



A demonstration of athermal effects of continuous microwave irradiation on the growth and antibiotic sensitivity of *Pseudomonas aeruginosa* PAO1

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3 1 **A demonstration of athermal effects of continuous microwave irradiation on the growth**
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5 2 **and antibiotic sensitivity of *Pseudomonas aeruginosa* PAO1**
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3 20 **Abstract:**
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6 21 Stress, caused by exposure to microwaves (2.45GHz) at constant temperature ($37 \pm 0.5^\circ\text{C}$),
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8 22 alters the growth profile of *Pseudomonas aeruginosa* PAO1. In the absence of microwave
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10 23 treatment a simple, highly reproducible growth curve was observed over 24 hours or more.
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12 24 Microwave treatment caused no reduction in growth during the first 6 hours, but at a later
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14 25 stage (>12hours) the growth was markedly different to the controls. Secondary growth,
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16 26 typical of the presence of persisters clearly became apparent, as judged by both the
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18 27 dissolved oxygen and the cell density profiles. These treated cells showed distinct
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20 28 morphological changes, but on re-growth these cells reverted to normal. The Microwave
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22 29 Induced Persisters were subject to antibiotic challenge (tobramycin) and showed increased
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24 30 sensitivity when compared to the un-stressed planktonic cells. This is in marked contrast to
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26 31 antibiotic induced persisters which show increased resistance. This provides evidence for
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28 32 both a non-thermal effect of microwaves and a previously undescribed route to a novel
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30 33 form of antibiotic susceptible persister cells.
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36 34 **Key words:** *Pseudomonas aeruginosa* PAO1, microwave, 2.45GHz, constant temperature,
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43 36 **Abbreviations:** MIPs: Microwave Induces persisters.
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1. Introduction

1.1 Microwave effects on bacteria

Microwave heating has been extensively used for pasteurisation and sterilisation^{1, 2}. It is well known that microwaves used in this way adversely affect the growth of bacteria, but this is usually ascribed solely to a thermal effect^{1, 3, 4}. However, there is a continuing interest in exploring a possible non-thermal or “microwave component” particularly for any sub-lethal effects. Unfortunately, it has proved remarkably difficult to come to any firm conclusion, not least because of the practical difficulties of such experiments⁵⁻⁸.

The argument for a “microwave effect” has been much better rehearsed in the attempts to understand the well-known microwave acceleration of chemical reactions⁹⁻¹¹. Again, no entirely satisfactory conclusion has yet been reached. At the moment the balance of opinion favours understanding the effect of microwaves as a purely thermal phenomenon, with changes in reaction rate coming from differences in heat transfer. Indeed, even for chemical reactions it is very difficult to critically compare different experiments as they have been carried out under a wide range of conditions and are often reported in insufficient detail to allow reliable replication. However, there are a few claims that microwaves affect the activation energy for a chemical reaction in way not easily attributable to purely thermal effects as predicted by the Arrhenius equation¹²⁻¹⁵. Interestingly, there are claims that microwaves do affect the rate of enzyme catalysed reactions conducted athermally *in vitro*¹⁶⁻¹⁹ which could have consequences for viability.

1.2 Persister cells

Persisters are a sub-population of cells which survive a variety of adverse conditions. They represent a small fraction (0.1-1 %) of the bacterial population and increase significantly

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3 64 when the cell culture enter stationary phase²⁰. The vast majority of studies on bacteria
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5 65 refer to persisters arising from bactericidal doses of an antibiotic and this is of particular
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7 66 importance in the aetiology of drug-resistant microbial infections.^{21, 22}. Persisters are
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10 67 phenotypic, not genotypic, variants of the cell population and hence their drug resistance is
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12 68 non-heritable. They will give rise to a new population that is identical to the original one and
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14 69 equally sensitive to the antibiotic²³. However it is now recognised that persisters
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17 70 themselves are not uniform and need to be considered stochastically to understand their
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19 71 phenotypic heterogeneity and the stability of cellular proliferation²⁴. It is thought that
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21 72 persisters are selected for their resistance to stress through a period of dormancy in the
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23 73 early stage of population growth, coupled with a low growth rate, though the detailed
24
25 74 mechanism is still subject of much discussion. A variety of effects at both environmental and
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27 75 cellular levels have been considered^{20, 22, 23}.
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30 76 This study has been undertaken here whereby conditions are well defined. This is part of a
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32 77 programme aimed at examining the effects of microwaves on microbial growth. In this case
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34 78 the effect of continuous exposure to constant (non-pulsed) microwave field (2.45GHz) on a
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36 79 series of batch fermentations of *Pseudomonas aeruginosa* (*P. aeruginosa*) PAO1 have been
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38 80 examined. No attempt was made to use other frequencies. In an attempt to decouple any
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40 81 thermal effect from any microwave induced changes particular attention was paid to
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42 82 operating at constant bulk temperature (37±0.5 °C) with other conditions being typical for
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44 83 the growth of this organism. The scale of the experiments (2 litres working volume) was
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46 84 such that, with stirring, bulk averaging ensured the homogeneity of the treatment of the
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48 85 contents, a feature lacking in many smaller scale experiments²⁵. Here we report that non-
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50 86 thermal stress caused by continuous exposure of *P. aeruginosa* PAO1 to microwave (100 W)
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3 87 at constant temperature causes the appearance of persisters. These cells demonstrate
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5 88 increased susceptibility to tobramycin, the aminoglycoside antibiotic typically used to
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7 89 eliminate *Pseudomonas* infections²⁶.
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11 91 **2. Materials and methods**

12 92 2.1 Organism

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15 93 A pure culture of *P. aeruginosa* PAO1 was stored in a bacterial preservation kit (Technical
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17 94 Service Consultants Ltd., Lancashire) at -80⁰ C.
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20 95 2.2 Culture media and fermentation

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22 96 The *P. aeruginosa* PAO1 strain was maintained on nutrient agar (CM0309, Oxoid) at 37⁰ C.
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24 97 Cultures were grown overnight in 50 ml of sterile nutrient broth (CM001, Oxoid) at 37⁰ C in
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26 98 an orbital shaker (200 rpm). The main fermentation was carried out in an autoclavable 3
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28 99 litre fermenter, (e-z controller, Applikon Biotechnology, UK) equipped with stirrer, heating
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30 100 jacket, cooling loop, condenser, pH, temperature and dissolved oxygen (DO) probes. Cooling
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32 101 was available by circulation of a coolant through the loop at 15⁰C with the coolant
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34 102 maintained at that temperature by means of a Huber cooler (Polystat Control). Cell density
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36 103 was monitored on line using the non-invasive biomass monitor, "BugLab" (BugLab, LLC Ca).
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38 104 The monitor was calibrated in arbitrary units according to the manufacturer's instructions.
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40 105 Figure 1_{a, b} shows the equipment.
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42 106 In a typical experiment the bioreactor containing 2 litres of nutrient broth (Oxoid) was
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44 107 assembled and sterilised by autoclaving at 120⁰ C for 30 minutes. Following cooling the
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46 108 fermenter vessel was mounted on the microwave block, stirred at 200 rpm and sterile
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48 109 filtered air was supplied at a rate of 1 litre per minute. The microwaves were applied at the
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3 110 required energy as described below. The system was then allowed to reach the pre-set
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5 111 temperature of 37°C, matching the heating effect of the microwaves with the cooling under
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7 112 the control of the Applikon Biotechnology software.

