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Improving the specificity of *E. coli* acetate/propionate exclusion biosensors via iterative engineering

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ABSTRACT

Real-time monitoring of key performance indicator analytes such as acetate and propionate within anaerobic digestors (AD) is required for optimum biogas production. In this paper the further refinement of acetate and propionate whole cell (*E. coli*) exclusion biosensors is reported following an iterative process in which key metabolites that might interfere with O_2-uptake measurements are identified and genes required for their catabolism are knocked out (exclusion). Analysis of biological leachate from an AD reactor treating lignocellulosic material revealed the presence of formate, which was subsequently shown to elicit a response in previously developed *E. coli* biosensor strains. P1 phage transduction was employed to delete two genes encoding formate dehydrogenase, *fdoH* and *fdnH*, to eliminate formate catabolism. Deletion of these genes from the propionate biosensor strain W:ldgyepak abolished interference from formate and enabled accurate determination of propionate concentrations in biological leachate. However, the acetate biosensing strain E1/pGDR11-*acs*, despite not having any response to formate, responded to propionate. It was likely that this was a result of the promiscuity of the wild type acetyl CoA synthetase, which was replaced with Acs2 from *Saccharomyces cerevisiae*, resolving the problem and enabling acetate determination with the biosensor. Acetate and propionate concentrations in authentic leachate influent were estimated to be 26.5 mM and 65.5 mM, respectively, using the biosensor, and 26.6 and 70 mM, respectively, by HPLC, demonstrating the accuracy and specificity of the refined biosensor.

1. Introduction

Biogas is an important renewable energy source obtained from anaerobic digestion. Optimal production of biogas in anaerobic digestors (AD) requires careful monitoring of the metabolites acetate and propionate as markers of the AD health [1]. However, currently employed technologies are off-line and require specialised chromatographic equipment, commonly HPLC and GC. This means that in the time required to determine the status of the AD, the conditions may have changed and impacted the production of biogas. Thus, a rapid and easy-to-use monitoring system is required if biogas production from AD is to be realised as a renewable energy source.

One potential solution to the monitoring and control of AD is to employ biosensors. Examples of electrochemical enzymatic biosensors that detect a single analyte in solution such as lactic acid, glucose and ethanol are extensive [2–4]. The biosensor required for AD monitoring needs to measure more than one metabolite simultaneously, yet there are only a limited number of amperometric biosensors with which multiple organic acids can be detected within a single biological sample at the same time [5,6]. Additionally, reagents such as coenzymes required for enzymatic biosensors are expensive, further limiting their application to AD monitoring. Whole cell biosensors do not have the same limitations, but because of their extensive metabolism, are perceived to be too non-specific and their application thus far has been limited to measurement of biological oxygen demand. One way to improve the selectivity of whole cell biosensors, thereby broadening their application, is to genetically tailor the catabolism of a bacterium to ensure precise removal and addition of catabolic activities. Thus, highly specific whole cell biosensors that are inexpensive and simple to operate are feasible.

Previously we reported the genetic modification of *Escherichia coli* to generate propionate and acetate exclusion biosensors, which, when attached to an oxygen electrode, accurately determined the concentration of the metabolites in a synthetic leachate [7,8]. However, to fully

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assess their capabilities beyond the laboratory setting, testing on authentic AD biological leachates is required, since the chemical composition of these will vary depending on both the input substrate used and the biological process applied. These differences result in biological leachates possessing diverse catabolite profiles, which might interfere with any whole cell biosensor. We now report on the further development of reference exclusion biosensors that were designed in an iterative manner as shown in Fig. 1, following chemical analysis of biological leachate. To determine if the compounds interfered with the whole cell biosensor, they were either employed as potential growth substrates, or their catabolism directly assessed by measuring oxygen uptake by *E. coli* cells. If a compound was identified as one that interfered with the biosensor, the key gene(s) required for its catabolism were deleted from the bacterial genome. In this paper we report the improvement of our previously developed acetate and propionate biosensors by modifying *E. coli* to prevent formate catabolism. Furthermore, the native promiscuous acetyl CoA synthase was replaced with a more stringent one from *Saccharomyces cerevisiae*, resulting in a highly specific biosensor.

