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Demonstration of In Situ Product Recovery of Butyric Acid via CO_2-Facilitated pH Swings and Medium Development in Two-Phase Partitioning Bioreactors

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ABSTRACT: Production of organic acids in solid–liquid two-phase partitioning bioreactors (TPPBs) is challenging, and highly pH-dependent, as cell growth occurs near neutral pH, while acid sorption occurs only at low pH conditions. CO_2 sparging was used to achieve acidic pH swings, facilitating undissociated organic acid uptake without generating osmotic stress inherent in traditional acid/base pH control. A modified cultivation medium was formulated to permit greater pH reduction by CO_2 sparging (pH 4.8) compared to typical media (pH 5.3), while still possessing adequate nutrients for extensive cell growth. In situ product recovery (ISPR) of butyric acid (pKa = 4.8) produced by *Clostridium tyrobutyricum* was achieved through intermittent CO_2 sparging while recycling reactor contents through a column packed with absorptive polymer Hytrel® 3078. This polymer was selected on the basis of its composition as a polyether copolymer, and the use of solubility parameters for predicting solute polymer affinity, and was found to have a partition coefficient for butyric acid of 3. Total polymeric extraction of 3.2 g butyric acid with no CO_2 mediated pH swings was increased to 4.5 g via CO_2-facilitated pH shifting, despite the buffering capacity of butyric acid, which resists pH shifting. This work shows that CO_2-mediated pH swings have an observable positive effect on organic acid extraction, with improvements well over 150% under optimal conditions in early stage fermentation compared to CO_2-free controls, and this technique can be applied other organic acid fermentations to achieve or improve ISPR.

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KEYWORDS: *Clostridium tyrobutyricum*; butyric acid; two-phase partitioning bioreactor (TPPB); in situ product recovery (ISPR); carbon dioxide

Introduction

Interest in biologically produced feedstocks as an alternative to petrochemicals has spurred substantial research into products of microbial fermentation, with the hope of improving process efficiency and reducing production costs, which would aid in overcoming the economic challenges required to attain industrial feasibility. In the case of organic acids, one of the most prominent costs incurred is product recovery, which can represent up to 60% of process costs, and typically employs techniques such as precipitation, ion-exchange, and reactive extraction (Kurzrock and Weuster-Botz, 2010). However, these approaches cannot easily be performed during fermentation, and largely represent downstream separation processes only. Two-phase partitioning bioreactors (TPPBs) represent another technique wherein an immiscible phase is included in the fermentation vessel, effectively partitioning target molecules away from fermentation broth, achieving in situ product recovery (ISPR) (Daugulis et al., 2011). Recently, research has focused on the use of absorptive commodity polymers as the sequestering phase in TPPBs as an alternative to employing organic solvents (Amsden et al., 2003; Daugulis et al., 2011; Morrish and Daugulis, 2008), as effective commodity polymers have been shown to be relatively more affordable (e.g., 5$ kg^{-}) compared to absorptive resins (e.g., 170$ kg^{-}) (Nielsen and Prather, 2009) and easier to handle compare to other liquid extractants, which are substantially more expensive (e.g. >150$ kg^{-}) (Quijano et al., 2010). To date polymer selection for use in TPPBs has been largely heuristic, which can restrict application of TPPBs to production of new molecules, as no straightforward method for predicting polymer-solute affinity has been available. Thus, development of a rational polymer selection strategy for use in TPPBs based on first principles thermodynamics is underway (Parent et al., 2012), and use of accessible polymer properties such as Hildebrand solubility parameters may provide methods of ranking polymers for solute affinity.

Regardless of phase selection, an important process concern in the extraction of organic acids is pH. It is well

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accepted that organic acids will partition into sequestering phases only as the undissociated species (Garcia, 1999; Kertes and King, 1986; Yang et al., 1991). As optimal uptake occurs below the lowest pK_a of an organic acid, extractive and bioproductive operations are therefore often exclusive due to differences in optimal pH ranges. This exclusion implies that product recovery of organic acids cannot be achieved in a manner similar to target molecules that partition independently of pH, wherein a second phase is simply added directly to a reactor. Online extraction of dissociable species thus requires both cyclic pH changes to alternate between bioproduction and extraction, and physical separation to ensure extraction efficiency.

