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Editorial

Special Issue on "Bioreactor System: Design, Modeling and Continuous Production Process"

Francesca Raganati [1,*] and Alessandra Procentese [2]

[1] Dipartimento di Ingegneria Chimica, dei Materiali e della Produzione Industriale–Università degli Studi di Napoli Federico II, P.le V. Tecchio 80, 80125 Naples, Italy
[2] Dipartimento di Ingegneria Industriale–Università degli Studi di Salerno, Via Giovanni Paolo II 132, 84084 Salerno, Italy
* Correspondence: francesca.raganati@unina.it

Biochemical engineering deals with the processing of biological or chemical materials using enzymes or living cells as biological catalysts. At a central position in a biotechnological process is the bioreactor. The term bioreactor refers to a device, or system that contains substrates and enzymes or cells as biocatalysts and provides an environment in which the biocatalysts can perform their functions. The characteristics of enzyme biocatalysts resemble more or less those of chemical catalysts in that their activities degrade with time, whereas cells are self-multiplying living systems. Both types of biocatalysts have undergone successful developments in producing various products.

The bioreactor role is frequently dominant on the overall technical and economical performance of the process. The characteristics of the biological reaction can also affect the requirements on the other steps of the process, such as the preparation of the media and the downstream operations for product recovery and purification. Bioreactions can be conducted in bioreactor systems with many diverse characteristics. Once a product is selected, we must consider various aspects of production using microbial cells, plant or animal cells, including the sterilization processes, bioreactor operation modes, product location (intracellular or extracellular) and separation methods or may even compare bioreactor production with competing manufacturing methods by biological or chemical means or a combination of both.

Each bioconversion process is dependent on many factors including growth conditions, homogeneity of fermentation medium, cell density, etc. The decisions made in the design of bioreactors might have a significant impact on the overall process performance; in fact, bioreactor design/operation mode is an important key factor to achieve optimum conditions for maximum yield/productivity in fermentation; the main function of a properly designed bioreactor is to provide a controlled environment to achieve optimal growth and/or product formation in the particular cell system employed. In this regard, knowledge of reaction kinetics is essential to gain an understanding of the workings of a biological reactor. Other areas of bioprocess engineering, such as mass and energy balances, mixing, mass transfer, and heat transfer, are also required.

Moreover, qualitative and quantitative descriptions of a production process through the analysis of various parameters by automatic or manual methods are necessary for process control and optimization. The objects of process monitoring can be the environmental status or the varied values of operational variables. Through analysis, the cellular or engineering problems of a bioreactor on different scales can be identified. Inter-scale observation and operation are crucial in bioprocess optimization.

This Special Issue on "Bioreactor System: Design, Modeling and Continuous Production Process" of Processes collects the recent work of leading researchers in a single forum, and the contents cover a variety of experimental applications and theoretical studies, focusing on bioreactor systems used for the microorganism culture and bio-chemical production.

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The SI cover biology and engineering research. Despite the interdisciplinary nature of the different applications involved, there are some common issues in bioreactor development, set-up and optimization connecting the areas together which we seek to capture in this issue. We believe that the advances described by the contributors have significantly helped accomplish this target. In addition to the research articles, the issue features a number of reviews, covering a range of topics, which highlight the versatility of the area.

Research Articles

Four papers in the Special Issue focused on the optimization of the bioreactor configuration with the aim to increase metabolite production and or wastewater treatment efficiency. Lanzillo et al. [1] presented a work dealing with syngas fermentation by *Clostridium carboxidivorans* for production of acids and alcohols in batch pressurized bioreactors. The authors assessed the effects of constant CO partial pressure (ranging from 0.5 to 2.5 atm) on cell growth, acid and solvent production. The work also focused on the effect of the liquid to gas volume ratio (0.28 and 0.92) on fermentation performances.

Johonson and Rehmann [2] revealed the self-synchronized oscillatory metabolism of *Clostridium pasteurianum* in continuous fermentation by using real time monitoring of CO_2 and H_2 production and redox potential. The oscillations in CO_2 and H_2 production were reported to be in sync with each other and with both redox potential and glycerol in a continuous stirred tank reactor (CSTR). The importance of understanding such an "oscillatory metabolism" is for developing a stable and highly productive industrial fermentation process for butanol production, as unstable oscillations are unproductive. It was shown that the oscillatory metabolism can be eradicated and reinstated and that the period of oscillations can be altered by modification of the operating parameters. Synchronized oscillatory metabolism impacted the product profile such that it lowered the selectivity for butanol and increased the selectivity for ethanol. This permitted to elucidate a possible cause for the variability in the product profile of *C. pasteurianum* that has been reported in many previous studies.

