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Research paper

Development of a bacterial propionate-biosensor for anaerobic digestion monitoring

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ABSTRACT

Monitoring anaerobic digestion (AD) leachate for changes in acetate and propionate concentrations is essential for effective AD operation. In this paper the development of a novel propionate cell-based biosensor is described. A previously designed *E. coli* mutant (IMD Wldgy) that could selectively determine acetate concentrations in synthetic leachates, based on oxygen uptake measurements, was used as a starting point in the development of a propionate biosensor. However, the propionate-grown IMD Wldgy cells exhibited extremely low propionate:acetate O_2 consumption ratios (1:2.4). Screening for alternative propionate-grown *E. coli* strains naturally possessing a more favourable propionate:acetate O_2 consumption ratio identified strain IMD 1, which exhibited a positive ratio (1.6:1). To improve the selectivity of the strain, successive gene knockouts were performed generating the IMD 1 hldgyep mutant. However, propionate-grown IMD 1hdlgyep's O_2 consumption ratio was deemed too low to be considered as a propionate detecting bio-element. It was reasoned that the mechanisms by which *E. coli* activates acetate had to be removed. Deleting *acs* (acetyl-CoA synthesase) and *ackA* (acetate kinase) from IMD Wldgyep, resulted in an *E. coli* IMD Wldgyepak knockout mutant that, when grown on propionate, produced a mean propionate:acetate O_2 consumption ratio of approx. 13:1. The resulting IMD Wldgyep and IMD Wldgyepak strains, which formed the acetate- and propionate-biosensor, respectively, were capable of detecting acetate and propionate concentrations ranging from 0.05 mM to 4.5 mM within two-phase AD synthetic leachates.

1. Introduction

The primary objective of any Anaerobic Digestion (AD) plant is to maximise methane yields, organic loading rates (OLR) and the destruction of volatile solids (VS), while minimising reactor volume sizes and hydraulic retention times [1–3]. High throughput two-phase AD systems adhere most closely to this primary objective but require extensive monitoring and control (MC), which currently requires specialised instrumentation and its assessments are generally subsequent to the activities in the reactor, so are not done in most instances. Therefore, many commercial AD systems function sub-optimally [3]. It has been demonstrated that the deviation of the propionate degradation rate in relation to that of acetate in a two-phase AD reactor occurs before a change in chemical oxygen demand (COD) reduction efficiency or drop in pH is observed [4], which are the parameters current MC technologies monitor to determine the efficient functioning of an AD system. Thus the rapid, accurate measurement of the utilisation of these two compounds could be used to indicate reactor performance. The use of acetate and propionate concentrations as indicators of performance was further supported since AD batch reactors that were supplied with acetate and propionate concentration ratios below 2:1 required extended HRTs to produce comparable VS destruction and methane yields, when compared to ratios above this value [5]. There have been repeated calls for a means to accurately measure an AD system's individual volatile fatty acid (VFA) concentrations as a means to identifying near real time AD system health [4,6]; however, current monitoring techniques are not suitable for in-situ deployment. A sensor capable of detecting an AD system's acetate and propionate concentrations is required to be accurate, possess a suitably long operational lifespan (stability) and not require extensive sample pre-treatment.

An *E. coli* acetate-biosensor based on whole cell biological oxygen demand sensors [7,8], capable of catabolising acetate with the exclusion of all other organic acids in synthetic leachate (SL), has been developed [9]. It was shown to be accurate, remained stable for three to four days and did not require sample filtration. However, the *E. coli* strain, although capable of differentiating between propionate concentrations in propionate-only samples, was incapable of selectively

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catabolising propionate when acetate was present in solution. Thus, further development of a selective propionate biosensor is required. In this paper two approaches to this were explored: firstly, the identification of an *E. coli* strain that has a naturally low acetate utilisation when grown on propionate and secondly, the deletion of key genes associated with acetate activation in the cell. Acetate- and propionate-selective *E. coli* strains were further optimised by immobilisation and the removal of metabolic pathways that are necessary for the catabolism of organic acids present in two-phase AD system-biological leachates (BL). Immobilisation significantly extended the biosensors stability periods, while the removal of organic acid-specific metabolic pathways allowed for changes in the ratio of acetate and propionate to be accurately measured by oxygen electrode in the presence of additional organic acids. It is proposed that the ability to identify a two-phase AD's individual acetate and propionate concentrations will allow for timely adjustments to the operational parameters to be made, thus ensuring AD system health is maintained.

