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Evaluating the performance of carboxylate platform fermentations across diverse inocula originating as sediments from extreme environments

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HIGHLIGHTS

- 501 geographically diverse sediments varied for carboxylate platform performance.
- Multivariate analysis revealed complex relationships among parameters measured.
- Established trade-offs among fermentation outcomes.
- Identified optimal sediment characters for desirable fermentation outcomes.

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ABSTRACT

To test the hypothesis that microbial communities from saline and thermal sediment environments are pre-adapted to exhibit superior fermentation performances, 501 saline and thermal samples were collected from a wide geographic range. Each sediment sample was screened as inoculum in a 30-day batch fermentation. Using multivariate statistics, the capacity of each community was assessed to determine its ability to degrade a cellulosic substrate and produce carboxylic acids in the context of the inoculum sediment chemistry. Conductance of soils was positively associated with production of particular acids, but negatively associated with conversion efficiency. *In situ* sediment temperature and conversion efficiency were consistently positively related. Because inoculum characteristics influence carboxylate platform productivity, optimization of the inoculum is an important and realistic goal.

1. Introduction

The MixAlco™ process, which was developed at Texas A&M University, is an example of the carboxylate platform for biofuel production. It involves the conversion of lignocellulosic biomass into carboxylate salts by fermentation with a mixed microbial community isolated from sediment (Hollister et al., 2011; Fu and Holtzapple, 2010b). Carboxylate platform fermentations produce small-chain (C2–C7) carboxylic acids, which convert to ethanol, gasoline, jet fuel, or industrial chemicals via well-established chemistry (Holtzapple and Granda, 2009). As feedstocks, the carboxylate platform can use many non-food biomass materials including landfill-targeted wastes such as yard clippings and kitchen waste, agricultural residues such as sugarcane bagasse (Fu and Holtzapple, 2011), and industrial byproducts such as paper fines and industrial biosludge (Domke et al., 2004). Furthermore, unlike the more common sugar platform, the carboxylate platform is a non-sterile fermentation process in which an initial mixed microbial inoculum overtakes microbial populations in the feedstock and nutrient sources used in the fermentation, eliminating any energy or material costs associated with sterilization.

In the carboxylate platform, carboxylic acids are buffered to carboxylate salts (Aiello-Mazzarri et al., 2006). Prior to initiating this project, the few attempts to manipulate the inoculum successfully improved fermentation performance of the carboxylate platform. Terrestrial inocula from environments expected to favor rapid degradation of biomass (e.g., compost pile or ruminant gut) (Fu, 2007) were the original microbial communities for the platform. A noteworthy aspect of these early studies of carboxylate platform fermentations is that the productivity of these original

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communities slows as the fermentation reaches high product concentration, a well-established issue in industry (Taylor et al., 2008). Therefore, it seems reasonable to expect that the original terrestrial microbial communities are sensitive to product concentrations. Because the fermentation products are salts, initial attempts to optimize inocula included microbial communities from saline environments. Specifically, switching inocula to a marine community from Galveston Island, TX sediment resulted in more than double acid yield relative to the original terrestrial (non-saline) soil community (Thanakoses et al., 2003a,b). Furthermore, a community from a hypersaline environment (Great Salt Lake, Salt Lake City, UT) boosted performance another 20% relative to the Galveston Island community (Fu, 2007). Thus, it is reasonable to expect that other microbial communities from natural environments with conditions similar to those in industrial fermentations will exhibit continued optimization of fermentation performance.

Microbes found in extreme environments have physiological adaptations that allow them to live normally in adverse conditions, including high temperatures and high salt concentrations (Mesbah et al., 2007; Meyer-Dombard et al., 2005; Porter et al., 2007). As a general rule, industrial bioprocesses operating at higher temperatures run at faster rates, thus providing shorter residence times and greater profitability (Aitken and Mullennix, 1992). Microbes in industrial processes tend to perform optimally at lower product concentrations (Heipieper et al., 2007; Taylor et al., 2008); however, efficient recovery requires high product concentrations. Thus, it seems reasonable to expect that microbial communities from thermal (Rastogi et al., 2010) and high-salinity (Mesbah and Wiegel, 2008) natural environments should possess adaptations that favor superior performance in high-temperature industrial processes that also accumulate high concentrations of salts. Recent studies of mixed microbial communities from soils that degrade lignocellulose (Haruta et al., 2002; DeAngelis et al., 2010, 2012) and from compost (Izquierdo et al., 2010; Sizova et al., 2011) reveal that fermentation-capable communities are stable after prolonged fermentation (Werner et al., 2011) including maintenance of cellulose degradation despite exposure to heat, cold, and sub-culturing (Haruta et al., 2002). Further, a direct study of community acclimation in the presence of elevated salts and ammonia reveal improved waste hydrolysis rates, leading to the suggestion that microbial communities from natural saline environments allow for additional optimization (Wilson et al., 2013).

