported liquid in the membrane support, with one recent study reporting orders of magnitude increases in ethanol fluxes and selectivity compared to other in-situ ethanol recovery methods [161].

3.5.2. Membrane bioreactor (MBR)

A membrane bioreactor (MBR) combines a bioreactor with membrane filtration for cell/enzyme retention or in-situ separation of products from substrates and biocatalysts. The technology has evolved significantly over the last couple of decades [162,163] and has been implemented in a variety of important applications involving biofuels and biorefineries, amongst many others [164]. Both whole cell microorganisms and enzymes have been employed as biocatalysts in MBRs, as highlighted in the most recent reviews focusing on each type of biocatalyst [165,166]. The intensification potential of a number of membrane-based bioreactors has already been covered elsewhere in this review for whole cell biotransformations under perfusion for cell retention, pervaporation and perstraction for in-situ product separation, all of which are key technologies for enhancing bioprocess productivity. In enzymatic reactions involving hydrolysis, continuous MBRs have also demonstrated promising potential for bioprocess intensification in comparison with batch processing with off-line membrane filtration, with several fold enhancement in productivity and yield being reported in a number of applications [165]. More effort has been directed recently at finding effective enzyme immobilisation strategies while

retaining enzyme activity for different membrane materials [167,168]. One other limitation of MBRs is the propensity for membrane fouling which requires intermittent back flushing and impacts on the long-term use of continuously operated membrane bioreactors. Solving this problem requires development of new, robust membrane materials [162]. Some of the techniques for mitigating fouling that have been developed for application in wastewater treatment (e.g. in-situ cleaning techniques using ultrasound [169]) may be used in enzymatic and whole cell biotransformations if their impact on the biocatalyst can be minimised.

3.5.3. Reactive distillation

Reactive distillation in the context of bioprocessing typically combines an equilibrium-based enzyme catalysed reaction with a distillation process to separate relevant products. In physical terms, a packed column is divided into a reaction stage housing the immobilised enzymes on or within the packing structures near the feed entry point with the rest of the stages in the column used for separation of product components Fig. 6. Careful selection of operating temperatures and pressures in such a process is important to maintain optimal activity of temperature-sensitive enzymes in the reaction zone while enabling sufficient vaporisation of components in the separation zones [170]. An insightful review of the historical development of enzymatic reactive distillation research over the last 20 years is given by Fellechner et al. [116]. The merits of the process have been practically demonstrated and simulated in applications relating to lipase-catalysed equilibrium-controlled transesterification of ethyl butyrate (EtBu) with n-butanol (BuOH) to butyl butyrate (BuBu) operated in batch and continuous modes [170–172]. At least 50% enhancement in conversion of n-butanol is reported in the batch reactive distillation compared to a conventional stirred tank reactor [172], highlighting the benefits of reaction equilibrium shift in favour of the desired product in such a system. Similar benefits have also been highlighted in enzymatic reactive distillation of chiral compounds, where transesterification of racemic 1-phenylethanol with isopropenyl acetate was successfully carried out at high conversion of the R-enantiomer [173].

3.6. Advances in external fields and alternative energy processing

External field applications involve some kind of energy input and this type of intensification is referred to as active techniques. Energy input can directly impact the fluid dynamics; for example, energy to rotate a surface or a body creates centrifugal fields which give thin film flow and pulsating motion can result in oscillatory flow. Electromagnetic energy input in the form of microwave, ultraviolet, infrared, ultrasound can also be used as an alternative to thermal energy to enhance molecular interactions and transformations. Often, such energy input are more targeted (e.g., microwaves and light energy) and therefore 'cleaner' in that they produce less by-products.

