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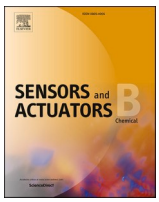

Simple, reusable, *solid-state* system for measuring total (aerobic) viable count, TVC, using the micro-respirometry method (μRM)

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ABSTRACT

Liquid micro-respirometry, Liq-μRM, is a popular, more rapid alternative method for measuring Total Viable Count of aerobes, i.e., TVC, units: colony forming units (CFU)/mL, to the traditional, time-consuming, plate counting method. Liq-μRM is based on monitoring the consumption of dissolved O_2 in a *liquid* growth medium as a function of incubation time. However, in Liq-μRM, the O_2 indicator is difficult to make, non-reusable and not well suited for use in well-plates, due to agitation issues. In this work a new, Solid-State micro-respirometry method, i.e., SS-μRM, is described based on a *solid*, rather than *liquid*, growth medium, in which the consumption of O_2 in the headspace is monitored, using a 3D printed O_2 indicator set in the lid of a small Petri dish or well plate containing the solid growth medium. The performance of the SS-μRM in a small Petri dish is compared with that of a commercial Liq-μRM system, using *Escherichia coli*, i.e., *E. coli*, as the test bacterium over the range 10^1 – 10^8 CFU/mL and demonstrated to be equivalent. The same system is used to measure the TVC of *Pseudomonas aeruginosa*, *P. aeruginosa,* and *Enterobacter cloacae*, *E. cloacae,* over the range 10^1 – 10^8 CFU/mL. When used to measure the TVC of *E. coli*, in a well plate, SS-μRM is shown to be insensitive to agitation, in contrast to Liq-μRM. The ability of the SS-μRM to address the main concerns regarding Liq-μRM are discussed.

1. Introduction

The identification and enumeration of viable bacteria in complex samples is a critical feature in food safety, environmental monitoring, and medicine, especially with regard to the detection of common and emerging foodborne pathogens, tracing outbreaks of microbial contamination and diagnosis of diseases [1]. The 'gold standard' method for measuring the total viable count of aerobes, TVC, (typical units: colony forming units (CFU)/mL) is the aerobic plate counting method, APC [2–4], which today is still widely and routinely used by industry and in microbiological research. Unfortunately, APC is time consuming and laborious, involving multiple dilutions and counting, with a time to result of up to 72 h [5,6]. It also requires a significant number of bulky consumables, such as disposable plasticware, a dedicated lab, and microbiological technical support [6]. Thus, APC is not ideal, especially when dealing with samples requiring rapid testing [7], and so there is a real demand for more rapid, high-throughput, cost-efficient methods, that are amenable to automation, for measuring TVC [8]. Recent studies suggest that one such method is based on monitoring the consumption of O_2 as the bacteria grow, i.e., micro-respirometry [9,10].

In the micro-respirometry method (for measuring TVC), μRM, the aerobic bacteria in the sample under test actively consume O_2 as they metabolise, so that the loss of dissolved O_2 provides an indicator of cell growth and proliferation [11,12]. In recent years it has been shown that μRM is able to provide an automated, user-friendly, and faster alternative to the conventional plate-counting method for measuring TVC [4, 11–14]. In μRM, the $\%O_2$ dissolved in the growth medium, initially inoculated with the sample under test, is measured as a function of incubation time, *t*, using an O_2-sensitive lumophore in the form of a polymer 'dot' or soluble dye [10,15]. Since the luminescence lifetime, τ, of the O_2 indicator is inversely related to the $\%O_2$, via the Stern-Volmer equation [10], in μRM, τ increases with increasing *t*, as the level of dissolved O_2 in the growth medium is depleted due to bacteria growth and respiration [13]. A typical τ vs incubation *t* profile, recorded using a commercial TVC system based on μRM, is illustrated in Fig. 1 for a sample comprising initially a 1 mL dilution of 10^5 CFU/mL *E. coli* in 9 mL of a growth medium, incubated at 30 °C. In μRM a set lifetime value is selected, τ_{TT}, which in Fig. 1 is 32.5 μs, and the time taken for the O_2 indicator to reach this value, the threshold time, TT, is determined. This process is then repeated for a series of inoculums of the same bacterium

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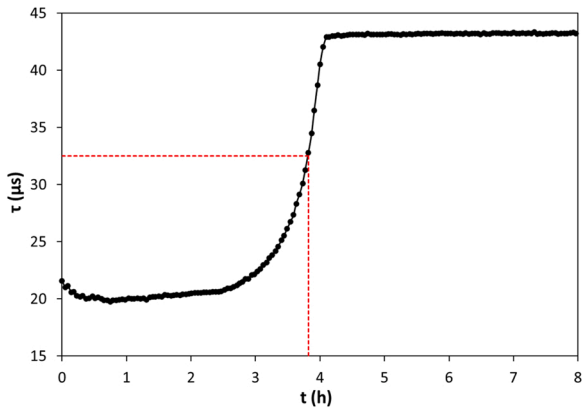

Fig. 1. Typical lifetime, τ, vs incubation time, t, trace for 10^5 CFU/mL *E. coli* recorded using the Oculer Rapid 930 instrument [9]. Red dashed line represents the τ_{TT} value of 32.5 μs, which is used to determine the TT value (3.82 h) associated with 10^5 CFU/mL *E. coli*.

at different, known concentrations, typically from 10^8 – 10^1 CFU/mL, and the results used to generate a straight-line calibration graph plot of Log (TVC) vs TT. This calibration graph is then used in all subsequent work to determine the TVC value in test samples containing the same bacterial species [11,13].

