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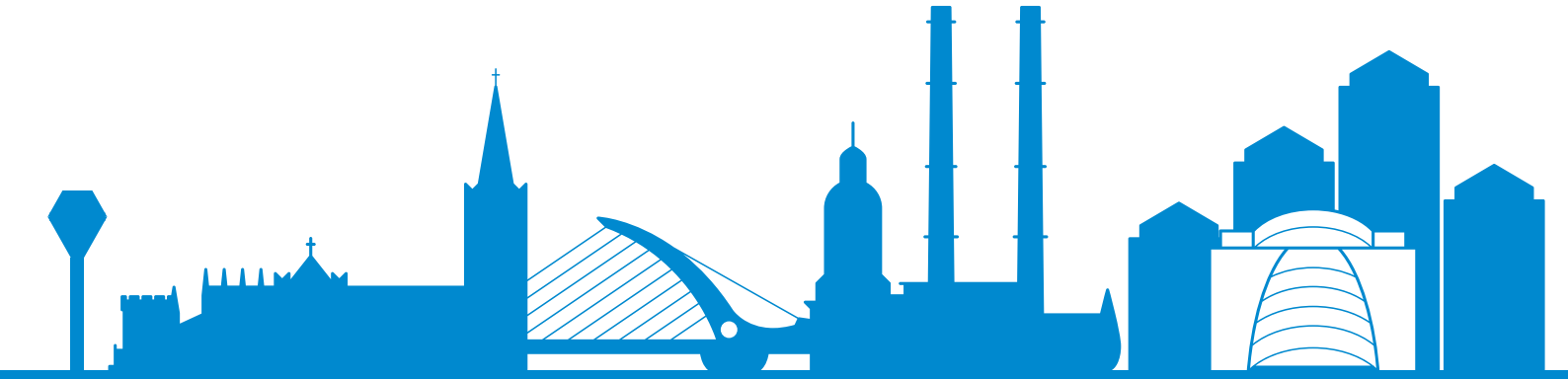

SCBE 3RD ANNUAL SEMINAR DAY

22 MARCH 2024

ROOM L143
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BOOK OF ABSTRACTS

UCD SCHOOL OF CHEMICAL & BIOPROCESS ENGINEERING
3rd ANNUAL SEMINAR DAY
22 MARCH 2024

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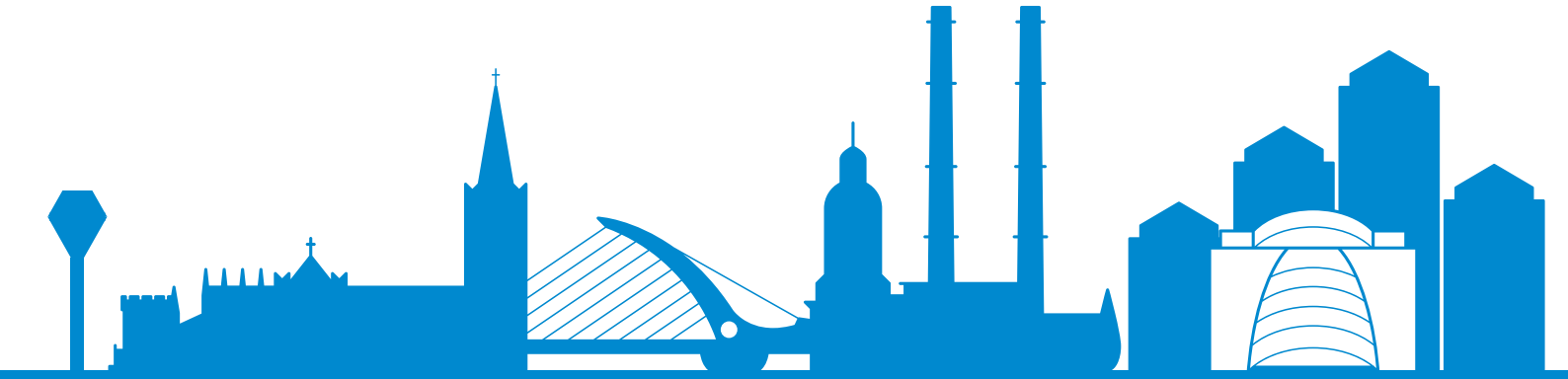

ORAL PRESENTATIONS

BOOK OF ABSTRACTS

O1

Design of Experiment (DoE) Enabled Scale-Up for Adeno-associated Virus (AAV) Production

[1, 2]*Bogdanovic, A., [2, 3]Whelan, J., [2]McDonnell, S., [1, 4] Donohue, N., & [1, 2]Glennon, B.
*Lead Presenter: alexandra.bogdanovic@approcess.com
[1] Applied Process Company Limited (APC Ltd), Cherrywood, Dublin;
[2] School of Chemical and Bioprocess Engineering, University College Dublin;
[3] Conway Institute of Biomolecular & Biomedical Research, University College Dublin;
[4] National Institute for Bioprocessing Research and Training (NIBRT), Dublin
Funded by the Irish Research Council Employment Based Postgraduate Programme

Adeno-associated virus (AAV) therapies are produced through triple transfection into a single cell line, where three plasmids are combined with a transfection reagent that undergoes interactions with DNA. While transfection of multiple plasmids gives flexibility and allows modification of transgene regulatory elements, there are complexities associated with the triple transfection that result in challenges around reproducibility of process performance in terms of titre and product quality. Additionally, there are often difficulties encountered when scaling up.

This study describes a 2 stage DoE approach to optimize triple transfection using FectoVir-AAV and a HEK293F cell line. The three plasmids allowed for production of an AAV5 containing the transgene encoding Green Fluorescent Protein (GFP). An initial screening design was implemented due to the large number of factors with potential to have significant effects on transfection efficiency, genome titre, capsid titre, and ratio of full to empty capsids. The factors screened included DNA amount, plasmid ratios, volume of FectoVir-AAV, complexation volume, complexation time and cell culture density. Across the conditions screened, transfection efficiency ranged from 8.8 - 61.6%, genome titres from 1.6E+09 – 3.0E+10 VG/mL and capsid titres from 4.6E+09 – 3.9E+11 capsids/mL. Analysis indicated that DNA amount, complexation time and the volume of FectoVir-AAV had the greatest effect. These factors were taken forward to the second stage which implemented a central composite design. This design was chosen to allow accurate estimation of quadratic terms in responses and expand the design space beyond levels included in the screening. The output of the second stage of the DoE was used to identify the optimum conditions for the triple transfection. The optimized conditions were scaled up from 125 ml shake flasks to a 50 L Wave reactor. Success of the scale up was determined by assessing genome titre, capsid titre, and ratio of full to empty capsids.

