THESIS

REWIRING ANAEROBIC DIGESTION: PRODUCTION OF BIOFUEL INTERMEDIATES AND HIGH-VALUE CHEMICALS FROM CELLULOSIC WASTES

Submitted by

Jorge L. Rico Reyes

Department of Civil and Environmental Engineering

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Master's Committee:

Advisor: Susan De Long

Sybil Sharvelle
Kenneth Reardon
Terry Engle

ABSTRACT

REWIRING ANAEROBIC DIGESTION: PRODUCTION OF BIOFUEL INTERMEDIATES AND HIGH-VALUE CHEMICALS FROM CELLULOSIC WASTES

Anaerobic Digestion (AD) is a mature biotechnology for the valorization of organic residues, and AD is one of the most popular technologies for organic carbon recovery and waste stabilization. Research and applications for this process have been focused on the production of methane-containing biogas. However, the recent drop in natural gas prices has affected the economic value and market for this biofuel. Existing AD applications for the management of organic wastes (municipal and agricultural) are not economically attractive. Furthermore, it is unclear if methane biogas is the most economically advantageous product. Promising opportunities for AD have emerged in the production of chemical intermediates, such as short-chain fatty acids (SCFA). The market for these chemicals is growing, and more sustainable practices could replace their current petrochemical-based production. AD for the production of SCFA is an alternative approach with attractive market and economic opportunities. This approach is known as the carboxylate platform and relies on the beneficial features of using undefined mixed microbial cultures (also known as microbiomes) for fermentation of heterogeneous organic residues. One of the main identified technological barriers to the carboxylate platform is the inability to control the product spectrum and achieve high yields. AD is a complex biological system, and advances in the fundamental understanding of the microbial ecology associated with SCFA production in these systems are still needed. The identification of specific taxonomic

groups involved in the synthesis of certain products could provide insights for novel microbial shaping methods (e.g., bioaugmentation) to improve SCFA selectivity and production yield.

This study investigated the relationships between the production of SCFA and the microbial composition from three inoculum sources (anaerobic digester sludge, beef cattle rumen, and bison rumen), with cellulose as a carbon source. Results from the present work found associations between specific taxonomic groups within each of the microbial communities, and the production of particular SCFA. *Clostridium lentocellum DSM 5427* and the genus *Bacteroides* were selectively enriched, and these microbial taxa dominated in anaerobic sludge-inoculated cellulose-fed reactors; these taxa were strongly correlated with acetic acid, caproic acid, and enanthic acid production. On the other hand, propionic acid production was strongly related to the abundance of Prevotella *ruminicola*, *Fibrobacter succinogenes*, and members of the family *Rikenellaceae*. Further investigations at the molecular level (metagenome, metatranscriptome, and proteome) are suggested to expand current knowledge and better understand the microbiological factors that dictate the fermentation of cellulosic material within the context of the carboxylate platform. By expanding this understanding, microbiome shaping methods could be designed and evaluated to optimize and scale-up alternative bioprocessing approaches.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

1.1 Motivation

Anaerobic digestion (AD) is one of the oldest practices for the valorization of organic residues. Historical evidence suggests that the ancient Assyrians and Persians utilized the gas generated from accumulated waste piles for heating purposes. In the mid-nineteenth century, AD was established as a formal industrial process, and years later (the 1890s), wastewater treatment plants incorporated the technology into their treatment trains (Bond & Templeton, 2011). Currently, AD is one of the most popular technologies for the generation of methane-containing biogas for combined heat-power and electricity production (EPA, 2019a). In the United States (U.S.), the process has been extensively adopted as a waste stabilization and bioenergy procedure in wastewater treatment plants. More than 1000 digesters have been constructed in these facilities; additionally, around 260 have been reported to digest livestock manure (ARPA-E, 2016); and 131 for food waste processing (24 million tons per year) (EPA, 2019b). Under anaerobic conditions, methane is produced spontaneously by microbial communities and can be utilized for heat production without significant downstream processing. Despite these benefits, the economic value of methane biogas has been challenged by current low natural gas prices. Existing AD applications for the management of organic wastes (municipal and agricultural) are not economically attractive. Furthermore, it is unclear, if methane biogas is the most economically advantageous product (Kleerebezem, Joosse, Rozendal, & Van Loosdrecht, 2015).

An alternative biomass treatment process could significantly improve the economic viability of AD. One option is to "rewire" AD favoring the production of higher-value end products (e.g., short-chain fatty acids). Short-chain fatty acids (SCFA) are intermediate metabolites (2–5 carbon atoms) synthesized by the hydrolysis and fermentation of organic-waste

biopolymers; these molecules can be upgraded via biological or physical-chemical processing into a variety of biochemicals useful in several markets, including pharmaceuticals, food and beverages, biopolymers, and biofuels, among others (Atasoy, Owusu-Agyeman, Plaza, & Cetecioglu, 2018). Table 1 summarizes some features for the first three SCFA; the economic value for propionic and butyric (longer SCFA) are higher than acetic acid. On the other hand, market opportunities are different and specific for each product. The ability to control production for desired products plays a fundamental role in the successful process implementation (Agler, Wrenn, Zinder, & Angenent, 2011).

Table 1. SCFA general properties adapted from (Atasoy et al., 2018).

SCFA	Market Size kton/year	Market Price USD/ton	Main Applications
Acetic Acid	14000-17000	400- 800	Synthesis of vinyl acetic acid acetate (polymer industry); textiles, acetic anhydride (cellulose acetate precursor), and acetate esters (used as solvents and diluents for the food industry
Propionic Acid	90-150	2200-2700	Additive to preserve both animal feeds and human food
Butyric Acid	350-470	1650-1830	Animal feed sector; pharmaceuticals; animal feeds as a supplements and antibiotic.

Developing resource recovery and waste valorization technologies to produce specific organic acids is an area of increasing research interest. Researchers have explored the potential of organic waste feedstocks under different operating parameters. One study conducted on food waste investigated the effects of pH, temperature, and organic loading rates (OLR) using batch reactors (bench-scale); optimum conditions for total SCFA production were found to be pH 6.0, 35°C and 11 g/L*day (OLR). In that study, acetate and butyrate accounted for 60% of the total products (Jiang et al., 2013). High conversion and SCFA concentrations (30 g/L) were achieved

in another study using municipal solid waste combined with human biosolids; this work suggested that "rewired" AD could be an effective solution for municipal solid waste disposal in developed and developing countries (Lonkar, Fu, Wales, & Holtzapple, 2017). Other feedstocks, including lignocellulosic biomass (Xiong, Richard, & Kumar, 2015), bovine manure (Chan, Fu, & Holtzapple, 2011), and sewage sludge (Cagnetta, Coma, Vlaeminck, & Rabaey, 2016), have been investigated as well.

Mixed microbial communities, also known as microbiomes, can be manipulated or shaped by reactor inocula and operating conditions. For example, animal nutrition investigations have explored the production of particular SCFA, while simultaneously decreasing methane production (mostly in ruminants). A study conducted in vitro showed that grain-rich diets (readily fermentable carbon) supplemented with lauric acid decreased methane production, enhanced total SCFA production, and increased proportions of propionate (Yabuuchi, Matsushita, Otsuka, Fukamachi, & Kobayashi, 2006). Studies translating these findings to optimize the selective production of fatty acids in AD systems, where feedstocks are waste and composition depends on waste treatment needs, are limited. Further, the bioproduction of these acids currently, relies on the utilization of pure cultures and homogeneous feedstocks; for example, butyric acid can be produced using *Clostridium sp* and fermentable carbohydrates such as glucose, lactose, glycerol, and xylose, among others (Zigova & Šturdík, 2000). Production SCFA from complex non-sterile feedstocks remains challenging and demands research attention (Agler et al., 2011).

Very few published studies have characterized microbial communities in response to operating alterations for the fermentation of organic residues using mixed cultures. Critically, how microbial community structure is associated with the selective production of fatty acids is

not fully understood, and thus rational approaches to control or manipulate AD reactor microbiomes for specific acid production remain unattainable. Advances in fundamental understandings of the microbial ecology associated with fatty acid production in AD systems are needed. The identification of specific microorganisms involved in the synthesis of certain products would provide insights for novel microbiome reactor shaping methods (e.g., bioaugmentation). This knowledge is expected to be useful for the development of innovative microbial management and monitoring methods in anaerobic fermenters, for example, a set of microbial indicators for the diagnosis of reactor performance and process stability (Carballa, Regueiro, & Lema, 2015).

1.2 Research Objectives

The present work aims to establish associations between microbial community structure and the production of SCFA within the context of the carboxylate platform. By comparing productions responses for different microbial communities, potential taxonomic groups associated with the production of particular fatty acids could be identified. These findings would be expected to facilitate the development of microbiome shaping methods for the enhanced production of fatty acids from organic wastes. Thus, the objectives of this study were to: (1) investigate relationships between the production of SFCA and the microbial composition for three inoculum sources; and (2) identify taxonomic groups associated with the production of particular fatty acids.

1.3 Thesis overview

Chapter 1 presents the motivations and objectives for the present work. To familiarize the reader with metabolic processes, market opportunities, current studies, and outlook perspectives, an overview of the carboxylate platform is presented in Chapter 2. Chapter 3 presents the materials and methods used for the experimental setup, the SCFA characterization, the microbial analysis (using next-generation sequencing technologies), and the applied statistical tools. Results are presented in Chapter 4, describing findings associated with the production of SCFA and the microbiome structure. Chapter 5 summarizes the main findings of this work and contrast them with the literature; in particular, links between some of specific taxonomic groups and their fermentation capabilities are discussed. Finally, future research directions are suggested at the end of this section. The appendices include graphs for the production of every SCFA and tables with the regression coefficients, a representation of substrate affinity and product specificity for some ruminant microorganisms and the Tukey pairwise comparisons with their respective models used for the SCFA comparisons.

2. BACKGROUND AND LITERATURE REVIEW

Human population growth and unsustainable consumption behaviors have led to an unprecedented over-accumulation of solid waste in the biosphere. According to the World Bank (2019), over 2.01 billion tons of solid waste were produced in 2016; this amount is expected to reach 3.4 billion tons by 20250 (World-Bank, 2019). The organic fraction of municipal and agricultural solid waste represents more than 50% of the total produced (EPA, 2016). In spite of the added value opportunities for organic waste valorization (e.g., compost or biogas production), a significant proportion is not utilized and ends up in landfills. In 2015, more than 30 million tons of food waste went to landfills in the U.S.; according to the EPA this amount represents around 22% percent of all municipal solid waste (MSW) landfilled in 2015. Only a small fraction of total food waste was utilized for either compost (2.1 million tons, 5.3% percent of total food waste) or combustion for energy recovery (7.38 million tons, 22% percent of all MSW combusted with energy recovery) (EPA, 2015).

Underutilization of organic waste is due to the current focus of waste treatment procedures on only meeting environmental waste management regulations, and the limited economic viability of current AD technologies. Incentives for waste valorization to stakeholders, therefore, could promote the appropriate beneficial use of organic residues avoiding landfill accumulation. However, improving economics could potentially drive adoption without incentives. The carboxylate platform is a promising alternative because shows promise for improved economic viability. The process relies on AD of organic residues using undefined mixed microbial cultures for the production of SCFA (instead of methane biogas). SCFA are AD intermediates that can be upgraded into energy pipeline chemicals or biochemicals with versatile

uses in several markets. The economic value of SCFA is higher than methane biogas, and which leads to more favorable economics for the overall process (Kleerebezem et al., 2015).

In this chapter, a state of the art of the carboxylate platform is presented. The section starts by providing an overview of metabolic processes within these systems. Then contrasts between benefits and challenges in AD for the production of methane biogas and SCFA are presented. A summary of market opportunities for the first three SCFA and finally an overview of current studies, relevant developments, and outlook perspectives are presented as well.

2.1 Metabolic processes within the carboxylate platform

From an energetic perspective, metabolic processes progress towards a state of thermodynamic equilibrium. Biological systems, such as microbiomes, catabolize organic carbon for energy utilization. Without external perturbations, for example, the addition of an external electron acceptor, these systems achieve the thermodynamic equilibrium when the least energetic form of organic carbon is produced, methane (Table 2) (Kleerebezem et al., 2015). This methane-producing process is commonly known as anaerobic digestion (AD), and consists of a set of metabolic transformations where organic carbon molecules are transformed into a variety of intermediates, and then reduced and oxidized to produce methane and carbon dioxide, respectively.

Table 2. Gibbs energy change per electron (kJ/mol e-) upon oxidation to carbon dioxide (Kleerebezem & Van Loosdrecht, 2010).

Compound	Structure	ΔGe (kJ/mol e)
Methane	CH4	−23.0
Acetate	C2H3O2−1	−26.9
Propionate	C3H5O2−1	−27.0
Valerate	C5H9O2−1	−27.1
Caproate	C6H11O2−1	−27.1
N-butanol	C4H10O	−28.6
Ethanol	C2H6O	−30.4
Lactate	C3H5O3−1	−31.6
Hydrogen	H2	−40.0

Many authors have agreed to represent the AD system as a four-stage process catalyzed by physiologically distinct groups of microorganisms. First, extracellular enzymes secreted by fermentative bacteria hydrolyze (breakdown) insoluble organic polymers (cellulose, proteins, polysaccharides, and lipids) into soluble organic monomers (amino acids, sugars, and fatty acids) (Figure 1). These monomers are then oxidized to the central intermediate pyruvate and then reduced to low-molecular-weight organic acids (SCFA) and alcohols by fermentative bacteria; in the absence of oxygen, the oxidized and reduced forms of the electron carrier Nicotinamide Adenine Dinucleotide (NAD+/NADH) serve as an electron donor and electron acceptor in these transformations (Agler et al., 2011). Fermentation products (SCFA and alcohols) are then utilized as electron donors and electron acceptors in secondary fermentation reactions. These molecules could be either transformed into acetic acid, carbon dioxide, and hydrogen by acetogenic bacteria (acetogenesis), or transformed into longer molecules such as butyrate, caproate, butanol, and hexanol (chain elongation of carboxylates with ethanol). The production

of these larger molecules is driven by the presence or absence of the reactants and microbial catalysts involved. In the absence of perturbations, for example, the addition of a particular substrate, the system will progress towards the production of the organic carbon compound with the lowest energy content (Table 2). Since these longer carbon chain molecules, in general, have high energetic content, their synthesis is less thermodynamically favorable; therefore, they might not dominate the product profile (in an unperturbed AD system). A summary of some of the substrates and microorganisms involved in some secondary fermentation reactions; Gibbs energy changes per reactions could be found in the following reference (Agler et al., 2011). Finally, hydrogenotrophic and acetogenotrophic methanogens transform hydrogen (hydrogenotrophic methanogenesis) and acetate (acetogenotrophic methanogenesis) into methane and carbon dioxide.

Syntrophic associations can dictate the direction of specific pathways (e.g., methanogenesis or acetogenesis) within the AD metabolic network, even if these pathways are not thermodynamically favorable (Hattori, 2008). For example, anaerobic acetate oxidation (carried out by acetate-oxidizing bacteria) has a standard-state positive Gibbs free energy change (ΔG^{o}) and can only happen when products and reactants are at certain concentrations (Dolfing, 2014) (Nüsslein, Chin, Eckert, & Conrad, 2001). Hydrogen-producing acetogenic oxidizers can establish syntrophic interactions with acetate-producing acetogenic bacteria and methanogens to regulate the hydrogen partial pressure levels and allow the thermodynamic favorability of acetate oxidation and methanogenesis (Figure 1). These syntrophic interactions are also fundamental in the oxidation of other fermentation intermediates (SCFA, lactate, ethanol). Fiebig & Gottschalk demonstrated that *Desulfovibrio* sp. oxidizes lactate to acetate when co-cultured with methanogens (Fiebig & Gottschalk, 1983). Other studies have shown obligate syntrophic

relationships between propionate degraders, butyrate oxidizers, hydrogenotrophic methanogens, and acetoclastic methanogens (J. Li, Ban, Zhang, & Jha, 2012). Butyrate and propionate oxidizers produce substrates used by methanogens (hydrogen, acetate and carbon dioxide). The removal or addition of these metabolic intermediates (e.g., acetate or hydrogen) could alter these relationships. For example, removing acetate from the system might promote an increased metabolic activity in propionate and butyrate oxidizers, resulting in higher production of hydrogen, formate and carbon dioxide, and therefore, increased methanogenesis fluxes. On the other hand, the addition of acetate could inhibit propionate and butyrate oxidation (Ahring & Westermann, 1988).

Microbial networks are also fundamental for the utilization of heterogeneous organic carbon sources and the synthesis of SCFA in AD systems. Specific bacterial phylotypes are often highly selective to particular substrates, and studies on rumen fermentations have revealed that various bacterial species can build micro-networks (Czerkawski, 2013). For example, *Ruminococcus* can break down cellulose but not xylose or glucose*, Bacteriodetes amylophilus* can metabolize starch (maltose) but not pectin or glucose, and *Lachnospira* can degrade xylose but not cellulose. Acetate is a common fermentation product in many species; however, other fatty acids like propionate, butyrate, and lactate are selectively synthesized by specific organisms such as *Holotricha*, *Succinomonas amylolytica, Entodinium* and *Peptostreptococcus Elsdenii* (See Appendix A) (Czerkawski, 2013).

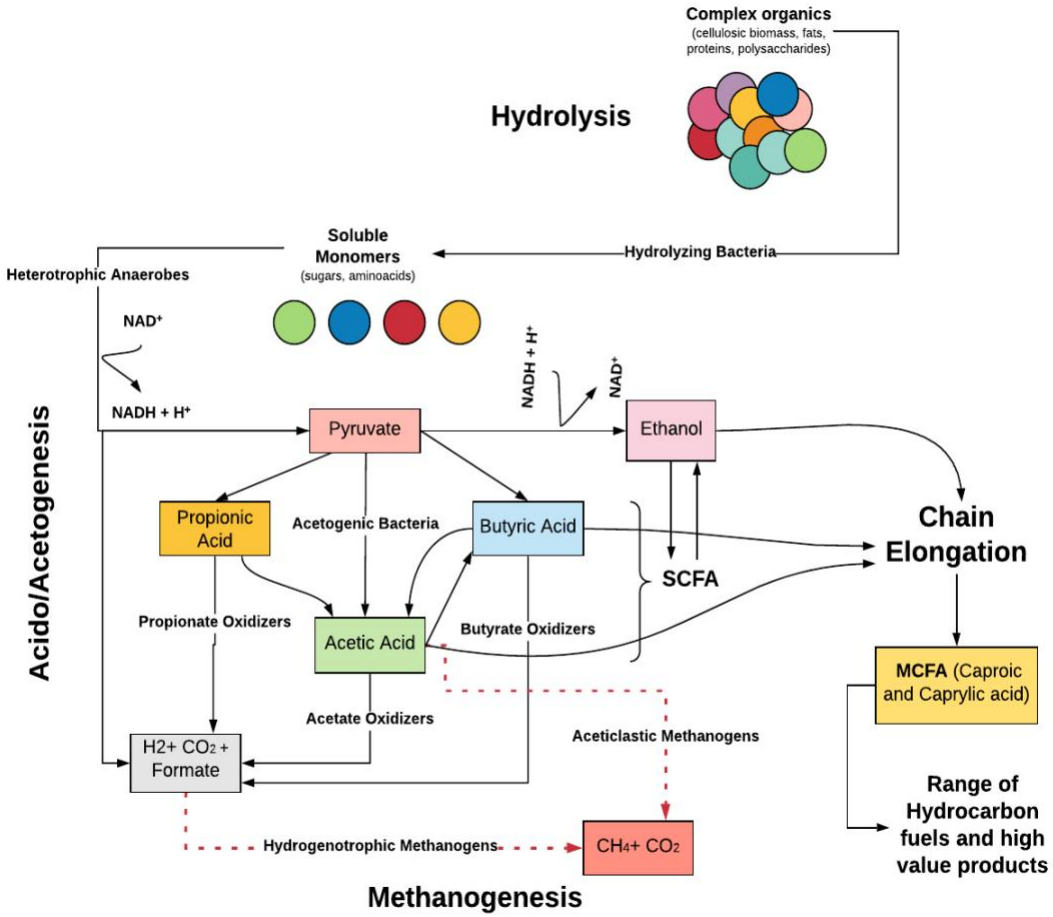


Figure 1. AD metabolic pathways in the context of the carboxylate platform.

2.2. Methane vs. SCFA production

AD for the production of methane-containing biogas has been a standard procedure in environmental engineering. The technology is advantageous over other waste treatment procedures, such as aerobic degradation or composting, because AD does not require aeration (which is energy intensive), produces energy, and offers both benefits of waste stabilization and valorization. Furthermore, AD processes yield low waste biomass, which is desired in the treatment of wastewater. Another advantage is related to the thermodynamic properties of the

final product; methane has the lowest Gibb's energy change per electron value when compared to other organic compounds (Table2) in the absence of external electron acceptors methane is produced spontaneously and methane automatically separates from the liquid production matrix (gaseous substance poorly soluble in water). Moreover, methane can be used as energy in the form of heat or electricity in a combined heat and power plant. Typically, AD for methane production is also highly attractive in terms of biomass conversion efficiency; the process can yield two or three times more energy per unit of biomass than other biological processes such as biodiesel or bioethanol production (Kleerebezem et al., 2015). This is due mainly to the intrinsic low energy requirements of the technology and the synthesis of a homogeneous product regardless of the heterogeneity of feedstock composition. Nevertheless, current methane production is low when compared to other biochemicals (Kleerebezem et al., 2015). As a result of the drop in natural gas prices, biogas production has been limited and is less likely to be adopted widely as a bioenergy technology (due to the low economic profit that can be gained). Another important disadvantage is related to sustainability; if biogas is not handled correctly, releases to the atmosphere could have negative impacts considering that methane global warming potential is 28–36 times higher than carbon dioxide (EPA).

Although AD for methane production is not highly profitable the AD technology still has potential if higher value products are yielded (Kleerebezem et al., 2015). The production of SCFA (the carboxylate platform) is a promising alternative. Two clear benefits can be highlighted in this approach: one related to the economic viability of AD (because SCFA have more economic value than methane) and the other related to the sustainable production of biochemicals. The current SCFA production industry is unsustainable; chemical production uses toxic chemicals (heavy metals), consumes fossil fuels, and relies on processes with high energy

requirements (W. S. Lee, Chua, Yeoh, & Ngoh, 2014). The carboxylate platform addresses these challenges; AD relies on microbial mixed cultures, processes operate at low pressures and temperatures, do not require sterile conditions, and can process heterogeneous waste feedstocks (which do not compete for production with human food). Furthermore, SCFA can be upgraded into higher-value products, for example, liquid biofuels, biopolymers, and pharmaceuticals, among others.

2.3 Methanogenesis inhibition with iodoform

Effective inhibition of methanogenesis prevent transformation of SCFA into methane which could be critical in achieving high SCFA yields. Different strategies has been implemented in several contexts. Among these inhibition approaches, the addition of iodoform has succeeded in AD systems. Aiello-Mazzarri, Agbogbo, & Holtzapple used iodoform in the conversion of municipal solid waste into SCFA using mesophilic microbiomes, the study reported high product concentrations (up to 25 g/L), and productivity (up to 1.4 g total acid/(L liquid d) (Aiello-Mazzarri, Agbogbo, & Holtzapple, 2006). Another study tested the effect of four different concentrations of iodoform (0, 30 50 and 70 ppm) on the SCFA production from marine macroalgae. The researchers observed that the SCFA productivity increased with the addition of iodoform (30 and 50 ppm), however, less SCFA product resulted at 70 ppm, suggesting that high iodoform concentrations could have negative impacts in the SCFA production yield (Pham, Nam, Jeon, & Yoon, 2012). Iodoform has been preferred among other inhibitors such as (2-bromoethanesulfonic acid or monensin) because does not have any regulation (by the Montreal Protocol) or known negative impacts into the ozone layer. Iodoform is a high reactive chemical very unlikely to enter the stratosphere (Holtzapple et al., 1999).

However, iodoform is a light and air sensitive chemical, and this could be a costly operating and disadvantage for scaling-up approaches.

2.4 Market opportunities

2.4.1 Acetic Acid

Acetic acid is a two-carbon carboxylic acid with a growing global market and demand. Market size for this chemical was at USD 6.4 billion in 2014 and is expected to grow 9% by 2022. Global acetate demand is currently estimated at around 6.5 million metric tons per year (Mt/a), primarily dominated by the synthesis of vinyl acetic acid for its applications in the polymer industry. Other essential drivers in the acetate market are textiles, acetic anhydride (cellulose acetate precursor), and acetate esters (used as solvents and diluents for the food industry) (Grandviewresearch, 2015a). The acetate industry currently depends on the petrochemical sector. Around 60% percent of worldwide production is based on methanol (fossil fuel feedstock) carbonylation. This process is catalyzed by costly catalysts such as rhodium or iridium (30.000 USD/mol catalyst) and operates at high temperatures and pressures (20-60 atm; 150-200 ºC). Capacities for full-scale acetic acid plants are at around 100,000 tons/year or more. Cost components in these processes (methanol carbonylation based) are dominated by operating parameters (approximately 40%), feedstocks (30%) and capital costs 30% driven mainly catalyst costs (Dionisi & Silva, 2016).

Currently, only around 10% of total acetate manufacturing is bio-based (Vidra & Németh, 2018); this process follows ancient practices to obtain vinegar from souring wine or beer via bacterial ethanol fermentation. Bio-based ethanol production and subsequent oxidation to acetate are less energy-intensive than petrochemical processes; however, feedstocks utilized in

these processes (sugar cane and corn) have environmental issues associated with the competition for human food and the extensive land use for monoculture crops. Second-generation bioethanol (using lignocellulosic waste feedstocks) has been demonstrated to be feasible using pure cultures of *Clostridium* species. Pilot plants have been established, or are at least are under construction, with prospective capacities of 1 ton/year to 1100 tons/year. Nevertheless, effective feedstock pretreatments and final product separation are still challenges for a full-scale up (Gnansounou, 2010).

Acetic acid synthesis via mixed culture fermentation of organic residues is also feasible. When hydrogen partial pressure levels are kept low within the system, acetate fluxes are driven by the syntrophic association of acetogens (acetate producers) with acetate oxidizers and methanogens (hydrogenotrophic and acetoclastic). As mentioned previously, AD systems progress towards the thermodynamic equilibrium; without perturbations, methane synthesis is therefore spontaneous. However, if methanogenesis is inhibited, acetate could accumulate, since it has the second-lowest energy content among other AD metabolites (Table 2). Both primary fermentation reactions and oxidation of longer chain fatty acids end up in acetate synthesis. If acetate accumulates, hydrogen levels also rise (due to inhibition of hydrogenotrophic methanogens) and fluxes from these reactions could are reduced resulting in an overall yield decrease (Figure 1).

High acetate yields, however, could be obtained by keeping low concentrations of acetate and hydrogen in the system, as is done by the conventional AD metabolic network. Acetate must be extracted by a continuous separation process, instead of allowing it to transform into methane or to accumulate. Membrane liquid-liquid extraction separation technology could be suitable for this approach (Agler et al., 2011). On the other hand, optimization of operational parameters

including pH, temperature, solids retention time, feedstock composition, and microbial inoculum could also provide higher acetate yields than have been heretofore obtained. Mixed culture fermentation experiments with various feedstocks and operating parameters have shown that acetate is generally always a major component in the fatty acid profile. Acetate concentrations ranging from 0.3 to 19 kg/m$_3$ have been obtained from the fermentation of different feedstocks, including food waste, rice, wastewater, and molasses, among others (Dionisi & Silva, 2016).

2.4.2 Propionic Acid

Propionic acid is a three-carbon carboxylate with applications as a food preservative. Due to its antimicrobial and antifungal properties, it is highly used as an additive to preserve both animal feeds and human foods. The propionic acid market demand was estimated at 399.4 kilotons in 2013 and is expected to grow up to 470.0 kilotons by 2020 (2.7% CAGR from 2014 to 2020). The market for this chemical is growing and expected to reach USD 1.53 billion by 2020 (dominated primarily by the animal feed industry) (Grandviewresearch, 2015b). Production of propionic acid is mainly by petrochemical routes. Hydrocarboxylation of ethylene using nickel carbonyl catalyst is one of the most used processes (Kilner & Winter, 1996). Biological production of this chemical has been demonstrated via fermentation of lactic acid, glucose and other sugars using bacteria of the genus *Propionibacterium* (Himmi, Bories, Boussaid, & Hassani, 2000). Other recent metabolic engineering approaches, also have explored the viability of engineered *Propionibacterium* species to synthesize propionic acid from glycerol (Zhang & Yang, 2009). Large scale biological production of propionic acid is currently a challenge due to associated costs in product separation, and low yields due to product inhibition (Agler et al., 2011).

2.4.3 Butyric Acid

Butyric acid (BA) production is currently dominated by petrochemical processes via oxidation of n-butyraldehyde. Synthesis for this chemical (n-butyraldehyde) relies on crude oil derived materials and operates at high temperatures and pressure conditions (0 - 150°C and 15 - 50 atm) (Dionisi & Silva, 2016). On the other hand, butyric acid can be obtained from butter (contains 2 - 4 % BA); however, this method is expensive and difficult from a process engineering perspective (Zigova & Šturdík, 2000). The butyric acid market is driven primarily by the animal feed sector (40% of the market share in 2014), followed by chemical intermediates and pharmaceuticals (Research-And-Markets, 2016). Butyric acid has anti-pathogen properties and is being used in animal feeds as a supplement and antibiotic. From the health and nutrition perspective, this compound is also known for its anticarcinogenic properties and for being an efficient energy source for animals. Another interesting application is biodiesel production. In fact, butyric acid has been identified as one of the most valuable sources for biodiesel manufacturing. Bioproduction of butyric acid has been recently catalyzed by the food industry sector because of its approval as a food flavoring agent by the U.S. Food and Drug Administration (Atasoy et al., 2018). Biotechnologies for butyric acid production have been demonstrated using *Clostridium* species grown on fermentable carbohydrates such as glucose, lactose, glycerol, and xylose, among others (Zigova & Šturdík, 2000). Mixed culture fermentation of lignocellulosic biomass (rice straw) also has been reported for BA production. Using a mixture of manures and other organic residues as an inoculum, about 6 g/l of BA was produced and BA accounted for ~76% of the total SCFA profile (Ai et al., 2014). However, to my knowledge, no industrial-scale production has been developed using this approach.

2.5 Recent advances and future perspectives

The production of SCFA from organic wastes, using undefined mixed microbial cultures is a current topic of research and development. To my knowledge, successful industrial SCFA production from organic wastes has not been formally reported. Only Terrabon Inc. scale up a technology called MixAlco for the bioproduction of mixed alcohols and carboxylic acids from organic wastes. However, the company went out of business in 2017 and liquidated under bankruptcy, due to lack of additional funding (Pulsinelli, 2012). Our inability to control the product spectrum, lack of efficient processes for the separation of desired acid products and challenges with achieving high yields are some of the main limitations that have affected the successful scale-up (Agler et al., 2011). Although several studies have attempted to optimize operating parameters (pH, temperature, organic loading rates, and feedstock pre-treatment) to improve SCFA production yields, the complex variability of these systems remains challenging (Atasoy et al., 2018). A "unique" set of optimum parameters is hard to establish and these optimum parameters are likely to depend on specific conditions, such as the feedstock composition, the source of microbial inoculum, or the desired product. Studies have reported different outputs for the same operating conditions. For example, food waste fermentations have produced differing SCFA product spectrums in experiments carried out under the same pH and with similar sources of microbial inocula. Wang, Yin, Shen, & Li (2014) evaluated the effects of pH on fermentation of food waste using aerobic and anaerobic sludge as an inoculum; the study found optimum total SCFA concentrations at pH 6.0 using anaerobic sludge but found higher proportions of BA (above 80%) at pH 5.0 for both microbial inocula (K. Wang, Yin, Shen, & Li, 2014). In contrast, with similar conditions (fermentation of food waste using mesophilic anaerobic digester sludge), Jiang et al. (2013) found acetic acid to be the dominant product

(60%), followed by butyric, propionic, and valeric acid (pH 5.0); when the pH was at 6.0 and 7.0, butyric acid was the main product in the spectrum profile (Jiang et al., 2013). Kim, Kim, Jung, Kim, & Shin (2011) tested the effect of pH (ranging from 5.0 - 9.0) on fermentation for food waste for SCFA production; acetic acid was the predominant product in all tested conditions, and butyric acid production was highest at pH 8.0 (Kim, Kim, Jung, Kim, & Shin, 2011).