To address the hypothesis that microbial communities from saline and thermal environments should exhibit improved fermentation performance, samples from saline and thermal sediments across a wide geographic range (Supplemental Tables 1 and 2) were collected and screened for performance in a 30-day batch carboxylate platform fermentation. The capacity of each community to degrade a cellulosic substrate and produce carboxylic acids was measured. Further, with the resulting performance data, the features of particular soil environments that favored the most successful communities were identified. Specifically, a multivariate statistical analysis was performed to examine soil chemistry data and fermentation performance data, including acid profiles and biomass conversion. Using this large-scale analysis of communities, the prediction was that associations among soil characteristics and process performance parameters would inform efforts to optimize the carboxylate platform for producing biofuels and industrial chemicals.

2. Methods

2.1. Study design and site selection

This study was a large-scale effort to examine variation among soil microbial communities as inocula for fermentations in the carboxylate platform. Frequent collection trips were conducted from October 2008 to May 2010. In most cases, at a given geographic location (site) multiple samples were collected; sample locations were chosen based on variation in physical and ecological features or presumed gradients (e.g., moisture, visible salt accumulation, temperature). In total, 501 samples (Supplemental Table 1) from 75 sites (Supplemental Table 2) were collected. Sites were identified via literature, database (Boyd, 2002), internet searches, and by personal communications with site stakeholders.

This study had two stages (hereafter Stage I and Stage II) with distinct site selection criteria and fermentation experimental conditions (Supplemental Tables 1 and 2). In Stage I of this study, sampling sites were from within the southern central region of the United States with a history of salt accumulation or commercial salt production and/or sites known to be high in total dissolved solids (TDS). Stage I involved evaluating 102 inoculum samples from four collection trips to 17 sites conducted in 2008 (Supplemental Table 2). In Stage II of this study, site selection criteria were expanded to include greater ecological and geographic diversity and specific addition of sites with thermal features (Supplemental Table 2). Stage II involved evaluating of 399 inoculum samples from 58 sites and 14 collection trips across the continental United States, Puerto Rico, and Hawaii conducted in 2009 and 2010 (Supplemental Table 2).

2.2. Sediment sampling

In most cases, a single sample involved collecting three adjacent cores using a standard garden bulb-planting tool to a depth of 10–12 cm and with a width of 6.5 cm. Sediment temperatures (Splashproof Thermometer, VWR, PA, USA) at a depth of 5 cm were recorded. Each of the three cores was sealed in a separate zip-top plastic bag with the air removed. As soon after collection as possible, one core from each sample was flash frozen with dry ice and subsequently stored at −80 °C for use in future studies. The remaining two cores were vacuum-sealed (Sunbeam Products, Inc., FoodSaver Model V2220, DE, USA) and stored in insulated coolers allowing them to reach ambient temperature during transport to the lab. These ambient-temperature cores were used as inoculum sources for fermentation and as material for sediment characterization.

2.3. Sediment characterization

Upon return to the laboratory and immediately prior to inoculating the fermentation vessel, one sample core was homogenized by hand. Approximately 30 cm^3 (or around two tablespoons) of homogenized sediment mixture was used to measure volatile solids and moisture content and followed the method of Fu and Holtzapple (2010a). Additionally, sufficient sediment from this homogenized core was submitted to the Soil, Water, and Forage Testing Laboratory at Texas A&M University for chemical and physical characterization following the methods described by Hollister et al. (2010a). As long as the sample size permitted, these soil samples were analyzed for the following: (1) electrical conductivity of soluble salts; (2) soil water pH; (3) detailed salinity measures of potassium, calcium, magnesium, and sodium; (4) plant-available phosphorus and sulfur; (5) analysis of organic carbon, total carbon, total nitrogen; and (6) texture. All sediment remaining after this procedure was stored under vacuum-seal at 4 °C for use in further studies.

2.4. Fermentation experiments

Table 1 details the fermentation screens employed in Stage I and Stage II. Because the fermentation broth contained $\geqslant$2% carboxylate salt concentration, both approaches evaluated inoculum

Table 1
Conditions for Stage I and Stage II fermentation experiments conducted with environmental samples collected for this study.

