Development of an Intensified Plasmid Production Process based on Continuous Lysis and Membrane Chromatography

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1 Introduction

The growing demand for plasmid DNA (pDNA) in gene therapy and vaccine development requires efficient and scalable production methods. Traditional batch lysis methods face limitations with long mixing times, high shear forces, and inconsistent lysis control. Additionally, resin-based purification is time-consuming and requires extra steps like RNA precipitation and tangential flow filtration (TFF). This work presents a continuous cell lysis device with low-shear pumps and a loop reactor for precise lysis control and minimal shear damage. Combined with Natrix® Q membrane chromatography, it allows high flow rates and RNA reduction without chemicals, optimizing conductivity for faster, cost-effective pDNA production with high supercoiled pDNA purity.
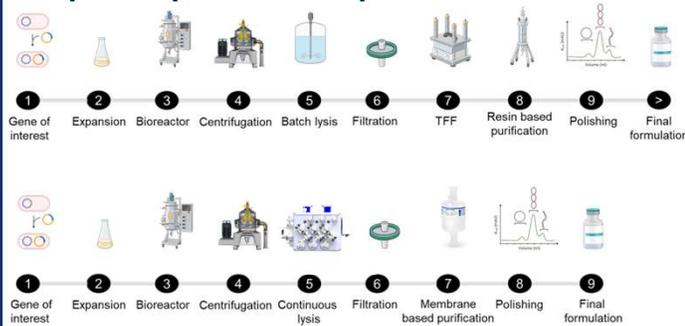
2 pDNA production process

Figure 1: Traditional versus intensified pDNA production process.
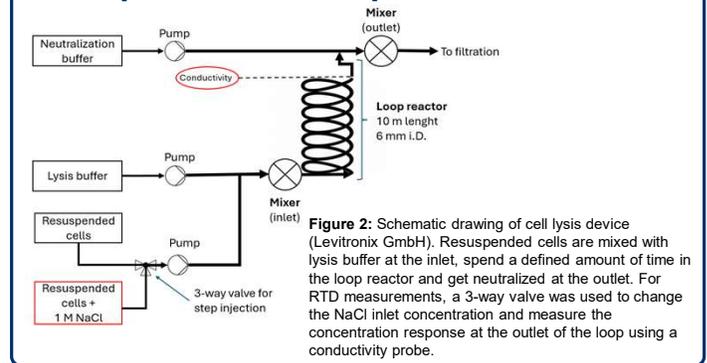
3 Experimental setup

Figure 2: Schematic drawing of cell lysis device (Levitronix GmbH). Resuspended cells are mixed with lysis buffer at the inlet, spend a defined amount of time in the loop reactor and get neutralized at the outlet. For RTD measurements, a 3-way valve was used to change the NaCl inlet concentration and measure the concentration response at the outlet of the loop using a conductivity probe.

4 Characterization of continuous cell lysis device
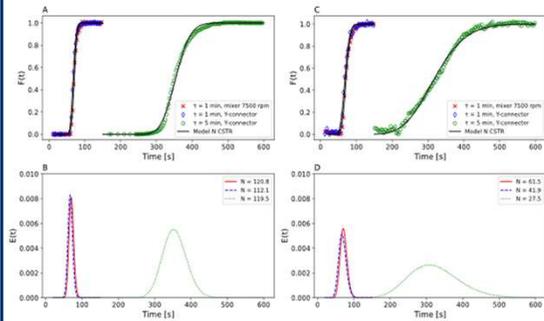

Figure 3: RTD measurement in loop reactor with water (A and B) and cells (C and D) using the number of continuous stirred tank reactor (N CSTR) model. The F(t)-curve represents experimental data and model fitting, the E(t)-curve represents the RTD.

Figure 3 illustrates RTD modelling in the loop reactor using water and cells. In both cases, NaCl was used as tracer to record the change of conductivity at the outlet of the loop reactor. The 3-way valve was used to create the step injection at t = 0. Short and long residence times were tested, and the effect of inlet mixing on the RTD was compared to a Y-connector.

Figure 4 demonstrates that while yields increase with extended lysis time, sample purity declines, due to contamination with proteins and nucleic fragments. Experiments incorporating active mixing prior to the loop reactor achieved superior yields and purity.
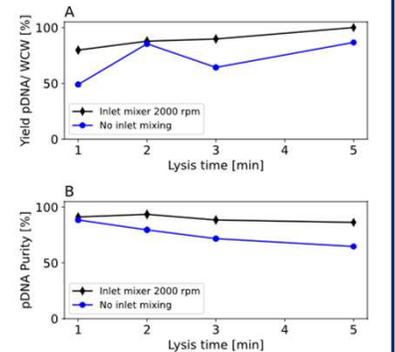

Figure 4: Influence of lysis time on pDNA yield (A) and final purity (B) using E. coli DH5-α.

5 Membrane based chromatography for pDNA purification

Figure 5 demonstrates that an optimal load conductivity of 76 mS/cm led to highest pDNA yield (A) and purity (B) for all plasmids tested. At lower conductivities, contaminants with lower charge densities, such as RNA and other impurities (proteins/ nucleic fragments), were able to bind to the membrane, resulting in reduced pDNA purity. In some instances, as shown in figure 6, there was an approximate 60% reduction of contaminants, primarily RNA. However, at higher load conductivities, the yield significantly decreased as plasmids were no longer able to bind effectively to the membrane.
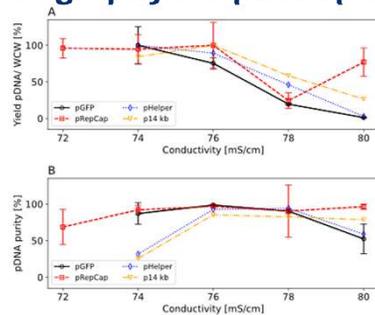

Figure 5: Influence of load conductivity on final pDNA yield (A) and purity (B) using membrane-based purification with Natrix® Q (Merck).
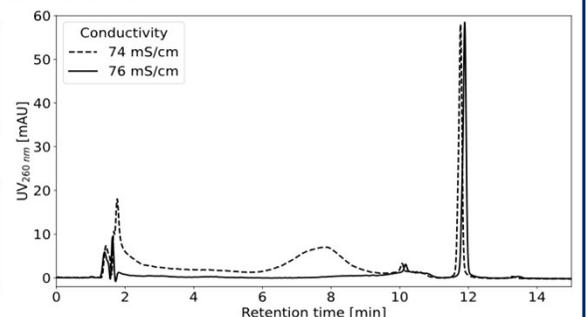

Figure 6: HPLC elution showing influence of load conductivity on RNA reduction for a 14 kb plasmid.

6 Conclusion

- Continuous cell lysis integrating dynamic inline mixers and a loop reactor for controlled alkaline lysis
- Membrane chromatography for faster, more efficient plasmid purification
- A process entirely free of RNase to meet regulatory requirements

7 Acknowledgement