1 Swirl flow bioreactor containing dendritic copper-containing alginate beads: A
2 potential rapid method for the eradication of *Escherichia coli* from waste water
3 streams.

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17

18 *Abstract*

19 Despite the increasing use of Decentralised Waste Water Systems (DEWATS) in the
20 developing world, which effectively dewater sludge, the problem of preventing the
21 pathogen-laden water produced by these systems from re-entering the food chain
22 constitutes a continuing burden on developing countries, which hinders subsequent
23 advancements. We report on a swirl flow reactor generating high mixing areas which,
24 in conjunction with Cu/alginate beads effectively reduces *Escherichia coli* numbers
25 by five orders of magnitude in ten minutes. The system is simple, low cost, portable
26 and modular; it can be assembled with simple plastic plumbing parts available in most
27 areas and, once further developed, may represent a useful adjunct for both existing
28 and new DEWATS facilities.

29

30 *1. Introduction*

31 The rapid expansion of mankind's endeavours and prosperity seen during the
32 industrial revolution was fuelled by the discovery of how to effectively separate
33 sewerage and potable water. The result of effective waste water treatment was the
34 ability for large numbers of people to co-exist in urban areas, due to the prevention of
35 the massive mortality and morbidity caused by pandemics of diseases such as Cholera
36 and Typhoid. This also resulted in a reduction in infant mortality, further fuelling
37 growth and prosperity, through an expanding and healthy workforce (Grady *et al.*
38 2011).

39 Unfortunately, there are still vast areas of the planet where humans are denied even
40 rudimentary toilet and waste water treatment facilities. For example, in Sub-Saharan
41 Africa an estimated 59% of the rural population has no access to clean drinking water
42 (Ngwenya *et al.* 2013), and even in the African economic powerhouse of South
43 Africa, an estimated 5 million people, over 5 % of the population, have no direct
44 access to clean drinking water (Victoria *et al.* 2000).

45 Despite a worldwide reduction in infant mortality during the last 30 years, the number
46 of premature infant deaths in Africa has reduced at a slower rate, with an estimated
47 mortality rate of 101 (per 1000 live births) reported in Sub-Saharan Africa during
48 2010 (Adjuik *et al.* 2010). Two thirds of these deaths were due to infectious diseases,
49 and although the increase in mortality due to Acquired Immunodeficiency Syndrome
50 (AIDS) accounts for many of these deaths (Muula 2008; Kleinschmidt *et al.* 2007),
51 diarrheal diseases still account for up to a third of mortalities, and up to 10.6 million
52 infant deaths worldwide per annum (Muula, 2008).

Although the introduction of new rehydration regimes has reduced mortality from diarrheal diseases in the under-fives by an estimated 66% over the last 20 years (Victoria *et al.* 2000), the cost of associated morbidity is vast, amounting to an estimated \$6.24 per household for every episode (Boschi-Pinto *et al.* 2006). Thus, the subsequent socioeconomic effect of the contamination of potable water supplies in many developing regions is a huge contribution to the continuation of poor living standards. While Rotavirus infection accounts for approximately 20% of cases (Neuzil *et al.* 2010; Sanchez-Padilla *et al.* 2009; Steele *et al.* 2009), *Escherichia coli* and the closely related *Shigella sp.* are the major causative agents of diarrheal diseases in Africa (Adjuick *et al.* 2010). The increasing prevalence of enterotoxigenic (ETEC), enteropathogenic (EPEC) and enterohemorrhagic (EHEC) serotypes which are associated with higher infant mortalities than other serotypes, and the lack of improvement in sanitation facilities in some areas of Africa has contributed to a slowing in the rate of decline in infant mortality observed in this area over the last 30 years (Darong *et al.* 2010; Okeke 2010; Qadri *et al.* 2005).