2. Materials and methods

2.1. Strains, culture conditions and gene knockouts

The strains, plasmids and $P1_{vir}$ phages that were used in the study are shown in Table 1. Methods for gene knockout were as described previously [7]. Briefly, a $P1_{vir}$ phage lysate was generated from a Keio Collection strain in which the target gene has been replaced with a kanamycin cassette flanked by flippase recognition targets (FRT). P1 phage transduction replaced the wild type gene in the host *E. coli* strain with the kanamycin cassette, which was subsequently removed by homologously recombining its FRT sites using the pCP20 helper plasmid. After the heat labile pCP20 plasmid was removed the knockout was confirmed by PCR using gene specific primers provided by Baba et al. [9]. Cell cultivation and cell extraction were similar to that described previously [8], with some modifications. The cell cultivation minimal media previously used was replaced with M9 minimal medium (6.8 g Na_2HPO_4, 3 g KH_2PO_4, 0.5 g NaCl, 1 g $(NH_4)_2SO_4$ and 1 mL SL-10 salts per litre) adjusted to pH 7 and autoclaved. Before being inoculated, the medium was supplemented with filter sterilised $MgSO_4$ (0.4 mM), $CaCl_2$ (0.1 mM) and one or more organic acids as the carbon source. Starter cultures in which 4 mL TSB was inoculated with a single colony and grown for 17–48 h at 37 °C were used as inocula. The volume of inoculum required varied depending on the experiments: for growth in minimal medium (100 mL) containing 30 mM organic acid or for E1/pGDR11-*acs*, which required 20 mM acetate and 5 mM succinate, 4 mL inoculum was required; 1 mL was used to inoculate 100 mL of TSB. Growth of E1/pGDR11-*acs2* in 10 mM acetate required an inoculum of 2 mL TSB supplemented with sodium citrate (20 mM) and thiamine (5 μg). Cultures were incubated at 37 °C, except those growing in acetate, propionate or acetate and succinate, which were incubated at 30 °C.

Saline PBS (pH 7), instead of Tris-buffer, was used to extract and resuspend biosensor cells, submerge biosensors (20 mL) and maintain them overnight (4 °C). Previously, cells were harvested at OD_{600} of 1.4 – 1.6 [7] with 20 mg wet cells mL^{-1} and immobilised onto the membrane surface [8], whereas here E1/pGDR11-*acs2* was harvested at OD_{600} 0.35 – 1.0 while all other cells were extracted between 0.9 and 1.4. Unless stated otherwise cell suspensions of 40 mg wet cells mL^{-1} were prepared and 200–500 μL immobilised onto the membrane surface.

2.2. Creating the E1/pGDR11-acs and E1/pGDR11-acs2 acetate biosensor "plug-in" strains

The acetyl-CoA Synthetase (Acs2) gene from *Saccharomyces cerevisiae* was codon optimised for *E. coli* and cloned into a pET-28b(+) expression vector by GenScript (Piscataway, NJ, USA). As *E. coli* E1 does not possess a T7 RNA polymerase, expression of this gene and *E. coli*'s native *acs* required an expression vector that possesses an IPTG inducible T5 promoter (pGdr-11). Creation of the pGDR11-*acs* and -*acs2* expression vectors required *acs* to be linearly amplified from *E. coli* W3110 by colony PCR, while *acs2* was linearly amplified using the pET-28b(+)-*acs2* plasmid as the template DNA. To clone the linear DNA fragments into pGDR-11, primers that provided flanking sequences for pGdr-11's *Bam*HI and *Hin*dIII restriction sites were used (Table S1). Cloned plasmids were transformed into E1 yielding the E1/pGDR11-*acs* and E1/pGDR11-*acs2* strains. E1/pGDR11-*acs* was cultivated in 4 mL TSB which was supplemented with sodium citrate (20 mM) and thiamine (5 μg) for E1/pGDR11-*acs2*; this was used to inoculate flasks containing 100 mL M9 medium plus organic acids. Expression of *acs* and *acs2* was induced by adding 50 μg L^{-1} isopropyl-β-D-thiogalactoside (IPTG) at time zero.

