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Production of rhamnolipid Biosurfactants by *Pseudomonas aeruginosa* DS10-129 in a microfluidic bioreactor

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**ABSTRACT**

A low-cost microbioreactor made polytetrafluoroethylene (PTFE) was used to cultivate a model organism *Pseudomonas aeruginosa* DS10-129. The progress of bioprocessing was monitored by comparing the growth of the organism in the microbioreactor, conventional bench scale bioreactor and shake flask methods. Under the microbioreactor conditions the organism produced 23mg/ml of pyocyanin that had antimicrobial effect against *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas teessidea* and *Pseudomonas clemancea*. Furthermore, 106µg/ml of effective biosurfactants was produced within 16h of cultivation in the microbioreactor. The biosurfactants reduced the surface tension of distilled water from 72 mN/m to 27.9mN/m and emulsified kerosene by 71.30%. The pyocyanin and rhamnolipids were produced during the exponential and stationary phase of growth respectively. The results of the microbioreactor were comparable to those obtained by using the conventional scale methods.

[Key Words: Microbioreactor, Biosurfactant, rhamnolipids, High-throughput, microfluidic, antimicrobial activity]

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**INTRODUCTION**

The development of microfluidic systems over the past decade has been remarkable world-wide, due to their potential to improve analytical performance, reduce costs, ease of use and above all speeding up processes. Reports have shown that the global markets for microfluidics technology are steadily rising to several billions of dollars as companies are actively pursuing the commercialisation of their systems [1]. Microbioreactors represents a form of a complex microfluidic system which are a scaled-down version mimicking the conventional scale grandiose bioreactor systems. They are low-cost modules providing data rich solutions useful to optimise conventional scale operations and offering highly parallel analytical platforms in high through-put bioprocessing. The attractive features of microbioreactors have resulted in their use in a number of fields such as drug discovery, tissue engineering, strain development, cell based screening and bioprocess optimisation. Their low volume has the advantage of supporting small bacterial populations which allows long-time monitoring of cell behaviour. Furthermore, microbioreactors have the added potential of lowering the cost of conducting research and increase process development rate. In view of these developments, microbioreactors are promising tools that can potentially overcome the bottle-necks (*non-integration of systems, intensity of labour, high operational costs, and prolonged time for cell cultivations*) that are currently encountered with macroscale operations. A number of researchers have reported various designs of microbioreactors in the literature. For example Kim et al. [2] reported a silicon microfermentor chip that used electrodes to measure cell density, pH and dissolved oxygen. Zanzotto et al. and Kostov et al. [3-4] developed and demonstrated a low cost microfluidic bioreactor for high throughput bioprocessing using *Escherichia coli* as the model organism. The results showed that *E. coli* cultivation in the microbioreactor was identical to the results obtained in conventional systems (flask shake and bench bioreactor). In other studies Szita et al. [5] demonstrated a 150µl membrane aerated well mixed polymer based microbioreactor system integrated with optical density, pH and dissolved oxygen sensors with real time measurements and concluded that a microchemostat was an effective tool in bioprocessing. Walther et
al. [6] developed a 3ml continuous bioreactor with integrated microelectronic sensors for biomass, pH and temperature to investigate physiological and morphological properties of yeast cells in microgravity environments in a space laboratory. Steinhaus et al. [7] developed a portable aerobic microfluidic bioreactor with microchannels of different widths to study the optimum growth conditions for the methanogen *Methanoseta concilii*, a methane-producing obligate anaerobic archaea microbe using microfluidic bioreactor systems. Under these conditions they were able to study the behaviour and responses of *M. concilii* as it grew in the channels under optimum conditions of temperature, nutrients and pH. Lamping et al. [8] reported on a bioreactor made from Plexiglas with a volume capacity of 6ml. Oxygenation and mixing in the bioreactor was achieved by sparging and impellation respectively, while measurements of cell density, dissolved oxygen and pH were performed using optical methods.

In another development DiLuzio et al. [9] used microfluidics to study the swimming behaviour of *E. coli* and showed that it sensed the presence of microchannel walls at a distance of 10µm. A microfluidic bioreactor which sustained oscillation in the cellular density of *E. coli* with morphological changes was further developed by Balagadde et al. [10].

