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Strategies to overcome sodium benzoate inhibition during ethanol production from expired soft drinks

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ABSTRACT

Disposal of expired, sugar-containing beverages by conventional wastewater treatment represents a waste of a potentially valuable resource that could instead be used for ethanol production. A key limitation is that many soft drinks contain sodium benzoate as a preservative, and this interferes with yeast fermentation. In this study various approaches were used to inactivate or remove sodium benzoate so that fermentation could proceed. Transformation of benzoic acid into its less toxic salt form, by adjusting the fermentation pH to 7, only marginally increased fermentation efficiency and ethanol yield of Mountain Dew. Treatment with the anion exchange resins Biorad UNO Q1 versus DowEX Monosphere 99CA/320, achieved high fermentation efficiencies and yields, but were far too expensive for commercial application ($10-85/L ethanol produced). Biorad UNO Q1 and DowEX Monosphere 99CA/320 resins achieved fermentation efficiencies near 100%, and the highest ethanol yields. However these treatments were prohibitively expensive. The highest performing and most economical treatment was activated carbon BG-HHM, with an ethanol yield of 82.5%, fermentation efficiency of 93.5%, and a treatment cost of only $0.02 per liter ethanol produced. Activated carbon WPH performed similarly, but at a 10-fold higher cost. Treatment with biochar was the least expensive ($0.01/L ethanol produced), but ethanol yields were only 45%.

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ABBREVIATIONS

ATP	:	adenosine triphosphate
CO_2	:	carbon dioxide
CCS	:	condensed corn solubles
HCl	:	hydrochloric acid
M	:	molarity
N	:	normality
PDA	:	potato dextrose agar
rpm	:	revolutions per minute
NaOH	:	sodium hydroxide
H_2SO_4	:	sulfuric acid
v/v	:	volume per volume
w/w	:	weight per weight

INTRODUCTION

Each year in the United States millions of liters (L) of beverages pass their expiration dates and must be disposed of, traditionally via conventional wastewater treatment [1]. Bottling companies have to adjust the pH of these beverage wastes before discharging to wastewater treatment plants. Disposal of expired soft drinks represents a waste of a valuable resource, and in some cases accrues treatment costs or fees.

An alternative to the disposal of expired beverages is ethanol production. Parallel Products, based in Louisville, KY and Ontario, CA, currently produces

approximately (~) 5.5 million gallons of waste-derived ethanol each year by converting certain sugar-based and alcohol-based liquids into ethanol. As a leader in America's unsaleable beverage industry, they process ~13 million cases and ~3 million bulk gallons of distressed or out of date beverage products annually [2]. Fuel ethanol produced from corn now accounts for approximately 10% of the US transportation fuel market, and facilities for producing and blending ethanol are located around the country. Therefore, it would be relatively easy to incorporate soft drink-derived ethanol into this marketing system. This would be more environment-friendly and could potentially be a more economical alternative.

Unfortunately, two characteristics of soft drinks must be addressed prior to ethanol production or any other microbe-based process. One is the low sugar concentration of typical soft drinks, which ranges from 0-118 grams per liter (g/L). Assuming 100% fermentation efficiency by yeast, the upper ethanol titer would be 60-65 g/L ethanol, which is far below the 150+ g/L levels typically found in corn beer prior to distillation. Options to boost sugar levels in expired pop could include multiple effect evaporation [3, 4] or blending with other expired products (e.g., candy). If a corn ethanol facility was located nearby, the expired pop could be used to replace part of the water in the initial corn mash preparation stage. Alternatively, it may be possible to economically recover dilute ethanol streams using membrane-based distillation systems [5, 6].