Developing a fundamental understanding of microbial processes associated with the production of specific SCFA within mixed microbial systems is a critical step in addressing the challenges previously mentioned. Certain taxonomic groups with potential fermentation capabilities have already been identified in various mixed microbial ecosystems. Sun, Liu, Müller, & Schnürer investigated the microbial community structure effects on the degradation rate of cellulose and straw from four co-digestion biogas plants (agricultural waste, source-separated organic municipal household waste, and slaughterhouse waste) and six wastewater treatment plants. The study identified the families *Firmicutes*, *Bacteriodetes* and the species *Clostridium cellulolyticum* as potential cellulose-degrading organisms. Other studies have explored the biological potential of alternative microbiomes, in particular, the gut microflora present in the digestive tract of certain animals (Sun, Liu, Müller, & Schnürer, 2016). Kangaroos are known for having an efficient metabolism with very little methane outputs (Godwin et al., 2014). These attributes have been associated with the composition of their gut microbiome and the capabilities of particular taxa. Godwin et al. investigated carbon dioxide and hydrogen metabolism in the kangaroo foregut, this work compared methane and SCFA productions *in vitro* using bovine rumen and kangaroo foregut as a microbial inocula. Results from this study demonstrated that the activity of the reductive acetogen *Blautia coccoides* was fundamental for

the reduced methane output and increased acetic acid production in kangaroo microbiomes (when compared to bovine rumen). This organism was suggested to serve as "sink" for hydrogen and carbon dioxide, other taxonomic groups including the genera *Prevotella, Oscillibacter,* and *Streptococcus* were also suggested to be associated with these fermentative capabilities (Godwin et al., 2014). Another potential microbiome is the termite gut microflora; termites are characterized for digesting a variety of lignocellulosic materials with high lignin content (e.g. wood). Auer et al. (2017) explored the potential of the termite gut microbiome, the researchers highlighted the biological potential of this microbial system for the bioconversion of lignocellulose within the context of the carboxylate platform. Reactors inoculated with termite gut degraded lignocellulose and up to 45% w/w (from wheat straw) and achieved total SCFA concentrations of up to 5 g/L. The phylum *Bacteriodetes*, *Firmicutes* and *Proteobacteria* were selectively enriched, in particular, researchers associated *Dysgonomonas* (*Bacteriodetes* genus) and *Clostridium termitidis* (*Firmicutes* species) with potential fermentation capabilities (Auer et al., 2017).

Connecting links between the microbial structure and certain functions within the carboxylate platform might promote the development of new strategies for optimizing the SCFA production in AD systems. Some studies have demonstrated the value of this fundamental understanding by designing microbiome shaping methods for increased fatty acid production (e.g. bioaugmentation or chain elongation). Shanmugam, Sun, Chen, & Wu (2019) improved butanol yield to 98-fold via bioaugmentation of a mangrove sediment microbiome reactor with a butanol-producing *Clostridium sp.* strain WST (Shanmugam, Sun, Chen, & Wu, 2019). Another study compared SCFA and medium chain fatty acids (MCFA) productions from food waste using an enriched food waste microbiome (non-bioaugmented) and bioaugmented microbiome

with *Clostridium kluyveri*; the study found higher productions of butyric (8.9 g/l) and caproic acid (8.1 g/l) in the bioaugmented communities (when compared to the non-bioaugmented) (Reddy, Hayashi, Choi, Cho, & Chang, 2018). Agler, Spirito, Usack, Werner, & Angenent, (2012) upgraded ethanol to n-caproic acid using sheep rumen on unstilled brewery wastewater. The study shaped the reactor microbiome for producing n-caproic acid via chain elongation of SCFA with ethanol. By using a continuous in-line product extraction system, the conducted experiment demonstrated high n-caproic acid productions (more than 2 g / L*day) without the use of methanogen inhibitors. Shotgun metagenomic analysis of this system found associations between the chain-elongation gene pool (for caproic acid production) and the abundances of *Clostridium spp*. and *Ruminococcaceae* OTU. On the other hand, associations for *Ethanoligenens* (*Ruminococcaceae* Family), *Bifidobacterium*, and *Desulfitobacterium*, were related to important gene pools for hydrolysis and ethanol oxidation (Agler, Spirito, Usack, Werner, & Angenent, 2012).

Although bioaugmentation and chain elongation have been demonstrated to be promising methods for the improvement of SCFA production yields and the synthesis of MCFA, the limited available information regarding the molecular mechanisms (metabolic pathways) and the microbial ecology associated with these strategies demands further attention . Most publicly available studies have reported strains of *Clostridium* species, for both bioaugmentation and chain elongation approaches in AD systems. The biological potential of other organisms remains unknown. Furthermore, researchers have reported failures in bioaugmentation attempts. The massive addition of a particular strain into a microbial community could result in an overall ecosystem imbalance and possible negative consequences on the desired output (e.g., decreased production yields or microbial growth inhibition) (Herrero & Stuckey, 2015).

On the other hand, lack of information regarding the specific role (functions) of particular microbes or hub of microbes in AD systems limits the ability to predict success or failure in the implementation of microbiome shaping strategies. Production of medium chain fatty acids in microbiomes using chain elongation has been associated with the reverse beta-oxidation pathway present in strains of *Clostridium sp* (Weimer, Nerdahl, & Brandl, 2015). Nevertheless, as mentioned before, limited or no information is available for other taxonomic groups. The discovery of new strains with potential capabilities for bioaugmentation and chain elongation is, therefore, a promising opportunity. Microbial composition analysis linked multi-omics approaches (metagenomics, metatranscriptomics, metaproteomics, and metabolomics) could facilitate these novel discoveries. The expected outcome from the implementation of these tools is the association of genes, enzymes, metabolites, and pathways with particular microbes or hubs of microbes in an AD system. This knowledge is also expected to facilitate the design and evaluation of novel microbiome shaping and monitoring methods to optimize and scale-up alternative bioprocessing approaches (Oleskowicz-Popiel, 2018).

3. METHODS

3.1 Experimental design

The goal of the experiment was to evaluate the production of SCFAs as a function of the reactor microbiome composition. The approach we used involved three different microbial inocula (Anaerobic Wastewater Sludge, Bison Rumen, and Beef cattle Rumen). A synthetic nutrient solution (Owen, Stuckey, Healy Jr, Young, & McCarty, 1979) with cellulose as a carbon source was used to simulate a cellulosic waste feedstock. Non-fed control reactors (without cellulose) were run to account for the effects of the inoculum itself on the production of SCFA. An abiotic control (without inoculum, but with cellulose) was included as well . In summary, a total of six treatments plus the abiotic control were analyzed in this study (Figure 2). The experiment consisted of three factors (microbial inocula) under two levels (with and without cellulose), and each of the treatments was performed in triplicate. The twenty-one reactors were incubated for 20 days, and measures were taken every five days (Days 0, 5, 10, 15 and 20); a total of 105 samples were collected and processed for chemical and microbial analysis.

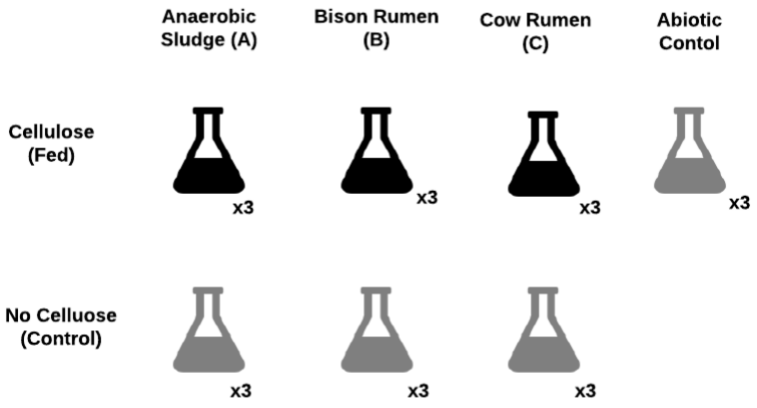


Figure 2. Experimental design 3x2 factorial design in triplicate.

3.2 Inoculum collection

Anaerobic wastewater sludge (A) was collected from Drake Wastewater Reclamation Facility (Fort Collins, CO). Bison rumen (B) was collected immediately after slaughtering at Brush Meat Processors (Brush, CO). Beef cattle rumen (C) was collected from a grass-fed fistulated beef cattle at the Colorado State University Agricultural Research, Development, and Education Center (ARDEC). All three microbial inocula were collected within 24h before inoculation and were kept under anaerobic conditions using sealed bottles at 35 ºC with continuous mixing (100 rpm). Rumen fluids were collected only from one animal and reproducibility of results might be compromised by this fact.

3.3 Biomass normalization

To normalize the amount of inoculum added to each reactor, DNA concentration was used as a measurement of the biomass present in each inocula. Microbial inocula were filtered using a cheesecloth (to remove large solid particles), and then 1 mL of filtered fluid was centrifuged at 10,000 x g for 10 min. Pellets were processed to extract DNA using a Qiagen DNeasy PowerSoil Kit (Hilden, Germany) according to the manufacturer's instructions. DNA concentrations were then measured by optical density at 260nm using a BioTek Synergy 2 Multi-Detection Multiplate Reader (BioTek Instruments, Inc., headquartered in Winooski, VT, USA). Microbial inocula were diluted using deionized water and equivalent biomass amounts were provided to each reactor (~10 µg DNA/ml).

3.4 Reactor setup

Twenty one 160-mL syringes (Sherwood Medical, Northern Ireland) equipped with three-way Luer lock valves (QUOSINA, Ronkonkoma, Long Island, NY, USA) and Precision Seal rubber septa (Sigma-Aldrich, St. Louis, MO, USA) were assembled and filled with 90 mL

of a nutrient solution (Owen et al., 1979) (Appendix C) and 15 ml of microbial inoculum (normalized inoculum) (Figure 3). Cellulose was used as a substrate to represent a cellulosic waste feedstock; 2 g of cellulose (Lab grade cellulose, Fisher Scientific, Hampton, NH) were added to each reactor (excluding the non-fed controls). Iodoform was added every other day (1.2 mg/L*day) to inhibit methanogenesis, promoting the accumulation of fatty acids in the liquid phase (Lonkar et al., 2017). Reactors were incubated at 35ºC for 20 days a 100 rpm.

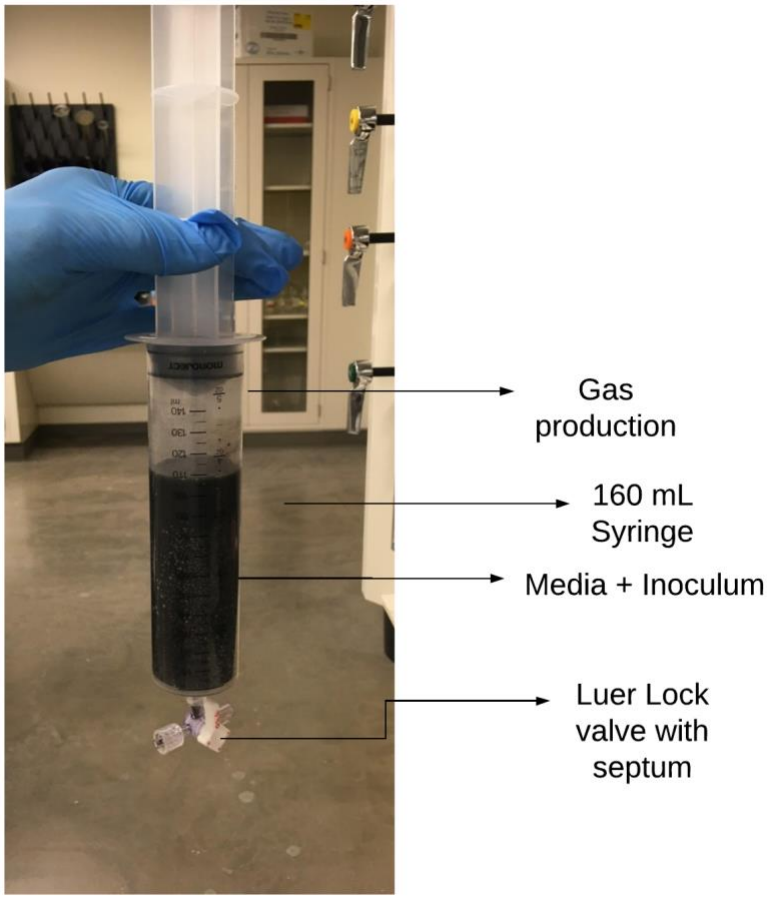


Figure 3. Syringe reactor setup.

3.5 Sample collection and preservation

2-mL samples from each reactor were collected every five days (0, 5, 10, 15 and 20). Samples were centrifuged at 10,000 x g for 10 min, and the supernatant and pellet were separately stored. Supernatants were used for chemical analysis of SFCA, and pellets were stored at -20°C for microbial analyses.

3.6 Chemical analyses

Samples were analyzed using gas chromatography with mass spectrometry (GC-MS) (Agilent 6890 with a 5973A MSD; column: Agilent DB-Wax UI 30m x 0.25mm x 0.25um). 2 mL of collected samples were filtered (0.22 μm) and acidified with 1N HCl to pH below 3 prior to chromatography. Gas chromatography was performed using the following protocol: injection volume: 1 μL; split mode 3:1; inlet temperature: 250 °C; carrier gas: Helium at 1 mL/min; oven temperature program: 60 °C (5 min), 5 °C/min to 150°C (5 min), 150 to 230 °C at 10 °C/min, 230 °C for an additional 10 min; detector transfer line temperature: 250 °C.

3.7 DNA extraction

DNA extractions for microbial community analysis over time were completed using the Qiagen DNeasy PowerSoil Kit (Hilden, Germany) according to the manufacturer's instructions. After thawing, the sample pellets were transferred to the PowerBead Tubes. DNA extracts were then processed for quantification by optical density at 260nm using a BioTek Synergy 2 Multi-Detection Multiplate Reader (BioTek Instruments, Inc., headquartered in Winooski, VT, USA).

3.8 Library preparation and DNA sequencing

16S rRNA gene Illumina amplicon libraries were constructed based on the Earth Microbiome Project protocol (Earth-Microbiome-Project, 2018). Polymerase Chain Reaction (PCR) was performed on each of the DNA extracts using single barcoded primers (515F and 926R) flanking the 16S rRNA gene V4 region. Unique barcodes were present on each of the forward primers (Single-index Illumina sequencing). The PCR was carried out on a Bio-Rad thermocycler (Bio-Rad Laboratories, location), using a Platinum Hot Start PCR Master Mix (2X) (ThermoFisher, Waltham, Massachusetts); 50 µL reactions were conducted by a denaturation step at 94°C for 3 min, followed by 30 cycles of 94°C for 45 s, 50°C for 60 s, and 72°C for 90 s, concluding with an extension step at 72°C for 10 min. Amplicons were kept at 4°C before further library processing. Reaction products (5 µl) were screened on a 1% agarose TAE (Tris-acetate EDTA) gel, using a Gel electrophoresis system (Bio-Rad Laboratories, Hercules, CA) to verify successful amplification. Blank amplicon controls (PCR reactions without DNA template) and DNA extraction negative controls (PCR reactions with a template from a blank DNA extraction) were screened to account for potential contamination either in the PCR master mix or in the DNA extraction kit. After the gel electrophoresis verification, 3 µL of each PCR amplification product were combined in a single microcentrifuge tube. The pooled library was then purified using SPRI (Solid Phase Reversible Immobilization) magnetic beads (Sera-mag SpeedBeads, Fisher, Hampton, NH). 280 µL of SPRI beads were combined with the pooled library and incubated at room temperature for 2 min. The mix was then transferred to a magnetic rack (Reference, location) to allow magnetic bead separation (4 min). Three 70% ethanol washes were performed followed by 15 min room temperature drying. The dried pellet was then resuspended in 50 µL of nuclease-free water and incubated at room temperature for 2 min. The

suspension was then transferred back to the magnetic rack for bead separation (~2 min). The DNA-containing supernatant was finally transferred into a new microcentrifuge tube. Sequencing was performed with the Illumina MiSeq platform (Illumina, Inc, San Diego, CA) at the Colorado State University Next Generation Sequencing Facility.

3.9 Microbial analysis

Demultiplexed reads from the Illumina MiSeq platform were processed on the QIIME 2.0 v2018.6 software pipeline. The files were imported using the Casava 1.8 paired-end demultiplexed fastq algorithm and then filtered for data quality control with DADA2 (Callahan et al., 2016).Taxonomy was assigned with Naive Bayes classifiers trained on the Silva v.132 database, and taxonomic barplots based on relative abundances were made to visualize differences across taxonomic levels (Quast et al., 2012). Microbial alpha and beta diversity were analyzed to evaluate significant differences in the microbiome composition across different operating conditions. Sequences were rarified at a sampling depth of 11797 (lowest among all samples). Phylogenetic trees were created with FastTree and then input into the q2-diversity QIIME plugin for alpha (Faith's Phylogenetic Diversity) (Chao et al., 2015) and beta diversity analysis (unweighted UniFrac distance) (Chang, Luan, & Sun, 2011).To visualize differences in the community structure, three-dimensional principal coordinate analysis (PCoA) plots of the unweighted UniFrac distance were generated using the Emperor tool (Vázquez-Baeza, Pirrung, Gonzalez, & Knight, 2013).

3.10 Statistical analysis

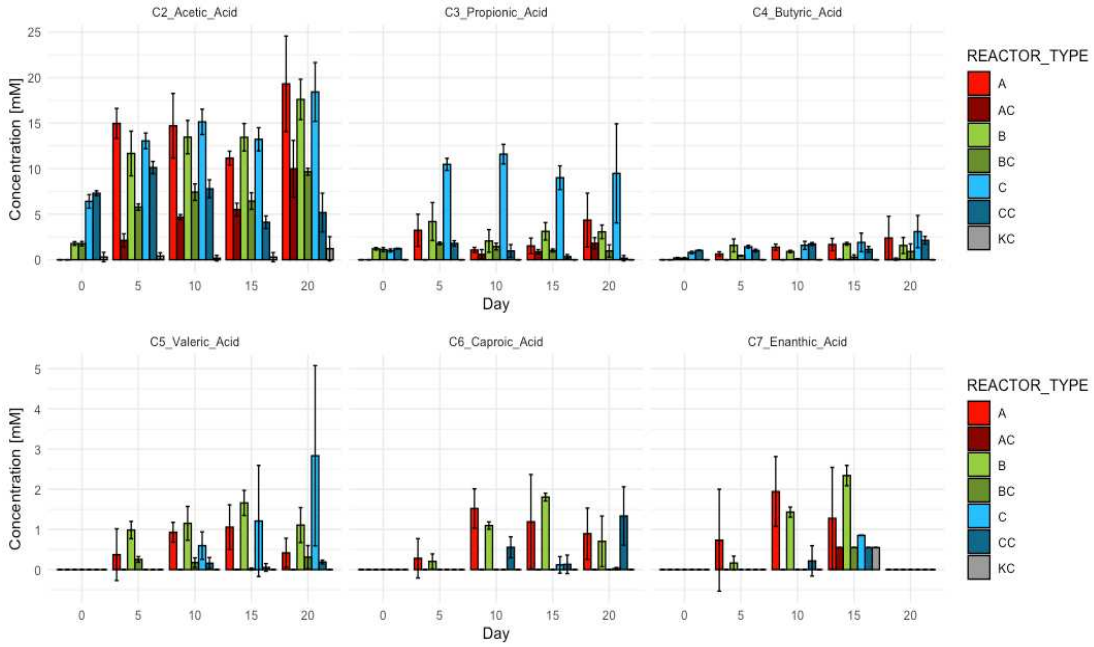
To compare differences between the SCFA concentrations and production rates, barplots showing the concentrations as well as the proportions of produced SCFA were constructed using RstudioVersion 1.1.463 and the package ggplot2. Additionally, Tukey pairwise comparisons between all the inoculum treatments were performed with using the functions emmeans and emtrends from the package emmeans v1.4.1(Lenth, 2019). The analysis was done separately for each of the days and day intervals (day 0-5, day 5-10, day 10-15 and day 15-20). Linear models were fitted for each of the individual acids in both fed and treatment controls; predictor variables (defined as factors) for these models were the inoculum source and day (interaction allowed: Acid ~ Inoculum*Day). Comparisons between SCFA production rates were estimated using the function emtrends, by contrasting the slope estimates within each of the day intervals. Additionally, to establish relationships between the SCFA concentrations and the presence of specific taxonomic groups, Pearson correlation analyses were performed by contrasting the concentrations and proportions of all SCFA products with the relative abundances of selected taxonomic groups (abundances higher 400 reads per sample). Significant correlations were selected and summarized (regression coefficient $|r| > 0.5$ for at least one correlation, p-value < 0.05 for at least one correlation).

4. RESULTS

4.1 The SCFA product spectrum was shaped by the source of microbial inoculum and the effects of cellulose as a carbon source

The three microbial inoculum sources exhibited differences in the SCFA product spectrum dynamics under the influence of cellulose as a carbon source (Figure 4). Fed reactors showed significant dissimilarities between inoculum treatments over time. Beef cattle rumen (C) fed microbiome reactors produced statistically more propionic acid than bison rumen (B) and anaerobic sludge (A) (Days 5 - 20) ($p<0.05$) (Table 3); furthermore, by day 20 an increase in valeric acid production was observed in these reactors (C) as well. In contrast, during days 10 and 15, concentrations of longer fatty acids such as caproic acid (C6) and enanthic acid (C7) were statistically higher in reactors A and B when compared to C. Acetic and butyric acid concentrations were not significantly different at any time point among the reactor conditions (with the exception of acetic acid at day 0, where C had higher levels presumably due to acetic acid presence in the inoculum source). However, rates of production for these acids were significantly higher in reactors A for specific timeframes: Day 0 - 5 (acetic acid, A higher than B and C), Day 5 - 10 (butyric acid, A higher than B) (Table 4). Production rates for other SCFA were also different for specific inoculum pairs; all pairwise comparisons within each time frame are shown in table 4. Additionally, appendices D and E present the regression coefficients and p-values for all pairwise comparisons.

a)



b)

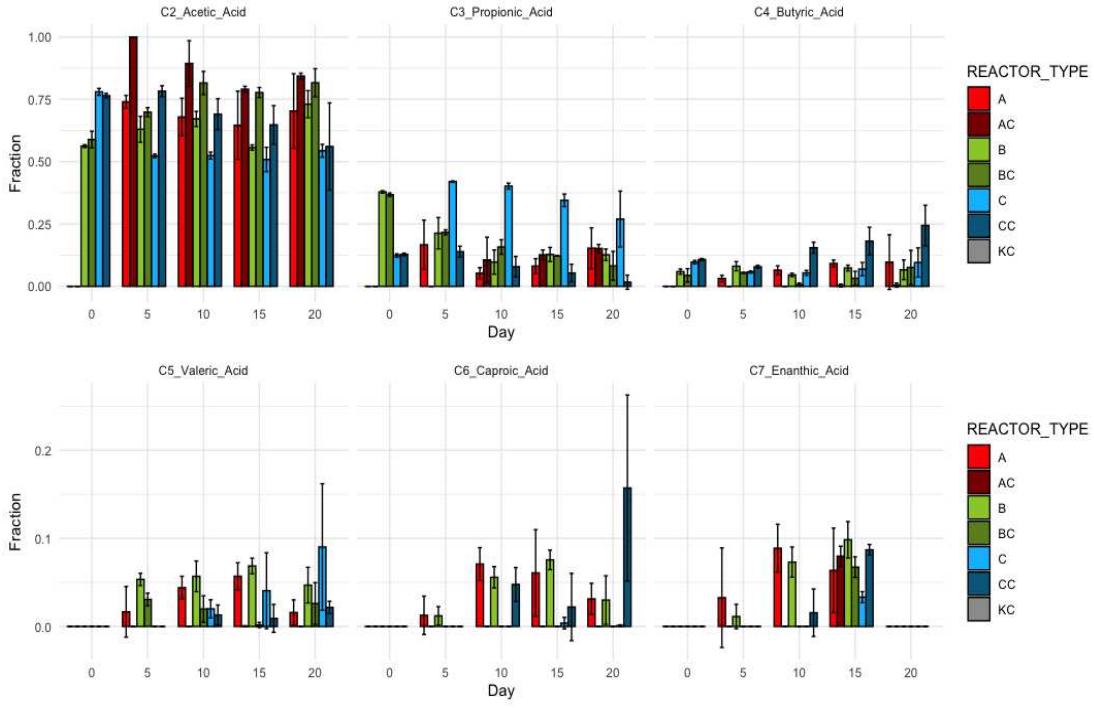


Figure 4. a) Concentration [mM] of SCFA for the different treatments. b) Relative proportions for SCFA production profiles.

A= Anaerobic Sludge (Fed), AC= Anaerobic Sludge (Control); B=Bison Rumen (Fed), BC=Bison Rumen (Control); C = Beef cattle Rumen (Fed), CC=Beef cattle Rumen (Control); KC= Abiotic Control.

Table 3. Tukey pairwise comparisons between the mean SCFA concentrations*.

	Day 0			Day 5			Day 10			Day 15			Day 20		
	A - B	**A - C**	**B - C**	**A - B**	**A - C**	**B - C**	**A - B**	**A - C**	**B - C**	**A - B**	**A - C**	**B - C**	**A - B**	**A - C**	**B - C**
Acetic Acid	-1.8	**-6.4**	**-4.6**	3.3	1.9	-1.4	1.3	-0.4	-1.7	-2.3	-2.1	0.2	1.7	0.9	-0.8
Propionic Acid	-1.2	-1.0	0.2	-1.0	**-7.2**	**-6.3**	-1.0	**-10.5**	**-9.5**	-1.6	**-7.5**	**-5.9**	1.3	**-5.1**	**-6.4**
Butyric Acid	-0.2	-0.8	-0.6	-0.9	-0.8	0.1	0.5	-0.2	-0.7	-0.1	-0.2	-0.1	0.8	-0.7	-1.5
Valeric Acid	0.0	0.0	0.0	-0.6	0.4	1.0	-0.2	0.3	0.6	-0.6	-0.2	0.5	-0.7	**-2.4**	**-1.7**
Caproic Acid	0.0	0.0	0.0	0.1	0.3	0.2	0.4	**1.5**	**1.1**	-0.6	**1.1**	**1.7**	0.2	**0.9**	0.7
Enanthic Acid	0.0	0.0	0.0	0.6	0.7	0.2	0.5	**1.9**	**1.4**	**-1.1**	0.4	**1.5**	0.0	0.0	0.0

**Significant comparisons are highlighted (p-value < 0.05). The numbers in the table represent the differences between the respective concentration means.*

Table 4. Tukey pairwise comparisons between the rates of SCFA production within a specific timeframe.*

	Day 0 - 5			Day 5 - 10			Day 10 - 15			Day 15 - 20		
	A - B	**A - C**	**B - C**	**A - B**	**A - C**	**B - C**	**A - B**	**A - C**	**B - C**	**A - B**	**A - C**	**B - C**
Acetic Acid	**1.0**	**1.7**	0.7	-0.4	-0.5	-0.1	-0.7	-0.3	0.4	-0.2	-0.1	0.1
Propionic Acid	0.0	**-1.2**	**-1.3**	0.0	-0.7	-0.7	-0.1	0.6	**0.7**	0.1	0.1	0.0
Butyric Acid	-0.2	0.0	0.2	**0.3**	0.1	-0.2	-0.1	0.0	0.1	0.1	0.0	-0.1
Valeric Acid	-0.1	0.1	**0.2**	0.1	0.0	-0.1	-0.1	-0.1	0.0	0.0	-0.2	-0.2
Caproic Acid	0.0	0.1	0.0	0.1	**0.2**	0.2	-0.2	-0.1	0.1	0.0	0.0	0.0
Enanthic Acid	0.1	0.1	0.0	0.0	0.2	0.3	-0.3	-0.3	0.0	-0.1	0.0	0.0

**Significant comparisons were highlighted (p-value < 0.05). The numbers in the table represent the differences between the respective slopes.*

Production responses for all the measured variables, including total SCFA production, DNA concentration (microbial growth), SCOD, and gas production (carbon dioxide mainly, since methane was not detected) were higher in the fed reactors. These production responses suggest that cellulose was metabolized either into biomass, SCFA, or gas by the different microbial communities (Figures 5, 6 and S1). The total SCFA production (normalized on acetic acid equivalents) (by day 10) was highest in beef cattle reactor microbiomes (Figure 5 and S4).

These C communities started with higher DNA concentrations (indicator of biomass) of ~1.6-fold higher than A and ~3.5-fold higher than B (despite normalization efforts), and DNA concentration remained higher during the fermentation (Figure S1). However, interestingly C produced less carbon dioxide than A and B (Figure 6). These results suggest that beef cattle rumen microbial communities metabolized cellulose more efficiently into SCFA or biomass, but product profiles were strongly influenced by the microbial composition and not only by biomass growth (Figure 4).

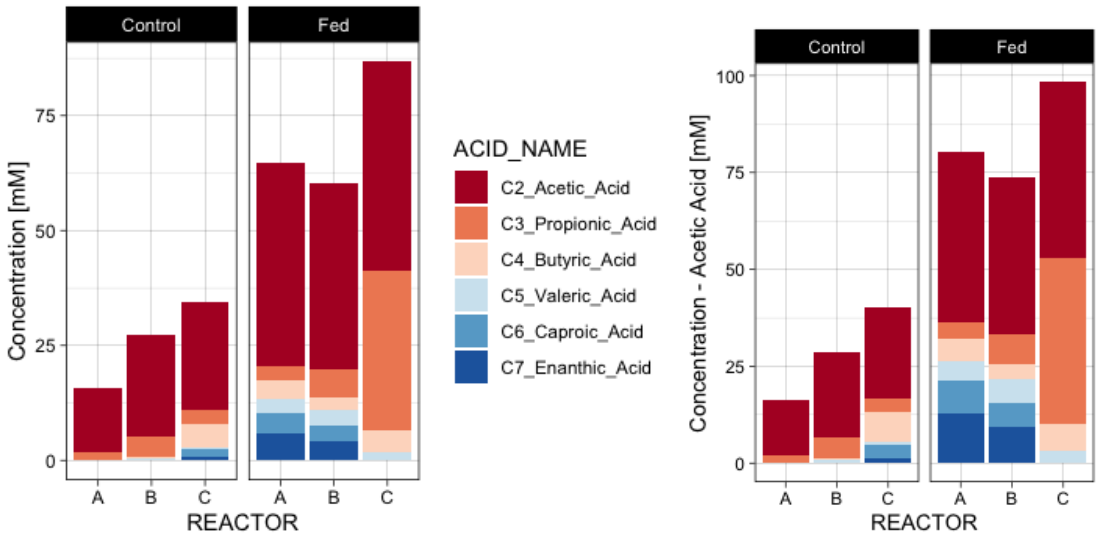


Figure 5. Stacked bar plots representing the total SCFA production for each of the inoculum sources at day 10. Concentration in mM (Left) ; Normalized concentration values in mM of acetic acid (Right).

The control reactors did not produce gas, and microbial biomass decreased (DNA concentration dropped over time) (Figure S1). However, fatty acids were produced, and concentrations showed interesting trends compared to fed reactors. Production of fatty acids in controls might be attributed to microbial metabolism of organic carbon present in the inoculum sources or endogenous decay (Droste, 1998). For example, the three controls produced acetic acid between days 0 and 5, but beef cattle rumen controls degraded some of the acetic acid after

day 5 (day 5 - day 15) while the production stayed relatively constant in anaerobic sludge and bison rumen controls (Figure S6). On the other hand, propionic acid was produced in anaerobic sludge (A) but consumed overtime in bison (B) and beef cattle (C) rumen controls (Figure S7). Additionally, between days 5 and 10, butyric acid was produced in beef cattle rumen but degraded in bison rumen and not observed in anaerobic sludge controls (Figure S8).

