ELECTROMAGNETIC ACTUATED STIRRING IN MICROBIOREACTOR ENABLING EASIER MULTIPLEXING & FLEXIBLE DEVICE DESIGN

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Abstract The development of a **novel electromagnetically (EM) actuated stirring** method, for use in microbioreactors, is reported. Mixing in microbioreactors is critical to ensure even distribution of nutrients to microorganisms and cells. Magnetically driven stirrer bars or peristaltic mixing are the most commonly utilised mixing methods employed in completely liquid-filled microbioreactors. However the circular reactor shape required for mixing with a stirrer bar and frequently used for peristaltically mixed microbioreactors presents difficulties for bubble-free priming in a microfluidic bioreactor. Moreover the circular shape and the hardware required for both types of mixing reduces the potential packing density of multiplexed reactors. We present a new method of mixing, displaying design flexibility by demonstrating mixing in circular and diamond-shaped reactors and a duplex diamond reactor and fermentation of the **gram-positive bacteria *S. carnosus*** in a **diamond-shaped microbioreactor system**. The results of the optimisation of this mixing method for performing fermentations alongside both batch and continuous culture fermentations are presented.

Keywords: MicroBioReactor, Stirring, Electromagnetic, Duplex

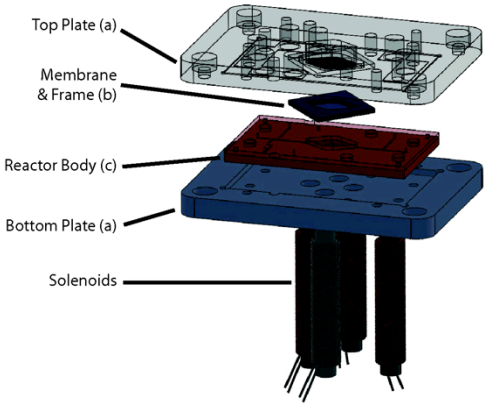
1. Introduction

Microbioreactors, initially developed to aid in bioprocess development (Marques et al., 2010; Szita et al., 2005), have also found application in strain optimisation for synthetic biology (Szita et al., 2010). The ability to *in situ* monitor fermentation variables and multiplex reactor chambers, relevant to both applications, is a direct result of the microscale dimensions of microbioreactors.

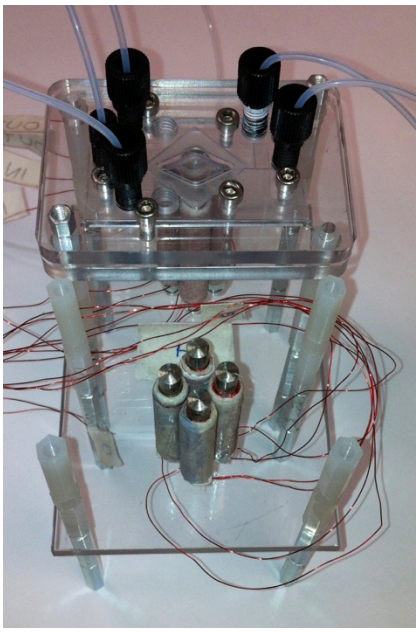
Many different passive and active (C.-Y. Lee et al., 2011) mixing methods have been developed for a wide variety of microfluidic devices. Suspension culture microbioreactors, being a special case in microfluidics as a result of the large culture chamber volume, relative to the surrounding fluid channels, require stirring rather than mixing. Several methods have been implemented, however most rely upon rotating a magnetically-actuated stirrer bar (Schäpper et al., 2010; Szita et al., 2005) or peristalsis implemented via a variety of actuation methods (H. L. T. Lee et al., 2006; K. S. Lee et al., 2011; Li et al., 2009). Despite these developments mixing in microbioreactors still provides some challenges as a result of the specific conditions required for microorganism growth, operational monitoring, and ease of multiplexing (Kirk and Szita, 2013). Additionally, rotating permanent magnets to drive stirrer bars, as previously implemented (Schäpper et al., 2010; Szita et al., 2005), require a circular reactor chamber to prevent stagnant zones, which makes reactor priming challenging. Here, we present for the first time an EM actuated stirring method, which is not hard-linked with a particular reactor shape, that facilely enables optical access for condition monitoring between the EMs, and has the potential for easy multiplexing.