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10 113 For control experiments, when no microwave energy was applied, heating was via an
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12 114 external electrical heating jacket. Temperature was monitored and controlled at all times.
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14 115 Only when the system had reached a steady state at the required temperature was the
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16 116 inoculum added to a final concentration of OD₅₅₀ of 0.1 (BugLab unit: 0.1). The process was
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18 117 then monitored for at least 36 hours. Each fermentation was at least duplicated to examine
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20 118 run-to-run variability.

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22 119 All parameters were logged on-line through the Applikon and Bug Lab proprietary software.

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24 120 Data was exported as Excel™ files in .csv format and further manipulated within Excel.

25 26 121 2.3 Microwaves

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29 122 Microwaves were generated in a Sairem solid state microwave generator (model number:
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31 123 GMS200WSM56MPFCFST1IRWF AIT) operating at 2.45GHz capable of a continuous, non-
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33 124 pulsed output of up to 200W, with measurement of both forward and reverse power. This
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35 125 was coupled to the fermenter through an aperture in a dished aluminium block, shaped to
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37 126 match the profile of the bottom of the fermenter vessel as shown in Figure 1_b. The
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39 127 fermenter was surrounded by an aluminium mesh to prevent re-radiation of the
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41 128 microwaves. The absorption of the microwaves into the fermenter was assessed by
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43 129 measuring the reflected power. Microwave absorption was complete as the reflected
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45 130 power was very low, typically about 1% of forward power, so that no further matching or
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47 131 tuning was required.

48 49 132 2.4 Scanning Electron Microscopy (SEM)

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3 133 A sample of *P. aeruginosa* PAO1 broth was treated with 1% gluteraldehyde overnight at 4⁰ C,
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5 134 washed ten times with distilled water by centrifugation (10000 rpm) and dehydrated in air
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8 135 (24h). The sample was coated using an Emitech k550x gold sputter coater at 25mA for 2.5
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10 136 mins, giving a thickness of 12nm and examined under the SEM (Fei quanta 200 esem).

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13 137 2.5 OxoPlate[®]/statistical analysis and antibiotic susceptibility test
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16 138 OxoPlate[®] (PreSens) is a 96 well microtitre plate, containing specific sensors that measure
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18 139 oxygen levels in each well using a fluorescence plate reader (BMG/OPTIMA.). Raw data was
19
20 140 collected using an indicator filter (540/650 nm) and a reference filter (540/590 nm). The
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22 141 effect of tobramycin was evaluated by mixing 180 µl of sterile nutrient broth, 10 µl of
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24 142 tobramycin and 10 µl of the cell culture onto each of the OxoPlate[®] wells. The plate was
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26 143 incubated at 37⁰ C in a horizontal shaking (2 g 5 mins⁻¹) fluorescent plate reader
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28 144 (BMG/OPTIMA). Six replicates were performed for each experiment and automatic
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30 145 fluorescent readings were obtained every 5 minutes. The data generated were collected
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32 146 over 24 hours and PO_2 (the percentage of O₂ saturation) was calculated using the following
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34 147 equation:
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$$P_{O_2} = 100 (K_0/I_r - 1) / (K_0/K_{100} - 1) \quad (1)$$

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46 150 Where K_0 was the highest I_r (relative) number and K_{100} was the lowest I_r value.
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$$I_r = I_{ind}/I_{ref} \quad (2)$$

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3 152 Where I_{ref} (reference) is the mean value of all the replicate samples measured at 540/590
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5 153 nm at each time point and I_{ind} (indicator) is the mean number of all the replicate samples
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7 154 measured at 540/645nm at each time point.
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10 155 2.5.1 Tobramycin

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14 156 Tobramycin (T4014, Sigma), an aminoglycoside, was selected for the antibiotic challenge as
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16 157 it is very effective against *Pseudomonas*. Each dilution was freshly prepared and filter
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18 158 sterilised prior to use. Concentrations of $0.312 \mu\text{g ml}^{-1}$, $0.625 \mu\text{g ml}^{-1}$, $1.250 \mu\text{g ml}^{-1}$, $2.500 \mu\text{g}$
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21 159 ml^{-1} , $5 \mu\text{g ml}^{-1}$ and $10 \mu\text{g ml}^{-1}$ were tested against all planktonic cells.
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24 160 3. Results

25 161 3.1 Microwave absorption

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28 162 With the fermenter filled, the microwave energy was completely absorbed, as judged by the
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30 163 reflected power which was less than 1W for an applied power of 100W. This was, of course,
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32 164 supported by the observation of a temperature rise of the contents when no cooling was
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34 165 applied.
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38 166 3.2 The normal growth profile of *P. aeruginosa* PAO1

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41 167 Typical results for the growth of the *P. aeruginosa* PAO1 without microwave exposure are
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43 168 shown in Fig. 2.
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45 169 As expected the DO began to decrease rapidly due to microbial respiration in the first
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47 170 growth phase. This was accompanied by an increase in cell density as evident from the
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49 171 BugLab data (Fig. 3). After about 2 hours the DO was effectively zero but the biomass was
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51 172 rapidly increasing under these microaerophilic conditions. This continued for a further 17
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53 173 hours after which the DO slowly rose above 0%, consistently reaching a plateau at about
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3 174 80% saturation after 24 hours (final OD₅₅₀ of 7). The final DO was less than 100%, but
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5 175 remarkably similar in every case, suggesting some endogenous metabolism rather than
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8 176 probe drift, which might be expected to be more random. However, this was not
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10 177 investigated further. During this phase the biomass level also reached a plateau, indicating
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12 178 metabolic rest. Catabolising an amino-acid based broth, such as nutrient broth, without an
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15 179 added energy source will result in an increased deamination and subsequent pH increase.
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17 180 Figure 4 demonstrates that the PH rose only marginally over pH 7 and hence is unlikely to
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19 181 have had adverse metabolic consequences.