μRM has since been extended to measuring TVC in small volume samples, such as well plates (WPs), although usually this is carried out using a dispersion of the O_2 indicator, rather than an indicator 'dot' set in the base of each WP [13].

The above *liquid-based*, micro-respirometry methodology for measuring TVC, Liq-μRM, has some notable limitations. For example, the current methods used to produce the O_2 indicators are 'slow and difficult to control and standardise' [16] and, therefore, expensive. Also, the O_2 indicators, in 'dot' or dispersed form, are not reusable, which creates a significant consumable cost. Finally, when used in a well-plate, solution agitation due to the regular plate reading process can produce a significant error in the measured value of TT due to re-aeration by the ambient air; a problem that is usually addressed (in part at least) by adding a layer of mineral oil [14,17–20]. As an alternative, in this work, a simple, *reusable*, *solid-state*, micro-respirometry system for measuring TVC, SS-μRM, has been developed and characterised, which utilises a novel, inexpensive, 3D printed O_2 indicator, and its performance as a method for measuring TVC compared with that of a commercial, Liq-μRM system.

2. Experimental

2.1. Materials

Unless otherwise stated, all chemicals and solvents were purchased from Merck (Dublin, Ireland) in the highest purity available, and all aqueous solutions were prepared using double distilled, deionized water. All gases were from BOC at the highest purity. KWIK STIK stock cultures of *Escherichia coli* (*E. coli,* ATCC 8739) and *Enterobacter cloacae* (*E. cloacae,* ATCC 13047) were purchased from Microbiologics (St Cloud, Minnesota USA), and *Pseudomonas aeruginosa* (*P. aeruginosa,* NCIMB 10548) from the National Collection of Industrial, Food and Marine Bacteria (NCIMB) (Scotland, UK). Pt (II) meso-tetraphenyl tetrabenzoporphine (PtBP) was from Inochem Ltd (Carnforth, UK) and hydrophilic fumed silica (Aerosil 130) from Evonik Industries (Essen, Germany). Low-density polyethylene (LDPE) powder, melt flow index (MFI) = 20, was from PW Hall UK Ltd (Glasgow, UK). The 35 × 10 mm Nunc EasYDish™ small plastic, lidded Petri dishes and Corning™ Costar™ 96-Well, cell culture, flat-bottom microplates were from Thermo Fischer Scientific™ (Warrington, UK). Water-absorbing pads (34 mm diameter) and Greiner multi-well plate sealers were purchased from Merck Millipore Ltd (Dorset, UK). The Oculer Rapid 930 Liq-μRM instrument and the sample Falcon® tubes with an O_2-indicator 'dot' deposited in each base for use with this instrument, were from Oculer Ltd. (Tipperary, Ireland) [9]. The *liquid* growth medium was a nutrient broth (NB) containing casein yeast peptone and meat extract and agar was used to prepare the *solid* growth medium used in the APC, and SS-μRM work. Further details regarding the preparation of both growth media and the preparation of liquid cultures of *E. coli, E. cloacae,* or *P. aeruginosa* are given in section S1 of the electronic supplementary information, ESI, file. Details regarding the preparation of the O_2 indicator used in this work and how it is able to measure both O_2 in the gas phase and that dissolved in solution, are given in section S2 in the ESI [21].

2.2. Methods

Different blends of O_2/Ar of known concentration were generated using a Cole-Parmer gas blender and an Anéolia Legend O_2/CO_2 gas analyser was used to confirm the $\%O_2$ in the gas blend. All photographs were taken using a Canon 7D digital camera. In all work carried out using the 3D printed PtBP O_2 indicator, its lifetime was measured using a Pyroscience FireSting-O_2 fibre-optic oxygen meter. Further details of this phase modulation, luminescence lifetime measuring system are given in section S3 of the ESI.

2.3. The liquid and solid-state micro-respirometric systems

The conventional format for using micro-respirometry to determine TVC in biological samples, the liquid-based, micro-respirometric method, Liq-μRM, relies on the measurement of the level of dissolved O_2 in a liquid growth medium as a function of incubation time, *t*. An example of a dedicated commercial system for Liq-μRM is the Oculer Rapid 930 instrument, a carousel-type benchtop combined reader and incubator which uses disposable vials, Falcon® tubes, containing a polymeric O_2 indicator 'dot' set in its base, as illustrated in Fig. 2(a); all Liq-μRM work reported here was carried out using this instrument.