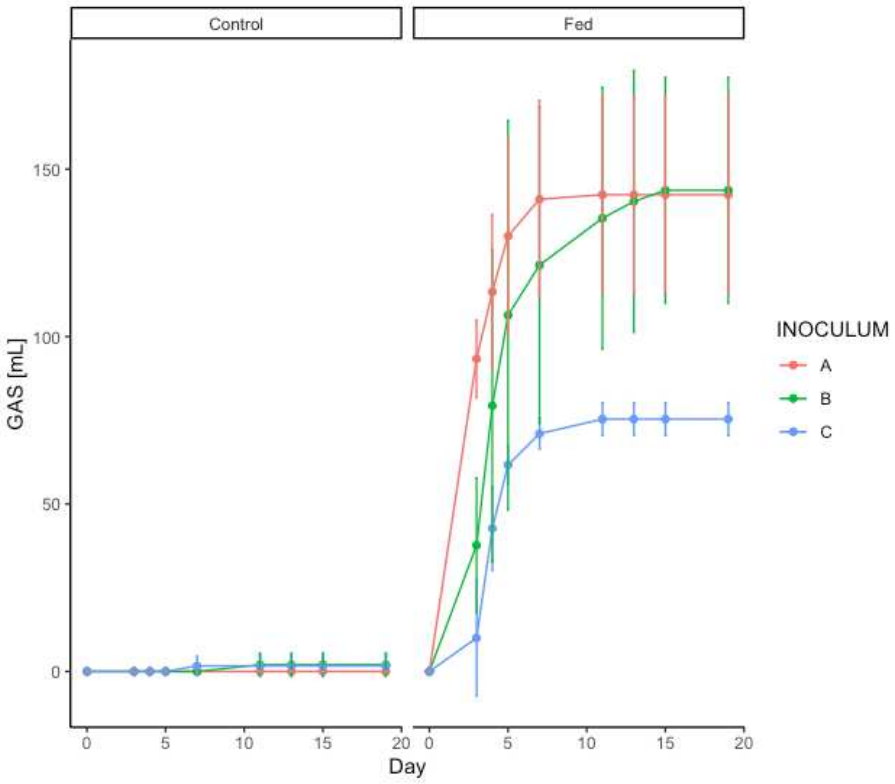


Figure 6. Gas production. Control - No cellulose addition; Fed - Cellulose addition.

4.2 Microbial community structure differed among inoculum sources over time and due to cellulose as a carbon source

Reactor microbiomes remained distinct over time among the inoculum sources despite identical reactor operating conditions (Figure 7). Additionally, differences were observed between fed and control reactors. The microbial compositions (represented by each marker in the plot) were shaped over time, especially in the fed reactors; replicates clustered together and reactor microbiomes grouped by inoculum source and whether they were fed. At day 0, the three reactor microbiomes started with three clearly distinct structures (represented by the smallest spheres in Figure 7); however, by day 5 drastic shifts were observed in all reactors, both fed and controls, with the exception of anaerobic sludge controls, which stayed relatively close to their starting microbial composition. After day 5, changes over time were more evident in beef cattle rumen controls and bison fed reactors. By contrast, the composition in bison rumen controls remained relatively stable .

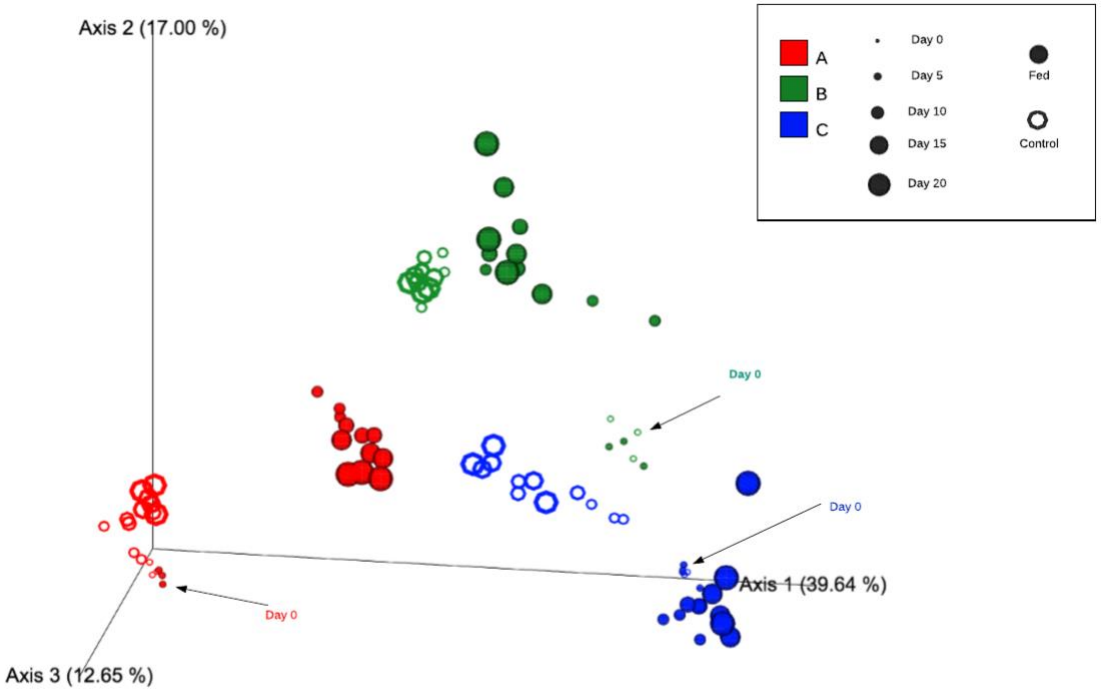


Figure 7. Principal Coordinate Analysis of the weighted UniFrac distance. Anaerobic Sludge (Red); Bison Rumen (Green); Beef cattle Rumen (Blue); Fed reactors (Sphere); Controls (Rings). Marker size is proportional to the sampling day.

The previously mentioned differences in the microbial community compositions were also evident in the taxonomic diversity analysis. Cellulose affected the microbial alpha diversity for all inoculum sources; controls were phylogenetically distinct and more diverse than fed reactors (Figure 8) due to the enrichment and decrease in the relative abundance of specific groups of microorganisms (Figures 9, 10 and 11). In particular, fed anaerobic sludge reactors exhibited a dramatic shift in the community structure by the enrichment of two particular taxonomic groups: the species *Clostridium lentocellum DSM 5427* and the genus *Bacteroides,* which dominated at relative abundances higher than 20% over the course of the fermentations. Other groups such as the family *Lachnospiraceae* and the genus *Erysipelatoclostridium* were also present at relative abundances higher than 2 % (Days 5-20) in A fed reactors. In contrast, the

relative abundance of other taxonomic groups decreased over time in fed reactors, while they either increased or remained relatively constant in the controls (e.g., the genus *DMER64* and members of the order *Cloacimonadales*) (See Figure 9).

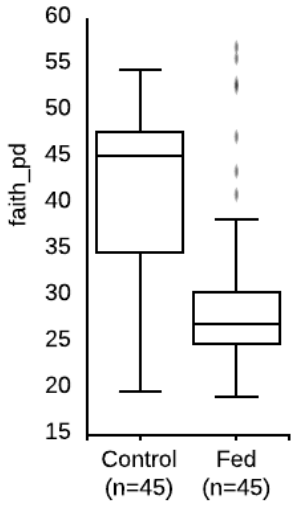


Figure 8. Alpha diversity comparison between fed reactors and controls.

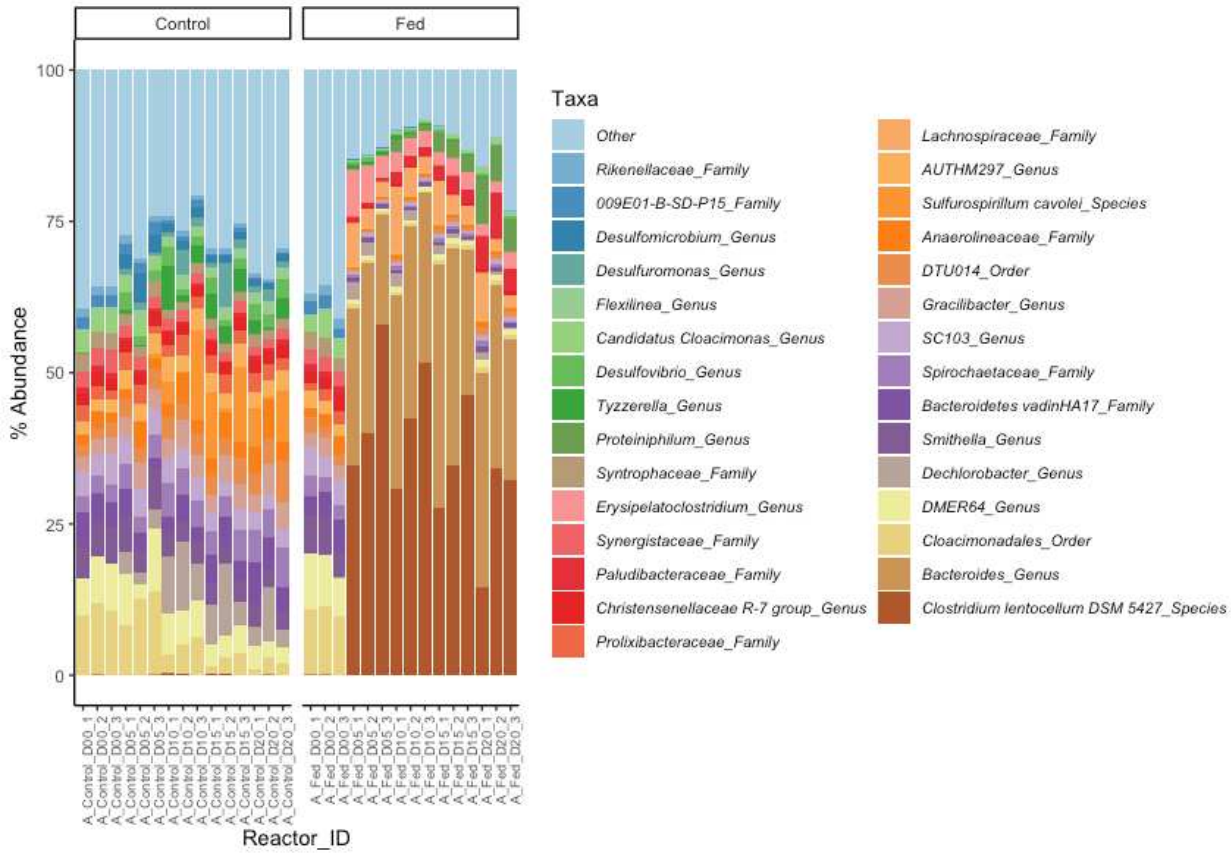


Figure 9. Taxonomic composition bar plots - Anaerobic Sludge reactors (A).

In fed beef cattle rumen reactors, the species *Prevotella ruminicola*, *Fibrobacter succinogenes* and the members of the family *Rikenellaceae* and *Fibrobacter* were present at relative abundances higher than 5% but were not present in the controls nor the other inoculum sources (Figure 10). On the other hand , fed bison rumen reactors favored the growth of the genus *Prevotella* and a member of the family *Muribaculaceae*; a peak in the relative abundance of these taxa was observed on days 5 and 10, respectively (Figure 11). In bison rumen fed reactors, other taxonomic groups, including *Clostridiales bacterium DJF_VP35* (species), *Muribaculaceae* (family), *[Eubacterium] nodatum group* (genus), *Prevotellaceae* UCG-004 (genus) and *Bacteroidales* (order), where present at relative abundances higher than 5% in fed reactors but were not observed at abundances below 5% the controls. Alternatively, the genera

Rubeoparvulum, Symbiobacterium, Tyzzerella, and *Desulfovibrio* were enriched in bison controls and not in fed reactors (Figure 11).

Some taxonomic groups that were enriched with cellulose addition were common among multiple treatments. For example, the family *Lachnospiraceae* was enriched in anaerobic sludge and bison rumen fed reactors (not seen in others); an uncultured rumen bacterium of the genus *Succiniclasticum* was present in both beef cattle and bison rumen; the genus *Proteiniborus* was present in both bison and beef cattle rumen controls. Alternatively, Archaea were more abundant in control reactors, and the bacterial genus *Christensenellaceae* R-7 group was enriched in all the control reactors (A, B and C).

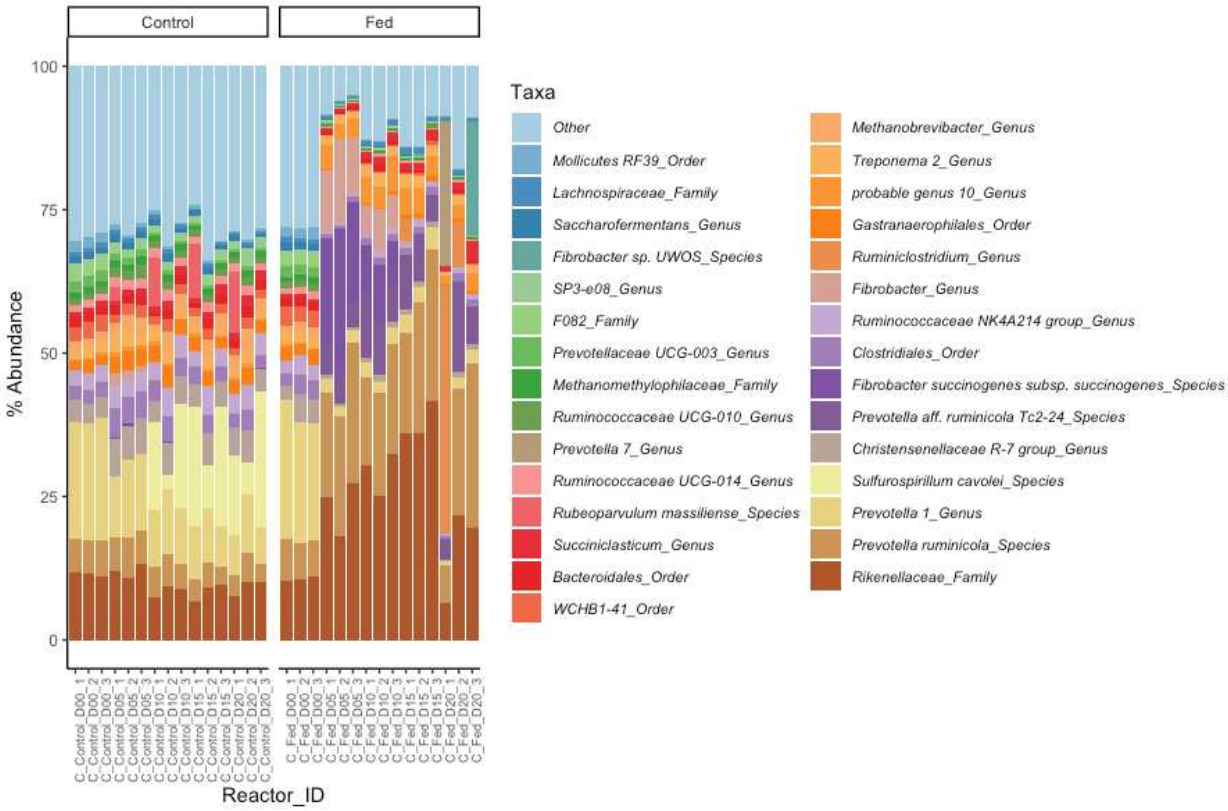


Figure 10. Taxonomic composition bar plots - Beef cattle Rumen reactors (C).

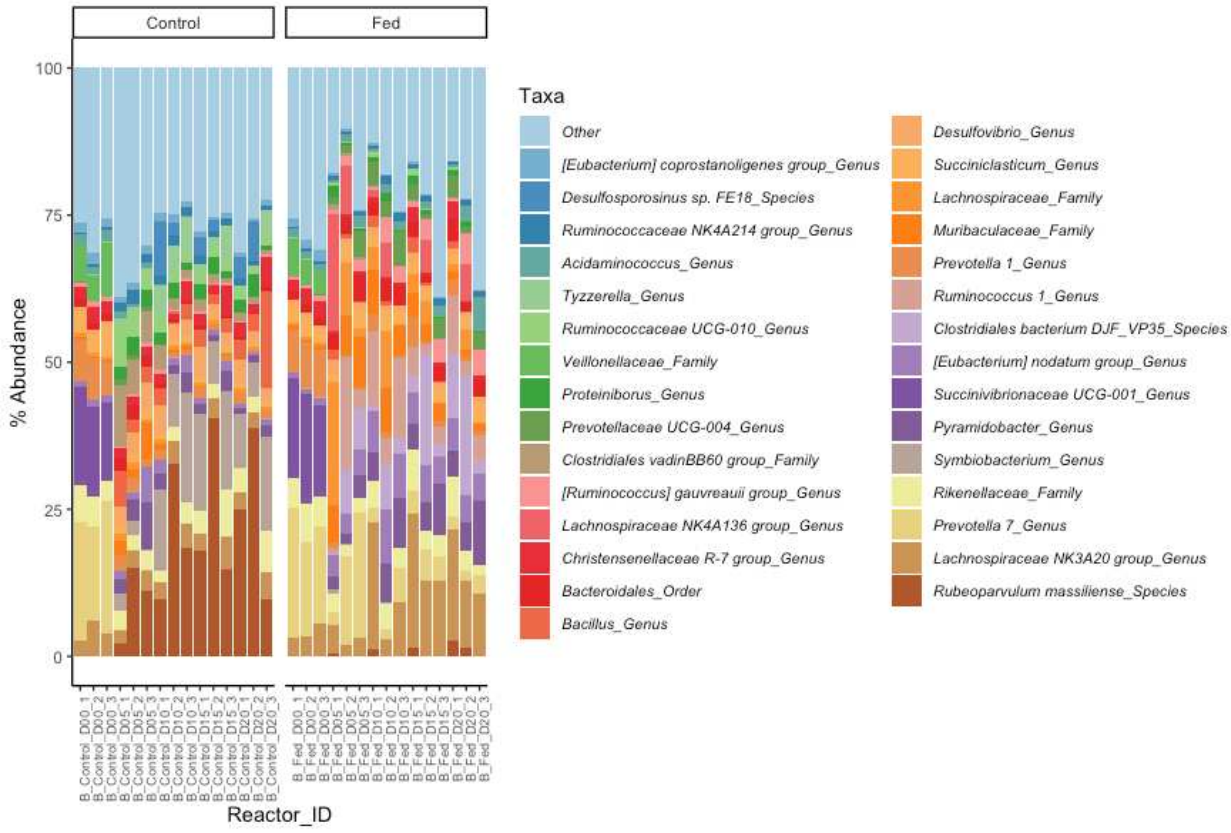


Figure 11. Taxonomic composition bar plots - Bison Rumen reactors (B).

4.3 Relationships between microbial community structure and the SCFA product spectrum

To establish relationships between the production of SCFA and the presence of specific taxonomic groups, a Pearson correlation analysis was done. Strong correlations ($|r| > 0.5$; p-value <0.05) were found for each of the SCFA products (Figure 12). In particular, four taxonomic groups (*Prevotella ruminicola* [species], *Prevotella aff. ruminicola Tc2-24* [species], *Probable Genus 10* [genus from *Lachnospiraceae*], and *Schwartzia* [genus]) showed the strongest Pearson correlations for propionic acid ($r > 0.75$); as expected these groups were only present in beef cattle fed reactor microbiomes where propionic acid production was higher (Section 4.1). Other taxa (*Erysipelotrichaceae UCG-009* [genus], *Fibrobacter succinogenes*

subsp. Succinogenes [species], *Fibrobacter* [genus], *Rikenellaceae* [family], *Rummeliibacillus* [genus], and *Veillonellaceae UCG-001* [genus]) also were positively correlated with propionic acid production at lower person coefficients ($0.5 < r < 0.75$), as shown in Figure 12. Acetic acid production was positively correlated with seventeen taxonomic groups; among these, seven groups (*Anaerocolumna* [Genus], *Bacteroides* [Genus], *Clostridium lentocellum DSM 5427* [Species], *Erysipelatoclostridium* [Genus], *Macellibacteroides* [Genus], *Oscillibacter* [Genus], *Phascolarctobacterium* [Genus]) were also correlated with production of caproic acid, enanthic acid (only the first six), and gas ($r > 0.57$). By day 5, when the acetic acid production rate was significantly higher in fed anaerobic sludge reactors*, Clostridium lentocellum DSM 5427, Bacteroides,* and *Erysipelatoclostridium* dominated primarily at relative abundances higher than 40%, 30%, and 2%, respectively and were not present in other microbiome reactors. Further, another ten taxa (*Erysipelotrichaceae UCG-009* [genus], *Fibrobacter succinogenes subsp. Succinogenes* [species], *Fibrobacter* [genus], *Prevotella aff. ruminicola Tc2-24* [Species], *Prevotella ruminicola* [Species], *probable genus 10* [Genus], *Rikenellaceae* [Family], *Rummeliibacillus* [Genus], *Schwartzia* [Genus], *Veillonellaceae UCG-001* [Genus]) were correlated with propionic, butyric and valeric acid production, and were only present in the rumen microbiomes (beef cattle and bison). Negative correlations were observed for eight taxonomic groups: *009E01-B-SD-P15* [Family], *Acholeplasma* [Genus], *Candidatus Cloacimonas* [Genus], *Cloacimonadales* [Order], *Family XIII* [Family], *Ruminococcaceae* [Family], *Christensenellaceae R-7 group* [Genus], *Romboutsia* [Genus]; the first six were strongly negatively correlated with acetic acid ($r < - 0.75$). The family *Ruminococcaceae* was negatively correlated with all the metabolic products (Figure 12).

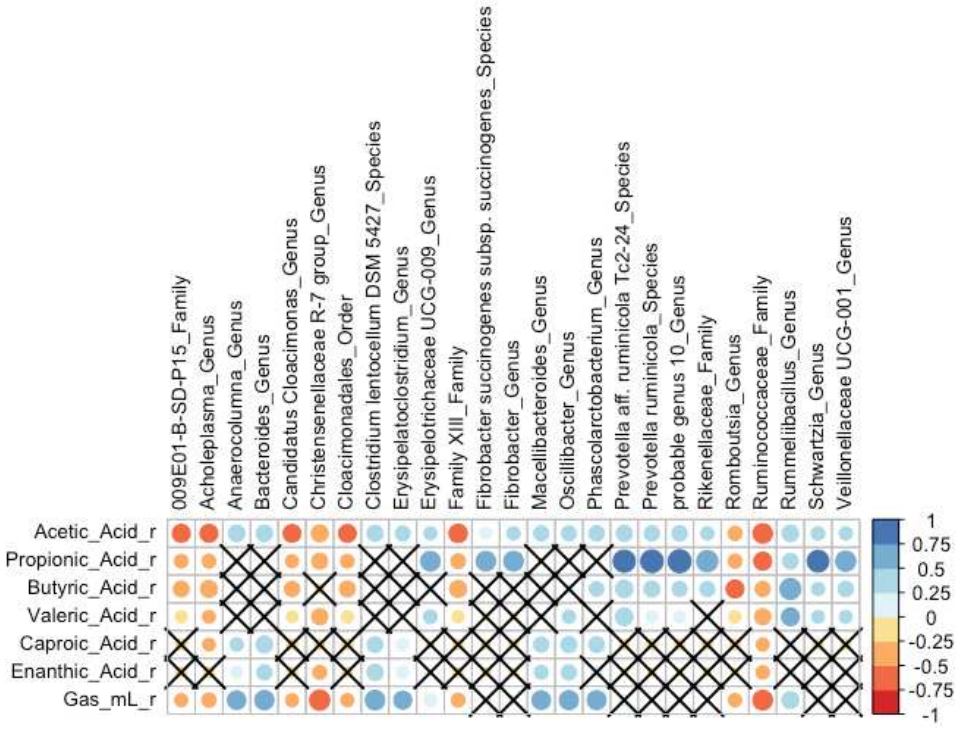


Figure 12. Pearson correlation analysis between SCFA concentrations, gas production and relevant taxonomic groups*.

*| r | > 0.5 for at least one correlation, p-value < 0.05 for at least one correlation). "X" represent correlations with p-values > 0.05.

5. DISCUSSION

Total SCFA production and composition were different between the three microbial inocula. Beef cattle rumen microbiome reactors yielded more total SCFAs, and propionic acid production was significantly higher. On the other hand, the rate of production of acetic acid was significantly higher in anaerobic sludge reactors (by day 5) (Section 4.1). This product was predominant in the spectrum profile (Figure 4). Since reactors operated under the same conditions, we attributed these differences to the effects of the microbial composition, which was different for each of the inoculum treatments. The three inoculum sources showed distinct microbial community structures since the beginning of the fermentation (Figure 7). These structures changed after cellulose addition and responses in the SCFA concentrations differed among the treatments (Inoculum / Cellulose addition) (Figure 5). The first changes in the microbial structure were observed by day 5 in fed reactors (Figure 7 and Figures 9 - 11); other variables including microbial growth (DNA) and gas production, also responded to these changes (between day 0 and day 5) and showed higher increasing rates (slopes) when compared to the rest of the experiment (day 10 - 20) (Figure S1 and 6). Since microbial communities were previously acclimated to their natural feeds (not pure cellulose), the observed changes can be attributed to the addition of cellulose to the fed reactors or the absence of a fed carbon source to the controls, as well as to reactor media components.

Not many published studies have explored the effects of pure cellulose on different microbial communities; however, different feedstock compositions and other operating variables have been studied for both SCFA productions and the microbial structure. Guo et al., (2015) investigated the effects of the carbon source composition from two cellulose-containing agricultural residues, straw and spent mushroom substrates (SMS, residues remaining in the soil

after mushroom harvest), on the production of SCFA using waste activated sludge as inoculum. This study concluded that straw resulted in higher SCFAs yield (1.2 times SMS) and was a more suitable substrate due to the higher cellulose content and particular carbon composition. Straw was composed by more than 50% cellulose; less than 3% protein and a C/N ratio greater than 80); on the other hand SMSs contained less than 50% cellulose, more than 4% protein and a C/N ratio less than 50.No iodoform addition was reported in the study, however, researchers reported shifts in the microbial structure due to the effects of the substrate conditioning. Carbohydrate-fermentation-related genera (*Clostridium IV*, *Xylanibacter*, and *Parabacteroides*) and the protein-fermentation-related genus *Lysinibacillus* were enriched by straw, while spent mushroom substrates enriched different fermentation genera (*Levilinea*, *Proteiniphilum*, and *Petrimonas*) (Z. Guo et al., 2015).

A study conducted by Wang et al., (2014) compared the SCFA productions from food waste under the effects of pH differences (pH 4 to 6) for two inocula, aerobic and anaerobic activated sludge. These fermentations were carried out under facultative anaerobic conditions (oxidation-reduction potential above −350 mV to inhibit methanogenesis), and differences in the SCFA spectra were reported by both the effects of pH and the inoculum source. Acetic and butyric acid were the dominant components of the product spectrum for all tested treatments. The concentration and SCFA yield were highest at pH 6.0 for anaerobic activated sludge reactors. Further, under this condition, acetic and butyric acids accounted for greater than 90% of the product spectrum. On the other hand, aerobic sludge reactors produced less total SCFA concentrations at pH 6.0; however, the spectrum showed higher proportions of propionic acid (around 15%). Although this study demonstrated how different microbial inocula have different responses under the same operating conditions, this work did not characterize microbial

communities, and their responses under the operational changes (pH). The microbial community composition for other waste activated sludge inoculum could be different and replication of these results could be challenging to achieve. On the other hand, it is still unclear why anaerobic sludge performed better in terms of SCFA yield than aerobic sludge under pH 6.0. Microbiome analysis could added evidence to answer these uncertainties (K. Wang et al., 2014).

Another study conducted by X. M. Li, Cheng, Selvam, & Wong, (2013) investigated the effects of three inoculum sources (domestic wastewater, aerobic sludge, and anaerobic sludge) under the same operating conditions on the acidogenic fermentation of food waste for bioelectricity production. This study reported that anaerobic sludge (as a co-inoculum with food waste leachate) provided the highest power output and attributed the better performance to the enrichment of both fermentative (*Clostridium sp.* and *Bacteroides sp.*) and electrogenic bacteria (*Magnetospirillum sp*. and *Geobacter sp.*) at the anode (X. M. Li, Cheng, Selvam, & Wong, 2013). This study utilized PCR-DGGE for the microbial structure characterization, which might not be accurate in characterizing species with low abundances. Furthermore, statistical correlations between the abundances of particular taxa and the reactor performance are not possible since the method is based on image based gel extraction approach, that do not characterize all the members of a community (Xie et al., 2016).

Results from the presented work demonstrated statistical correlations between the microbial structure and the SCFA product spectrum. Specific taxonomic groups were selectively enriched by the use of cellulose as a carbon source in each of the three microbial inocula (Section 4.2). The Pearson correlation analysis suggested potential candidates involved in influencing SCFA productions in their respective communities. The previously mentioned study conducted by X. M. Li, Cheng, Selvam, & Wong, (2013) attributed performance to the

enrichment of the fermentative taxa *Clostridium sp.* and *Bacteroides sp.*, found in their anaerobic sludge reactors. Interestingly, similar taxa, the species *Clostridium lentocellum DSM 5427* and the genus *Bacteroides*, were selectively enriched and dominated at relative abundances higher than 20% in our anaerobic sludge reactors (Figure 9). Furthermore, the abundance of these taxa positively correlated with acetic acid, caproic acid and enanthic acid production (Figure 12). Production of these acids might be related to the fermentation capabilities of these organisms.

The complete whole-genome sequence for *Cellulosilyticum lentocellum DSM 5427* (also known as *Clostridium lentocellum*) has been reported (Miller et al., 2011). This organism was characterized as a cellulose-degrading bacterium with potential features for biofuel production. Our taxonomic assignment (using the Silva v132 database) for the species *Clostridium lentocellum DSM 5427* reported confidence of 76%. However, further BLAST of the respective sequence with the NCBI database confirmed a 100% query cover and identity with the species *Cellulosilyticum lentocellum DSM 5427* (accession NR_074536.1). Discrepancy in the percentage confidence for the taxonomic between both databases could be attributed to the different algorithms used in each platform (QIIME vs NCBI BLAST website) and the publicly available information that each of the databases (Balvočiūtė & Huson, 2017). Ravinder, T. et al. (2001) reported one of the highest acetic acid fermentation yields (0.67 g/g cellulose) in monoculture fermentation for direct conversion of cellulose to acetic acid using a strain of *Clostridium lentocellum* identified as *Clostridium lentocellum SG6.* A very recent study performed by Zhang et al. (2019) isolated strain *Clostridium lentocellum Cel10* from giant panda (*Ailuropoda melanoleuca*) excrement and demonstrated the efficient conversion of cellulose to hydrogen; the authors suggested its potential use as a novel method for direct

hydrogen production from lignocellulosic materials (Ravinder, Swamy, Seenayya, & Reddy, 2001).

On the other hand, species from the genus *Bacteroides*, which also dominated in anaerobic sludge reactors and positively correlated with acetic acid production (Figures 9 and 12), have been known for their cellulolytic activity and cellulase producing capabilities. These enzymes metabolize cellulose into cellobiose and glucose and play a crucial role in the fermentation of lignocellulosic material (Giuliano & Khan, 1984). Our results suggest that the increased acetic acid production rate from cellulose in the anaerobic sludge reactors might be related to the activity of *C. lentocellum DSM 5427* and species of *Bacteroides.* These organisms could also establish syntrophic interactions due to the cellulolytic capabilities of *Bacteroides* for promoting beneficial growth for *Clostridium*. Murray (1986) studied the symbiotic relationship between *Bacteroides cellulosolvens* and *Clostridium saccharolyticum* using cellulose as a carbon source. The authors highlighted that *B. cellulosolvens* hydrolyzes cellulose and supplies *C. saccharolyticum* with sugars and a growth factor, the symbiotic relationship resulted in 33% more cellulose fermented than *B. cellulosolvens* alone (Murray, 1986).

In terms of performance, beef cattle reactor microbiomes yielded more total SCFAs and sCOD (Figures 5, S3. and S5). These reactors also produced less carbon dioxide, suggesting that the communities fermented cellulose more efficiently into SCFA or biomass instead of undesired gas (Figure 6). While anaerobic sludge might have been previously exposed to a different variety of substrates, not primarily composed of a high cellulose content, one could explain that the increased performance in beef cattle rumen reactors was due to the inoculum source's previous familiarity with cellulosic substrates, since the ruminal fluid was collected from a grass-fed beef cattle. However, this hypothesis does not explain why bison rumen (grass-fed) did not

performed as good as beef cattle rumen (in terms of total SCFA yield). Metabolism effects from the previous host animal for each of the ruminant microbial communities could also have a significant impact as well. Furthermore, the literature suggests that beef cattle rumen, as an inoculum source for bioprocess engineering, has the potential to achieve increased performance in the fermentation of lignocellulosic material, when compared to other inoculum sources. S. Wang et al., (2018) demonstrated that rumen fluid effectively enhanced hydrolysis and acidification of grass clippings, achieving high total SCFA concentrations (10.2 g/L) (S. Wang et al., 2018). The researchers attributed the performance to the presence of particular taxa (*Firmicutes* and *Fibrobacteres*) and enzymes (CMCase and cellobiase) during the *in vitro* rumen fluid fermentations. Another study compared the effects of six inoculum sources (digested dairy manure, digested swine manure, digested chicken manure, digested municipal sludge, anaerobic granular sludge, and paper mill sludge) on the anaerobic digestion of rice straw for biogas production. This study found the highest lignocellulose degradation in reactors inoculated with digested manures and attributed the result to the high cellulase and xylanase activity of beef cattle rumen related microorganisms contained in the digested manure inoculum (Gu, Chen, Liu, Zhou, & Zhang, 2014).