In this work we describe a simple low cost microbioreactor (µBR) of working volume 1.5ml made from polytetrafluoroethylene (PTFE). PTFE was used as the material of choice because of its low cost and biocompatibility [11]. To assess the performance and effectiveness of the microbioreactor we used a fast growing organism *Pseudomonas aeruginosa* DS10-129 sampled from sites contaminated with diesel as described by Rahman et al. [12-14] as the model organism to assess the effectiveness of the microbioreactor. Previous research has shown that *P. aeruginosa* produces factors that enhance its competitiveness and survival in many environments. In view of these developments, we investigated the ability of the µBR to sustain the growth of *P. aeruginosa* DS10-129 resulting in the production of two principal metabolites namely biosurfactants (rhamnolipids) and pyocyanins. We report the cultivation of *P. aeruginosa* in the microbioreactor in which the optical density (OD) as a measure of growth, rhamnolipids and pyocyanins production were continuously monitored and the results compared with the cultivation in conventional scale systems such as bench scale bioreactor (BSBR) and shake flask (SF).

**MATERIALS AND METHODS**

**Microbioreactor Design**
The microbioreactor (µBR) was made from polytetrafluoroethylene (PTFE) tubes. The PTFE tube (3m, 0.8 mm diameter) was made into coils of 4.2cm in diameter, creating a bioreactor of working volume 1.5 ml (Fig 1).

**Microorganism and culture medium conditions**
*Pseudomonas aeruginosa* DS10-129 (EBI accession no. AM419153) isolated from diesel-contaminated sites using methods described in literature [12-14] was used for all the experiments. The stock cultures of DS10-129 were maintained on Nutrient Agar (NA) (Oxoid, UK) slants in glycerol. Working cultures of the organism were prepared by streaking NA plates with the reference stock cultures. The inoculated plates were incubated for 18-24h at 30°C. To promote pyocyanin and rhamnolipid production the Nutrient Broth (NB) (Oxoid, UK) was modified with glycerol to produce Glycerol Supplemented Nutrient Broth (GSNB) medium. The composition of the GSNB medium was (g/l): Lab Lemco Powder 1.0, yeast extract 2.0, Peptone 5.0, Sodium Chloride 5.0, 30 ml glycerol and 1litre of deionised water. Single colonies of DS10-129 were used to inoculate the sterile GSNB medium. The pH of the medium was adjusted to 6.5±0.2 and sterilised under steam at 120°C for 15minutes. The medium was inoculated and incubated on a rotary shaker set at 30°C and 150 rpm. When the culture reached an optical density of 0.5-0.6 (600nm). Approximately 6x10^8 cells per ml were used to inoculate the µBR, BSBR and SF respectively.

**Bacterial growth in the microbioreactor**
A 5 ml syringe containing the GSNB culture of DS10-129 was used to inoculate the µBR through a syringe pump (Razell Scientific, USA) Fig 1. The inlet and outlet ports of the microbioreactor were connected to 0.2 µm Whatman sterile syringe filters after inoculation to prevent microbial contamination. A total of three µBR’s were prepared and inoculated in the same manner and allowed to run in parallel. The µBR’s were incubated on a rotary shaker set at 30°C and 150 rpm. The batch cultivation run for the µBR was 24h. At
periodic intervals all the three µBR’s had their contents sacrificed and monitored for cell growth by measuring the optical density of the metabolised GSNB medium at 600nm wavelengths using a Pharmacia UV-Vis Spectrophotometer. All experiments were carried out in triplicates and variations were not greater than 5%.

The metabolites produced by the organism were collected by centrifuging the culture broth at 8000g at 4 °C for 20 minutes to remove bacterial cells. The supernatant was filtered through a 0.2 µm syringe filter, protected from light and kept refrigerated for subsequent analysis.

**Bacterial growth in a bench scale bioreactor and shake flask**
Parallel batch cultivations of DS10-129 were carried out in a BSBR and SF respectively. A 7.5 L bioreactor (BioFlo 110, New Brunswick Scientific, USA) was filled with 3L of sterile GSNB medium which was subsequently inoculated with a 10% suspension of DS10-129. The operating conditions of the BSBR notably temperature, pH and agitation speed were maintained at 30°C, 7 and 200 rpm respectively. Samples of the metabolised culture medium (5ml) were periodically withdrawn for optical density measurements and subsequent down-stream processing as described for the microbioreactor. Similarly a 250ml conical flask containing 100ml of sterile GSNB medium was inoculated with DS10-129 and incubated on a stirred rotary shaker set at 30°C and 150 rpm for 120 h. Samples were withdrawn periodically and treated as previously described for the microbioreactor and the BSBR.