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22 182 During the whole process the DO profile was well matched by the cell density data from the
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24 183 “Bug Lab”, as far as could be observed. This latter data has the advantage of reporting the
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27 184 growth, whilst the system was still at 0% DO. The profile of each run was typical of normal
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29 185 growth and between-run variation for the controls was small. This is obvious from the data
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31 186 shown in Fig. 2 and 3.

32 187 3.3 Growth under continuous microwave exposure

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36 188 When *P. aeruginosa* PAO1 was grown under the continuous exposure to microwaves
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38 189 (2.45GHz) at constant temperature (37±0.5°C) the growth profile was markedly altered
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40 190 when compared to the controls, as is shown in Fig. 5 but the pH profile hasn't changed (Fig.
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43 191 6). Treatment with microwaves at constant temperature did not stop the *P. aeruginosa*
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45 192 PAO1 from growing, but overall the results of the fermentations with and without
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47 193 microwave treatment were substantially different.

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50 194 Between-run variation for the runs carried out with microwave application was again small
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52 195 during the first 2 hours. Moreover at this stage the DO and cell density profiles for the
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55 196 microwave treated growth closely matched the controls.

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3 197 The next stage of growth for both treated and untreated samples occurred at 0 % DO,
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5 198 indicating a very active respiration demand. However, whilst the reactions could not be
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7 199 monitored via DO during this period, the Bug-Lab data showed a continuing increase in cell
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9 200 density for both treated and untreated samples. Particularly though, it was clear that cell
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11 201 growth was notably smaller for the microwave treated sample (OD_{550} of 5) when compared
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13 202 with the untreated sample (OD_{550} of 7) (Fig. 7).
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17 203 After the period at 0% DO the fermentation profiles for the treated samples diverged a little
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19 204 more between runs and very noticeably in comparison with the controls. In this stage the
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21 205 DO increased, indicating that the rate of oxygen consumption had fallen, as expected. Here
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23 206 there was a much bigger difference between treated and control samples. The increase in
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25 207 DO occurred at about 19 hours in the untreated samples and proceeded to a stationary
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27 208 level. However, for the microwave exposed samples the increase in DO commenced
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29 209 markedly earlier, at about 9 hours. This can be related to the cell-density measurements
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31 210 (Fig. 5). The microwave treated fermentation produced lower cell growth than the control,
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33 211 hence the lower cell density created a lower oxygen demand, allowing oxygenation to raise
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35 212 the DO above 0% at an earlier stage. However, importantly for the treated cells, after a
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37 213 period of about two to three hours during which the DO increased the DO then began to
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39 214 decrease again for about 3-4 hours, indicating renewed secondary growth. This was never
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41 215 observed with the untreated samples.
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47 216 A sample was taken at that point in order to investigate the cells' morphology and antibiotic
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49 217 susceptibility. These cells will be called MIPs (Microwave Induced Persisters) from now on as
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51 218 they appeared stochastically during the fermentation and were tolerant to the microwave
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53 219 irradiation.
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220 3.4 Cell morphology

221 After fixing, a sample of *P. aeruginosa* PAO1 the MIPs were examined under the SEM at a
222 magnification of 13000 x as shown in Figs. 8-10. For the controls (untreated cells), a typical
223 cell length was between 1-1.4µm whereas in comparison the persister cells were
224 elongated to between 1.6-1.7 µm. After re-growth the cells returned to their normal size,
225 which is a typical, non-inheritable trait of persister cells. The difference between persisters
226 versus non microwaved cells was significant (Mann-Whitney p=0.012, n_{1,2}=5).

227 3.5 Antibiotic challenge

228 The effect of microwave treatment on subsequent growth under antibiotic challenge is
229 shown in Fig. 9.

230 In order to assess the antibiotic sensitivity of the MIPs, the cells were exposed to an
231 antibiotic challenge. Negative controls of both the planktonic cells and the isolated MIPs,
232 with tobramycin omitted, rapidly consumed the oxygen reaching a PO₂ of zero in over 3
233 hours. These results are very similar for both types of cells and demonstrate a very active
234 metabolic rate. Data shown in Fig. 11 represents a mean of six replicates.

235 The introduction of 0.3 µg ml⁻¹ of tobramycin to MIPs produced a completely different
236 metabolic profile for these treated cells. Oxygen levels diminished only partially then
237 remains constant, indicating that the cells were being exposed to bacteriostatic levels of the
238 antibiotic²⁷. In contrast, planktonic cells were completely unaffected.

239 At higher levels, exposing the MIPs to 0.625 µg/ ml⁻¹ of tobramycin and above caused cell
240 death with PO₂ levels remaining at 100 %, whereas with the same treatment, planktonic
241 cells carried on rapidly consuming and depleting oxygen for the first three hours. In contrast

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3 242 planktonic cells were only affected at concentrations of tobramycin well above $0.625 \mu\text{g ml}^{-1}$
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5 243 of tobramycin, with growth completely inhibited at $1.25 \mu\text{g ml}^{-1}$ of tobramycin.
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7 244 Thus when MIPs were re-cultivated although they reverted back to their original shape, they
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9 245 demonstrated lowered drug tolerance. This is entirely different to antibiotic induced
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11 246 persisters which show an increased drug resistance, a major challenge for infection control
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13 247 ^{20, 22, 28, 29}. To our knowledge this is the first report of the synergetic impact of tobramycin on
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16 248 the microwave exposed *P.aeruginosa* PAO1 cells.
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23 250 **4 Discussion**