	Stage I	Stage II
Year of experiment	2008	2009–10
Polypropylene bottle volume (mL)	1000	250
Mixing method	Horizontal rolling	Shaken upright
Speed of mixing (rpm)	10	100
Fermentation temperature (°C)	55	55
Deionized water (mL)	400	150
Shredded office paper (g)	36	9
Yeast extract (g)	4	1
Calcium acetate (g)	6.4	3.2
Butyric acid (g)	1.2	0.5
Calcium propionate (g)	0.4	0.2
Sediment (g)	10	2.5
Calcium carbonate (g)	10–15	2.2
Initial carboxylate salt concentration ($g\,L^{-1}$)	20	26
Volatile solids concentration ($g\,L^{-1}$)	90	60

performance in the presence of high product concentrations. Furthermore, in an attempt to favor shorter residence times, these screens were conducted at the high process temperature of 55 °C. The screens were conducted in polypropylene centrifuge bottles with screw-top caps (Thermo Fisher Scientific Inc., Waltham, MA, USA). Immediately after homogenizing the sample, the indicated amount of sediment (Table 1) was used to inoculate each fermentation. Additionally, 100 μL and 20 μL iodoform (20 $g\,L^{-1}$ in ethanol solution) was added every other day to inhibit methanogenesis in Stage I and Stage II fermentations, respectively.

2.5. Fermentation characterization

All fermentations were harvested 30 days post-inoculation and the remaining volatile solids and acid products were determined (Golub et al., 2012; Hollister et al., 2010b). *Conversion* is defined as the grams of volatile solids digested divided by the grams of volatile solids fed. Conversion indicates fermentation performance without regard to acid production. White office paper served as the carbon source and yeast extract as the nutrient source at a 10:1 ratio. Mixed acid product concentrations are characterized by acetic acid equivalents, which is defined as the acetic acid concentration with the same reducing power as the mixed acids (Datta, 1981). *Selectivity* is defined as the grams acetic acid equivalents per gram volatile solids digested. *Yield* is defined as the grams total change in acids (final − initial) per grams volatile solids fed. For this study, selectivity and yield data were not used in the analyses; however, these values are available in Supplementary Table 1. Both parameters indicate fermentation performance across multiple variables and are useful to quickly assess how a particular fermentation degrades biomass into different products (i.e., those targeted, and those not).

2.6. Data transformation and statistical analyses

Only samples with complete data were included in the statistical analyses. For a complete list of all samples, see Supplementary Table 1. Data for Stage I and II fermentations were analyzed separately.

To better approximate normal distributions and to achieve even scaling, sample data were transformed; log(1 + value) or log(3 + value) for discrete quantity data or arcsine [square root (proportion)] for percentage data. The pH data were not transformed because of the logarithmic nature of this variable. Within each analysis, two blocks of data were established: the sediment data block (S-data), and the fermentation data block (F-data). The S-data blocks included the percent total volatile compounds, percent total moisture content, pH, levels of cation concentration, percent total nitrogen, percent total carbon, percent organic carbon, and the Mehlich III measured phosphorus and sulfur levels (showing plant-available quantities). F-data blocks included measured individual changes in acid concentration (acetic, butyric, iso-propionic, propionic, isovaleric, valeric, caproic, and heptanoic) and the conversion for each sample.

To test for correspondence of fermentation products and soil environmental data, multivariate analysis of variance (MANOVA) was performed using JMP Pro (version 10.0 by SAS). The MANOVA provided an overall significance test for the correspondence and indicated which soil variables were significantly related to fermentation data. Model strength was estimated using eta-squared, which is the multivariate analog of R^2 in univariate analysis (Tabachnick and Fidell, 2000). Because of the differences between Stage I and Stage II experiments (Table 1), the data were analyzed separately. However, a comparison of the effects from the two stages was conducted by assessing the correlation among respective F statistics.

To explore the relationships among the soil and fermentation variables, two-block partial least squares (PLS) analysis was performed using Microsoft Office Excel 2007 and the add-in PopTools (Hood, 2010). PLS is a technique that performs both regression and dimensional reduction simultaneously, by performing eigendecomposition on the cross-variance (i.e., covariance *across* data blocks), and is popular in a growing number of fields, especially in chemometrics (Wold et al., 2001). For this analysis, all the transformed variables were standardized, so interpretation of eigenvalues would be comparable across different data types. The F-data comprised one block and the S-data formed the second block. PLS was performed on the matrix multiplication product of the blocks of standardized F-data and S-data (Wold et al., 2001). Each row of the F-data contained the results of a single fermentation, related to the respective row of S-data. The effect strength was calculated as the sum of cross-variance (variance in common between the two data blocks) as a fraction of total variance in fermentation products. Singular vector (eigenvector) loadings for Stage I and Stage II were plotted graphically for comparison, and scores on these multivariate constructs (termed "singular value scores") were graphed and presented as regressions to indicate the magnitude of relationships between F-data and S-data.