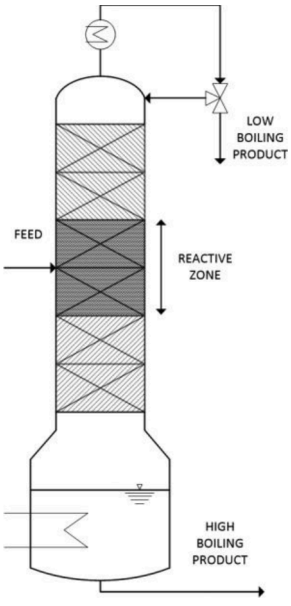

Fig. 6. Typical configuration of an enzymatic reactive distillation column operating in continuous mode [116].

3.6.1. Centrifugal fields

Centrifugal fields are created by rotation of surfaces or enclosed volumes; high gravity fields, typically of the order of 10-1000g, are thus formed which impact on fluid dynamics, mixing and transport processes [18,174–176]. In bioprocessing applications, shear sensitivity of the biocatalyst is an important consideration in selecting the optimum rotational speed to operate at. However, as highlighted below, the strength of the support matrix used for immobilisation could play an important role in protecting the immobilised catalysts against the detrimental effects of shear. Selected examples of the most recent advances in rotating technologies applied to enzymatic and whole cell biotransformations are highlighted here, following a more detailed review [177]. It is to be noted that only high gravity applications i.e., those subjected to rotational speeds >100 rpm are considered here; rotating biological contactors for wastewater treatment which typically operate at less than 50 rpm to maintain a stable biofilm on their surface during rotation are therefore outside the scope of this review.

Spinning disc bioreactor. The application of a spinning disc bioreactor (SDBR) to light driven CO_2 absorption via *C. vulgaris* microalgae cells immobilized in a biocomposite paper (Fig. 7) has recently been highlighted by Ekins-Coward et al. [50]. The capability of the SDBR to maintain high photoactivity at a spin speed of 300 rpm (equivalent to 5g at disc edge) throughout the 15 h of operation is demonstrated. A 73% enhancement in CO_2 biofixation was demonstrated compared to conventional photobioreactors operating with suspended cells. The performance of the relatively compact biocomposite-integrated SDBR, having a much smaller surface area of ~62 cm^2 packed with a high concentration of living cells (~8.8 × 10^{12} cell loading/m^2) highlights the bioprocess intensification potential of this concept. The scaleability of this process can be envisaged by having a significantly larger surface area and higher throughput

Enzymatic transformations have been conducted in a spinning mesh reactor where lipase immobilised on a wool support has been shown to give enhanced activity in the hydrolysis of tributyrin [178] and in the kinetic resolution of racemic 1-phenylethanol [179] compared to processing with immobilised and free enzymes in a conventional agitated batch vessel. Reaction was ~5x faster in spinning cloth system at 450 rpm (equivalent to 13g at the edge of the 12 cm diameter disc) than in batch reactor in the enzymatic resolution reaction whilst rate was almost doubled from 0.16 mmol min^{-1} to 0.28 mmol min^{-1} by using a stack of cloths to increase catalyst loading [179]. Similar rate enhancements were observed in the hydrolysis process [178]. The rate enhancement is

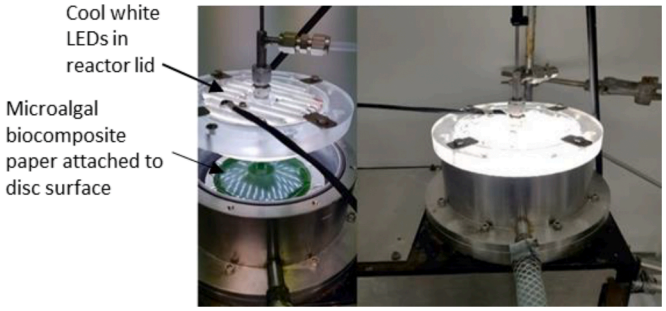

Fig. 7. C. Vulgaris microalgae biocomposite in a spinning disc bioreactor [50].

attributed to improved solid-liquid mass transfer between the reactants and the immobilised lipase enzyme arising from increased shear in the thin film flow. The wool support protects the enzyme from shear forces, resulting in high reusability (high activity in > 15 cycles).

