

Evaluation of batch vs. fed-batch strategy for biomass formation

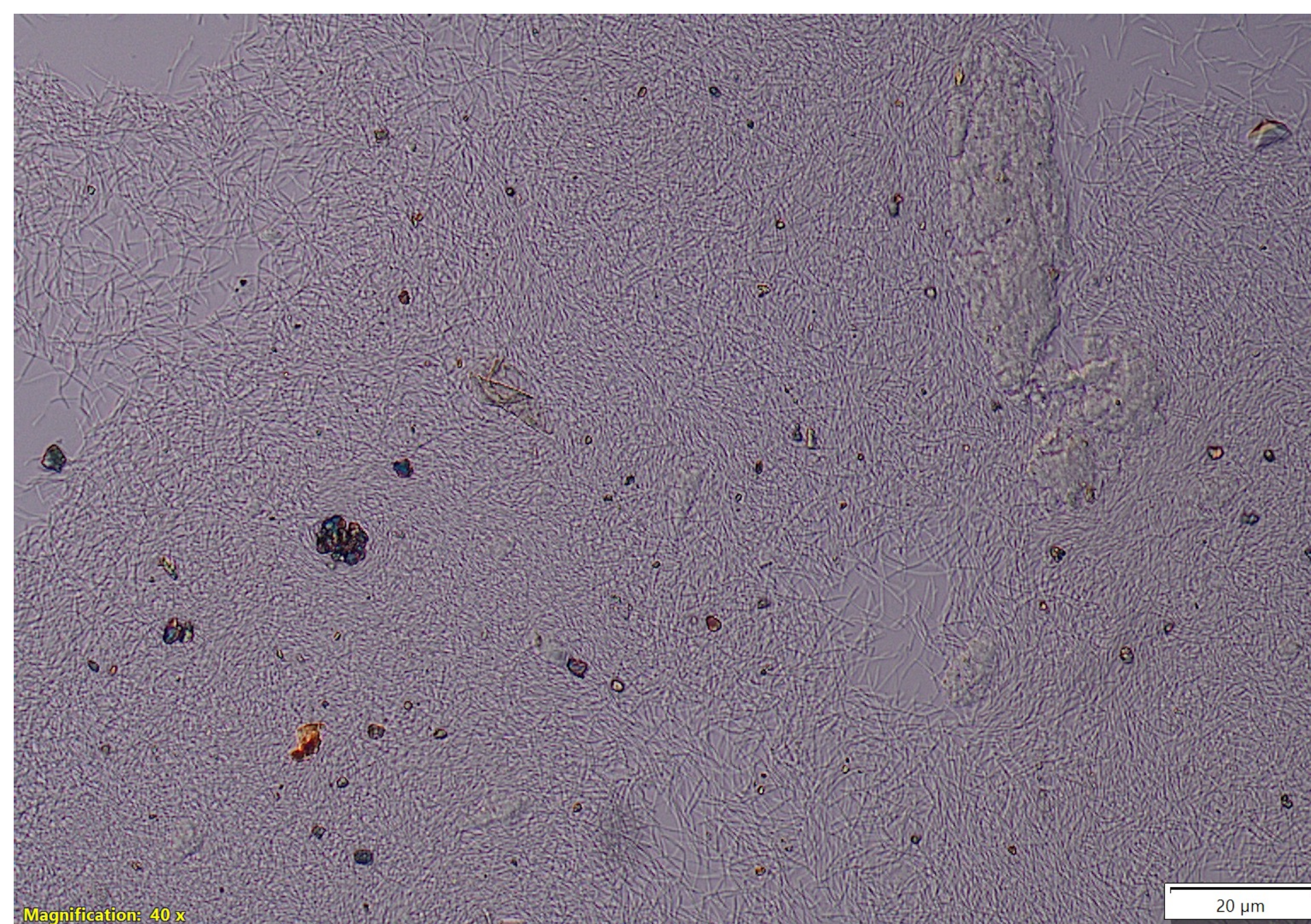
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Introduction