An interesting finding in our study was the higher production of propionic acid in beef cattle rumen fed reactors over the other treatments (Tables 3 and 4; Figure S7), and their relationships with the microbial structure (Figures 10 and 12). In our beef cattle fed reactors, the abundance of specific taxonomic groups dominated and was positively correlated with the production of propionic acid (Section 4.3). In particular, *P. ruminicola*, *F. succinogenes* and members of the family *Rikenellaceae* showed positive Pearson correlations with propionic acid production and dominated at relative abundances higher than 15%. Other studies on both *in vivo*

and *in vitro* rumen fluid fermentations also have attributed fermentation capabilities to these taxonomic groups (propionic acid production and hydrolysis of cellulosic material). Agematu, Takahashi, & Hamano, (2017) studied the SFCA production from lignocellulosic biomass by a "novel rumen-mimetic bioprocess" utilizing rumen fluid as a microbial inoculum; interestingly their study highlighted the fibrolytic capabilities of *P. ruminicola* and *F. succinogenes* in plant cell wall degradation and cellulose digestion (Agematu, Takahashi, & Hamano, 2017). The dominance of these two taxa (*P. ruminicola* and *F. succinogenes*) was also reported on dairy bovine (*in vivo* rumen fermentation); the study attributed fibrolytic capabilities to these taxa and referenced a study that identified *P. ruminicola* as a propionic acid producer (Strobel, 1992). Another recent investigation of *in situ* corn stover ruminal fermentation found a predominant abundance of the genus *Prevotella,* the researches attributed potential corn stover fermentations capabilities to this taxa (W. Guo, Guo, Zhu, Guo, & Zhou, 2019).

F. succinogenes has been identified as one of the most actively fibrolytic bacteria in the rumen ecosystem and a potential candidate for biofuel production (Burnet et al., 2015). This organism possesses a variety of fibrolytic enzymes and plays a fundamental role in the degradation of several substrates for the production of SCFA (KOIKE et al., 2004). Models to represent the cellulose degradation and ecological importance of *F. succinogenes* within the rumen ecosystem were evaluated by (Burnet et al., 2015)). Results from this modeling approach support other findings where *F. succinogenes* has been suggested to have important synergistic role in the rumen microbiota (Shinkai, Ueki, & Kobayashi, 2010). *F. succinogenes* produces xylanases (enzymes that metabolize xylan into xylose) and releases cellulolytic vesicles into the extracellular matrix. The activity of these enzymes can produce mono- and di-saccharides that

can be used by other microorganisms lacking cellulose-degrading capabilities, enabling their ability to grow in the presence of cellulose-containing feeds.

Members of the family *Rikenellaceae* are characteristic from the gastrointestinal tract of different animals (Rosenberg, DeLong, Lory, Stackebrandt, & Thompson, 2014). In the presented work, the family *Rikenellaceae* was positively correlated with propionic and acetic acid production. A particular sequence variant in this taxonomic group was highly abundant (150.374 reads in 44 samples) in beef cattle rumen fed reactors. BLAST of this sequence against the NCBI database gave undefined taxonomic lower assignments (at genus or species level). The presence of *Rikenellaceae* also has been reported in anaerobic digestion studies (Ozbayram, Akyol, Ince, Karakoç, & Ince, 2018; Zou, Xu, Li, Yang, & Zhang, 2018). However, the links between this taxonomic group and the production of specific fatty acids from cellulosic materials have not been established to our knowledge.

Negative correlations were observed for eight taxonomic groups (Figure 12). These trends could be attributed to contrast effects between the decreased relative abundances and the production of some SCFA. For example, relative abundances for *Acholeplasma***,** *Cloacimonas* and *009E01-B-SD-P15* decreased over time (from ~2% to less than 0.05%) in anaerobic sludge fed reactors. *Ruminococcaceae*, *Christensenellaceae R-7* and *Family* XIII were present in all reactor treatments at the beginning of the fermentations, however, the relative abundance of these groups decreased over time in fed reactors and increased or remained constant in controls. These results suggest that cellulose might not be an optimum substrate for the growth and microbial dominance of these taxa in the microbiome, however, their role could still be important in the production of SCFA. Studies have reported the presence of some of these taxonomic groups in anaerobic digestion studies. Furthermore, some of them have been associated potential

fermentation capabilities. *Ruminococcaceae* has been associated with fibrolytic capabilities and found in diverse gut microbial communities (Biddle, Stewart, Blanchard, & Leschine, 2013). A study reported positive correlations between *Ruminococcaceae* and butyric acid production, from selected dietary fibers and human feces. This study, suggested a potential role for *Ruminococcaceae* in butyrate production in the human colon (J. Yang, Martínez, Walter, Keshavarzian, & Rose, 2013). Another study, reported the complete genome sequence for a *Ruminococcaceae* strain and highlighted their potential capabilities for n-caproic acid production from lactate. Oher taxa such as *Candidatus Cloacimonas* and *Cloacimonadales* (also negatively correlated with certain SCFA) have been reported in anaerobic digestion systems for the production of methane biogas (Solli, Håvelsrud, Horn, & Rike, 2014) (J. Lee et al., 2018).

The production of other fatty acids such as butyric, valeric, caproic acid, and enanthic was positively correlated to the presence taxonomic groups listed in Figure 12. The genus *Veillonellaceae UCG-001* was related to butyric acid production in our work and on a very recent rumen fermentation study (Lu, Shen, & Shen, 2019). *Schwartzia* was strongly correlated with propionic acid production in our study, and a species from this genus has been reported as succinate utilizing rumen bacterium (Van Gylswyk, Hippe, & Rainey, 1997). *Anaerocolumna*, *Erysipelatoclostridium*, *Macellibacteroides* were positively correlated with acetic, caproic and enanthic acid production. Literature search on these taxonomic groups found associations with fermentation capabilities. Species from *Anaerocolumna* have been isolated from cattle waste methanogenic reactors, and associated with cellulolytic activity (Ueki, Ohtaki, Kaku, & Ueki, 2016). Han et al., (2018) found positives correlations between Erysipelatoclostridium and iso-valerate in a microbiome study on gut rats. In the present work, *Erysipelotrichaceae UCG-009* and *Oscillibacter* were positively correlated with valeric acid production. Similar taxonomic

groups were reported by other authors and associated with medium chain medium-chain carboxylate production (*Erysipelotrichaceae UCG-009*) and valeric acid production. (*Oscillibacter valericigenes*) (Lambrecht et al., 2019) (Iino, Mori, Tanaka, Suzuki, & Harayama, 2007). *Rummeliibacillus* was strongly correlated with valeric and caproic acid production (Figure 12) this genus has been associated with potential fermentation capabilities for biohydrogen production (G. Yang, Hu, & Wang, 2019).

Further research is required to elucidate the mechanistic underpinnings of these correlative relationships found in our work. A multi-omics approach at the metagenome, metatranscriptome, and metaproteome level could, for example, provide more evidence to correlate microbial structure with specific functions. The activity of specific genes and enzymes might be crucial to expand our current knowledge about the pathways governing the fermentation of cellulosic material when methanogenesis is inhibited. The identification of these pathways, and their connection with the microbial structure, is expected to facilitate the selection of novel strains for bioaugmentation strategies (which are currently limited). On the other hand, multi-omics and process performance data coupled with machine learning algorithms could also facilitate the development of prediction and diagnosis tools in AD systems (in the context of the carboxylate platform). Prediction and diagnosis for success or failure in the production of a particular fatty acid, given the genomic potential of a specific microbial community is a promising opportunity. Nevertheless, the robustness and accuracy of this advanced tools depends on the availability of enough data, which is also currently limited. On the other hand, the identification of genes associated with synthesis of particular fatty acids could also serve for designing other microbiome manipulation strategies such as genetic improvement through horizontal gene transfer (Smillie et al., 2011). By expanding our understanding of the

microbiome, new technological improvements could be designed and evaluated to optimize and scale-up alternative bioprocessing approaches, an outlook infographic summarizing this approach is presented in figure 13 as a concluding remark for this work.

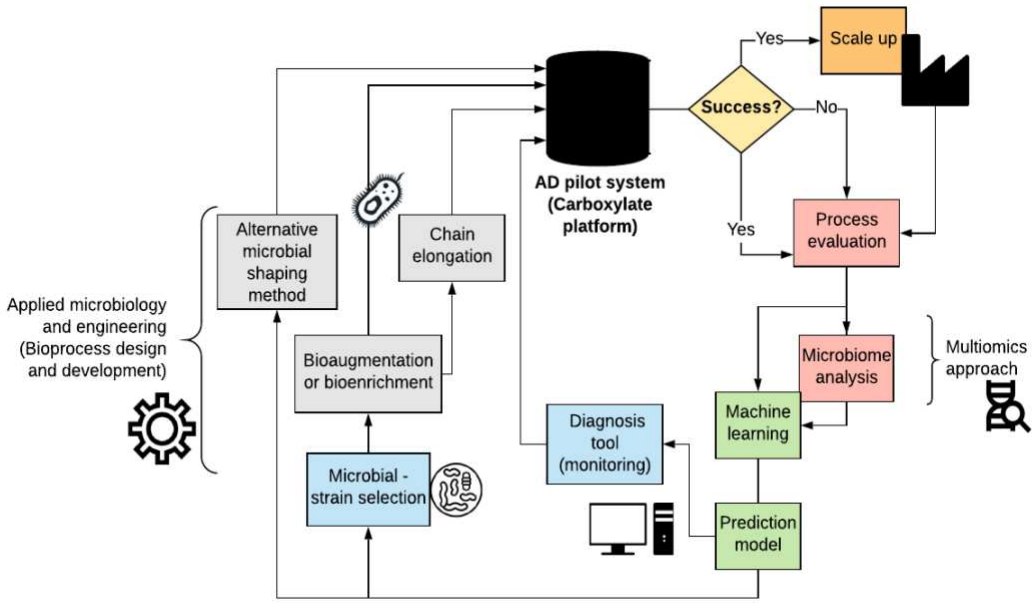


Figure 13. Application of microbiome analysis in the development context of the carboxylate platform.

7. CONCLUSIONS

The first objective of this study was to investigate relationships between production of SFCA and the microbial composition for three microbial inoculum sources. Results from the present work suggests that the microbial community composition strongly influences responses in the production of SCFA from cellulose. Total SCFA production was higher in beef cattle rumen reactors; these communities also produced more propionic acid. Alternatively, higher production rates of acetic acid were found in anaerobic sludge reactors. Butyric, valeric, caproic and enanthic acid production also differed among the treatments; however, concentrations and differences were lower in magnitude when compared to acetic and propionic acid. Since reactors operated under the same conditions, we attributed these differences due to the effects of the microbial composition, which differed among inoculum sources over time and due to the use of cellulose as a carbon source.

The second objective of this study was to identify taxonomic groups associated with the production of particular SCFA. Findings in this work suggest that specific microorganisms were selectively enriched by the use of cellulose as a carbon source within each of the microbial communities. The Pearson correlation analysis identified potential candidates involved in the synthesis of certain SCFA. The further literature review confirmed the fermentative capabilities of these organisms and their possible role in the microbial ecosystem. The species *Clostridium lentocellum DSM 5427* and the genus *Bacteroides* were selectively enriched and dominated in anaerobic sludge fed reactors. The abundance of these taxa positively correlated with acetic acid, caproic acid and enanthic acid production. Our results and the literature reports suggest that the production of acetic acid in fed anaerobic sludge reactors might be attributed to the cellulolytic capabilities of *C. lentocellum DSM 5427* and *Bacteroides.* On the other hand, propionic acid

production was strongly related with abundance of *P. ruminicola, F. succinogenes* and members of the Family *Rikenellaceae*. These taxonomic groups have been found primarily in rumen fluids and literature reported have attributed fermentation capabilities to these taxonomic groups (propionic acid production and hydrolysis of cellulosic material). In particular, *P. ruminicola* has been identified as a propionic acid producer and *F. succinogenes* as one of the most actively fibrolytic bacteria in the rumen microflora.

This study established relationships between the microbial structure and the production of SCFA within the context of the carboxylate platform. Results from this work confirmed previous findings in the literature and provided insights for a better understanding of these complex systems. Here further investigations at the molecular level (metagenome, metatranscriptome, and metaproteome) are suggested to expand current knowledge and better understand the biological factors that dictate the fermentation of cellulosic material when methanogenesis is inhibited. By expanding this understanding, microbiome shaping methods could be designed and evaluated to optimize and scale-up alternative bioprocessing approaches.

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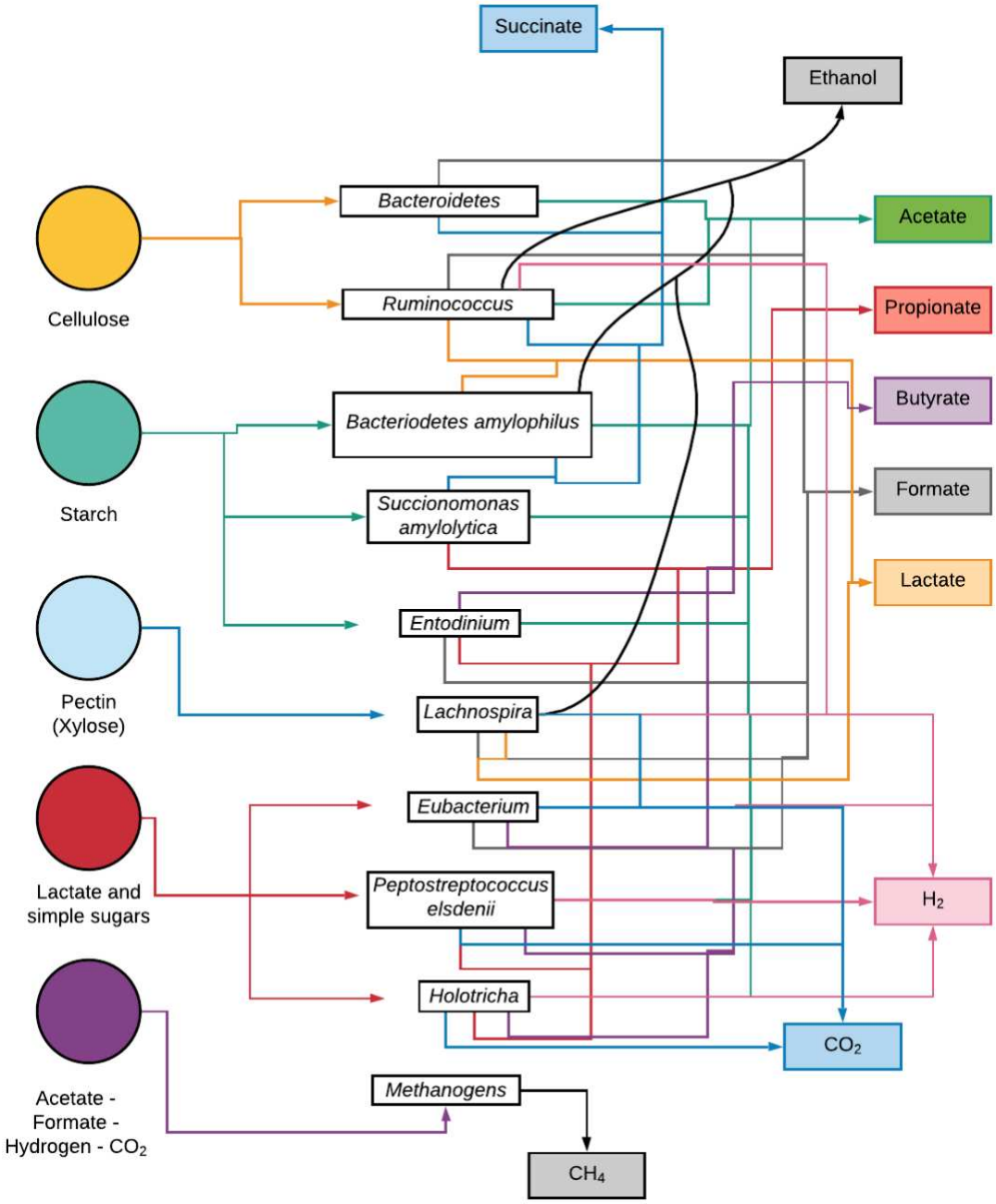
APPENDICES

Appendix A. Substrate and product specificity in some ruminant microbial species (Czerkawski, 2013)**.**



Appendix B. Supplementary figures for SCFA production and DNA (microbial growth).

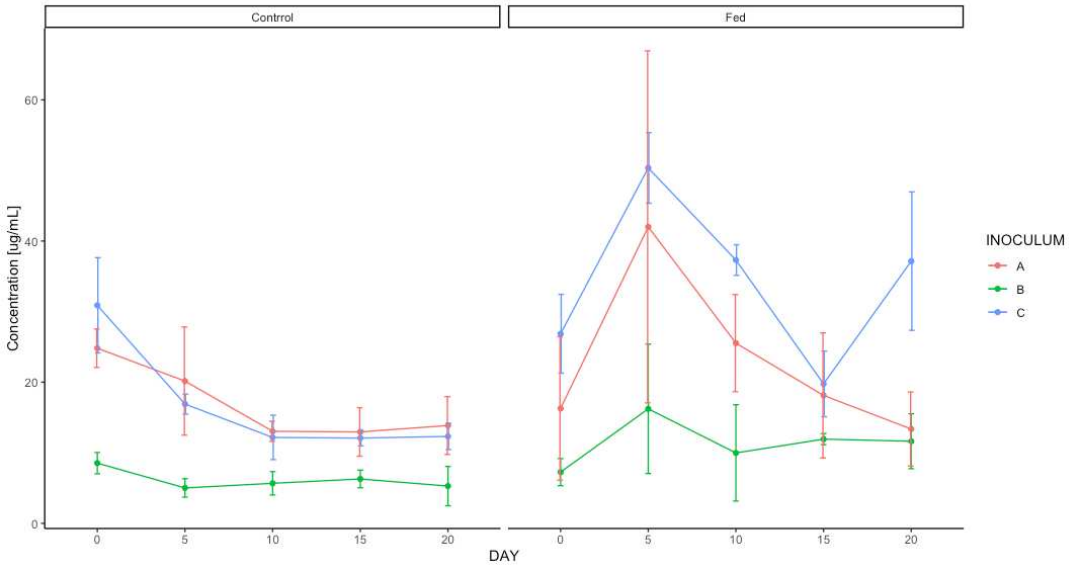


Figure S 1. DNA Concentrations (biomass growth).

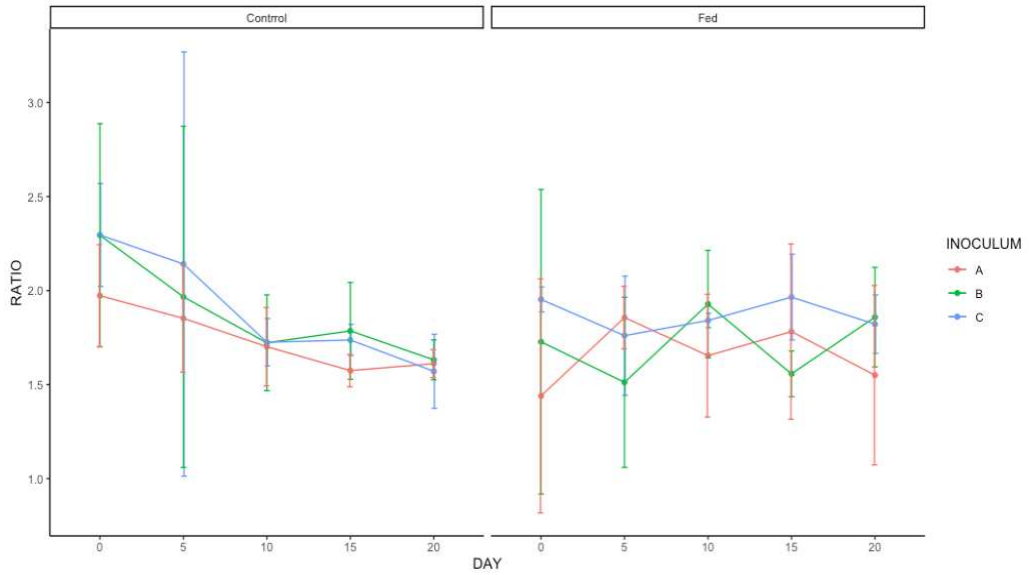


Figure S 2. DNA quality ratios.

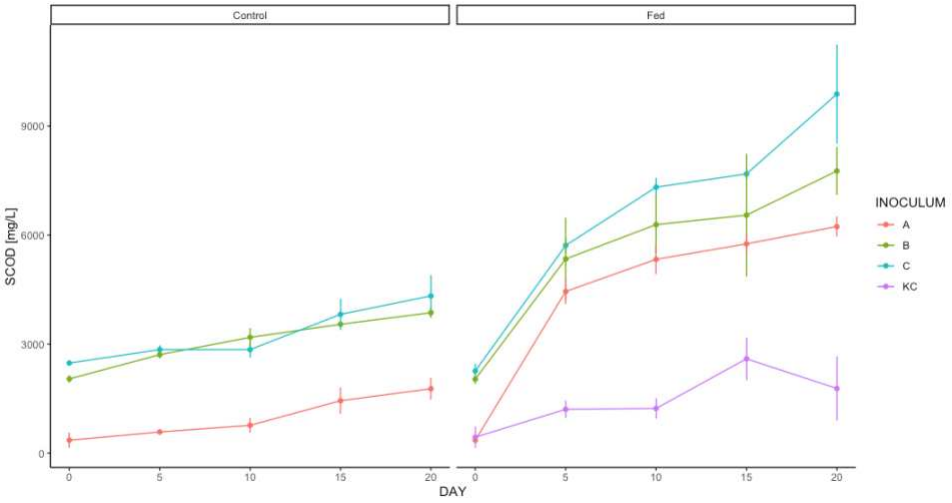


Figure S 3. Soluble COD (sCOD).

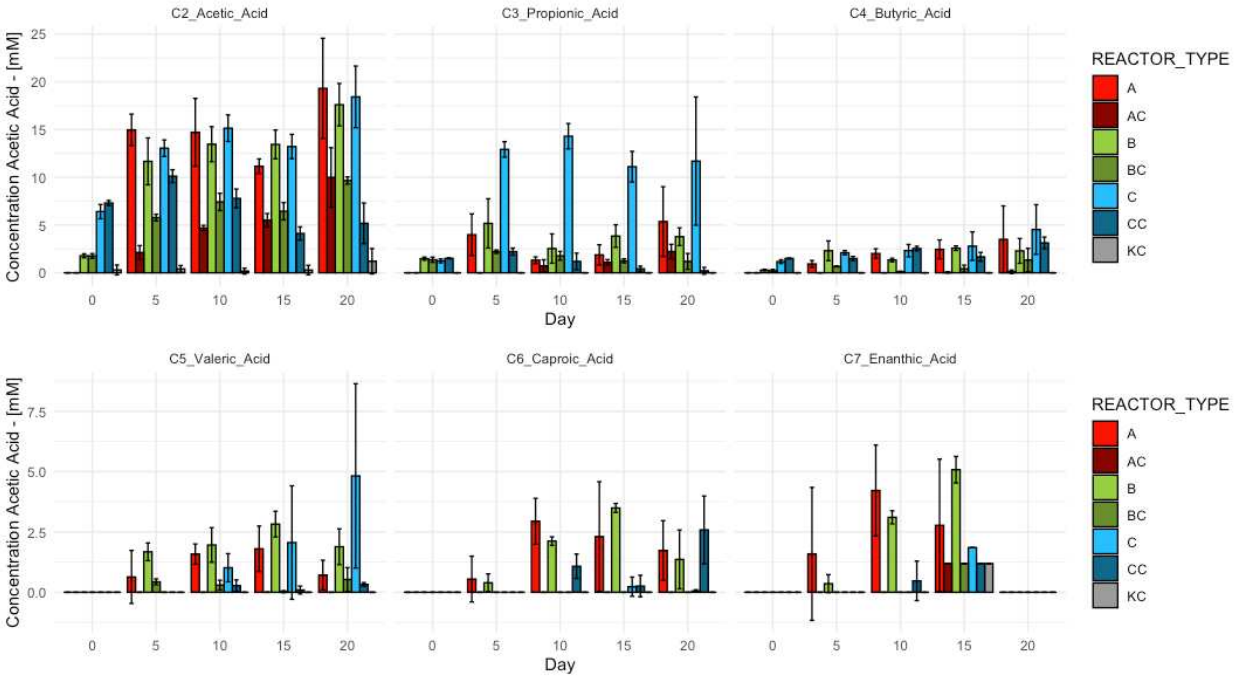


Figure S 4. SCFA production normalized in terms of Acetic Acid.

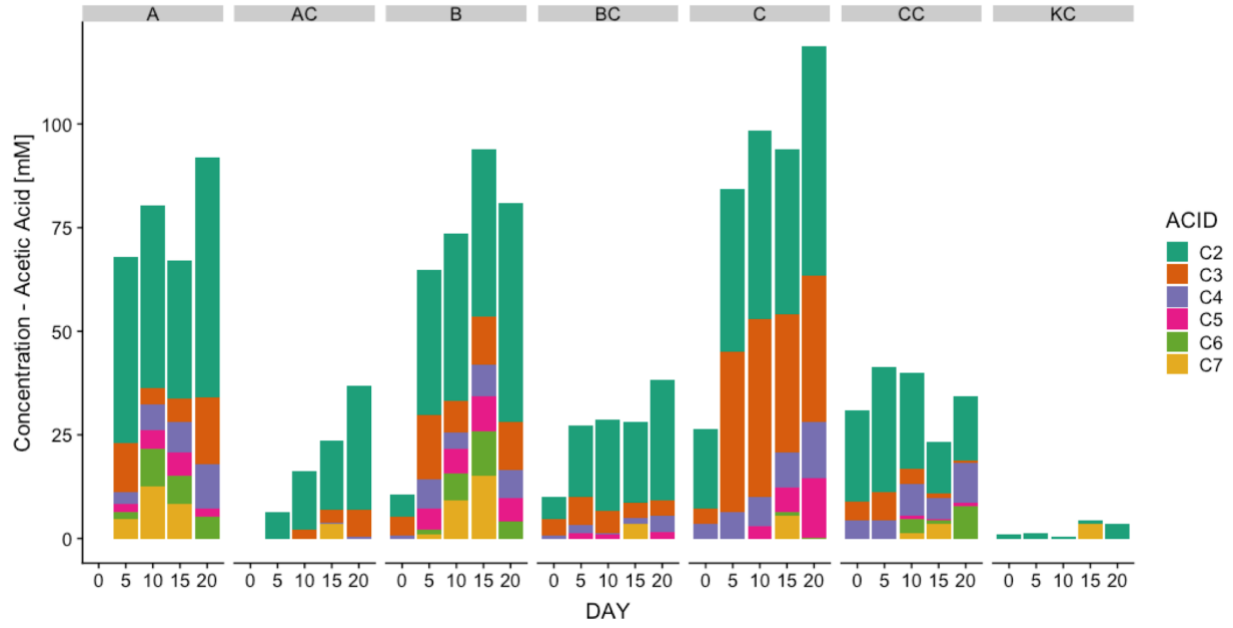


Figure S 5. Stacked bar plot representing total SCFA concentrations and composition.

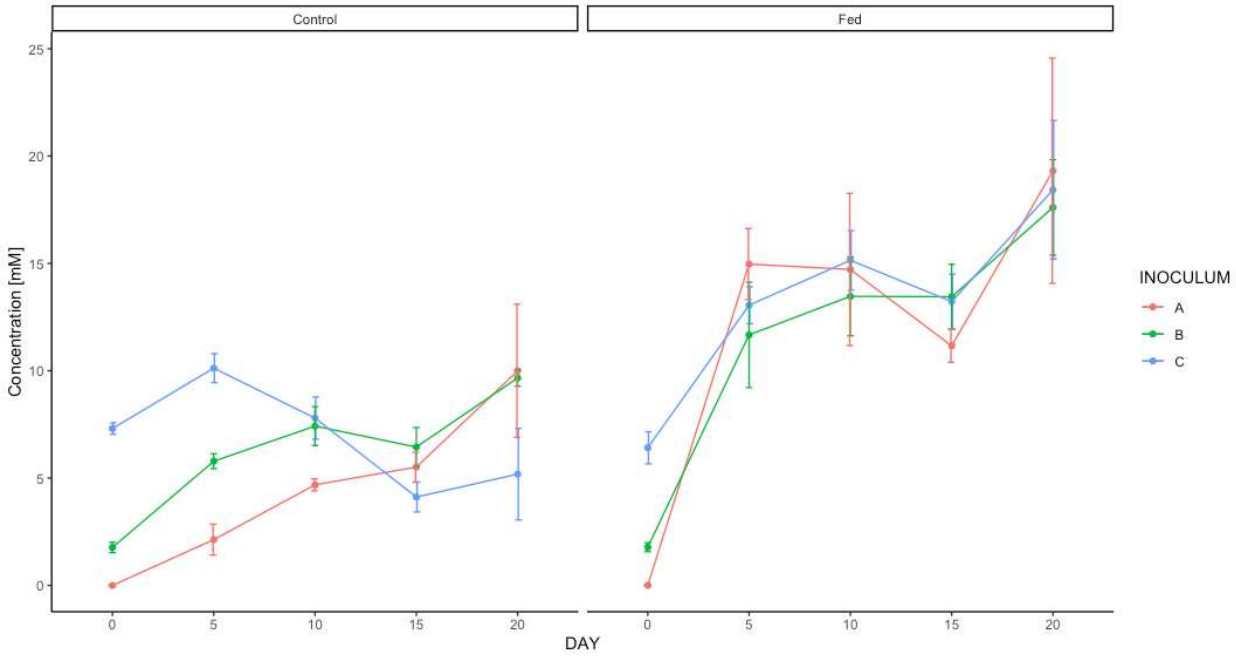


Figure S 6. Acetic acid production.

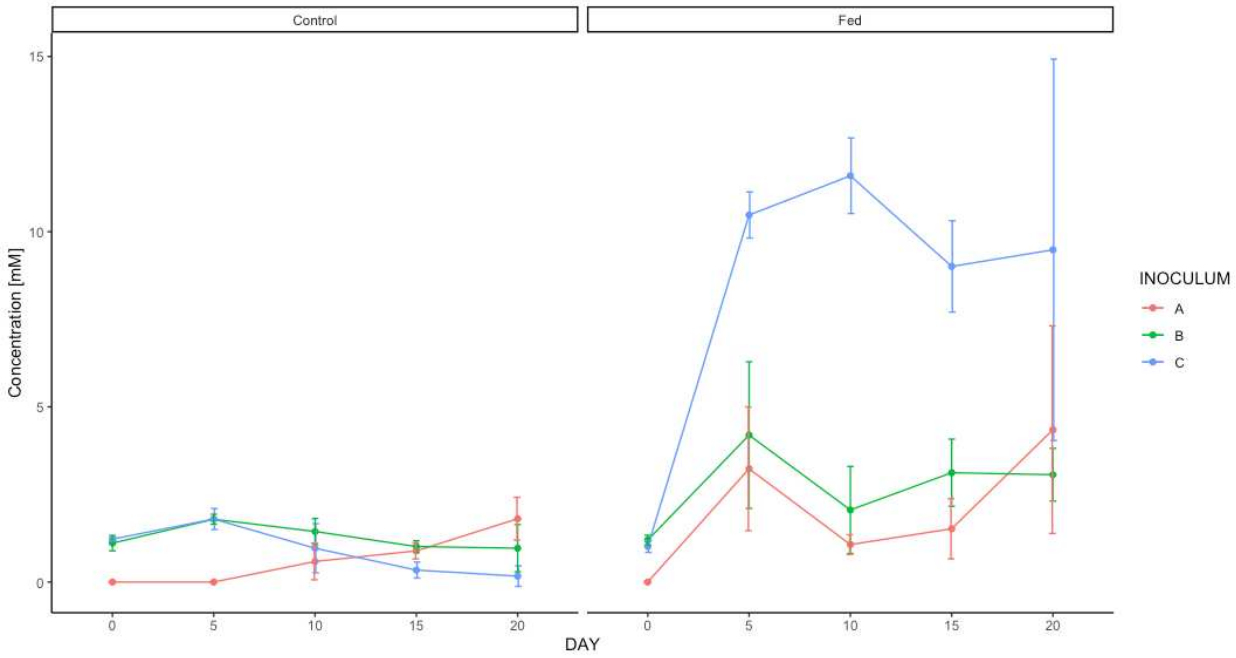


Figure S 7. Propionic Acid production.