24 251 The effect of microwaves on microbial growth, or more commonly on microbial death, has
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26 252 been quite widely studied, usually in connection with disinfection, particularly with
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28 253 foodstuffs ³⁰. In general the microwave energy is regarded as a source of heat, albeit with
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30 254 markedly different heat transfer characteristics to normal “thermal” process ². A few
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32 255 experiments have been described in which attempts have been made to disentangle the
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34 256 “thermal” component of microwave disinfection from a putative “microwave” component
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36 257 which might act in some as yet unspecified manner, but no definitive answer has been
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38 258 reached ³¹. In part this is because the experiments are widely varied with parameters such
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41 259 as microwave frequency, energy density, field profile.
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45 260 In the experiments described here a further attempt has been made to examine the
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47 261 athermal effect of microwaves on a culture actively growing at constant temperature. To
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49 262 our knowledge this is the first report of a fully automated batch system operated at
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51 263 constant temperature with continuous microwave irradiation. The measurements of bulk
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53 264 temperature were such that it is certain that the bacteria were growing at constant
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3 265 temperature of $37 \pm 0.5^\circ\text{C}$, within the accuracy of the measuring system. Complete transfer
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5 266 of energy from microwave radiation to the fermenter contents occurred. The energy was
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7 267 absorbed directly by the fermenter contents and not indirectly via microwave heating of the
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10 268 fermenter vessel, followed by heat transfer to the contents via conduction and diffusion.
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12 269 Glass is largely transparent to microwaves whilst water is extremely lossy and readily
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15 270 absorbs microwave energy. This was supported by measurements of the surface
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17 271 temperature of the vessel using a fibre optic contact probe which showed the exterior
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19 272 surface was at 37°C . Moreover, even if some indirect heating of this sort had occurred, it
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21 273 would then only resemble the heating via the electric wrapper used when microwaves were
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23 274 not applied and could not be responsible for the significant change in growth seen here. It is
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26 275 important to emphasise that in these experiments microwave energy was supplied
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28 276 continuously at the constant, i.e. non-pulsed, power level quoted here. This is unlike the
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31 277 continuous application of microwaves from pulsed fields where the power is a time-
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33 278 weighted average of high and low power states e.g. as in the domestic oven^{32, 33}. Under
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36 279 those conditions average power figures may disguise very high instantaneous powers.
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38 280 Moreover, in our experiments the microwaves were applied during the whole of each
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41 281 fermentation so that during the run any transient effects would be eliminated.
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43 282 The changes in both growth profile and morphology between treated and untreated
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45 283 samples observed in these experiments lead to the conclusion that at the energy used there
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48 284 is a distinct, sub-lethal, adverse effect of microwaves on growth. Growth under microwave
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50 285 irradiation certainly occurs. In the first 6 hours the rate of growth for treated and untreated
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52 286 samples is broadly the same but at a reduced cell density, which cannot be ascribed to any
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55 287 change in bulk temperature. The next stage for both treated and untreated samples

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3 288 occurred at 0 % DO, indicating a high metabolic rate. During this period the cell density of
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5 289 the cultures increased although it was obvious that growth was much smaller for the
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7 290 microwave treated cells compared to the untreated one. As the DO started to increase the
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9 291 treated cells demonstrated a decrease in DO, which was indicative of secondary growth
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11 292 phase. This was never observed with the untreated cells. It was this point that the culture
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13 293 broth was sampled and we called these cells MIPS. The MIPS were observed under the SEM
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15 294 and appeared elongated. Rosenberg has previously reported that *Escherichia coli* cells
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17 295 become elongated following treatment with electrical currents but to our knowledge there
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19 296 are no reports of a similar microwave effect³⁴. The persisters were ephemeral and on re-
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21 297 growth in the absence of microwave stress they reverted to normal size and metabolic
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23 298 active stage as seen in the electron micrographs and the Oxoplate data. This observation
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25 299 supports the theory that persister cells are a small population that express a temporary
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27 300 phenotypic characteristic, a variant of the planktonic cells, and revert back to their original
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29 301 growing state once the environmental stress is removed^{20, 23}.

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31 302 When challenged with antibiotics during re-growth the treated cells appeared to be more
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33 303 susceptible to antibiotics. More specifically introducing 0.3 $\mu\text{g ml}^{-1}$ of tobramycin had
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35 304 bacteriostatic effect to the MIPS compared to the untreated cells that continued growing
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37 305 with no obvious effect on their metabolism. Therefore we conclude that microwave
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39 306 treatment leads to the appearance of *P. aeruginosa* PAO1 persisters with decreased
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41 307 tolerance to tobramycin. Phenotypic resistance presents a major challenge to the
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43 308 development of anti-persister drugs.

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45 309 Previously it has been reported by Niepa *et al* that drug tolerance of viable planktonic *P.*
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47 310 *aeruginosa* PAO1 persister cells can be eradicated by a combination of weak

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3 311 electrochemical currents and tobramycin ($1.5\mu\text{g ml}^{-1}$)³⁵. This mechanism, known as
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5 312 bioelectric effect, is poorly understood and has also been reported to be efficient against *P.*
6
7 313 *aeruginosa* biofilms³⁶⁻³⁹. However this is the first report of electromagnetic waves applied
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9 314 to eliminate *P. aeruginosa* PAO1 persister cells in synergy with tobramycin. The effect of
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11 315 microwaves on the membrane of the MIPS and the permeability to tobramycin is part of on-
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13 316 going research.

17 317 **5 Conclusions**

18
19 318 Distinct changes in growth compared to untreated controls are induced by microwave
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21 319 treatment of *P. aeruginosa* PAO1 under the conditions used. However these changes only
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23 320 become apparent at later stage in the growth cycle. Cells treated with microwaves show a
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25 321 distinct change in morphology which disappears on re-growth. They also demonstrated
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27 322 increased sensitivity to tobramycin compared to the planktonic cells.
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33 324 **Conflict of interest:**

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36 325 There is no conflict of interest related to this work.

37 326 **Permission statements:**

38
39 327 The manuscript does not contain human or animal studies.
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34 437 **Figure legends:**

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36 438 **Figures 1 a, b** Fermenter assembly showing microwave cavity and tuning section beneath
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38 439 (left) and the dished coupling section (right).

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41 440 **Figure 2** The growth profile of *P. aeruginosa* PAO1 during control fermentations.

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43 441 **Figure 3** BugLab data for *P. aeruginosa* PAO1 growth profile. The culture reached an average
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45 442 final OD₅₅₀ of 7.

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47 443 **Figure 4** The relationship between dissolved oxygen and pH during control fermentations.