O2

Boosting the thermochemical energy storage performance of Limestone by adding Mayenite

[1]*Anwar, R., [1]Kape, A.A., [2]Lusi, M., [3]Voutsas, E., [4]Cammarata, A., & [1]Sofianos, M.V.
*Lead presenter: rehan.anwar@ucdconnect.ie
[1] School of Chemical and Bioprocess Engineering, University College Dublin, Dublin, Ireland
[2]Department of Chemical Sciences & Bernal Institute, University of Limerick, Limerick, Ireland
[3]School of Chemical Engineering, National Technical University of Athens, Athens, Greece
[4]Department of Control Engineering, Faculty of Electrical Engineering, Czech Technical University in Prague, Prague, Czech Republic

Long-duration energy storage (LDES) systems play a critical role in the integration of intermittent renewable energy sources into the grid. Thermochemical energy storage (TCES) systems, particularly those based on Limestone, offer promising solutions due to their high energy density and cost-effectiveness. However, the cycling performance of Limestone-based TCES systems is hindered by sintering phenomena and pore plugging. This paper explores the enhancement of Limestone's TCES performance by incorporating Mayenite, a mesoporous ternary oxide, as an additive. Mayenite improves cycling performance by mitigating sintering and enhancing reaction kinetics. Three different Mayenite samples with unique Ca to Al ratios were synthesized and added to Limestone in varying concentrations. The study reveals that incorporating Mayenite significantly enhances Limestone's energy storage performance, with a 1.5 times increase in energy storage density observed with 5% Mayenite (with a lower Ca/Al ratio) addition after 40 cycles. Characterization techniques including X-ray diffraction, scanning electron microscopy, and thermogravimetric analysis provide insights into the structural and kinetic changes induced by Mayenite addition. The results demonstrate the potential of Mayenite as an effective additive for improving the performance of Limestone-based TCES systems, paving the way for more efficient and reliable long-duration energy storage solutions.

O3

PEGylation as a therapeutic protein aggregation prevention strategy

[1,2]*Tugrul, A.D., [1]Barros, C.H., & [1]Topp, E.
*Lead presenter: arda.deniz@nibrt.ie
[1]National Institute of Bioprocessing Research and Training (NIBRT), Dublin, Ireland;
[2]School of Chemical and Bioprocess Engineering, University College Dublin, Dublin, Ireland

Protein aggregation poses a significant challenge in the development of therapeutic proteins, due to its potential immunogenicity, toxicity, and the bioprocessing costs associated with its removal [1, 2]. Protein drugs require continuous formulation monitoring and improvements to ensure consistent aggregate-free purity. This research investigates the efficacy of polyethylene glycol (PEG) modification (PEG length 24 and 33), commonly known as PEGylation, in mitigating aggregation of therapeutic proteins, with a particular focus on monobody fragments (Mobs) of monoclonal antibodies. Mobs represent a novel platform in the development of antivirals which have not been thoroughly studied or modified previously, and has the potential to exhibit high affinity towards a specific viral antigen, thus offering a ground-breaking approach to antiviral drug development [3]. By leveraging this evolvable and rapidly adaptable mechanism in conjunction with computational tools, Mobs remain a potential innovative platform to be further studied. Through the use of Circular Dichroism (CD), Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE), Microfluidic Capillary Electrophoresis, Qubit protein quantification, and FITC-specific fluorescence plate reader analysis following stress-inducing assays; this study aims to elucidate the propensity for aggregation, understand the degree of substitution, and explore the benefits of PEGylation. Initial findings reveal that PEGylation preserves the secondary structure of Mob, in addition to elevating the protein's melting point, which suggests an increased thermal stability. Quantification methods have demonstrated a significant reduction in monomer loss, highlighting the effectiveness of PEGylation in conserving the monobody against aggregation when subjected to physical agitation. This was further corroborated by results from SDS-PAGE, which confirm that non-PEGylated Mob fragments readily aggregate upon exposure to stress, illustrating the protective effect of PEGylation against such conditions. These findings show dual benefit of PEGylation in maintaining the structural integrity and enhancing the stability of the model monobody, thereby potentially improving their therapeutic efficacy and shelf life. This research not only contributes to the fundamental understanding of antiviral Monobody aggregation but also outlines a practical approach to mitigating one of the major concerns in the biopharmaceutical industry.

References

1. Roberts CJ. Protein aggregation and its impact on product quality. Curr Opin Biotechnol. 2014 Dec;30:211-7. doi: 10.1016/j.copbio.2014.08.001. Epub 2014 Aug 28. PMID: 25173826; PMCID: PMC4266928.
2. Pham NB, Meng WS. Protein aggregation and immunogenicity of biotherapeutics. Int J Pharm. 2020 Jul 30;585:119523. doi: 10.1016/j.ijpharm.2020.119523. Epub 2020 Jun 9. PMID: 32531452; PMCID: PMC7362938.
3. Evamobs. (2022). Innovative Mobility Solutions. Evamobs.eu. Retrieved March 12, 2024, from https://www.evamobs.eu/

O4

Advanced Imaging of Biofilm-Particle Interactions

[1*]Dunne, C., [1]Brink, J., [1]Singh, D., [1]Hiebner, D., & [1]Casey, E.
*Lead presenter: ciaran.dunne3@ucdconnect.ie
[1] School of Chemical and Bioprocess Engineering, University College Dublin, Ireland

Biofilms are complex, heterogenous communities of microorganisms in which diverse resident species or phenotypes coexist and produce an extracellular polymeric matrix. This matrix is composed of extracellular proteins, carbohydrates and nucleic acids with a host of functions ranging from surface attachment and cellular cohesion to environmental resistance or structural stabilisation. Biofilms are also transient, constant dispersion and attachment events occur in which resident planktonic cells attach or exit to the aqueous environment in response to localized environmental changes within the biofilm. Whole biofilm masses of both cells and matrix can also be sloughed off the biofilm as aggregates due to shear stress or other interactions. Both dispersed planktonic cells and biofilm aggregates exhibit the capacity for further surface colonisation and biofilm development. In industrial wastewater treatment bacterial biofilms are employed for their nutrient depletion capabilities and flocculation. In this setting biofilms can be subjected to high shear flow which can induce aggregate sloughing while also facilitating diverse interactions with suspended particulate matter. While biofilm attachment and development are well characterised, a knowledge gap exists regarding dispersion, recolonisation ad particulate-biofilm interactions.

Using confocal laser scanning microscopy (CLSM), advanced microscopic three-dimensional images can be obtained of fluorescently labelled biofilms to probe their structural and population dynamics. Using CLSM and flow cell technology the group is investigating how dispersed biofilm cells and aggregates recolonise surfaces and establish secondary biofilm biomass and how surface associated biofilms interact with suspended particulate matter. The first objective of the project is to develop a model system for repeatable biofilm development investigation. *Escherichia coli* strains that have been transformed to express the fluorescent proteins mCherry, GFP and mVenus are being used to investigate biofilm population dynamics. A low motility strain (K12 MG1655) of *E. coli* that has been transformed to express complementary surface associated blue light inducible aggregating proteins (nMagHigh and pMagHigh) is being employed for biofilm aggregate investigations. In a mixed culture of two strains expressing either nMagHigh or pMagHigh on the cell surface, blue light exposure induces aggregation. mCherry expressing *Pseudomonas flourescens* and *Staphylococcus epidermidis* are also employed alongside fluorescent labels that target extracellular matrix components. Once biofilm development has been optimised methods for the dispersion of biofilm into both planktonic cells and biofilm aggregates of varying size will be used for subsequent secondary colonisation studies. Fluorescent nanoparticles are being employed to investigate how particles penetrate biofilm mass, how particles and biofilm interact in flow conditions and how these particle interactions impact biofilm development. The future prospects of the overall project will include implications for water treatment process optimisation and elucidation of specific biofilm-particulate interactions under dynamic flow conditions.

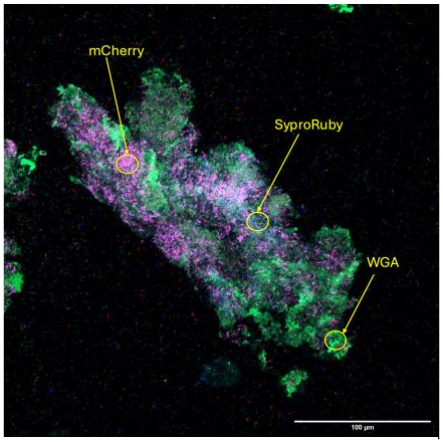

Figure 1: *P. aeruginosa* biofilm aggregate image obtained with CLSM. Stains used are mCherry (cells) SyproRuby (protein) and WGA (polysaccharides).