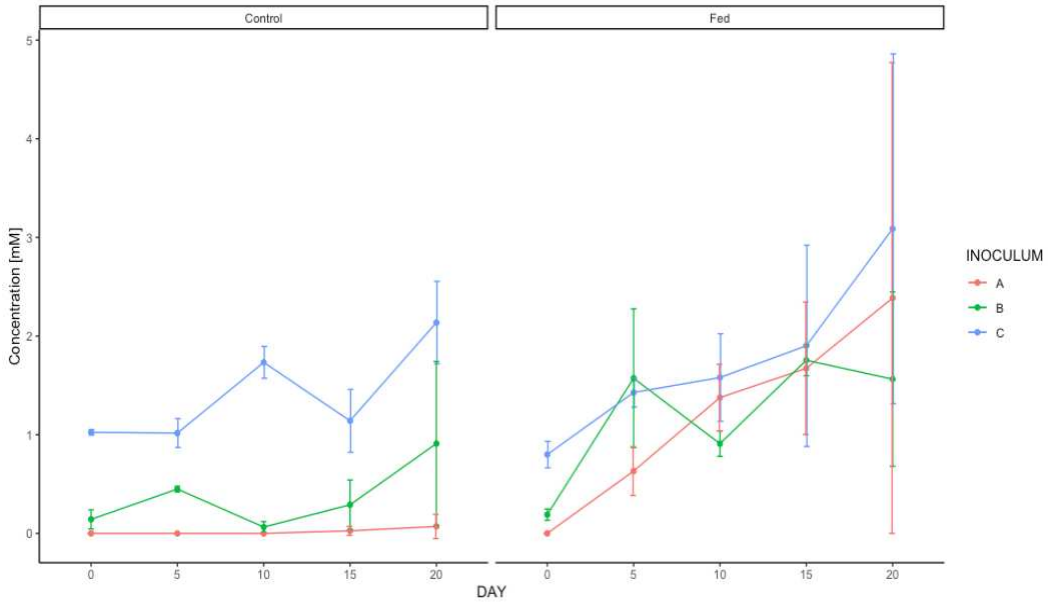


Figure S 8. Butyric Acid production.

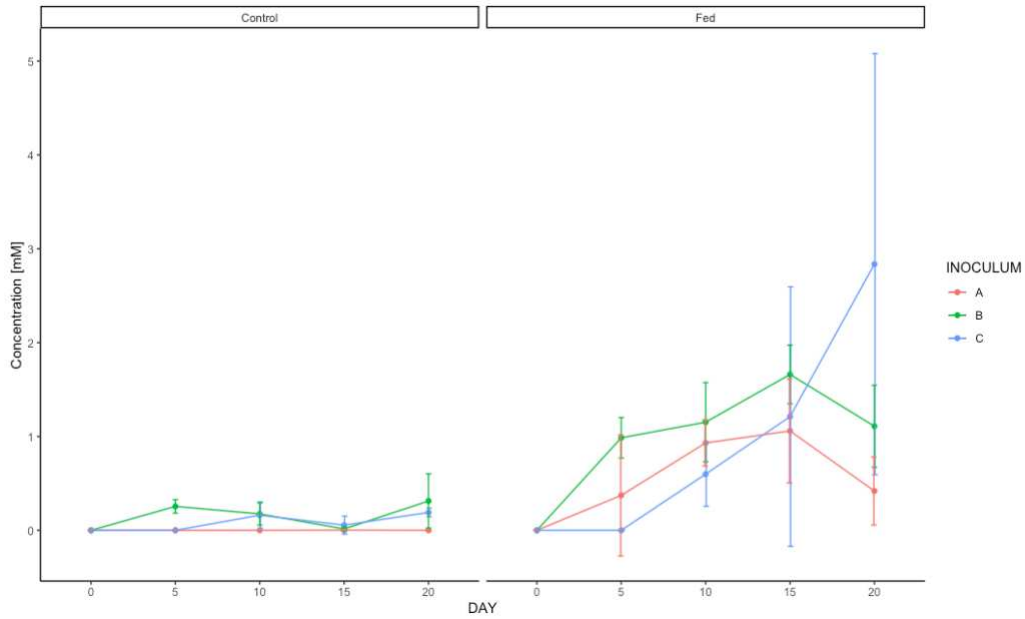


Figure S 9. Valeric Acid production.

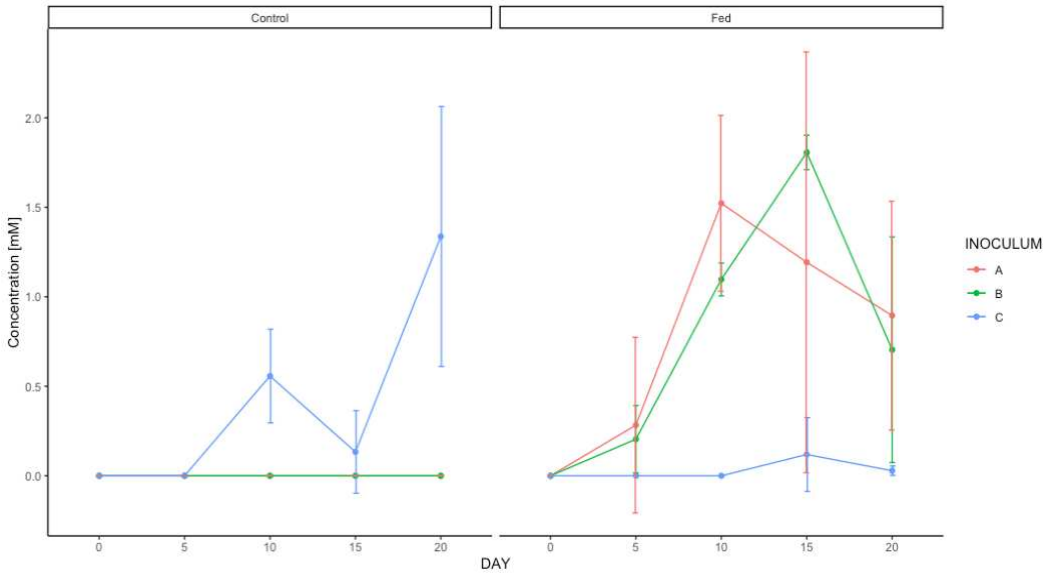


Figure S 10. Caproic Acid production.

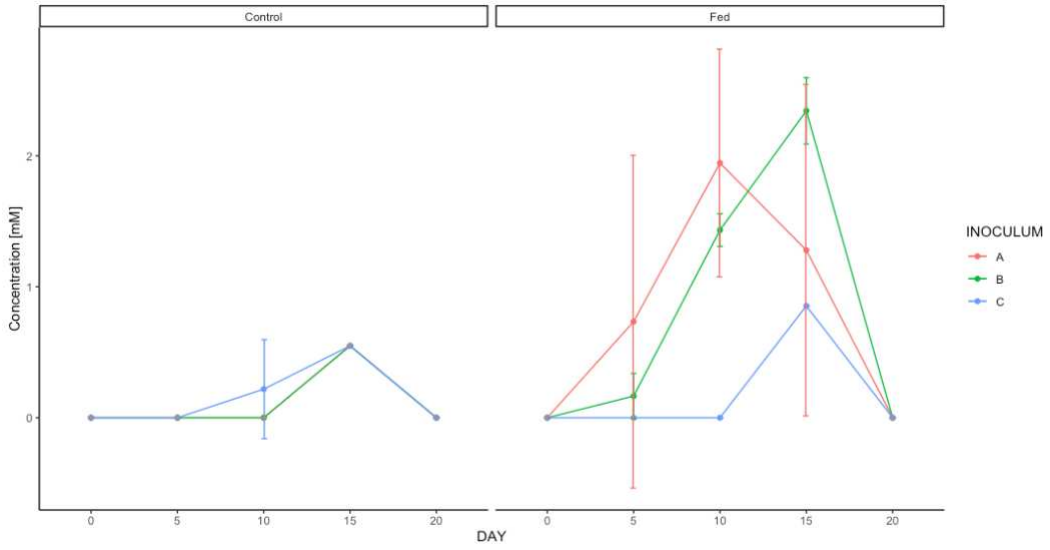


Figure S 11. Enanthic Acid production

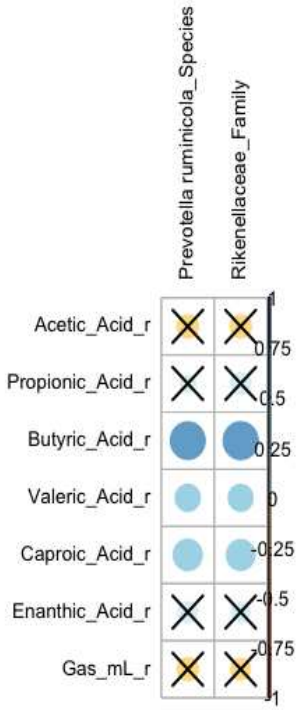


Figure S 12. Pearson correlation analysis between SCFA proportions, gas production and relevant taxonomic groups*.

*| r | > 0.5 for at least one correlation, p-value < 0.05 for at least one correlation). “X” represents correlations with p-values > 0.05.

Appendix C. Recipe for nutrient solution from (Owen et al., 1979)

Solution	Compound	Concentration [g/L]
S2	Resazurin	1
S3	$(NH_4)2HPO_4$	26.7
S4	$CaCl_2 – 2H_2O$	16.7
	NH_4Cl	26.6
	$MgCl_2 – 6H_2O$	120
	KCl	86.7
	$MnCl_2 – 4H_2O$	1.33
	$CoCl2 – 6H_2O$	2
	H_3BO_3	0.38
	$CuCl_2 – 2H_2O$	0.18
	$Na2MoO_4 – 2H_2O$	0.17
	$ZnCl_2$	0.14
S5	$FeCl_2 – 4H_2O$	370
S6	$Na2S – 9H_2O$	500
S7	Biotin	0.002
	Folic Acid	0.002
	Pyridoxine hydrochloride	0.01
	Riboflavin	0.005
	Thiamin	0.005
	Nicotinic acid	0.005
	Pantothenic acid	0.005
	B_{12}	0.0001
	p-aminobenzoic acid	0.005
	Thioctic acid	0.005

Defined Media Preparation adapted from (Owen et al., 1979)

1. Add one liter of deionized water to a two-liter volumetric flask.
2. Add the following:
    a. 1.8 ml S2
    b. 5.4 ml S3
    c. 27 ml S4
3. Add deionized water up to the 1800 ml mark.
4. Boil for 15 minutes while flushing with nitrogen gas at approximately 1L/min.
5. Cool to room temperature while continuing to flush with nitrogen gas.
6. Add the following:
    a. 18 ml S7
    b. 1.8 ml S5
    c. 1.8 ml S6
7. Add 8.40g $NaHCO_3$ as powder.
8. Bubble the CO_2: N_2 gas mixture until media pH stabilizes at approximately 7.1.
9. Carefully seal volumetric flask while minimizing the introduction of air into the container.

Appendix D. Pairwise comparison between SCFA production rates.

Day 0 - 5 (Fed Reactors)

```
      > #C2 ACETIC ACID
> summary (FullModelC2_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C2_Yes)

Residuals:
    Min      1Q  Median      3Q     Max
-2.8180 -0.2582  0.0000  0.7468  1.6797

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)    1.675e-15  7.495e-01   0.000 1.000000
DAY            2.994e+00  2.120e-01  14.121 7.74e-09 ***
INOCULUMB      1.780e+00  1.060e+00   1.679 0.118918
INOCULUMC      6.411e+00  1.060e+00   6.048 5.78e-05 ***
DAY:INOCULUMB -1.015e+00  2.998e-01  -3.387 0.005398 **
DAY:INOCULUMC -1.666e+00  2.998e-01  -5.556 0.000125 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 1.298 on 12 degrees of freedom
Multiple R-squared:  0.9662,  Adjusted R-squared:  0.9521
F-statistic: 68.62 on 5 and 12 DF,  p-value: 2.09e-08

> emtrends(FullModelC2_Yes, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend    SE df lower.CL upper.CL
 A             2.99 0.212 12    2.532     3.46
 B             1.98 0.212 12    1.516     2.44
 C             1.33 0.212 12    0.866     1.79

Confidence level used: 0.95

$contrasts
 contrast estimate  SE df t.ratio p.value
 A - B        1.02 0.3 12 3.387   0.0138
 A - C        1.67 0.3 12 5.556   0.0003
 B - C        0.65 0.3 12 2.169   0.1175

P value adjustment: tukey method for comparing a family of 3 estimates

>
      > #C3 PROPIONIC ACID
> summary(FullModelC3_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C3_Yes)

Residuals:
    Min      1Q  Median      3Q     Max
```

```
-2.2574 -0.1379  0.0000  0.3344  1.8757

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)   -2.093e-15  6.655e-01   0.000 1.000000
DAY            6.468e-01  1.882e-01   3.436 0.004933 **
INOCULUMB      1.198e+00  9.412e-01   1.273 0.227097
INOCULUMC      1.016e+00  9.412e-01   1.079 0.301753
DAY:INOCULUMB -4.772e-02  2.662e-01  -0.179 0.860730
DAY:INOCULUMC  1.245e+00  2.662e-01   4.677 0.000535 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 1.153 on 12 degrees of freedom
Multiple R-squared:  0.932,   Adjusted R-squared:  0.9036
F-statistic: 32.87 on 5 and 12 DF,  p-value: 1.33e-06

> emtrends(FullModelC3_Yes, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend    SE df lower.CL upper.CL
 A            0.647 0.188 12    0.237     1.06
 B            0.599 0.188 12    0.189     1.01
 C            1.892 0.188 12    1.482     2.30

Confidence level used: 0.95

$contrasts
 contrast estimate    SE df t.ratio p.value
 A - B      0.0477 0.266 12  0.179  0.9825
 A - C     -1.2452 0.266 12 -4.677  0.0014
 B - C     -1.2929 0.266 12 -4.857  0.0011

P value adjustment: tukey method for comparing a family of 3 estimates

>
      > #C4 BUTYTIC ACID
> summary(FullModelC4_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C4_Yes)

Residuals:
     Min       1Q   Median       3Q      Max
-0.78313 -0.05055  0.00000  0.14416  0.57808

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)   -4.187e-16  1.824e-01   0.000  1.00000
DAY            1.264e-01  5.159e-02   2.449  0.03062 *
INOCULUMB      1.899e-01  2.580e-01   0.736  0.47573
INOCULUMC      7.989e-01  2.580e-01   3.097  0.00925 **
DAY:INOCULUMB  1.502e-01  7.297e-02   2.058  0.06194 .
DAY:INOCULUMC -5.852e-04  7.297e-02  -0.008  0.99373
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.316 on 12 degrees of freedom
```

```
Multiple R-squared:  0.8354,  Adjusted R-squared:  0.7668
F-statistic: 12.18 on 5 and 12 DF,  p-value: 0.0002322

> emtrends(FullModelC4_Yes, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A            0.126 0.0516 12   0.0140    0.239
 B            0.277 0.0516 12   0.1642    0.389
 C            0.126 0.0516 12   0.0134    0.238

Confidence level used: 0.95

$contrasts
 contrast  estimate    SE df t.ratio p.value
 A - B    -0.150200 0.073 12 -2.058  0.1407
 A - C     0.000585 0.073 12  0.008  1.0000
 B - C     0.150785 0.073 12  2.067  0.1389

P value adjustment: tukey method for comparing a family of 3 estimates

>
      > #C5 VALERIC ACID
> summary(FullModelC5_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C5_Yes)

Residuals:
    Min      1Q  Median      3Q     Max
-0.3727  0.0000  0.0000  0.0000  0.7455

Coefficients:
               Estimate Std. Error t value Pr(>|t|)
(Intercept)   -4.449e-16  1.604e-01   0.000   1.0000
DAY            7.455e-02  4.537e-02   1.643   0.1263
INOCULUMB      4.029e-16  2.268e-01   0.000   1.0000
INOCULUMC      3.616e-16  2.268e-01   0.000   1.0000
DAY:INOCULUMB  1.226e-01  6.416e-02   1.912   0.0801 .
DAY:INOCULUMC -7.455e-02  6.416e-02  -1.162   0.2679
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.2778 on 12 degrees of freedom
Multiple R-squared:  0.7224,  Adjusted R-squared:  0.6067
F-statistic: 6.245 on 5 and 12 DF,  p-value: 0.004469

> emtrends(FullModelC5_Yes, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A           0.0745 0.0454 12  -0.0243   0.1734
 B           0.1972 0.0454 12   0.0983   0.2960
 C           0.0000 0.0454 12  -0.0989   0.0989

Confidence level used: 0.95

$contrasts
 contrast estimate     SE df t.ratio p.value
```

```
 A - B     -0.1226 0.0642 12 -1.912  0.1778
 A - C      0.0745 0.0642 12  1.162  0.4967
 B - C      0.1972 0.0642 12  3.073  0.0243

P value adjustment: tukey method for comparing a family of 3 estimates

>
      > #C6 CAPROIC ACID
> summary(FullModelC6_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C6_Yes)

Residuals:
    Min      1Q  Median      3Q     Max
-0.2832  0.0000  0.0000  0.0000  0.5663

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)   -5.234e-17  1.238e-01   0.000    1.000
DAY            5.663e-02  3.502e-02   1.617    0.132
INOCULUMB      6.119e-17  1.751e-01   0.000    1.000
INOCULUMC      3.912e-17  1.751e-01   0.000    1.000
DAY:INOCULUMB -1.575e-02  4.953e-02  -0.318    0.756
DAY:INOCULUMC -5.663e-02  4.953e-02  -1.143    0.275

Residual standard error: 0.2145 on 12 degrees of freedom
Multiple R-squared:  0.3092,  Adjusted R-squared:  0.02132
F-statistic: 1.074 on 5 and 12 DF,  p-value: 0.4218

> emtrends(FullModelC6_Yes, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend    SE df lower.CL upper.CL
 A           0.0566 0.035 12  -0.0197   0.1329
 B           0.0409 0.035 12  -0.0354   0.1172
 C           0.0000 0.035 12  -0.0763   0.0763

Confidence level used: 0.95

$contrasts
 contrast estimate     SE df t.ratio p.value
 A - B      0.0158 0.0495 12 0.318   0.9460
 A - C      0.0566 0.0495 12 1.143   0.5072
 B - C      0.0409 0.0495 12 0.825   0.6951

P value adjustment: tukey method for comparing a family of 3 estimates

>
      > #C7 ENANTHIC ACID
> summary(FullModelC7_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C7_Yes)

Residuals:
    Min      1Q  Median      3Q     Max
-0.7335  0.0000  0.0000  0.0000  1.4669
```

```
Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)   -2.617e-16  3.022e-01   0.000    1.000
DAY            1.467e-01  8.548e-02   1.716    0.112
INOCULUMB      2.499e-16  4.274e-01   0.000    1.000
INOCULUMC      4.997e-16  4.274e-01   0.000    1.000
DAY:INOCULUMB -1.137e-01  1.209e-01  -0.941    0.365
DAY:INOCULUMC -1.467e-01  1.209e-01  -1.214    0.248

Residual standard error: 0.5234 on 12 degrees of freedom
Multiple R-squared:  0.2821,  Adjusted R-squared:  -0.01702
F-statistic: 0.9431 on 5 and 12 DF,  p-value: 0.4881

> emtrends(FullModelC7_Yes, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A            0.147 0.0855 12  -0.0395    0.333
 B            0.033 0.0855 12  -0.1533    0.219
 C            0.000 0.0855 12  -0.1862    0.186

Confidence level used: 0.95

$contrasts
 contrast estimate    SE df t.ratio p.value
 A - B       0.114 0.121 12 0.941   0.6261
 A - C       0.147 0.121 12 1.214   0.4680
 B - C       0.033 0.121 12 0.273   0.9600

P value adjustment: tukey method for comparing a family of 3 estimates
```

Day 5 - 10 (Fed Reactors)

```
> #C2 ACETIC ACID
> summary(FullModelC2_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C2_Yes)

Residuals:
    Min      1Q  Median      3Q     Max
-4.0060 -1.3724  0.4809  1.2336  2.7406

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)   15.21971    2.75827   5.518 0.000132 ***
DAY           -0.05035    0.34890  -0.144 0.887646
INOCULUMB     -5.34313    3.90078  -1.370 0.195848
INOCULUMC     -4.26234    3.90078  -1.093 0.295981
DAY:INOCULUMB  0.40919    0.49341   0.829 0.423117
DAY:INOCULUMC  0.46889    0.49341   0.950 0.360713
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 2.137 on 12 degrees of freedom
```

```
Multiple R-squared:  0.3352,  Adjusted R-squared:  0.05822
F-statistic:  1.21 on 5 and 12 DF,  p-value: 0.3618

> emtrends(FullModelC2_Yes, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend    SE df lower.CL upper.CL
 A          -0.0504 0.349 12   -0.811     0.71
 B           0.3588 0.349 12   -0.401     1.12
 C           0.4185 0.349 12   -0.342     1.18

Confidence level used: 0.95

$contrasts
 contrast estimate    SE df t.ratio p.value
 A - B     -0.4092 0.493 12 -0.829  0.6927
 A - C     -0.4689 0.493 12 -0.950  0.6204
 B - C     -0.0597 0.493 12 -0.121  0.9920

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C3 PROPIONIC ACID
> summary(FullModelC3_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C3_Yes)

Residuals:
    Min      1Q  Median      3Q     Max
-2.2574 -0.4042  0.1643  0.8072  1.8757

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)    5.398331   1.724857   3.130   0.0087 **
DAY           -0.432897   0.218179  -1.984   0.0706 .
INOCULUMB      0.930673   2.439316   0.382   0.7095
INOCULUMC      3.956972   2.439316   1.622   0.1307
DAY:INOCULUMB  0.005798   0.308552   0.019   0.9853
DAY:INOCULUMC  0.656948   0.308552   2.129   0.0546 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 1.336 on 12 degrees of freedom
Multiple R-squared:  0.9335,  Adjusted R-squared:  0.9058
F-statistic: 33.68 on 5 and 12 DF,  p-value: 1.163e-06

> emtrends(FullModelC3_Yes, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend    SE df lower.CL upper.CL
 A          -0.433 0.218 12   -0.908   0.0425
 B          -0.427 0.218 12   -0.902   0.0483
 C           0.224 0.218 12   -0.251   0.6994

Confidence level used: 0.95

$contrasts
 contrast estimate    SE df t.ratio p.value
```

```
 A - B     -0.0058 0.309 12 -0.019  0.9998
 A - C     -0.6569 0.309 12 -2.129  0.1254
 B - C     -0.6512 0.309 12 -2.110  0.1293

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C4 BUTYTIC ACID
> summary(FullModelC4_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C4_Yes)

Residuals:
    Min      1Q  Median      3Q     Max
-0.7831 -0.1384  0.1047  0.1911  0.5781

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)   -0.11272    0.50145  -0.225  0.82592
DAY            0.14893    0.06343   2.348  0.03685 *
INOCULUMB      2.34858    0.70915   3.312  0.00620 **
INOCULUMC      1.38849    0.70915   1.958  0.07390 .
DAY:INOCULUMB -0.28153    0.08970  -3.139  0.00856 **
DAY:INOCULUMC -0.11851    0.08970  -1.321  0.21110
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.3884 on 12 degrees of freedom
Multiple R-squared:  0.5569,  Adjusted R-squared:  0.3723
F-statistic: 3.017 on 5 and 12 DF,  p-value: 0.05435

> emtrends(FullModelC4_Yes, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A           0.1489 0.0634 12   0.0107  0.28712
 B          -0.1326 0.0634 12  -0.2708  0.00559
 C           0.0304 0.0634 12  -0.1078  0.16862

Confidence level used: 0.95

$contrasts
 contrast estimate     SE df t.ratio p.value
 A - B       0.282 0.0897 12  3.139  0.0216
 A - C       0.119 0.0897 12  1.321  0.4109
 B - C      -0.163 0.0897 12 -1.817  0.2056

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C5 VALERIC ACID
> summary(FullModelC5_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C5_Yes)

Residuals:
```

```
    Min      1Q  Median      3Q     Max
-0.3928 -0.2623  0.0000  0.1883  0.7455

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)   -0.185954   0.477195  -0.390   0.7036
DAY            0.111739   0.060361   1.851   0.0889 .
INOCULUMB      1.005318   0.674856   1.490   0.1621
INOCULUMC     -0.412637   0.674856  -0.611   0.5523
DAY:INOCULUMB -0.078416   0.085363  -0.919   0.3764
DAY:INOCULUMC  0.007979   0.085363   0.093   0.9271
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.3696 on 12 degrees of freedom
Multiple R-squared:  0.6332,  Adjusted R-squared:  0.4803
F-statistic: 4.143 on 5 and 12 DF,  p-value: 0.02031

> emtrends(FullModelC5_Yes, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A           0.1117 0.0604 12  -0.0198    0.243
 B           0.0333 0.0604 12  -0.0982    0.165
 C           0.1197 0.0604 12  -0.0118    0.251

Confidence level used: 0.95

$contrasts
 contrast estimate     SE df t.ratio p.value
 A - B     0.07842 0.0854 12  0.919  0.6394
 A - C    -0.00798 0.0854 12 -0.093  0.9952
 B - C    -0.08640 0.0854 12 -1.012  0.5836

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C6 CAPROIC ACID
> summary(FullModelC6_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C6_Yes)

Residuals:
     Min       1Q   Median       3Q      Max
-0.47581 -0.08489  0.00000  0.03633  0.56633

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)   -0.95583    0.38213  -2.501 0.027849 *
DAY            0.24780    0.04834   5.127 0.000251 ***
INOCULUMB      0.26758    0.54042   0.495 0.629450
INOCULUMC      0.95583    0.54042   1.769 0.102334
DAY:INOCULUMB -0.06927    0.06836  -1.013 0.330898
DAY:INOCULUMC -0.24780    0.06836  -3.625 0.003482 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
Residual standard error: 0.296 on 12 degrees of freedom
Multiple R-squared:  0.853,   Adjusted R-squared:  0.7918
F-statistic: 13.93 on 5 and 12 DF,  p-value: 0.0001209

> emtrends(FullModelC6_Yes, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A            0.248 0.0483 12   0.1425    0.353
 B            0.179 0.0483 12   0.0732    0.284
 C            0.000 0.0483 12  -0.1053    0.105

Confidence level used: 0.95

$contrasts
 contrast estimate     SE df t.ratio p.value
 A - B      0.0693 0.0684 12 1.013   0.5829
 A - C      0.2478 0.0684 12 3.625   0.0090
 B - C      0.1785 0.0684 12 2.612   0.0552

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C7 ENANTHIC ACID
> summary(FullModelC7_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C7_Yes)

Residuals:
    Min      1Q  Median      3Q     Max
-0.7335 -0.1597  0.0000  0.0504  1.4669

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)   -0.47769    0.81917  -0.583   0.5706
DAY            0.24223    0.10362   2.338   0.0375 *
INOCULUMB     -0.62538    1.15847  -0.540   0.5992
INOCULUMC      0.47769    1.15847   0.412   0.6874
DAY:INOCULUMB  0.01135    0.14654   0.077   0.9395
DAY:INOCULUMC -0.24223    0.14654  -1.653   0.1242
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.6345 on 12 degrees of freedom
Multiple R-squared:  0.6755,  Adjusted R-squared:  0.5403
F-statistic: 4.996 on 5 and 12 DF,  p-value: 0.01051

> emtrends(FullModelC7_Yes, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend    SE df lower.CL upper.CL
 A            0.242 0.104 12   0.0165    0.468
 B            0.254 0.104 12   0.0278    0.479
 C            0.000 0.104 12  -0.2258    0.226

Confidence level used: 0.95

$contrasts
```

```
 contrast estimate    SE df t.ratio p.value
 A - B     -0.0113 0.147 12 -0.077  0.9967
 A - C      0.2422 0.147 12  1.653  0.2624
 B - C      0.2536 0.147 12  1.731  0.2343

P value adjustment: tukey method for comparing a family of 3 estimates
```