An important operational consideration is how reversible pH swings can be achieved, especially if online extraction for ISPR is implemented as an alternative to downstream processing. Traditional acid/base addition for pH control results in elevated ion concentrations, increasing osmotic stress and hindering conventional batch fermentation (Liu et al., 2008), and this effect is exacerbated if this type of pH control is used to facilitate the necessary pH swings for extraction. An alternative is the use of carbon dioxide sparging, reducing the pH via carbonic acid dissociation. While it is well known that CO_2 lowers pH by formation and dissociation of carbonic acid, the use of CO_2 to lower pH for facilitating absorption represents a novel application of this well-known phenomenon. Previous work (Hepburn and Daugulis, 2012) has shown that alternate sparging between CO_2 and N_2 permits pH shifting between 7 and 3.5 with no ion accumulation in reverse osmosis (RO) water. In the case of media, however, the buffering capacity of medium components limited pH swings, and careful consideration of medium composition is required. This work focuses on the extraction of butyric acid produced by *Clostridium tyrobutyricum*, and the threefold objective of this study is to:

(1) test suitability of Hildebrand solubility parameters for prediction of polymer affinity for an organic acid;
(2) reformulate a medium for *C. tyrobutyricum* that minimizes buffering capacity, while not adversely affecting growth or butyric acid production; and
(3) investigate the use of intermittent CO_2-mediated pH swings to facilitate organic acid ISPR.

This approach would theoretically apply to a range of organic acids and extractive techniques, and could potentially be used for widespread application in organic acid fermentation, particularly through the use of elevated CO_2 pressures.

Materials and Methods

Organism, Polymers, and Chemicals

C. tyrobutyricum (ATCC 25755) was initially grown on media described elsewhere (Wu and Yang, 2003) and cryopreserved in 15% glycerol at −75°C until needed. All polymers were kindly donated by Arkema and DuPont, and are listed in Table I. All polymers were washed with agitation three times, first with hot tap water on a stir plate, twice with RO water, and allowed to air dry overnight to remove processing contaminants. All chemicals used in this study were purchased from Fisher Scientific Company, Ltd (Ottawa, ON).

Partitioning Coefficient Determination

Partitioning coefficients (P) for butyric acid were determined using methods outlined elsewhere (Dafoe and Daugulis, 2011), with the additional step of weighing polymers after partitioning tests to determine water absorption to allow for correction of aqueous volume at equilibrium. Hildebrand solubility parameters of the soft segments of all polymers (Brandrup et al., 1999) were used as a measure to compare predicted polymer affinity for butyric acid to observed partitioning.

Culture Conditions and Medium Formulation

C. tyrobutyricum was cultured under anaerobic conditions in sealed 150 mL serum bottles, or in sealed 500 mL shake flasks with closable vents and a spargeline, which were sparged aseptically with N_2 for 20 min post and prior to autoclaving. All bottles, flasks, and reactors described herein were autoclaved for at least 20 min at 15 psi and 121°C.

A medium formulation with minimized buffering capacity was developed capable of enhanced CO_2-pH swings. Medium A represents a typical medium found in the literature (Wu and Yang, 2003), which consists of yeast extract, $5\,g\,L^{-1}$; $(NH_4)_2SO_4$, $3\,g\,L^{-1}$; K_2HPO_4, $1.5\,g\,L^{-1}$; $MgSO_4 \cdot 7H_2O$, $0.6\,g\,L^{-1}$; $FeSO_4 \cdot 7H_2O$, $0.3\,g\,L^{-1}$. Peptone was omitted from Medium A to simplify the effect of a single complex medium component on pH swing. Medium B represents a modified formulation for improved CO_2-pH swing capacity wherein the above formulation remains unchanged except for a reduction of K_2HPO_4 to $0.3\,g\,L^{-1}$. All bottles and flasks contained $10\,g\,L^{-1}$ glucose, and were incubated at 37°C and 180 rpm. Growth and pH shifting studies were performed in

Table I. Polymer properties for Pebax® and Hytrel®.

Polymer grade	Pebax® 2533[a]	Pebax® 1074[a]	Pebax® 1657[a]	Hytrel® 3078[b]
Hard segment	Nylon 12	Nylon 12	Nylon 6	PBT
% Hard segment	20	45	40	n/a.
Soft segment	PBO	PEO	PEO	PBO
% Soft segment	80	55	60	n/a
Soft segment T_g (°C)	−77	−55	−55	−77
% Water absorption	1.2	50	120	0.8

PBT, polybutylene terephthalate; PBO, polybutylene oxide; PEO, polyethylene oxide.
[a]Yampolskii and Freeman (2010) and respective Arkema, Inc., datasheets.
[b]Dupont, personal correspondence and datasheet.

serum bottles and in bioreactors, respectively, with decreasing K_2HPO_4 and yeast extract concentrations.