In a typical Liq-μRM experiment, the micro-respirometry process is initiated by dispensing 1 mL of each dilution of the bacterial culture under test, of known concentration, say 10^5 CFU/mL, into a 15 mL Falcon® tube containing 9 mL of a sterile liquid growth medium to a final volume of 10 mL, and an O_2 indicator 'dot' set in its base. The inoculated tube is incubated at 30 °C and the Oculer Rapid 930 instrument used to record the subsequent variation in lifetime of the O_2 indicator, τ, as a function of incubation time, *t*; Fig. 1 illustrates a typical τ vs *t* plot for an initial inoculum of 10^5 CFU/mL of *E. coli*.

In contrast to Liq-μRM, in SS-μRM a solid (agar) growth medium is

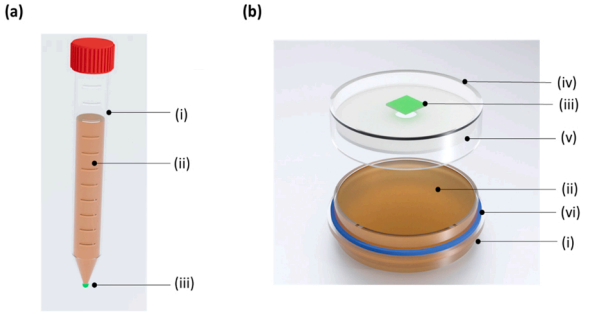

Fig. 2. Schematic illustration of the 'reactor' used in (a) the Liq-μRM system, where; (i) is a 15 mL Falcon® tube; (ii) liquid growth medium and (iii) an Oculer O_2 indicator 'dot'; (b) the SS-μRM system comprising; (i) a 35 × 10 mm mini Petri dish; (ii) solid agar growth medium; (iii) a 3D-printed PtBP/silica in LDPE O_2 indicator; (iv) lid of the dish; (v) an absorbent pad and (vi) a thin 'O' ring of Blutac® used to provide a gas-tight seal between the lid and the dish.

used, as in APC, and the sample under test is simply spread over its surface, the system sealed using a lid containing the O_2 indicator, and the $\%O_2$ in the headspace monitored as a function of incubation time, *t*. A schematic illustration of the SS-μRM system used in this work is illustrated in Fig. 2(b) and comprises a 3D printed PtBP O_2 indicator set in the lid of a plastic (35×10 mm) mini-Petri dish, with the latter filled with 8 mL of solid agar growth medium. To prevent water condensing on the lid, which otherwise interferes with the measurement of $\%O_2$, a thin, 34 mm diameter water-absorbing cellulose pad, is also set inside the lid with a 6 mm diameter hole at its centre to ensure the O_2 indicator has unrestricted access to the headspace. In a typical experiment, 20 μL of the bacteria-containing solution under test, generated from a stock solution, the concentration of which had been determined using APC, is deposited over the surface of the agar film, and then quickly sealed by pressing the rim of the lid into the Blutac®- based sealing gasket, see Fig. 2(b). Crucially, with the lid in place, a small, reproducible, fixed volume headspace (ca. 0.5 mL), is created. The lifetime of the O_2 indicator, τ, is then measured as a function of incubation time, *t*, and profiles like that illustrated in Fig. 1 are generated.

Unless stated otherwise, all microbiological assays were carried out in triplicate and the average value taken; in all cases the standard deviation, σ, was ≤ 10%, which equated to a 95% confidence interval (CI), = 1.96xσ, of ≤ ± 0.1 Log (CFU/mL). The SS-μRM and Liq-μRM methods were compared using Pearson correlation analysis, Blant-Altman analysis, and a Shapiro-Wilk normality test.

3. Results and discussion

3.1. Characterisation of the 3D-printed indicator in air

Since current methods used to produce O_2 indicators are 'slow and difficult to control and standardise' [16], such indicators exhibit significant batch-to-batch variability, limited potential for scaled-up production, and a high cost for the indicators of ca. $3–36 per indicator [16]. In contrast, 3D-printing is an ideal method for the low cost, scaled production of plastic materials, including indicators for O_2 and CO_2 [21, 22].

The sensitivity of the green coloured, 3D-printed PtBP O_2-sensitive indicator was probed by measuring its lifetime, τ, in a variety of different blends of O_2/Ar gas of known $\%O_2$, under humid conditions at 30 °C to replicate the humid conditions of the SS-μRM system. The resulting plot of the results in the form of τ vs $\%O_2$, is illustrated in Fig. 3.

A Stern-Volmer plot of the data in Fig. 3 is illustrated in the insert plot from which a value for the Stern-Volmer constant, Ksv, of $0.37\%O_2^{-1}$ was calculated. When the same experiment was carried out using the Oculer O_2 indicator, the results of which are given in section S4 of the ESI, the value of Ksv was found to be $0.090\%O_2^{-1}$. The difference between the K_{sv} values of the two indicators is most likely due to the difference in the O_2 permeability of the two different polymeric encapsulation materials, namely, that of LDPE for the 3D-printed indicator and the unknown polymer that is used to encapsulate the same PtBP dye in the polymeric 'dot' of the Oculer O_2 indicator.