Another limitation is that preservatives are added to some soft drinks, and these could inhibit fermenting organisms. Sodium benzoate is used as a food preservative in acidic foods to control bacteria, mold, yeasts, and other microbes [7]. This includes products such as fruit juices, ketchup, peanut butter, soft drinks, and many others [8]. At low pH, sodium benzoate dissociates into benzoic acid, and it is generally accepted that benzoic acid is the active antimicrobial agent [9, 10, 11]. Yeast cells are permeable to weak acids like benzoic acid [12]. It is theorized that benzoic acid is toxic to *Saccharomyces cerevisiae* (*S. cerevisiae*) because the reduced pH deactivates phosphofructokinase, thereby inhibiting glycolysis and reducing adenosine triphosphate (ATP) production, which restricts cell growth [13, 14]. In most cases, a concentration of 0.2-0.3 g/L benzoic acid is enough to prevent growth of *S. cerevisiae* [14]. However, one study had shown that *S. cerevisiae* could be acclimated and grown in the presence of benzoic acid to enhance resistance to weak acid preservatives [15]. Sodium or potassium benzoate are typically the forms added to foods because they are ~200 times more soluble than benzoic acid [16].

The objective of this study was to explore methods to alleviate the inhibitory effects of sodium benzoate. We investigated inactivation of benzoic acid by increasing pH, as well as testing several adsorbents (anion exchange resins, activated carbon, and biochar) to remove sodium benzoate to determine if this would permit successful yeast fermentation.

MATERIALS AND METHODS

Culture, maintenance, and preparation

Saccharomyces cerevisiae NRRL Y-2034 was obtained from the National Center for Agricultural Utilization Research (Peoria, IL). Short term maintenance cultures were stored on Potato Dextrose Agar (PDA) plates and slants at 4 degrees Celsius (ºC). Lyophilization was used for long term storage. Inoculum for all experiments was prepared by transferring colonies into a 5% glucose, 0.5% yeast extract medium in 100 milliliter (ml) in 250 ml Erlenmeyer flasks, then incubating for 24 hours (h) at 35 ºC in a rotary shaker at 150 revolutions per minute (rpm).

Materials

Condensed corn solubles (CCS)

CCS was used as a nutrient source for yeast metabolism. CCS was obtained from a local dry grind corn ethanol plant and was refrigerated until use. CCS composition is listed in Table 1.

Soft drinks

Soft drinks evaluated in this study included Coke, Diet Coke, Pepsi, Diet Pepsi, Mountain Dew, and Sierra Mist. These samples were all past their recommended consumption date. Total sugar concentrations and the presence of other components are listed in Table 2. Total sugars represent the combined concentrations of glucose and fructose. Mountain Dew, which contains sodium benzoate, was used as the model system to test various methods of alleviating benzoic acid inhibition.

Table 1. Composition of CCS.

Component	Component amount[a]
Total dry matter (%)	27.7
Calcium (%)	0.03
Magnesium (%)	0.22
Phosphorus (%)	0.46
Potassium (%)	0.77
Sodium (%)	0.20
Copper (ppm)	1.20
Zinc (ppm)	29.9
Crude protein (%)	5.25
Ash total (%)	3.28
Crude fiber (%)	0.48
Crude fat, Base hydrolysis (%)	5.71
Nitrogen free extract (%)	13.0

[a]Dry matter basis.

Adsorbents

Various types of adsorbents were tested to remove sodium benzoate from Mountain Dew, including anion exchange, activated carbon, and biochar. Anion exchange resins included Bio-Rad UNO Q1 (Bio-Rad Laboratories, Philadelphia, PA, USA) and DowEX Monosphere 99CA/320 (DOW, Philadelphia, PA, USA). These resins were packed into columns and Mountain Dew was passed through the columns as described in a subsequent section. Activated carbon adsorbents included BG-HHM (Calgon, Pittsburgh, PA, USA) and WPH (Calgon, Pittsburgh, PA, USA). BG-HHM is derived from wood and is typically used for decolorizing in the sugar or soft drink industries. WPH is a high performance activated carbon specifically designed to treat potable water. Biochar was obtained from Chippewa Valley Ethanol of Benson, MN, which is the residue from the gasification of corn cobs and wood chips. Activated carbon and biochar were used in powder form and were mixed with

Table 2. Composition of untreated soft drinks.