CO_2–N_2 Sparging for pH Shifting in Media, With or Without Butyric Acid

To determine CO_2-pH swing capacity for media, CO_2 sparging tests were performed in 5 L Bioflo III reactors (New Brunswick Scientific, Edison, NJ), with sparging performed at 1 VVM, 500 rpm, and 37°C with no pH control. As optimal growth of *C. tyrobutyricum* occurs at pH of 6.0 (Wu and Yang, 2003), this value was used as the initial pH in all sparging tests. CO_2 was sparged for 5 min, followed by 15 min of sparging with N_2 to return the pH to its starting level. To determine the effect of butyric acid on pH swings, sparging tests were also performed on Medium B with either 5 or 10 g L^{-1} butyric acid and compared to Medium B sparge tests in RO water as a control.

Batch Reactor Culture Conditions

C. tyrobutyricum was grown in batch on Medium B in 5 L BioFlo III reactors with a 2 L working volume under anaerobic conditions at 37°C, 200 rpm agitation and 0.25 VVM N_2 sparging, while pH was controlled to 6.0 by addition of 3 M KOH and H_2SO_4. Inoculum was generated on Medium B over 12 h first in serum bottles, then flasks as described above and added anaerobically to reactors (10% v/v). All batch reactors were grown on 60 g L^{-1} glucose.

CO_2-pH Swing Mediated Online Extraction of Butyric Acid

To demonstrate that pH dependent extraction of an organic acid can be facilitated by CO_2 during fermentation without the use of strong acid addition, batch reactors were prepared with modified medium and inoculated as described above. Once automatic pH control was initiated and 50 mL 3 M KOH were added, one reactor was sparged with 1 VVM CO_2 at 500 rpm, with the resultant pH decrease recorded using TracerDAQ data acquisition software (MicroDAQ.com, Ltd, Contoocook, NH), while the second reactor was left as a CO_2-free control. After sparging, the contents of both reactors were then cycled anaerobically at 3 L h^{-1} for 1 h through a 1-L glass column packed with 700 g of Hytrel 3078, corresponding to a polymer fraction of 35% (w/v) within the total system. The packed column was autoclaved and sparged with N_2 to drive of excess oxygen prior to extraction. CO_2-mediated pH swing extractions were performed four times over the course of the fermentation, separated by 3-h intervals. To characterize extraction, fresh polymers were employed for each extraction event, and triplicate overnight desorptions in 2 L of 0.1 N KOH were performed on polymer masses post extraction to determine butyric acid absorbed at each point over the course of the fermentation. To determine what effect both extractive runs exerted on microbial activity, a batch reactor without extraction was run as a control.

Analytical Methods

Aqueous samples were analyzed using HPLC (Varian Prostar, Mississauga, ON) with a Varian Hi-Plex H column (300 × 7.7 mm) at 60°C with a 10 mmol L^{-1} H_2SO_4 mobile phase at 0.7 mL min^{-1}, and a UV–Vis detector (Varian Prostar, PS325) at 220 nm. Cell concentration was measured using optical density at 600 nm. Glucose was measured using the dinitrosalicylic (DNS) assay (Miller, 1959) at 540 nm.

Results and Discussion

Polymer Selection and Partition Coefficient Calculation

If polymers are to demonstrate good absorption of any target molecule, important polymer properties to consider are glass transition temperature (T_g), crystallinity, and polymer-solute affinity. Recent work has identified T_g and crystallinity as key initial determinants for selection of absorptive properties, citing the need for chain mobility and intermolecular free space to permit diffusion of the target molecule into a polymer matrix (Parent et al., 2012). While such physical polymer properties are relatively straightforward as criteria for polymer selection, determination of polymer-solute affinity is more challenging.

In terms of solute affinity, previous work has highlighted good partitioning of hydrophilic target molecules into polyether copolymers such as Pebax® (Arkema) and Hytrel® (DuPont), both polyether block copolymers with polyamides and polybutylene terephthalate (PBT) as respective hard segments (Dafoe and Daugulis, 2011; Gao and Daugulis, 2010; Hepburn and Daugulis, 2012; Prpich and Daugulis, 2004). In this study, three grades of Pebax® (Grades 2533, 1074, and 1657) were tested based on differences in their hard and soft segments, as well Hytrel 3078. All selected hard segments (nylon-6, nylon 12, and PBT) are semicrystalline with glass transition temperatures higher than room temperature, and thus it is likely that any absorption will be achieved only by the polyether soft segment, whether polyethylene oxide (PEO) or polybutylene oxide (PBO). To try to explain this affinity, use of Hildebrand solubility parameters (Hildebrand and Scott, 1962) have been suggested as a potential tool (Parent et al., 2012). Solubility parameters are commonly used terms widely available for most polymers and solvents, and can be used to predict binary polymer–solvent interactions, wherein the absolute difference between the parameters for two given materials can predict solubilization, and the minimization of this difference results in improved solubility. By comparing the difference between solubility parameters of respective soft segments and that of butyric acid, it may be possible to explain differences in partitioning across polymer grades.