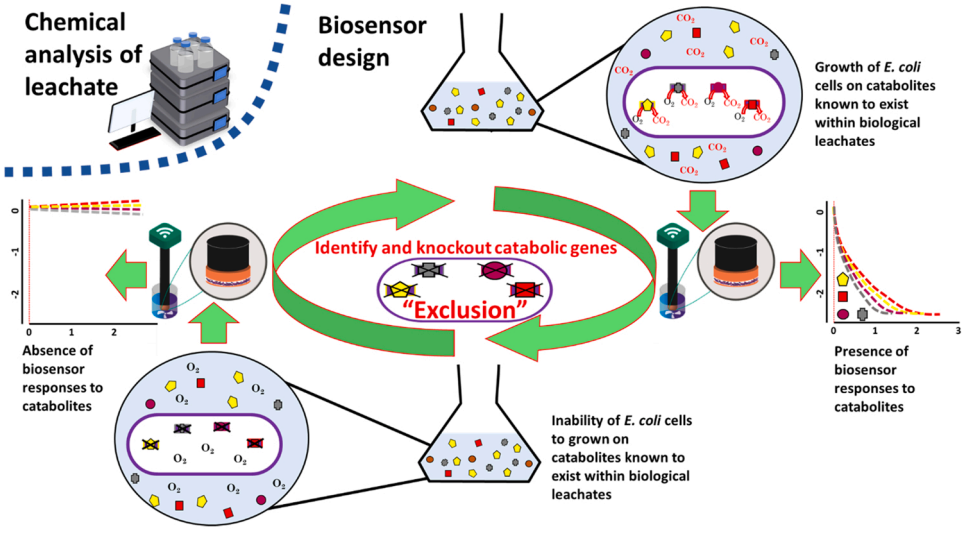

Fig. 1. Iterative design of biosensors. Following chemical analysis of leachate, *E. coli* is immobilised and assessed for its response to the different leachate components by assessing oxygen uptake. A positive response means the biosensor needs to be genetically manipulated and the success of is determined by remeasuring the oxygen uptake. The process is repeated until the biosensor responds only to the key metabolites in the leachate.

Table 1
Strains, plasmids, phages used in this study.

Strain	Genotype	Source
A1 (W: ldgyep)	W3110:: Δ*lldD, dld, glcD, ykgF, adhP, adhE*$^{(Kan\ Removed)}$	Previous study [8]
W:ldgyepak	W3110:: Δ*lldD, dld, glcD, ykgF, adhP, adhE, acs, ackA*$^{(Kan\ Removed)}$	Previous study [8]
A1::*FdoH*	W3110:: Δ*lldD, dld, glcD, ykgF, adhP, adhE, fdoH*$^{(+Kan)}$	This study
E3	W3110:: Δ*lldD, dld, glcD, ykgF, adhP, adhE, acs, ackA, prpE*$^{(Kan\ Removed)}$	This study
E3::*FdoH*	W3110:: Δ*lldD, dld, glcD, ykgF, adhP, adhE, acs, ackA, prpE, fdoH*$^{(Kan\ Removed)}$	This study
E1	W3110:: Δ*lldD, dld, glcD, ykgF, adhP, adhE, acs, ackA, prpE, fdoH, fdnH*$^{(Kan\ Removed)}$	This study
P1	W3110:: Δ*lldD, dld, glcD, ykgF, adhP, adhE, acs, ackA, fdoH, fdnH*$^{(Kan\ Removed)}$	This study
E1/ pGDR11-*acs*	W3110:: Δ*lldD, dld, glcD, ykgF, adhP, adhE, acs, ackA, prpE, fdoH, fdnH*$^{(Kan\ Removed)}$/pGdr11-*acs*	This study
E1/ pGDR11-*acs2*	W3110:: Δ*lldD, dld, glcD, ykgF, adhP, adhE, acs, ackA, prpE, fdoH, fdnH*$^{(Kan\ Removed)}$/pGdr11-*acs2*	This study
W:: Δ*prpE*	JW0326 prpE776(del)::kan acquired from CGSC	[9]
W::Δ*fdoH*	JW3864 fdoH756(del)::kan acquired from CGSC	[9]
W::Δ*fdnH*	JW1471 fdnH768(del)::kan acquired from CGSC	[9]
Plasmid	**Description**	**Source**
pGdr11	IPTG induced pQE31 expression vector derivative	[21]
pGDR11-*acs*	*acs* was linearly amplified from W3110 and cloned into the pGDR11 expression vector using *Bam*HI and *Hin*dIII restriction sites.	This study
pET-28b (+)-*acs2*	Acetyl-CoA Synthetase (*acs2*) from *S. cerevisiae* was codon optimised for *E. coli* and cloned into a pET-28b(+) expression vector	GenScript
pGDR11-*acs2*	*acs2* was linearly amplified from pET-28b (+)-*acs2* and cloned into the pGDR11 expression vector using *Bam*HI and *Hin*dIII restriction sites.	This study
pCP20	Extracted from *E. coli* BT340 which was acquired from CGSC	[22]
Phage	**Description**	**Source**
$P1_{vir}$ lysate	Generalised transduction of *E. coli* strains.	Coli Genetic Stock Center
K12 Δ*prpE*	JW0326 prpE776(del)::kan – $P1_{vir}$ lysate	This study
K12 Δ*fdoH*	JW3864 fdoH756(del)::kan – $P1_{vir}$ lysate	This study
K12 Δ*fdnH*	JW1471 fdnH768(del)::kan – $P1_{vir}$ lysate	This study