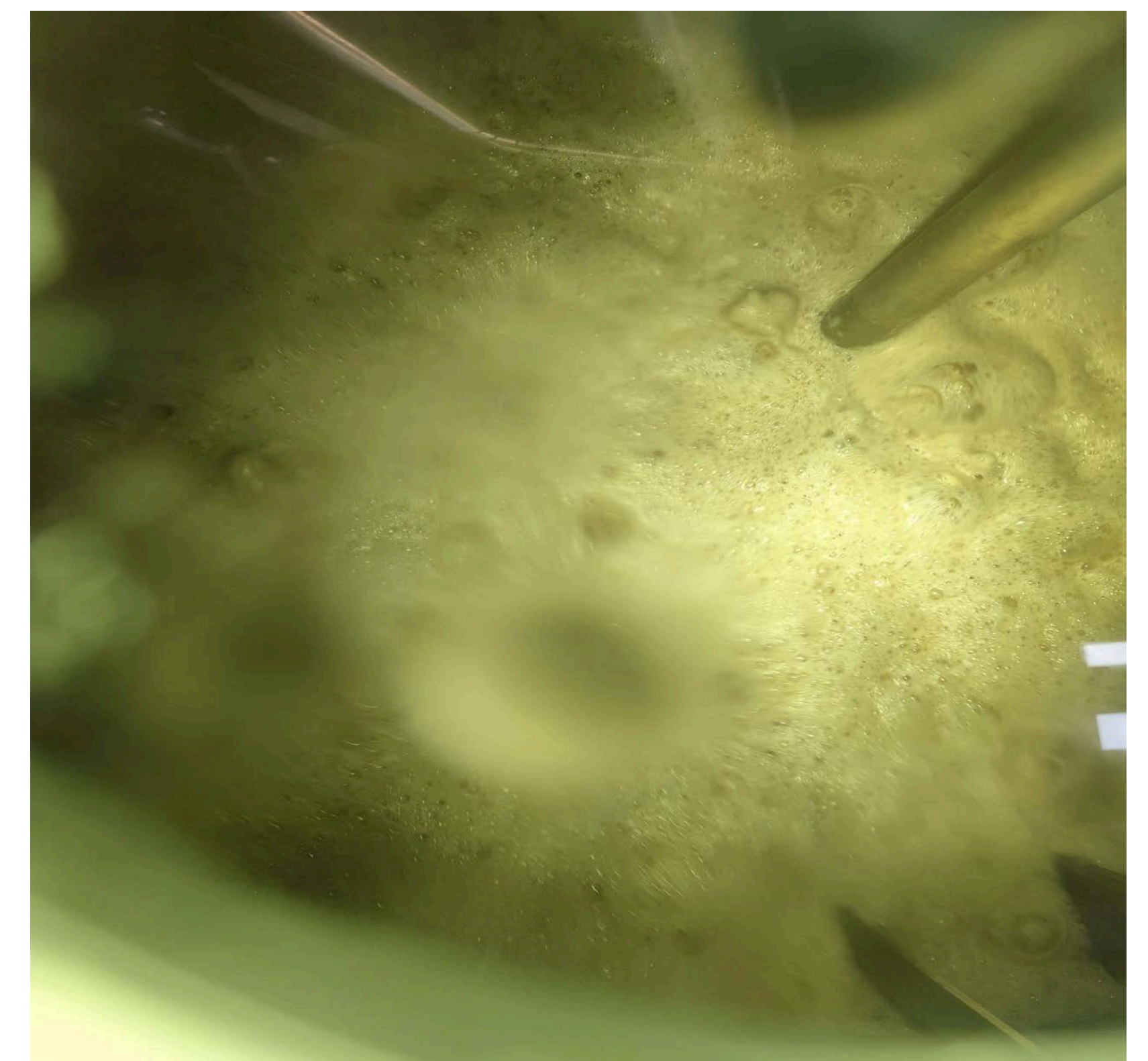
Industrial fermentation processes are widely used to produce enzymes and microbial products, relying heavily on well-balanced nutrient media and robust process control. In batch and fed-batch fermentation modes, the concentrations of salts, nutrients, and metabolic by-products change dynamically, requiring both off-line and on-line analytical approaches to monitor and optimize process performance. The master's project was conducted at **Novonosis**, focusing on both **fermentation strategy for seed tanks and media development**. A key objective was to design and evaluate a new fermentation medium based on potato protein, replacing the previously used soy protein, which showed variability and contamination risk. The alternative medium aimed to provide greater consistency and improve fermentation performance at production scale. This integrated approach provides valuable insight into how medium composition and fermentation strategy jointly influence process efficiency and product quality in industrial biotechnology.