**Pyocyanin Production**
The presence of pyocyanin was detected by visual observation of the production of a green pigment in the culture medium, UV-VIS spectroscopy measurements and antimicrobial activity against a selected number of bacterial species.

**UV-Vis spectroscopy**
Pyocyanin production was determined by spectroscopy measurements using a double beam UV-visible spectrophotometer (Jasco V-630). A series of pyocyanin standards were prepared and used to construct a standard calibration curve. The pyocyanin concentration produced in the bioreactors was quantified using standard pyocyanin calibration curve.

**Antimicrobial susceptibility testing**
The antimicrobial testing of the green pigment pyocyanin was carried out using the method described by Bonev et al. and Rahman et al. [15-16] against the following bacterial species: *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Pseudomonas teessidea* and *Pseudomonas clemancea*. Antibiobic Medium Number No.1 (ANTBM1) with the following composition (g/l); Peptone 6.0; Tryptone 4.0; Yeast extract 3.0; Lab- Lemco1.5; Glucose 1.0; and Agar No.1, 11.5 was used for all the experiments. Equidistant holes were punched on the solidified inoculated ATNBM1 using a standard cork borer (diam 7mm) to cut wells on the agar surface. Sixty microlitres (60µL) of cell-free supernatant harvested at different incubation times was added to each agar well respectively. The treated agar plates were allowed to stand at room temperature for 2h and thereafter they were incubated at 37°C for 18-24h. For *Pseudomonas teessidea PR 6.5, P. clemancea PR22.1* and *P. aeruginosa*, the plates were incubated at 30°C for 18-24h. The suspension of *B. subtilis* was prepared from a culture that had been previously cultured for 5-7 days to allow for the formation of spores. The antimicrobial activity of the pyocyanin was determined by measuring the zone of inhibition against each organism using a pair of vernier callipers.

**Biosurfactant production**
Rhamnolipids were detected by surface tension measurements, haemolysis of red blood cells, and precipitation of Methylene blue agar (MBA). The molecular structure of the rhamnolipids was confirmed by infrared spectroscopy.

**Surface tension**
The surface tension of the cell-free supernatant medium was measured at different time intervals using a Tensiometer (Kruss Digital Tensiometer - Model K9, Germany), following the method described by Rahman et al. [17].

**Critical Micelle Concentration**
The Critical Micelle Concentration (CMC) for rhamnolipid was estimated from the intercept of two straight lines from the concentration-dependent and concentration-independent sections of the graph [18]. A series of rhamnolipid solutions were prepared in deionised water and the surface tension of each solution was measured at room temperature and plotted against the surfactant concentration.

**Quantification of rhamnolipids**
The concentration of rhamnolipid in the sample was estimated by the Orcinol method [17]. 333 µl of the filtered supernatant was extracted twice with 1ml of diethyl ether. The ether was evaporated to dryness and dissolved in 0.5ml of deionised water. To 100µl of each sample 900 µl of a solution containing 0.19% Orcinol (in 53% Sulphuric acid) was added. The samples were heated at 80°C in a water bath for 30 minutes and cooled for 15 minutes at room temperature and the absorbance measured at 420. The rhamnolipid concentration was quantified from the standard L-rhamnose calibration curve between 0 and 50 µg/ml and the result were expressed as rhamnose equivalents (RE) (mg/ml) by multiplying rhamnose values by a coefficient of 3.4, obtained from the correlation of pure rhamnolipids/rhamnose [17, 19-20].

**Haemolytic activity - Blood agar assay**
Equidistant holes (7mm diameter) were punched on petri dishes containing 5% Sheep Blood Agar. 60 µl of the cell-free supernatant was introduced in each well respectively. The inoculated plates were allowed to stand at room temperature for 24-48h and the zones of haemolysis were measured.

**CTAB Methylene blue agar assay**
The concentration of anionic surfactants in aqueous solution can be determined by the formation of insoluble ion pair with cationic substances. The insoluble ion precipitates in the MBA by forming a deep dark blue colour against a light blue background [21-22]. The composition of the MBA is provided in Table 1. MBA plates were prepared and treated as described for antimicrobial and haemolysis agar assays. The zones of precipitation were measured for each process and the results recorded.