2.3. Biological leachate

Sludge collected from an anaerobic digestor configured to digest lignocellulosic material [10] was filter pressed and re-digested at lab-scale. The influent (P15a) and effluent (P15b) from this re-digestion process was kindly provided with corresponding chemical analysis by Dr. Andreas Wagner, University of Innsbruck, Austria. Volatile fatty acids (VFAs), lactate and formate concentrations were assessed by HPLC as described previously [11].

2.4. Assembly of dissolved oxygen probes

Dissolved oxygen measurements were taken using either a Thermo Scientific Orion® 5 star 083010MD (Waltham, MA, USA)- or DO-BTA Vernier® (Beaverton, OR, USA)-dissolved oxygen probe, onto which bacterial cells that were initially trapped under vacuum on cellulose membranes, were carefully cut and fixed onto the top of the probe tips using parafilm wrap as previously described [8]. For both probe types, mixed cellulose esters membranes with a pore size of 0.45 μm (GE Healthcare) were used. For the Vernier dissolved oxygen probe the protective bevels were removed to facilitate membrane attachment and the exposed tip was protected using perforated steel guards (Fig. S1).

The raw voltage signals that were produced by the Vernier DO probes were captured at one second intervals by an Arduino Uno Rev3 using Vernier's Arduino BT-ARD interface shield (Fig. S1). The raw voltage signals were then converted into mg O_2 L^{-1} using Vernier's "VernierLib" Arduino library which provides a linear calibration sketch, specific to DO-BTA raw voltage signals. For each biosensor sample, O_2 readings that were produced at one second intervals were logged by a PC to a delaminated.TXT file. The data contained within the.TXT files for specific time intervals were then plotted together via a R program plotting function, which enabled a variable delay factor (DF) to be applied, thus ensuring that O_2 consumption rates between catabolites could be compared. As previously described, O_2 consumption rates were calculated from the linear portion of the O_2 consumption response, which occurred at two- to three-fifths the difference between the initial O_2 consumption response (maximum) and minimum dissolved oxygen concentrations, in mg L^{-1} [8]. Means were calculated from at least duplicate measurements while standard deviations were calculated from at least triplicates.

2.5. Calculating biologically available acetate and propionate within biological leachates

To be able to efficiently estimate the mM of biologically available catabolite within a biological leachate sample, the following equation was applied.

$$mM_{BL\ catabolite\ sample} = mM_{standard} \times \frac{mg\ O2\ min^{-1}{}_{BL\ catabolite\ sample}}{mg\ O2\ min^{-1}{}_{standard}} \div \mathrm{mL}_{BL\ catabolite\ sample} \times 20\ mL \quad (1)$$

Where.
$mM_{standard}$ is the concentration of the standard.
$mg\ O2\ min^{-1}{}_{BL\ Catabolite}$ is the O_2 consumption rate of the BL sample.
$mg\ O2\ min^{-1}{}_{standard}$ is the O_2 consumption rate of the standard.
$mL_{BL\ catabolite}$ is the volume of the BL sample.
The total working volume is 20 mL.