2. Materials and methods

2.1. Bacterial growth and immobilisation

The strains and plasmids used in this study are listed in Table 1; all new strains were deposited in the Industrial Microbiology Dublin (IMD) culture collection, School of Biomolecular and Biomedical Science, University College Dublin, Ireland. Acetate- and propionate-grown cell suspensions (20 mg wet cell mL^{-1}) were grown and extracted as described in a previous study [9]. Cell immobilisations were performed by adsorbing 1 mL of a cell suspension under a moderate vacuum (3.2×10^{-2} Torr) onto a 16 mm diameter cellulose acetate membrane (0.8 µm, GE Healthcare; Fig. 1) which had been cut to fit a 20 mL Coors™ Hirsch funnel (Fig. 1c). The cellulose acetate membrane which possessed a visible cell surface layer, was carefully removed and cut to fit the tip (Fig. 1b) of an Orion 5-Star Plus Dissolved Oxygen (DO) probe (Fig. 1a). The cell surface layer facilitated the adhesion of the cellulose acetate membrane onto the DO probe's tip (Fig. 1d), after which the joined edges were fixed into position with a tight Parafilm M seal (Fig. 1e). The immobilised-DO probe was submerged and clamped into position in Tris-buffer pH 7 solution (20 mL), and stirred at room temperature (Fig. 1g). To ensure measurement repeatability and differentiability, organic acids were added when a steady DO of at least 1.5 mg L^{-1} was observed. After each measurement the cellulose acetate membrane was carefully washed with deionised water and dried with blotting paper (Fig. 1f). Immobilised cells were stored overnight in Tris-buffer at 4 °C in between sampling and remained stable for six days.

2.2. Gene knockouts

E. coli IMD R and W3110 gene knockouts and all $P1_{vir}$ phage lysate generations were performed and confirmed using techniques described in a previous study [9]. The strains, plasmids and $P1_{vir}$ phage lysates created and used in the current study are listed in Table 1.

2.3. E. coli IMD1 $P1_{vir}$ phage transduction and $P1_{vir}$ phage generation

Successive IMD 1 transductions required *E. coli* B $P1_{vir}$ phage lysates, which had to be generated from Rel606 knockout mutants that had been transduced with a respective K-12 $P1_{vir}$ phage lysate. All transduction and $P1_{vir}$ phage generation techniques used were those reported previously [9]. However, the TSA-kanamycin concentration was increased to 100 µg mL^{-1} for IMD 1 transductions as the large number of satellite colonies made the identification of successful transductants difficult.

2.4. E. coli IMD 1 electro-transformation with the pcp20 plasmid

E. coli IMD 1 h (Δ*hsdR*) cells were made electro-competent [10] and electro-transformed using a modified electroporation protocol [11], whereby the electroporator was set to 1.8 mv (1 mm Molecular Bio-Products cuvette), 200 Ω and 25 µF. However, no successful transformations were observed until; (i) a high titre pcp20 (75 µg mL^{-1}) plasmid prep was prepared (QIAGEN plasmid midi-kit); (ii) the recovery period was extended from one to four hours; (iii) electroporated cells were centrifuged (11,000 rpm for 5 min), re-suspended in 150 µL TSB and plated on TSA plates with ampicillin concentrations that had been reduced from 100 to 50 µg mL^{-1}. Successive gene knockouts using the modified transduction and electro-transformation techniques outlined above were verified using the PCR protocol described in a previous study [9], with the exception, that Phusion® High-Fidelity DNA Polymerase (New England Biolabs) was used.

2.5. Synthetic leachate composition

The synthetic leachate (SL) used in a previous study [9] was modified and comprised of D-lactate, L-lactate, ethanol, butyrate, valerate and hexanoate in a 15:15:15:1:1:1 ratio, plus acetate and/or propionate as required.

2.6. Calculating initial O_2 consumption rates

The initial O_2 consumption rate was calculated from the time required to achieve two-fifths of the difference between the starting and minimum DO mg L^{-1} value, which corresponded to the linear portion of the O_2 response curve, after an initial DO response was observed. The time required to observe an initial response was experiment specific, cell suspensions required 15–20 s, whereas immobilised cells, due to reduced diffusion rates, required 45–65 s. Mean (µ) and standard deviation (SD) values were calculated from a minimum of four initial O_2 consumption rates unless otherwise specified. Statistical significance was determined with a two-tailed unequal variance *t*-test (Microsoft Excel).