In another experiment, the 90% response (0 to 21%), $t(90)_{\downarrow}$, and recovery (21 to 0%), $t(90)_{\uparrow}$, times of the 3D-printed PtBP O_2 indicator were measured by monitoring the lifetime of the indicator as a function of time, as it is exposed to a continuous cycle of air and argon. The results of this work are illustrated in Fig. 4, and reveal, $t(90)_{\downarrow}$ and $t(90)_{\uparrow}$, values of 18 and 96 s, respectively. Given in a typical μRM experiment the transition from 21% to 0% O_2 occurs over ca. 60 min, see Fig. 1, it follows that the indicator is well suited to monitor this transition.

In comparison, the 90% response (0 to 21%), $t(90)_{\downarrow}$, and recovery (21 to 0%), $t(90)_{\uparrow}$, times of the Oculer O_2 indicator were found to be 11 and 18 s, respectively (see Fig. S8 in section S4 in the ESI).

Finally, the reproducibility of the 3D printing method of production for the O_2 indicator was examined by printing twenty such indicators and measuring their lifetime in air at 20 °C, which yielded an average value of 6.1 ± 0.1 μs, i.e., a coefficient of variation of 1.6% which is slightly better than that (1.7%) reported by others using the O_2 indicators also based on PtBP and produced by Agilent Ireland (Cork, ROI) for use with their μRM instrumentation [11].

3.2. Comparison of the SS-μRM and Liq-μRM systems using E. coli

The solid state micro-respirometry, SS-μRM, system, illustrated in Fig. 2(b), was used to determine the bacterial loads in a series of dispersions of *E. coli* of known, different concentrations, as measured using APC. Thus, the *E. coli* inoculum concentration was systematically varied over the range 10^8-10^1 CFU/mL and, in each case, a 20 μL inoculum was spread over the surface of the solid agar growth medium, the system sealed, and the lifetime of the 3D printed O_2 indicator in the lid recorded as a function of incubation time, *t*. The results arising from this work are illustrated in Fig. 5(a), and the data therein used to generate the Log (CFU/mL) vs TT calibration straight line shown in Fig. 5(b).

A similar set of experiments were carried out based on Liq-μRM, using the Oculer Rapid 930 system, and the equivalent set of results generated are illustrated in Fig. 6. A quick comparison of the two data sets reveals that both methods have a similar sensitivity, gradient (*m*) = −0.64 and − 0.68 Log (CFU/mL)/h, respectively, although the SS-μRM has a larger delay time, by ca. 5 h, for each inoculum. In Liq-μRM or SS-μRM, it appears reasonable to assume that the measured value of TT will be proportional to the ratio of the number of moles of O_2, N_{O2}, to the

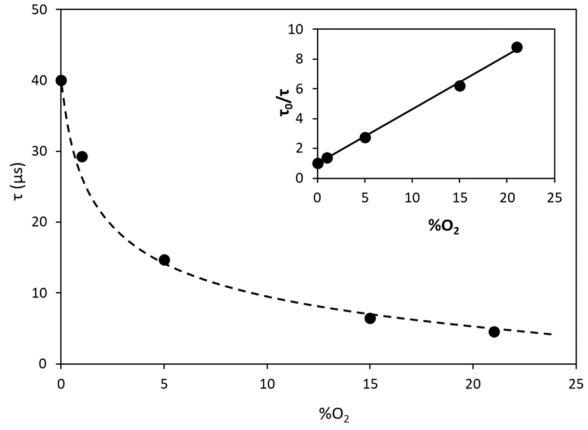

Fig. 3. Decay plot of τ (μs) for a 3D-printed PtBP O_2 indicator exposed to a range of different O_2/Ar mixtures under 100% humidity at 30 °C with [O_2] varying from 0–21%O_2. Insert is a Stern-Volmer plot of lifetime, τ_0/τ vs $\%O_2$ for the data illustrated in the main diagram, revealing a Ksv of $0.37\%O_2^{-1}$.

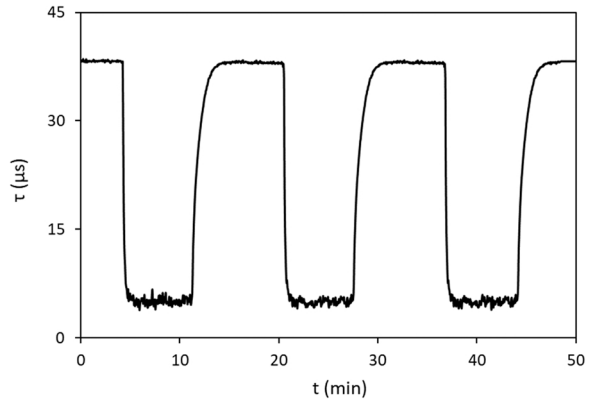

Fig. 4. Response and recovery spectra of the 3D printed PtBP O_2 indicator recorded under an alternative gas stream of argon and air under 100% humidity at 30 °C revealing a 90% response time of t90↓ = 18 s and 90% recovery time of t90↑ = 96 s.