A few studies also reported fermentations [176,180] and enzymatic transformations [181] in a rotating packed bed system. In the fermentations, the difficulty of achieving good heat transfer throughout the packing bed highlighted some challenges in maintaining a high enough viable cell density over long durations [176].

Taylor-Couette reactor. The Taylor-Couette reactor (TCR) is another technology which operates by imposing centrifugal forces on fluids flowing between the annular space of the two differentially rotating cylinders. A schematic of a typical TCR is shown in Fig. 8, where the axially flowing fluid is supplied from one end of the cylinder and the rotational flow is superimposed on the axial flow by the inner rotating cylinder. In some designs both cylinders can rotate although it is more common for the inner cylinder to rotate while the outer cylinder is stationary. Taylor vortices generated as a result of rotational flow give rise to enhanced mixing of the fluid. The ability to generate uniform, yet gentle mixing in shear sensitive applications is one of the attractive features of the TCR in bioprocessing applications. The degree of hydrodynamic instability in the fluid is primarily controlled by the rotational speed of the cylinder, with the Taylor-vortex flow regime at lower Reynolds numbers being best suited for biotransformations [182]. The increased mixing and interphase G-L-S mass transfer have been exploited in a range of bioprocessing applications involving enzymatic transformations [183,184]. More recently, the application of TCR as an algal photobioreactor has been studied [185] where dry algal biomass concentration has been shown to be approximately doubled when inner cylinder rotation increased from 0 rpm and 600 rpm after 100 h of batch mode operation. More efficient and uniform exposure of the algal cells to the incident light energy as a result of their movement within the circulating vortices is suggested to be responsible for the biomass growth enhancement. An up-to-date review of other bio-related applications for the TCR has been presented most recently by Schrimpf et al. [182].

3.6.2. Oscillatory flow reactor

Oscillatory baffled reactors (OBRs) are another type of intensified technology relying on input of energy in the form of flow pulsations or oscillations within tubes fitted with orifice plate baffles. The pulsations, superimposed upon the net flow of the process fluid, generate vortices between successive baffles which are responsible for relatively gentle mixing within the fluid. This attribute has been demonstrated to be beneficial for productivity enhancements in a number of biological processing applications. Examples include shear sensitive bioprocesses such as bioethanol production [186], enzymatic hydrolysis [187] and the cultivation of microalgae in an OBR photobioreactor [188]. Interestingly, in the latter system, the capability for the technology to be used as a flotation system for harvesting cells without additional surfactant is suggested, whereby the hybridization of the OBR photobioreactor as an integrated cell culture/cell separation can be envisaged for further bioprocess intensification advantages.

3.6.3. Ultrasound

Ultrasound refers to sound waves in the frequency range of approximately 20 kHz to 500 MHz, with frequencies typically applied to chemical processing being generally no higher than 2 MHz [189]. When applied to a liquid medium or to a solid-liquid interface, ultrasonic energy induces cavitation effects whereby the formation, expansion and destruction of microbubbles in successive compression cycles release large amounts of heat and pressure energy. This results in mixing or 'micro-streaming' in the surrounding medium environment. Ultrasound has been the subject of significant research interest as a bioprocess intensification technique over many years. Because the energy release from high power ultrasound can result in local temperatures and pressures as high as 5000 °C and 2000 atmospheres, respectively, [189], such operating conditions are typically reserved for cell lysis and intracellular metabolite recovery in downstream bioprocessing applications. In the context of productivity enhancement in bioprocess intensification, ultrasound is generally limited to low power inputs to limit biocatalyst damage whilst still improving the mixing and mass transfer rates of the process. Much research effort has been directed at studying the effects of ultrasound and its cavitation impacts on whole cell fermentations and enzymatic reactions, many of which are discussed in detail in recently published reviews on these subjects [190–194]. A few pertinent and recent examples will be highlighted here to demonstrate the extent of intensification that can be realised by the ultrasound technique in bioprocessing.