3. Results and discussion

3.1. Propionate-grown E. coli K-12 strains as the propionate-biosensor

Acetate grown IMD W cell suspension experiments (acetate-biosensor), produced propionate:aceate O_2 consumption ratios that ranged from 1:8–1:39; however, to be able to estimate propionate concentration with the exclusion of all other organic acids, a propionate-specific propionate-biosensor is required. Previous observations [12–14] indicated that propionate-grown *E. coli* could preferentially catabolise propionate over acetate owing to expression of 2-methylcitrate synthase. However, the O_2 consumption of propionate-grown *E. coli* W3110 was greater with acetate than propionate (Table 2). This high O_2 consumption with acetate was thought to be as a consequence of an active glyoxylate shunt or citrate synthase. Therefore, two knockout strains, IMD Wi and IMD Wc, in which the genes for isocitrate lyase (*aceA*) and citrate synthase (*gltA)* were, respectively, deleted, were assessed for their ability to catabolise acetate and propionate. However, only a minor improvement in the ratio of propionate:acetate catabolism was observed in IMD Wi (Table 2), therefore attention then focussed on selecting another *E. coli* strain that might differentiate between the two acids.

3.2. Propionate grown E. coli B strains as the propionate detecting bio-element

E. coli B strain Rel606 was screened for its ability to catabolise propionate as it was hoped that the variances regarding the expression

Table 1
Bacteria, plasmids and $P1_{vir}$ phages used in the study.

Bacterium, Plasmid or Phage:	Description:	Reference or Source:
Bacterium:		
W3110	*E.coli* Wild Type Strain from which various JW derivatives were created.	J.Lederberg, Laboratory of Molecular Genetics and Informatics, The Rockefeller University, NY
IMD Wi	JW3975 *aceA782*(del)::*kan E. Coli ΔaceA* single mutant	Keio Collection [19]
IMD Wc	JW0710 *gltA770*(del)::*kan E. coli ΔgltA* single mutant	Keio Collection [19]
IMD Wh	JW4313 *hsdR748*(del)::*kan E. coli ΔhsdR* single mutant	Keio Collection [19]
IMD We	JW1228 *adhE748*(del)::*kan E. coli ΔadhE* single mutant	Keio Collection [19]
IMD Wp	JW1474 *adhP771*(del)::*kan E. coli ΔadhP* single mutant	Keio Collection [19]
IMD Wa	JW4030 *acs-763*(del)::*kan E. coli Δacs* single mutant	Keio Collection [19]
IMD Wk	JW2293 *ackA778*(del)::*kan E. coli ΔackA* single mutant	Keio Collection [19]
IMD Wldgy	W3110 *ΔlldD dld glcD ykgF* - quadruple mutant	[9]
IMD Wldgye	W3110 *ΔlldD dld glcD ykgF adhE* - quintuple mutant	This study.
IMD Wldgyep	W3110 *ΔlldD dld glcD ykgF adhE adhP* - sextuple mutant	This study.
IMD Wldgyepa	W3110 *ΔlldD dld glcD ykgF adhE adhP acs* - septuple mutant	This study.
IMD Wldgyepk	W3110 *ΔlldD dld glcD ykgF adhE adhP ackA* - septuple mutant	This study.
IMD Wldgyepak	W3110 *ΔlldD dld glcD ykgF adhE adhP acs ackA* - octuple mutant	This study.
IMD R	Rel606 wild type strain	*E.coli* Genetic Stock Centre, Yale University.
IMD Ri	Rel606 *ΔaceA* – single mutant	This study.
IMD Rc	Rel606 *ΔgltA* – single mutant	This study.
IMD 1	Putative *E. coli* B (medical isolate)	IMD culture collection, University College Dublin
IMD 1h	IMD 1 *ΔhsdR* – single mutant	
IMD 1hldgy	IMD 1 *ΔhsdR lldD dld glcD ykgF* – quintuple mutant	This study.
IMD 1ldgye	IMD 1 *ΔhsdR lldD dld glcD ykgF adhE* – sextuple mutant	This study.
IMD 1ldgyep	IMD 1 *ΔhsdR lldD dld glcD ykgF adhE adhP* – septuple mutant	This study.
Plasmids:		
pCP20	Extracted from the *E. coli* BT340 strain which was acquired from CGSC.	[21]
pUC19	Transformation protocol positive control	Thermo scientific
$P1_{vir}$ Phage lysates:		
$P1_{vir}$ stock	Generalised transduction of E. coli strains.	E.coli Genetic Stock Centre, Yale University.
K-12 *ΔlldD*	JW3580 *lldD789(del)*::*kan* - $P1_{vir}$ lysate	[9]
K-12 *ΔdlD*	JW2121 *dld759*(del)::*kan* - $P1_{vir}$ lysate	[9]
K-12 *ΔglcD*	JW2946 *glcD753*(del)::*kan* - $P1_{vir}$ lysate	[9]
K-12 *ΔykgF*	JW0300 ykgF750(del)::kan - $P1_{vir}$ lysate	[9]
K-12 *ΔaceA*	JW3975 *aceA782*(del)::*kan* - $P1_{vir}$ lysate	This Study.
K-12 *ΔgltA*	JW0710 *gltA770*(del)::*kan* - $P1_{vir}$ lysate	This Study.
K-12 *ΔhsdR*	JW4313 *hsdR748*(del)::*kan* - $P1_{vir}$ lysate	This Study.
K-12 *ΔadhE*	JW1228 *adhE748*(del)::*kan* - $P1_{vir}$ lysate	This Study.
K-12 *ΔadhP*	JW1474 *adhP771*(del)::*kan* - $P1_{vir}$ lysate	This Study.
K-12 *Δacs*	JW4030 *acs-763*(del)::kan - $P1_{vir}$ lysate	This Study.
K-12 *ΔackA*	JW2293 *ackA778*(del)::*kan* - $P1_{vir}$ lysate	This Study.
B *ΔlldD*	IMD Rl *ΔlldD* - $P1_{vir}$ lysate	This Study.
B *ΔdlD*	IMD Rd *Δdld* - $P1_{vir}$ lysate	This Study.
B *ΔglcD*	IMD Rg *ΔglcD* - $P1_{vir}$ lysate	This Study.
B *ΔykgF*	IMD Ry *ΔykgF* - $P1_{vir}$ lysate	This Study.
B *ΔhsdR*	IMD Rh *ΔhsdR* - $P1_{vir}$ lysate	This Study.
B *ΔadhE*	IMD Re *ΔadhE* - $P1_{vir}$ lysate	This Study.
B *ΔadhP*	IMD Rp *ΔadhP* $P1_{vir}$ lysate	This Study.