Component	Coke	Diet Coke	Pepsi	Diet Pepsi	Mountain Dew	Sierra Mist	Root Beer
Total sugars (g/L)	98.0	0.6	107.3	0.6	117.7	100.4	109.0
Initial pH	1.54	1.93	1.54	1.91	2.16	2.10	2.98
Aspartame		*		*			
Concentrated orange juice					*		
High fructose corn syrup	*		*		*	*	*
Modified food starch							*
Calcium disodium EDTA					*	*	*
Potassium benzoate		*		*		*	
Sodium benzoate					*		*
Brominated vegetable oil					*		
Gum arabic					*		
Potassium citrate						*	
Sodium citrate					*		
Ascorbic acid						*	
Citric acid		*	*	*	*	*	*
Erythobic acid					*		
Phosphoric acid	*	*	*	*			
Caffeine	*	*	*	*	*		
Yellow 5					*		

*Represents ingredient present or components for which concentrations were not available.

Mountain Dew in a batch method. Table 3 provides the sources and characteristics of these adsorbents.

Experimental procedure

Fermentation of untreated soft drinks

Untreated samples of soft drinks were tested by mixing 150 ml quantities of each with 5% (w/w) CCS (as a nutritional supplement) in 250 ml Erlenmeyer flasks. The pH was adjusted to 4-5 with either 10 M sodium hydroxide (NaOH) or 15 N hydrochloric acid (HCl). The flasks were then sealed with rubber stoppers and pierced with 12 gauge needles attached to 0.2 micrometer (μm) filters to allow for carbon dioxide (CO_2) release. After autoclaving and cooling, 1 ml of a tetracycline solution (0.01 g in 1 ml of 70% ethanol) was added, along with 1 ml of a 24 h *S. cerevisiae* culture. Flasks were incubated for 96 h at 35 ºC in a 150 rpm rotary shaker. Trials were completed in triplicate.

Fermentation of pH neutralized Mountain Dew

To determine the effectiveness of pH adjustment in neutralizing the toxicity of benzoate, samples of 75 ml of Mountain Dew and 5% CCS were adjusted to an initial pH of 7 using 10 molar (M) NaOH. Flasks were then autoclaved, inoculated, and incubated as described above. At 12 h intervals the pH was checked and adjusted to 7 as needed. For a control, Pepsi (which doesn't contain benzoate) was also tested at a pH level of 7. Trials were completed in triplicate.

Adsorbent treatment of Mountain Dew prior to fermentation

Mountain Dew was treated with anion exchange, activated carbon, and biochar adsorbents by the methods listed in Tables 4-6. All adsorption treatments were done at 20–25 ºC. Treated Mountain Dew samples (75 ml) were then dispensed into 250 ml Erlenmeyer flask with 5% (w/w) CCS as a nutritional supplement. The pH was adjusted to 4-5 with either 10 M NaOH or 15 M HCl, and the flasks were sealed, autoclaved, and cooled as described above. Flasks were then inoculated with 1 ml of a 24 h *S. cerevisiae* culture and were incubated for 132 h at 35 ºC in a 150 rpm rotary shaker. Trials were completed in triplicate. Biochar fermentations were also completed with Pepsi to observe the effects of biochar on a non-sodium benzoate containing soft drink.

Analytical methods

Samples (5 ml) were aseptically collected before and after the various treatment processes and then at 12 h intervals throughout fermentation. Sample

Table 3. Properties of adsorbents.

Adsorbent		Vendors	Surface area (m^2/g)	Ash (%)	Volatile-leachable (%)	Particle size (μm)
Anion exchange	Biorad UNO Q1	Bio-Rad	-	-	-	50
	DowEX Monosphere 99CA/320	Dow	-	-	-	150-500
Activated carbon	BG-HHM	Calgon	850	< 5	< 3	20-150
	WPH	Calgon	1050	< 5	< 3	20-150
Biochar	Biochar	Chippewa Valley Ethanol	120	35	19	30-300

Table 4. Anion exchange treatment conditions.

