POLITECNICO DI MILANO

PhD in CHIMICA INDUSTRIALE E INGEGNERIA CHIMICA / INDUSTRIAL CHEMISTRY AND CHEMICAL ENGINEERING - 41st cycle

THEMATIC Research Field: INTEGRATED BIOMANUFACTURING OF THERAPEUTIC OLIGONUCLEOTIDES

Monthly net income of PhDscholarship (max 36 months)
1400.0 In case of a change of the welfare rates during the three-year period, the amount could be modified.

Context of the research activity	
Motivation and objectives of the research in this field	Oligonucleotides, short-chain RNAs or DNAs, are introducing a change in the paradigm in the way of treating diseases. In fact, unlike conventional therapeutics targeting the proteins associated to a specific condition, oligonucleotides intervene at an earlier stage of the process, by regulating gene expression. This introduces the appealing opportunity of developing new therapies for complex diseases. Unfortunately, the processes currently in use for the production of oligonucleotides, mainly exploiting the phosphoramidite-based solid-phase synthesis, are associated to very low yield and huge waste generation. In order to overcome these limits, a recent approach has been proposed based on the recombinant manufacturing of oligonucleotides from the marine phototrophic bacterium Rhodovolum sulfidophilum. Still, the synthesis and chromatographic purification of the target nucleic acid is reported with reference to batch operations, associated to lot-to-lot variability and poor performance. On the other hand, an integrated framework combining the continuous biomanufacturing of oligonucleotides and their multicolumn continuous chromatographic purification is still missing. This, at the same time, would be crucial in the direction of improved process performance and environmental sustainability, required to sustain the expansion of the oligonucleotides marked expected in the

	near future. To overcome these limitations, the objectives of this research can be articulated in three levels: 1. Development of perfusion cultures of R. sulfidophilum and of a hybrid model of the operation. 2. Development of the multicolumn countercurrent solvent gradient purification (MCSGP) for the target oligonucleotide. 3. Integration of perfusion bioreactor and MCSGP to obtain a fully continuous end-to-end process for the biomanufacturing of oligonucleotides and development of a digital twin for the model-based process control.
Methods and techniques that will be developed and used to carry out the research	The first objective of this research is the establishment of perfusion cultures of R. sulfidophilum aimed at the biomanufacturing of the pre-miRNA-29b, as a model oligonucleotide, and will be carried out in collaboration with Jacobs Italia s.p.a. (https://www.jacobs.com/). This will require the investigation of the growth kinetics for the bacterium and product accumulation in the extracellular environment. The analysis will be first performed on scale-down models with the advantage of parallelizing the experiments with reduced consumption of materials. The results obtained will be then validated in a bench-top 2 L bioreactor. A hybrid kinetic model for the description of cell growth and product accumulation combining conservation equations and machine learning algorithms will be developed. The model will be trained with the experimental data obtained in both scale-down experiments and in the bench-top bioreactor and validated on experiments not used for model training. An additional aspect to be covered is the development of a soft sensor based on in situ analytics, e.g. Raman spectroscopy, for the real-time measurement of cell density and oligonucleotide concentration. A multivariate data analysis (MVD) model will be established based on an experimental training set and then validated by comparison of the results provided by the soft sensor with offline characterization of the bioreactor broth. In the second part of the project, MCSGP will be developed for the purification of the same oligonucleotide.

	Starting from single-column experiments, a step-by-step procedure will be followed to assess the influence of gradient slope, loading time and gradient boundaries on the chromatographic resolution, yield and productivity at a fixed purity specification. By taking advantage of the results achieved, a mechanistic model for the separation will be established. After validation in selected experimental tests, the model will be exploited in the design and optimization of MCSGP. A dynamic process control based on the UV signal recorded at the outlet of each of the two columns will be established to automatically modulate the characteristic times of MCSGP in the direction of improved process stability. Finally, the two sections of the process, i.e. perfusion bioreactor and MCSGP, will be integrated for a fully continuous end-to-end biomanufacturing of the pre-miRNA-29b. A digital twin of the process will be developed, with the aim of its model-based supervision and control. In this direction, information will be acquired in real-time from the soft sensor developed for the perfusion bioreactor and from the UV sensors in the chromatographic unit. These will require interpretation and elaboration to be used in the direction of a model update, as well as in the development of a predictive control strategy to be implemented on the process (e.g. by modulation of inlet flow rates and loading time for MCSGP).
Educational objectives	• In-depth understanding of biotechnology processes. • Awareness about the new challenges and trends characterizing the biopharmaceutical sector. • Experience in design and conduction of perfusion operations and multicolumn chromatographic processes. • Self-dependency and analytic thinking
Job opportunities	The candidate, after the PhD, will have the opportunity to operate in both academia as well as in companies focused on the manufacturing of biopharmaceuticals. The combination of modeling and laboratory experience allows the candidate to be versatile and particularly suitable for R&D sectors.

Composition of the research group	4 Full Professors 5 Associated Professors 3 Assistant Professors 20 PhD Students
Name of the research directors	Prof. Mattia Sponchioni

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Additional support - Financial aid per PhD student per year (gross amount)	
Housing - Foreign Students	--
Housing - Out-of-town residents	--

Scholarship Increase for a period abroad	
Amount monthly	700.0 €
By number of months	6

Additional information: educational activity, teaching assistantship, computer availability, desk availability, any other information
Confidentiality: since this is a thematic scholarship, the management of Confidential Information, Results and their publication is subordinate to the restrictions agreed upon with the funding company. Upon acceptance of the scholarship, the beneficiary may sign a specific commitment. **Educational activities** (funding for participation in courses, summer schools, workshops and conferences) - financial aid per PhD student per year: 1st year: around 1.900 euros 2nd year: around 1.900 euros 3rd year: around 1.900 euros **Teaching assistantship**: availability of funding in recognition of supporting teaching activities by the PhD student: There are various forms of financial support for activities in assistance to the teaching practice. The PhD student is encouraged to take part in these activities within the limits allowed by the regulation.