Table II shows that the soft segment solubility parameter for PEO-bearing polymers Pebax® 1074 and 1657 (18.5 $MPa^{1/2}$) is closer to that of butyric acid (20.3 $MPa^{1/2}$), compared to the soft segment solubility parameter of PBO-bearing Pebax® 2533 and Hytrel® 3078 (18.1 $MPa^{1/2}$), and thus it would be

Table II. Polymer soft segment solubility parameters (SS δ, $MPa^{1/2}$), respective difference to butyric acid solubility parameter ($\delta - \delta$) (Brandrup et al., 1999) and partitioning coefficients (P) of butyric acid into Pebax®, with additional corrected *P*-values, with subscripts H_2O and % SS representing water and soft segment fraction.

Polymer	SS δ	$(\delta - \delta)$	*P*	P_{H_2O}	$P_{\%SS}$	$P_{H_2O+\%SS}$
Pebax® 1074	18.5	1.8	3.5	4.0	6.4	7.3
Pebax® 1657	18.5	1.8	2.3	3.5	3.8	5.8
Pebax® 2533	18.1	2.2	4.1	4.1	5.1	5.1
Hytrel® 3078	18.1	2.2	3	3	n/a	n/a

expected that PEO-bearing grades would yield better partitioning. However, Pebax® 2533 and Hytrel® 3078 yield partitioning coefficients of 4.1 and 3, respectively, compared to that of PEO-bearing Pebax® 1074 ($P = 3.5$) and 1657 ($P = 2.3$), suggesting limitations in the use of solubility parameters, if conventional approaches to calculating partitioning are employed. In general, partitioning is calculated through observation of equilibrated aqueous concentrations, with mass balance calculations used to determine solute concentration in the polymer phase. However, inaccuracies can exist, if mass balances assume only uptake of the solute, while ignoring other parameters such as water transport. Specifically, PEO-bearing grades have high moisture absorption (Table I), and this water absorption must be taken into account when calculating partition coefficients, as the decrease in volume skews aqueous solute concentration to appear higher, resulting in underestimation of partitioning. An accurate aqueous phase volume is necessary to calculate mass balance and is easily determined by observing changes in polymer mass. If volume decreases in the aqueous phase are accounted for, partitioning coefficients for water absorbing Pebax® 1074 and 1657 are increased to 4.0 and 3.5, respectively. Thus, if corrections are made to reflect water uptake, similar partitioning is observed between PBO-bearing 2533 and PEO bearing 1074, which is still not entirely consistent with solubility parameter predictions.

Another important consideration is polymer soft segment fraction, if it is assumed that only the soft segment plays a role in partitioning. The PBO fraction of Pebax® 2533 is substantially higher than that of PEO grades (Table I), and thus presents more absorptive mass. If partitioning is normalized to reflect only absorptive mass, PEO-bearing Pebax® 1074 and 1657 show mixed improvements in uptake ($P = 6.4$ and $P = 3.8$, respectively) to that of PBO-bearing Pebax® 2533 ($P = 5.1$), and thus does not support solubility parameter predictions. If corrections for water uptake and soft segment fraction are combined, however, Pebax® 1074 and 1657 surpass 2533, yielding partitioning coefficients of 7.3 and 5.8, which fits with solubility parameter predictions. However, although both PEO grades contain similar soft segment proportions, partitioning is significantly different, which brings into question the assumption that solely soft segment is responsible for uptake. A final consideration may be a second effect of water, which can exhibit a plasticizing effect on polymers, and it is possible that this permits uptake in the hard segments as well. Overall, through the use of solubility parameters to predict affinity, while also correcting for water uptake and soft segment amount, qualitative uptake prediction was achieved. However, further work must be done to confirm the validity of these corrections before they are fully adopted. Regardless, the polymers selected using these approaches have shown a marked improvement in partitioning compared to previous studies with organic acids (Hepburn and Daugulis, 2012), and further study of these strategies is merited. In the case of these experiments, as the low melting temperature of Pebax® 2533 (130°C, Arkema datasheet) could prove problematic during steam sterilization, Hytrel® 3078 was ultimately used for the ISPR studies as it shares the same soft segment (PBO), while possessing a higher melt temperature (177°C, DuPont datasheet; DuPont Co, 2013) making it suitable for autoclaving and thus was used for all subsequent experiments.