O5

Cold Plasma Deposition of Topotecan as a Novel Technology for Cancer Drug delivery to Glioblastoma Cells

[1,2]*Pinheiro Lopes, B., [3]O'Neill, L., [3]O'Neill, F., [1,4,5]Bourke, P., & [1,2]Boehm, D.
*Lead presenter: beatriz.pinheirolopes@ucdconnect.ie
[1]School of Chemical and Bioprocess Engineering, University College Dublin, Dublin, Ireland;
[2]Sustainability and Health Research Hub and School of Food Science and Environmental Health, Technological University Dublin, Dublin, Ireland;
[3]TheraDep Ltd., QUESTUM Innovation Centre, Limerick Institute of Technology, Clonmel, Ireland;
[4]Plasma Research Group, School of Biosystems and Food Engineering, University College Dublin, Dublin, Ireland;
[5]Conway Institute, University College Dublin, Dublin, Ireland

Glioblastoma multiforme (GBM) is the most common, malignant, and aggressive brain cancer. Despite many innovations regarding GBM treatment, the outcome is still very poor, making it necessary to develop new therapeutic approaches [1]. Cold Atmospheric Plasma (CAP) based technologies are being studied as new possible approaches against cancer, including direct plasma treatment, Plasma-Activated Liquids (PAL) [2], as well as plasma deposition (PD) of therapeutics for local delivery of oncology drugs to cancerous tissue. Possible combinatory effects with conventional therapies, such as chemotherapeutics may expand the potential of plasma-based interventions.
Topotecan (TPT), a water-soluble topoisomerase I inhibitor with major cytotoxic effects during S-phase of the cell cycle, possesses potent antitumor activity. However, systemic administration of TPT is still limited for cancer types such as Glioblastoma due to low levels of blood-brain barrier crossing [3]. For these reasons, TPT may be repurposed for local combined therapies [4]. The overall research aim is to explore the therapeutic properties of a combination between plasma-based technologies and TPT on a human brain cancer cell line (U-251mg).
Combined treatments with Plasma Activated Water (PAW) and TPT showed a reduction of the metabolic activity and cell mass and an increase of apoptotic cell death. PAW+TPT treatments also pointed to a possible arrest of cell proliferation, also affecting the long-term survival of U-251mg [4].
Evaluation of direct TPT plasma deposition onto U251mg cells grown in 2D or 3D culture indicated synergistic effects between the drug and the plasma treatment. Even though the cancer spheroids showed much lower sensitivity to the plasma deposited TPT than the planar cell culture, a loss of long-term repopulation capacity of the treated spheroids was observed.
These results can open the doors to a wide variety of new combinations and approaches to local drug application for a range of cancers.

This work was supported by the Irish Research Council under the Enterprise Partnership Scheme under grant number EPSPG/2020/277 and Science Foundation Ireland grant number 15/SIRG/3466, and is performed in partnership with TheraDep Ltd.

References

1. van Solinge, Thomas S., et al. Nature Reviews Neurology 18.4 (2022): 221-236
2. Volotskova, Olga, et al. Scientific reports 2.1 (2012): 636
3. D'Amico, Randy S., et al. Journal of neurosurgery 133.3 (2019): 614-623
4. Pinheiro Lopes, Beatriz, et al. Cancers 15.19 (2023): 4858

O6

The impact of temperature and pH on ammonia recovery from animal manure using air stripping analysed by process simulation

[1*]Azizi, N., [2]Connolly, S., [2]Krol, D., & [1]Syron, E.
*Lead presenter: niloufar.azizi@ucdconnect.ie
[1]School of Chemical and Bioprocess Engineering, University College Dublin, Ireland;
[2]Teagasc, Environment, Soils, and Land Use Department, Johnstown Castle, Co. Wexford, Y35 TC97, Ireland

Animal manure is the major source of Ammonia emissions in Ireland, contributing to environmental issues such as air pollution, water contamination, and greenhouse gas emissions. Although ammonia is essential as a fertilizer for agricultural production, it is released during the pumping and land application of animal manure slurries. One way to reduce emissions and minimise the need for fossil sources of ammonia for fertilizer production is to remove and recover ammonia from animal manure wastes. However, due to the nature of production, animal manure volumes are dispersed with relatively low ammonia concentrations compared to industrial solutions. Therefore, the economics of ammonia recovery are not promising. To investigate the critical factors regarding the feasibility of ammonia recovery, a model was generated in Aspen PlusV11 to simulate the recovery of ammonia using air stripping and subsequent production of Ammonium Nitrate fertiliser using an absorber. This was then combined with a cost-of-collection model to determine the overall economic feasibility of the process. Factors, including the size of the operation, energy and chemical costs, and operating temperature, were investigated using Aspen Plus. In contrast, manure transport cost and production intensity were examined using the cost collection model. Air stripping simulations are supported by experimental results to validate the assumptions being made and provide confidence in the results. Initial results indicate that while the transport cost of manure to a production facility is significant, the scale of the process recovery facility has a more considerable impact on the overall feasibility of such a recovery process. A benefit of 1.11 $ per kilogram of recovered nitrogen was obtained for the high scale of production, and -1.01 and 0.22 were obtained for the low and medium scale of production, respectively. Future work will consider the influence of pretreatment technologies on process economics and examine other recovery technologies, including gas-permeable membrane recovery.

O7

SialMAX: Maximizing Biopharmaceutical α-2,6-Sialylation in CHO Cells

[1]*Abascal Ruiz, C., & [1]Del Val, I.J.
*Lead presenter: cristina.abascalruiz@ucdconnect.ie
[1] School of Chemical and Bioprocess Engineering, University College Dublin, Ireland

Controlling and enhancing sialylation is crucial for optimizing the functionality of monoclonal antibodies (mAbs) and ensuring consistent, high-quality manufacturing, thereby promoting therapeutic efficacy. Our innovative approach strategically shifts sialylation from α-2,3 to α-2,6-sialic acid, pioneering precision optimization in biopharmaceutical manufacturing. As mAbs play a pivotal role in treating diseases like cancer and autoimmune disorders, addressing challenges in glycosylation variability becomes imperative. Our research focuses on unravelling the intricate landscape of glycosylation, with sialylation emerging as a key factor influencing mAb therapeutic mechanisms.

Building upon the GalMAX technology, we employ CRISPR/Cas9 genome editing to target α-2,6-sialylation in CHO cells by eliminating α-2,3-sialylation. By knocking out the ST3GAL3, ST3GAL4, and ST3GAL6 genes encoding α-2,3-sialyltransferase, we pave the way for a sophisticated glycoengineering approach. Our progress includes the successful removal of these genes, a pivotal step toward achieving optimal α-2,6 sialylation. Additionally, we introduce a novel lectin-assisted confocal laser scanning microscopy technique for efficient screening and phenotypic characterization of clones, streamlining the assessment of 2,3 K.O. lines and ensuring effective screening of diverse cell lines with varied Glyco-profiles. As we advance to the characterization phase, our approach holds significant promise for enhancing the therapeutic potential of mAbs, setting a new standard in precision glycoengineering and revolutionizing biopharmaceutical manufacturing.