**Characterisation of rhamnolipids by infrared spectroscopy**
Fourier Transform Infrared spectrophotometer (FTIR) Perkin Elmer 100 series was used to determine the molecular structure of the rhamnolipids. The cell-free supernatant was acidified to pH 2 by adding drops of 2M Sulphuric acid to precipitate the rhamnolipids. The precipitated rhamnolipids were extracted with an equal volume of 2:1 dichloromethane/methanol. The organic phase was dried with anhydrous Sodium Sulphate (Na₂SO₄) and evaporated on a rotary evaporator (Buchi, rota vapour R-200 Germany) set at 60-70°C. Approximately 2-5mg of the concentrated rhamnolipids were analysed on the FTIR spectrophotometer.

**Results and Discussion**

**Growth of organism**
The growth profile of DS10-129 in the bioreactors (µBR, BSBR and SF) was monitored by measuring the optical density of the inoculated media (Fig. 2). Production of pyocyanin was evident during the exponential phase of growth of the organism in all the three bioreactors and this was identified by visual appearance of a green pigment in the culture medium whose intensity increased with incubation time. The production of pyocyanin was confirmed by UV-VIS spectroscopy measurements and antimicrobial activity against a selected number of bacterial species. In the microbioreactor pyocyanins were produced as early as 4h and for the shake flask and the bench scale bioreactor it was after 6h (figure not included). This was attributed to the fact that miniaturised devices, due to their small cross sectional dimensions there is rapid heat and mass transfer because of the very large surface area to volume ratio. In view of this development
the production of pyocyanins could have been caused by an increase in temperature in the microbioreactor channels. Previous studies [23] have demonstrated that the production of pyocyanin was dependent on an increase in temperature.

The results of UV absorption spectroscopic measurements (Fig. 3) are consistent with those previously reported by [24]. Maximum pyocyanin production occurred during the exponential phase of growth in all the three scales. The estimated amount of pyocyanin produced after 24h of cultivation was 23mg/ml, 28mg/ml and 35mg/ml in the µBR, SF and BSBR respectively.

The produced pyocyanin inhibited the growth of a number of bacterial species and most susceptible organism to the bactericidal effect was \textit{S. epidermidis}; with larger zones of inhibition measuring up to 31mm in diameter (result not shown). \textit{Pseudomonas clemencea} PR22.1 was the least susceptible with the smallest zone of inhibition of 8 mm. The bactericidal effect of the pyocyanin was more effective on Gram-positive species as earlier reported by Stephen et al.[25].

**Biosurfactant production**

Biosurfactants were produced by the organism in all the three bioreactor scales. Biosurfactant production by \textit{P. aeruginosa} DS10-129, started as early as 10h and reached the maximum level after 16h of incubation in the microbioreactor, while for the SF and BSBR the maximum production was achieved at 72h respectively when the organism was in its stationary phase of growth. Previous reports [26-27] have shown that production of biosurfactants in shake flasks experiments occurred after 14h of incubation and reached its maximum level after 58h.

**Surface tension reduction and critical micelle concentration**

The surface tension measurements of the cell-free supernatant harvested from spent medium in the three scales (µBR, BSBR and SF) was used to estimate the extent of biosurfactant production. Results showed that DS10-129 produced effective biosurfactants that lowered the surface tension of water from 72 to 27mN/m (Table 2 and Fig. 3).

The critical micelle concentration (CMC) is that at which the individual surfactant monomers start to aggregate to form micelles. This concentration is the lowest surface tension at equilibrium or surface tension at CMC, which typically ranges from 1 to 200 mg/l [28]. The surface tension of water decreased gradually with increasing concentration of biosurfactant from 72 to 27 mN/m. The CMC for this rhamnolipid extract was estimated to be about 22 mg/l from the intercept of two straight lines from the concentration-dependent and concentration-independent sections (Fig. 4). This estimate is slightly lower than that of a CMC of 30 mg/l for rhamnolipid produced by \textit{Pseudomonas aeruginosa} ATCC 9027 [29]. The different CMC values may have resulted from differences in biosurfactant purity and composition. A range of CMC values between 10 and 230 mg/l have been reported for rhamnolipids from other microbial sources [28, 30]. It is known that the properties of rhamnolipids depend on the distribution of their homologues. The di-rhamnolipid shows lower CMC values (5 mg/l) than the mono-rhamnolipid, which showed a CMC of 40 mg/l [31]. The CMC of rhamnolipid produced by \textit{P. aeruginosa} DS10-129 was 22 mg/l and this was due to the presence of abundant di-rhamnolipids in the biosurfactant [17].