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49 444 **Figure 5** The profile of *P. aeruginosa* PAO1 during constant exposure to microwaves
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51 445 (2.45GHz). Temperature was controlled at (37± 0.5°C).
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54 446 **Figure 6** The relationship between dissolved oxygen and pH during microwave exposure.
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3 447 **Figure 7** Growth of *P. aeruginosa* PAO1 during microwave treatment.
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5 448 **Figure 8** Scanning electron micrograph of *P. aeruginosa* PAO1 grown without microwave
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7 449 treatment.
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10 450 **Figure 9** A scanning electron micrograph of the *P. aeruginosa* PAO1 persisters.
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12 451 **Figure 10** Persisters re-grown.
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14 452 **Figure 11** Metabolic profile of planktonic cells and persisters in the presence of tobramycin.
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17 453 When the persisters were subjected to an antibiotic challenge (tobramycin), they
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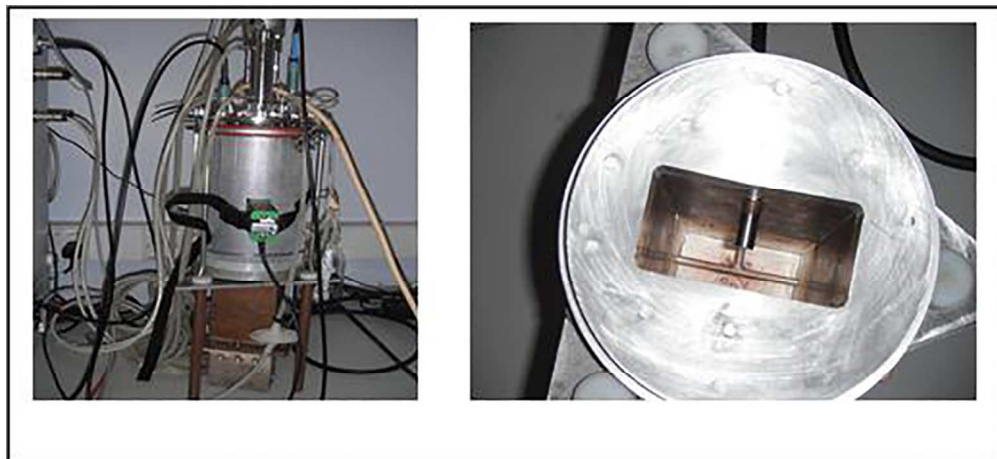
19 454 demonstrated increased sensitivity (evident from the slow metabolic profile) compared to
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21 455 the un-stressed planktonic cells.
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24 456 W: planktonic cells with no tobramycin present. P: Isolated persisters with no tobramycin
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26 457 present. Negative: Un-inoculated sterile media.
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Figures 1 a, b Fermenter assembly showing microwave cavity and tuning section beneath (left) and the dished coupling section (right).

468x215mm (72 x 72 DPI)

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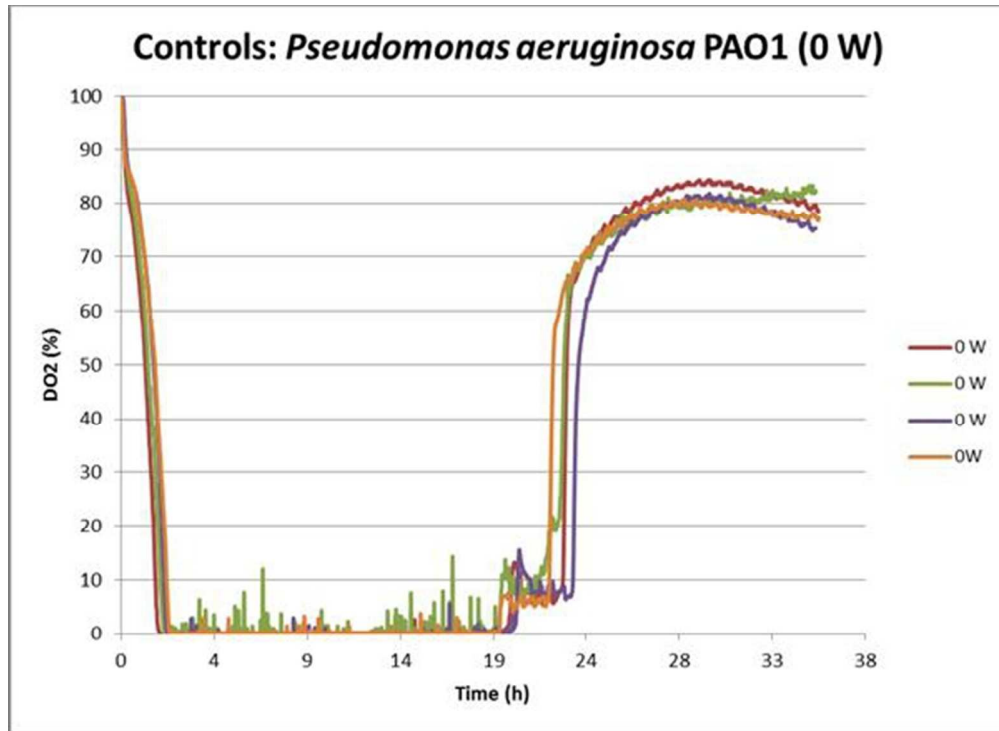


Fig. 2 The growth profile of *P. aeruginosa* PAO1 during control fermentations.

159x116mm (96 x 96 DPI)

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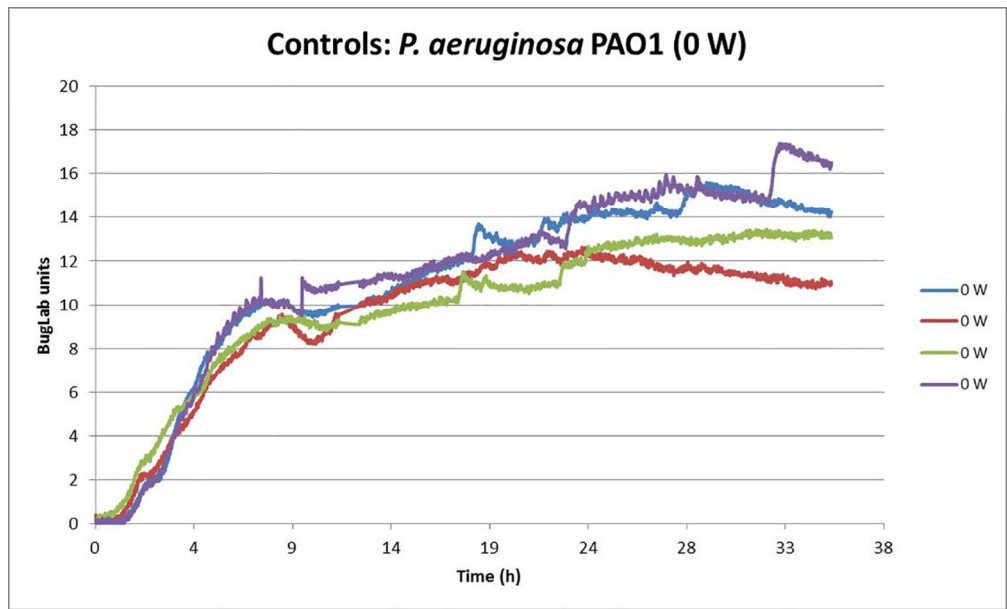


Figure 3 BugLab data for *P. aeruginosa* PAO1 growth profile.