O8

Digital Design for Advanced Biotherapeutics: A Model-enabled Framework for the Development and Optimisation of Biopharmaceutical Cell Culture Processes

[1,2*]Doyle, K., [1,2]Glennon, B., & [1]Del Val, I.J.
*Lead presenter: kallum.doyle@ucdconnect.ie
[1]School of Chemical and Bioprocess Engineering, University College Dublin, Ireland;
[2]Applied Process Company Limited, Ireland

Driven by scientific innovation and apposite regulatory guidance, the biopharmaceutical industry continues to evolve and adapt to meet the growing needs of patient populations worldwide. Prospective pipelines for investigative biotherapeutic candidates continue to expand, while the advent of biosimilars and further promise of advanced therapy medicinal products (ATMPs) place greater economic uncertainty and commercial pressure on the development of new therapeutic entities. Clinical and commercial success is largely contingent upon the timely and efficient development of quality biotherapeutic candidates. Of central importance, the development of a robust manufacturing strategy is paramount to ensure the therapeutic candidate can be reliably produced with the desired quality profile to consistently deliver demonstrated clinical performance.

Implementation of the Quality by Design framework greatly contributes towards assurance of product quality through the systematic identification of critical quality attributes (CQAs), and subsequent linking of process inputs to CQAs though critical process parameters (CPPs) and critical material attributes (CMAs). By closely integrating quality attributes within the manufacturing process, product quality may be designed into the process during development, providing greater confidence and assurance in the established manufacturing strategy.

To effectively link product quality with the manufacturing process, statistical and mathematical models provide an effective approach for quantitatively identifying the relationship between the manufacturing process and product quality. While limitations have been identified across both statistical and mechanistic modelling approaches, novel hybrid modelling strategies, which combine statistical malleability with mechanistic certainty, are expected to advance process development programs through reduced model development effort, improved predictive performance, extended extrapolative capability, and capacity to capture the influence of process inputs and state variables on cell culture dynamics. Hybrid models thus have the potential to accelerate bioprocess development while ensuring the most efficient use of experimental resources during an investigative campaign.

In this presentation, a brief overview of model-enabled bioprocess development for mammalian cell culture processes will be provided. Commencing with an introduction to high-throughput cell culture technology, automated strategies for experimental data analysis and dynamic model assembly will be subsequently reviewed. To finalise, establishment of hybrid dynamic models utilising Gaussian Process Regression algorithms will be presented, in alignment with addressing current challenges in design space characterisation, process scale-up, and resource-effective experimental design.

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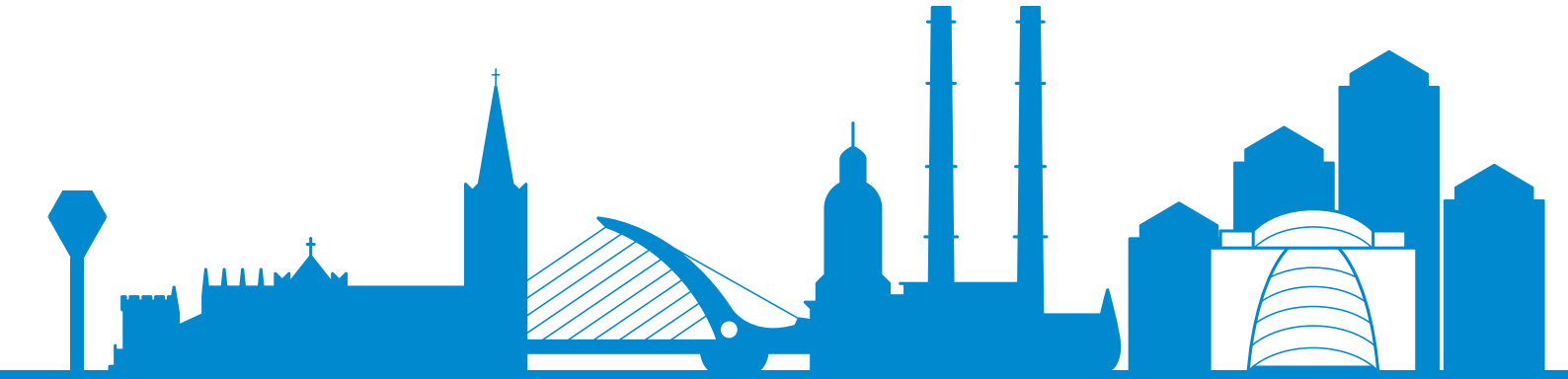

POSTER PRESENTATIONS

BOOK OF ABSTRACTS

P1

Development of Approaches that Enable Demand Response in Wastewater Treatment Plants

[1]*Aghdam Tabar, S., [1]Casey, E., [1]Dereli, R.K., & [2]Cotterill, S.
*Lead presenter: saba.aghdamtabar@ucdconnect.ie
[1]School of Chemical and Bioprocess Engineering, University College Dublin, Ireland
[2]School of Civil Engineering, University College Dublin, Ireland

Wastewater treatment plants (WWTPs) are significant energy consumers but also hold potential to produce energy through anaerobic digester process. WWTPs possess the capability to stabilize energy grids with their existing infrastructure and promote the integration of renewable energy sources into the energy system. WWTPs can handle various energy forms i.e. electricity, heat, biogas, etc., making them flexible energy prosumers. However, opportunities for cooperation between WWTPs and energy systems remain largely underexplored. This study explores innovative technologies and methodologies to optimise the synergetic relationship between WWTPs and energy systems. One promising approach involves utilising surplus energy to power the electrolysis process, thereby generating hydrogen for biogas upgrading within WWTPs. This process enhances the calorific value of biogas and valorises it. Furthermore, the oxygen by-product of the electrolysis process can be used for aeration in biological treatment within WWTPs, enhancing energy efficiency. This dual-purpose utilisation highlights the multifaceted benefits of integrating innovative technologies into WWTP operations. Through mathematical modelling, this research assesses the impacts of integrating such technologies, both within the WWTP sector and on the broader energy grid. As an initial step, this study investigates the integration of power-to-hydrogen technology with anaerobic digestion using the Benchmark Simulation Model No. 2 (BSM2), a widely accepted model for testing control and operational strategies in WWTPs. By incorporating hydrogen injection into the BSM2 anaerobic digester model, a grid-synchronized approach is presented for optimizing the anaerobic digestion process. This approach dynamically responds to Ireland's surplus renewable energy to produce the required amount of hydrogen for converting biogenic CO_2 into CH_4 gas in the anaerobic digester. Over the simulation period, H_2 injection leads to an average 10% increase in the biomethane content of biogas demonstrating the potential of grid-synchronized Power-to-Gas technology to contribute to Ireland's decarbonization and biomethane production goals.