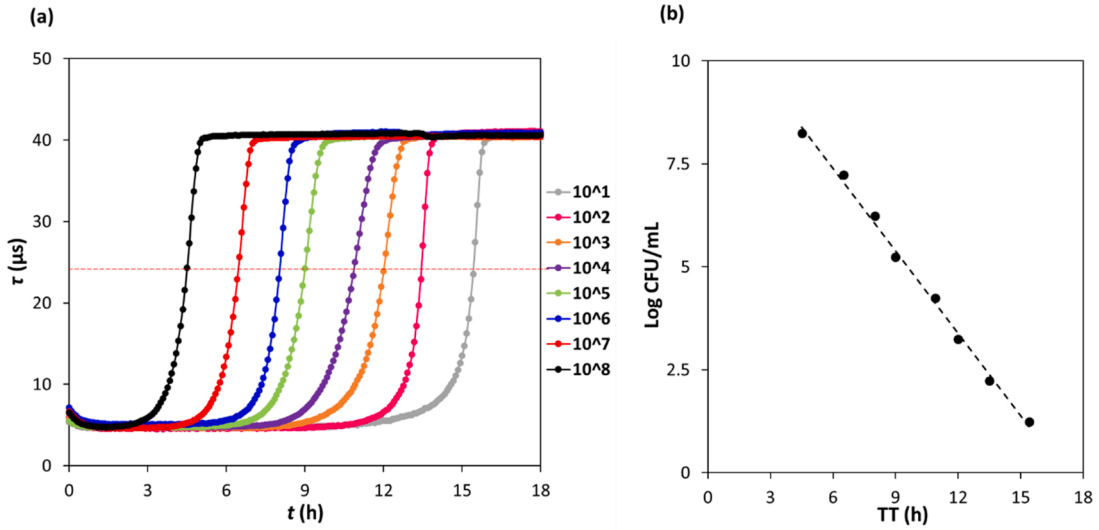

Fig. 5. (a) Lifetime vs incubation time profiles for serial dilutions of E. coli cultures ranging from 10^8– 10^1 CFU/mL) recorded using the SS-μRM system illustrated in Fig. 2(b); inoculum volume = 20 μL. The broken red line represents the lifetime threshold value of 25 μs, used to determine a TT value for each different inoculum; (b) plot of initial inoculum concentration, Log (CFU/mL), vs TT, based on the data from (a). The line of best fit (broken line) had a gradient and intercept of − 0.64 ± 0.01 and 10.8 ± 0.1, respectively.

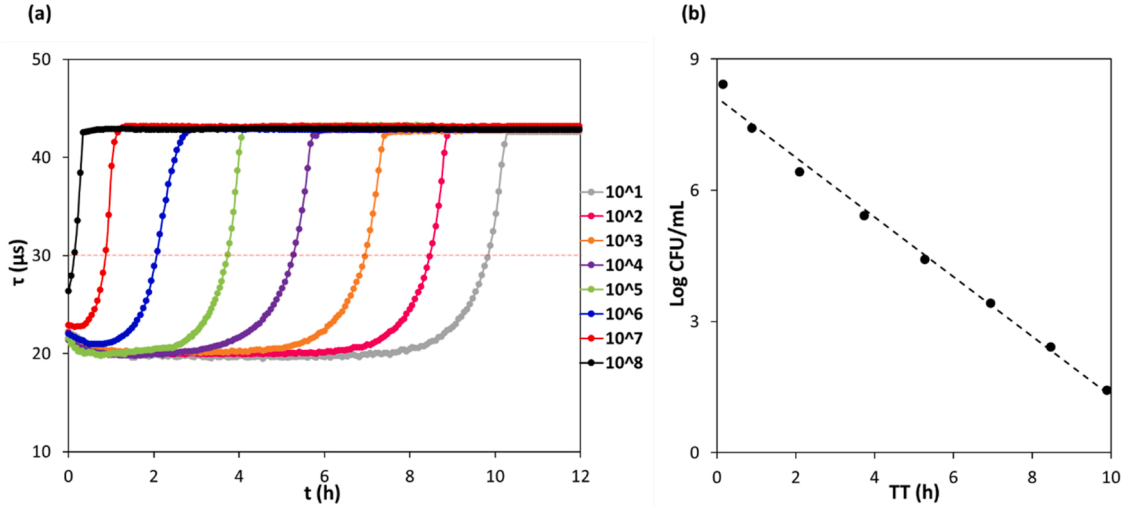

Fig. 6. (a) Lifetime vs incubation time profiles for serial dilutions of *E. coli* cultures ranging from 10^8– 10^1 CFU/mL) recorded using the Liq-μRM system; inoculum volume = 1 mL. The broken red line represents the τ_{TT} value of 30 μs, which was used to determine a TT value for each different inoculum; (b) plot of initial inoculum concentration, Log (CFU/mL), vs TT, based on the data from (a). The line of best fit (broken line) had a gradient and intercept of - 0.68 ± 0.02 and 7.69 ± 0.10, respectively.

number of bacteria, N_{bac}, present in the system. Consequently, the larger delay exhibited by SS-μRM is because of a greater value of N_{O2}, and smaller value of N_{bac}, see S5 in ESI for calculated values. It follows also that the larger delay time exhibited by SS-μRM could be reduced by either using a smaller headspace volume than 0.5 mL and/or, more easily, by increasing the sample volume from 20 μL. In addition, since the bacterial cells are trans-inoculated from a liquid one to the surface of a solid one in SS-μRM, in contrast to the all-liquid trans-inoculation in Liq-μRM, such a marked difference in growth conditions could be responsible, at least in part, for the longer lag phase associated with SS-μRM.