3. Results and discussion

3.1. Knock out of key genes for formate catabolism is necessary to create AD biological leachate reference biosensors

In previous studies a synthetic leachate was used to assess oxygen consumption by acetate and propionate biosensors [7,8]. However, authentic biological leachate is likely to contain other compounds that might interfere with the biosensor. Biological leachate from an AD reactor treating lignocellulosic material, before and after re-digestion was collected and its composition determined by HPLC (Table 2), providing a realistic model leachate that was used to refine the biosensor further. There were several compounds detected that were not present in the previously used synthetic leachate. The VFAs isobutyrate, isovalerate and valerate were present in relatively high concentrations;

Table 2
The concentrations of volatile fatty acids (VFA) in biological leachate influent (P15a) and effluent (P15b) from an AD reactor treating lignocellulosic material.

VFA	Influent (mM)	Effluent (mM)
Acetate	26.6	1.76
Propionate	70.2	0.88
Iso-butyrate	17.88	0.36
Iso-valerate	32.04	2.84
Valerate	58.24	6.64
Lactate	0.56	0.44
Formate	0.6	1.16

however, wild type *E. coli* cannot catabolise these compounds [12], which is one reason why this bacterium is a promising whole cell biosensor. However, formate, which is a significant component of a number of anaerobic digestion leachates [1,13,14], was detected and its ability to cause reference biosensor interference needed to be assessed. Following the steps outlined in Fig. 1, *E. coli* strains A1, which was developed previously as an acetate biosensor strain [7,8] and E3, which is incapable of catabolising either acetate or propionate (Table 1), were initially investigated for their ability to grow on formate as a sole carbon and energy source. Both failed to grow, which is consistent for wild type *E. coli* [15] but this did not rule out the possibility of the cells co-metabolising formate and consuming oxygen. Therefore, E3 and A1 cells were grown on alternative carbon sources (TSB and acetate, respectively) and used as the biological element of biosensors that were then exposed to formate. As shown in Table 3, Fig. S2.a and S2.b, O_2 uptake was greater for both biosensors when formate was present compared to either acetate or glucose. The E3 biosensor had O_2 consumption rates of 1.96 and 1.99 mg O_2 min^{-1} for 1 mM glucose and formate, respectively and the A1 acetate biosensor had O_2 consumption rates of 2.38 and 2.50 mg O_2 min^{-1} for 0.45 mM acetate and 0.5 mM formate, respectively. Therefore, these biosensors are not suitable to use for the measurement of other catabolites in biological leachates that contain even small concentrations of formate. To enable the application of *E. coli* biosensors to measure catabolites, such as acetate, in biological leachates, their ability to catabolise formate must be removed.

E. coli possess three membrane-bound formate dehydogenases FDH-O, FDH-N and FDH-H, which are encoded by *fdoGHI*, *fdnGHI* and *fdhF*, respectively. As FDH-O has been proposed as being the primary mechanism by which *E. coli* oxidises formate (FdOx) [16], E3 and A1 *fdoH* knockout mutants designated E3::Δ*fdoH* and A1::Δ*fdoH*, were created, grown on TSB or 30 mM acetate, and immobilised as glucose and acetate exclusion biosensors, respectively. The A1::Δ*fdoH* acetate exclusion biosensor exhibited little or no formate:O_2 consumption response even when formate was provided at 20 times the concentration of acetate (Table 3, Fig. S2.d). In contrast, the E3::Δ*fdoH* glucose exclusion biosensor (Fig. S2.c) performed almost identically to that of E3, with O_2 consumption rates of 1.87 and 2.32 mg O_2 min^{-1} for 1 mM glucose and 1 mM formate, respectively. Therefore, a further analysis of the catabolic pathways responsible for eliciting formate:O_2 consumption responses in E3::Δ*fdoH* cells was required.

FDH-O and FDH-N's high sequence homology and their comparable formate Km values (120 μM and 160 ± 20 μM [16,17]) meant that the removal of FDN-H was intuitively the next step in removing glucose-grown E3::Δ*fdoH* cells' ability to catabolise formate. A double E3::Δ*fdoH*Δ*fdnH* knockout mutant, designated E1, was created, grown on TSB and immobilised as before. The formate:O_2 consumption responses in the glucose grown E1 cells were completely abolished even when formate was present in excess of 20 times the concentration of glucose (Table 3, Fig. S2e). The remaining formate dehydrogenase, FDH-H, did not interfere with the assay. A possible explanation for this is that the enzyme functions under anaerobic conditions and the *fdhf* gene is repressed in the presence of oxygen [18].