Treatment	Resin amount (ml)	Sample volume (ml)	Flow rate (ml/min)
Biorad UNO Q1[a]	5	75 ml	2
DowEX Monosphere 99CA/320[a]	20	75 ml	5

[a]Biorad and DowEX resins were packed into columns for anion exchange.

Table 5. Activated carbon treatment conditions.

Activated carbon	Adsorption (h)	Activated carbon concentration (g/L)
BG-HHM[a]	2	5
BG-HHM[a]	2	2
BG-HHM[a]	2	1
BG-HHM[a]	2	0.5
BG-HHM[a]	2	0.25
WPH[b]	2	5

[a]BG-HHM is activated carbon from wood for sugar or soft drink industry (decolor).
[b]WPH is activated carbon from coal for water purification.

Table 6. Biochar treatment conditions.

Adsorption time (h)	Biochar concentration (g/L)
2	5
2	10
2	15
12	5

pH was measured and then samples were filtered through 0.2 µm filters into high performance liquid chromatography (HPLC) vials, which were frozen until analysis. Carbohydrates, organic acids, and ethanol were measured in a Waters HPLC, with a refractive index detector. The system used an Aminex HPX-87H column (Bio-rad, Hercules, CA, USA) operated at 65 ºC, with a mobile phase of 0.01 normal (N) sulfuric acid (H_2SO_4) at a flow rate of 0.6 milliliters per minute (ml/min).

RESULTS AND DISCUSSION

Fermentation of untreated soft drinks

Untreated soft drink samples were fermented for 96 h and the results from three replicate trials are shown in Table 7. The sugar containing soft drinks (Coke, Pepsi, Mountain Dew, Sierra Mist and Root Beer) contained both glucose and fructose. Glucose was completely consumed in the Coke and Pepsi samples, but only small amounts of glucose were used in the other three samples due to the presence of benzoate. Similar trends were observed with fructose consumption. Overall fermentation efficiency was 85.4 ± 8.3 and 89.0 ± 10.0% in Coke and Pepsi, respectively, compared to low fermentation efficiency in Mountain Dew, Sierra Mist and Root Beer. Overall sugar utilization levels correlated well with ethanol production parameters. For example, ethanol yields were 72.4 ± 8.6 and 73.7 ± 8.7% for Coke and Pepsi, respectively, but only ranged from 0.4 ± 0.3 to 13.5 ± 11.8% for the benzoate-containing soft drinks. Trials with Diet Coke and Diet Pepsi only contained trace amounts of sugars (contributed by the CCS) and therefore produced only negligible amounts of ethanol. The ethanol yield of 166% for diet Coke was due to variability in HPLC performance of one replication. These results demonstrate that the sugars in expired soft drinks can be efficiently fermented to ethanol, except when the soft drink contains the preservative benzoate.

Fermentation of pH neutralized Mountain Dew

One approach to reducing the toxicity of benzoate is to increase the pH level to convert benzoic acid into the salt form, since the latter is much less toxic [10]. Therefore we evaluated fermentation of Mountain Dew at a pH of 7 compared to pH 4, along with controls testing Pepsi at the same pH levels (Table 8). In Pepsi, a neutral pH negatively affected yeast performance and reduced the ethanol yield from 73.7 ± 8.7% down to 35.3 ± 1.5 g/L. Fermentation efficiency was similarly reduced from 89.0 ± 10.0 at pH 4 to 50.2 ± 2.4% at pH 7. Meanwhile, neutralizing pH in Mountain Dew containing benzoic acid increased both the ethanol yield (from 2.6 ± 2.2% to 28.9 ± 0.9% g/L) and fermentation efficiency (from 2.7 ± 3.7 to 39.8 ± 3.2%). These results are in agreement with

Table 7. Fermentation of untreated soft drinks.