levels of genes responsible for *E. coli* K-12 and *E. coli* B strains' energy production pathways [15–17] could have a larger impact on propionate catabolism than the slight reduction in acetate metabolism observed in IMD Wi. *E. coli* B Rel606 propionate grown cell suspensions were supplied with acetate and propionate over forty eight hour periods and the resulting propionate:acetate O_2 consumption ratios are listed in Table 2. These varied wildly between 2.2:1 and 1:2.74, probably due to unpredictable expression of acetate and propionate catabolic genes. It was proposed that the two Rel606 mutant strains IMD Ri and IMD Rc, which possessed a defective glyoxylate pathway and TCA cycle, respectively, would inhibit the key acetate-catabolising pathways. However, propionate grown IMD Ri and IMD Rc strains only produced propionate:acetate O_2 consumption values that were comparable to the lower Rel606 wild type (WT) values observed (Table 2).

3.3. *E. coli IMD 1 as the propionate detecting bio-element*

It was thought that the wide variability in the propionate:acetate O_2 consumption values observed in Rel606 may have been strain-specific and that another *E. coli* B strain may have more favourable characteristics. A second *E. coli* B strain, IMD 1, was therefore screened. When grown on propionate this strain gave positive propionate:acetate O_2 consumption values, which unlike Rel606, were consistent for the full forty-eight hour experiment period (Table 2). *E. coli* IMD 1 cells were selected for further knockout experiments.

3.4. *Genetic manipulation of IMD1 as a potential propionate-biosensor*

Central to the development of a selective propionate-biosensor is the removal of the ability of the bacterium to catabolise other components of leachate. Thus, based on a previous study [9] a *ΔlldD dlD glcD* and *ykgF* quadruple IMD 1 knockout mutant was sought. *E. coli* K-12 and *E. coli* B strains possess strain specific restriction-modification systems called EcoK and EcoB, respectively. DNA modified by an EcoK or EcoB methylase (HsdM) transformed into an *E. coli* B or *E. coli* K-12 strain, respectively, is cleaved by the cognate restriction-endonuclease (HsdR). However, deletion of EcoK and EcoB's cognate *hsdR* genes ($HsdR^-$) ensures modified DNA can be successfully transformed between