Day 10 - 15 (Fed Reactors)

```
> #C2 ACETIC ACID
> summary(FullModelC2_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C2_Yes)

Residuals:
    Min      1Q  Median      3Q     Max
-4.0060 -1.2305  0.3235  1.1390  2.7406

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)    21.8355     4.0203   5.431 0.000152 ***
DAY            -0.7119     0.3154  -2.257 0.043415 *
INOCULUMB      -8.3316     5.6855  -1.465 0.168515
INOCULUMC      -2.8482     5.6855  -0.501 0.625460
DAY:INOCULUMB   0.7080     0.4460   1.588 0.138382
DAY:INOCULUMC   0.3275     0.4460   0.734 0.476909
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 1.931 on 12 degrees of freedom
Multiple R-squared:  0.3952,  Adjusted R-squared:  0.1432
F-statistic: 1.568 on 5 and 12 DF,  p-value: 0.242

> emtrends(FullModelC2_Yes, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend    SE df lower.CL upper.CL
 A         -0.71193 0.315 12   -1.399  -0.0248
 B         -0.00388 0.315 12   -0.691   0.6833
 C         -0.38445 0.315 12   -1.072   0.3027

Confidence level used: 0.95

$contrasts
 contrast estimate    SE df t.ratio p.value
 A - B      -0.708 0.446 12 -1.588  0.2882
 A - C      -0.327 0.446 12 -0.734  0.7485
 B - C       0.381 0.446 12  0.853  0.6785

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C3 PROPIONIC ACID
> summary(FullModelC3_Yes)
```

```
Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C3_Yes)

Residuals:
     Min       1Q   Median       3Q      Max
-1.28858 -0.76355  0.01693  0.71713  1.49216

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)    0.16599    2.10545   0.079  0.93846
DAY            0.09034    0.16517   0.547  0.59444
INOCULUMB     -0.23332    2.97756  -0.078  0.93883
INOCULUMC     16.60572    2.97756   5.577  0.00012 ***
DAY:INOCULUMB  0.12220    0.23358   0.523  0.61039
DAY:INOCULUMC -0.60793    0.23358  -2.603  0.02311 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 1.011 on 12 degrees of freedom
Multiple R-squared:  0.9603,  Adjusted R-squared:  0.9437
F-statistic: 57.99 on 5 and 12 DF,  p-value: 5.486e-08

> emtrends(FullModelC3_Yes, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend    SE df lower.CL upper.CL
 A           0.0903 0.165 12   -0.270    0.450
 B           0.2125 0.165 12   -0.147    0.572
 C          -0.5176 0.165 12   -0.877   -0.158

Confidence level used: 0.95

$contrasts
 contrast estimate    SE df t.ratio p.value
 A - B      -0.122 0.234 12 -0.523  0.8616
 A - C       0.608 0.234 12  2.603  0.0561
 B - C       0.730 0.234 12  3.126  0.0221

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C4 BUTYTIC ACID
> summary(FullModelC4_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C4_Yes)

Residuals:
    Min      1Q  Median      3Q     Max
-1.0388 -0.3181  0.0399  0.2243  1.0000

Coefficients:
               Estimate Std. Error t value Pr(>|t|)
(Intercept)    0.783158   1.153720   0.679    0.510
DAY            0.059337   0.090505   0.656    0.524
INOCULUMB     -1.563887   1.631606  -0.958    0.357
INOCULUMC      0.154952   1.631606   0.095    0.926
DAY:INOCULUMB  0.109717   0.127994   0.857    0.408
```

```
DAY:INOCULUMC  0.004848   0.127994   0.038    0.970

Residual standard error: 0.5542 on 12 degrees of freedom
Multiple R-squared:  0.3351,  Adjusted R-squared:  0.05813
F-statistic:  1.21 on 5 and 12 DF,  p-value: 0.362

> emtrends(FullModelC4_Yes, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A           0.0593 0.0905 12  -0.1379    0.257
 B           0.1691 0.0905 12  -0.0281    0.366
 C           0.0642 0.0905 12  -0.1330    0.261

Confidence level used: 0.95

$contrasts
 contrast estimate    SE df t.ratio p.value
 A - B    -0.10972 0.128 12 -0.857  0.6761
 A - C    -0.00485 0.128 12 -0.038  0.9992
 B - C     0.10487 0.128 12  0.819  0.6986

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C5 VALERIC ACID
> summary(FullModelC5_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C5_Yes)

Residuals:
     Min       1Q   Median       3Q      Max
-1.21167 -0.32761 -0.00222  0.23128  1.50566

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)   0.67572    1.38933   0.486    0.635
DAY           0.02557    0.10899   0.235    0.818
INOCULUMB    -0.54083    1.96482  -0.275    0.788
INOCULUMC    -1.30329    1.96482  -0.663    0.520
DAY:INOCULUMB 0.07620    0.15413   0.494    0.630
DAY:INOCULUMC 0.09704    0.15413   0.630    0.541

Residual standard error: 0.6674 on 12 degrees of freedom
Multiple R-squared:  0.2556,  Adjusted R-squared:  -0.05452
F-statistic: 0.8242 on 5 and 12 DF,  p-value: 0.5559

> emtrends(FullModelC5_Yes, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend    SE df lower.CL upper.CL
 A           0.0256 0.109 12   -0.212    0.263
 B           0.1018 0.109 12   -0.136    0.339
 C           0.1226 0.109 12   -0.115    0.360

Confidence level used: 0.95

$contrasts
```

```
 contrast estimate    SE df t.ratio p.value
 A - B     -0.0762 0.154 12 -0.494  0.8753
 A - C     -0.0970 0.154 12 -0.630  0.8070
 B - C     -0.0208 0.154 12 -0.135  0.9900

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C6 CAPROIC ACID
> summary(FullModelC6_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C6_Yes)

Residuals:
     Min       1Q   Median       3Q      Max
-1.04602 -0.11504 -0.00659  0.06202  1.27213

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)    2.18105    1.10255   1.978   0.0713 .
DAY           -0.06589    0.08649  -0.762   0.4609
INOCULUMB     -2.50276    1.55924  -1.605   0.1344
INOCULUMC     -2.41890    1.55924  -1.551   0.1468
DAY:INOCULUMB  0.20776    0.12232   1.699   0.1152
DAY:INOCULUMC  0.08967    0.12232   0.733   0.4776
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.5296 on 12 degrees of freedom
Multiple R-squared:  0.709,   Adjusted R-squared:  0.5878
F-statistic: 5.848 on 5 and 12 DF,  p-value: 0.005792

> emtrends(FullModelC6_Yes, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A          -0.0659 0.0865 12  -0.2543    0.123
 B           0.1419 0.0865 12  -0.0466    0.330
 C           0.0238 0.0865 12  -0.1647    0.212

Confidence level used: 0.95

$contrasts
 contrast estimate    SE df t.ratio p.value
 A - B     -0.2078 0.122 12 -1.699  0.2456
 A - C     -0.0897 0.122 12 -0.733  0.7491
 B - C      0.1181 0.122 12  0.965  0.6114

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C7 ENANTHIC ACID
> summary(FullModelC7_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C7_Yes)
```

```
Residuals:
    Min      1Q  Median      3Q     Max
-0.7307 -0.1756  0.0000  0.0504  1.4613

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)     3.2747     1.3269   2.468   0.0296 *
DAY            -0.1330     0.1041  -1.278   0.2255
INOCULUMB      -3.6623     1.8765  -1.952   0.0747 .
INOCULUMC      -4.9819     1.8765  -2.655   0.0210 *
DAY:INOCULUMB   0.3150     0.1472   2.140   0.0536 .
DAY:INOCULUMC   0.3037     0.1472   2.063   0.0614 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.6374 on 12 degrees of freedom
Multiple R-squared:  0.6772,  Adjusted R-squared:  0.5428
F-statistic: 5.036 on 5 and 12 DF,  p-value: 0.01021

> emtrends(FullModelC7_Yes, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend    SE df lower.CL upper.CL
 A           -0.133 0.104 12  -0.3598   0.0938
 B            0.182 0.104 12  -0.0448   0.4088
 C            0.171 0.104 12  -0.0561   0.3975

Confidence level used: 0.95

$contrasts
 contrast estimate    SE df t.ratio p.value
 A - B     -0.3150 0.147 12 -2.140  0.1232
 A - C     -0.3037 0.147 12 -2.063  0.1396
 B - C      0.0113 0.147 12  0.077  0.9968

P value adjustment: tukey method for comparing a family of 3 estimates


                          Day 15 - 20  (Fed Reactors)

> #C2 ACETIC ACID
> summary(FullModelC2_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C2_Yes)

Residuals:
    Min      1Q  Median      3Q     Max
-5.1302 -2.1703 -0.3512  1.7780  7.4537

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)    12.5600     2.6171   4.799 9.64e-05 ***
DAY             0.1940     0.1778   1.091    0.288
INOCULUMB      -3.1787     3.7012  -0.859    0.400
INOCULUMC      -1.7902     3.7012  -0.484    0.634
DAY:INOCULUMB   0.1704     0.2514   0.678    0.505
DAY:INOCULUMC   0.1157     0.2514   0.460    0.650
```

```
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 3.326 on 21 degrees of freedom
Multiple R-squared:  0.2949,  Adjusted R-squared:  0.127
F-statistic: 1.757 on 5 and 21 DF,  p-value: 0.1657

> emtrends(FullModelC2_Yes, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend    SE df lower.CL upper.CL
 A            0.194 0.178 21 -0.17573    0.564
 B            0.364 0.178 21 -0.00532    0.734
 C            0.310 0.178 21 -0.06004    0.679

Confidence level used: 0.95

$contrasts
 contrast estimate    SE df t.ratio p.value
 A - B     -0.1704 0.251 21 -0.678  0.7788
 A - C     -0.1157 0.251 21 -0.460  0.8905
 B - C      0.0547 0.251 21  0.218  0.9743

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C3 PROPIONIC ACID
> summary(FullModelC3_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C3_Yes)

Residuals:
    Min      1Q  Median      3Q     Max
-3.2890 -1.2808 -0.2804  0.9517  6.6041

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)    2.51082    1.82986   1.372  0.18450
DAY            0.03931    0.12431   0.316  0.75496
INOCULUMB      2.01346    2.58781   0.778  0.44521
INOCULUMC      8.18057    2.58781   3.161  0.00471 **
DAY:INOCULUMB -0.11917    0.17581  -0.678  0.50525
DAY:INOCULUMC -0.11701    0.17581  -0.666  0.51293
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 2.326 on 21 degrees of freedom
Multiple R-squared:  0.6894,  Adjusted R-squared:  0.6154
F-statistic: 9.321 on 5 and 21 DF,  p-value: 8.562e-05

> emtrends(FullModelC3_Yes, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend    SE df lower.CL upper.CL
 A           0.0393 0.124 21   -0.219    0.298
 B          -0.0799 0.124 21   -0.338    0.179
 C          -0.0777 0.124 21   -0.336    0.181
```

```
Confidence level used: 0.95

$contrasts
 contrast estimate    SE df t.ratio p.value
 A - B     0.11917 0.176 21  0.678  0.7787
 A - C     0.11701 0.176 21  0.666  0.7857
 B - C    -0.00216 0.176 21 -0.012  0.9999

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C4 BUTYTIC ACID
> summary(FullModelC4_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C4_Yes)

Residuals:
     Min       1Q   Median       3Q      Max
-1.65247 -0.46995  0.01503  0.26807  2.78184

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)    0.02874    0.84630   0.034   0.9732
DAY            0.11513    0.05749   2.003   0.0583 .
INOCULUMB      1.57417    1.19684   1.315   0.2026
INOCULUMC      0.75520    1.19684   0.631   0.5349
DAY:INOCULUMB -0.11307    0.08131  -1.391   0.1789
DAY:INOCULUMC -0.01351    0.08131  -0.166   0.8696
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 1.076 on 21 degrees of freedom
Multiple R-squared:  0.2923,  Adjusted R-squared:  0.1238
F-statistic: 1.735 on 5 and 21 DF,  p-value: 0.1705

> emtrends(FullModelC4_Yes, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A          0.11513 0.0575 21 -0.00443    0.235
 B          0.00207 0.0575 21 -0.11750    0.122
 C          0.10162 0.0575 21 -0.01794    0.221

Confidence level used: 0.95

$contrasts
 contrast estimate     SE df t.ratio p.value
 A - B      0.1131 0.0813 21  1.391  0.3636
 A - C      0.0135 0.0813 21  0.166  0.9849
 B - C     -0.0996 0.0813 21 -1.224  0.4524

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C5 VALERIC ACID
> summary(FullModelC5_Yes)
```

```
Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C5_Yes)

Residuals:
     Min       1Q   Median       3Q      Max
-2.12444 -0.43225  0.05864  0.32085  2.31113

Coefficients:
               Estimate Std. Error t value Pr(>|t|)
(Intercept)    0.451033   0.741900   0.608   0.5497
DAY            0.012447   0.050402   0.247   0.8073
INOCULUMB      0.578428   1.049204   0.551   0.5872
INOCULUMC     -1.493575   1.049204  -1.424   0.1693
DAY:INOCULUMB  0.004272   0.071279   0.060   0.9528
DAY:INOCULUMC  0.166948   0.071279   2.342   0.0291 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.9429 on 21 degrees of freedom
Multiple R-squared:  0.4331,  Adjusted R-squared:  0.2981
F-statistic: 3.209 on 5 and 21 DF,  p-value: 0.02627

> emtrends(FullModelC5_Yes, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A           0.0124 0.0504 21  -0.0924    0.117
 B           0.0167 0.0504 21  -0.0881    0.122
 C           0.1794 0.0504 21   0.0746    0.284

Confidence level used: 0.95

$contrasts
 contrast estimate     SE df t.ratio p.value
 A - B    -0.00427 0.0713 21 -0.060  0.9980
 A - C    -0.16695 0.0713 21 -2.342  0.0716
 B - C    -0.16268 0.0713 21 -2.282  0.0806

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C6 CAPROIC ACID
> summary(FullModelC6_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C6_Yes)

Residuals:
    Min      1Q  Median      3Q     Max
-0.9684 -0.3907 -0.0381  0.2414  1.5946

Coefficients:
               Estimate Std. Error t value Pr(>|t|)
(Intercept)    0.150919   0.504716   0.299    0.768
DAY            0.047951   0.034289   1.398    0.177
INOCULUMB      0.068120   0.713776   0.095    0.925
INOCULUMC     -0.146120   0.713776  -0.205    0.840
DAY:INOCULUMB  0.003497   0.048492   0.072    0.943
```

```
DAY:INOCULUMC -0.044627   0.048492  -0.920    0.368

Residual standard error: 0.6415 on 21 degrees of freedom
Multiple R-squared:  0.3941,  Adjusted R-squared:  0.2498
F-statistic: 2.732 on 5 and 21 DF,  p-value: 0.04716

> emtrends(FullModelC6_Yes, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A          0.04795 0.0343 21  -0.0234   0.1193
 B          0.05145 0.0343 21  -0.0199   0.1228
 C          0.00332 0.0343 21  -0.0680   0.0746

Confidence level used: 0.95

$contrasts
 contrast estimate     SE df t.ratio p.value
 A - B     -0.0035 0.0485 21 -0.072  0.9971
 A - C      0.0446 0.0485 21  0.920  0.6338
 B - C      0.0481 0.0485 21  0.992  0.5896

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C7 ENANTHIC ACID
> summary(FullModelC7_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C7_Yes)

Residuals:
    Min      1Q  Median      3Q     Max
-0.9806 -0.4747 -0.3100  0.5488  2.1268

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)    1.12584    0.77142   1.459    0.159
DAY           -0.03411    0.05241  -0.651    0.522
INOCULUMB     -0.57920    1.09095  -0.531    0.601
INOCULUMC     -1.00389    1.09095  -0.920    0.368
DAY:INOCULUMB  0.05581    0.07412   0.753    0.460
DAY:INOCULUMC  0.04631    0.07412   0.625    0.539

Residual standard error: 0.9805 on 21 degrees of freedom
Multiple R-squared:  0.09283, Adjusted R-squared:  -0.1232
F-statistic: 0.4298 on 5 and 21 DF,  p-value: 0.8227

> emtrends(FullModelC7_Yes, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A          -0.0341 0.0524 21  -0.1431   0.0749
 B           0.0217 0.0524 21  -0.0873   0.1307
 C           0.0122 0.0524 21  -0.0968   0.1212

Confidence level used: 0.95

$contrasts
```

```
 contrast estimate     SE df t.ratio p.value
 A - B     -0.0558 0.0741 21 -0.753  0.7352
 A - C     -0.0463 0.0741 21 -0.625  0.8083
 B - C      0.0095 0.0741 21  0.128  0.9910

P value adjustment: tukey method for comparing a family of 3 estimates
```

Day 0 - 5 (Controls)

```
> #C2 ACETIC ACID
> summary(FullModelC2_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C2_Control)

Residuals:
    Min      1Q  Median      3Q     Max
-2.6540 -1.1751  0.2094  0.9552  3.2483

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)    -2.5459     0.9666  -2.634  0.01204 *
DAY             2.3366     0.2914   8.018 8.97e-10 ***
INOCULUMB       3.8254     1.3669   2.799  0.00794 **
INOCULUMC      12.5268     1.3669   9.164 2.85e-11 ***
DAY:INOCULUMB  -0.6912     0.4121  -1.677  0.10154
DAY:INOCULUMC  -3.3622     0.4121  -8.158 5.84e-10 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 1.596 on 39 degrees of freedom
Multiple R-squared:  0.7653,  Adjusted R-squared:  0.7352
F-statistic: 25.44 on 5 and 39 DF,  p-value: 2.58e-11

> emtrends(FullModelC2_Control, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend    SE df lower.CL upper.CL
 A             2.34 0.291 39     1.75    2.926
 B             1.65 0.291 39     1.06    2.235
 C            -1.03 0.291 39    -1.62   -0.436

Confidence level used: 0.95

$contrasts
 contrast estimate    SE df t.ratio p.value
 A - B       0.691 0.412 39 1.677   0.2267
 A - C       3.362 0.412 39 8.158   <.0001
 B - C       2.671 0.412 39 6.481   <.0001

P value adjustment: tukey method for comparing a family of 3 estimates
```

```
>
> #C3 PROPIONIC ACID
> summary(FullModelC3_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C3_Control)

Residuals:
     Min       1Q   Median       3Q      Max
-0.69403 -0.27310 -0.08319  0.32134  0.85902

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)   -0.69431    0.26459  -2.624   0.0123 *
DAY            0.45041    0.07978   5.646 1.61e-06 ***
INOCULUMB      2.28060    0.37419   6.095 3.82e-07 ***
INOCULUMC      2.66447    0.37419   7.121 1.46e-08 ***
DAY:INOCULUMB -0.55735    0.11282  -4.940 1.51e-05 ***
DAY:INOCULUMC -0.80720    0.11282  -7.155 1.32e-08 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.437 on 39 degrees of freedom
Multiple R-squared:  0.6369,  Adjusted R-squared:  0.5904
F-statistic: 13.68 on 5 and 39 DF,  p-value: 9.901e-08

> emtrends(FullModelC3_Control, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A            0.450 0.0798 39    0.289   0.6118
 B           -0.107 0.0798 39   -0.268   0.0544
 C           -0.357 0.0798 39   -0.518  -0.1954

Confidence level used: 0.95

$contrasts
 contrast estimate    SE df t.ratio p.value
 A - B       0.557 0.113 39 4.940   <.0001
 A - C       0.807 0.113 39 7.155   <.0001
 B - C       0.250 0.113 39 2.215   0.0812

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C4 BUTYTIC ACID
> summary(FullModelC4_Control)

Call:
```

```
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C4_Control)

Residuals:
     Min       1Q   Median       3Q      Max
-0.80663 -0.06761 -0.00261  0.10903  0.98083

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)   -0.03090    0.20274  -0.152   0.8797
DAY            0.01675    0.06113   0.274   0.7855
INOCULUMB     -0.01124    0.28671  -0.039   0.9689
INOCULUMC      0.73676    0.28671   2.570   0.0141 *
DAY:INOCULUMB  0.12105    0.08645   1.400   0.1694
DAY:INOCULUMC  0.21814    0.08645   2.523   0.0158 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.3348 on 39 degrees of freedom
Multiple R-squared:  0.804,   Adjusted R-squared:  0.7788
F-statistic: 31.99 on 5 and 39 DF,  p-value: 8.272e-13

> emtrends(FullModelC4_Control, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A           0.0168 0.0611 39  -0.1069    0.140
 B           0.1378 0.0611 39   0.0142    0.261
 C           0.2349 0.0611 39   0.1113    0.359

Confidence level used: 0.95

$contrasts
 contrast estimate     SE df t.ratio p.value
 A - B     -0.1210 0.0864 39 -1.400  0.3507
 A - C     -0.2181 0.0864 39 -2.523  0.0410
 B - C     -0.0971 0.0864 39 -1.123  0.5058

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C5 VALERIC ACID
> summary(FullModelC5_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C5_Control)

Residuals:
     Min       1Q   Median       3Q      Max
-0.22818 -0.03779 -0.00047  0.00636  0.34183
```

```
Coefficients:
               Estimate Std. Error t value Pr(>|t|)
(Intercept)   -0.0002355  0.0685907  -0.003    0.997
DAY            0.0002355  0.0206809   0.011    0.991
INOCULUMB      0.0362821  0.0970019   0.374    0.710
INOCULUMC     -0.0492787  0.0970019  -0.508    0.614
DAY:INOCULUMB  0.0381911  0.0292472   1.306    0.199
DAY:INOCULUMC  0.0434163  0.0292472   1.484    0.146

Residual standard error: 0.1133 on 39 degrees of freedom
Multiple R-squared:  0.3525,  Adjusted R-squared:  0.2695
F-statistic: 4.247 on 5 and 39 DF,  p-value: 0.003535

> emtrends(FullModelC5_Control, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A         0.000235 0.0207 39 -0.04160   0.0421
 B         0.038427 0.0207 39 -0.00340   0.0803
 C         0.043652 0.0207 39  0.00182   0.0855

Confidence level used: 0.95

$contrasts
 contrast estimate     SE df t.ratio p.value
 A - B    -0.03819 0.0292 39 -1.306  0.4005
 A - C    -0.04342 0.0292 39 -1.484  0.3093
 B - C    -0.00523 0.0292 39 -0.179  0.9826

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C6 CAPROIC ACID
> summary(FullModelC6_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C6_Control)

Residuals:
    Min      1Q  Median      3Q     Max
-0.6862  0.0000  0.0000  0.0000  1.2033

Coefficients:
               Estimate Std. Error t value Pr(>|t|)
(Intercept)    2.201e-16  1.627e-01   0.000 1.000000
DAY           -8.696e-17  4.904e-02   0.000 1.000000
INOCULUMB     -1.755e-16  2.300e-01   0.000 1.000000
INOCULUMC     -4.366e-01  2.300e-01  -1.898 0.065118 .
DAY:INOCULUMB  7.448e-17  6.936e-02   0.000 1.000000
DAY:INOCULUMC  2.807e-01  6.936e-02   4.047 0.000238 ***
```

```
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.2686 on 39 degrees of freedom
Multiple R-squared:  0.5875,  Adjusted R-squared:  0.5346
F-statistic: 11.11 on 5 and 39 DF,  p-value: 1.068e-06

> emtrends(FullModelC6_Control, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend    SE df lower.CL upper.CL
 A            0.000 0.049 39  -0.0992   0.0992
 B            0.000 0.049 39  -0.0992   0.0992
 C            0.281 0.049 39   0.1815   0.3799

Confidence level used: 0.95

$contrasts
 contrast estimate     SE df t.ratio p.value
 A - B       0.000 0.0694 39  0.000  1.0000
 A - C      -0.281 0.0694 39 -4.047  0.0007
 B - C      -0.281 0.0694 39 -4.047  0.0007

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C7 ENANTHIC ACID
> summary(FullModelC7_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C7_Control)

Residuals:
     Min       1Q   Median       3Q      Max
-0.26322 -0.10987 -0.05498  0.00000  0.50122

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)   -5.490e-02  1.423e-01  -0.386    0.702
DAY            5.490e-02  4.291e-02   1.279    0.208
INOCULUMB      8.394e-05  2.013e-01   0.000    1.000
INOCULUMC      4.364e-02  2.013e-01   0.217    0.829
DAY:INOCULUMB -6.518e-17  6.069e-02   0.000    1.000
DAY:INOCULUMC -4.658e-17  6.069e-02   0.000    1.000

Residual standard error: 0.235 on 39 degrees of freedom
Multiple R-squared:  0.1187,  Adjusted R-squared:  0.005731
F-statistic: 1.051 on 5 and 39 DF,  p-value: 0.4022

> emtrends(FullModelC7_Control, pairwise ~ INOCULUM, var = "DAY")
```

```
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A           0.0549 0.0429 39  -0.0319    0.142
 B           0.0549 0.0429 39  -0.0319    0.142
 C           0.0549 0.0429 39  -0.0319    0.142

Confidence level used: 0.95

$contrasts
 contrast  estimate     SE df t.ratio p.value
 A - B     6.52e-17 0.0607 39 0.000   1.0000
 A - C     4.66e-17 0.0607 39 0.000   1.0000
 B - C    -1.86e-17 0.0607 39 0.000   1.0000

P value adjustment: tukey method for comparing a family of 3 estimates
```

Day 5 - 10 (Controls)

```
> #C2 ACETIC ACID
> summary(FullModelC2_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C2_Control)

Residuals:
    Min      1Q  Median      3Q     Max
-0.9518 -0.3782  0.1067  0.2243  1.0824

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)    -0.4220     0.9059  -0.466 0.649701
DAY             0.5106     0.1146   4.456 0.000784 ***
INOCULUMB       4.5680     1.2811   3.566 0.003884 **
INOCULUMC      12.8728     1.2811  10.048 3.40e-07 ***
DAY:INOCULUMB  -0.1832     0.1621  -1.130 0.280477
DAY:INOCULUMC  -0.9764     0.1621  -6.026 5.98e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.7017 on 12 degrees of freedom
Multiple R-squared:  0.9511,  Adjusted R-squared:  0.9308
F-statistic: 46.72 on 5 and 12 DF,  p-value: 1.87e-07

> emtrends(FullModelC2_Control, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend    SE df lower.CL upper.CL
 A            0.511 0.115 12   0.2610    0.760
 B            0.327 0.115 12   0.0778    0.577
 C           -0.466 0.115 12  -0.7155   -0.216
```

```
Confidence level used: 0.95

$contrasts
 contrast estimate    SE df t.ratio p.value
 A - B       0.183 0.162 12 1.130   0.5147
 A - C       0.976 0.162 12 6.026   0.0002
 B - C       0.793 0.162 12 4.895   0.0010

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C3 PROPIONIC ACID
> summary(FullModelC3_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C3_Control)

Residuals:
    Min      1Q  Median      3Q     Max
-0.5731 -0.2234  0.0000  0.1525  0.7937

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)   -0.58784    0.52922  -1.111  0.28843
DAY            0.11757    0.06694   1.756  0.10451
INOCULUMB      2.73087    0.74843   3.649  0.00333 **
INOCULUMC      3.22047    0.74843   4.303  0.00103 **
DAY:INOCULUMB -0.18754    0.09467  -1.981  0.07098 .
DAY:INOCULUMC -0.28432    0.09467  -3.003  0.01100 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.4099 on 12 degrees of freedom
Multiple R-squared:  0.7931,  Adjusted R-squared:  0.7069
F-statistic: 9.202 on 5 and 12 DF,  p-value: 0.0008577

> emtrends(FullModelC3_Control, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A            0.118 0.0669 12  -0.0283   0.2634
 B           -0.070 0.0669 12  -0.2158   0.0759
 C           -0.167 0.0669 12  -0.3126  -0.0209

Confidence level used: 0.95

$contrasts
 contrast estimate     SE df t.ratio p.value
 A - B      0.1875 0.0947 12 1.981   0.1593
```

```
 A - C      0.2843 0.0947 12 3.003   0.0276
 B - C      0.0968 0.0947 12 1.022   0.5775

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C4 BUTYTIC ACID
> summary(FullModelC4_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C4_Control)

Residuals:
     Min       1Q   Median       3Q      Max
-0.18521 -0.01752  0.00000  0.03238  0.16191

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)    4.813e-16  1.194e-01   0.000 1.000000
DAY           -3.626e-17  1.510e-02   0.000 1.000000
INOCULUMB      8.343e-01  1.688e-01   4.942 0.000341 ***
INOCULUMC      3.002e-01  1.688e-01   1.778 0.100762
DAY:INOCULUMB -7.703e-02  2.136e-02  -3.607 0.003599 **
DAY:INOCULUMC  1.433e-01  2.136e-02   6.711 2.16e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.09247 on 12 degrees of freedom
Multiple R-squared:  0.9863,  Adjusted R-squared:  0.9806
F-statistic: 173.2 on 5 and 12 DF,  p-value: 9.375e-11

> emtrends(FullModelC4_Control, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A            0.000 0.0151 12  -0.0329   0.0329
 B           -0.077 0.0151 12  -0.1099  -0.0441
 C            0.143 0.0151 12   0.1104   0.1762

Confidence level used: 0.95

$contrasts
 contrast estimate     SE df t.ratio p.value
 A - B       0.077 0.0214 12   3.607 0.0093
 A - C      -0.143 0.0214 12  -6.711 0.0001
 B - C      -0.220 0.0214 12 -10.318 <.0001

P value adjustment: tukey method for comparing a family of 3 estimates

>
```

```
> #C5 VALERIC ACID
> summary(FullModelC5_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C5_Control)

Residuals:
     Min       1Q   Median       3Q      Max
-0.16134 -0.01947  0.00000  0.00000  0.13093

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)   -4.517e-17  1.040e-01   0.000   1.0000
DAY            4.278e-18  1.315e-02   0.000   1.0000
INOCULUMB      3.353e-01  1.470e-01   2.280   0.0417 *
INOCULUMC     -1.613e-01  1.470e-01  -1.097   0.2941
DAY:INOCULUMB -1.606e-02  1.860e-02  -0.863   0.4048
DAY:INOCULUMC  3.227e-02  1.860e-02   1.735   0.1083
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.08054 on 12 degrees of freedom
Multiple R-squared:  0.7094,  Adjusted R-squared:  0.5884
F-statistic:  5.86 on 5 and 12 DF,  p-value: 0.005746

> emtrends(FullModelC5_Control, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A           0.0000 0.0132 12 -0.02866   0.0287
 B          -0.0161 0.0132 12 -0.04472   0.0126
 C           0.0323 0.0132 12  0.00361   0.0609

Confidence level used: 0.95

$contrasts
 contrast estimate     SE df t.ratio p.value
 A - B      0.0161 0.0186 12  0.863  0.6724
 A - C     -0.0323 0.0186 12 -1.735  0.2328
 B - C     -0.0483 0.0186 12 -2.598  0.0565

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C6 CAPROIC ACID
> summary(FullModelC6_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C6_Control)
```

```
Residuals:
    Min      1Q  Median      3Q     Max
-0.3003  0.0000  0.0000  0.0000  0.1768

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)    1.687e-16  1.378e-01   0.000 1.000000
DAY           -1.552e-17  1.743e-02   0.000 1.000000
INOCULUMB      0.000e+00  1.949e-01   0.000 1.000000
INOCULUMC     -5.573e-01  1.949e-01  -2.860 0.014350 *
DAY:INOCULUMB -1.110e-17  2.465e-02   0.000 1.000000
DAY:INOCULUMC  1.115e-01  2.465e-02   4.522 0.000699 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.1067 on 12 degrees of freedom
Multiple R-squared:  0.8503,  Adjusted R-squared:  0.788
F-statistic: 13.63 on 5 and 12 DF,  p-value: 0.0001342

> emtrends(FullModelC6_Control, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A            0.000 0.0174 12  -0.0380    0.038
 B            0.000 0.0174 12  -0.0380    0.038
 C            0.111 0.0174 12   0.0735    0.149

Confidence level used: 0.95

$contrasts
 contrast estimate     SE df t.ratio p.value
 A - B       0.000 0.0246 12  0.000  1.0000
 A - C      -0.111 0.0246 12 -4.522  0.0019
 B - C      -0.111 0.0246 12 -4.522  0.0019

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C7 ENANTHIC ACID
> summary(FullModelC7_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C7_Control)

Residuals:
    Min      1Q  Median      3Q     Max
-0.2182  0.0000  0.0000  0.0000  0.4364

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
```

```
(Intercept)    1.666e-16  1.992e-01   0.000    1.000
DAY           -1.316e-17  2.520e-02   0.000    1.000
INOCULUMB     -4.197e-04  2.817e-01  -0.001    0.999
INOCULUMC     -2.182e-01  2.817e-01  -0.775    0.454
DAY:INOCULUMB  8.394e-05  3.563e-02   0.002    0.998
DAY:INOCULUMC  4.364e-02  3.563e-02   1.225    0.244

Residual standard error: 0.1543 on 12 degrees of freedom
Multiple R-squared:  0.294,   Adjusted R-squared:  -0.0002263
F-statistic: 0.9992 on 5 and 12 DF,  p-value: 0.4586

> emtrends(FullModelC7_Control, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A         0.00e+00 0.0252 12  -0.0549   0.0549
 B         8.39e-05 0.0252 12  -0.0548   0.0550
 C         4.36e-02 0.0252 12  -0.0113   0.0985

Confidence level used: 0.95

$contrasts
 contrast  estimate     SE df t.ratio p.value
 A - B    -8.39e-05 0.0356 12 -0.002  1.0000
 A - C    -4.36e-02 0.0356 12 -1.225  0.4619
 B - C    -4.36e-02 0.0356 12 -1.222  0.4632

P value adjustment: tukey method for comparing a family of 3 estimates


                              Day 10 - 15  (Controls)

> #C2 ACETIC ACID
> summary(FullModelC2_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C2_Control)

Residuals:
    Min      1Q  Median      3Q     Max
-1.0071 -0.6238  0.1067  0.5336  1.0824

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)     3.0344     1.6274   1.865 0.086867 .
DAY             0.1650     0.1277   1.292 0.220519
INOCULUMB       6.3381     2.3014   2.754 0.017472 *
INOCULUMC      12.1094     2.3014   5.262 0.000201 ***
DAY:INOCULUMB  -0.3602     0.1805  -1.995 0.069266 .
DAY:INOCULUMC  -0.9001     0.1805  -4.986 0.000317 ***
---
```

```
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.7818 on 12 degrees of freedom
Multiple R-squared:  0.8175,  Adjusted R-squared:  0.7414
F-statistic: 10.75 on 5 and 12 DF,  p-value: 0.0004204

> emtrends(FullModelC2_Control, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend    SE df lower.CL upper.CL
 A            0.165 0.128 12   -0.113    0.443
 B           -0.195 0.128 12   -0.473    0.083
 C           -0.735 0.128 12   -1.013   -0.457

Confidence level used: 0.95

$contrasts
 contrast estimate    SE df t.ratio p.value
 A - B        0.36 0.181 12 1.995   0.1558
 A - C        0.90 0.181 12 4.986   0.0009
 B - C        0.54 0.181 12 2.991   0.0282

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C3 PROPIONIC ACID
> summary(FullModelC3_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C3_Control)

Residuals:
     Min       1Q   Median       3Q      Max
-0.57306 -0.26523  0.09478  0.13399  0.79373

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)   -0.01523    0.86343  -0.018   0.9862
DAY            0.06031    0.06773   0.890   0.3908
INOCULUMB      2.31792    1.22108   1.898   0.0820 .
INOCULUMC      2.22769    1.22108   1.824   0.0931 .
DAY:INOCULUMB -0.14625    0.09579  -1.527   0.1527
DAY:INOCULUMC -0.18505    0.09579  -1.932   0.0773 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.4148 on 12 degrees of freedom
Multiple R-squared:  0.5105,  Adjusted R-squared:  0.3065
F-statistic: 2.503 on 5 and 12 DF,  p-value: 0.08956
```

```
> emtrends(FullModelC3_Control, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A           0.0603 0.0677 12  -0.0873   0.2079
 B          -0.0859 0.0677 12  -0.2335   0.0616
 C          -0.1247 0.0677 12  -0.2723   0.0228

Confidence level used: 0.95

$contrasts
 contrast estimate     SE df t.ratio p.value
 A - B      0.1462 0.0958 12 1.527   0.3136
 A - C      0.1850 0.0958 12 1.932   0.1722
 B - C      0.0388 0.0958 12 0.405   0.9142

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C4 BUTYRIC ACID
> summary(FullModelC4_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C4_Control)

Residuals:
     Min       1Q   Median       3Q      Max
-0.30246 -0.02902  0.00000  0.07029  0.33245

Coefficients:
               Estimate Std. Error t value Pr(>|t|)
(Intercept)   -0.052245   0.376115  -0.139 0.891827
DAY            0.005225   0.029505   0.177 0.862404
INOCULUMB     -0.335418   0.531907  -0.631 0.540129
INOCULUMC      2.969621   0.531907   5.583 0.000119 ***
DAY:INOCULUMB  0.039942   0.041726   0.957 0.357333
DAY:INOCULUMC -0.123631   0.041726  -2.963 0.011856 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.1807 on 12 degrees of freedom
Multiple R-squared:  0.9527,  Adjusted R-squared:  0.933
F-statistic: 48.34 on 5 and 12 DF,  p-value: 1.543e-07

> emtrends(FullModelC4_Control, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A          0.00522 0.0295 12  -0.0591   0.0695
 B          0.04517 0.0295 12  -0.0191   0.1095
 C         -0.11841 0.0295 12  -0.1827  -0.0541
```

```
Confidence level used: 0.95

$contrasts
 contrast estimate     SE df t.ratio p.value
 A - B     -0.0399 0.0417 12 -0.957  0.6163
 A - C      0.1236 0.0417 12  2.963  0.0296
 B - C      0.1636 0.0417 12  3.920  0.0053

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C5 VALERIC ACID
> summary(FullModelC5_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C5_Control)

Residuals:
      Min        1Q    Median        3Q       Max
-0.161342 -0.026095 -0.001177  0.023065  0.130933

Coefficients:
               Estimate Std. Error t value Pr(>|t|)
(Intercept)   -0.0047093  0.1773360  -0.027   0.9793
DAY            0.0004709  0.0139114   0.034   0.9736
INOCULUMB      0.4996323  0.2507910   1.992   0.0696 .
INOCULUMC      0.3783155  0.2507910   1.508   0.1573
DAY:INOCULUMB -0.0324930  0.0196737  -1.652   0.1245
DAY:INOCULUMC -0.0216973  0.0196737  -1.103   0.2917
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.08519 on 12 degrees of freedom
Multiple R-squared:  0.5247,  Adjusted R-squared:  0.3267
F-statistic:  2.65 on 5 and 12 DF,  p-value: 0.07739

> emtrends(FullModelC5_Control, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A         0.000471 0.0139 12  -0.0298  0.03078
 B        -0.032022 0.0139 12  -0.0623 -0.00171
 C        -0.021226 0.0139 12  -0.0515  0.00908

Confidence level used: 0.95

$contrasts
 contrast estimate     SE df t.ratio p.value
 A - B      0.0325 0.0197 12  1.652  0.2630
```

```
 A - C      0.0217 0.0197 12  1.103  0.5304
 B - C     -0.0108 0.0197 12 -0.549  0.8490

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C6 CAPROIC ACID
> summary(FullModelC6_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C6_Control)

Residuals:
    Min      1Q  Median      3Q     Max
-0.3003  0.0000  0.0000  0.0000  0.2662

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)   -6.516e-16  2.962e-01   0.000  1.00000
DAY            4.202e-17  2.324e-02   0.000  1.00000
INOCULUMB      2.220e-16  4.189e-01   0.000  1.00000
INOCULUMC      1.406e+00  4.189e-01   3.356  0.00572 **
DAY:INOCULUMB  0.000e+00  3.286e-02   0.000  1.00000
DAY:INOCULUMC -8.485e-02  3.286e-02  -2.582  0.02403 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.1423 on 12 degrees of freedom
Multiple R-squared:  0.7545,  Adjusted R-squared:  0.6521
F-statistic: 7.374 on 5 and 12 DF,  p-value: 0.002256

> emtrends(FullModelC6_Control, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A           0.0000 0.0232 12  -0.0506   0.0506
 B           0.0000 0.0232 12  -0.0506   0.0506
 C          -0.0848 0.0232 12  -0.1355  -0.0342

Confidence level used: 0.95

$contrasts
 contrast estimate     SE df t.ratio p.value
 A - B      0.0000 0.0329 12 0.000   1.0000
 A - C      0.0848 0.0329 12 2.582   0.0582
 B - C      0.0848 0.0329 12 2.582   0.0582

P value adjustment: tukey method for comparing a family of 3 estimates

>
```

```
> #C7 ENANTHIC ACID
> summary(FullModelC7_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C7_Control)

Residuals:
    Min      1Q  Median      3Q     Max
-0.2182  0.0000  0.0000  0.0000  0.4364

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)   -1.098e+00  3.212e-01  -3.418 0.005097 **
DAY            1.098e-01  2.520e-02   4.357 0.000933 ***
INOCULUMB      1.259e-03  4.543e-01   0.003 0.997834
INOCULUMC      6.547e-01  4.543e-01   1.441 0.175124
DAY:INOCULUMB -8.394e-05  3.563e-02  -0.002 0.998159
DAY:INOCULUMC -4.364e-02  3.563e-02  -1.225 0.244171
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.1543 on 12 degrees of freedom
Multiple R-squared:  0.796,   Adjusted R-squared:  0.711
F-statistic: 9.366 on 5 and 12 DF,  p-value: 0.0007921

> emtrends(FullModelC7_Control, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A           0.1098 0.0252 12   0.0549    0.165
 B           0.1097 0.0252 12   0.0548    0.165
 C           0.0661 0.0252 12   0.0112    0.121

Confidence level used: 0.95

$contrasts
 contrast estimate     SE df t.ratio p.value
 A - B    8.39e-05 0.0356 12 0.002   1.0000
 A - C    4.36e-02 0.0356 12 1.225   0.4619
 B - C    4.36e-02 0.0356 12 1.222   0.4632

P value adjustment: tukey method for comparing a family of 3 estimates
```

