Master of Science HES-SO in Life Sciences

Fermentative production of optically pure D-(-)-Lactic Acid on modified de Man Rogosa Sharpe medium and kinetic studies on the influence of various neutralizing agents

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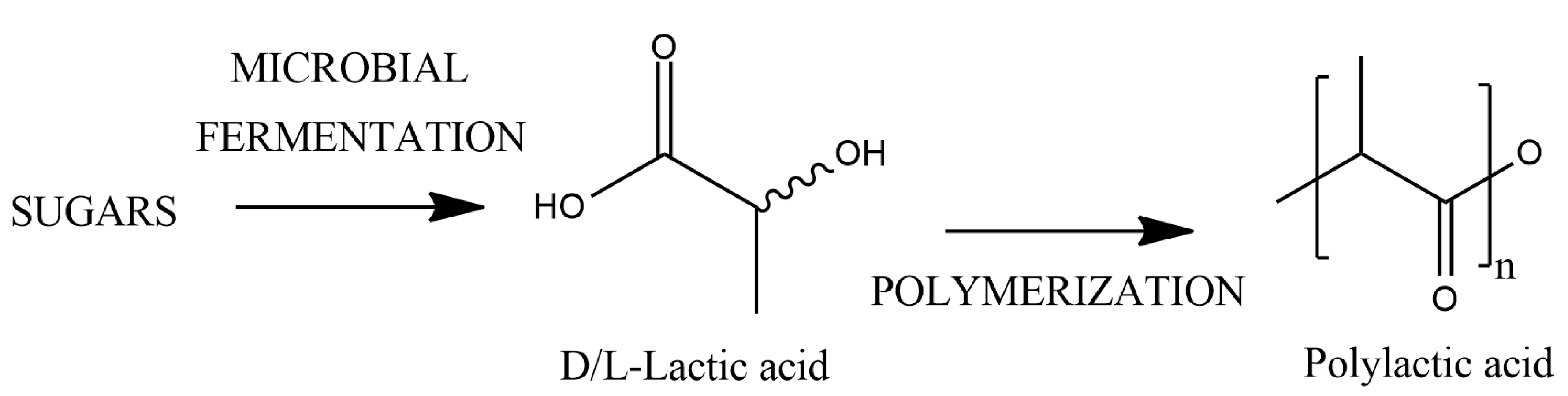
PROJECT BACKGROUND