Holder et al. [3] focused on production of bio-methane via anaerobic co-digestion of optimized mixture of river tamarind and dolphin fish offal. Fish offal and other high protein substrates are generally not suitable for anaerobic digestion because of the high levels of ammonia produced as a result of their biodegradation; in these regards, co-digestion of substrates could be a solution in nutrient balance and could increase the overall methane yield and productivity. The authors tested a total of 25 substrate combinations in batch bioreactors; the optimum of these tested combinations tested was 50% fish offal to 50% river tamarind; this combination gave much improvement when compared with the fish offal alone performances. The reported results were very significant when considering the implementation of the process in a larger scale.

Feng et al. [4] designed a bioreactor for restricting oversize of aerobic granular sludge. Indeed, aerobic granular sludge (AGS) with oversized diameter commonly is reported to affect its stability and pollutant removal. The authors designed a sequencing batch reactor (SBR) with a spiny aeration device with the aim to effectively restrict the particle size of AGS. The authors compared the performances of their innovative bioreactor configuration with a conventional SBR treating tannery wastewater. The oversized AGS was more likely to collide and abrase with the spines and air bubbles. Indeed, the authors succeeded to restrict the average particle size of AGS at about 300 micrometers.

One paper in the Special Issue was linked to the production of vaccines. Indeed, Ugwu et al. [5] discussed on the important topic of large volume production of vaccine virus. Developing a vaccine could be a very challenging process, and one of the major drawbacks to the availability of vaccines over the years has been technical manufacturing scale-up obstacles. In particular, the authors reported the possibility for primary chicken embryo liver (CEL) cells to be adapted to Cytodex^{TM} 1 microcarrier culture and to be used to propagate Fowl adenovirus (FAdV) isolate in a stirred tank bioreactor.

One paper of the Special Issue was related to the field of monitoring and control technologies. With the rapid expansion of biotechnology in fields traditionally limited

to chemistry, the development of these technologies becomes urgent. In this regards, Kottelat et al. [6] stated that Biocalorimetry is a very promising PAT tool for the monitoring of various strains including Crabtree-positive microorganisms, and its implementation at large scale in the industry can be carried out without prohibitive investments. In particular, Kottelat et al. [6] reported on a novel strategy for the calorimetry-based control of fed-batch cultivations of *Saccharomyces cerevisiae*. The authors developed a novel calorimetry-based feed-forward/feedback PI controller that allowed to precisely and accurately control fed-batch cultures of *Saccharomyces cerevisiae* and to prevent the Crabtree effect. In particular, the heat produced by the cells was successfully applied as the control variable and the controller noise was noticeably decreased.

Three papers of the Special Issue regarded the topic of bioreactor modeling and design. Due to the heterogeneous nature of large-scale fermentation processes they cannot be always modeled as ideally mixed reactors, and therefore flow models are necessary to accurately represent the processes. In this context, Bisgaard et al. [7] reported on a new approach for the development of data-based flow models in the form of compartment models (flows are defined between a network of ideally mixed zones called compartments), which utilized axial-flow rates obtained from flow-following sensor devices in combination with a proposed procedure for automatic zoning of volume. The proposed approach required little experimental effort and eliminated the necessity for computational determination of inter-compartmental flow rates and manual zoning. The authors demonstrated the concept in a 580 L stirred bioreactor. This approach provided a versatile and automated flow modeling platform to be applied to large-scale bioreactors

Ramírez et al. [8] focused on the importance of mixing operations in biological processes, related to its effect on scaling-up and heat and mass transfer. In particular, the authors characterized a bench-top bioreactor with different impeller configurations, agitation and oxygen transfer rates, using CFD simulations and experimental procedures. The authors demonstrated that factors such as the type of impeller and the flow regime could drastically vary the operation as in the preparation of cultures. Moreover, the authors compared the k_La values obtained for the different configurations with the maximum shear rate values of different cell cultures to highlight the impact of their study and its applicability to different industries that use agitation processes for cell growth.

As discussed previously, bioreactors mimic the natural environment of cells and tissues by providing a controlled micro-environment. However, their design is often expensive and complex. In this regards, Dursun et al. [9] developed a low-cost compression bioreactor which enabled the application of different mechanical stimulation regimes to in vitro tissue models and provided the information of applied stress and strain in real-time. The in-house software of the bioreactor provided an opportunity for faster implementation of desirable changes to the controlling unit, as well as transmitted instant experimental data to the cloud. Moreover, the bioreactor mobile application enabled users to display and control the experiment information remotely through smartphones. This study proved that the unique design of the compression bioreactor is a powerful and real-time controllable tool for mechanobiology studies.

Review Articles

Two reviews articles are published in the SI. Carpine et al. [10] reported main issues regarding the PHB (poly-beta-hydroxybutyrate) production and the challenging issues to be investigated/developed. The PHB structure as well as its formation/accumulation in the cyanobacteria is presented. The biotechnological route to produce the PHB is presented with the potential solution to improve the productivity and reduce the cost of the production. The effects of the PHB structure in the cell on the recovery performance is discussed. The authors stated that so far, the main drawback of the commercial production and application of PHB in the industry is its high production cost compared with conventional plastics. The recovery of PHB granules from cytoplasm significantly increases total processing costs. The other big challenge of the PHB production process from cyanobacteria is to find a low-cost and environmentally friendly strategy to recover it.