Untreated soft drink	Initial glucose (g/L)[a]	Initial fructose (g/L)[a]	Fermentation efficiency (%)	Net ethanol produced (g/L)[b]	Ethanol productivity (g/L/h)	Ethanol yield (%)
Coke	40.3 ± 1.4	57.7 ± 2.2	85.4 ± 8.3	36.1 ± 2.6	0.4 ± 0.0	72.4 ± 8.6
Diet Coke	0.4 ± 0.1	0.2 ± 0.1	51.7 ± 17.2	0.4 ± 0.4	0.0 ± 0.0	166.6 ± 178.8[c]
Pepsi	44.5 ± 1.1	62.8 ± 1.6	89.0 ± 10.0	40.3 ± 3.6	0.5 ± 0.1	73.7 ± 8.7
Diet Pepsi	0.4 ± 0.1	0.2 ± 0.2	36.6 ± 32.6	0.2 ± 0.1	0.0 ± 0.0	56.7 ± 35.6
Mountain Dew	48.8 ± 1.2	68.9 ± 1.7	3.2 ± 2.8	1.6 ± 1.1	0.0 ± 0.0	2.6 ± 2.2
Sierra Mist	41.7 ± 0.5	58.7 ± 1.2	15.1 ± 13.1	7.0 ± 4.9	0.1 ± 0.1	13.5 ± 11.8
Root Beer	44.6 ± 0.9	64.1 ± 2.1	0.4 ± 0.6	0.2 ± 0.1	0.0 ± 0.0	0.4 ± 0.3

[a]Initial sugar levels at 0 h after CCS nutrient added and pH adjusted to 4-5.
[b]Net ethanol was calculated by subtracting the initial ethanol concentrations at 0 h from the final ethanol concentration. Initial ethanol was due to the ethanol added with the tetracycline antibiotic that was added at 0 h.
[c]Values for the three triplicates varied from 0 to 355.5% of the theoretical ethanol yield. This may be due to low ethanol titers (< 3 g/L) and normal variability in HPLC measurements of the 0 h ethanol titers due to the addition of tetracycline solution.

Table 8. Fermentation of pH neutralized soft drinks.

Soft drink, pH	Net ethanol (g/L)	Ethanol productivity (g/L/h)	Fermentation efficiency (%)	Ethanol yield (%)
Pepsi, pH 7	20.5 ± 0.8	0.2 ± 0.0	50.2 ± 2.4	35.3 ± 1.5
Pepsi, pH 4	40.3 ± 3.6	0.5 ± 0.1	89.0 ± 10.0	73.7 ± 8.7
Mountain Dew, pH 7	19.3 ± 1.2	0.2 ± 0.0	39.8 ± 3.2	28.9 ± 0.9
Mountain Dew, pH 4	1.6 ± 1.1	0.0 ± 0.0	3.2 ± 2.8	2.6 ± 2.2

prior studies that have shown that adjusting the pH to 7 can reduce the inhibitory effects of benzoic acid by converting it to the less toxic sodium benzoate form. Unfortunately, this higher pH itself inhibited yeast metabolism (as evidenced by the reduced performance in the pH 7 Pepsi), as it is commonly known that the optimum pH of *S. cerevisiae* is 4-6 [17, 18, 19]. Therefore pH adjustment does not appear to be a solution, since only ~40% of the sugar was consumed and the ethanol yield was less that 30%.