Trentin et al. [195] and Badgujar et al. [196] demonstrated enhanced activities in immobilized lipase under ultrasound-assisted conditions compared to conventional mixing methods. For instance, in the synthesis of furfuryl acetate from immobilised lipase *Candida rugosa*, the conversion of furfuryl alcohol was doubled from 48% to 99% in the ultrasonicated system at 50% duty cycle, 25 kHz frequency and 100 W power input vs. the non-sonicated set-up [196]. Increased turbulence induced by the cavitation effects of ultrasound and the associated mass transfer to and from the immobilised enzyme are highlighted as contributory factors for the observed enhancement. Furthermore, the protection of the enzyme afforded by the support matrix is thought to play an important part in maintaining greater activity of the immobilised enzyme when subjected to ultrasound, compared to free enzymes.

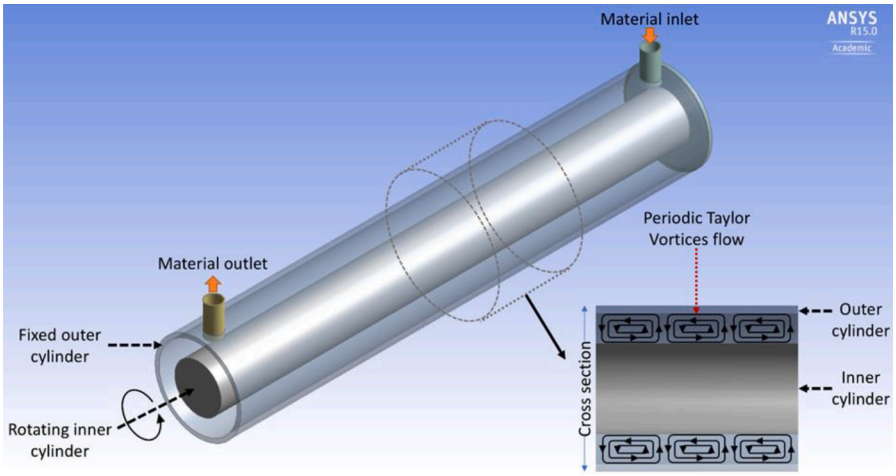

Fig. 8. 3D view of a Taylor-Couette device with inset showing Taylor vortices formed in fluid flowing in annular space.

The effects of ultrasound on whole cell biotransformations involving bacterial or fungal cell cultures have been widely studied in an attempt to understand and elucidate the enhancement mechanism in these systems [197,198]. Significant increases in substrate conversion, biomass growth rate and metabolite productivity have been reported in a number of recent studies [199–201]. Aside from the turbulence effects resulting from the cavitation phenomenon described above for enzymatic transformations, notable observations specific to the presence of cells include enhanced permeability of cell membrane allowing increased uptake rates of substrates, nutrients and product release by cells and therefore higher productivity [199–201]. There is also an effect of declustering of bacterial cells under ultrasonic treatment, enabling more uniform distribution of microorganisms and activity throughout the medium [202]. The extent to which these enhancements occur is highly dependent on frequency, intensity/power input and duration of US treatment applied. Moderate frequency in the range 20-40 kHz, amplitude below 15 μm (or up to 60-80% amplitude), mild power input and short, intermittent duration of US exposure are generally found to yield the best outcomes for productivity enhancement [199–201]. Beyond these limits, excessive cell disruption and loss of activity become apparent due to protein structure breakdown when subjected to intense acoustic waves [203], although the applicable thresholds are very much dependent on the type of cells subjected to ultrasonication, with some being more fragile/-sensitive to cavitation effects than others [199].