Interestingly, because of the longer delay in the τ vs t profile exhibited by the SS-μRM compared to the Liq-μRM, it follows that the former is better suited to measure the TVC of samples with bacterial levels, ≥ 10^8 CFU/mL. In addition, even with a ca. 5 h delay the SS-μRM system can measure TVC well within a day, and so is notably faster than APC.

The reusable nature of the O_2-indicator lid of the SS-μRM was demonstrated by recording near identical τ vs *t* profiles in four consecutive runs of SS-μRM using the same O_2-indicator lid, but with different Petri dishes filled with the same amount of solid agar growth medium, so as to create a final headspace volume of 0.5 mL and inoculated with 10^6 CFU/mL of *E. coli*; see S5 in the ESI.

To compare the performance of the SS-μRM (for measuring TVC) with that of a commercial Liq-μRM system (the Oculer Rapid 930), 16 solutions of *E. coli* of different concentration, spanning the range 10^8 to 10^1 CFU/mL, were prepared and their TVC measured using both SS-μRM and Liq-μRM. The latter is taken as the reference method given it is a well-established alternative to APC. The results of this work were then plotted in the form of Log $(CFU/mL)_{SS\text{-}\mu RM}$ vs Log $(CFU/mL)_{Liq\text{-}\mu RM}$, which yielded an excellent straight line with a gradient, *m*, = 1.00 ± 0.04 and intercept, *c*, = −0.06 ± 0.20, with a high correlation

(Pearson) coefficient, r, = 0.9948, P < 0.05. Note, the errors for m and c are the 95% confidence interval (CI) values (i.e., 1.96 x standard deviation, σ). Thus, on first inspection, the two methods appear to yield near identical results, suggesting that the SS-μRM is an appropriate alternative to Liq-μRM in the measurement of the TVC of samples containing *E. coli* at least. Tanaka *et al.* [4], when also investigating different alternative rapid methods to APC, notes that 'if the two methods behave equivalently, the slope and *y*-intercept do not significantly differ from 1 and 0, respectively'. It follows that, since the 95% CI of m of the plot in Fig. 7(a) contains 1 and the 95% CI of c contains 0, the two methods, SS-μRM and Liq-μRM, can be considered equivalent.

However, when comparing two analytical methods such a simple plot is generally not considered a sufficient demonstration of equivalence, as it does not probe any undesirable trend in the differences [23]. Thus, a more rigorous test was employed, in the form of an Altman-Bland (A-B) plot of the differences (d = (Log (CFU/mL)$_{Liq\text{-}\mu RM}$ − Log (CFU/mL)$_{SS\text{-}\mu RM}$) vs the mean, Log (CFU)$_{Av}$, = (Log (CFU/mL)$_{SS\text{-}\mu RM}$ + Log (CFU/mL)$_{Liq\text{-}\mu RM}$)/2). The results of an Altman-Bland plot of the data in Fig. 7(a) is illustrated in Fig. 7(b), and reveals a mean difference, $\bar{d}$, = 0.046 with a standard deviation, σ, = 0.17 and all points lying within the limits of agreement ($\bar{d} \pm 1.96x\sigma$ = 0.046 ± 0.34). A Shapiro-Wilk normality test on the differences showed the data were normally distributed (P = 0.566). The confidence intervals associated with $\bar{d}$, (= $\bar{d}$ +/- (σ^2/n)$^{0.5}$), has the line of equality (i.e., d = 0) as one of its borders, see Fig. 7(b), thereby indicating little, or no, significant bias exits between the two methods [24]. The Bland-Altman plot of the data, Fig. 7(b), coupled with the narrow range covered by the limits of agreement, ≤ 0.34 Log (CFU/mL), highlighted in that same plot, provide strong additional support to the analysis of the data in Fig. 7(a) and the conclusion that SS-μRM and Liq-μRM can be considered equivalent methods.

In another set of experiments, SS-μRM was also shown to work in a well-plate and proven to be indifferent to agitation, unlike its Liq-μRM counterpart. The results of this work are given in section S6 in the ESI. Note that by scaling down the system for this WP study, small differences in the culture volume (5 μL) would alter the effective headspace (60 μL) available, thus particular care must be taken to dispense accurately the 5 μL inoculum.