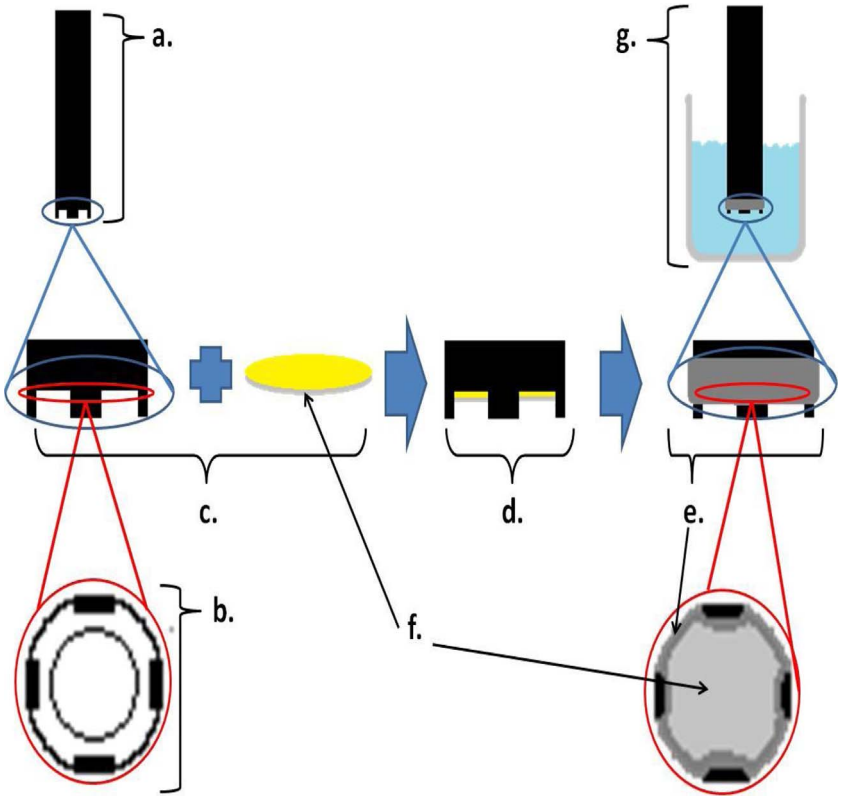

Fig. 1. Cell suspension immobilisation technique schematic; **a.** DO probe, **b.** DO probe tip viewed from underneath, **c.** Cells (yellow) adsorbed onto cellulose acetate membrane (grey) being cut to fit DO probe tip, **d.** Immobilised cells (yellow) facilitating adhesion onto tip, **e.** PARAFILM seal between edges of probe and cellulose acetate membrane, **f.** Exposed cellulose acetate membrane and **g.** Immobilised biosensor suspended in Tris-buffer.

Table 2
Propionate grown *E. coli* cell suspension screened for their mean propionate:acetate O_2 consumption ratios (initial O_2 consumption rates mg L^{-1} min^{-1}).

Strain	propionate:acetate O_2 consumption ratios
W3110	1:2.4
IMD Wi	1:2.2
IMD Wc	1:1.25
IMD R	2.2:1–1:2.74
IMD Ri	1:1.93
IMD Rc	1:1.96
IMD 1	1.6:1[a]
IMD Wldgyepa	11:1[a]
IMD Wldgyepk	1.75:1[a]
IMD Wldgyepak	12.8:1[a]

[a] The mean propionate:acetate O_2 consumption ratio was calculated from immobilised cells as opposed to cell-suspensions.

different restriction-modification systems [18]. The various *E. coli* $P1_{vir}$ phage lysates were generated from the Keio collection EcoK strains [19], thus the putative *E. coli* B strain IMD 1 could not be transduced as its HsdR restricted all EcoK generated DNA, including the *ΔhsdR* K-12 $P1_{vir}$ phage lysate. Casali [20] proposed that EcoK- or EcoB- modified DNA can be transformed into $HsdR^+$ *E. coli* B and *E. coli* K-12 strains, respectively, if it is passed through a corresponding $HsdM^+$ $HsdR^-$ *E. coli* strain first. The *E. coli* B strain Rel606, which is $HsdM^+$ $HsdR^-$, was successfully transduced with the *ΔhsdR* K-12 $P1_{vir}$ phage lysate, and a B *ΔhsdR* $P1_{vir}$ phage lysate generated. IMD 1 was successfully transduced with B *ΔhsdR*, although at a fifty fold reduction when compared to the Rel606 control. Interestingly, the IMD 1 h:*Kan*^{R} strain could not be transduced with any of the K-12 $P1_{vir}$ phage lysates and as such all successive IMD 1 h transductions required the B $P1_{vir}$ phage lysate to be generated first (Table 1).

Successive gene knockouts required that the IMD 1 h strain's *ΔhsdR:Kan* cassette be FLP-FRT excised [21]. However, all attempts to transform the IMD 1 h with the required pcp20 plasmid using a $CaCl_2$-heat-shock method [22] were unsuccessful. It was assumed that IMD 1 h could be transformed due to the puc19 positive control successful transformations but that the larger pcp20 required a higher transformation efficiency technique when compared to K-12 strains. Successful transformations were observed when a modified electroporation transformation technique was used (materials and methods) which allowed the successive gene knockouts to produce the IMD 1hldgy quintuple mutant to be applied.