3.6.4. Electric field

The application of electric fields in bioprocessing has largely focused on extraction of intracellular molecules from cells through the process of electroporesis, whereby nanoscale, expandable pores are reversibly created in the cell membranes to allow more efficient recovery of molecules [204,205]. Electric field strength, pulse frequency and long enough resting phase (of 30 min minimum) have been found to be key parameters dictating improvement in cell permeability without loss in viability [204]. Pulsed electric field extraction enhancement of lipids, proteins and pigments from microalgal and cyanobacterial cultures have been demonstrated in food applications without having to mechanically destroy the cells [206,207]. This intracellular product recovery method is deemed to be more advantageous than other conventional methods of extraction such as solvent-based processes, high energy homogenisation etc. as it is more environmentally friendly, cleaner and enables selective extraction without destroying the cells.

Enzymatic transformations subjected to electric fields in aqueous phase have been demonstrated to result in enhanced enzyme activity [208]. In processes involving immiscible liquid phases- one such process being the enzymatic hydrolysis of tri-glyceride esters to free fatty acids and glycerol- electrostatic fields have been used to intensify the dispersion of the aqueous phase into the oil substrate [209,210]. The increase in interfacial area is thought to be one of several beneficial effects caused by the application of electric fields. More recently, increase in enzyme activity and enhancement in mass transfer of substrate and product molecules to and from the active sites on and within the enzyme have been found to play an important role in the observed enzymatic reaction rate enhancement [209].

In a recent study, a microextractor driven by electric field has been described for intensified separation of electrically charge product molecules from an enzyme across a membrane, giving fast, continuous, and selective separation of electrically charged molecules [211]. Separation efficiency is doubled from 50% to 100% at short residence time under the electric field, allowing recovery and re-use of enzyme.

3.6.5. Electromagnetic fields

Amongst the array of energy fields in the electromagnetic spectrum spanning high energy X-rays and γ rays through to lower energy radio waves, the most studied for intensification of bioprocessing applications is microwaves and light (solar) energy. A brief overview of the latest bioprocessing advances using these technologies is presented here.

Microwaves. Microwaves are a form of electromagnetic energy with frequencies in the range of 300 MHz to 300 GHz, with 2.45 GHz being the commonly used frequency for microwave heating and chemical processing. Microwave heating of materials relies on dipole interactions or ionic conduction depending on the chemical species involved [212], unlike conventional heating where conduction and convection throughout the whole medium are the main mechanisms for the transfer of heat. Dipole interactions occur with polar molecules having high dielectric constants such as water and alcohols whilst migration of dissolved ions in the electric field takes place in ionic conduction. Both mechanisms require effective coupling between components of the target material and the rapidly oscillating electrical field of the microwaves. Heat generated by molecular collision and friction is thus transmitted in the medium. Heating by microwave is more efficient and selective than conventional thermal energy input as the heat energy can be targeted to the desired molecules rather than the surrounding medium [213–215]. In this way, more rapid, controlled and uniform heating rates afforded by microwave exposure results not only in higher rates of reactions rates than conventional heating methods but also in better product quality through improved selectivity.

In biocatalysis applications, a key consideration has long been to operate within a narrow temperature range for optimum biocatalyst activity, which has somewhat limited the scope of microwave application in biotransformations. Increasingly, though, great strides are being made in engineering biocatalysts capable of withstanding higher temperatures with minimal degradation [216,217]. Combined with the rapid heating characteristics of microwave processing and the associated reduced reaction times of microwave processing, there should be limited damage to the biocatalysts involved, especially if they are in immobilised form. Recent reviews highlight the technological developments and achievements in microwave processing of a range of industrially relevant enzymatic transformations [218,219], including lipase catalysed transesterification process to make biodiesel [220–222]. There are reports of processing times of enzymatic reactions being reduced from several hours to under one hour or even a few minutes to achieve comparable product yield in excess of 80% [223,224], although it should be noted that much of the reported work has been developed in small scale, batch processes. Although continuous reactor systems with microwave irradiation have been widely studied in biotechnology applications related to food processing [225,226], there remains much scope still to investigate such designs for their intensification potential in enhancing enzyme activity and yields of biotransformations of interest.