Day 15 - 20 (Controls)

```
> #C2 ACETIC ACID
> summary(FullModelC2_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C2_Control)
```

```
Residuals:
   Min     1Q Median     3Q    Max
-2.712 -1.127  0.014  1.041  3.190

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)   -0.75580    1.35692  -0.557   0.5834
DAY            0.49754    0.09218   5.397 2.36e-05 ***
INOCULUMB      4.97134    1.91897   2.591   0.0171 *
INOCULUMC     12.13710    1.91897   6.325 2.85e-06 ***
DAY:INOCULUMB -0.26643    0.13037  -2.044   0.0537 .
DAY:INOCULUMC -0.86564    0.13037  -6.640 1.42e-06 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 1.725 on 21 degrees of freedom
Multiple R-squared:  0.7216,  Adjusted R-squared:  0.6553
F-statistic: 10.89 on 5 and 21 DF,  p-value: 2.879e-05

> emtrends(FullModelC2_Control, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A            0.498 0.0922 21   0.3058    0.689
 B            0.231 0.0922 21   0.0394    0.423
 C           -0.368 0.0922 21  -0.5598   -0.176

Confidence level used: 0.95

$contrasts
 contrast estimate   SE df t.ratio p.value
 A - B       0.266 0.13 21 2.044   0.1265
 A - C       0.866 0.13 21 6.640   <.0001
 B - C       0.599 0.13 21 4.596   0.0004

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C3 PROPIONIC ACID
> summary(FullModelC3_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C3_Control)

Residuals:
     Min       1Q   Median       3Q      Max
-0.55660 -0.10667 -0.01764  0.13393  0.82137

Coefficients:
```

```
              Estimate Std. Error t value Pr(>|t|)
(Intercept)   -0.64752    0.29070  -2.228    0.037 *
DAY            0.11598    0.01975   5.873 7.89e-06 ***
INOCULUMB      2.68374    0.41110   6.528 1.82e-06 ***
INOCULUMC      2.93660    0.41110   7.143 4.82e-07 ***
DAY:INOCULUMB -0.17438    0.02793  -6.244 3.42e-06 ***
DAY:INOCULUMC -0.22993    0.02793  -8.233 5.19e-08 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.3695 on 21 degrees of freedom
Multiple R-squared:  0.8018,  Adjusted R-squared:  0.7546
F-statistic: 16.99 on 5 and 21 DF,  p-value: 9.354e-07

> emtrends(FullModelC3_Control, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A           0.1160 0.0197 21   0.0749   0.1571
 B          -0.0584 0.0197 21  -0.0995  -0.0173
 C          -0.1139 0.0197 21  -0.1550  -0.0729

Confidence level used: 0.95

$contrasts
 contrast estimate     SE df t.ratio p.value
 A - B      0.1744 0.0279 21 6.244   <.0001
 A - C      0.2299 0.0279 21 8.233   <.0001
 B - C      0.0556 0.0279 21 1.989   0.1396

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C4 BUTYTIC ACID
> summary(FullModelC4_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C4_Control)

Residuals:
     Min       1Q   Median       3Q      Max
-0.71076 -0.06461  0.00450  0.10670  0.91694

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)   -0.026573   0.312209  -0.085   0.9330
DAY            0.004414   0.021210   0.208   0.8372
INOCULUMB      0.255048   0.441531   0.578   0.5696
INOCULUMC      0.581066   0.441531   1.316   0.2024
DAY:INOCULUMB  0.019700   0.029996   0.657   0.5185
```

```
DAY:INOCULUMC  0.061370   0.029996   2.046   0.0535 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.3968 on 21 degrees of freedom
Multiple R-squared:  0.7645,  Adjusted R-squared:  0.7084
F-statistic: 13.64 on 5 and 21 DF,  p-value: 5.36e-06

> emtrends(FullModelC4_Control, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A          0.00441 0.0212 21  -0.0397   0.0485
 B          0.02411 0.0212 21  -0.0200   0.0682
 C          0.06578 0.0212 21   0.0217   0.1099

Confidence level used: 0.95

$contrasts
 contrast estimate   SE df t.ratio p.value
 A - B     -0.0197 0.03 21 -0.657  0.7906
 A - C     -0.0614 0.03 21 -2.046  0.1260
 B - C     -0.0417 0.03 21 -1.389  0.3644

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C5 VALERIC ACID
> summary(FullModelC5_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C5_Control)

Residuals:
     Min       1Q   Median       3Q      Max
-0.19371 -0.01068 -0.00050  0.02458  0.37709

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)    3.364e-04  1.031e-01   0.003    0.997
DAY            3.364e-05  7.007e-03   0.005    0.996
INOCULUMB      1.957e-01  1.459e-01   1.342    0.194
INOCULUMC     -7.416e-02  1.459e-01  -0.508    0.616
DAY:INOCULUMB -1.917e-04  9.909e-03  -0.019    0.985
DAY:INOCULUMC  1.165e-02  9.909e-03   1.176    0.253

Residual standard error: 0.1311 on 21 degrees of freedom
Multiple R-squared:  0.3757,  Adjusted R-squared:  0.2271
F-statistic: 2.528 on 5 and 21 DF,  p-value: 0.06095
```

```
> emtrends(FullModelC5_Control, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend      SE df lower.CL upper.CL
 A         3.36e-05 0.00701 21 -0.01454   0.0146
 B        -1.58e-04 0.00701 21 -0.01473   0.0144
 C         1.17e-02 0.00701 21 -0.00289   0.0263

Confidence level used: 0.95

$contrasts
 contrast  estimate      SE df t.ratio p.value
 A - B     0.000192 0.00991 21  0.019  0.9998
 A - C    -0.011650 0.00991 21 -1.176  0.4802
 B - C    -0.011841 0.00991 21 -1.195  0.4691

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C6 CAPROIC ACID
> summary(FullModelC6_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C6_Control)

Residuals:
    Min      1Q  Median      3Q     Max
-0.6205  0.0000  0.0000  0.0000  1.1582

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)    2.232e-17  2.586e-01   0.000  1.00000
DAY            1.623e-17  1.757e-02   0.000  1.00000
INOCULUMB      1.813e-16  3.657e-01   0.000  1.00000
INOCULUMC     -5.539e-01  3.657e-01  -1.515  0.14478
DAY:INOCULUMB -2.180e-17  2.485e-02   0.000  1.00000
DAY:INOCULUMC  7.829e-02  2.485e-02   3.151  0.00482 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.3287 on 21 degrees of freedom
Multiple R-squared:  0.6125,  Adjusted R-squared:  0.5202
F-statistic: 6.639 on 5 and 21 DF,  p-value: 0.0007479

> emtrends(FullModelC6_Control, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A           0.0000 0.0176 21  -0.0365   0.0365
 B           0.0000 0.0176 21  -0.0365   0.0365
 C           0.0783 0.0176 21   0.0418   0.1148
```

```
Confidence level used: 0.95

$contrasts
 contrast estimate     SE df t.ratio p.value
 A - B      0.0000 0.0248 21  0.000  1.0000
 A - C     -0.0783 0.0248 21 -3.151  0.0128
 B - C     -0.0783 0.0248 21 -3.151  0.0128

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C7 ENANTHIC ACID
> summary(FullModelC7_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C7_Control)

Residuals:
    Min      1Q  Median      3Q     Max
-0.2353 -0.2353 -0.1176  0.3529  0.3529

Coefficients:
               Estimate Std. Error t value Pr(>|t|)
(Intercept)    7.842e-02  2.267e-01   0.346    0.733
DAY            7.842e-03  1.540e-02   0.509    0.616
INOCULUMB      2.425e-16  3.206e-01   0.000    1.000
INOCULUMC      2.572e-16  3.206e-01   0.000    1.000
DAY:INOCULUMB -1.986e-17  2.178e-02   0.000    1.000
DAY:INOCULUMC -2.518e-17  2.178e-02   0.000    1.000

Residual standard error: 0.2881 on 21 degrees of freedom
Multiple R-squared:  0.03571, Adjusted R-squared:  -0.1939
F-statistic: 0.1556 on 5 and 21 DF,  p-value: 0.976

> emtrends(FullModelC7_Control, pairwise ~ INOCULUM, var = "DAY")
$emtrends
 INOCULUM DAY.trend     SE df lower.CL upper.CL
 A          0.00784 0.0154 21  -0.0242   0.0399
 B          0.00784 0.0154 21  -0.0242   0.0399
 C          0.00784 0.0154 21  -0.0242   0.0399

Confidence level used: 0.95
$contrasts
 contrast estimate     SE df t.ratio p.value
 A - B    1.99e-17 0.0218 21 0.000   1.0000
 A - C    2.52e-17 0.0218 21 0.000   1.0000
 B - C    5.32e-18 0.0218 21 0.000   1.0000
```

Appendix E. Pairwise comparisons between SCFA production means (concentrations).

Fed Reactors

```
> #C2 ACETIC ACID
> summary(FullModelC2_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C2_Yes)

Residuals:
    Min      1Q  Median      3Q     Max
-5.7269 -0.8165  0.0175  1.1383  4.5779

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)     -3.707e-15  1.294e+00   0.000  1.00000
DAY5             1.497e+01  1.831e+00   8.176 3.98e-09 ***
DAY10            1.472e+01  1.831e+00   8.039 5.67e-09 ***
DAY15            1.116e+01  1.831e+00   6.094 1.07e-06 ***
DAY20            1.932e+01  1.831e+00  10.551 1.29e-11 ***
INOCULUMB        1.780e+00  1.831e+00   0.972  0.33865
INOCULUMC        6.411e+00  1.831e+00   3.502  0.00147 **
DAY5:INOCULUMB  -5.077e+00  2.589e+00  -1.961  0.05921 .
DAY10:INOCULUMB -3.031e+00  2.589e+00  -1.171  0.25088
DAY15:INOCULUMB  5.090e-01  2.589e+00   0.197  0.84546
DAY20:INOCULUMB -3.492e+00  2.589e+00  -1.349  0.18755
DAY5:INOCULUMC  -8.328e+00  2.589e+00  -3.217  0.00310 **
DAY10:INOCULUMC -5.984e+00  2.589e+00  -2.311  0.02786 *
DAY15:INOCULUMC -4.347e+00  2.589e+00  -1.679  0.10355
DAY20:INOCULUMC -7.299e+00  2.589e+00  -2.819  0.00844 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 2.242 on 30 degrees of freedom
Multiple R-squared:  0.8971,  Adjusted R-squared:  0.8491
F-statistic: 18.69 on 14 and 30 DF,  p-value: 4.521e-11

> emmeans(FullModelC2_Yes, pairwise~INOCULUM|DAY)
$emmeans
DAY = 0:
 INOCULUM emmean   SE df lower.CL upper.CL
 A          0.00 1.29 30   -2.644     2.64
 B          1.78 1.29 30   -0.864     4.42
 C          6.41 1.29 30    3.767     9.05

DAY = 5:
 INOCULUM emmean   SE df lower.CL upper.CL
 A         14.97 1.29 30   12.324    17.61
 B         11.67 1.29 30    9.027    14.31
 C         13.05 1.29 30   10.406    15.69

DAY = 10:
 INOCULUM emmean   SE df lower.CL upper.CL
 A         14.72 1.29 30   12.073    17.36
 B         13.47 1.29 30   10.821    16.11
```

```
 C         15.14 1.29 30   12.499    17.79

DAY = 15:
 INOCULUM emmean   SE df lower.CL upper.CL
 A         11.16 1.29 30    8.513    13.80
 B         13.45 1.29 30   10.802    16.09
 C         13.22 1.29 30   10.577    15.86

DAY = 20:
 INOCULUM emmean   SE df lower.CL upper.CL
 A         19.32 1.29 30   16.672    21.96
 B         17.60 1.29 30   14.961    20.25
 C         18.43 1.29 30   15.784    21.07

Confidence level used: 0.95

$contrasts
DAY = 0:
 contrast estimate   SE df t.ratio p.value
 A - B      -1.780 1.83 30 -0.972  0.5997
 A - C      -6.411 1.83 30 -3.502  0.0041
 B - C      -4.630 1.83 30 -2.529  0.0434

DAY = 5:
 contrast estimate   SE df t.ratio p.value
 A - B       3.297 1.83 30  1.801  0.1864
 A - C       1.918 1.83 30  1.048  0.5533
 B - C      -1.379 1.83 30 -0.753  0.7339

DAY = 10:
 contrast estimate   SE df t.ratio p.value
 A - B       1.251 1.83 30  0.683  0.7748
 A - C      -0.427 1.83 30 -0.233  0.9705
 B - C      -1.678 1.83 30 -0.916  0.6343

DAY = 15:
 contrast estimate   SE df t.ratio p.value
 A - B      -2.289 1.83 30 -1.250  0.4337
 A - C      -2.064 1.83 30 -1.127  0.5051
 B - C       0.225 1.83 30  0.123  0.9917

DAY = 20:
 contrast estimate   SE df t.ratio p.value
 A - B       1.712 1.83 30  0.935  0.6229
 A - C       0.889 1.83 30  0.485  0.8787
 B - C      -0.823 1.83 30 -0.449  0.8950

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C3 PROPIONIC ACID
> summary(FullModelC3_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C3_Yes)

Residuals:
```

```
    Min      1Q  Median      3Q     Max
-3.6343 -0.5850  0.0000  0.7566  6.2588

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)     -2.118e-15  1.086e+00   0.000 1.000000
DAY5             3.234e+00  1.536e+00   2.105 0.043732 *
DAY10            1.069e+00  1.536e+00   0.696 0.491650
DAY15            1.521e+00  1.536e+00   0.990 0.329950
DAY20            4.350e+00  1.536e+00   2.832 0.008183 **
INOCULUMB        1.198e+00  1.536e+00   0.780 0.441419
INOCULUMC        1.016e+00  1.536e+00   0.661 0.513486
DAY5:INOCULUMB  -2.386e-01  2.172e+00  -0.110 0.913264
DAY10:INOCULUMB -2.096e-01  2.172e+00  -0.096 0.923766
DAY15:INOCULUMB  4.014e-01  2.172e+00   0.185 0.854645
DAY20:INOCULUMB -2.484e+00  2.172e+00  -1.144 0.261823
DAY5:INOCULUMC   6.226e+00  2.172e+00   2.866 0.007522 **
DAY10:INOCULUMC  9.511e+00  2.172e+00   4.378 0.000134 ***
DAY15:INOCULUMC  6.471e+00  2.172e+00   2.979 0.005680 **
DAY20:INOCULUMC  4.117e+00  2.172e+00   1.895 0.067715 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 1.881 on 30 degrees of freedom
Multiple R-squared:  0.8537,  Adjusted R-squared:  0.7854
F-statistic:  12.5 on 14 and 30 DF,  p-value: 6.753e-09

> emmeans(FullModelC3_Yes, pairwise~INOCULUM|DAY)
$emmeans
DAY = 0:
 INOCULUM emmean   SE df lower.CL upper.CL
 A          0.00 1.09 30   -2.218     2.22
 B          1.20 1.09 30   -1.020     3.42
 C          1.02 1.09 30   -1.202     3.23

DAY = 5:
 INOCULUM emmean   SE df lower.CL upper.CL
 A          3.23 1.09 30    1.016     5.45
 B          4.19 1.09 30    1.975     6.41
 C         10.48 1.09 30    8.257    12.69

DAY = 10:
 INOCULUM emmean   SE df lower.CL upper.CL
 A          1.07 1.09 30   -1.149     3.29
 B          2.06 1.09 30   -0.160     4.28
 C         11.60 1.09 30    9.378    13.81

DAY = 15:
 INOCULUM emmean   SE df lower.CL upper.CL
 A          1.52 1.09 30   -0.697     3.74
 B          3.12 1.09 30    0.903     5.34
 C          9.01 1.09 30    6.790    11.23

DAY = 20:
 INOCULUM emmean   SE df lower.CL upper.CL
 A          4.35 1.09 30    2.132     6.57
 B          3.06 1.09 30    0.846     5.28
```

```
 C          9.48 1.09 30    7.265    11.70

Confidence level used: 0.95

$contrasts
DAY = 0:
 contrast estimate   SE df t.ratio p.value
 A - B      -1.198 1.54 30 -0.780  0.7179
 A - C      -1.016 1.54 30 -0.661  0.7875
 B - C       0.183 1.54 30  0.119  0.9922

DAY = 5:
 contrast estimate   SE df t.ratio p.value
 A - B      -0.960 1.54 30 -0.625  0.8077
 A - C      -7.242 1.54 30 -4.715  0.0002
 B - C      -6.282 1.54 30 -4.090  0.0008

DAY = 10:
 contrast estimate   SE df t.ratio p.value
 A - B      -0.989 1.54 30 -0.644  0.7973
 A - C     -10.526 1.54 30 -6.853  <.0001
 B - C      -9.538 1.54 30 -6.210  <.0001

DAY = 15:
 contrast estimate   SE df t.ratio p.value
 A - B      -1.600 1.54 30 -1.041  0.5571
 A - C      -7.487 1.54 30 -4.874  0.0001
 B - C      -5.887 1.54 30 -3.833  0.0017

DAY = 20:
 contrast estimate   SE df t.ratio p.value
 A - B       1.286 1.54 30  0.837  0.6832
 A - C      -5.133 1.54 30 -3.342  0.0062
 B - C      -6.419 1.54 30 -4.179  0.0007

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C4 BUTYRIC ACID
> summary(FullModelC4_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C4_Yes)

Residuals:
    Min      1Q  Median      3Q     Max
-1.7075 -0.2566  0.0000  0.1472  2.7268

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)     -1.324e-16  5.177e-01   0.000  1.00000
DAY5             6.319e-01  7.321e-01   0.863  0.39490
DAY10            1.377e+00  7.321e-01   1.880  0.06981 .
DAY15            1.673e+00  7.321e-01   2.286  0.02952 *
DAY20            2.386e+00  7.321e-01   3.260  0.00278 **
INOCULUMB        1.899e-01  7.321e-01   0.259  0.79707
INOCULUMC        7.989e-01  7.321e-01   1.091  0.28386
```

```
DAY5:INOCULUMB   7.510e-01  1.035e+00   0.725  0.47385
DAY10:INOCULUMB -6.566e-01  1.035e+00  -0.634  0.53073
DAY15:INOCULUMB -1.081e-01  1.035e+00  -0.104  0.91757
DAY20:INOCULUMB -1.013e+00  1.035e+00  -0.978  0.33573
DAY5:INOCULUMC  -2.926e-03  1.035e+00  -0.003  0.99776
DAY10:INOCULUMC -5.955e-01  1.035e+00  -0.575  0.56949
DAY15:INOCULUMC -5.712e-01  1.035e+00  -0.552  0.58523
DAY20:INOCULUMC -9.726e-02  1.035e+00  -0.094  0.92578
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.8966 on 30 degrees of freedom
Multiple R-squared:  0.5276,  Adjusted R-squared:  0.3071
F-statistic: 2.393 on 14 and 30 DF,  p-value: 0.02201

> emmeans(FullModelC4_Yes, pairwise~INOCULUM|DAY)
$emmeans
DAY = 0:
 INOCULUM emmean    SE df lower.CL upper.CL
 A         0.000 0.518 30   -1.057     1.06
 B         0.190 0.518 30   -0.867     1.25
 C         0.799 0.518 30   -0.258     1.86

DAY = 5:
 INOCULUM emmean    SE df lower.CL upper.CL
 A         0.632 0.518 30   -0.425     1.69
 B         1.573 0.518 30    0.516     2.63
 C         1.428 0.518 30    0.371     2.49

DAY = 10:
 INOCULUM emmean    SE df lower.CL upper.CL
 A         1.377 0.518 30    0.319     2.43
 B         0.910 0.518 30   -0.147     1.97
 C         1.580 0.518 30    0.523     2.64

DAY = 15:
 INOCULUM emmean    SE df lower.CL upper.CL
 A         1.673 0.518 30    0.616     2.73
 B         1.755 0.518 30    0.698     2.81
 C         1.901 0.518 30    0.844     2.96

DAY = 20:
 INOCULUM emmean    SE df lower.CL upper.CL
 A         2.386 0.518 30    1.329     3.44
 B         1.563 0.518 30    0.506     2.62
 C         3.088 0.518 30    2.031     4.15

Confidence level used: 0.95

$contrasts
DAY = 0:
 contrast estimate    SE df t.ratio p.value
 A - B     -0.1899 0.732 30 -0.259  0.9636
 A - C     -0.7989 0.732 30 -1.091  0.5268
 B - C     -0.6090 0.732 30 -0.832  0.6865

DAY = 5:
```

```
 contrast estimate    SE df t.ratio p.value
 A - B     -0.9409 0.732 30 -1.285  0.4143
 A - C     -0.7960 0.732 30 -1.087  0.5292
 B - C      0.1450 0.732 30  0.198  0.9786

DAY = 10:
 contrast estimate    SE df t.ratio p.value
 A - B      0.4667 0.732 30  0.638  0.8007
 A - C     -0.2034 0.732 30 -0.278  0.9584
 B - C     -0.6701 0.732 30 -0.915  0.6350

DAY = 15:
 contrast estimate    SE df t.ratio p.value
 A - B     -0.0819 0.732 30 -0.112  0.9931
 A - C     -0.2277 0.732 30 -0.311  0.9482
 B - C     -0.1458 0.732 30 -0.199  0.9784

DAY = 20:
 contrast estimate    SE df t.ratio p.value
 A - B      0.8230 0.732 30  1.124  0.5070
 A - C     -0.7016 0.732 30 -0.958  0.6083
 B - C     -1.5246 0.732 30 -2.083  0.1106

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C5 VALERIC ACID
> summary(FullModelC5_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C5_Yes)

Residuals:
    Min      1Q  Median      3Q     Max
-2.4156 -0.2752  0.0000  0.2006  2.0200

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)     -3.145e-16  4.345e-01   0.000  1.00000
DAY5             3.727e-01  6.144e-01   0.607  0.54864
DAY10            9.314e-01  6.144e-01   1.516  0.13999
DAY15            1.059e+00  6.144e-01   1.724  0.09498 .
DAY20            4.189e-01  6.144e-01   0.682  0.50058
INOCULUMB        3.790e-16  6.144e-01   0.000  1.00000
INOCULUMC        7.932e-16  6.144e-01   0.000  1.00000
DAY5:INOCULUMB   6.132e-01  8.689e-01   0.706  0.48578
DAY10:INOCULUMB  2.212e-01  8.689e-01   0.255  0.80083
DAY15:INOCULUMB  6.021e-01  8.689e-01   0.693  0.49364
DAY20:INOCULUMB  6.908e-01  8.689e-01   0.795  0.43287
DAY5:INOCULUMC  -3.727e-01  8.689e-01  -0.429  0.67100
DAY10:INOCULUMC -3.328e-01  8.689e-01  -0.383  0.70437
DAY15:INOCULUMC  1.524e-01  8.689e-01   0.175  0.86198
DAY20:INOCULUMC  2.418e+00  8.689e-01   2.782  0.00924 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.7525 on 30 degrees of freedom
```

```
Multiple R-squared:  0.595,   Adjusted R-squared:  0.406
F-statistic: 3.148 on 14 and 30 DF,  p-value: 0.004096

> emmeans(FullModelC5_Yes, pairwise~INOCULUM|DAY)
$emmeans
DAY = 0:
 INOCULUM emmean    SE df lower.CL upper.CL
 A         0.000 0.434 30  -0.8873    0.887
 B         0.000 0.434 30  -0.8873    0.887
 C         0.000 0.434 30  -0.8873    0.887

DAY = 5:
 INOCULUM emmean    SE df lower.CL upper.CL
 A         0.373 0.434 30  -0.5145    1.260
 B         0.986 0.434 30   0.0987    1.873
 C         0.000 0.434 30  -0.8873    0.887

DAY = 10:
 INOCULUM emmean    SE df lower.CL upper.CL
 A         0.931 0.434 30   0.0442    1.819
 B         1.153 0.434 30   0.2653    2.040
 C         0.599 0.434 30  -0.2887    1.486

DAY = 15:
 INOCULUM emmean    SE df lower.CL upper.CL
 A         1.059 0.434 30   0.1720    1.947
 B         1.661 0.434 30   0.7742    2.549
 C         1.212 0.434 30   0.3244    2.099

DAY = 20:
 INOCULUM emmean    SE df lower.CL upper.CL
 A         0.419 0.434 30  -0.4684    1.306
 B         1.110 0.434 30   0.2224    1.997
 C         2.836 0.434 30   1.9492    3.724

Confidence level used: 0.95

$contrasts
DAY = 0:
 contrast estimate    SE df t.ratio p.value
 A - B       0.000 0.614 30  0.000  1.0000
 A - C       0.000 0.614 30  0.000  1.0000
 B - C       0.000 0.614 30  0.000  1.0000

DAY = 5:
 contrast estimate    SE df t.ratio p.value
 A - B      -0.613 0.614 30 -0.998  0.5838
 A - C       0.373 0.614 30  0.607  0.8176
 B - C       0.986 0.614 30  1.605  0.2593

DAY = 10:
 contrast estimate    SE df t.ratio p.value
 A - B      -0.221 0.614 30 -0.360  0.9312
 A - C       0.333 0.614 30  0.542  0.8514
 B - C       0.554 0.614 30  0.902  0.6434

DAY = 15:
```

```
 contrast estimate    SE df t.ratio p.value
 A - B      -0.602 0.614 30 -0.980  0.5949
 A - C      -0.152 0.614 30 -0.248  0.9667
 B - C       0.450 0.614 30  0.732  0.7466

DAY = 20:
 contrast estimate    SE df t.ratio p.value
 A - B      -0.691 0.614 30 -1.124  0.5069
 A - C      -2.418 0.614 30 -3.935  0.0013
 B - C      -1.727 0.614 30 -2.811  0.0228

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C6 CAPROIC ACID
> summary(FullModelC6_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C6_Yes)

Residuals:
    Min      1Q  Median      3Q     Max
-1.0460 -0.1189  0.0000  0.0237  1.2721

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)     -4.882e-16  2.479e-01   0.000 1.000000
DAY5             2.832e-01  3.506e-01   0.808 0.425649
DAY10            1.522e+00  3.506e-01   4.342 0.000148 ***
DAY15            1.193e+00  3.506e-01   3.402 0.001914 **
DAY20            8.949e-01  3.506e-01   2.553 0.016024 *
INOCULUMB       -6.452e-16  3.506e-01   0.000 1.000000
INOCULUMC       -2.021e-16  3.506e-01   0.000 1.000000
DAY5:INOCULUMB  -7.877e-02  4.958e-01  -0.159 0.874840
DAY10:INOCULUMB -4.251e-01  4.958e-01  -0.857 0.398030
DAY15:INOCULUMB  6.137e-01  4.958e-01   1.238 0.225411
DAY20:INOCULUMB -1.907e-01  4.958e-01  -0.385 0.703258
DAY5:INOCULUMC  -2.832e-01  4.958e-01  -0.571 0.572188
DAY10:INOCULUMC -1.522e+00  4.958e-01  -3.070 0.004517 **
DAY15:INOCULUMC -1.074e+00  4.958e-01  -2.166 0.038417 *
DAY20:INOCULUMC -8.665e-01  4.958e-01  -1.748 0.090774 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.4294 on 30 degrees of freedom
Multiple R-squared:  0.7499,  Adjusted R-squared:  0.6332
F-statistic: 6.426 on 14 and 30 DF,  p-value: 1.028e-05

> emmeans(FullModelC6_Yes, pairwise~INOCULUM|DAY)
$emmeans
DAY = 0:
 INOCULUM emmean    SE df lower.CL upper.CL
 A        0.0000 0.248 30   -0.506    0.506
 B        0.0000 0.248 30   -0.506    0.506
 C        0.0000 0.248 30   -0.506    0.506

DAY = 5:
```

```
 INOCULUM emmean    SE df lower.CL upper.CL
 A        0.2832 0.248 30   -0.223    0.789
 B        0.2044 0.248 30   -0.302    0.711
 C        0.0000 0.248 30   -0.506    0.506

DAY = 10:
 INOCULUM emmean    SE df lower.CL upper.CL
 A        1.5222 0.248 30    1.016    2.028
 B        1.0970 0.248 30    0.591    1.603
 C        0.0000 0.248 30   -0.506    0.506

DAY = 15:
 INOCULUM emmean    SE df lower.CL upper.CL
 A        1.1927 0.248 30    0.686    1.699
 B        1.8064 0.248 30    1.300    2.313
 C        0.1189 0.248 30   -0.387    0.625

DAY = 20:
 INOCULUM emmean    SE df lower.CL upper.CL
 A        0.8949 0.248 30    0.389    1.401
 B        0.7042 0.248 30    0.198    1.211
 C        0.0284 0.248 30   -0.478    0.535

Confidence level used: 0.95

$contrasts
DAY = 0:
 contrast estimate    SE df t.ratio p.value
 A - B      0.0000 0.351 30  0.000  1.0000
 A - C      0.0000 0.351 30  0.000  1.0000
 B - C      0.0000 0.351 30  0.000  1.0000

DAY = 5:
 contrast estimate    SE df t.ratio p.value
 A - B      0.0788 0.351 30  0.225  0.9726
 A - C      0.2832 0.351 30  0.808  0.7013
 B - C      0.2044 0.351 30  0.583  0.8302

DAY = 10:
 contrast estimate    SE df t.ratio p.value
 A - B      0.4251 0.351 30  1.213  0.4552
 A - C      1.5222 0.351 30  4.342  0.0004
 B - C      1.0970 0.351 30  3.129  0.0105

DAY = 15:
 contrast estimate    SE df t.ratio p.value
 A - B     -0.6137 0.351 30 -1.750  0.2036
 A - C      1.0738 0.351 30  3.063  0.0124
 B - C      1.6875 0.351 30  4.813  0.0001

DAY = 20:
 contrast estimate    SE df t.ratio p.value
 A - B      0.1907 0.351 30  0.544  0.8504
 A - C      0.8665 0.351 30  2.471  0.0493
 B - C      0.6758 0.351 30  1.928  0.1485

P value adjustment: tukey method for comparing a family of 3 estimates
```

```
>
> #C7 ENANTHIC ACID
> summary(FullModelC7_Yes)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C7_Yes)

Residuals:
    Min      1Q  Median      3Q     Max
-0.7335  0.0000  0.0000  0.0000  1.4669

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)     -8.441e-16  3.012e-01   0.000  1.00000
DAY5             7.335e-01  4.259e-01   1.722  0.09535 .
DAY10            1.945e+00  4.259e-01   4.566 7.92e-05 ***
DAY15            1.280e+00  4.259e-01   3.004  0.00533 **
DAY20            6.369e-16  4.259e-01   0.000  1.00000
INOCULUMB        2.036e-16  4.259e-01   0.000  1.00000
INOCULUMC        1.250e-15  4.259e-01   0.000  1.00000
DAY5:INOCULUMB  -5.686e-01  6.023e-01  -0.944  0.35269
DAY10:INOCULUMB -5.119e-01  6.023e-01  -0.850  0.40215
DAY15:INOCULUMB  1.063e+00  6.023e-01   1.765  0.08769 .
DAY20:INOCULUMB -4.800e-16  6.023e-01   0.000  1.00000
DAY5:INOCULUMC  -7.335e-01  6.023e-01  -1.218  0.23283
DAY10:INOCULUMC -1.945e+00  6.023e-01  -3.228  0.00301 **
DAY15:INOCULUMC -4.260e-01  6.023e-01  -0.707  0.48488
DAY20:INOCULUMC -1.122e-15  6.023e-01   0.000  1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.5216 on 30 degrees of freedom
Multiple R-squared:  0.7708,  Adjusted R-squared:  0.6638
F-statistic: 7.205 on 14 and 30 DF,  p-value: 3.24e-06

> emmeans(FullModelC7_Yes, pairwise~INOCULUM|DAY)
$emmeans
DAY = 0:
 INOCULUM emmean    SE df lower.CL upper.CL
 A         0.000 0.301 30   -0.615    0.615
 B         0.000 0.301 30   -0.615    0.615
 C         0.000 0.301 30   -0.615    0.615

DAY = 5:
 INOCULUM emmean    SE df lower.CL upper.CL
 A         0.733 0.301 30    0.118    1.349
 B         0.165 0.301 30   -0.450    0.780
 C         0.000 0.301 30   -0.615    0.615

DAY = 10:
 INOCULUM emmean    SE df lower.CL upper.CL
 A         1.945 0.301 30    1.330    2.560
 B         1.433 0.301 30    0.818    2.048
 C         0.000 0.301 30   -0.615    0.615

DAY = 15:
```

```
 INOCULUM emmean    SE df lower.CL upper.CL
 A         1.280 0.301 30    0.665    1.895
 B         2.343 0.301 30    1.728    2.958
 C         0.854 0.301 30    0.239    1.469

DAY = 20:
 INOCULUM emmean    SE df lower.CL upper.CL
 A         0.000 0.301 30   -0.615    0.615
 B         0.000 0.301 30   -0.615    0.615
 C         0.000 0.301 30   -0.615    0.615

Confidence level used: 0.95

$contrasts
DAY = 0:
 contrast estimate    SE df t.ratio p.value
 A - B       0.000 0.426 30  0.000  1.0000
 A - C       0.000 0.426 30  0.000  1.0000
 B - C       0.000 0.426 30  0.000  1.0000

DAY = 5:
 contrast estimate    SE df t.ratio p.value
 A - B       0.569 0.426 30  1.335  0.3873
 A - C       0.733 0.426 30  1.722  0.2137
 B - C       0.165 0.426 30  0.387  0.9210

DAY = 10:
 contrast estimate    SE df t.ratio p.value
 A - B       0.512 0.426 30  1.202  0.4613
 A - C       1.945 0.426 30  4.566  0.0002
 B - C       1.433 0.426 30  3.364  0.0058

DAY = 15:
 contrast estimate    SE df t.ratio p.value
 A - B      -1.063 0.426 30 -2.497  0.0467
 A - C       0.426 0.426 30  1.000  0.5825
 B - C       1.489 0.426 30  3.497  0.0041

DAY = 20:
 contrast estimate    SE df t.ratio p.value
 A - B       0.000 0.426 30  0.000  1.0000
 A - C       0.000 0.426 30  0.000  1.0000
 B - C       0.000 0.426 30  0.000  1.0000

P value adjustment: tukey method for comparing a family of 3 estimates
```