P2

The Effect of Cold Atmospheric Plasma (CAP) and Plasma Activated Liquid (PAL) on Mesenchymal Stem Cells (MSCs) and MSC extracellular vesicles (EVs)

[1*]Alsiyabi, S.,[1]Boehm, D.
*Lead presenter: sara.a.alsiyabi@ucdconnect.ie
[1] School of Chemical and Bioprocess Engineering, University College Dublin, Ireland

Mesenchymal Stem Cells (MSCs) are multipotent cells that have the ability self-renew and to differentiate into multiple lineages. Due to their unique capabilities, MSCs have been intensely researched as a potential regenerative therapeutic agent. MSCs Extracellular vesicles (EVs) are membrane-bound functional cargo transporters that play a vital role in proliferation, regeneration and immune regulation. Several studies have shown that MSCs-EVs combat the limitation that comes with utilizing MSCs such as aging and tumorgenic risks [1]. Cold Atmospheric Plasma (CAP) is ionized gas that contains ions, electromagnetic fields, UV and Reactive Oxygen and Nitrogen Species (RONS) has wide applications in medicine, which include wound healing, anti-tumour activity, disinfection as well as its ability to promote cell proliferation [2]. In addition to CAP, studies have also reported that Plasma-Activated Liquid (PAL) provides a more stable alternative to direct CAP [3]. This study aims to investigate the effect of CAP/PAL on human MSC viability and proliferation as well as exploring changes in MSC-EV cargo and production inflicted by exposure to CAP/PAL. Experiments are conducted using MSCs obtained from commercial suppliers and cultured as per supplier's protocol. Plasma treatment is conducted using a helium-based Atmospheric Pressure Plasma Jet (APPJ) device (J-Plasma, Apyx Medical). For direct CAP experiments, cells are exposed directly to plasma at predefined parameters. For PAL treatment, liquids are exposed to direct CAP for different exposure times and reactive species are characterized using colorimetric assays for determination of hydrogen peroxide, nitrites and nitrates, following which cells are treated with PAL. Changes in cell metabolic activity are measured using Resazurin colorimetric analysis and changes in cellular mass are measured using Crystal Violet staining. EVs are isolated from treated MSCs using ultracentrifugation and Size Exclusion Chromatography (SEC). Subsequently, they are analysed through flow cytometry and Nano-particle tracking (NTA). This work determines safe CAP/PAL parameters at which further investigations of cellular changes can be conducted to further understand how CAP/PAL-generated RONS induce cellular proliferation. Additionally, it investigates changes in cargo and production of MSCs-EVs. Both of which could potentially be used to optimize and enhance the use of MSCs and their EVs in regenerative cellular and cell-free treatments.

This work is supported by funding from University College Dublin Ad Astra Fellowship. Extracellular Vesicles work is supported by the UCD STEM Funding (ref no: 80672).

References:

1. Kou, M., Huang, L., Yang, J. et al. Mesenchymal stem cell-derived extracellular vesicles for immunomodulation and regeneration: a next generation therapeutic tool?. Cell Death Dis 13, 580 (2022).
2. Dušan Braný, Dana Dvorská, Erika Halašová and Henrieta Škovierová. Cold Atmospheric Plasma: A Powerful Tool for Modern Medicine, International Journal of Molecular Science, 21, 29-30 (2020).
3. Vlad, I.-E. & Anghel, S. D. Time stability of water activated by different on-liquid atmospheric pressure plasmas. Journal of Electrostatics 87, 284–292 (2017).

P3

The monitoring and optimization of cell culture medium preparation to support bioprocess intensification

[1*]Boles, C.M., Jones, R.C., & Whelan, J.
*Lead presenter: claire.boles@ucdconnect.ie
[1]School of Chemical and Bioprocess Engineering, University College Dublin, Ireland

The aim of this research project is to investigate the application of an image-based process analytical technology (PAT) tool to the real-time monitoring of media prep processes, which can be used to develop a fundamental understanding of the process and inform development and optimization activities. The on cell culture medium are ever increasing from process intensification in order to meet current and future pharmaceutical demands. Cell culture medium must supply all nutrients to support cellular growth and production. The greater the cell density and product titre in a process, the greater the nutrient demand and the more challenging it is to achieve and maintain dissolution of the required concentrations of nutrients. Medium preparation is affected by parameters such as mixing time, temperature, pH and addition order of the constituent components. Currently process development relies on trial and error due to the challenges associated with monitoring dissolution endpoint. This project aims to develop the application of image analysis-based process monitoring using Canty's image analysis system as a PAT tool to track the progression and endpoint of dissolution of medium components during the preparation process. The application will support the intensification of cell culture processes requiring media enrichment. The process understanding provided by the Canty technology will be used to develop a process model for media prep which will be used in order to optimize cell culture media processes. This will be used to perform in silico experiments in order to propose an optimization strategy that will ensure robust, reliable media prep at scale. Technologies such as Malvern and focussed beam reflectance measurement will be used to measure particle size for comparison to the Canty system's output. The information will be used both to develop and validate the Canty method and ensure confidence in its validity and performance. The optimized conditions determined using the model will be experimentally verified. The project will primarily focus on the use of the NISTCHO cell line which produces an IgG monoclonal antibody and its associated basal and feed medias for cell culture. The raw materials i.e. media components will be characterized. The quality of the media prepared will be determined using a combination of analytical methods e.g. HPLC for the determination of amino acid concentration and functional testing. A Design-Of-Experiment approach will be taken to the experimental mapping of the media prep design space in order to maximise the information gained per experiment.

P4

Aggregates in Action: Dynamics and Spatial Organization in Biofilm Formation

[1]*Brink, J., [1]Dunne, C., [1]Singh, D., [1]Hiebner, D., & [1]Casey, E.
*Lead presenter: jacobus.brink@ucdconnect.ie
[1]School of Chemical and Bioprocess Engineering, University College Dublin, Ireland

Bacteria have typically been known to exhibit two principal lifestyles: either as free-floating planktonic individuals or as surface-attached biofilms. However, suspended bacterial aggregates are increasingly being highlighted as significant forms of bacterial communities distinct from surface-attached biofilms and are frequently found in clinical and environmental settings. Despite similarities, these non-attached cell clumps exhibit varying sizes and distinct formation mechanisms. This study aims to compare biofilm characteristics depending on whether they originate from either planktonic cells or bacterial aggregates. Our focus is on spatial organization, cell viability, and the interplay between growth rates, detachment processes, and consequential recolonization. To this end, elongated culture times are used to control aggregate size. Aggregates will be introduced under both static and flow conditions to monitor their fate as nuclei for biofilm development as well as their interaction with already established biofilms. Confocal laser scanning microscopy (CLSM) and advanced image processing will be used to provide a comprehensive understanding of the dynamics of this biofilm system. To date, changes have been observed in the initial phase of biofilm formation, specifically concerning the attachment rates of planktonic cells, across varied environmental conditions. There was a lack of significant detachment of surface-attached planktonic cells at the initial stages of biofilm formation under varying flow conditions (e.g., flow rates and media composition). However, further experiments are ongoing to further substantiate these observations and to assess the biofilms structural characteristics under the different formation conditions over longer time periods. Characterizing and understanding the dynamics of biofilm-aggregate interactions will enable a better understanding of the biofilm life-cycle in order to develop new technologies for the exploitation of biofilms as well as new modalities for biofilm removal and control.

P5

Model-based optimization of control in glycosylation control in antibody-producing CHO cells

[1]*Gialamoidou, S., & [1]Del Val, I.J.
*Lead presenter: sofia.gialamoidou@ucdconnect.ie
[1]School of Chemical and Bioprocess Engineering, University College Dublin, Ireland

Monoclonal antibodies (mAbs) are the highest-grossing class of pharmaceuticals and had worldwide revenues of over US$180 billion in 2022 [1]. The success of these therapeutic proteins arises from their effectiveness in treating prevalent autoimmune disorders as well pervasive types of cancer. Glycosylation is a critical quality attribute of monoclonal antibodies (mAbs) because it defines their safety, serum half-life and efficacy. Even small changes in bioprocess conditions can lead to significant changes and have a negative impact on safety and efficacy of mAbs [2]. Controlling biopharmaceutical processes to produce mAbs with optimal and consistent glycosylation is imperative and computational modelling have been widely used to facilitate this goal [3]. Dial-A-Sugar aims to control the biomanufacturing processes by integrating computational modelling with cutting-edge synthetic biology tools to enable the manufacture of mAbs with targeted and consistent glycosylation profiles.