Development of a pH-Shiftable Medium

To maximize the ability of CO_2 to decrease pH, minimization of buffering medium component concentrations is important; however, any reduction in component concentrations cannot negatively affect cell growth and acid production. Previous studies in our group determined that yeast extract and dibasic phosphate were responsible for the majority of the buffering capacity observed in media used for organic acid production (Hepburn and Daugulis, 2012). As shown in Figure 1, medium studies investigating the effect of yeast extract on growth and butyric acid production showed a direct relationship between yeast extract and microbial activity, with any reduction in yeast extract concentration showing a negative impact on both response variables. To reduce potential buffering contributed from excess dibasic phosphate, it was found that concentrations could be reduced from 1.5 to 0.3 $g\,L^{-1}$ with no observable difference to original Medium A. It is likely that any phosphate over this concentration is in excess of the cellular requirements of *C. tyrobutyricum*. Thus Figure 1 shows that while no reduction in yeast extract was possible, medium formulation B with reduced phosphate was determined to have no observable effect on growth or acid production.

Sparging tests were performed on both Media A and B to determine the extent to which phosphate reduction improved pH swing capacity. As can be seen in Figure 2, Medium B achieved a lower pH value of 4.8 in sparging tests compared to Medium A (pH 5.25), which represents a significant increase in pH swing capacity, especially considering the logarithmic nature of the pH scale. Thus, a medium formulation for *C. tyrobutyricum* was developed which resulted in lower pH values when sparged with CO_2 while not adversely growth and butyric acid production, satisfying both objectives for medium suitability in this application.

Buffering Effect of Organic Acids on pH Swing

Butyric acid, like many other organic acids, is a weak acid with a relatively high pKa (4.8), and thus is a good buffer

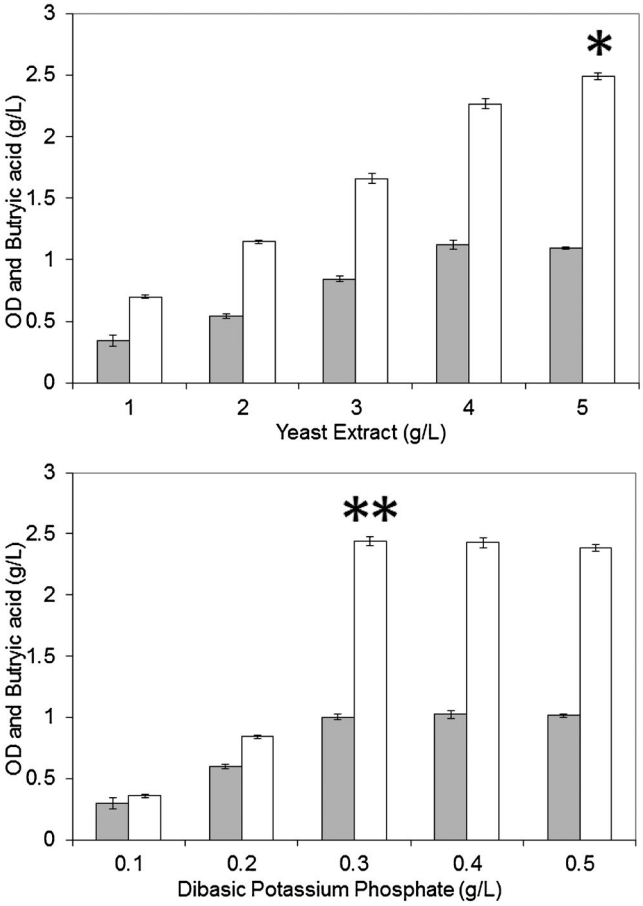

Figure 1. Growth studies of decreasing dibasic potassium phosphate and yeast extract concentrations. * represents Medium A, while ** represents modified Medium B. Gray bars represent optical density mean values, white bars represent butyric acid concentration mean values. Error bars represent standard deviation (n = 3).

itself. It is likely that the combination of pH control using base and butyric acid production would result in an increased buffering capacity as the fermentation proceeds, thus decreasing pH swing capacity and ultimately reducing extraction. It was found that butyrate has a negative effect on total pH swing (Fig. 3), with 5 g L^{-1} of butyric acid limiting pH swing in medium sparging tests to pH 5.4, while 10 g L^{-1} limited pH swing even further and only achieved a pH of 5.6, compared to a pH of 4.8, achieved on butyrate-free Medium B. At a pH of 5.6 only 14% butyric acid would be protonated compared to a 39% at a pH of 5.0, which is much closer to the pKa for butyric acid (4.8). As only the neutral protonated species will partition, early extraction would result in better uptake, preferably below or near a concentration of 5 g L^{-1}, in the case of butyrate. However, in the case of heterofermentations, by-product acids (e.g., acetic acid) would also have a similar buffering effect, and consideration of total acid production is important. Thus, base addition was used as a general measure of acid production with extractions initiated prior to the creation of elevated overall buffering capacity.

