



Advancing Biofuels Research

NCERC at SIUE

Lab Scale Fermentation Options (100 mL to 5 L fermentors)

1. Introduction

The Laboratory Division at NCERC can perform fermentation in a variety of sizes.

The front end process in the lab is comparable to runs in the NCERC pilot plant; this has been demonstrated by successful simulations.

The process typically starts by grinding the grain in a Wiley mill to a fine particle size and mixed with deionized (DI) water. The slurry is then treated with a commercial alpha-amylase enzyme and placed in the Labomat (model BFL-12 and/or BFA-24) for cooking/liquefaction, which simulates the temperature achieved by a jet cooker or pick heater. The dextrins produced during liquefaction are further hydrolyzed to glucose in a process called saccharification with the aid of a commercial glucoamylase enzyme and the glucose produced is utilized by yeast in a process called Simultaneous Saccharification and Fermentation (SSF) and converted into ethanol. Mass loss is monitored by weighing either online for the bigger vessels, or by weighing at several time intervals (in flasks). The back end processes can be modeled, but are not representative of pilot processes, due to the small and heterogeneous nature of the post-fermentation residues.

Options for each phase of the process are outlined in the tables below. These tables list our most usual protocol settings; however, the Client can specify desired amounts for most variables.



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2. Liquefaction

Variable	Options		
Replicates	Triplicates (recommended)	Client-selected	
Grain	Corn, milo, barley	Cellulosic feedstock	Client-provided
Grain grind size	1 mm	2 mm	
% Total solids	20 – 32% w/w		
Alpha-amylase enzyme	Recommended dosage (manufacturer)	Client-specified	
Water source for liquefaction	City water from tap	Deionized (DI) water	Waste water, stillage or other waste stream
Liquefaction instrument*	Labomat BFL-12, BFA-24 (any flask size)		
Liquefaction cook time	90 minutes	Client-specified	
Liquefaction temperature	Enzyme-specific (e.g. 83 °C)		
Mass of material to be liquefied	160 g (small flask)	500 g (large flask)	



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3. Fermentation

Variable	Options		
Gluco-amylase enzyme	Recommended dosage (manufacturer)	Client-specified	
Other enzymes (lipases, cellulases, etc.)	Recommended dosage (manufacturer)	Client-requested	
Nitrogen source (i.e. urea)	500 ppm nitrogen	Client-specified	
Yeast nutrients	Client-specified		
Yeast*	Hydrated yeast (in warm water), 10 – 20 million cells/ml		
Yeast propagation	Propagate in nutrient media from plate or vial (1-5 days)	Propagate in slurry (overnight prep)	
Bacteria	Inoculate fermentation with bacteria (Client-specified)		
Antibiotic	0.5 ppm	Client-Specified	
Fermentation duration time	48 – 72 hours		
Sampling frequency	During “workday” (7am – midnight)	Twice a day	Client-specified
Analysis throughout fermentation or at final time point	Mass loss	HPLC (sugar conversion, organic acids production, glycerol, ethanol)	Density
	Total solids	Dissolved solids	Yeast counts
	Bacteria colony counts (plating)		
	Other analyses as specified by Client can be negotiated		

* The Client can select between Yeast hydration and yeast propagation but not both.



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Backend (whole stillage, syrup, DDGS)

Backend products are prepared only if specified by client. Once fermentation is complete, we can prepare whole stillage, syrup as long as the individual fermentation unit was at least 1-L (500 g). DDGS can be made after fermentation is completed in the Bioreactor (>10,000 g of fermented material).

4. Yield Determination

Ethanol yield is based on: moisture content of grain, mass of initial grain, mass loss at end of fermentation, final suspended solids, final density, and final ethanol concentration. NCERC also performs calculations on % starch conversion compared to theoretical values.

5. Cost

The cost of an experimental run at the laboratory scale depends on a variety of variables:

1. The number of flasks or runs in the experimental design
2. The length of fermentation
3. The size of the experimental unit (flask or bioreactor vessel)
4. The sampling frequency
5. The number and type of analyses requested

The price of a test is set after discussing the experimental design with the Client.