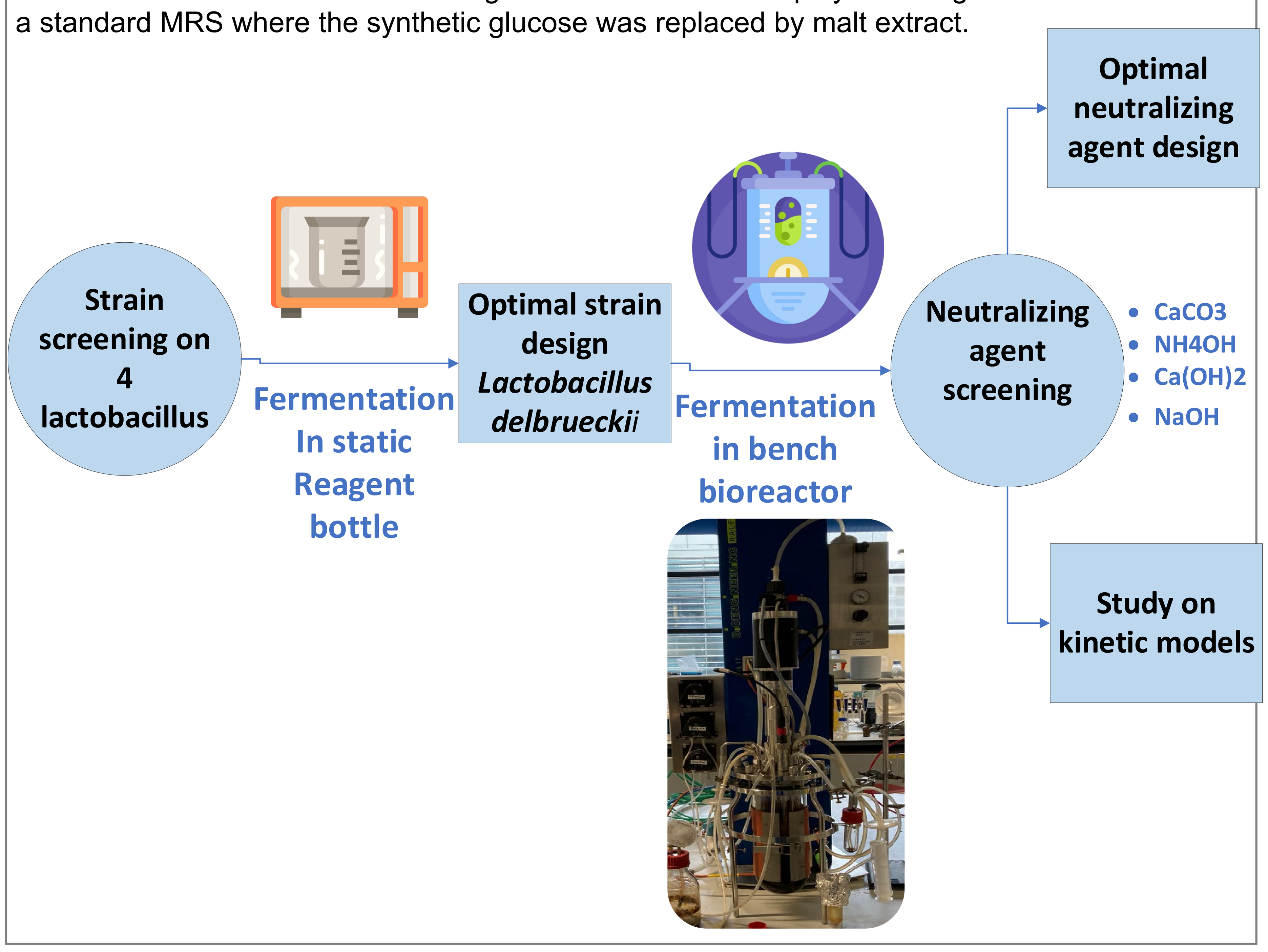
The industrial manufacture of plastics and their widespread use have created huge waste disposal issues. As a result, bioplastics have piqued the scientific community's interest in recent years. Among them is the PLA polymer, which is composed of lactic acid monomers and exhibits grwat thermophysical characteristics. Not least, this plastic is biodegradable, and its constituent monomer can be synthesized via bacterial fermentation rather than pure chemical synthesis. Thus, It is fundamental to optimize the bioprocess in sense of lactic acid productivity, in order to produce PLA in the most economical way possible.



Depending on the ratio between the two enantiomers of lactic acid, bioplastics with different thermal and physical properties can be produced.

