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Carboxylic acid production from brewer's spent grain via mixed culture fermentation

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HIGHLIGHTS

- pH affected carboxylic acid production with mixed culture fermentation.
- Lactic acid and ethanol were produced and quickly consumed at the neutral pH.
- Neutral pH favored longer chain carboxylic acid production.
- Ethanol addition enhanced valeric acid and caproic acid production.
- Lactic acid addition enhanced propionic acid and butyric acid production.

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ABSTRACT

This study aimed at investigating carboxylic acid production from brewer's spent grain (BSG) via mixed culture fermentation. The results showed that the distribution of fermentation products was significantly affected by pH conditions and the addition of electron donors. Lactic acid was the dominant component under acidic and alkaline conditions while volatile fatty acids (VFAs) became dominant under the neutral condition. Furthermore, the neutral condition favored the chain elongation of carboxylic acids, especially with ethanol as the electron donor. Ethanol addition enhanced valeric acid and caproic acid production by 44% and 167%, respectively. Lactic acid addition also had positive effects on VFAs production under the neutral condition but limited to C2–C4 products. As a result, propionic acid and butyric acid production was increased by 109% and 152%, respectively. These findings provide substantial evidence for regulating carboxylic acid production during mixed culture fermentation of BSG by controlling pH and adding electron donors.

1. Introduction

The global climate change is highly influenced by greenhouse gas emissions from human activities on the utilization of fossil fuels. In the United States alone, more than 78% of greenhouse gas emissions are derived from the burning of petroleum, coal and natural gas for electricity, heat and transportation (EPA, 2014). Sustainable energy from biomass provides a promising alternative to fossil fuels, and its supply mediates climate issues and reduces the dependency on fossil fuels. Biomass and organic wastes generated from agricultural and industrial sectors have great potential as low-cost feedstocks for the production of value-added bioproducts, such as sugars, alcohols, and carboxylic acids. Three platforms have been proposed for biomass conversion through biological, chemical, or integrated reaction pathways: sugar, syngas, or carboxylate platforms (Agler et al., 2011), of which the carboxylate platform is regarded as the most efficient platform for carboxylic acid (especially volatile fatty acids (VFAs)) production (Holtzapple and Granda, 2009).

VFAs can be produced from various organic wastes with pure or mixed cultures under anaerobic conditions. Undefined mixed cultures (or open microbiomes) have advantages over pure cultures because open microbiomes can tolerate the complexity and variability of substrates due to the metabolic flexibility (Agler et al., 2011; Spirito et al., 2014). Furthermore, sterilization and aeration can be eliminated since undefined mixed cultures can grow under non-sterile and anaerobic conditions. The metabolic pathways of open microbiomes can be regulated by multiple factors, such as pH, substrate types, and hydrogen donor agents (Hoelzle et al., 2014; Lee et al., 2014). The fermentation products generated by open microbiomes are generally a mixture of alcohols, VFAs and other carboxylic acids (Lee et al., 2014; Liang et al., 2014).

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Compared to C2–C4 carboxylic acids, longer chain carboxylic acids are superior in terms of energy content and hydrophobic nature that facilitates the downstream processing (Spirito et al., 2014). Chain elongation of carboxylic acids typically takes place in a bioreactor when both carbon source and reducing agent are available. For example, mixed cultures utilize ethanol as a carbon source and reducing equivalent to elongate the chain of carboxylic acids via reversed β oxidation (Agler et al., 2012; Grootscholten et al., 2013b; Steinbusch et al., 2011). Lactic acid also plays a similar role in the chain elongation of carboxylic acids via reversed β oxidation, which was revealed by an anaerobic bacterium *Megasphaera elsdenii* (Prabhu et al., 2012; Weimer and Moen, 2013). However, no research has ever been reported in regards to lactic acid conversion by mixed cultures for the chain elongation of carboxylic acids.

As a brewing byproduct, brewer's spent grain (BSG) is mainly composed of fiber, starch, and protein, and its global production is estimated at approximately 38.6 million tons annually (Mussatto, 2014). Currently, BSG is mainly used as animal feed due to its high fiber and protein contents as well as operational convenience (Ben-Hamed et al., 2011). Although BGS has great potential as a low-cost feedstock for biorefineries, only a few studies have been reported to use BSG for producing biofuels and bioproducts, such as ethanol, xylitol, and lactic acid production (Mussatto, 2014; Xiros and Christakopoulos, 2009; Xiros et al., 2008). Recently, the Food and Drug Administration (FDA) proposed a rule on current good manufacture practice that restricts the handling and utilization of BSG as animal feed (Byrne, 2014), which may promote the utilization of BSG as a feedstock for the production of other value-added bioproducts.