The facility of this mixing method for application in microbioreactors operating in a variety of fermentation modes is demonstrated by performing both batch and continuous culture fermentations using the gram-positive bacteria *S. carnosus*. Moreover, the ease with which EM-actuated mixing enables reactors to be multiplexed is displayed using a duplex

reactor format.



a).



b).

Figure 1a). Schematic of the single reactor chamber microbioreactor cassette and b). the duplex electromagnetic device, with one reactor removed, to demonstrate the placement of the electromagnets.

Materials and Methods

The microbioreactor is designed in a cassette format comprised of 4 separate components (Fig 1a) with the reactor body formed of 2 layers thermally bonded together. Three of the four components are micromilled in polycarbonate (RS components, UK), with the fourth cast in PDMS (Sylgard 184, Dow Corning, UK). Manually fabricated EMs, with a 5 mm diameter stainless steel core wound with 480 turns of 0.25 mm copper wire, are mounted axially underneath each corner of the reactor chamber (Fig 1b). The EMs direct the movement of a 1 mm diameter NiCuNi coated NdFeB permanent magnetic bead (Earthmag GmbH, Germany) within the reactor chamber. EM activation is controlled using an Arduino Mega2560. Fluid was supplied to the microbioreactor using a syringe pump (neMESYS, Cetoni GmbH, Germany) equipped with glass syringes (Hamilton company, USA) and PEEK tubing (Upchurch Scientific, USA). Mixing results from the motion of the magnetic bead moving to each activated EM in turn. Stirring was visualised using dye, water blue (Sigma-Aldrich, UK), and a USB microscope (VMS-004D, Veho, UK). Video was analyzed by eye to determine mixing time.

For fermentations the microbioreactor was sterilised using a 70% ethanol solution. The batch fermentation was performed using *S. carnosus* TM300 grown in a defined medium (Medaglia and Panke, 2010) while the continuous culture fermentation was performed using the same defined medium with the lactose concentration reduced from 1% to 0.5% and with medium supplied at a flow rate of 0.8 µl/min. Optical density was monitored, at a wavelength of 600 nm, as an indirect measure of bacterial growth. The duplex reactor was formed using two, fluidically independently addressed, reactor chambers with the Arduino code duplicating electromagnet timing for both microbioreactors.

Results

Bubble-free priming of the diamond-shaped reactor device was achieved without any user interaction. Different mixing patterns, formed using either a loop or figure of 8 (F8) EM actuation sequence or some combination of the two, were tested with the optimum mixing pattern consisting of two loops followed by a F8 actuation sequence. Mixing time was measured for different EM actuation timings. The EM ‘on’ time and ‘off’ time was varied (total actuation time equals the sum of the EM ‘on’ and ‘off’ time), with the timings additionally varying between the loop and F8 actuation sequences. A mixing time of 3.4 seconds, resulting from a loop total actuation

time of 60 ms followed by an F8 total actuation time of 100 ms, was found to be optimum for fermentation due to a combination of low heat production and a short mixing time. Experiments demonstrated that mixing in both chambers of the duplex microbioreactor was almost identical (Fig 2).

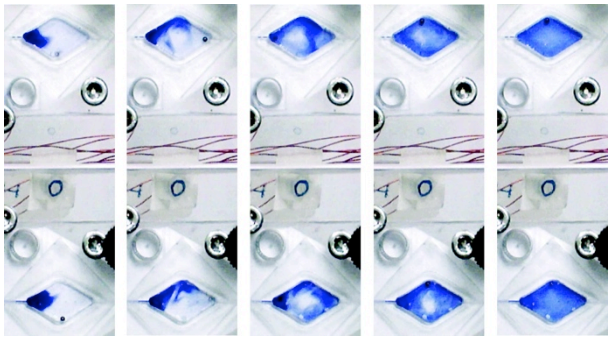


Figure 2. Sequence of stills, of the, electromagnetically mixed duplex microbioreactor showing close agreement in the mixing behaviour between the two chambers.