468x282mm (72 x 72 DPI)

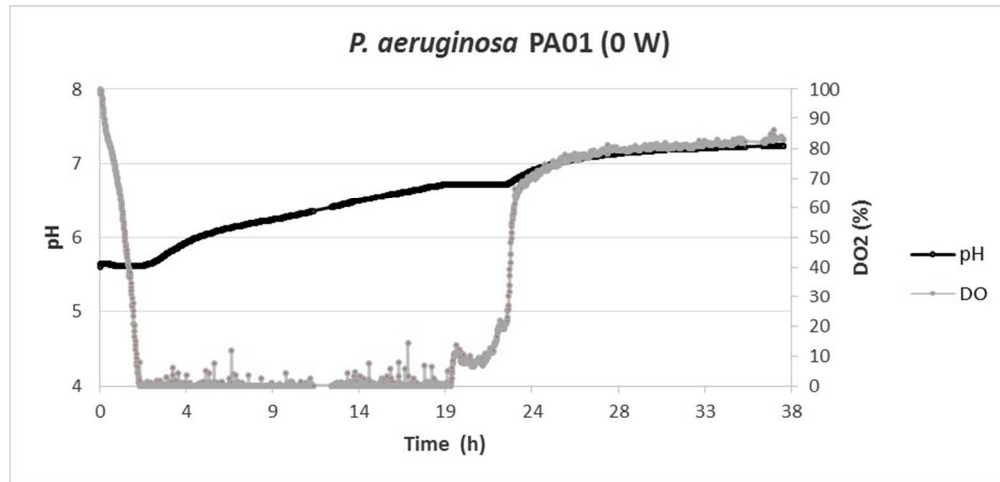


Figure 4 The relationship between dissolved oxygen and pH during control fermentations.

159x76mm (150 x 150 DPI)

Peer Review

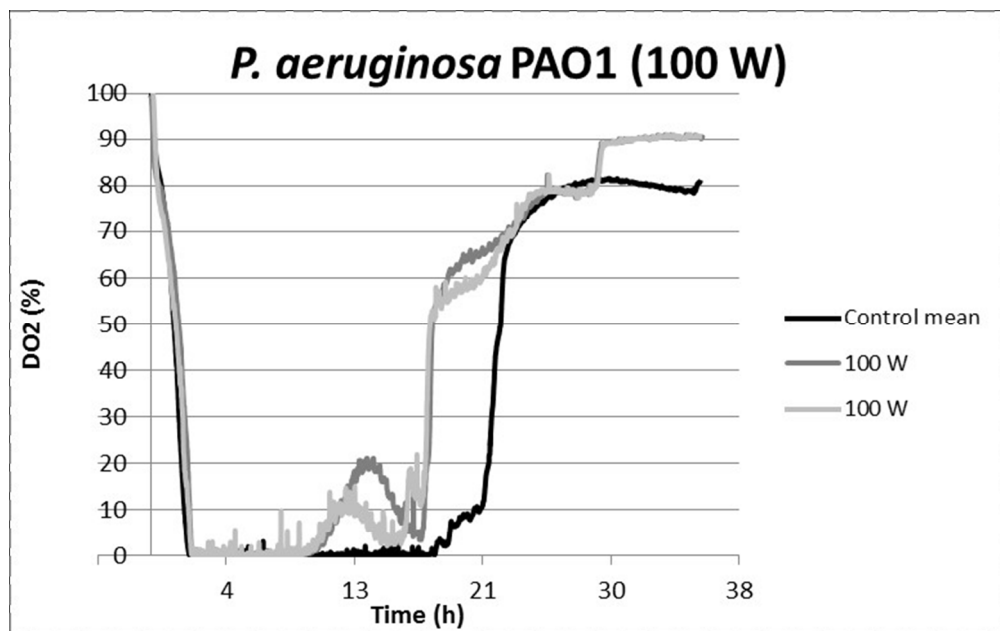


Figure 5 The profile of *P. aeruginosa* PAO1 during constant exposure to microwaves (2.45GHz). Temperature was controlled at (37+ 0.5oC).

131x82mm (150 x 150 DPI)

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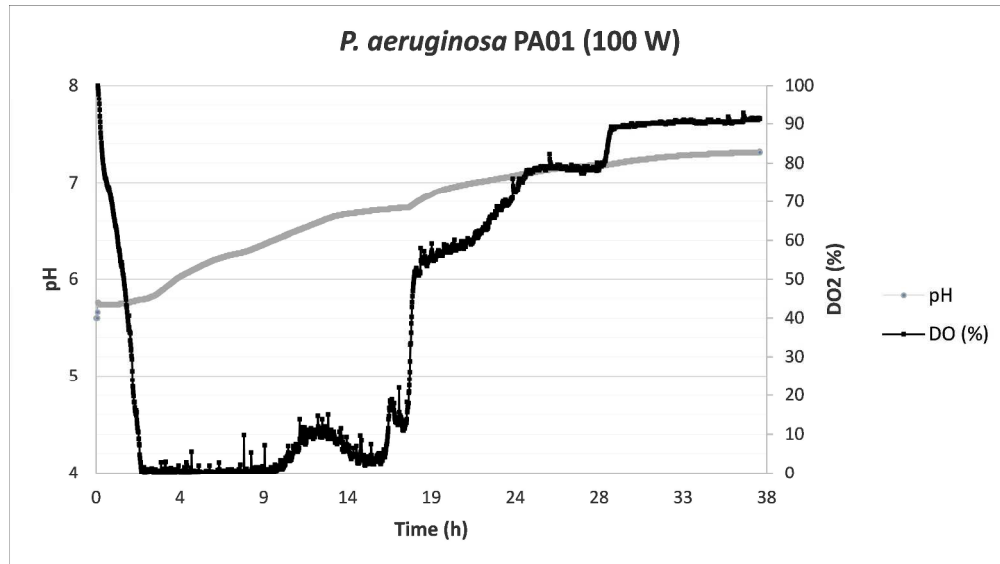
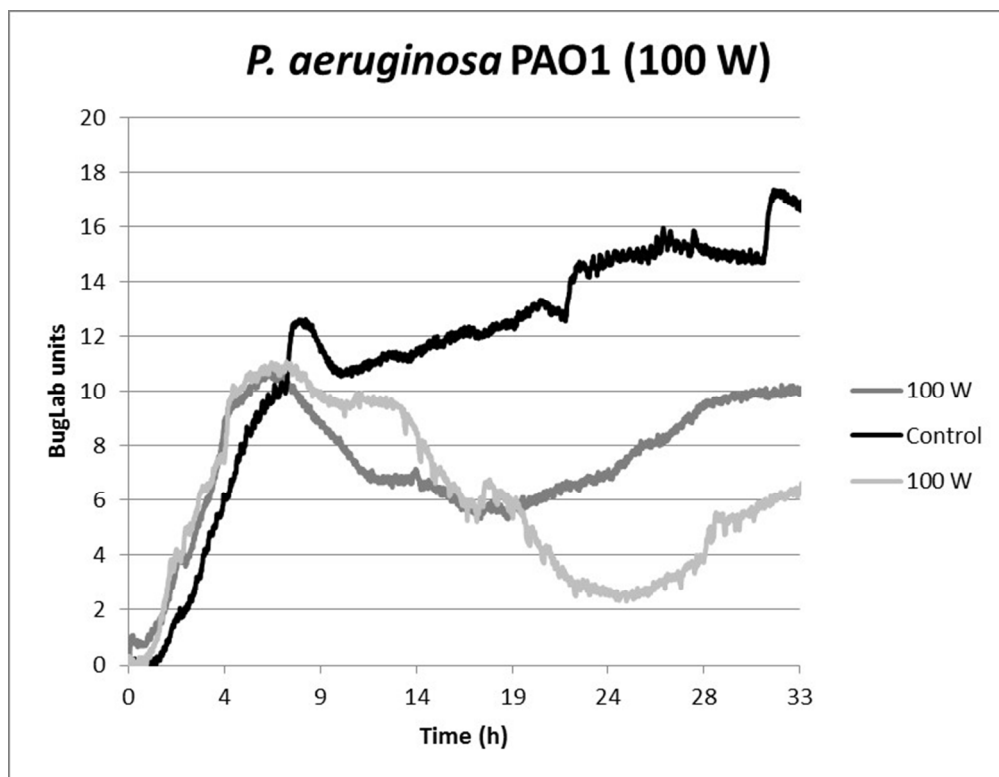