3.5. Ethanol and lactate catabolism

Initial O_2-consumption rates for *E. coli* IMD1 hldgy and IMD Wldgy with butyrate, valerate and hexanoate were insignificant (Table 3). However, measurable rates were observed in the presence of ethanol and lactate, indicating that IMD1 and IMD W had other mechanisms to catabolise these substrates that were not apparent in previously designed IMD Wldgy cell-suspension experiments [9]. The response to ethanol is substantially greater when both cell strains were immobilised (materials and methods) compared to suspended cultures and this response increased as the cells aged. It was thought that the bacterium

Table 3
Immobilised acetate grown IMD Wldgy and IMD hldgy cells supplied individual Synthetic leachate organic acid components.

Organic acid conc. (mM)		O_2 mg L^{-1} min^{-1}
Acetate	0.3	0.38
Ethanol	5	0.672
D-,L-lactate	4.5	0.285[a]
D-,L-lactate	4.5	0.05[b]
Butyrate	1	0.05
Valerate	1	0.05
Hexanoate	1	0.05

[a] This was the largest single value observed when DL-lactate was first added to IMD Wldgy cells.
[b] The mean value (from at least duplicate samples) of subsequent DL-lactate additions.

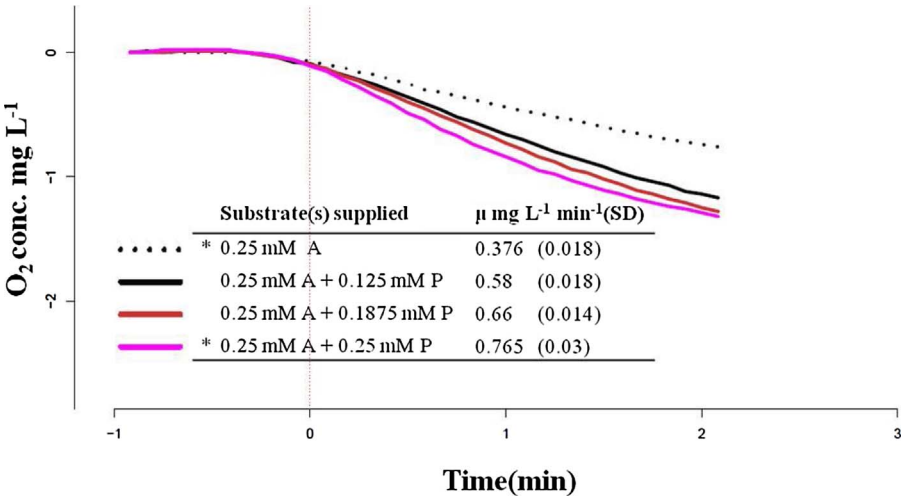

Fig. 2. The respiration response curves and mean (μ) initial O_2 consumption rates of immobilised propionate-grown IMD 1hldgyep cells supplied combined acetate and propionate concentrations. All samples were supplied with an initial SL concentration, while half were supplemented with a second aliquot of SL. *The means are statistically different ($p < 0.001$).

switched to fermentative catabolism under the immobilisation conditions, which would result in reduced oxygen transfer over time. Under these conditions *adhE*, which codes for an alcohol dehydrogenase, might be expressed [23]. When this gene was knocked out (IMD Whldgye) the mutant behaved in much the same way as the WT strain, suggesting the expression of another alcohol dehydrogenase. Shafqat et al. [24] identified AdhP that could oxidise ethanol, thus this gene was knocked out and the resulting mutants (IMD1 hdlgyep, IMD Wldgyep) were incapable of catabolising ethanol.

When freshly immobilised cells were incubated with lactate, an unexpected O_2 consumption was measured; however, subsequent lactate additions to the same cells elicited a much reduced response (Table 3). Similarly, when cells were starved of lactate, the initial lactate incubation also elicited a large O_2 consumption. To avoid any complications from lactate in leachate, all cell samples were initially incubated with SL (materials and methods). Experiments in which a second aliquot of SL was added to cells to determine whether a higher SL concentration would affect repeatability, demonstrated that there was no increase in O_2 consumption (Figs. 2–4).

3.6. Creating the propionate-biosensor's propionate-detecting bioelement

Immobilised propionate-grown IMD 1 hldgyep cells were effective in differentiating between increasing propionate concentrations when propionate-only samples were provided (Fig. S1). However, their ability to differentiate between increasing propionate concentrations when acetate was present was poor (Fig. 2) and as such the number of increments that could be differentiated within the immobilised biosensor's initial O_2 consumption rate range (0–2 mg L^{-1} min^{-1}) was deemed too low to allow for IMD 1hdlgyep to be considered as a viable propionate-detecting bio-element. To this end the exact mechanisms by which propionate-grown *E. coli* cells' propionate:acetate O_2 consumption rate was being limited, was sought and the steps required to remove it, applied.