In the present work, a mathematical model describing N-linked glycosylation in CHO cells is proposed as a component for achieving real-time control of mAb glycosylation. Specifically, a dynamic mathematical model is developed that describes the glycosylation process based on an extensive reaction network which yield to highly complex glycan structures that have been observed in CHO cells. The model considers cisternal maturation and approximates the Golgi apparatus as a single Plug Flow reactor. The kinetic expressions in the present model were expanded by accounting for the structural and kinetic mechanisms by which these enzymes act. Optimization-based methodologies have been implemented for the estimation of enzyme concentration profile parameters.

The parameters that define enzyme distribution were estimated by seeking the minimum amount of total enzyme to obtain the desired oligosaccharide profile. With the obtained parameters from optimisation-based method, the model has been shown to generate results that are in good agreement with previously reported observations and enzyme distribution was found to follow expected patterns. Moving forward, this mathematical model can be integrated with models for cellular metabolism to identify the critical process parameters and aid in the control and optimization of biopharmaceutical processes.

References

1. Walsh, G., *Biopharmaceutical benchmarks 2018.* Nat Biotechnol, 2018. **36**(12): p. 1136-1145.
2. Zhang, P., et al., *Challenges of glycosylation analysis and control: an integrated approach to producing optimal and consistent therapeutic drugs.* Drug Discov Today, 2016. **21**(5): p. 740-65.
3. Schiestl, M., et al., *Acceptable changes in quality attributes of glycosylated biopharmaceuticals.* Nat Biotechnol, 2011. **29**(4): p. 310-2.

P6

Long-term Assessment of Hydrogen Technology Deployment for Large-scale Power Production: Impact of Electrolyser and Fuel Cell Efficiency

[1]*Khammadov, K., [2]Flynn, D., & [1]Syron, E.
*Lead presenter: kamran.khammadov@ucdconnect.ie
[1]School of Chemical and Bioprocess Engineering, University College Dublin, Ireland
[2]School of Electrical and Electronic Engineering, University College Dublin, Ireland

Currently in most countries electricity is generated via fossil fuels when there is insufficient renewable electricity. Hydrogen generated through electrolysis and low-carbon techniques could potentially be used to provide this back up electricity and reduce carbon emissions. Competitiveness of the hydrogen production, storage and utilisation technologies play a critical role in realising the potential of the hydrogen in the energy systems transition. While low-carbon hydrogen holds a competitive edge in present market conditions, it is crucial to adopt a long-term perspective, considering the potential impact of technology advancements and carbon policies. Hydrogen can be used to produce electricity via fuel cells which have a high cost and low lifespan, and gas turbines which can be modified to burn blended gases or 100% hydrogen, although these have a lower efficiency. Hence, the objective of this research is to assess how electrolyser and fuel efficiency improvements will influence the future roadmap of hydrogen technologies in helping the energy system meeting stringent carbon emission reduction targets.

A mathematical model of a large-scale power system was created for a long term energy transition period up to 2050. Power supply-demand and associated gas consumption balances were modelled using an hourly timeframe. Electricity generation included wind and solar technologies, gas turbines, and fuel cells. Energy storage options included in the model were compressed hydrogen storage and batteries. Hydrogen in the model can be sourced from electrolysers or the external procurement of low-carbon hydrogen. Biomethane was included in the model as an alternative renewable gas. All Ireland energy system data was used for power demand, existing infrastructure, renewable energy potential and carbon emission targets. The model objective was to minimise the net present cost of the overall system which included technology, fuel, and carbon cost. The mathematical model was built and solved in Python software with the Gurobi solver. Electrolyser and fuel cell efficiencies were the variable factors with 3 levels in the optimisation cases.

Results highlighted the significant impact of electrolyser efficiency on both electrolyser deployment and consequent hydrogen production. Increasing the electrolyser efficiency from 60% to 80% resulted in a rise in installed electrolyser capacity from 5.4 GW to 7.3 GW by 2050. Hydrogen production via electrolysis also was increased by 64%. Electrolyser deployment began in 2030, accounting for up to 90% of the overall hydrogen supply during the transition period. The results indicated that approximately 4.5 GW of the existing conventional gas turbine capacity should be upgraded to burn hydrogen in all cases. Fuel cell deployment increased from 0.1 GW to 0.5 GW due to the improvement in fuel cell efficiency from 50% to 70%. Gas turbine upgrades were observed to occur before 2030, while fuel cell deployment was delayed until 2050. Levelised cost of the energy is not influenced significantly with the efficiency improvements in electrolyser and fuel cell. The overall results indicate that electrolyser efficiency significantly impacts the competitiveness of hydrogen production via electrolysis in the long term. Gas turbine upgrades are more cost-competitive than fuel cells despite the enhancements in fuel cell efficiency.

P7

Dial-A-Sugar: Engineering CHO Cells For Deployment of Actuators for Real-Time mAb Glycosylation Control

[1]*Lim, S.L.Y., [1]Kaur, R., [1]Tsopanoglou, A., [2]Blanco, A., [3]Carillo, S., & [3]Bones, J., [1]Del Val, I.J.

*Lead presenter: sheryl.lim@ucdconnect.ie

[1]School of Chemical and Bioprocess Engineering, University College Dublin, Ireland;

[2]Flow Cytometry, Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Ireland;

[3]Characterisation and Comparability Laboratory, NIBRT, Ireland

Glycosylation is defined as the attachment of carbohydrates or sugars to the backbone of a protein through an enzymatic reaction and is a form of co-translational or post-translational modification [4]. Due to the numerous variations at the end of glycosylation reactions, the macroheterogeneity (i.e., presence or absence of glycans) and microheterogeneity (i.e., glycosidic linkages and varying degrees of mannosylation, antennarity, core fucosylation, galactosylation, and sialylation) on mAbs heavily influences the safety and efficacy of protein therapeutics [5]. Therefore, Dial-A-Sugar aims to control the biopharmaceutical manufacturing processes by integrating sophisticated computational modelling with cutting-edge synthetic biology tools to produce mAbs containing optimal and consistent glycosylation for safe, efficacious, and cost-effective treatments.

The framework combines all components of Model Predictive Control (MPC): (i) Actuators that enable real-time tuning of mAb glycosylation; (ii) Sensors that analyse cell surface glycosylation; and (iii) a computational model which relates cell culture inputs with mAb synthesis and glycosylation. The focus of this doctorate is on the actuators, where the expression of two glycosylation enzymes, α-1,6 Fucosyltransferase (Fut8) and β1,4-Galactosyltransferase (β4GalT1), will be controlled in real-time using lineariser inducible transcriptional circuits.

Dial-A-Sugar will utilise two negatively autoregulated lineariser gene circuits: (i) TetR_Lin; and (ii) PhlF_Lin [6-8]. Both linearisers were chosen as they display highly favourable fold induction and linear dose response [6]. The linearisers will contain a gene of interest (GOI) (either Fut8 or β4GalT1) and a repressor protein gene (either TetR or PhlF). Addition of small molecular inducers (SMI) will unbind repressor proteins from the operons to enable transcription of itself and the GOI.