3.3. SS-μRM TVC measurements of other bacteria

To help validate the efficacy of the SS-μRM system as a general method for assessing TVC, a further two bacterial species, namely, *P. aeruginosa* and *E. cloacae*, were tested under otherwise the same reaction conditions as used earlier for *E. coli*, see Fig. 5. Thus, for each, the lifetime of the O_2 indicator was monitored as a function of incubation time, *t,* for a series of different initial loadings of the bacterial species under test and the resulting collection of τ vs *t* profiles were then used to construct a Log (CFU/mL) vs TT calibration curve. The results of this work are illustrated in Figs. 8(a) and (b) and 9(a) and (b) for *P. aeruginosa* and *E. cloacae*, respectively.

As with *E. coli,* the SS-μRM system works well with the two other bacteria, producing linear calibration plots with gradients, m, of − 0.34 ± 0.02 Log (CFU/mL)/h and − 0.50 ± 0.03 Log (CFU/mL)/h for *P. aeruginosa* and *E. cloacae*, respectively. The low measured values of m, for *P. aeruginosa* and *E. cloacae*, compared to that determined for *E. coli* (−0.64), are due to slower growth kinetics of *P. aeruginosa* and *E. cloacae*, compared to *E. coli,* although they can be increased, if required, by optimizing the growth medium and/or increasing the incubation temperature.

4. Conclusions

The solid state micro-respirometry method for measuring TVC, SS-μRM, appears able to address some of the concerns associated with its liquid growth medium counterpart, Liq-μRM, which is increasingly used as an alternative to APC for measuring (aerobic) TVC levels. Thus, it utilises an inexpensive, 3D printed O_2 indicator, and so is amenable to mass production. Since the O_2-indicator is located inside the *lid* of the incubation vessel (a Petri dish or well plate), rather than in the growth-media containing vessel, it is reusable. Finally, SS-μRM is indifferent to agitation, unlike Liq-μRM. This μRM micro-respirometry system addresses the demands of industry, for a rapid, simple, and user-friendly method for determining microbial growth in samples. SS-μRM, like Liq-μRM, is reliable, accurate and versatile using different bacteria and, although the SS-μRM appears slower than the Liq-μRM, it is still quicker than APC and may be of particular use when studying samples with a high (> 10^8 CFU/mL) bacterial load. Overall, SS-μRM appears to offer notable benefits over Liq-μRM, which may see its subsequent adoption as a rapid, alternative method to APC for measuring TVC.

CRediT authorship contribution statement

Yusufu dilidaer: Writing – review & editing, Methodology, Investigation, Data curation. **Mills Andrew:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Funding

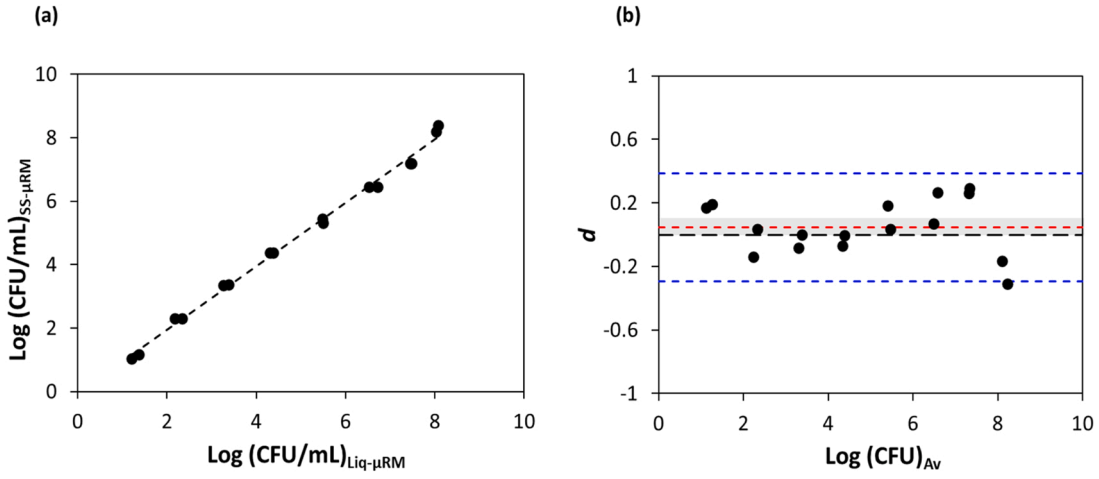

Fig. 7. Measurement of TVC in 16 different samples of *E.coli* using SS-μRM and a commercial Liq-μRM system and subsequent plot of (a) Log (CFU/mL)$_{SS\text{-}\mu RM}$ vs Log (CFU/mL)$_{Liq\text{-}\mu RM}$ and (b) d (= (Log (CFU/mL)$_{Liq\text{-}\mu RM}$ − Log (CFU/mL)$_{SS\text{-}\mu RM}$)) vs the mean, (= Log (CFU)$_{Av}$, = (Log (CFU/mL)$_{Liq\text{-}\mu RM}$ + Log (CFU/mL)$_{SS\text{-}\mu RM}$)/2)). The broken red line is the mean difference, $\bar{d}$, and the broken blue lines are the limits of agreement. The grey band highlights the confidence limits of $\bar{d}$, which touch on the line of equality, broken black line, d = 0 [23,24].