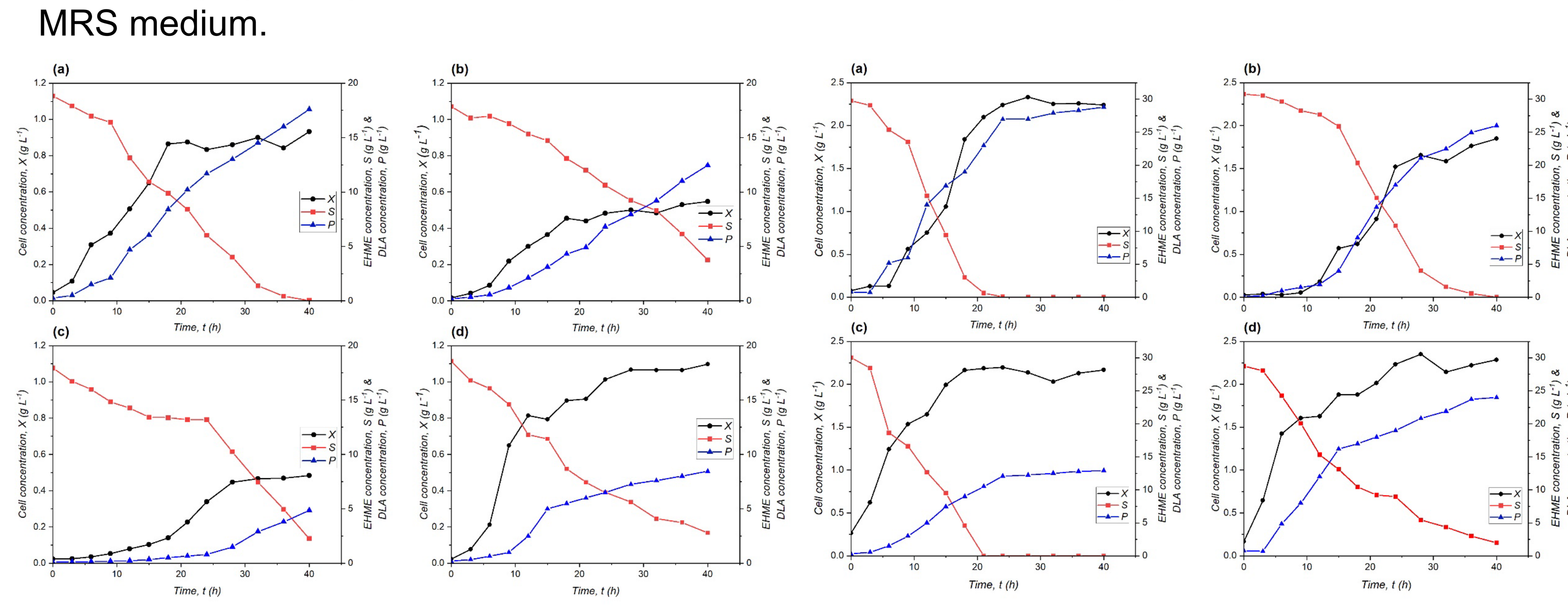
EXPERIMENTAL

The purpose of this project was to optimize the production of high optically pure D-LA by using *lactobacilli strains* as fermentative organisms. The media employed during the fermentation was a standard MRS where the synthetic glucose was replaced by malt extract.



RESULTS

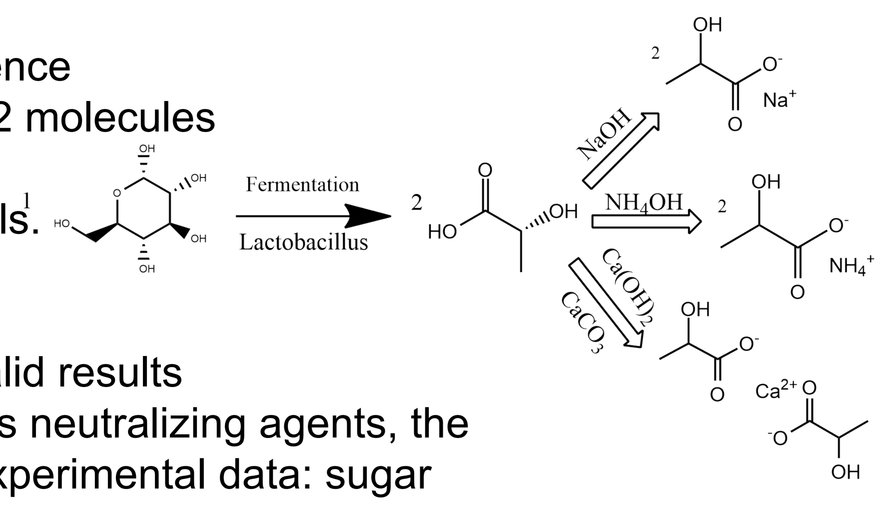
→ *Lactobacillus delbrueckii* designed as optimal strain for production of D-LA. Figures below illustrate graphically the results achieved for the strain screening in standard and modified MRS medium.