CO_2-Mediated pH Swings for Online Extraction During Fermentation

In order to determine what effect CO_2-mediated extraction has on batch performance, a comparison was made between a conventional batch run (Fig. 4A), a CO_2-free extractive control run (Fig. 4B) and an extractive run with CO_2 sparging (Fig. 4C). While observable butyric acid recovery was achieved for the CO_2 sparged treatment, no significant improvements were observed in titre or yield compared to conventional or CO_2-free runs (Table III), with approximately 40 g butyric acid produced and yields of 0.33–0.34 g of product per gram of glucose added achieved in all runs. While benefits from extraction might be expected due to the alleviation of end-product inhibition as butyric acid is known

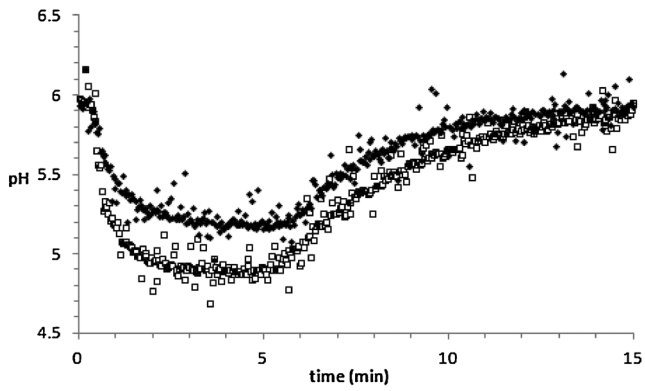

Figure 2. pH adjustment of media tested using CO_2–N_2 sparging tests at atmospheric pressure. Closed diamonds represent an original Medium A, and open squares represent improved pH swingable Medium B.

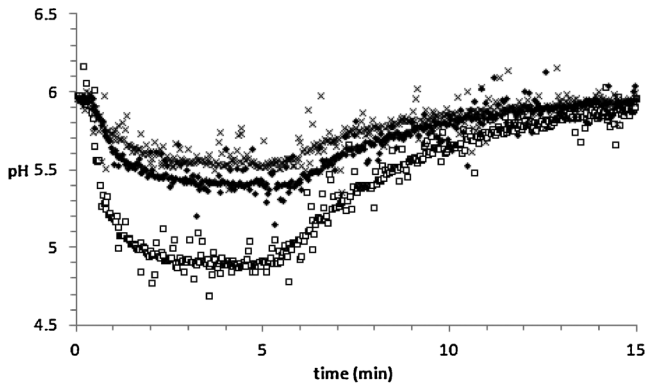

Figure 3. pH adjustment of modified Medium B containing varying amounts of butyric acid using CO_2–N_2 sparging tests at atmospheric pressure. Crosses represent medium with 10 g L^{-1}, Closed diamonds represent medium with 5 g L^{-1} butyric acid, and open squares represent Medium B.