Figure 6 The relationship between dissolved oxygen and pH during microwave exposure.

1032x580mm (96 x 96 DPI)



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Figure 7 Growth of *P. aeruginosa* PAO1 during microwave treatment.

139x107mm (150 x 150 DPI)

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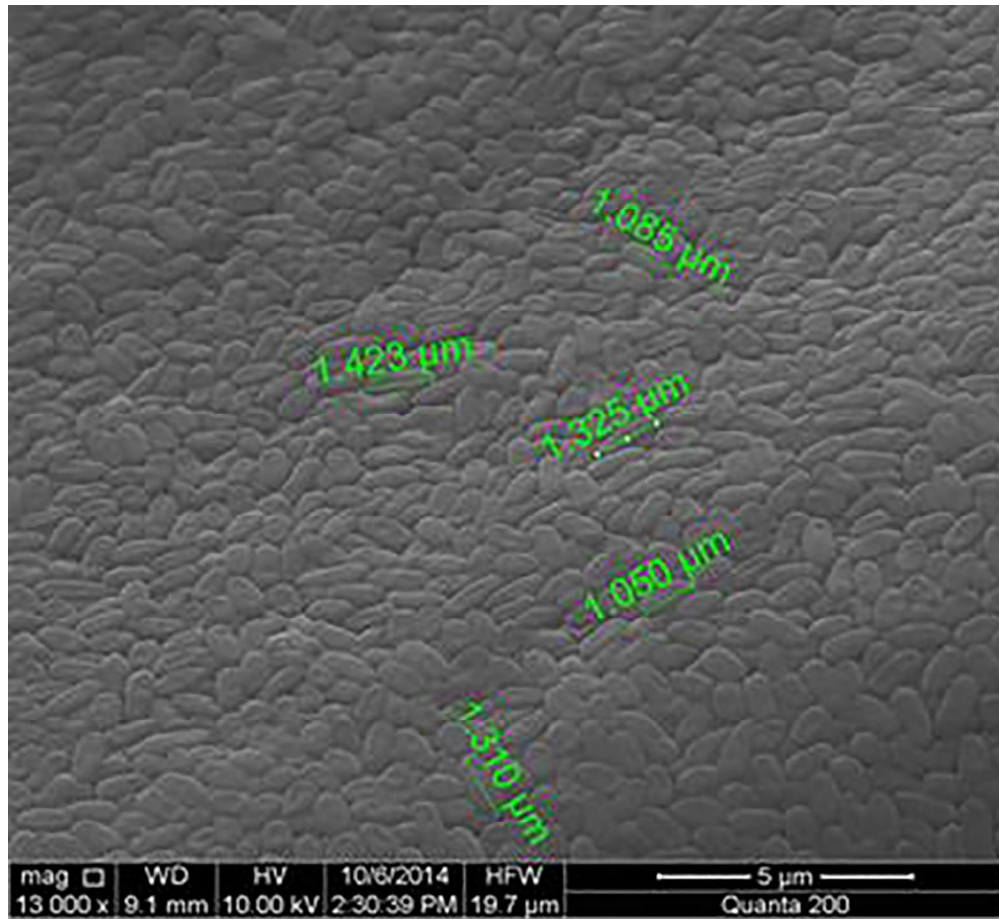


Figure 8 Scanning electron micrograph of *P. aeruginosa* PAO1 grown without microwave treatment.

468x429mm (72 x 72 DPI)



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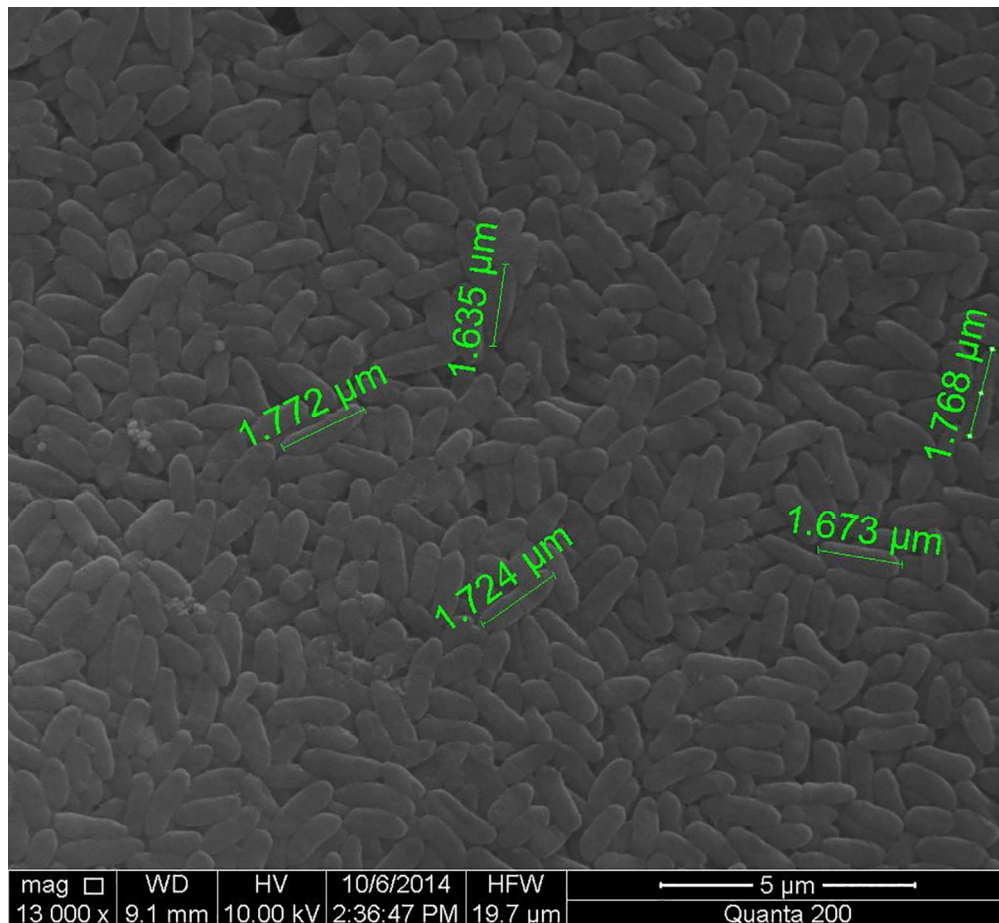