3. Results and discussion

3.1. Variation among sediments

As expected, the geographically and ecologically diverse sediments used as inocula in this study exhibited extremely wide ranges of measured characteristics (e.g., temperature, texture, conductivity, pH) (Supplementary Tables 1 and 2). Across the 501 samples in this study, 494 were analyzed for electrical conductivity used as an indicator of salinity and pH resulting in the following: 53.5% strongly saline (>16 $dS\,m^{-1}$), 11.3% moderately saline (8–15.9 $dS\,m^{-1}$), 9.0% slightly saline (4–7.9 $dS\,m^{-1}$), 12.1% very slightly saline (2–3.9 $dS\,m^{-1}$), 13.9% non-saline (<2 $dS\,m^{-1}$) (Staff, 1993). Based on pH measurements taken during analysis for detailed salinity, 8.3% of samples were ultra acidic (pH < 3.5), 2.4% extremely acidic (pH 3.5–4.4), 2.8% very strongly acidic (pH 4.5–5.0), 1.4% strongly acidic (pH 5.1–5.5), 4.0% moderately acidic (pH 5.6–6.0), 7.1% slightly acidic (pH 6.1–6.5), 24.8% neutral (pH 6.6–7.3), 28.3% slightly alkaline (pH 7.4–7.8), 16.4% moderately alkaline (pH 7.9–8.4), 4.6% strongly alkaline (pH 8.5–9.0), and 1.8% very strongly alkaline (pH > 9.0) (Staff, 1993). The 459 samples with temperature data available exhibited correspondence to a range

Table 2
Distribution of sediment and fermentation variables across the Stage I and Stage II fermentation experiments in this study.

	Stage I		Stage II	
	Min	Max	Min	Max
Sediment data				
Moisture in sediment (%)	1.1	62.9	0.0	100.0
Volatiles in sediment (%)	0.4	47.9	0.0	70.6
Sand (%)	11	97	5	100
Silt (%)	0	48	0	79
Clay (%)	1	86	0	67
pH	5.6	9.5	1.7	10.2
Conductance (dS m^{-1})	0	215	0	202
Total dissolved solids (mg/L)	5	137,600	0	129,280
Sodium (Na^{+} mg kg^{-1})	0	149,756	24	186,457
Potassium (K^{+} mg kg^{-1})	6	29,952	1	9974
Calcium (Ca^{++} mg kg^{-1})	11	1696	1	10,414
Magnesium (Mg^{++} mg kg^{-1})	6	10,354	1	15,003
Phosphorus (P mg kg^{-1})	1	124	0	384
Sulfur (S mg kg^{-1})	16	24,437	19	32,916
Organic carbon (%)	0.1	12.7	0.0	46.4
Total nitrogen (%)	0.1	1.0	0.0	20.8
Total carbon (%)	0.1	13.0	0.1	45.7
Sediment temperature (°C)	6.7	30.1	7.6	92.7
Fermentation data				
Acetic acid (change in g L^{-1})	−2.71	7.27	−1.06	6.16
Propionic acid (change in g L^{-1})	−0.21	0.86	−0.73	1.91
Isobutyric acid (change in g L^{-1})	0.00	0.35	−0.03	0.14
Butyric acid (change in g L^{-1})	−0.44	3.93	−0.30	1.43
Isovaleric acid (change in g L^{-1})	0.00	0.66	0.00	0.42
Valeric acid (change in g L^{-1})	0.00	0.00	0.00	0.05
Caproic acid (change in g L^{-1})	0.00	0.16	−0.05	0.14
Heptanoic acid (change in g L^{-1})	0.00	0.43	0.00	0.15
Total acids (change in g L^{-1})	−1.27	9.14	0.00	8.08
Conversion (% VS digested/fed in g)	15	49	2	57
Selectivity (% g acid/g VS dig)	−6	35	0	46
Yield (% g acid/g VS fed)	−1	9	0	13
AEQ (mol/L)	−0.03	0.19	0.00	0.16
AEQ (g L^{-1})	−1.55	11.17	0.00	9.32

of spring categories: 34% to cold springs (<20 °C), 54% warm springs (20–50 °C), 11.5% hot springs (>50 °C). Although it is helpful to have a standard nomenclature for reference sake, it is important to recognize that not all samples were from springs. Finally, for the 501 samples with organic carbon data, 14 (2.7%) contained greater than 12% organic carbon, a level considered as "high" organic carbon (Staff, 1993).