Table 3
O_2 consumption by *E. coli* biosensors upon exposure to different substrates after growth on either acetate (A1) or tryptone soya broth (E3) and immobilised on the Thermo Scientific Orion® 5 star dissolved oxygen probe. The concentrations of the substrates varied in the experiment and are detailed in the text. The O_2 uptake curves are shown in the SI.

	O_2 uptake mg min^{-1} (SD)		
Strain/substrate	Acetate	Glucose	Formate
E3	NM[a]	1.962 (0.01)	1.999 (0.001)
E3::ΔfdoH	NM	1.87 (0.03)	2.32 (0.01)
E1	NM	2.00 (0.06)	0
A1	2.38 (0.37)	NM[a]	2.50 (0.274)
A1::ΔfdoH	2.22 (0.05)	NM	0

[a] Not measured.

3.2. Formate exclusion enabled accurate propionate detection in biological leachate

Further manipulation of the previously reported propionate biosensor strain (W:ldgyepak, Table 1) was needed to be able to accurately measure propionate concentrations in the biological leachate. By applying the double FDH-O and FDH-N knockouts described above to W:ldgyepak, the propionate biosensor strain P1 was created (Table 1), which was grown on 30 mM propionate, harvested and immobilised as a propionate biosensor using the Vernier dissolved oxygen probe. The O_2 uptake was measured with varying concentrations of propionate and acetate, which enabled calibration of the probe and the concentration of acetate and propionate in the biological leachate influent (P15a), determined.

Fig. 2 shows the O_2 uptake response when the P1 biosensor was exposed to propionate, a mixture of propionate and acetate and two different volumes of influent leachate (P15a). The biosensor gave no response when exposed to much larger amount of acetate (data not shown) and produced identical responses with the propionate only and a mixture of propionate and acetate. Two different volumes were employed to provide a greater degree of confidence in the biosensor measurement. Each volume was aligned with the closest standard to allow for calculation of the propionate concentration using Eq. (1); the average concentration of the two measurements (65.5 mM) compares favourably to the concentration detected by HPLC (70 mM, Table 2) thereby validating the biosensor for measuring propionate in biological leachates. It should be acknowledged that by already knowing the concentration of propionate in leachate it was possible to gauge what volume to use for the biosensing in the current experiments, but for on-site application it would be necessary to titrate the leachate so that an appropriate volume is employed. The ability to conduct biosensor readings every thirty minutes coupled with the biosensor's stability of two to four days, means that for on-site applications where the concentration of analytes present within biological leachates is unknown, the application of a titration step to assess the volume of leachate that will fall within a biosensor's detection range is practically possible.

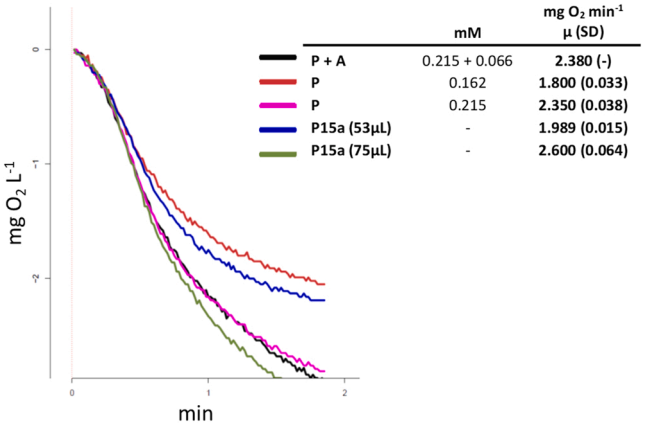

Fig. 2. O_2 uptake with P1 biosensor using the Vernier dissolved oxygen probe, when exposed to propionate (P), a mixture of propionate and acetate (P + A) and two different volumes of biological leachate influent (P15a). The concentration of propionate in P15a, calculated using Eq. 1, matches that determined by HPLC and validates the biosensor accuracy and specificity.