To evaluate the performance of the linearisers, a master cell line with: (i) the endogenous glycosyltransferase genes knocked-out; and (ii) an orthogonal landing pad system, must be established first. The genes Fut8 and β4GalT1 were disrupted or removed by CRISPR/Cas9 [9]. Cells were subsequently selected through: (i) lectin-aided flow cytometry for positive Fut8 knockout phenotype; and (ii) non-deletion/deletion PCR after single cell cloning for β4GalT1 knockout confirmation. In addition, the mAb product harvested from the double knockout cell lines were sent for glycan analysis to ensure complete abrogation of both glycosyltransferase genes [10]. Following the establishment of the double knockout cell line, the orthogonal landing pad system can be integrated into the safe harbour sites (sites A and T9) aided by CRISPR/Cas9 as well. A landing pad system was deemed to be the best solution to aid in controlling the location of gene integration and copy number [11] to ensure stoichiometric equivalence of the repressor protein gene and the GOI. Both landing pad vectors were constructed by Gibson Assembly [12], and landing pad vector Site A has been integrated into the double knockout cell line. Single cell isolation and characterisation of the landing pad cell line is still ongoing before the second landing pad can be introduced into the cells.

References

1. Walsh, G., *Biopharmaceutical benchmarks 2018.* Nat Biotechnol, 2018. **36**(12): p. 1136-1145.
2. Zhang, P., et al., *Challenges of glycosylation analysis and control: an integrated approach to producing optimal and consistent therapeutic drugs.* Drug Discov Today, 2016. **21**(5): p. 740-65.
3. Schiestl, M., et al., *Acceptable changes in quality attributes of glycosylated biopharmaceuticals.* Nat Biotechnol, 2011. **29**(4): p. 310-2.
4. Varki, A., et al., in *Essentials of Glycobiology*. 2009, Cold Spring Harbor Laboratory Press

Copyright © 2009, The Consortium of Glycobiology Editors, La Jolla, California.: Cold Spring Harbor (NY).

5. Kontoravdi, C. and I.J. del Val, *Computational tools for predicting and controlling the glycosylation of biopharmaceuticals.* Current Opinion in Chemical Engineering, 2018. **22**: p. 89-97.
6. Szenk, M., T. Yim, and G. Balázsi, *Multiplexed Gene Expression Tuning with Orthogonal Synthetic Gene Circuits.* ACS Synthetic Biology, 2020. **9**(4): p. 930-939.
7. Nevozhay, D., T. Zal, and G. Balázsi, *Transferring a synthetic gene circuit from yeast to mammalian cells.* Nature Communications, 2013. **4**(1): p. 1451.
8. Farquhar, K.S., et al., *Role of network-mediated stochasticity in mammalian drug resistance.* Nature Communications, 2019. **10**(1): p. 2766.
9. Ronda, C., et al., *Accelerating genome editing in CHO cells using CRISPR Cas9 and CRISPy, a web-based target finding tool.* Biotechnol Bioeng, 2014. **111**(8): p. 1604-16.
10. Carillo, S., et al., *Comparing different domains of analysis for the characterisation of N-glycans on monoclonal antibodies.* J Pharm Anal, 2020. **10**(1): p. 23-34.
11. Grav, L.M., et al., *Minimizing Clonal Variation during Mammalian Cell Line Engineering for Improved Systems Biology Data Generation.* ACS Synthetic Biology, 2018. **7**(9): p. 2148-2159.
12. Sergeeva, D., et al., *Multicopy Targeted Integration for Accelerated Development of High-Producing Chinese Hamster Ovary Cells.* ACS Synthetic Biology, 2020. **9**(9): p. 2546-2561.

P8

Upstream Process Platform Development for Therapeutic Exosome Production

[1*]Luo, J.P., [1]Whelan, J., & [1]McDonnell, S.
*Lead presenter: junpeng.luo@ucdconnect.ie
[1]School of Chemical and Bioprocess Engineering, University College Dublin, Ireland

Exosomes are secreted Extracellular Vesicles (EVs) approximately 40-160 nm distinguished by their endosomal origin. Their biological role as natural messengers in intercellular communication endows them with significant therapeutic potential. Human Mesenchymal Stem Cells (hMSCs) is one of the most prevalent cell types for Cell Therapy Products (CTP) due to their remarkable immunomodulatory and regenerative effects. Recent research has shown that MSC's therapeutic efficacy is primarily conveyed through their secreted exosomes (accurately termed MSC-EVs), which have emerged as a safer and off-the-shelf substitution of direct MSC injection. However, there are significant challenges in the large-scale production of MSC-EVs which limits their therapeutic potential: (i) current production processes typically yield 10^9–10^{11} EVs per liter of conditioned media, whereas clinical applications are estimated to require up to 10^{13} EVs per dose [1]; (ii) 2D culture systems face scalability limitations, failing to meet the high sample demands for late-phase clinical trials and subsequent commercial-scale production; (iii) the understanding of how cell culture processes impact the yield and quality of MSC-EVs remains in nascent stages, leading to slow progress and a lack of consensus in process development.

This project endeavors to establish a leading platform for standardizing upstream process (USP) development and conquering the identified hurdles. The hTERT immortalized adipose-derived MSC (hTERT-AT-MSC) is chosen as the model cell line as it retains properties of hMSC while its infinite proliferation capacity is suitable for consistent large-scale production. The impact of different cultivation systems (e.g. multilayer flasks, microcarriers culture, hollow-fiber bioreactors and fluidized-bed bioreactors), modes of operation and process conditions on the yield, quality attributes, and Cost of Goods of MSC-EVs will be systematically investigated through a Design of Experiment (DoE) approach. The platform is expected to expedite the robust production and scale-up of safe and effective MSC-EV products.

References

1. Pincela Lins PM, Pirlet E, Szymonik M, Bronckaers A, Nelissen I. Manufacture of extracellular vesicles derived from mesenchymal stromal cells. *Trends Biotechnol*. 2023;41(7):965-981. doi:10.1016/j.tibtech.2023.01.003

P9

Separating End-of-Life Battery Materials using Dielectrophoretic Filtration

[1*]O'Donnell, M.C., & [1]Pesch, G.
*Lead presenter: mary.c.odonnell@ucdconnect.ie
[1]School of Chemical and Bioprocess Engineering, University College Dublin, Ireland

Lithium-ion batteries (LIBs) offer a reliable energy storage solution and remain the most common rechargeable battery type on the market. They are particularly relevant in the electric vehicle (EV) sector. EV sales are forecasted to rise from 7.3 million in 2022 to 44 million units in 2035, resulting in a greater demand for battery materials such as Co, Mn, Li and graphite [1]. LIBs have a life span of 10 years and vast quantities of end-of-life batteries will accumulate in waste streams in the future. Current recycling technologies are energy intensive and cannot recover all materials, especially graphite, responsible for ~ 50 % of the mass of each battery, is lost. Therefore, the battery recycling industry is in need of cleaner, more efficient recycling methods [2].

Dielectrophoretic (DEP) filtration offers an efficient and environmentally-friendly method to separate microparticles based on their physical and chemical properties. Particle mixtures can be separated based on their differences in particle material, size, shape and polarizability [3]. Crushed battery material, called black mass, is a particle mixture composed of graphite (anode) and lithium metal oxide (LMO, cathode). By applying an electric field at low AC frequency, particles with a greater conductivity than the suspension medium (water), experience a DEP trapping force within a DEP filter. In comparison, non-conductive particles are largely unaffected and flow through the filter as normal. Graphite particles are more conductive than LMOs therefore, graphite is expected to experience DEP trapping whereas, LMO particles continue to flow through resulting in two separated particle streams. This was demonstrated in a study by Kepper et al. in 2024, in which graphite and lithium iron phosphate (LFP) were successfully separated using polarisability-dependent DEP filtration [4].