Table 2 provides the ranges for the soil characteristics measured as they distributed across Stage I and Stage II of this study. During Stage I, 102 samples were collected from grasslands, fresh- and salt-water marshes, salt lakes, playas, spring-fed lakes, and oil brine or potash slurry evaporation ponds sites in Texas, New Mexico, and Oklahoma. These initial sites were selected based on the presumed variation in salinity and total dissolved solids. In fact, these 102 samples span the entire range of conductance (0–215 dS m^{-1}) exhibited across all samples studied. The Stage I sampling trips did not include any sites with thermal features; as such, soil temperatures for these samples spanned a non-thermal range of 6.7–30.1 °C.

The 399 samples collected during Stage II were from sites representing a much broader geographic and ecological range (Supplementary Tables 1 and 2), selected based on presumed variation in ecology, salinity, total dissolved solids, and/or presence of thermal features. The ecological diversity for these sites included fresh, salt, and alkaline lakes, warm and hot springs, marshes, salt flats, coastal mangroves, and anchialine ponds (Supplementary Table 2). The broad ranges for most of the sediment data demonstrated this diversity (Table 2). The broad range of soil temperatures was particularly noteworthy (7.6–92.7 °C) but not surprising, in as much as, some soils were collected from thermal features.

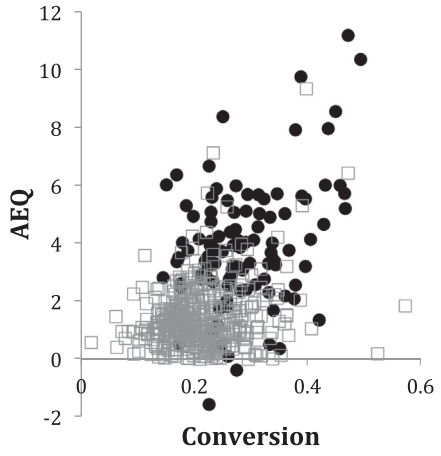

Fig. 1. Distribution of acetic acid equivalent concentration (AEQ) by conversion performance for each sample. Because fermentations with different inocula yield different acid profiles, AEQ (g L^{-1}) is used to standardize reporting the acid production across fermentations. Conversion is calculated as g volatile solids digested/g volatile solids fed. Black circles represent Stage I, and grey unfilled boxes represent Stage II.

3.2. Fermentation performance

Supplementary Table 1 provides all the sediment and fermentation data generated for all samples collected. The inocula were highly variable for fermentation performance for both Stage I and Stage II of this study (Fig. 1). In general, if microbial communities exhibited higher conversion of the substrate, then those samples produced more acid (Fig. 1). Presumably, in those more rare fermentations with high conversion but low acid production, some other unmeasured product was favored (e.g., H_2, CO_2, ethanol, or lactic acid). The wide range in fermentation performances across the diversity of soil samples lends credence to the idea that soil environments harbor different communities and thus it is reasonable to expect identification of soil parameters associated with community fermentation performance.

There was strong multivariate correspondence between fermentation products and environmental factors of the original soil samples. However, this result did not present as clear univariate responses (e.g., Soil Factor 1 increases concentration of Acid Species 1). Instead, strong effects associated with suites of correlated variables in one data block were related to suites of variables in the other block.

Substantial effect sizes were found in the MANOVA (Table 3) for both Stage I and Stage II experiments (η^2 = 0.921 and 0.566, respectively). Although the effect size was particularly strong for Stage I, note the statistical tests were not powerful because there were only six times the number of samples (N = 95) relative to the number of predictor variables. Although the only significant effect noted for Stage I resulted from sediment temperature (Table 3), some of the cations (K, Ca, S) might also be important determinants or correlates of acid composition, as suggested by the marginally significant p-values. It is worth noting that our other analyses confirmed these suggested effects. Stage II results, because of the greater sample size (N = 356), revealed more significant effects, despite the fact that the overall effect strength was less than for Stage I results. For Stage II, there were significant effects of soil temperature, pH, total N, Mg, Ca, moisture, and P on fermentation variables, with a suggestion (marginal significance) that organic carbon also may have played a causal or correlative role (Table 3). Although these significance tests indicate which independent variables are related to fermentation, the MANOVA framework treats each dependent variable separately, so it is difficult to interpret associations among variables.