standard MRS ***modified MRS***

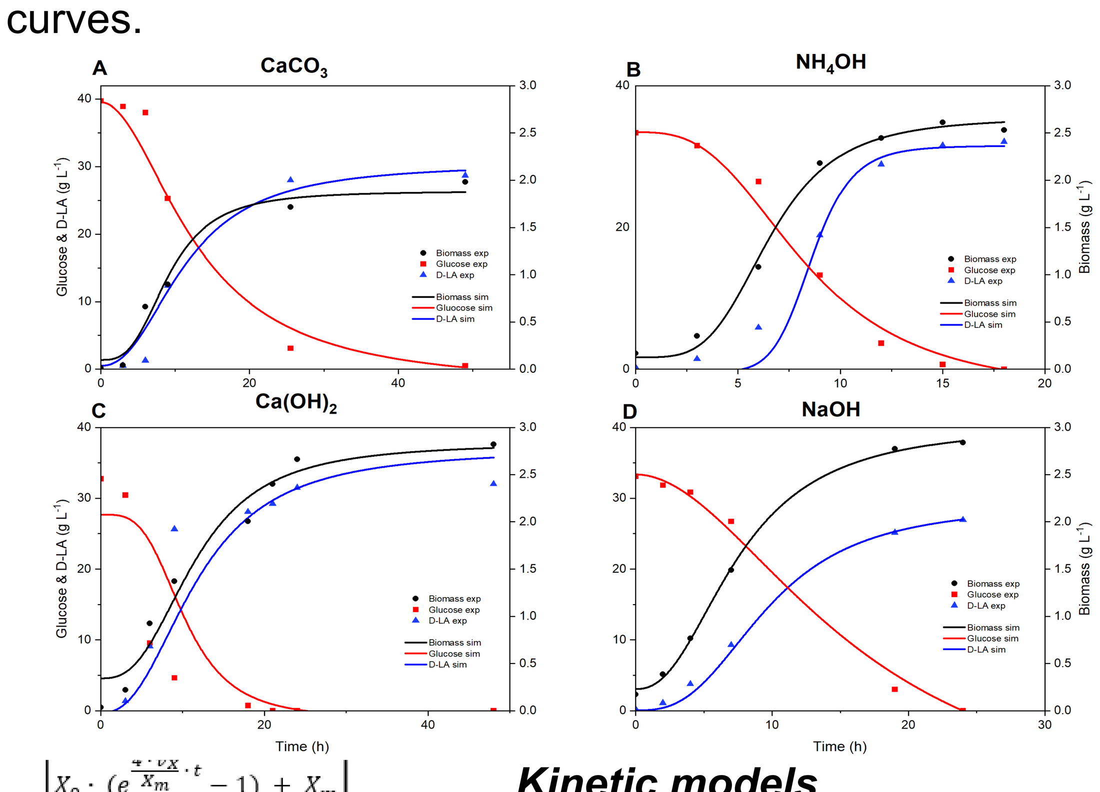
→ $Ca(OH)_2$ showed better properties in terms of neutralizing effect: highest specific growth rate (0.41 h^{-1}), volumetric productivity (4.04 g L^{-1} h^{-1}) and $Y_{P/S}$ (0.92 g g^{-1}) were reported.

The neutralizing effect depends strongly on the valence of the cationic ion of the base: Ca^{2+} can neutralize 2 molecules of lactate, leading to less cationic accumulation in the medium and less osmotic pressure on the cells.



Kinetics based on logistic models gave statistical valid results (p-values < 0.05 & R^2 > 0.95). In case of $Ca(OH)_2$ as neutralizing agents, the model does not correlate mathematically with the experimental data: sugar consumption and D-LA formation were extremely fast at this fermentation condition therefore could not be modelled using sigmoidal curves.

The following equations based on logistic models were used to simulate the cell concentration (X), the substrate consumption (S) and the product formation (P) during the time. Kinetic parameters were estimated by fitting the experimental data into the Models.



Kinetic models

$$X(t) = \frac{X_m}{1 + e^{\left[2 + \frac{4 \cdot v_X}{X_m} \cdot (\lambda_X - t)\right]}}$$

$$S(t) = S_0 - \frac{1}{Y_{X/S}} \cdot \left[\frac{X_m}{1 + e^{\left[2 + \frac{4 \cdot v_X}{X_m} \cdot (\lambda_X - t)\right]}} - X_0\right] - \frac{m \cdot X_m^2}{4 \cdot v_X} \cdot ln\left[\frac{X_0 \cdot (e^{\frac{4 \cdot v_X}{X_m} \cdot t} - 1) + X_m}{X_m}\right]$$

$$P(t) = \left[\frac{\alpha \cdot X_m}{1 + e^{\left[2 + \frac{4 \cdot v_X}{X_m} \cdot (\lambda_X - t)\right]}} - \alpha \cdot X_0\right] + \frac{\beta \cdot X_m^2}{4 \cdot v_X} \cdot ln\left[\frac{X_0 \cdot (e^{\frac{4 \cdot v_X}{X_m} \cdot t} - 1) + X_m}{X_m}\right]$$

CONCLUSION

Lactobacillus delbrueckii reported a better production in terms of volumetric productivity and optical purity of the enantiomer D-LA. Among the 4 neutralizing agents studied, calcium hydroxide was found to be the best option: higher volumetric productivity, specific growth rate and optical purity were achieved during the fermentation. Kinetic models on substrate consumption, biomass and D-LA formation correlated well to the experimental data and are statically valid. Due to the high rapidity on D-LA formation and substrate consumption in the fermentation employing calcium hydroxide, the simulated data did not correlated mathematically to the experimental data: a further model should be studied at this particular fermentation conditions.

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