The objective of this study was to investigate carboxylic acid production via mixed culture fermentation using BSG as a feedstock. The carboxylic acid production was first determined under different pH conditions. The metabolism of ethanol and lactic acid toward longer chain VFAs production was elucidated with temporal change of fermentation products in response to ethanol and lactic acid addition.

2. Methods

2.1. BSG and inoculum

The BSG samples were collected from the same batch at Flat Branch Pub & Brewing in Columbia, MO and stored at −20 °C prior to use. Activated sludge was collected from a single point at Columbia Municipal Wastewater Treatment Plant (Columbia, MO) and used as inoculum. Table 1 presents proximate and chemical characteristics of both BSG and inoculum.

2.2. Fermentation

Batch fermentation was carried out in 250 mL Erlenmeyer flasks that contained 35 g BSG (wet basis), 15 mL inoculum and 100 mL DI water. No additional nutrients were added. The total solid loading of the mixture in each flask was 5% (w/w). All flasks were sealed with rubber stoppers with each attached with a brewing airlock which allows gases to escape while preventing air from entering the flasks. The flasks were incubated in an orbital shaker at 37 °C and 160 rpm for 20 days. Three pH levels (uncontrolled, 7, and 10) were tested and 6 M HCl or NaOH solution was used to adjust pH levels to 7 or 10. The pH 7 was further used to study the effects of ethanol and lactic acid addition on the chain elongation of carboxylic acids. A certain amount of ethanol or lactic acid was added accordingly when their levels dropped markedly. 1 mL of fermentation broth was collected at each sampling time with up to 20 sampling times, centrifuged at 13,300 rpm for 5 min, and filtered through 0.45 μm nylon membrane filter for further analysis. All fermentation tests and measurements were conducted in triplicate.

Table 1
Characterization of brewer's spent grain (BSG) and inoculum.

Parameters*	BSG	Inoculum
Total solid (%)	20.88 ± 0.28	0.30 ± 0.01
Volatile solid (%)	96.13 ± 0.08	67.62 ± 0.74
Glucan (%)	27.36 ± 1.36	ND
Arabinoxylan (%)	9.69 ± 0.61	ND
Lignin (%)	26.22 ± 0.88	ND
Extractive (%)	9.26 ± 0.10	ND

* Except total solid which is based on wet matter (% w/w), all other parameters are based on dry matter (% w/w). ND denotes not determined. All measurements were performed in triplicate, and the average value and standard deviation were reported.

2.3. Analytical methods

The extractives in BSG were determined using Soxhlet extraction, and carbohydrates and lignin in BSG were determined by two-stage sulfuric acid hydrolysis according to the methods described previously (Liang and McDonald, 2014). The pH value was measured with a Meter Toledo FEP 20 portable meter (Toledo, OH). Total solid (TS) and volatile solid (VS) were measured according to the Standard Methods of the American Public Health Association (APHA, 2005). Lactic acid and sugars were analyzed using a Perkin–Elmer high performance liquid chromatography (HPLC) system equipped with a 410 LC pump, Bio-Rad Aminex HPX-87H column (300 × 7.8 mm), Perkin–Elmer UV–vis (220 nm), and Shimadzu RID-6A refractive index detectors. The temperatures of both column and RID detector were maintained at 55 °C. The mobile phase of 0.045 N sulfuric acid mixed with 6% acetonitrile eluted at 0.5 mL min^{-1}. Ethanol and VFAs were analyzed using a gas chromatography (GC) system equipped with a flame ionization detector (FID) and Alltech AT-1000 column (30 m × 0.25 mm, 0.25 μm film thickness). All samples were acidified to the pH 2 with HCl and then used for GC analysis. Helium was used as the carrier gas with the flow rate of 1 mL min^{-1} and split ratio of 20:1. VFAs were analyzed by maintaining the temperatures of both injector and detector at 250 °C and the temperature of column at 150 °C. Ethanol was analyzed using different temperature programming for column with its initial temperature held at 60 °C for 5 min, heated to 210 °C with a rate of 20 °C min^{-1}, and then held at this temperature for 5 min.

2.4. Statistical analysis

All fermentation tests and measurements were conducted in triplicate, and the average values and standard deviation were reported. Analysis of variance (ANOVA) and *t*-test were performed with SAS 9.2 software (SAS Institute Inc., Cary, NC, USA) with a threshold *p-value* of 0.05.

3. Results and discussion

3.1. Effect of pH on the mixed culture fermentation

The results of fermentation products at different pH levels are depicted in Fig. 1. The initial pH of the mixture in the fermentation flasks was around 6.5 and only small amounts of acetic acid, lactic acid, and ethanol (all <0.1 g L^{-1}) were detected at the beginning of the fermentation. When the pH was uncontrolled, its value dropped to 3.8 on day 1 and kept around this level through the rest of the fermentation process. Fig. 1a shows the profile of the mixed