Table 3
Multivariate analysis of variance (MANOVA) results for sediment verses fermentation variables across Stage I and Stage II of this study.

	Stage I			Stage II		
	F-stat	Degrees of freedom (Num, Den)[a]	*p*-Value	*F*-stat	Degrees of freedom (Num, Den)[a]	*p*-Value
Whole model	2.0226	(112, 523.5)	<0.0001	2.3420	(126, 2558.1)	<0.0001
Sediment temperature (°C)	3.2971	(8, 73)	0.0029	5.4991	(9, 333)	<0.0001
pH	0.9687	(8, 73)	0.4672	5.2717	(9, 333)	<0.0001
Total nitrogen (%)	0.6035	(8, 73)	0.7720	4.8577	(9, 333)	<0.0001
Magnesium (Mg^{++} mg kg^{-1})	0.9346	(8, 73)	0.4936	2.9892	(9, 333)	0.0019
Calcium (Ca^{++} mg kg^{-1})	1.8960	(8, 73)	0.0735	2.2190	(9, 333)	0.0206
Organic carbon (%)	0.7560	(8, 73)	0.6422	2.0485	(9, 333)	0.0337
Phosphorus (P mg kg^{-1})	1.4973	(8, 73)	0.1731	1.9655	(9, 333)	0.0426
Moisture in sediment (%)	1.0986	(8, 73)	0.3742	1.5265	(9, 333)	0.1372
Volatiles in sediment (%)	1.4177	(8, 73)	0.2037	1.5265	(9, 333)	0.1372
Sodium (Na^{+} mg kg^{-1})	0.7332	(8, 73)	0.6619	1.4944	(9, 333)	0.1485
Sulfur (S mg kg^{-1})	1.8564	(8, 73)	0.0802	0.8267	(9, 333)	0.5919
Conductance (dS m^{-1})	1.2548	(8, 73)	0.2806	0.7658	(9, 333)	0.6482
Total carbon (%)	0.8502	(8, 73)	0.5621	0.6825	(9, 333)	0.7248
Potassium (K^{+} mg kg^{-1})	1.9809	(8, 73)	0.0608	0.5897	(9, 333)	0.8055
Number of samples (*N*)		*N* = 95			*N* = 356	
Effect Strength (η^2)		η^2 = 0.921			η^2 = 0.566	

[a] Numerator (Num), Denominator (Den).

PLS constructs distilled axes for both the dependent and independent variables that can be related as multivariate pairs of axes, termed *singular axes* (Wold et al., 2001). Loadings of variables on these axes indicate the relative contribution of variables, as well as the direction of relationships, and thus provide a way to interpret two blocks of multivariate data as suites of associated variables. Again, to ensure the loadings could be compared within and between data blocks, this analysis was performed on standardized data. Fig. 3 shows the degree of relatedness of F-data and S-data expressed as the multivariate suites *within* data blocks that correspond to covariance *across* data blocks, which represents the majority of the predictable cross-variance. Fig. 2 illustrates the loadings of all the variables on each singular vector. The Stage I samples exhibited large correlation coefficients for both the first (R^2 = 0.2682; Fig. 3A) and second (R^2 = 0.23158; Fig. 3B) axis pairs, which is consistent with the strong effect size for Stage I in the MANOVA. The correlation coefficients for the Stage II first (R^2 = 0.11282; Fig. 3C) and second (R^2 = 0.06528; Fig. 3D) singular axis pairs were much lower for Stage II data, as was the effect strength in the MANOVA. In fact, the R^2 value for the second singular axis pair for Stage II was so low there was no justification for interpreting the loadings for this axis pair.