Finally, growth of the *S. carnosus* in a continuous culture fermentation demonstrated the ability of the EM stirring to sustain bacterial growth (Fig 3). A batch fermentation growth rate of 1.15 hr^{-1} was similar to that obtained in shake flask fermentations, while the continuous culture was maintained at a growth rate of 0.32 hr^{-1} for approximately 16 hours.

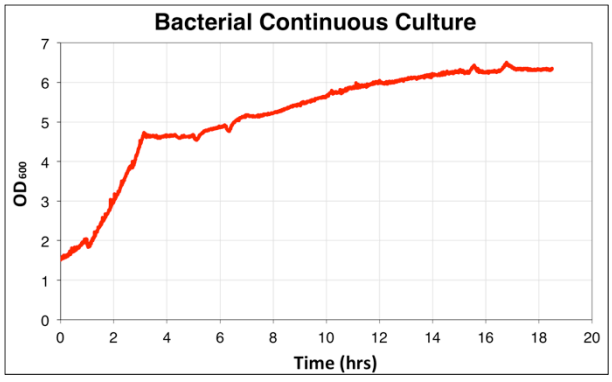


Figure 3. Plot of OD against time for a continuous culture fermentation of *S. carnosus*.

Discussion

EM actuated mixing provides flexibility both for the platform into which it is integrated and in the variety of actuation sequences and timing options possible. Following the observation of multiple sequences including solely loop actuation, solely F8 and many combinations of both it was observed that the mixing occurred more rapidly throughtout the entire reactor when a 'vortex' was created and then rapidly disrupted. The 'vortex' was formed by pulling the bead in a loop around the reactor. Disrupting the 'vortex' was then achieved by pulling the bead in a F8 pattern. Two loop sequences was sufficient to create a 'vortex' in which fluid throughout the reactor appeared to be moving in the same direction. A single F8 pattern was then sufficient to disrupt this.

Both a short mixing time and the amount of heat generated, by Ohmic heating of the EMs, needed to be accounted for in determining the optimum EM actuation timing. A combination of a short 'on' time and extended 'off' time was demonstrated to be the most effective for both. However the longer distance between the EMs when the bead is crossing the middle of the reactor requires that the total actuation time for the F8 sequence is longer than that of the loop sequence. A minimum 'on' time is also required to ensure that the bead was pulled sufficiently close to the active EM that when the EM was deactivated the bead is not pulled back towards the previous EM by the bead's own magnetic field. As a result of these considerations, an actuation sequence of 20, 40, 20 and 80 ms (loop 'on' and 'off' and figure of 8 'on' and 'off' respectively) giving a mixing time of 3.4 s and a temperature increase of only 3ºC, was developed.

This timing was demonstrated to be effective by applying it to fermentations of *S. carnosus* in the microbioreactor. Achieving similar batch fermentation growth rates in the microbioreactor and shake flask indicate either that mixing in both shake flasks and the EM actuated microbioreactor are sufficient to maintain maximum growth under these conditions or that both fermentation vessels have similar medium and oxygen transfer characteristics.

Finally the maintenance of bacterial growth under continuous culture conditions for over five generations establishes the versatility of this new mixing method by applying it to multiple fermentation modes. Further, demonstrating stirring in a duplex microbioreactor, in which similar patterns of mixing are observed, displayed the potential application of EM actuated stirring of microbioreactors to both bioprocess and strain development as a result of the ease with which multiplexing can be implemented.

Conclusion

The stirring method presented here facilitates **flexible implementation** of microfluidic chamber designs, **rapid mixing**, and **straightforward multiplexing** of microbioreactors, while still enabling the microbioreactor to **sustain, and monitor, bacterial growth**. Future work will include CFD modelling of the bead and fluid motion, to enable directed mixing optimisation, and further integration of growth condition monitoring and multiplexing.

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