Light energy. The deployment of light as an abundant, renewable energy source tool for biotransformations in cyanobacteria and microalgae has its origins in photosynthesis, a natural process that sustains life on earth [227]. Since the turn of the century, the development of efficient photobioreactors has received considerable attention for their potential not only to produce ‘green’ bioenergy and biopolymers but also and, perhaps more crucially, for their capability to capture and valorise CO_2 in the drive to reduce greenhouse gases in the atmosphere and mitigate climate change impacts. The effective assimilation of such inorganic carbon by microalgae or cyanobacteria in the presence of light energy and other nutrients results in biofuels including biohydrogen, biodiesel, bioethanol and biomethane [227,228] and biopolymers such as polyhydroxyalkanoates (PHAs) [229]. The large scale, high productivity cultivation of these photosynthetically active cells is therefore of tremendous interest as a potential solution to reducing our dependence on fossil-based energy and material resources.

Many parameters influence productivity in a photobioreactor, amongst which are light absorption and its conversion efficiency (referred to as quantum yield) and the fluid hydrodynamics to enable good mixing and mass transfer of gaseous CO_2 and nutrients to cells, especially important in immobilised cell systems. A detailed description

of all these aspects and their impact on photobioreactor productivity has been recently been published [230]. Effective light usage has been a particularly challenging problem in photobioreactors, especially in large scale system. When using solar energy, conversion efficiency is typically up to 3% with microalgal cultures although efficiencies of 10% are theoretically feasible under optimised operating conditions [231]. Light attenuation in dense cell cultures in conventional large vessels can negatively impact on productivity due to low surface area per unit volume ratios and extremely limited photons penetration depths into the fluid (a few centimeters at most). To mitigate the problems of the ineffective and non-uniform exposure of the cells to the light source, novel ways of introducing light energy are being developed. For example, the use of fibre optic probes that deliver the light from within rather than from the surface have been proposed as viable solutions [230,232,233]. Various designs of photobioreactors have also been proposed to eliminate light attenuation and maximise productivity. Indeed, photobiocatalysis in continuous flow reactor technologies has very recently been highlighted as a rapidly growing area of interest for biotransformations [78]. Designs based on film flow principles such as gravity-driven falling film reactors [232] and controlled thin film flow generated on spinning surfaces offer the unique advantage of very large surface areas to volume ratios, as high as 30000 m^2/m^3 in the spinning surface thin film [174] for instance. The Algofilm photobioreactor, which also applies the principles of falling film technology has recently been evaluated as intensified photobioreactor where biomass concentrations up to 100 times higher than in a raceway-type photobioreactor, is achieved under conditions of thin film flow (up to 1.5 mm thickness) of substrate over the immobilised cell structure [232]. Reduced water use is highlighted as an important benefit of this bioreactor technology, although the issue of light attenuation through the immobilised cell system remains to be solved.

An overview of the reviewed technologies, with their quantitative intensification characteristics illustrated by selected examples and potential limitations in the bioprocessing context is highlighted in Table 2.

3.7. Bioprocess intensification modelling tools

It is clear that one of the most important tools available to assist in the intensification of bioprocesses is to model and simulate novel configurations, novel equipment and novel biocatalysts. This enables an evaluation of the benefits of intensification in a given case and to establish the cost-benefit of implementing a particular technology [24,234]. This also means that processes can be assessed ahead of experimentation, which can clearly save expensive experiments. While modelling cannot replace experiments, it can reduce the number required, saving both time and cost. A lot of work has been done modelling intensified bioprocesses and only a limited selection of publications are cited here to illustrate the main developments while keeping within the scope of this review.