Controls

```
> #C2 ACETIC ACID
```

```
> summary(FullModelC2_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C2_Control)

Residuals:
    Min      1Q  Median      3Q     Max
-3.5108 -0.3175  0.1109  0.2782  2.3915

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)      7.481e-15  6.535e-01   0.000  1.00000
DAY5             2.131e+00  9.242e-01   2.306  0.02820 *
DAY10            4.684e+00  9.242e-01   5.069 1.92e-05 ***
DAY15            5.509e+00  9.242e-01   5.961 1.55e-06 ***
DAY20            9.994e+00  9.242e-01  10.813 7.18e-12 ***
INOCULUMB        1.766e+00  9.242e-01   1.911  0.06557 .
INOCULUMC        7.307e+00  9.242e-01   7.906 7.99e-09 ***
DAY5:INOCULUMB   1.886e+00  1.307e+00   1.443  0.15941
DAY10:INOCULUMB  9.701e-01  1.307e+00   0.742  0.46370
DAY15:INOCULUMB -8.307e-01  1.307e+00  -0.636  0.52989
DAY20:INOCULUMB -2.098e+00  1.307e+00  -1.605  0.11902
DAY5:INOCULUMC   6.835e-01  1.307e+00   0.523  0.60487
DAY10:INOCULUMC -4.199e+00  1.307e+00  -3.212  0.00314 **
DAY15:INOCULUMC -8.699e+00  1.307e+00  -6.656 2.27e-07 ***
DAY20:INOCULUMC -1.212e+01  1.307e+00  -9.273 2.58e-10 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 1.132 on 30 degrees of freedom
Multiple R-squared:  0.9092,  Adjusted R-squared:  0.8669
F-statistic: 21.46 on 14 and 30 DF,  p-value: 7.458e-12

> emmeans(FullModelC2_Control, pairwise~INOCULUM|DAY)
$emmeans
DAY = 0:
 INOCULUM emmean    SE df lower.CL upper.CL
 A          0.00 0.654 30   -1.335     1.33
 B          1.77 0.654 30    0.432     3.10
 C          7.31 0.654 30    5.972     8.64

DAY = 5:
 INOCULUM emmean    SE df lower.CL upper.CL
 A          2.13 0.654 30    0.797     3.47
 B          5.78 0.654 30    4.449     7.12
 C         10.12 0.654 30    8.787    11.46

DAY = 10:
 INOCULUM emmean    SE df lower.CL upper.CL
 A          4.68 0.654 30    3.350     6.02
 B          7.42 0.654 30    6.086     8.76
 C          7.79 0.654 30    6.458     9.13

DAY = 15:
 INOCULUM emmean    SE df lower.CL upper.CL
 A          5.51 0.654 30    4.175     6.84
 B          6.45 0.654 30    5.110     7.78
```

```
 C          4.12 0.654 30    2.783     5.45

DAY = 20:
 INOCULUM emmean    SE df lower.CL upper.CL
 A          9.99 0.654 30    8.659    11.33
 B          9.66 0.654 30    8.328    11.00
 C          5.18 0.654 30    3.847     6.52

Confidence level used: 0.95

$contrasts
DAY = 0:
 contrast estimate    SE df t.ratio p.value
 A - B      -1.766 0.924 30 -1.911  0.1530
 A - C      -7.307 0.924 30 -7.906  <.0001
 B - C      -5.541 0.924 30 -5.995  <.0001

DAY = 5:
 contrast estimate    SE df t.ratio p.value
 A - B      -3.652 0.924 30 -3.952  0.0012
 A - C      -7.991 0.924 30 -8.646  <.0001
 B - C      -4.338 0.924 30 -4.694  0.0002

DAY = 10:
 contrast estimate    SE df t.ratio p.value
 A - B      -2.736 0.924 30 -2.961  0.0159
 A - C      -3.108 0.924 30 -3.363  0.0058
 B - C      -0.372 0.924 30 -0.402  0.9149

DAY = 15:
 contrast estimate    SE df t.ratio p.value
 A - B      -0.936 0.924 30 -1.012  0.5749
 A - C       1.392 0.924 30  1.506  0.3023
 B - C       2.328 0.924 30  2.519  0.0444

DAY = 20:
 contrast estimate    SE df t.ratio p.value
 A - B       0.331 0.924 30  0.358  0.9318
 A - C       4.812 0.924 30  5.207  <.0001
 B - C       4.481 0.924 30  4.849  0.0001

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C3 PROPIONIC ACID
> summary(FullModelC3_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C3_Control)

Residuals:
    Min      1Q  Median      3Q     Max
-0.6918 -0.1692  0.0000  0.1348  0.7937

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)      2.069e-17  2.162e-01   0.000 1.000000
```

```
DAY5            -8.482e-16  3.058e-01   0.000 1.000000
DAY10            5.878e-01  3.058e-01   1.923 0.064077 .
DAY15            8.894e-01  3.058e-01   2.909 0.006771 **
DAY20            1.807e+00  3.058e-01   5.911 1.79e-06 ***
INOCULUMB        1.111e+00  3.058e-01   3.634 0.001033 **
INOCULUMC        1.224e+00  3.058e-01   4.005 0.000377 ***
DAY5:INOCULUMB   6.821e-01  4.324e-01   1.577 0.125203
DAY10:INOCULUMB -2.557e-01  4.324e-01  -0.591 0.558797
DAY15:INOCULUMB -9.869e-01  4.324e-01  -2.282 0.029731 *
DAY20:INOCULUMB -1.952e+00  4.324e-01  -4.515 9.13e-05 ***
DAY5:INOCULUMC   5.744e-01  4.324e-01   1.328 0.194045
DAY10:INOCULUMC -8.472e-01  4.324e-01  -1.959 0.059437 .
DAY15:INOCULUMC -1.772e+00  4.324e-01  -4.099 0.000291 ***
DAY20:INOCULUMC -2.863e+00  4.324e-01  -6.620 2.50e-07 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.3745 on 30 degrees of freedom
Multiple R-squared:  0.7949,  Adjusted R-squared:  0.6992
F-statistic: 8.304 on 14 and 30 DF,  p-value: 7.238e-07

> emmeans(FullModelC3_Control, pairwise~INOCULUM|DAY)
$emmeans
DAY = 0:
 INOCULUM emmean    SE df lower.CL upper.CL
 A         0.000 0.216 30   -0.442    0.442
 B         1.111 0.216 30    0.670    1.553
 C         1.224 0.216 30    0.783    1.666

DAY = 5:
 INOCULUM emmean    SE df lower.CL upper.CL
 A         0.000 0.216 30   -0.442    0.442
 B         1.793 0.216 30    1.352    2.235
 C         1.799 0.216 30    1.357    2.240

DAY = 10:
 INOCULUM emmean    SE df lower.CL upper.CL
 A         0.588 0.216 30    0.146    1.029
 B         1.443 0.216 30    1.002    1.885
 C         0.965 0.216 30    0.524    1.407

DAY = 15:
 INOCULUM emmean    SE df lower.CL upper.CL
 A         0.889 0.216 30    0.448    1.331
 B         1.014 0.216 30    0.572    1.455
 C         0.341 0.216 30   -0.100    0.783

DAY = 20:
 INOCULUM emmean    SE df lower.CL upper.CL
 A         1.807 0.216 30    1.366    2.249
 B         0.966 0.216 30    0.525    1.408
 C         0.169 0.216 30   -0.272    0.611

Confidence level used: 0.95

$contrasts
DAY = 0:
```

```
 contrast estimate    SE df t.ratio p.value
 A - B    -1.11111 0.306 30 -3.634  0.0029
 A - C    -1.22442 0.306 30 -4.005  0.0011
 B - C    -0.11331 0.306 30 -0.371  0.9273

DAY = 5:
 contrast estimate    SE df t.ratio p.value
 A - B    -1.79316 0.306 30 -5.865  <.0001
 A - C    -1.79885 0.306 30 -5.883  <.0001
 B - C    -0.00569 0.306 30 -0.019  0.9998

DAY = 10:
 contrast estimate    SE df t.ratio p.value
 A - B    -0.85545 0.306 30 -2.798  0.0235
 A - C    -0.37723 0.306 30 -1.234  0.4431
 B - C     0.47822 0.306 30  1.564  0.2765

DAY = 15:
 contrast estimate    SE df t.ratio p.value
 A - B    -0.12422 0.306 30 -0.406  0.9133
 A - C     0.54799 0.306 30  1.792  0.1894
 B - C     0.67221 0.306 30  2.199  0.0878

DAY = 20:
 contrast estimate    SE df t.ratio p.value
 A - B     0.84117 0.306 30  2.751  0.0262
 A - C     1.63817 0.306 30  5.358  <.0001
 B - C     0.79701 0.306 30  2.607  0.0365

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C4 BUTYRIC ACID
> summary(FullModelC4_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C4_Control)

Residuals:
     Min       1Q   Median       3Q      Max
-0.91099 -0.03931  0.00000  0.05225  0.71671

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)      8.665e-16  1.569e-01   0.000  1.00000
DAY5            -5.458e-16  2.219e-01   0.000  1.00000
DAY10           -1.456e-16  2.219e-01   0.000  1.00000
DAY15            2.612e-02  2.219e-01   0.118  0.90709
DAY20            7.071e-02  2.219e-01   0.319  0.75224
INOCULUMB        1.423e-01  2.219e-01   0.641  0.52622
INOCULUMC        1.025e+00  2.219e-01   4.616 6.87e-05 ***
DAY5:INOCULUMB   3.068e-01  3.139e-01   0.978  0.33611
DAY10:INOCULUMB -7.832e-02  3.139e-01  -0.250  0.80465
DAY15:INOCULUMB  1.214e-01  3.139e-01   0.387  0.70168
DAY20:INOCULUMB  6.980e-01  3.139e-01   2.224  0.03385 *
DAY5:INOCULUMC  -7.879e-03  3.139e-01  -0.025  0.98014
DAY10:INOCULUMC  7.087e-01  3.139e-01   2.258  0.03139 *
```

```
DAY15:INOCULUMC  9.054e-02  3.139e-01   0.288  0.77497
DAY20:INOCULUMC  1.041e+00  3.139e-01   3.318  0.00238 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.2718 on 30 degrees of freedom
Multiple R-squared:  0.9006,  Adjusted R-squared:  0.8542
F-statistic: 19.42 on 14 and 30 DF,  p-value: 2.757e-11

> emmeans(FullModelC4_Control, pairwise~INOCULUM|DAY)
$emmeans
DAY = 0:
 INOCULUM emmean    SE df lower.CL upper.CL
 A        0.0000 0.157 30  -0.3205    0.321
 B        0.1423 0.157 30  -0.1782    0.463
 C        1.0246 0.157 30   0.7041    1.345

DAY = 5:
 INOCULUM emmean    SE df lower.CL upper.CL
 A        0.0000 0.157 30  -0.3205    0.321
 B        0.4492 0.157 30   0.1286    0.770
 C        1.0167 0.157 30   0.6962    1.337

DAY = 10:
 INOCULUM emmean    SE df lower.CL upper.CL
 A        0.0000 0.157 30  -0.3205    0.321
 B        0.0640 0.157 30  -0.2565    0.385
 C        1.7333 0.157 30   1.4128    2.054

DAY = 15:
 INOCULUM emmean    SE df lower.CL upper.CL
 A        0.0261 0.157 30  -0.2944    0.347
 B        0.2898 0.157 30  -0.0307    0.610
 C        1.1413 0.157 30   0.8208    1.462

DAY = 20:
 INOCULUM emmean    SE df lower.CL upper.CL
 A        0.0707 0.157 30  -0.2498    0.391
 B        0.9110 0.157 30   0.5905    1.232
 C        2.1368 0.157 30   1.8163    2.457

Confidence level used: 0.95

$contrasts
DAY = 0:
 contrast estimate    SE df t.ratio p.value
 A - B      -0.142 0.222 30 -0.641  0.7987
 A - C      -1.025 0.222 30 -4.616  0.0002
 B - C      -0.882 0.222 30 -3.975  0.0012

DAY = 5:
 contrast estimate    SE df t.ratio p.value
 A - B      -0.449 0.222 30 -2.024  0.1239
 A - C      -1.017 0.222 30 -4.581  0.0002
 B - C      -0.568 0.222 30 -2.557  0.0408

DAY = 10:
```

```
 contrast estimate    SE df t.ratio p.value
 A - B      -0.064 0.222 30 -0.288  0.9553
 A - C      -1.733 0.222 30 -7.810  <.0001
 B - C      -1.669 0.222 30 -7.521  <.0001

DAY = 15:
 contrast estimate    SE df t.ratio p.value
 A - B      -0.264 0.222 30 -1.188  0.4692
 A - C      -1.115 0.222 30 -5.024  0.0001
 B - C      -0.851 0.222 30 -3.836  0.0017

DAY = 20:
 contrast estimate    SE df t.ratio p.value
 A - B      -0.840 0.222 30 -3.786  0.0019
 A - C      -2.066 0.222 30 -9.309  <.0001
 B - C      -1.226 0.222 30 -5.523  <.0001

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C5 VALERIC ACID
> summary(FullModelC5_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C5_Control)

Residuals:
      Min        1Q    Median        3Q       Max
-0.312337 -0.002355  0.000000  0.000000  0.257672

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)      1.529e-16  5.463e-02   0.000  1.00000
DAY5            -1.333e-16  7.726e-02   0.000  1.00000
DAY10           -8.830e-17  7.726e-02   0.000  1.00000
DAY15            2.355e-03  7.726e-02   0.030  0.97589
DAY20           -1.189e-16  7.726e-02   0.000  1.00000
INOCULUMB       -1.401e-16  7.726e-02   0.000  1.00000
INOCULUMC       -1.047e-16  7.726e-02   0.000  1.00000
DAY5:INOCULUMB   2.550e-01  1.093e-01   2.334  0.02650 *
DAY10:INOCULUMB  1.747e-01  1.093e-01   1.599  0.12032
DAY15:INOCULUMB  1.224e-02  1.093e-01   0.112  0.91157
DAY20:INOCULUMB  3.123e-01  1.093e-01   2.859  0.00767 **
DAY5:INOCULUMC   4.434e-17  1.093e-01   0.000  1.00000
DAY10:INOCULUMC  1.613e-01  1.093e-01   1.477  0.15020
DAY15:INOCULUMC  5.286e-02  1.093e-01   0.484  0.63208
DAY20:INOCULUMC  1.907e-01  1.093e-01   1.745  0.09124 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.09463 on 30 degrees of freedom
Multiple R-squared:  0.6524,  Adjusted R-squared:  0.4902
F-statistic: 4.023 on 14 and 30 DF,  p-value: 0.0006745

> emmeans(FullModelC5_Control, pairwise~INOCULUM|DAY)
$emmeans
DAY = 0:
```

```
 INOCULUM  emmean     SE df lower.CL upper.CL
 A        0.00000 0.0546 30  -0.1116    0.112
 B        0.00000 0.0546 30  -0.1116    0.112
 C        0.00000 0.0546 30  -0.1116    0.112

DAY = 5:
 INOCULUM  emmean     SE df lower.CL upper.CL
 A        0.00000 0.0546 30  -0.1116    0.112
 B        0.25500 0.0546 30   0.1434    0.367
 C        0.00000 0.0546 30  -0.1116    0.112

DAY = 10:
 INOCULUM  emmean     SE df lower.CL upper.CL
 A        0.00000 0.0546 30  -0.1116    0.112
 B        0.17470 0.0546 30   0.0631    0.286
 C        0.16134 0.0546 30   0.0498    0.273

DAY = 15:
 INOCULUM  emmean     SE df lower.CL upper.CL
 A        0.00235 0.0546 30  -0.1092    0.114
 B        0.01459 0.0546 30  -0.0970    0.126
 C        0.05521 0.0546 30  -0.0564    0.167

DAY = 20:
 INOCULUM  emmean     SE df lower.CL upper.CL
 A        0.00000 0.0546 30  -0.1116    0.112
 B        0.31234 0.0546 30   0.2008    0.424
 C        0.19065 0.0546 30   0.0791    0.302

Confidence level used: 0.95

$contrasts
DAY = 0:
 contrast estimate     SE df t.ratio p.value
 A - B      0.0000 0.0773 30  0.000  1.0000
 A - C      0.0000 0.0773 30  0.000  1.0000
 B - C      0.0000 0.0773 30  0.000  1.0000

DAY = 5:
 contrast estimate     SE df t.ratio p.value
 A - B     -0.2550 0.0773 30 -3.301  0.0068
 A - C      0.0000 0.0773 30  0.000  1.0000
 B - C      0.2550 0.0773 30  3.301  0.0068

DAY = 10:
 contrast estimate     SE df t.ratio p.value
 A - B     -0.1747 0.0773 30 -2.261  0.0772
 A - C     -0.1613 0.0773 30 -2.088  0.1093
 B - C      0.0134 0.0773 30  0.173  0.9837

DAY = 15:
 contrast estimate     SE df t.ratio p.value
 A - B     -0.0122 0.0773 30 -0.158  0.9863
 A - C     -0.0529 0.0773 30 -0.684  0.7745
 B - C     -0.0406 0.0773 30 -0.526  0.8594

DAY = 20:
```

```
 contrast estimate     SE df t.ratio p.value
 A - B     -0.3123 0.0773 30 -4.043  0.0010
 A - C     -0.1907 0.0773 30 -2.468  0.0497
 B - C      0.1217 0.0773 30  1.575  0.2718

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C6 CAPROIC ACID
> summary(FullModelC6_Control)

Call:
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C6_Control)

Residuals:
    Min      1Q  Median      3Q     Max
-0.4951  0.0000  0.0000  0.0000  0.8333

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)     -1.650e-16  1.200e-01   0.000   1.0000
DAY5             1.433e-16  1.698e-01   0.000   1.0000
DAY10           -4.802e-17  1.698e-01   0.000   1.0000
DAY15            2.861e-16  1.698e-01   0.000   1.0000
DAY20            3.999e-16  1.698e-01   0.000   1.0000
INOCULUMB        2.533e-16  1.698e-01   0.000   1.0000
INOCULUMC        4.206e-16  1.698e-01   0.000   1.0000
DAY5:INOCULUMB  -3.052e-16  2.401e-01   0.000   1.0000
DAY10:INOCULUMB -3.764e-17  2.401e-01   0.000   1.0000
DAY15:INOCULUMB -4.323e-16  2.401e-01   0.000   1.0000
DAY20:INOCULUMB -6.325e-16  2.401e-01   0.000   1.0000
DAY5:INOCULUMC  -2.787e-16  2.401e-01   0.000   1.0000
DAY10:INOCULUMC  5.573e-01  2.401e-01   2.321   0.0272 *
DAY15:INOCULUMC  1.331e-01  2.401e-01   0.554   0.5834
DAY20:INOCULUMC  1.337e+00  2.401e-01   5.568 4.68e-06 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.2079 on 30 degrees of freedom
Multiple R-squared:  0.8099,  Adjusted R-squared:  0.7212
F-statistic: 9.129 on 14 and 30 DF,  p-value: 2.567e-07

> emmeans(FullModelC6_Control, pairwise~INOCULUM|DAY)
$emmeans
DAY = 0:
 INOCULUM emmean   SE df lower.CL upper.CL
 A         0.000 0.12 30   -0.245    0.245
 B         0.000 0.12 30   -0.245    0.245
 C         0.000 0.12 30   -0.245    0.245

DAY = 5:
 INOCULUM emmean   SE df lower.CL upper.CL
 A         0.000 0.12 30   -0.245    0.245
 B         0.000 0.12 30   -0.245    0.245
 C         0.000 0.12 30   -0.245    0.245

DAY = 10:
```

```
 INOCULUM emmean   SE df lower.CL upper.CL
 A         0.000 0.12 30   -0.245    0.245
 B         0.000 0.12 30   -0.245    0.245
 C         0.557 0.12 30    0.312    0.803

DAY = 15:
 INOCULUM emmean   SE df lower.CL upper.CL
 A         0.000 0.12 30   -0.245    0.245
 B         0.000 0.12 30   -0.245    0.245
 C         0.133 0.12 30   -0.112    0.378

DAY = 20:
 INOCULUM emmean   SE df lower.CL upper.CL
 A         0.000 0.12 30   -0.245    0.245
 B         0.000 0.12 30   -0.245    0.245
 C         1.337 0.12 30    1.092    1.582

Confidence level used: 0.95

$contrasts
DAY = 0:
 contrast estimate   SE df t.ratio p.value
 A - B       0.000 0.17 30  0.000  1.0000
 A - C       0.000 0.17 30  0.000  1.0000
 B - C       0.000 0.17 30  0.000  1.0000

DAY = 5:
 contrast estimate   SE df t.ratio p.value
 A - B       0.000 0.17 30  0.000  1.0000
 A - C       0.000 0.17 30  0.000  1.0000
 B - C       0.000 0.17 30  0.000  1.0000

DAY = 10:
 contrast estimate   SE df t.ratio p.value
 A - B       0.000 0.17 30  0.000  1.0000
 A - C      -0.557 0.17 30 -3.283  0.0072
 B - C      -0.557 0.17 30 -3.283  0.0072

DAY = 15:
 contrast estimate   SE df t.ratio p.value
 A - B       0.000 0.17 30  0.000  1.0000
 A - C      -0.133 0.17 30 -0.784  0.7155
 B - C      -0.133 0.17 30 -0.784  0.7155

DAY = 20:
 contrast estimate   SE df t.ratio p.value
 A - B       0.000 0.17 30  0.000  1.0000
 A - C      -1.337 0.17 30 -7.875  <.0001
 B - C      -1.337 0.17 30 -7.875  <.0001

P value adjustment: tukey method for comparing a family of 3 estimates

>
> #C7 ENANTHIC ACID
> summary(FullModelC7_Control)

Call:
```

```
lm(formula = CONCENTRATION ~ DAY * INOCULUM, data = EXP3_C7_Control)

Residuals:
    Min      1Q  Median      3Q     Max
-0.2182  0.0000  0.0000  0.0000  0.4364

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)      1.526e-16  5.634e-02   0.000   1.0000
DAY5            -1.195e-16  7.968e-02   0.000   1.0000
DAY10           -6.410e-17  7.968e-02   0.000   1.0000
DAY15            5.490e-01  7.968e-02   6.889  1.2e-07 ***
DAY20           -1.463e-16  7.968e-02   0.000   1.0000
INOCULUMB       -1.375e-16  7.968e-02   0.000   1.0000
INOCULUMC       -1.686e-16  7.968e-02   0.000   1.0000
DAY5:INOCULUMB   5.927e-17  1.127e-01   0.000   1.0000
DAY10:INOCULUMB  4.197e-04  1.127e-01   0.004   0.9971
DAY15:INOCULUMB  9.919e-17  1.127e-01   0.000   1.0000
DAY20:INOCULUMB  1.130e-16  1.127e-01   0.000   1.0000
DAY5:INOCULUMC   8.235e-17  1.127e-01   0.000   1.0000
DAY10:INOCULUMC  2.182e-01  1.127e-01   1.936   0.0623 .
DAY15:INOCULUMC  1.202e-16  1.127e-01   0.000   1.0000
DAY20:INOCULUMC  1.687e-16  1.127e-01   0.000   1.0000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.09759 on 30 degrees of freedom
Multiple R-squared:  0.8831,  Adjusted R-squared:  0.8286
F-statistic: 16.19 on 14 and 30 DF,  p-value: 2.807e-10

> emmeans(FullModelC7_Control, pairwise~INOCULUM|DAY)
$emmeans
DAY = 0:
 INOCULUM  emmean     SE df lower.CL upper.CL
 A        0.00000 0.0563 30   -0.115    0.115
 B        0.00000 0.0563 30   -0.115    0.115
 C        0.00000 0.0563 30   -0.115    0.115

DAY = 5:
 INOCULUM  emmean     SE df lower.CL upper.CL
 A        0.00000 0.0563 30   -0.115    0.115
 B        0.00000 0.0563 30   -0.115    0.115
 C        0.00000 0.0563 30   -0.115    0.115

DAY = 10:
 INOCULUM  emmean     SE df lower.CL upper.CL
 A        0.00000 0.0563 30   -0.115    0.115
 B        0.00042 0.0563 30   -0.115    0.115
 C        0.21822 0.0563 30    0.103    0.333

DAY = 15:
 INOCULUM  emmean     SE df lower.CL upper.CL
 A        0.54895 0.0563 30    0.434    0.664
 B        0.54895 0.0563 30    0.434    0.664
 C        0.54895 0.0563 30    0.434    0.664

DAY = 20:
```

```
 INOCULUM  emmean     SE df lower.CL upper.CL
 A        0.00000 0.0563 30   -0.115    0.115
 B        0.00000 0.0563 30   -0.115    0.115
 C        0.00000 0.0563 30   -0.115    0.115

Confidence level used: 0.95

$contrasts
DAY = 0:
 contrast estimate     SE df t.ratio p.value
 A - B     0.00000 0.0797 30  0.000  1.0000
 A - C     0.00000 0.0797 30  0.000  1.0000
 B - C     0.00000 0.0797 30  0.000  1.0000

DAY = 5:
 contrast estimate     SE df t.ratio p.value
 A - B     0.00000 0.0797 30  0.000  1.0000
 A - C     0.00000 0.0797 30  0.000  1.0000
 B - C     0.00000 0.0797 30  0.000  1.0000

DAY = 10:
 contrast estimate     SE df t.ratio p.value
 A - B    -0.00042 0.0797 30 -0.005  1.0000
 A - C    -0.21822 0.0797 30 -2.739  0.0270
 B - C    -0.21780 0.0797 30 -2.733  0.0273

DAY = 15:
 contrast estimate     SE df t.ratio p.value
 A - B     0.00000 0.0797 30  0.000  1.0000
 A - C     0.00000 0.0797 30  0.000  1.0000
 B - C     0.00000 0.0797 30  0.000  1.0000

DAY = 20:
 contrast estimate     SE df t.ratio p.value
 A - B     0.00000 0.0797 30  0.000  1.0000
 A - C     0.00000 0.0797 30  0.000  1.0000
 B - C     0.00000 0.0797 30  0.000  1.0000

P value adjustment: tukey method for comparing a family of 3 estimates
```