In this poster, the selective separation and scalability of various battery components using DEP filtration will be examined. Firstly, the DEP behaviour of several battery materials at varying medium conductivities is observed using an interdigitated electrode DEP device. A DEP filter is then employed to separate a binary artificial black mass mixture of graphite and different LMO materials based on their distinct conductivities. The effect of applied voltage, medium conductivity, flow rate and frequency on separation efficiency is determined. In addition, the scalability of the filter is addressed by increasing the flow rate and electrode cross-sectional area.

At low AC frequency, (~15 kHz), graphite is predicted to become trapped within the filter whilst the LMO fraction flows through the filter unaffected. Particle trapping is expected to increase with voltage as well as the likelihood of Joule Heating which will be managed by adjusting fluid flow. Experimental parameters resulting in optimal separation efficiency and purity of particle streams will be determined to demonstrate the viability of employing DEP filtration as a reliable separation technique for black mass.

References

1. Power Technology, https://www.power-technology.com/news/globaldata-global-ev-growth-forecast-report/?cf-view, accessed Feb 2024
2. A. Hogan, ME Thesis, University College Dublin, 2023
3. G. Pesch, F. Du, 2021, Electrophoresis, 42, 134–152
4. M. Kepper et. al, 2024, Results in Engineering 21, 101854

P10

Post-dispersion dynamics of biofilm: deciphering the fate of a disrupted biofilm matrix

[1*]Singh, D., [1]Dunne, C., [1]Brink, J., [1]Hiebner, D., & [1]Casey, E.

*Lead presenter: dibyangshee.singh@ucdconnect.ie

[1]School of Chemical and Bioprocess Engineering, University College Dublin, Ireland

Biofilms are a complex community of microorganisms encased within a self-generated matrix of extracellular polymeric substances (EPS). The EPS provides protection to the microorganisms contained within, making them more resilient to environmental stresses and antimicrobial agents, surpassing the resilience of free-floating cells. The detachment of biofilms often leads to its partial disintegration into fragments of extracellular polymeric substance (EPS) matrix, biofilm-associated cells, and detached biofilm clusters, occurring over a significant time scale. Understanding the underlying mechanisms governing the relocalization efficiency of detached biofilm-associated cells and clusters, along with its impact on population dynamics in an established biofilm model, is impeded by inadequate research and a limited number of publications. The primary goals of the current study are to optimize the growth conditions of model bacteria, develop a platform for biofilm characterization, and investigate the interaction between particulates and biofilm. Additionally, understanding specific biofilm-particulate interactions under dynamic flow conditions can provide valuable insights on microbial dispersion kinetics. The methodological approaches include assessing proportionality of live and dead cell populations using confocal laser scanning microscopy (CLSM) and flow cytometry. A qualitative investigation of population dynamics will be conducted using CLSM and advanced image analysis tools, can be employed to monitor the discrepancy in morphological characteristics, spatial distribution of cells, changes in biochemical property, and the structural integrity of the biofilm matrix. Fluorescent tagged polystyrene particles of varied sizes and model bacteria for instance, *Pseudomonas fluorescence* and *Escherichia coli* have been selected for particle tracking study. These techniques collectively can provide insights into the fate, viability, and kinetics of the dispersed biofilm components. It is expected that dispersion events influenced by the introduction of particles will likely exert noticeable impact on the relocalization efficiency and viability of the dispersed entities, suggesting a potential disruption in the long-term dispersion kinetics. An anticipated shift in the dispersion rate is expected to be observed, elucidating the velocity and effectiveness of the dispersion process under varying environmental conditions. The present study establishes the framework for further research on the impact of biofilm dispersion on microbial ecology in mixed species biofilm models which have practical implications in several domains such as in biofilm-associated infections, healthcare settings, water treatment, and environmental science.

P11

Characterization, modelling and optimization of the cryopreservation and revival of mammalian cells

[1]*Zamprognio, M.S., & [1]Whelan, J.
* Lead presenter: maycou.soareszamprognio@ucdconnect.ie
[1] School of Chemical and Bioprocess Engineering, University College Dublin, Ireland

The cryopreservation and revival of mammalian cells represent a fundamental step for the manufacturing of biopharmaceutical products and Advanced Therapy Medicinal Products (ATMPs). However, while efforts in intensification of biopharmaceutical processes have been motivated by the need to reduce associated costs, increasing efficiency and the shortening of product cycle-time, the cryopreservation process faces challenges regarding the overall efficiency and capacity. This is due to ice formation and crystallization which can mechanically damage the cells; the usage of cryoprotective agents (CPAs) which can be cytotoxic, and the fluctuations of osmotic balance throughout the phase change which can affect the viability and integrity of cells [1].

Even though the empirical optimization of cryopreservation protocols has been widely discussed in literature for different cell lines, there is still a lack in fundamental understanding as to the relationship between cryopreservation conditions and the physiological state of the cell upon subsequent thaw.

Therefore, the aim of this project is to apply a blended approach of modelling and experimental results to understand the effect of key variables (i.e., cooling and warming rates, sample volume and freezing cell density) on CHO-K1 cell's viability attributes. The cell's viability attributes are measured by the trypan blue exclusion method as well as by flow cytometry with triple-staining of JC-1, YO-PRO3 and DRAQ7 dyes. The experimental data will inform the development of a predictive model by combining deterministic and data-driven methods i.e., hybrid modelling in MatlabR2022a®, as given by Figure 1. Ultimately the model developed with be used for the *in silico* optimization of the cryopreservation step, accelerating the development of a robust high-performing freezing process.

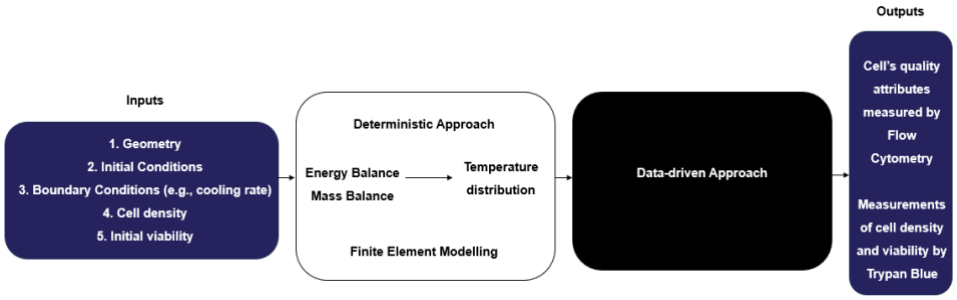

Figure 2: Hybrid model's structure

The preliminary results indicate the maximum relative residue of 2.5 % (node 84) for the freezing simulation of 1 mL solution (approx. 89 % water, 0.8 % NaCl and 10 % DMSO), when compared to a freezing profile of a cryovial with cell suspension (1.05×10^6 cells/ml) and 10 % DMSO. Additionally, the characterization of CHO-K1 cells indicates a drop of viability (by trypan blue staining) at day 7, when seeded with 0.4×10^6 cells/ml. This finding was supported by the reduction of subpopulation ratio of alive cells and their mitochondrial activity in day 6.

References

1. Pegg, D.E. (2007). Principles of Cryopreservation. Cryopreservation and Freeze-Drying Protocols, 368, pp.39–57. doi:https://doi.org/10.1007/978-1-59745-362-2_3.