Anion exchange resins for sodium benzoate removal

Mountain Dew samples were passed through an anion exchange column to compare benzoate removal via two different resins, Biorad UNO Q1 and Dow Ex Monosphere 99CA/320. Anion exchange resins have been shown to successfully remove acidic compounds, such as benzoic acid [11]. The treated Mountain Dew was then fermented with *S. cerevisiae* for 132 h, and the results of triplicate replications are shown in Table 9. Both resins effectively reduced benzoate levels such that *S. cerevisiae* metabolized nearly all sugars (95.1-98.5% fermentation efficiency). However, the Mountain Dew processed through the Biorad resin had higher ethanol titer and yield compared to the Dow resin. In fact the ethanol yield exceeded 100%, due to the metabolism of maltose or dextrins (and other unquantifiable nutrients) in the CCS. Trials with the DOW resin resulted in slightly higher fermentation efficiency, indicating that more sugars were consumed for yeast growth and maintenance.

Table 9. Fermentation performance following anion exchange treatment of Mountain Dew.

Treatments	Net ethanol (g/L)	Ethanol productivity (g/L/h)	Fermentation efficiency (%)	Ethanol yield (%)
Biorad UNO Q1	66.1 ± 14.6	0.6 ± 0.1	95.1 ± 1.8	104.1 ± 11.9
Dow Monosphere 99CA/320	56.0 ± 5.8	0.6 ± 0.1	98.5 ± 2.7	97.8 ± 16.0

Table 10. Fermentation performance following activated carbon treatment of Mountain Dew.

Activated carbon concentration	Net ethanol (g/L)	Ethanol productivity (g/L/h)	Fermentation efficiency (%)	Ethanol yield (%)
BG-HHM 0.25 g/L	42.9 ± 12.9	0.3 ± 0.1	82.0 ± 18.5	73.1 ± 23.5
BG-HHM 0.5 g/L	50.2 ± 7.3	0.5 ± 0.1	93.5 ± 9.7	82.5 ± 12.3
BG-HHM 1 g/L	46.4 ± 1.2	0.6 ± 0.2	94.2 ± 2.8	81.3 ± 3.6
BG-HHM 2 g/L	50.0 ± 5.3	0.5 ± 0.0	94.4 ± 2.0	89.5 ± 11.4
BG-HHM 5 g/L	43.0 ± 0.7	0.6 ± 0.0	94.9 ± 1.4	78.0 ± 2.4
WPH 5 g/L	43.3 ± 8.3	0.6 ± 0.1	99.0 ± 1.8	76.7 ± 16.1

Table 11. Fermentation performance following biochar adsorption treatment of Mountain Dew and Pepsi.

Biochar treatment (Adsorption time-concentration)	Net ethanol (g/L)	Ethanol productivity (g/L/h)	Fermentation efficiency (%)	Ethanol yield (%)
Mountain Dew				
2 hours – 5 g/L	25.6 ± 0.7	0.2 ± 0.0	54.1 ± 2.4	45.4 ± 4.3
2 hours – 10 g/L	19.4 ± 2.0	0.2 ± 0.0	65.4 ± 11.8	40.7 ± 0.7
2 hours – 15 g/L	15.6 ± 1.6	0.1 ± 0.0	57.1 ± 9.6	34.6 ± 2.6
12 hours – 5 g/L	23.1 ± 1.8	0.2 ± 0.0	45.6 ± 4.6	41.3 ± 1.9
Pepsi				
2 hours – 5 g/L	43.0 ± 3.1	0.4 ± 0.0	93.9 ± 7.6	71.0 ± 4.1
2 hours – 10 g/L	34.1 ± 5.7	0.4 ± 0.1	99.0 ± 0.06	68.9 ± 14.4
2 hours – 15 g/L	44.2 ± 0.3	0.4 ± 0.0	97.2 ± 1.5	73.3 ± 1.8
12 hours – 5 g/L	45.1 ± 0.3	0.5 ± 0.1	99.3 ± 0.1	73.6 ± 2.7
Control	40.3 ± 3.6	0.5 ± 0.1	89.0 ± 10.0	73.7 ± 8.7

Activated carbon adsorption for sodium benzoate removal

Several concentrations of BG-HHM activated carbon and one level of WPH activated carbon were tested as adsorbents during a 2 h batch adsorption stage. Subsequent fermentation of the recovered fluid yielded the data shown in Table 10. BG-HHM concentrations of 0.5-5 g/L and the 5 g/L WPH level resulted in similar sugar consumption and ethanol productivity. However ethanol titers and yields were highest in the 0.5 and 2 g/L trials with BG-HHM. Evidently the 0.25 g/L BG-HHM did not remove sufficient benzoate, since sugar consumption and ethanol production were lower.