For Stage I, the predominant correspondence across fermentation and soil data was that lower temperatures and higher cation concentrations (especially Mg, S, and Na) were associated with greater acid production, especially for acetic, isobutyric, and isovaleric acids (Fig. 2). The second major axis of cross-variance indicated that conversion (particularly that resulting in propionic and acetic acids) was driven by, or correlated with, variables typically associated with soil fertility (relatively low pH and high

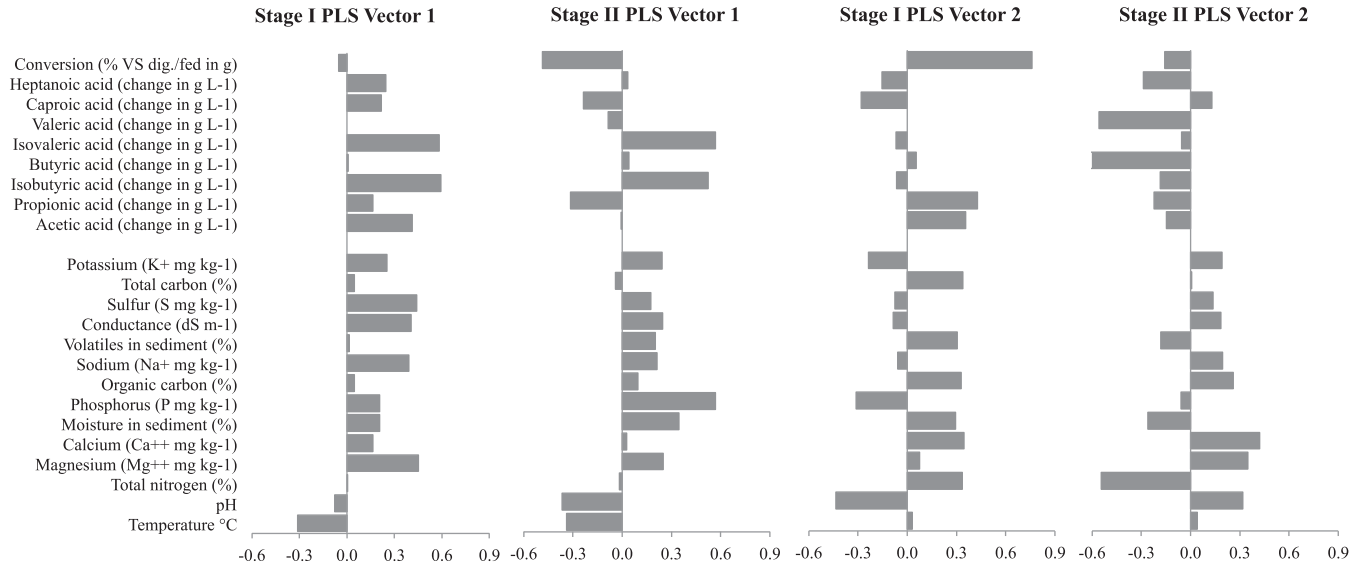

Fig. 2. Bar graphs illustrate the loading of each variable onto the major multivariate summary axes (singular vectors) that, taken across F-data and S-data, describe covariation of variable suites. Stage I and Stage II data largely evince congruent patterns for the major axis. Number of samples: 93 for Stage I fermentation, 356 for Stage II fermentation; sum of all variance: 3.7470 for Stage I, 2.1776 for Stage II. Valeric acid excluded in the Stage I fermentation analysis.

