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Comparison of three screening methods to select mixed-microbial inoculum for mixed-acid fermentations

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HIGHLIGHTS

- Carboxylate platform converts biomass into hydrocarbons and chemicals.
- Developed method to identify highest performing inoculum.
- Five bacterial communities were screened and ranked by three fermentation performance tests.
- Three screens are a useful and predictive method for choosing optimal inocula sources.
- Three screens do no predict worst-performing communities.

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ABSTRACT

Using a mixed culture of microorganisms, the carboxylate platform converts biomass into hydrocarbons and chemicals. To develop a method that identifies the highest performing inoculum for carboxylate fermentations, five bacterial communities were screened and ranked by three fermentation performance tests: (1) 30-day batch screen, (2) 28-day continuum particle distribution model (CPDM), and (3) 5-month continuous countercurrent fermentation trains. To screen numerous inocula sources, these tests were used sequentially in an aseptic environment. For the batch-fermentation screen, Inoculum 1 achieved the highest conversion. For the CPDM evaluation, the operating map for Inoculum 1 had the highest performance. For the continuous countercurrent fermentation, the train resulting from Inoculum 1 was among the best performers. This study suggests that the three screens are a useful and predictive method for choosing optimal inocula sources. The bacterial community with optimal performance in these three screens could be considered for use in commercial-scale fermentations.

1. Introduction

From 2004 to 2009, gross energy production increased 10% and global population increased 5% (IEA, 2011). To create a sustainable future, a new energy path is necessary. One approach is to convert over a billion tons of agricultural, municipal, and industrial biowastes generated annually in the United States into liquid biofuels (Perlack et al., 2005). Currently, bioethanol and biodiesel production are the primary biomass-to-liquid fuel routes; they provide about 3% of global road transport fuels (REN21, 2011). Unfortunately, these fuels are produced from high-value food crops. An alternative is the carboxylate platform, which can convert waste lignocellulose into liquid fuels (Agler et al., 2011; Holtzapple et al., 1999).

The carboxylate platform is a low-cost, nonsterile, flexible, and continuous technology that does not need added enzymes to convert nearly any biomass feedstock into chemicals and liquid fuels (Forrest et al., 2010; Granda et al., 2009). The carboxylate platform employs a mixed culture of naturally occurring microorganisms to ferment biomass into carboxylic salts, which are subsequently converted into a wide array of chemicals (e.g., ketones, alcohols) and hydrocarbons (e.g., jet fuel, gasoline) (Aiello-Mazzarri et al., 2006; Landoll and Holtzapple, 2011). To respond to varying market demands, the acid spectrum in the fermentations can be varied by using temperature as a control variable (Chan and Holtzapple, 2003). A commercial example of the carboxylate platform is the MixAlco™ process[1].

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Historically, to convert biomass to mixed volatile carboxylic acids (C_2–C_7), inocula for carboxylate fermentations have been collected from terrestrial environments, such as rumen fluid or compost piles (Aiello-Mazzarri et al., 2006; Ross and Holtzapple, 2001). Recent attempts to improve the fermentation community have greatly increased product yields. For example, a community isolated from a marine ecology (Galveston, TX) replaced the previous terrestrial community (College Station, TX) and doubled acid yields (Thanakoses, 2002). Replacing marine microorganisms with those from the hypersaline Great Salt Lake increased acid concentration by 30% (Fu, 2007). Natural saline environments provide microbial communities better suited to the salty environment in carboxylate fermentations.

Inoculating a biological process with a mixed culture is helpful in nonsterile environments that constantly change due to the nature of the substrates (e.g., agricultural residues) and nutrients (e.g., manure), offering advantages over a monoculture (Angenent et al., 2004; Das and Veziroglu, 2001). At the laboratory scale, it is fairly easy to maintain a monoculture. However, at the industrial scale, maintaining a monoculture is difficult and expensive; it requires specialized fermentors, pipes, pumps, heat exchangers, and filtration equipment. An economic evaluation of a lignocellulose-to-ethanol process that employs yeast monocultures assumes 7% sugars are lost to contaminants (NREL, 1999).

Previous studies have successfully screened natural inocula sources for improved fermentation production of ethanol and hydrogen, and for biocidal properties against potential pathogens (Casas et al., 2007; Koskinen et al., 2008). Bacterial DNA sequencing of different ecological sites is time intensive, expensive, and might not predict fermentation performance. Instead, screening requires laboratory-scale fermentations that simulate an industrial environment.