Biochar adsorption for sodium benzoate removal

Biochar is a co-product of some thermochemical biomass-to-fuel processes [20] and was evaluated

Table 12. Relative costs and performance of various treatments.

Treatment		Yield (%)	Fermentation efficiency (%)	Ethanol produced (ml) per L Mountain Dew	Treatment cost per L Mountain Dew ($)	Treatment cost per L ethanol produced ($)
Anion exchange	Biorad UNO Q1 resin	104.1 ± 11.9	95.1 ± 1.8	83.78	7.1[a]	84.75
	DowEX Monosphere 99CA/320 resin	97.8 ± 16.0	98.5 ± 2.7	70.98	0.71[b]	10.00
Activated carbon	BG-HHM 0.5 g/L	82.5 ± 12.3	93.5 ± 9.7	63.63	0.0014[c]	0.02
	BG-HHM 2.0 g/L	89.5 ± 11.4	94.4 ± 2.0	63.37	0.0055[c]	0.09
	WPH 5.0 g/L	76.7 ± 16.1	99.0 ± 1.8	54.88	0.014[d]	0.26
Biochar	5.0 g/L	45.4 ± 4.3	54.1 ± 2.4	32.45	0.0004[e]	0.01
pH Neutralized	pH 7.0	28.9 ± 0.9	39.8 ± 3.2	21.98	0.0093[f]	0.42

[a]Assuming resins can be regenerated for 70 cycles at $500/liter.
[b]Assuming resins can be regenerated for 70 cycles at $50/liter.
[c]Assuming use of a range of 0.5-2 g/L activated carbon BG-HHM at $2,500/ton.
[d]Assuming use of 5 g/L activated carbon WPH at $2,500/ton.
[e]Assuming use of 5 g/L biochar at an average of $72.50/ton.
[f]Assuming $0.925 per pound sodium hydroxide.

as a less expensive adsorbent to remove benzoate. Biochar is also commonly applied to soil to promote fertility and increased crop production [21]. Prior work has shown that biochar can absorb a range of chemicals, including pesticides in soil [22] and heavy metal such as lead [23].

Biochar treatments of Mountain Dew resulted in the lowest sugar utilization and ethanol production of any of the adsorbents tested, with fermentation efficiencies ranging from 45.6 ± 4.6 to 65.4 ± 11.8% and ethanol yields of 34.6 ± 2.6 to 45.4 ± 4.3% (Table 11). In fact, increasing the biochar concentration reduced ethanol production, suggesting that some compounds released by biochar may actually inhibit fermentation of *S. cerevisiae*. Extending the treatment time from 2 to 12 hours also reduced yeast performance. As a control we repeated these biochar treatments using Pepsi and found no significant impact of either biochar concentration or treatment time. Thus we could not confirm that biochar was leaching any inhibitory compounds into the pop, and have no explanation for the reduced performance observed in the Mountain Dew samples treated with higher levels of biochar.

CONCLUSIONS

Treatments were compared based on ethanol production and cost (Table 12). Biorad UNO Q1 and DowEX Monosphere 99CA/320 resins achieved fermentation efficiencies near 100%, and the highest ethanol yields. However these treatments were prohibitively expensive. The highest performing and most economical treatment was activated carbon BG-HHM, with an ethanol yield of 82.5%, fermentation efficiency of 93.5%, and a treatment cost of only $0.02 per liter ethanol produced. Activated carbon WPH performed similarly, but at a 10-fold higher cost. Biochar was the cheapest treatment, but ethanol yields were only 45%, while pH adjustment resulted in the lowest yields.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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