3.3. Acetyl CoA synthetase modification is essential for acetate-specific biosensing

The strain E1, from which FDH-O and FDH-N were removed, was assessed as an acetate biosensor after the native Acs (acetyl CoA synthetase) was cloned in a high expression plasmid and the strain transformed generating E1/pGDR11-*acs*. The strain grew poorly on acetate as its sole carbon source and as such was grown on 20 mM acetate supplemented with 5 mM succinate. These cells were harvested and used as a biosensor to measure O_2 uptake upon exposure to acetate and propionate. There was an unexpectedly high O_2-uptake recorded with 10 mM propionate (1.93 mg O_2 min^{-1}), which was between that of 0.4 mM (1.122 mg O_2 min^{-1}) and 1 mM (2.841 mg O_2 min^{-1}) acetate (Fig. 3.a). Our previous study reported that A1 cells (Table 1) immobilised as an acetate biosensor exhibited only slightly increased O_2 consumption responses for combined acetate and propionate [8]. The difference in the measurements observed in the current study might be as a result of modifications made to the DO probe set-up, as the same A1 cells tested under the current conditions now produced a comparable O_2 uptake with 0.4 mM acetate and 1 mM propionate (2.96 and 2.505 mg O_2 min^{-1}, respectively) (Fig. S3). The biochemical reason for the response to propionate was possibly a result of the promiscuity of *E. coli's* Acs, since Barak et al. [19] reported that propionate was also a substrate for the enzyme albeit with a rate that is 20 % that of acetate. This would account for the response that was observed in our experiments and since the biosensor cannot reliably detect acetate if propionate is also present, modifications to the Acs were undertaken to improve selectivity for acetate.

One possible approach to improving Acs selectivity was to replace the *E. coli* enzyme with one that was known to have a much lower activity towards propionate. A secondary acetyl-CoA synthetase in *Saccharomyces cerevisiae* has propionate activity that is only 0.1 % that of acetate [20], so this gene, *acs2*, was codon optimised, cloned into a pGDR11 and transformed into *E. coli* E1, generating a strain (E1 pGDR11-*acs2*) in which the wild type Acs is replaced with Acs2 from yeast. This strain was grown on acetate as a sole carbon source and extensively tested on acetate-only, propionate-only, combined acetate and propionate and P15a (Fig. 3.b). The response of E1/pGDR11-*acs2* to propionate (0.65 mM) was much less than that of the lowest concentration of acetate (0.15 mM), which is consistent with Acs2′s reduced ability to catabolise propionate. E1/pGDR11-*acs2* is a much more appropriate acetate exclusion biosensor strain than E1/pGDR11-*acs* and was employed to measure acetate in the biological leachate. The O_2 uptake response for 160 μL P15a (2.062 mg O_2 mL^{-1}) aligned almost exactly to that of 0.2 mM acetate (1.934 mg O_2 mL^{-1}) and as such only a single volume of P15a was assessed in triplicate. By applying Eq. (1) to 160 μL P15a and the 0.2 mM acetate standard, the concentration of biologically available acetate equated to 26.5 mM which is almost identical to what was detected using HPLC (26.6 mM, Table 2).

4. Conclusion

In this paper the modification of previously developed propionate and acetate biosensors to improve selectivity was described. The removal of formate dehydrogenase activity by knocking out *fdH-O* and *-N* ensured that interference by formate, which is present in biological leachate, was eliminated. This modification was sufficient to generate a propionate-specific biosensor (P1) that accurately measured propionate concentration in authentic biological leachate. However, both A1 and the newly created E1/pGDR11-*acs* acetate biosensor strains had an unacceptably high response to propionate. It was reasoned that this was because of the relaxed substrate specificity of acetyl CoA synthetase (Acs) in *E. coli* and was previously unrecognised owing to the physical set-up of the original biosensor. Thus, Acs that had been cloned into the high expression plasmid and transformed into E1 was replaced with the more stringent Acs2 from *S. cerevisiae*. The resulting strain was much less responsive to propionate and predicted the concentration of acetate in authentic biological leachate accurately.