This paper compares three methods to screen microbial community ecosystems as potential inocula in carboxylate fermentations: (1) a 30-d single-batch screen where the communities were used to inoculate 250-mL fermentors that were spiked with a high initial carboxylate concentration (~20 g/L), (2) a 28-d multi-batch screen where five 1-L fermentors with different substrate concentrations were fermented to generate performance maps produced by the continuum particle distribution model (CPDM) (Loescher, 1996), and (3) a continuous countercurrent fermentation where four-stage semi-continuous countercurrent trains were run in 1-L fermentors for several months.

When analyzing hundreds of inocula, the single-batch fermentation serves as the initial screen to determine the highest performing microbial communities. CPDM is the next level of screening used to predict the best performers from those chosen by the initial screen. Then, the continuous countercurrent fermentation is the final screen used to select the inocula that could be useful for industrial carboxylate fermentations. This study establishes the most accurate and efficient inoculum screening method for carboxylate fermentations. This paper compares three fermentation screens to evaluate microbial communities from saline and thermal ecosystems as potential inocula in the carboxylate platform.

2. Methods

2.1. Overview of microbial community screening

Four bacterial communities from Brazoria, TX and one from Galveston, TX were collected from their respective soil site and used as inocula in three simultaneous fermentation screens for carboxylate platform fermentations (Table 1). The performance variables for the bacterial communities were compared in three simultaneous fermentation performance tests: a 30-day batch screen, the continuum particle distribution model, and four-stage countercurrent fermentation trains (Fig. 1).

2.2. Inocula

Five inocula were selected and samples were collected from their respective soil sites (Table 1). Four Brazoria sediment inocula were collected with three 10-cm-deep and 7.5-cm-diameter core samples obtained with a soil corer. One Galveston, TX, sediment inocula was removed from the bottom of 0.5-m-deep shoreline pits. GPS coordinates recorded the exact location of soil collection (Table 1). These samples were immediately placed in separate sealed, air-free plastic bags at ambient temperature. All inocula were fresh and unfrozen when inoculating the fermentations.

2.3. Fermentor configuration

For the batch screening, the fermentors were 250-mL polypropylene centrifuge bottles capped by a polypropylene cap (Fig. 1). The fermentors for the CPDM and countercurrent trains were 1-L polypropylene centrifuge bottles capped by a rubber stopper inserted with a glass tube (Domke et al., 2004; Thanakoses et al., 2003). A rubber septum sealed the glass tube and allowed for gas sampling and release. Two lengths of ¼-in stainless steel pipe were inserted through the rubber stopper into the vessel, which mixed the contents of the fermentor as it rotated in the rolling incubator.

2.4. Substrate

Shredded recycled office paper was the substrate in all fermentations. Because commercial paper is lignin-free and has a specific composition, it does not need to be pretreated, which removes the variation that would have occurred from pretreating multiple batches of agricultural feedstock.

As a nutrient source, yeast extract was used rather than the more commonly used chicken manure. Chicken manure has been used as the nutrient source in the carboxylate platform because of its economic feasibility for commercial-scale implementation. Yeast extract is an aseptic nutrient, which minimizes the introduction of outside microorganisms from the nutrient source. Paper and yeast extract were fed at a ratio of 90:10 (Forrest, 2010), and were not autoclaved prior to fermentation. Where specified, urea was also added as a supplement.

2.5. Methanogen inhibition

Iodoform (CHI_3) inhibited methane in all fermentors. To ensure no methane was present, for continuous fermentations, the gas phase was monitored with gas chromatography (GC) analysis. Iodoform solution (20 g CHI_3/L 190-proof ethanol) was added to each fermentor every 48 h throughout the fermentation (Ross, 1998). Because iodoform is light, air, and temperature sensitive, the solution was kept in amber-colored glass bottles and special care was taken to replace the cap immediately after use to prevent degradation.

2.6. Analytical methods

2.6.1. Carboxylic acid concentration determination

Fermentation liquid was centrifuged (4,000 rpm, 3300 × g, 25 min) to remove insoluble solids. Then, it was mixed with equal parts of internal standard (1.162 g/L 4-methyl-*n*-valeric acid) and 3-M phosphoric acid (H_3PO_4), and then ultra-centrifuged (15,000 rpm, 18,363 × g, 8 min). The carboxylic acid concentration was measured using an Agilent 6890 Series Gas Chromatograph (GC) system with a flame ionization detector (FID) and an Agilent 7683 automatic liquid sampler. A 30-m fused-silica capillary