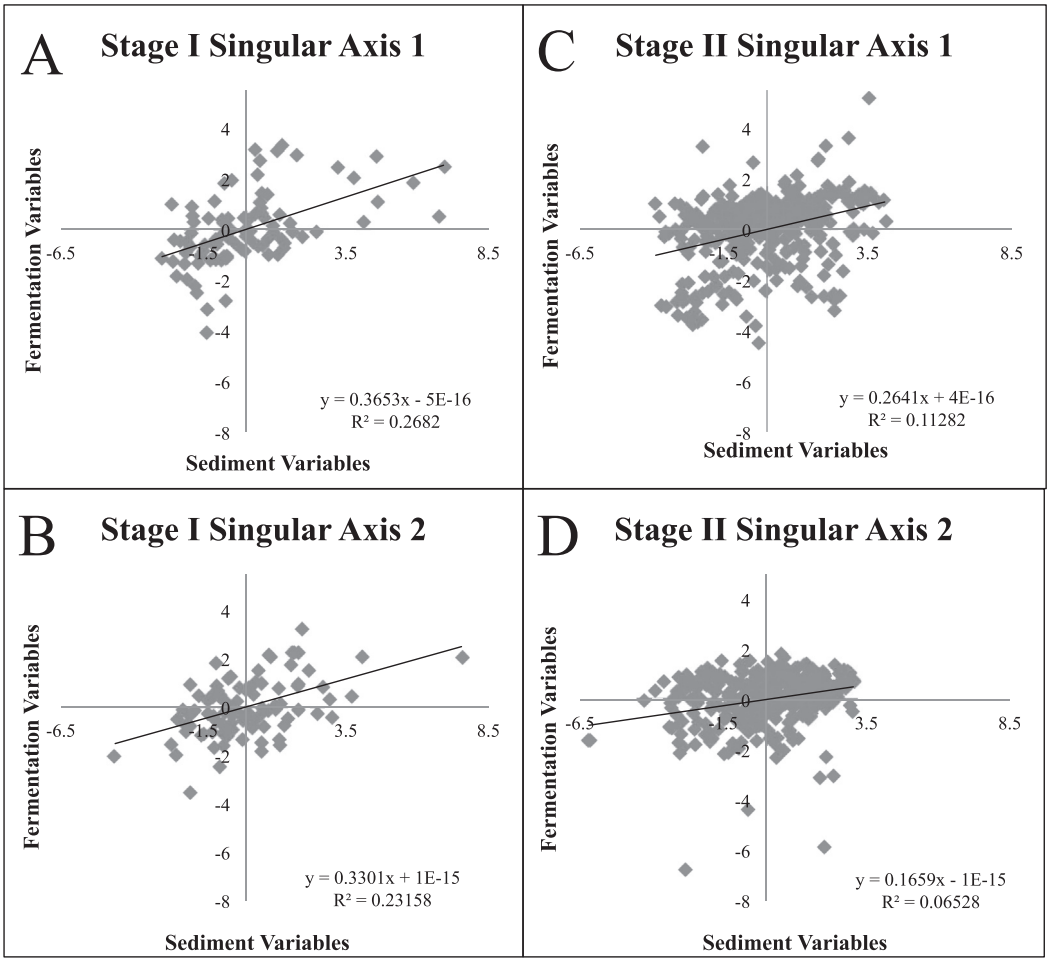

Fig. 3. Singular axis pairs for the two-block partial least squares (PLS) regressions conducted separately for the Stage I and Stage II data. (A) The Stage I singular axis pair 1 illustrates the majority of the cross-variance between the fermentation performance soil characteristics for Stage I. (B) Stage I singular axis pair 2 explains less cross-variance for Stage I. (C) Stage II singular axis pair 1 illustrates the majority of the cross-variance between the fermentation performance soil characteristics for Stage II. (D) Stage II singular axis pair 2 explains little cross-variance and is not interpreted in the text.

nitrogen, organic carbon, total carbon content, total volatile compounds, and moisture; Fig. 2). For Stage II, the soil variables associated with the first singular vector (Fig. 2) echo those identified for Stage I, namely sediment temperature, pH, and nutrients. The covariance of variables captured by the second major axis for Stage II was weak (see Fig. 3D), so there was no need to interpret the second axis.

The high effect sizes answer our central question about whether soil conditions can be used to predict fermentation products by the soil microbial community. Clearly they can; however, the relationships are complex because they do not emerge as a small number of isolated environmental parameters driving an isolated few aspects of organic chemistry. Soil conductance was consistently positively associated with production of some acids (namely isobutyric and isovaleric), but negatively associated with conversion (Fig. 2). Meanwhile, the relationship between sediment temperature and conversion was consistently positive (Fig. 2). The relationship between soil pH and conversion in Stage I was predominantly negative, whereas pH and conversion were positively associated in the Stage II experiments (Fig. 2). Taken together, the results from Stage I and Stage II suggest that one can maximize the desired fermentation products by sampling microbes from sediments relatively low in pH and temperature, but high in cations and nitrogen. These results apply to the fermentation conditions used in this study, and probably for more general conditions (e.g., sampling from other geographic regions).

This study shows that the productivity of the carboxylate platform is influenced by both fermentation conditions (Stage I and Stage II) and inocula; thus, it seems reasonable to expect both can be optimized to target desired outcomes (e.g., particular products and/or productivity levels). Also, this study demonstrated that fermentation performance is not as simple as finding one soil parameter that increases all performance parameters; rather, there are complex multivariate relationships that likely indicate trade-offs associated within the microbial communities.

4. Conclusions

In conclusion, it seems one should choose which performance parameter is of greatest interest and optimize by collecting inocula from soils with parameters that are positively correlated with the specific criteria. It might be justified to identify soils with multiple parameters that influence aspects of performance, exhibiting both optimal acid production and conversion. In absence of soils with both conductance and temperature at appropriate levels, perhaps one could optimize multiple performance parameters by combination of microbial communities from different environments. Future goals include testing these sorts of predictions, and identifying microbial community composition as related to fermentation with desirable performance outcomes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2013.12.105.

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