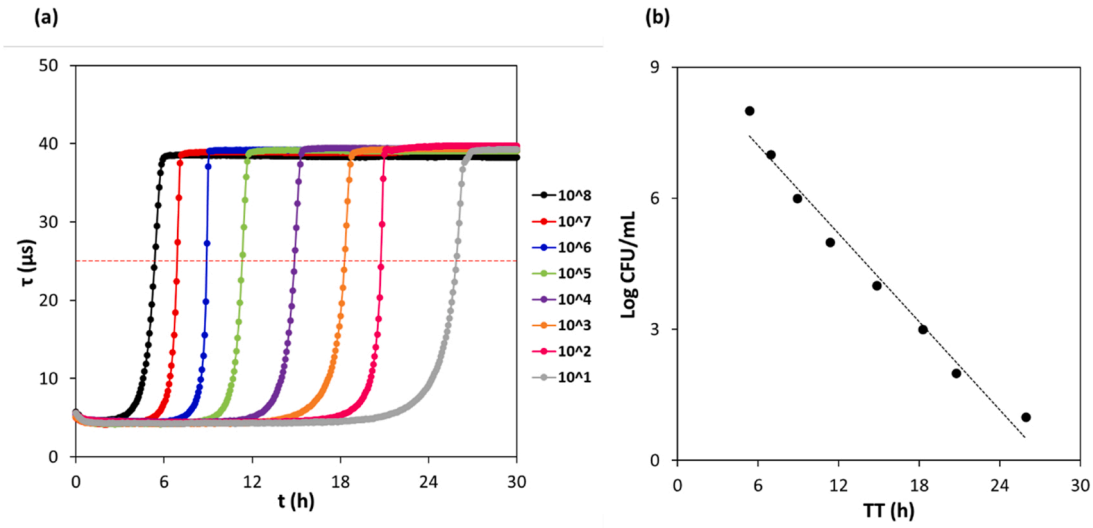

Fig. 8. (a) Lifetime vs incubation time profiles for serial dilutions of P. aeruginosa cultures ranging from 10^8– 10^1 CFU/mL recorded using the SS-µRM system illustrated in Fig. 2(b); inoculum volume = 5 µL. The broken red line represents the τ_{TT} value of 25 µs, used to determine a TT value for each different inoculum; (b) plot of initial inoculum concentration, Log (CFU/mL), vs TT, based on the data from (a). The line of best fit (broken line) had a gradient and intercept of − 0.34 ± 0.02 and 9.16 ± 0.30, respectively.

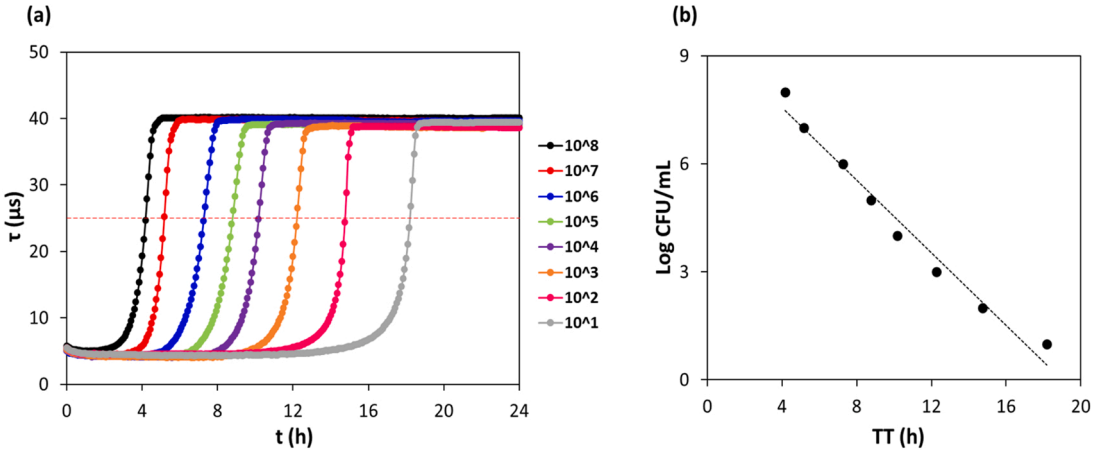

Fig. 9. (a) Lifetime vs incubation time profiles for serial dilutions of E. cloacae cultures ranging from 10^8– 10^1 CFU/mL) recorded using the SS-µRM system illustrated in Fig. 2(b) inoculum volume = 5 µL. The broken red line represents the lifetime threshold value of 25 µs, used to determine a TT value for each different inoculum; (b) plot of initial inoculum concentration, Log (CFU/mL), vs TT, based on the data from (a). The line of best fit (broken line) had a gradient and intercept of − 0.50 ± 0.03 and 9.59 ± 0.40, respectively.

acquisition, Formal analysis, Conceptualization. **Watson Michaella:** Writing – original draft, Investigation, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Data access statement

All data is provided in full in the results section of this paper and supplementary information accompanying this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.snb.2024.135435.

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