**Quantification of rhamnolipids**

The amount of biosurfactants produced by DS10-129 was estimated using the Orcinol method. The results for the orcinol assay showed that biosurfactant production occurred during the stationary phase of growth, consistent with earlier findings [32]. The results on the amount of biosurfactants produced by the organism in the bioreactors are consistent with surface tension measurements.

**Haemolytic Activity**

Haemolysis of blood agar was first described [33] to screen biosurfactant-producing bacteria and the method was further applied by Moran et al. [32] to quantify the amount of surfactin produced by \textit{B. Subtilis}. They demonstrated that the presence of the biosurfactants caused the lyses of the red blood cells in the agar. In this work the presence of biosurfactants in the samples was qualitatively detected by haemolysis of red blood cells in 5% Sheep blood agar. The results showed that the biosurfactants collected from the bioreactors contained fairly large amounts of factors with haemolytic activity as shown by the large diameters and areas of zone of clearing on red blood cells in sheep blood agar for various sampling times (Fig. 5.).
CTAB Methylene Blue Agar Assay
Like synthetic surfactants, rhamnolipids consists of a polar head and non-polar tail group, such that when they combine with different cationic substances, like CTAB they form insoluble ion pairs in aqueous solution, which precipitates as dark blue zones against a blue black ground in MBA [21]. The result shown indicates the production of effective rhamnolipids by DS10-129, which precipitates in MBA (Fig.6). The size of the zone of precipitation was proportional to the amount of biosurfactant present in the sample.

Emulsification Assay
Results in Table 2 shows that the biosurfactants produced by DS10-129 has good emulsifying activity. The higher emulsification index corresponds to the complete emulsification of the oil phase. A good bioemulsifier is the one that has an index of >50% [34]. Therefore our results suggest that the quality of biosurfactants produced by DS10-129 have higher emulsifying activity and would be very useful in bioremediation.

Identification of Rhamnolipid by FTIR spectroscopy
In this study the molecular structure of the rhamnolipids was confirmed by FTIR spectroscopy (Fig. 7). Strong and broad bands of the hydroxyl group free (-OH) stretch due to hydrogen bonding were observed in the region A (3368 cm⁻¹). The presence of carboxylic acid functional group in the molecule was confirmed by the bending of the hydroxyl (O-H) of medium intensity bands in the region D (1455-1380cm⁻¹). The aliphatic bonds CH₃, CH₂ and C-H stretching with strong bands are shown in region B and D (2925 -2856 and 1455-1380 cm⁻¹). The carbonyl (C=O) stretching was found in the region C (173 7cm⁻¹) with strong intensity bands. Two other strong peaks between 1300 and 1033 in region E due to C-O stretch are characteristic of an ester functional group in the molecule. The peaks in the range of 1121–1033 cm⁻¹ was also reported as C–O–C stretching in the rhamnose [35]. Moreover, we noticed stronger bands of pyranyl I sorption band in region E at 918 – 940cm⁻¹ and α- pyranyl II sorption band in region F at 838 – 844 cm⁻¹ that showed the presence of di-rhamnolipid in the mixture.

These characteristic adsorption bands together demonstrate that both hold chemical structures identical to those of rhamnolipids, which have rhamnose rings and long hydrocarbon chains. Thus, according to the results of the infrared spectra, the rhamnolipids produced by P. aeruginosa DS10-129 belong to the glycolipid group, which is made up of aliphatic acid and ester. The adsorption bands obtained are consistent with the report of Guo et al. [36] showing the presence of rhamnose rings and hydrocarbon chains. They have also provided a comparative study of mono and di-rhamnolipids (RL1 and RL2 and they found a shoulder in the ambient spectrum around 3006 cm⁻¹ resulting from the unsaturated C–H bonds’ stretching vibrations was detected for RL1 species. In the FTIR spectrum, we could observe only a minor shoulder; it might be because of the dirhamnolipid rich biosurfactant produced by DS10-129 [17]. The results obtained are consistent with the structure reported by Stanghellini and Miller [37] consisting of aliphatic acid and the glycolipid moiety.

Conclusion
This work has demonstrated that Pseudomonas aeruginosa DS10-129 can be cultured under microbioreactor conditions and produce two principal extracellular secondary metabolites; pyocyanins and biosurfactants. The results obtained by using the microbioreactor are comparable to conventional macroscale systems. The device could be a useful tool in the future of bioprocessing work, where high throughput processing and parallelism are required.