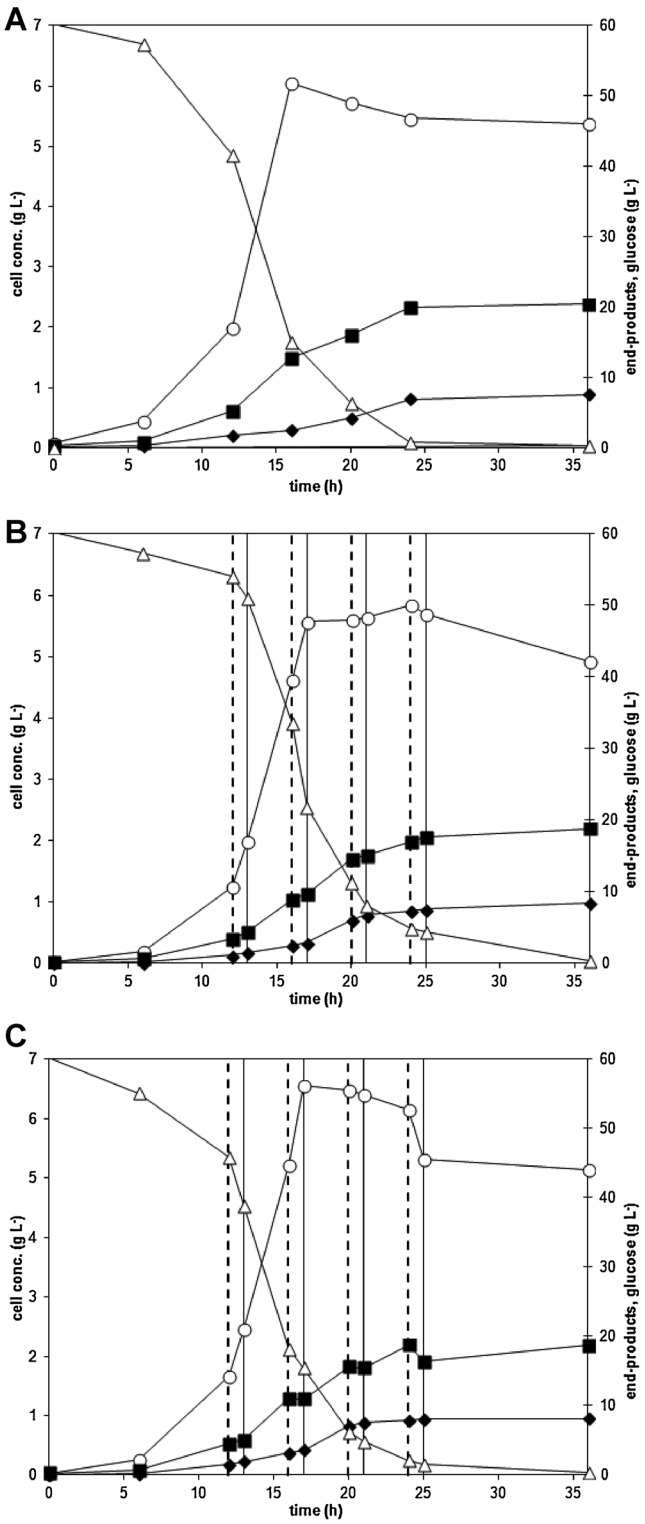

Figure 4. Batch fermentation runs of *C. tyrobutyricum* on modified Medium B for a conventional reactor (**A**) or reactors with recycle through a polymer-packed column in the absence (**B**) or presence (**C**) of CO_2 sparging. Open circles represent OD, solid squares represent butyric acid, solid diamonds represent acetic acid, and open triangles represent glucose. Dashed vertical lines indicate CO_2 sparging initiation and solid vertical lines represent CO_2 sparging termination.

Table III. Comparison of process parameters between conventional and extractive runs with or without CO_2 sparging.

Parameter	Conventional batch	CO_2-free extraction	CO_2 extraction
Total butyric acid (g)	40.9	41.1	42.0
Butyric acid yield (g/g)	0.34	0.34	0.35
Butyric: total acid ratio	0.73	0.71	0.72
Maximum cell conc. ($g\,L^{-1}$)	6.0	6.4	6.6
Base added (moles)	0.78	0.81	0.82

to be a strong non-competitive inhibitor of cell growth (Vandak et al., 1997; Zhu and Yang, 2004), the extraction achieved under these conditions proved insufficient to provide substantial product gains. However, both extractive runs demonstrated increased maximum cell growth (6.4–6.6 $g\,L^{-1}$ vs. 6.0 $g\,L^{-1}$), possibly as a result of butyric acid removal.

As direct quantification of butyric acid uptake was masked by simultaneous production of additional acid, butyric acid desorbed from the polymers was used to indicate the extent of extraction achieved. Table IV displays desorption values for each extraction, and CO_2-sparged extractions yielded 0.86, 1.20, 1.06, and 1.38 g butyric acid over the course of each extraction period, respectively, for a total of 4.51 g butyric acid recovered over the total fermentation compared to CO_2-free extractions, which yielded 0.30, 0.90, 0.82, and 1.2 g butyric acid for each respective extraction, resulting in a total recovery of 3.22 g butyric acid. Thus, CO_2 sparging improved butyric acid recovery by 186%, 33%, 29%, and 15% for each respective extraction, resulting in an overall improvement of 40%, clearly demonstrating CO_2-mediated pH swings exert a positive influence on extraction. However, as can be seen in Table IV, it is apparent that these improvements decrease during the course of the fermentation, as do the lowest pH values achieved by CO_2 sparging. This is likely due to the accumulation of neutralized butyrate salts present as a result of pH control, and this supports abiotic sparge tests demonstrating that increased butyrate has a significant negative effect on CO_2-pH swings due to increased buffering capacity, suggesting again that early extraction to maintain low butyrate concentrations would be beneficial.

As seen in Figure 5, in early extractions CO_2 was able to reduce the pH to 5.5 quickly, with a slower reduction to a pH

Table IV. Comparison of extraction parameters between extractions with or without CO_2 sparging.