Navarrete et al. [11] identified the main key issues associated with biorefinery platforms (included a lack of a reliable substrate supply, high water consumption rates and the metabolic burden associated with the heterologous pathways), and proposed possible solutions to overcome the issues in question. Novel cell factories and bioreactor design were discussed as possible solutions. In particular, novel cell factories such as *Yarrowia lipolytica*, *Trichosporon oleaginosus*, *Ustilago cynodontis*, *Debaryomyces hansenii* with promising natural characteristics of preferential glycerol catabolism, the ability to metabolize lignocellulosic inhibitors, broad pH tolerance and affinity for non-pure water sources, respectively, were reviewed. The authors also introduced an innovative bioreactor configuration using a sequential fermentation system. The solutions proposed could be adopted in a synergistic way in order to boost further biotechnology processes to an industrial level.

The above papers demonstrate the versatility and technical importance of the area of bioreactor control, optimization and applications, ranging from the formulation of fundamental theory to practical applications. Although the basic principles of bioprocess engineering, such as mass and energy balances, mass and heat transfer are fairly well understood, the articles address outstanding challenges related to different bioprocessing areas in terms of both application and theoretical perspectives, and much remains to be explored in the future. With the huge number and variety of the bioreactor applications currently under development, we feel confident for the longevity and future of this subject.

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References

1. Lanzillo, F.; Ruggiero, G.; Raganati, F.; Russo, M.E.; Marzocchella, A. Batch Syngas Fermentation by *Clostridium carboxidivorans* for Production of Acids and Alcohols. *Processes* **2020**, *8*, 1075. [CrossRef]
2. Johnson, E.E.; Rehmann, L. Self-Synchronized Oscillatory Metabolism of *Clostridium pasteurianum* in Continuous Culture. *Processes* **2020**, *8*, 137. [CrossRef]
3. Holder, N.; Mota-Meira, M.; Born, J.; Sutrina, S.L. Bio-Methane Production via Anaerobic Co-Digestion by Optimizing the Mixing Ratios of River Tamarind (*Leucaena leucocephala*) and Dolphin Fish (*Coryphaena hippurus*) Offal. *Processes* **2020**, *8*, 934. [CrossRef]
4. Feng, H.; Yang, H.; Sheng, J.; Pan, Z.; Li, J. A Bioreactor Designed for Restricting Oversize of Aerobic Granular Sludge. *Processes* **2021**, *9*, 374. [CrossRef]
5. Ugwu, C.C.; Hair-Bejo, M.; Nurulfiza, M.I.; Omar, A.R.; Ideris, A. Propagation and Molecular Characterization of Fowl Adenovirus Serotype 8b Isolates in Chicken Embryo Liver Cells Adapted on Cytodex™ 1 Microcarrier Using Stirred Tank Bioreactor. *Processes* **2020**, *8*, 1065. [CrossRef]
6. Kottelat, J.; Freeland, B.; Dabros, M. Novel Strategy for the Calorimetry-Based Control of Fed-Batch Cultivations of *Saccharomyces cerevisiae*. *Processes* **2021**, *9*, 723. [CrossRef]
7. Bisgaard, J.; Tajsoleiman, T.; Muldbak, M.; Rydal, T.; Rasmussen, T.; Huusom, J.K.; Gernaey, K.V. Automated Compartment Model Development Based on Data from Flow-Following Sensor Devices. *Processes* **2021**, *9*, 1651. [CrossRef]
8. Ramírez, L.A.; Pérez, E.L.; García Díaz, C.; Camacho Luengas, D.A.; Ratkovich, N.; Reyes, L.H. CFD and Experimental Characterization of a Bioreactor: Analysis via Power Curve, Flow Patterns and k L a. *Processes* **2020**, *8*, 878. [CrossRef]
9. Dursun, G.; Umer, M.; Markert, B.; Stoffel, M. Designing of an Advanced Compression Bioreactor with an Implementation of a Low-Cost Controlling System Connected to a Mobile Application. *Processes* **2021**, *9*, 915. [CrossRef]
10. Carpine, R.; Olivieri, G.; Hellingwerf, K.J.; Pollio, A.; Marzocchella, A. Industrial Production of Poly-β-hydroxybutyrate from CO_2: Can Cyanobacteria Meet this Challenge? *Processes* **2020**, *8*, 323. [CrossRef]
11. Navarrete, C.; Jacobsen, I.H.; Martínez, J.L.; Procentese, A. Cell Factories for Industrial Production Processes: Current Issues and Emerging Solutions. *Processes* **2020**, *8*, 768. [CrossRef]