Acknowledgements
Authors wish to thank the University of Teesside sponsored Research and Enterprise Development Fund and Higher Education Innovation Fund (HEIF) for their support towards the completion of this project. PKSMR wish to thank UK- Bioscience for Business KTN for the award of FROPTOP fund to further explore the biocatalytic study of biosurfactant production from renewable resources.
Table 1. Composition of CTAB methylene blue agar (g/l)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cetyl trimethyl ammonium bromide (CTAB)</td>
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</tr>
<tr>
<td>Methylene Blue</td>
<td>0.005</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0</td>
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</table>

**Mineral salt Medium**

<table>
<thead>
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<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
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</tr>
<tr>
<td>MgSO$_4$.7H$_2$O</td>
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</tr>
<tr>
<td>Na$_2$HPO$_4$.2H$_2$O</td>
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<tr>
<td>NaH$_2$PO$_4$.2H$_2$O</td>
<td>1.00</td>
</tr>
<tr>
<td>CaCl$_2$.2H$_2$O</td>
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</tr>
<tr>
<td>Glycerol</td>
<td>25ml</td>
</tr>
<tr>
<td>Deionised water</td>
<td>1L</td>
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</table>

Table 2. Surface Tension, Rhamnolipid concentration and $E_{24}$ measurements of cell free supernatant from different bioreactors

<table>
<thead>
<tr>
<th>Type of Bioreactor</th>
<th>Cell free Supernatant collected after</th>
<th>Surface Tension of water (mN/m)</th>
<th>Rhamnolipid Concentration (µg/ml)</th>
<th>Emulsification index ($E_{24}$) %</th>
</tr>
</thead>
<tbody>
<tr>
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<td>106</td>
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<tr>
<td>Shake Flask</td>
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<td>29.6</td>
<td>130</td>
<td>84.7</td>
</tr>
<tr>
<td>Bioreactor</td>
<td>96h</td>
<td>28.3</td>
<td>141</td>
<td>92.1</td>
</tr>
</tbody>
</table>
**Figure Legends**

**FIG. 1.** Schematic set up of the microbioreactor. The GSNB and the inoculum are introduced into the microbioreactor by a syringe pump as a batch feed. Sterile 2µm Whatman syringe filter are fitted at the inlet and outlet (when the syringe pump has been disconnected) of the microbioreactor. The purpose of the filters is to allow diffusion of gases in and out of the microbioreactor.

**FIG. 2.** Growth of *P. aeruginosa* DS10-129 in Glycerol Supplemented Nutrient Broth (GSNB) medium in µBR, SF and BSBR conditions. The OD$_{600}$ values of fermented culture from each bioreactor were determined at various time intervals at 30°C. The data represented is a mean of three cultivation measurements in each bioreaction. ▲ - Bioreactor; ♦ - Microbioreactor and ●- Shake Flask

**FIG. 3.** UV-Visible absorption spectra of pyocyanin produced by *P. aeruginosa* DS10-129

**FIG. 4.** Critical Micelle Concentrations of the biosurfactant concentrated from different cultivation trials. Indirect measurement of rhamnolipid concentration by determination of the critical micelle concentration (cmc). Samples of the cell free supernatant were diluted 500 fold and their surface tension measured for each dilution. Effect of increasing rhamnolipid concentrations on surface tension
FIG. 5. Precipitated CTAB in MBA after 24h; A-C represents µBR, BSBR and SF.

FIG. 6. Haemolysed red blood agar plates; A, B, C, D, E represents SF, BSBR, µBR, control and P. aeruginosa.

FIG. 7. Infrared Spectra of rhamnolipids produced by Pseudomonas aeruginosa DS10-129

A = 3368.10 cm\(^{-1}\) (-OH free stretch due to hydrogen bonding), B = 2925.42- 2856.40 cm\(^{-1}\), (Aliphatic bond stretch CH\(_3\) CH\(_2\) and CH), C = 1737.33 cm\(^{-1}\) (C=O stretch due to the ester functional group), D = 1455.52-1380.65 cm\(^{-1}\) (bending of O-H bands in carboxylic acid group ), E = 1300 – 1033.61 cm\(^{-1}\) (C–O–C stretching in the rhamnose), F = 918 – 940 cm\(^{-1}\) (pyranyl I sorption band) G = 838 – 844 cm\(^{-1}\) (α- pyranyl II sorption band)
Fig. 1.

Fig. 2.
Fig. 5.

Fig. 6.