Upstream, enzyme kinetics has been widely studied (see for example [235,236]), and in some cases linked to intensified integrated downstream processing, for example via in situ product crystallization [237] and reactive distillation [238]. The modelling of metabolic pathways also attracts great interest but remains difficult to integrate with process models. Efforts at mechanistic modelling of fermentation [239] however already start to form the basis for control. Likewise, modifications to operating mode have also been studied with respect to fermentation, for example for improved PHB production [240]. In all modelling of this type, it is clear that uncertainty needs to be taken into account in order to ensure decisions can be made on a secure footing [241]. Ultimately modelling scale-up is also required [242], although still many complexities exist here. One way to assist the development of such computationally demanding models may be to also use data from the plant itself, and recently a framework to examine such data from biologics manufacturing was proposed [243].

Emphasis on downstream processing models have also proved useful, particularly at the level of individual unit operations such as filtration [244], and also cell harvest [245]. New adsorbents have also been assessed [246]. Additionally, improved control strategies leading to intensified unit operations, for example for chromatography have also been explored through modelling [247].

Several challenges remain in the modelling field including the need to describe in a suitable mathematical notation the phenomena in a given system. Likewise, insufficiently processed (high quality) data are available to build an empirical model. Efforts at the microfluidic scale have also been used to try to collect suitable data [248]. In other cases the process is not understood sufficiently well to implement a first principles model. Another key issue is the need to validate models using experimental data, in order to bring confidence to the ability of models to predict intensified behaviour.

Finally, it is necessary to benchmark the models against costs, but also against conventional systems. It is perhaps this last aspect which is the hardest to achieve, but is also one of the most important to ensure that modelling is used as a valuable tool to reduce the number of experiments required when developing novel technologies. This is also what distinguishes modelling tools used mainly for optimization versus those that really help evaluate novel, intensified operations or processes. Recent examples have included work on the cost-benefit of switching to continuous manufacturing of monoclonal antibodies [249,250] and recombinant protein production [251]. A broader analysis of new bioprocessing concepts has also been modelled and assessed with respect to economics [252]. Likewise, a very useful common framework was recently published to assist the overall evaluation of novel biomanufacturing options in a combined industry-academic partnership [253]. Finally, short-cut calculations have also been used to assess product recovery options [254], where modelling in detail has been found too time-demanding.

4. Concluding remarks and future perspectives

Bioprocess intensification, a strategy that is based on the well-established principles of chemical process intensification, has captured the interest of industry and academic researchers in the quest for making sustainable processing a reality. Much of the attention to date has been on applications related to the pharmaceutical processing of drugs, vaccines etc. and large-scale manufacturing of biofuels, bio-based chemicals, biopolymers, food ingredients etc, all of which result in a market-driven product of value to society. Bioprocessing offers significant and as yet untapped potentials to address the environmental challenges of our times such as CO_2 capture but this is dependent on large scale bioprocesses becoming much more efficient.

In this review paper, we have highlighted the latest achievements in intensifying bioprocesses to make them more efficient and economically viable. The variety of techniques that have been explored in the last 10–20 years have covered the full spectrum of the bioprocessing train from upstream processing (biocatalyst engineering, immobilisation techniques, etc.), to innovations in fermenter/bioreactor design (continuous processing technologies including perfusion bioreactors, novel techniques of energy inputs for productivity enhancement and an array of in-situ product separation and recovery techniques for both whole cell and enzymatic transformations) through to developments in downstream purification. Whilst much of this activity has been undertaken as academic research in University laboratories and research institutions, industry interest and development has been particularly noticeable in areas such as continuous processing, perfusion bioreactors and improved downstream separation and purification techniques such as continuous chromatography.