Brock et al. [25] reported that propionate is catabolised to propionyl-CoA exclusively by acetyl-CoA sythetase (Acs) in *E. coli*, while Liu et al. [26] reported that their *E. coli* W3110 Δ*acs* knockout mutant was incapable of growing on propionate as a sole carbon source. However, Horswill and Escalante-Semerena [27] reported that *Salmonella enterecia's* propionyl-CoA synthetase (*prpE*) was capable of substituting for Acs in an acetate grown *Δacs Salmonella enterecia* knockout mutant. *E. coli* IMD Wldgyepa *Δacs* knockout mutant was created and contrary to observations made by Liu et al. [26], IMD Wldgyepa cells grew as well as the WT strain on propionate as a sole carbon source and produced a mean propionate:acetate O_2 consumption value of 11:1 (Table 2).

AckA which reversibly catabolises acetyl-P to acetate is an enzyme that, in combination with phosphotransacetylase (Pta), is the second pathway by which *E. coli* catabolises acetate to acetyl-CoA. An IMD Wldgyepk ΔackA mutant was created (Table 1) which grew as well as the WT strain on propionate and exhibited a mean propionate:acetate O_2 consumption value comparable to that of IMD 1 (Table 2). Castaño-Cerezo et al. [16] had reported that *acs* and *ackA* are expressed at higher levels in *E. coli* B when compared to E. coli K-12; however, Pta is

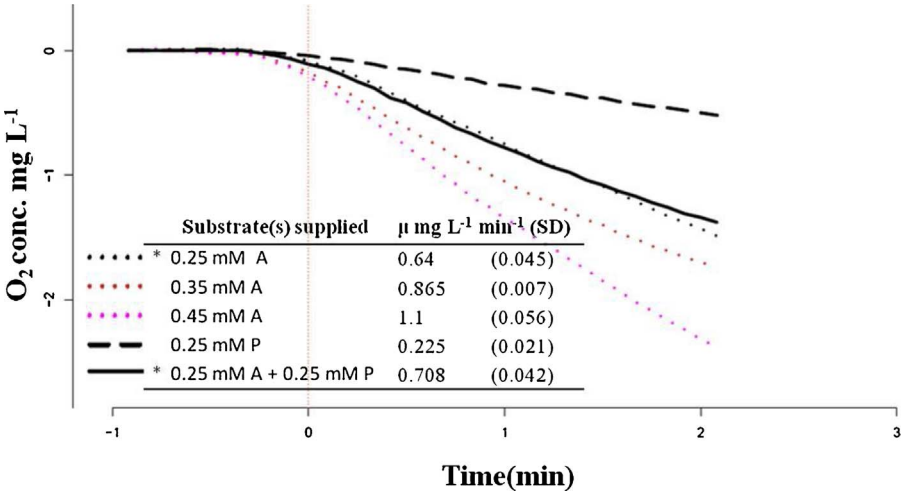

Fig. 3. The respiration response curves and mean (μ) initial O_2 consumption rates of immobilised acetate-grown IMD Wldgyep cells supplied acetate (A), propionate (P) or a combined acetate and propionate concentration. All samples were supplied with an initial SL concentration, while half were supplemented with a second aliquot of SL. * The means are not statistically different ($p > 0.05$).

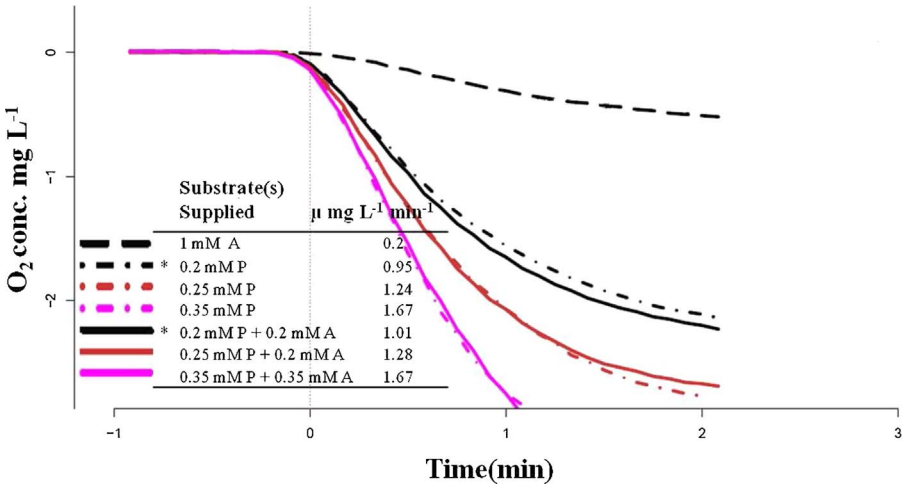

Fig. 4. The respiration response curves and mean (μ) initial O_2 consumption rate of immobilised propionate-grown IMD Wldgyepak cells supplied acetate (A), propionate (P) or a combined acetate and propionate concentration. The mean (μ) initial O_2 consumption rates were calculated from duplicate samples. The maximum difference between duplicates was 7%.* The means are not statistically different ($p > 0.05$).