	Butyric acid (g)			Initial extractive pH		Final extractive pH	
Extraction	CO_2 free	CO_2	% Improvement	CO_2 free	CO_2	CO_2 free	CO_2
1	0.30	0.86	186	6	5.5	5.5	5
2	0.90	1.20	33	6	5.6	5.3	5.2
3	0.82	1.06	29	6	5.7	5.7	5.6
4	1.2	1.38	15	6	5.7	5.8	5.6
Total	3.22	4.51	40				

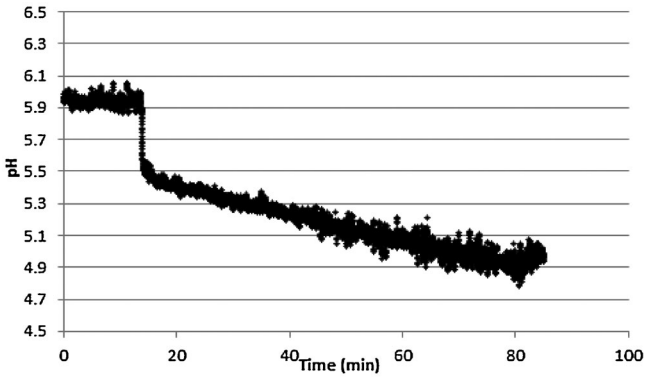

Figure 5. pH adjustment and subsequent butyric acid extraction from fermentation broth (*C. tyrobutyricum*) after 12 h using CO_2–N_2 sparging at atmospheric pressure.

of 5.0 following over the span of the extraction. As no similar secondary decrease in pH during abiotic sparging tests was observed, it is likely that this drop is a result of further acid production during extraction, facilitating a lower final pH value and further extraction. Use of acid production to achieve pH values necessary for extraction has been demonstrated in other works (Engel et al., 2011), but the use of CO_2 as an initial effector for decrease of pH reduces the time required to achieve a useful pH drop. Figure 5 also displays that N_2 sparging was not capable of returning the pH to original values, unlike sparge tests under abiotic conditions. It is likely the additional protonated acid produced during extraction buffers the system further, thus masking the stripping effects of N_2. However, as can be seen in Table III, no additional base was necessary to return pH values to fermentative conditions. Interestingly, no change in the ratio of acids was observed between conventional and sparged reactors, with the butyric:total acid ratio constant near 0.7 (Table III), indicating that short pH shifts do not result in metabolic shifts to by-product formation, which occurs with prolonged exposure to lowers pH values (Wu and Yang, 2003; Zhu and Yang, 2004).

It is important to note that the butyric acid extracted here reflects partitioning afforded by Hytrel® 3078, which shows a partition coefficient of 3. If a sequestering phase with a partitioning coefficient an order of magnitude higher was employed, and CO_2 sparging afforded a 40% increase in partitioning, this could make a marked improvement in extraction. ISPR of butyric acid has been demonstrated elsewhere (Wu and Yang, 2003), with similar studies proposed for succinic acid (Hepburn and Daugulis, 2012) and lactic acid (Krzyzaniak et al., 2013) and it is possible that the use of CO_2–N_2 sparging could generally improve ISPR of organic acids by facilitating further pH-dependent uptake. Previous work has shown that partitioning of an organic acid under acidified conditions can reach equilibrium in under 1 h (Hepburn and Daugulis, 2012) suggesting that this process is limited by pH and polymer-solute affinity. Thus in this specific case, improvements in yield and titre could be achieved through higher partitioning by both selecting polymers with increased polymer-solute affinity and improvement of pH swings through the use of elevated CO_2 pressure. Regardless, carbon dioxide sparging as described above presents a novel technique for facilitating ISPR of organic acids by allowing for rapid drops in pH to improve uptake, while avoiding osmotic stress and protracted exposure to low pH.

Conclusions

This work demonstrates CO_2-mediated pH swings result in improved organic acid ISPR, while modifying a medium to increase this effect at no expense to microbial activity, and this technique could be applied to other extractive processes to improve efficiency. This work further illustrates the ability of CO_2 to initiate rapid drops in pH during fermentation, significantly contributing to the pH drop achieved through acid production over the course of an extraction, while highlighting the importance of early extraction to mitigate buffering. The results shown here demonstrate initial attempts at CO_2-mediated ISPR, and require further steps before maximum capabilities of organic acid ISPR in TPPBs can be fulfilled. While this study demonstrates that Hildebrand solubility parameters qualitatively predict polymer-solute affinity with the use of corrections for soft segment and water uptake, Hansen solubility parameters and group-contribution activity coefficient models such as UNIFAC will be investigated with the goal of achieving superior partitioning. The identification of polymers with superior selective affinity would markedly improve extraction, not only through partitioning but by equilibrium favorability, as a superior partitioning phase would draw protonated acid away from the aqueous phase, permitting higher total amounts of undissociated acid in the system. Under these conditions, uptake would not rely on pKa, but on the partitioning-dependent "midpoint," and this would effectively reduce pH dependency. Importantly, preliminary research in our group has shown that elevated CO_2 pressures result in more effective pH swings and significant improvements in partitioning compared to atmospheric sparging, and thus future work will focus on use of pressurized vessels, rational polymer selection and equilibrium considerations to achieve effective ISPR for organic acids.

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