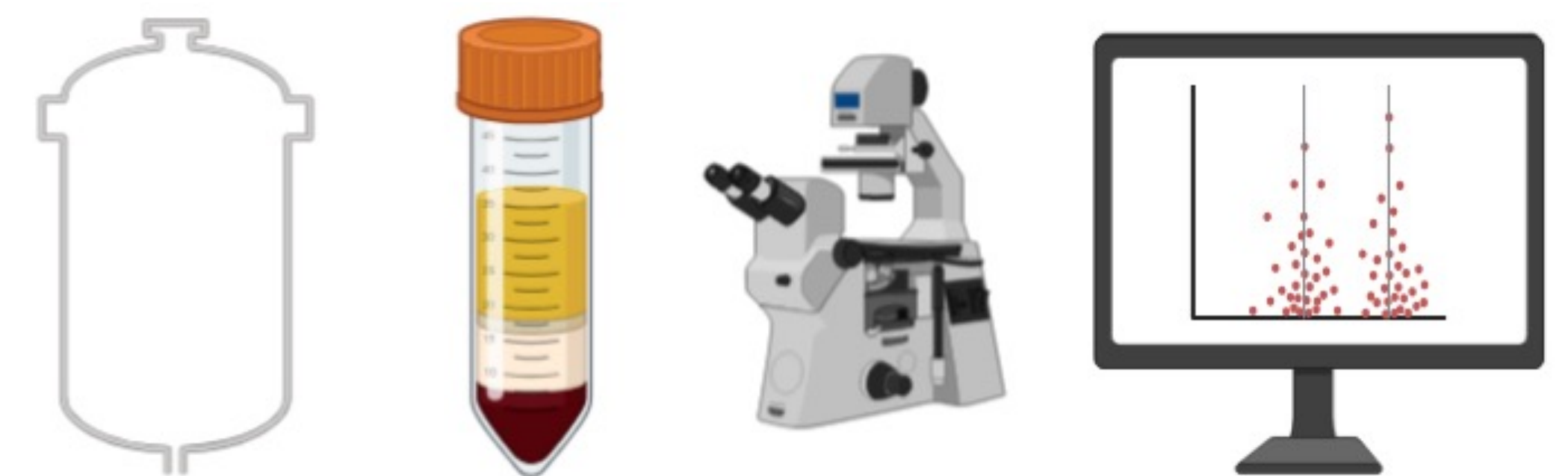
Samples taken from the bioreactor show the change in content of the different fractions during fermentation and the heterogeneity of the environment in the bioreactor.



The microscope photo shows large potato proteins, crystals of various salts and bacteria during fermentation in fed-batch mode.



Fermentation in a bioreactor results in significant amounts of foam due to the presence of proteins. It is necessary to use antifoam.



The project combined process trials, sampling preparations, light microscopy and data analysis to evaluate fermentation strategies and develop a new medium formulation based on potato protein.

Methods

The project combined process trials, lab-scale experiments and data analysis to evaluate fermentation strategies and develop a new medium formulation based on potato protein. Large-scale process trials were conducted in identical industrial bioreactors under both batch and fed-batch modes. Rather than applying traditional Design of Experiments (DoE), an iterative optimization strategy was followed, with incremental improvements made from one fermentation to the next. In the laboratory, biomass morphology was assessed using light microscopy, and samples were prepared for elemental composition analysis. Selected methods for biomass quantification were tested and compared to identify robust monitoring approaches. Data analysis integrated historical process data from Novonosis, real-time sensor outputs, and results from laboratory analyses. This enabled evaluation of biomass formation, nutrient dynamics, and process robustness across conditions.

Outcome

The project successfully demonstrated that soy protein can be replaced with potato protein in the fermentation medium for the investigated bacterial strain. In addition to this substitution, the composition of other medium components was optimized to support robust biomass growth. Production-scale fermentations were carried out in both batch and fed-batch modes, and biomass was obtained in both cases. Although fed-batch mode was expected to yield much better results, significant technical challenges were encountered during its implementation in the Novonosis production environment. Among all measured parameters, the carbon dioxide concentration at the bioreactor outlet proved to be the most reliable indicator of biomass quantity during fermentation. The project confirmed the feasibility of using potato protein as an alternative amino acids source and highlighted necessary steps for fed-batch implementation. **In conclusion**, this work provides a solid foundation for future improvements in industrial fermentation processes. It demonstrates that effective medium reformulation can be successfully achieved without compromising performance and highlights the key technical barriers that must be addressed to fully realize the advantages of fed-batch fermentation. It also identified potential directions for further research, including new process control strategies and technical improvements

	Batch mode (all sugar added at start)	Fed-batch mode (sugar dosing during fermentation)
Advantages	- significantly lower demands on equipment and process control	- up to 60% higher CO ₂ concentration at bioreactor outlet for the same substrate amount, indicating more biomass
	- higher repeatability in fermentation time and biomass yield (until fed-batch issues are resolved)	- higher biomass density and lower sugar content in the seed for the main tank
Disadvantages	- longer lag phase before bacterial growth	- technical problems with dosing system
	- no ability to maintain optimal sugar concentration	- high initial salt concentration leads to suboptimal ion conditions for bacteria
Shared challenges	- heterogeneity of process environment in bioreactors	
	- oxygen transport limitations	