Biosurfactant Production By Fermentation

Western Engineering

Chemical and Biochemical Engineering

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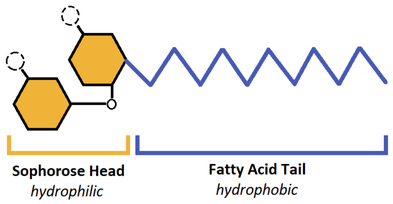
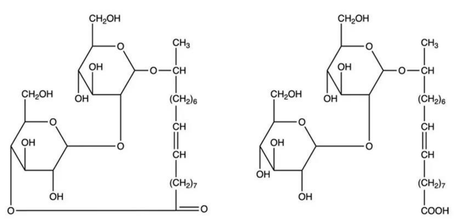
Motivation and Background

- The interest in using biosurfactants in replace of surfactants chemically derived from petrochemicals has provided a more environmentally conscious and sustainable approach for industrial application.
- Glycolipids are the most researched biosurfactants with promising industrial capabilities due to high production yields. Sophorolipds (SPL) are glycolipid biosurfactants produced by non-pathogenic yeast, *Candida bombicola.*



- SPLs can be produced as a mixture of acidic or lactone forms. The physical and chemical properties differ between the two structural SPLs, as well as their application in industry.



Lactone Acidic

- The strong antimicrobial properties of the lactone type SPLs make it a desirable active ingredient in cleaning products such as sanitizers, detergents, body washes, and shampoo.
- Industrial collaborator is exploring the use of SPLs in their surface cleaning products, detergents, odor removal agents, as well as antimicrobial cleaning products.

Objectives

- The main research objective is to optimize fermentation methodology, such that a consistent and reproduceable product can be achieved during large scale production.
- Sophorolipid product composition of at least 75-85% lactone and 25-15% acidic structure.
- Fermentation yield of at least 60% sophorolipid product.

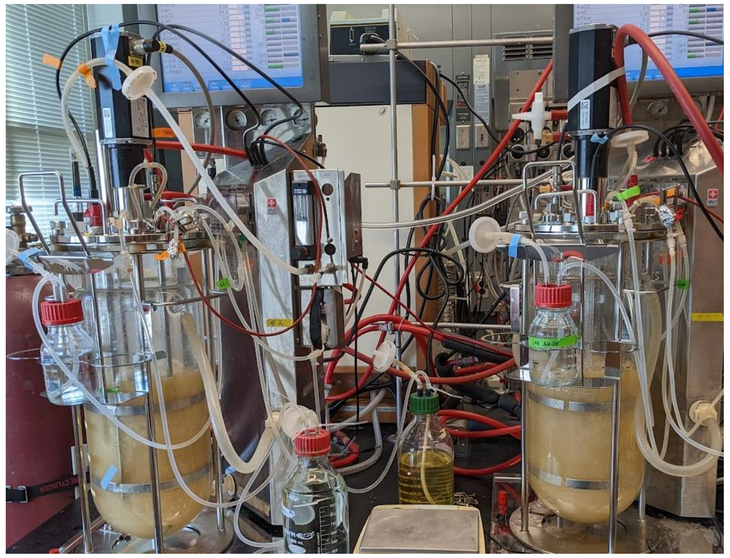
Research Methodology

- A series of fermentations were carried out on benchtop bioreactors of 7.5L to evaluate reproducibility.

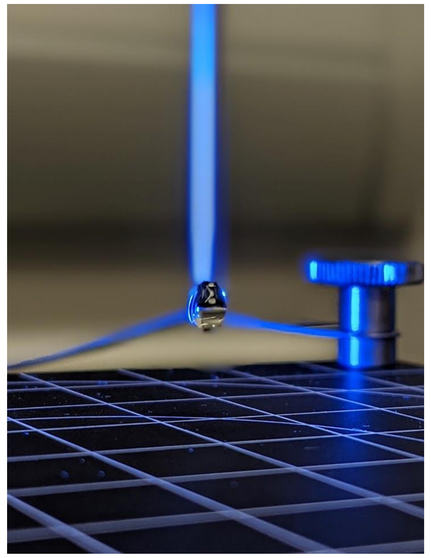
Parameter	Value	Notes
Temperature	25°C	
pH	3.5	Controlled with 6M NaOH
Aeration	5.6 L/min (1.4 vvm)	Changed to 1 L/min after approximately 96 h to mitigate foaming.
Limit of O_2	>30%	
Agitation	800 rpm	

- After harvesting the SPLs, product structure and properties were characterized using High Pressure Liquid Chromatography (HPLC) and pendant drop analyzer.
- The solubility and detergency power of the harvested biosurfactant is compared against commercially available biosurfactants.

Equipment



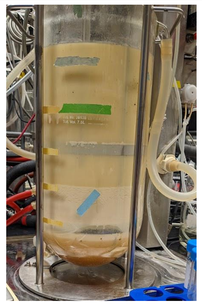
C. bombicola fermentation in 7.5L bioreactors

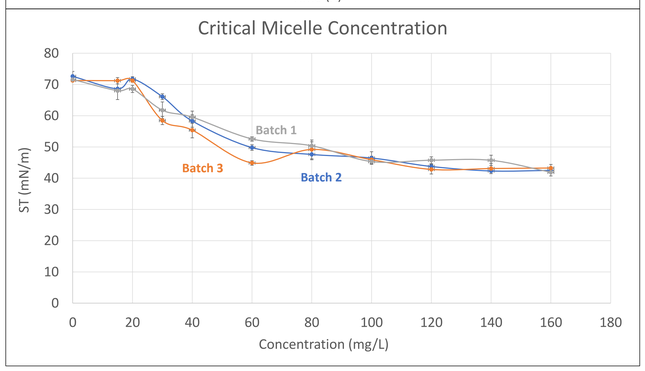


Pendant drop analyzer for critical micelle concentration

Key Results

Batch #	Feed Ratio(w/w) (Glucose : oil)	Yield (g product/g glucose)	Lactone : Acidic (%)	Critical micelle concentration (mg/L)
Replicate 1	2.99	0.80	83/17	100-120
Replicate 2	2.82	0.87	88/12	100-120
Replicate 3	2.81	0.88	87/13	100-120
Average	**2.87**	**0.85**	**86/14**	**N/A**
Stdev	**±0.10**	**±0.04**	**±2.64/±2.64**	**N/A**
Nitrogen rich media batch #	**Feed Ratio(w/w) (Glucose : oil)**	**Yield (g product/g glucose)**	**Lactone : Acidic (%)**	**Fermentation Temperature (°C)**
1	3.16	0.367	86/14	25
2	3.16	0.369	93/7	25
3	3.16	0.20	93/7	20
4	3.16	0.18	91/9	20





Summary

- A reproduceable fermentation methodology was achieved for a 10-day fermentation cycle.
- Investigation into further media optimization for increased product yield. Doubling the nitrogen source in the media to increase cell density, while operating at a lower temperature of 20°C for improved dissolved oxygen levels.
- With an increased cell density at the initial growth phase, the goal was to reduce the total fermentation time while producing adequate product yield. Reactor volume limited the fermentation duration as the substrate feeding rate increased.
- Ensuring glucose concentration is kept in excess (>30g/L) throughout fermentation may increase product yield. Feeding with a more concentrated feed solution will be further investigated.