We believe there remains much scope to drive even greater innovation bioprocess intensification in the short to medium term (next 10 years). Future prospects for advancing the field in bioprocess intensification include:

Next generation bioprocessing. The focus in the last few decades has

been on exploiting bioprocessing for market driven products such as biofuels, bio-based chemicals and polymers. The grand challenges of climate change, CO_2 capture and storage, access to water etc. that we now face require new thinking in how we tackle these issues. New nature-inspired biotechnologies capable of mimicking some of the elegant materials and extremely efficient processes in nature may provide the answer for what Coppens recently described as the "transformative technologies" [255] needed to address these grand challenges of our time. Some of the developments highlighted in this review paper are good starting points for this endeavour. One example is the novel micro-organism biocomposite structures capable of supporting high cell density in a compact matrix, which when applied to CO_2 biofixation application is the engineering equivalent of leaves absorbing CO_2 from air. More work needs to be undertaken in such novel materials in conjunction with new, large scale process technologies to fully exploit their promising potential in providing robust, stable, efficient, and cost-effective transformative biotechnologies.

1 *Biocatalyst engineering as a key intensification approach*. BPI is particularly important today given the enormous hope placed in biotechnology as a sustainable means of production for the future. On the one hand many bioprocesses are not particularly intense and therefore require new technology. On the other hand many bioprocesses have the nature endowed possibility of biocatalyst enhancement through recombinant DNA technology. Realising the potentials of biotechnology therefore rests heavily on engineering the biocatalysts at the heart of the bioprocess to enable their long-term stability and re-use for higher conversions, yield and productivity.
2 *Technical advancement*. Many of the novel processing techniques involving e.g. alternative energy inputs have been investigated at small scales and in some cases primarily in conventional batch technologies (e.g. microwave-assisted enzymatic transformations). These techniques would benefit from further development based on continuous flow processing to assess their potential for industrial scale application.
3 *Expanding industrial application*. In industry, many bioprocessing intensification developments have come about by shifting from batch to continuous processing and investigating hybrid technologies, particularly those related to in-situ product recovery. The industrial interest in continuous processing has been somewhat limited almost entirely to perfusion bioreactors. There is significant opportunity to explore other promising continuous flow technologies such as plug flow reactors with immobilised biocatalysts (enzymes or cells) for current and future (see point 1) industrially relevant applications.
4 *An integrated and holistic approach to education and practice.* The development of innovative and sustainable bioprocesses based on intensification principles necessarily includes *process* as well as *biocatalyst intensification* technologies and should consider both *upstream* as well as *downstream* stages as an integrated whole. Bioprocessing is quite unique in that biocatalyst engineering affects both upstream and downstream processing equally. Such a level of integration of these different aspects is a major challenge for the future. In research & development and education of the next generation of students, it is essential therefore that we integrate all four of these elements together and take a holistic view. With a number of Universities teaching process intensification as part of their engineering curriculum across the world, there are plenty of opportunities to embed these ideas and help the future (bio)process engineer develop a good understanding of these integrated principles.
5 *Process systems engineering tools*. New technologies based on bioprocess intensification philosophy should be assessed using modelling approaches to reduce experimental effort. This is especially important for evaluating the realistic outputs of simulated commercial scale processes designed on the basis of laboratory scale testing. This will require complex models for each step across the whole process chain coupled with the complex biological processes. For novel technologies especially, there is an urgent need to develop frameworks to enable implementation of reliable predictive models. The model outputs such as productivity and yield will enable the BPI technologies to be benchmarked against performance and costs of conventional approaches. As continuous processing becomes more widely implemented in the bioprocessing industries of today and the future, the digital manufacturing tools of Bioprocessing 4.0 will become indispensable and will require more development and integration of automated hardware and novel, on-line and in-line process analytics technologies (PAT) [99,256]
6 *A multidisplinary and collaborative challenge*. To further advance the field of bioprocess intensification will require effective collaboration across many disciplines including synthetic biology, biotechnology, material science, chemical and process engineering, process systems engineering, control engineering, amongst others. Most importantly, the development of new technologies should not only be the remit of University research, but also linked with industry to enable the testing of new concepts and ideas. Here, it is important that industry technical requirements and limitations are clearly understood by academics and that more strategies are put in place to enable long term, sustainable collaborations to develop promising technologies for deployment at higher TRL levels.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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