The approach to generate whole cell biosensors using an iterative approach of genetic modification (knock-out and plug in) and testing on

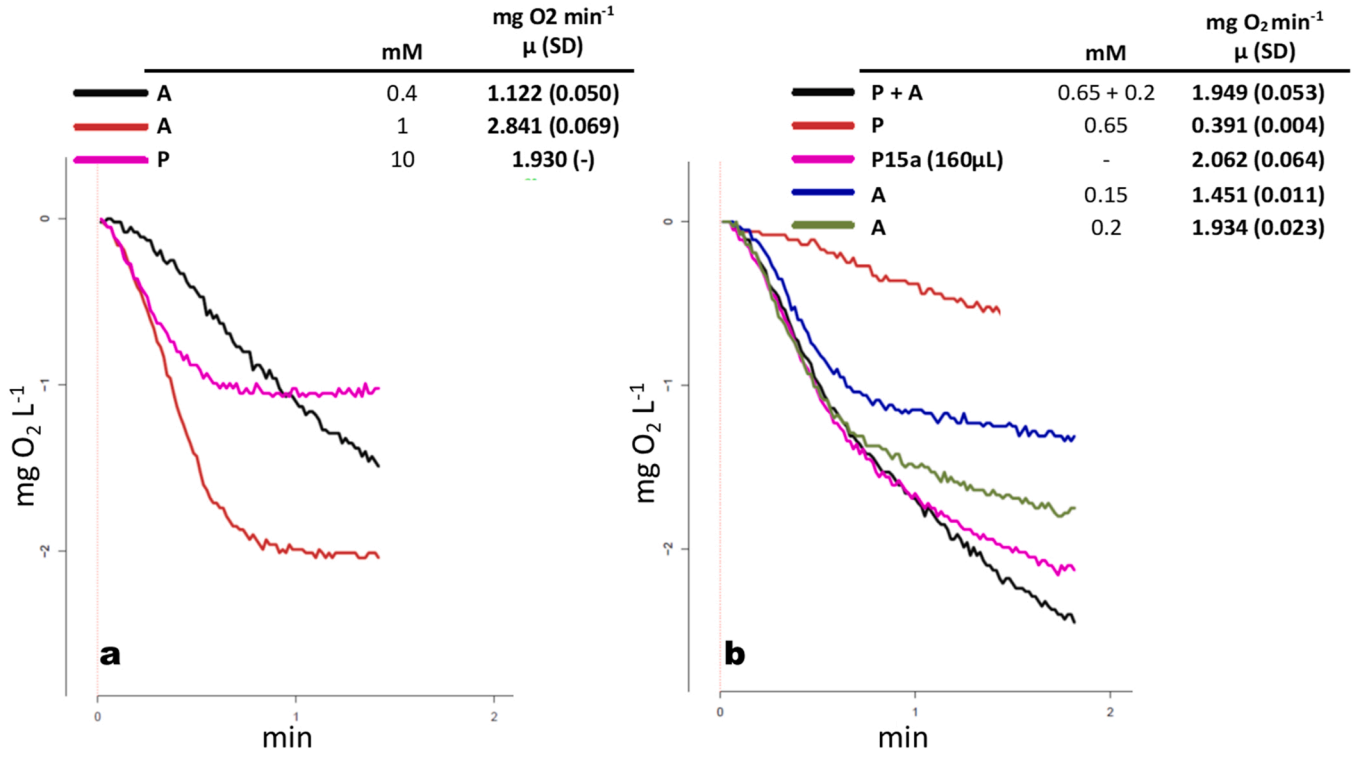

Fig. 3. O_2 uptake by the acetate biosensors (a) E1/pGDR11-*acs* and (b) E1/pGDR11-*acs2* using the Vernier dissolved oxygen probe. Replacement of *E. coli* Acs with Acs2 from *S. cerevisiae* improved the selectivity of the biosensor to acetate and enabled the accurate measurement of acetate in biological leachate (P15a).

various substrate combinations can be widely applied to a range of AD fermentations, provided the potential interfering compounds are known. Once in place an almost real-time monitoring of the fermentation enabling optimal production of biogas might be achieved.

CRediT authorship contribution statement

Joseph B. Sweeney: Conceptualization, Methodology, Software, Investigation, Resources, Data curation, Formal analysis, Writing – original draft, Visualization, Writing – review & editing, Project administration. **Kevin McDonnell:** Resources, Supervision, Project administration, Funding acquisition. **Cormac Murphy:** Resources, Writing – review & editing, Supervision, Visualization.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.enzmictec.2022.110091.

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