Identification of critical material attributes in lignin streams based on *Pseudomonas putida* performance

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INTRODUCTION

Variability in chemical composition of corn stover feedstocks has been suggested to play a role in biocatalyst performance during the conversion of lignocellulosic biomass to value-added compounds.

For example, we have found significant differences on muconate production by *Pseudomonas putida* CJ781 – an engineered bacterium that converts aromatic compounds to muconate – on different lignin streams produced from the deacetylation of corn stover.

To better understand the origin of these differences, we cultivated *P. putida* CJ781 in mock lignin streams supplemented with various concentrations of compounds most likely to be found in black liquors.

METHODS

- We performed a literature search to identify common chemical compounds found in black liquor streams (**Table 1**).
- Using a high-throughput microtiter plate pipeline, we cultivated *P. putida* CJ781 in mock black liquor streams supplemented with various concentrations of these compounds (Figure 1).
- Performance was measured by comparing maximum growth rates among conditions.
- EC25% was calculated using regression analysis of growth rate vs concentration plots.
- We then performed a chemical analysis of 12 different black liquor streams to identify typical concentrations of these compounds (Table 1).

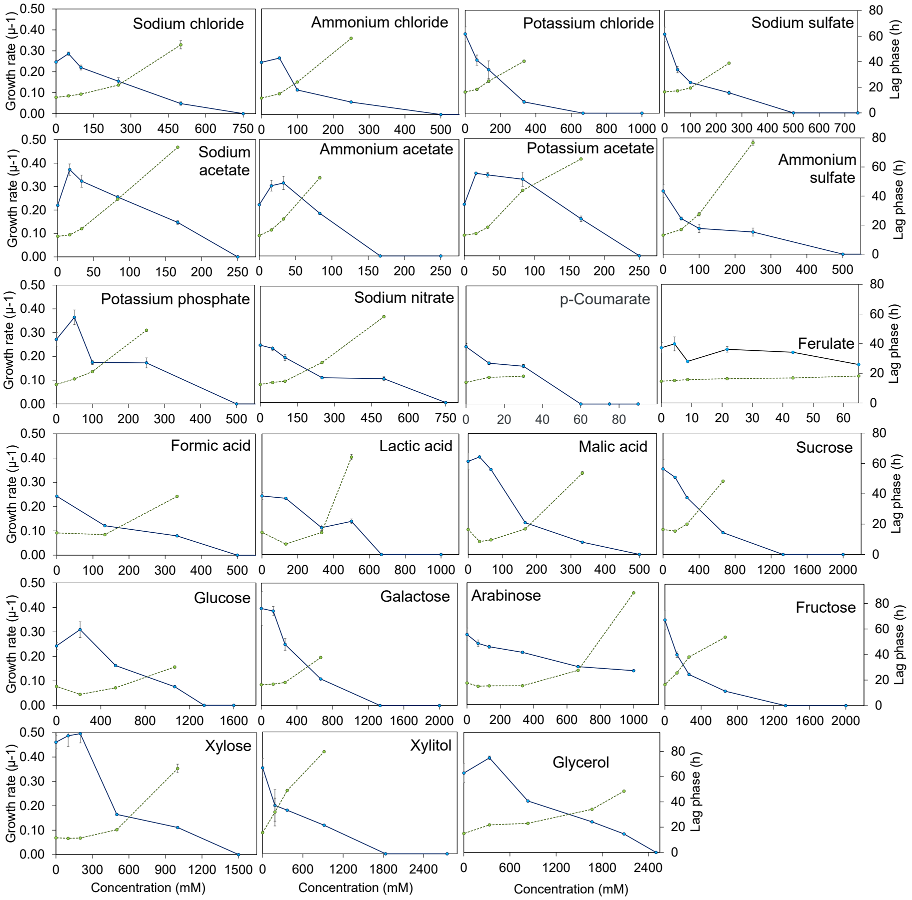

Figure 1. Growth rate and lag phase of *P. putida* strain CJ781 versus increasing concentrations of twenty-three different material attributes after 96 hours of cultivation. Circles represent the mean of four biological replicates; error bars represent the plus/minus SEM.

RESULTS

At maximum concentrations that may be found in lignin streams, sodium was the material attribute that limited growth rate to a greater extent, with a reduction in growth rate by 8 to 37%, depending on the counter ion, with sodium nitrate being the most inhibitory, followed by sodium acetate, sodium chloride, and sodium sulfate.

Ammonium acetate was also found to be inhibitory at average concentrations of ammonium in lignin streams, reducing maximum growth rate by 18%.

Table 1. Chemical compounds commonly detected in black liquor streams. For each compound, the concentration required to reduce growth rate by 25% (EC25) is shown. Additionally, the maximum concentration of each compound detected in tested 12 black liquor streams is also presented. Criticality of compounds is evaluated based on (1) the toxicity of each compound and (2) the concentration of each compound in typical black liquors. The most critical compounds, marked with asterisks, are those that are both toxic and present in black liquors in high concentrations.

Potentially Critical Material Attribute	EC25 in BL (mM)	Quantified in Black Liquor (g/L)		% of EC25 that is in BL	Criticality
Sodium chloride	155.7	Sodium	4.06	113.4	***
		Chloride	0.47	8.5	
Sodium sulfate	132.9	Sodium	4.06	66.5	**
		Sulfate	2.15	16.8	
Sodium nitrate	121.9	Sodium	4.06	144.9	***
		Nitrate	0.16	2.2	
Ammonium cloride	50.9	Ammonium	0.02	2.6	
		Chloride	0.47	26.0	
Ammonium sulfate	165.6	Ammonium	0.02	0.4	
		Sulfate	2.15	13.5	
Potassium chloride	148.4	Potassium	0.86	14.9	
		Chloride	0.47	8.9	
Potassium phosphate	148.1	Potassium	0.86	14.9	
		Phosphate	0.24	1.7	
Sodium acetate	161.7	Sodium	4.06	109.2	***
		Acetate	4.25	43.8	
Potassium acetate	183.1	Potassium	0.86	12.0	
		Acetate	4.25	38.6	
Ammonium acetate	84.4	Ammonium	0.02	1.6	
		Acetate	4.25	83.9	**
Formic acid	82.5	0.93		24.5	
Lactic acid	230.2	1.61		7.8	
p-Coumaric acid	14.4	0.85		35.9	
Ferulic acid	48.1	1.92		20.6	
Glucose	-	0		-	
Xylose	-	0		-	
Galactose	-	0		-	
Glycerol	230.2	0.7		0.8	
Xylitol	-	0		-	
Fructose	-	0		-	
Sucrose	-	0		-	
Arabinose	-	0		-	
Malic acid	149.4	0.83		4.1	

CONCLUSIONS AND FUTURE DRECTIONS

We are currently evaluating the effect of the most critical material attributes at bioreactor scale to assess the impact of these compounds on muconate production.

Overall, the data from this study has allowed to down select critical material attributes in lignin streams and process parameters that significantly affect the performance of *P. putida* and will be used as input to predictive models that aim to reduce risks attributed to feedstock variability in lignocellulosic biorefineries.

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