Figure 9 A scanning electron micrograph of the *P. aeruginosa* PAO1 persisters.

468x431mm (72 x 72 DPI)



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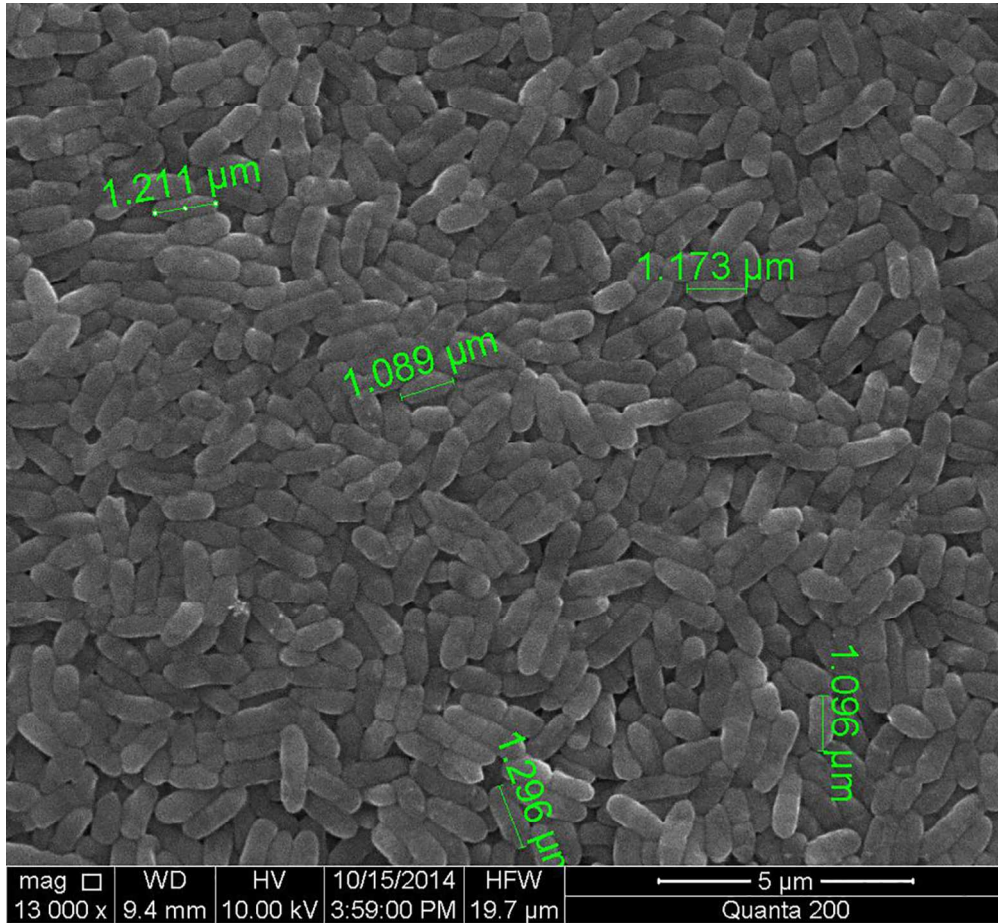


Figure 10 Persisters re-grown.

468x431mm (72 x 72 DPI)



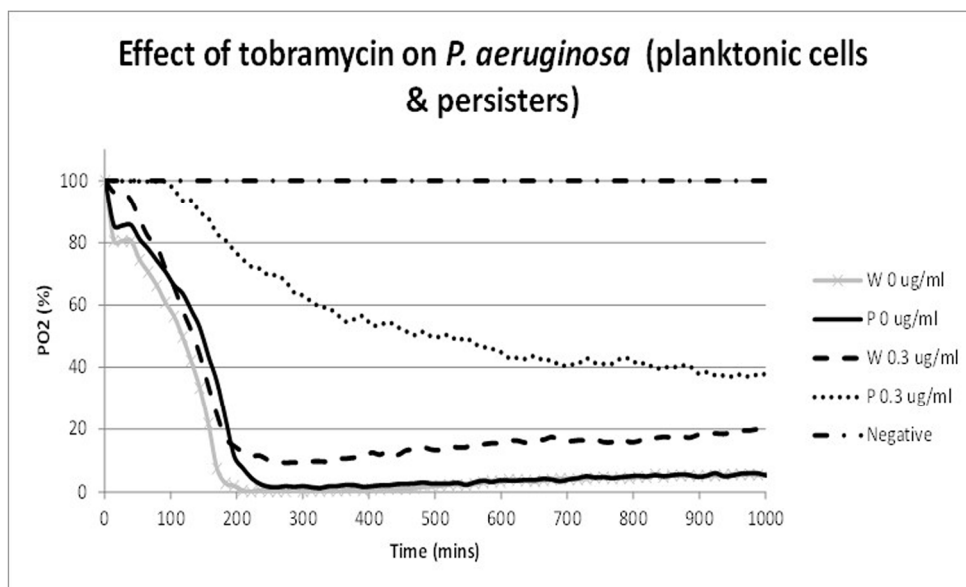


Figure 11 Metabolic profile of planktonic cells and persisters in the presence of tobramycin. When the persisters were subjected to an antibiotic challenge (tobramycin), they demonstrated increased sensitivity (evident from the slow metabolic profile) compared to the un-stressed planktonic cells. W: planktonic cells with no tobramycin present. P: Isolated persisters with no tobramycin present. Negative: Un-inoculated sterile media.