expressed at higher levels in *E. coli* K-12 cells when compared to *E. coli* B. The inability of IMD Wldgyepk to convert acetate to acetyl-P would have reduced but not eliminated the availability acetyl-P, which in turn could have resulted in a phenotype that is similar to a reduced level of *pta* expression.

It was thought that removal of both *acs* and *ackA* would further increase *E. coli*'s mean propionate:acetate O_2 consumption values; however, only a marginal improvement (Table 2) was observed from the resulting *E. coli* IMD Wldgyepak strain (Table 1). Although propionate is catabolised to propionyl-CoA exclusively by PrpE in propionate-grown IMD Wldgyepak cells, PrpE's ability to act as an Acs, at a reduced rate [28], is the limiting factor to further increases mean propionate:acetate O_2 consumption values.

3.7. Evaluating the (IMD Wldgyep) acetate- and (IMD Wldgyepak) propionate-biosensors

Immobilised IMD Wldgyep and IMD Wldgyepak cells' ability to catabolise acetate and propionate with the exclusion of all other SL organic acids has been established; however, the proposed dual acetate- and propionate-biosensor requires that acetate and propionate are identifiable from combined acetate, propionate and SL organic acid samples. The acetate-biosensor, which comprised of immobilised IMD Wldgyep cells, was assessed for its ability to catabolise acetate with the exclusion of propionate (Fig. 3), while the propionate-biosensor which comprised of immobilised IMD Wldgyepak cells, was assessed for its ability to catabolise propionate with the exclusion of acetate (Fig. 4).

It was evident that both the acetate- and propionate-biosensor could be used to selectively determine the acetate and propionate concentrations in solutions containing acetate and propionate, respectively. The presence of a comparable propionate concentration, in addition to acetate, had an O_2 consumption value in the acetate-biosensor that was only slightly greater than acetate alone, while the presence of a comparable acetate concentration, in addition to propionate, occasionally produced a slightly lower O_2 consumption value in the propionate-biosensor than propionate alone. The acetate-biosensor's slightly increased response when propionate is present could be as a result of Acs producing cumulative catabolic response, while the propionate-biosensor's slightly reduced response could be acetate acting as a weak competitive inhibitor of PrpE. The acetate- and propionate-biosensors could be operated individually to identify acetate and propionate respectively or in tandem with the view to making the necessary real time monitoring and control adjustments to a two-phase AD deployment, based on changes in both its acetate and propionate concentrations.

4. Conclusions

MC technologies currently available to two-phase AD systems can only detect AD overloading after it has occurred. Additionally, novel VFA determination techniques, although pre-emptive, require extensive sample pre-treatment and are thus far impractical for deployment on site. Immobilised IMD Wldgyepak and IMD Wldgyep *E. coli* cells, which constitute the acetate detecting acetate- and propionate detecting propionate-biosensor, respectively, were developed to enable straightforward AD sample monitoring.

The use of whole cell DO biosensors to determine individual organic acids with the exclusion of others requires that microorganisms that possess organic acid-specific metabolic pathways, which correspond to organic acids that are to be excluded, are removed through genetic modification. Microorganisms which are capable of catabolising a targeted organic acid by non-specific metabolic pathways are problematic and require genetic modification to remove them, or screening for strains which suppresses them. Biochemical pathways that can employ more than one substrate are particularly evident in propionate-grown *E. coli,* whereby the propionate:acetate O_2 consumption ratios vary significantly between strains. The higher organic acid concentrations used with immobilised *E. coli* cells when compared to cell suspensions and the increase in cell stability periods meant that SL components, which had elicited no previous catabolic activity, had to be adjusted for. The first steps towards developing a whole-cell acetate- and propionate-biosensor MC capable of detecting a BL's acetate and propionate concentrations have been made. However, automation and the complete removal of the biosensor's ability to catabolise lactate are required before application can be achieved.

Author agreement

We certify that all authors have seen and approved the final version of the manuscript being submitted. The article is the authors' original work, has not been published elsewhere and is not under consideration for publication elsewhere.

Contributions

CDM and KMcD conceived the research and supervised the progress.
JS conducted the experiments.
All of the authors contributed to the preparation of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.enzmictec.2017.09.011.

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