E. coli are Gram negative rod shaped bacteria, belonging to the gamma class of the proteobacteria. They are facultative anaerobes, capable of mixed-acid fermentation under anaerobic conditions, and are a ubiquitous commensal organism present in the gut of endothermic animals. Although most strains are non-pathogenic, certain virulent serotypes, such as ETEC and EPEC, have emerged in recent years (Okeke 2010; Qadri *et al.* 2005), and the spread of their associated pathogenic elements has accelerated due to the natural competence of the organisms - a feature which has facilitated their widespread use in molecular biology and fermentation laboratories (Sinha and Redfield, 2012). Infection is often by the faecal-oral route, and although infection in developed countries is often due to contaminated food, contaminated

78 water is still a major reservoir of infection in regions such as Africa, where an
79 estimated 345 million people are still unable to access clean drinking water (Adjuik *et*
80 *al.* 2010; Ngwenya *et al.* 2013).

81 Often the large centralised approaches to sewerage treatment employed in the
82 developed world are not feasible or suitable for many rural areas in developing
83 countries, with the logistics and costs associated with the transport of effluent to, and
84 potable water from, a centralised location totally prohibitive. In such situations,
85 innovative thinking is required, to lead to the development of DEWATS facilities that
86 allow the processing and sanitisation of sewerage on a local scale.

87 There is much ongoing research into cheap and robust methods of sewage treatment
88 in rural and densely populated urban areas of developing countries where
89 infrastructure and practicalities mitigate against traditional waste water treatment
90 methods. In areas such as Africa and the Indian subcontinent, DEWATS are
91 increasingly used as part of the solution for sewerage remediation (Schreiner &
92 Hassan 2011; Ngwenya *et al.* 2013). These modular systems use anaerobic digestion
93 coupled with the dewatering of the resultant sludge, followed by the subsequent
94 disposal of the solid content (Schreiner and Hassan, 2011). Unfortunately, there
95 remain large volumes of pathogen-laden water, and the release of this effluent into the
96 water course can constitute a considerable public health hazard (Ngwenya *et al.*
97 2013). Water treatment technologies suitable for use in developing countries have
98 been reviewed previously by Loo *et al* (2012), and the technologies typically utilised
99 in developed countries, such as ozonation, chlorination and UV treatment, are neither
100 cost effective nor efficient for this particular waste stream, due to factors such as high
101 solid loading, high colour content, remoteness of location or high capital and running
102 costs (Tree *et al.* 2003). Thus, there is an urgent requirement for new systems capable

of addressing both functional and financial problems unique to the environment of operation. Such systems will be required to be simple, effective, low cost and of appropriate scale and energy consumption.

Swirl is commonly employed in many engineering applications in order to increase fluid mixing and the heat and mass transfer characteristics of a process. The introduction of swirl results in vortex structures that promote mixing and turbulence production due to the presence of a third velocity component tangential to the flow, which results in additional velocity gradients. Combustion, mixing and separation are typical applications of swirling flow. In the context of bioreactors, swirling flows can be employed to effectively and efficiently promote mass transfer or to separate cells. Copper is inhibitory or lytic to many microorganisms, at amounts much lower than those known to be toxic to humans (Mathews *et al.*, 2013). The antimicrobial effect of copper has been known for millennia, and renewed interest has recently occurred in its properties, especially as a dry surface with antimicrobial properties, in areas of potentially high contamination, such as hospitals and nursing homes (Grass *et al.*, 2011). The use of copper or its salts in waste water treatment is restricted by factors such as difficulty in dispersion and collateral toxicity to aquatic organisms and commensal bacteria present in the treatment systems (Ochoa-Herrera *et al.*, 2011). During the following study, we describe a laboratory scale system, producing a turbulent swirl-type flow to bring copper alginate beads (previously shown by Thomas *et al.*, (2014) to reduce the minimum inhibitory concentration of dendritic copper to *Escherichia coli*) into contact with a simulated effluent containing *E. coli*, and demonstrate this as a means for the rapid disinfection of a contaminated water supply. We compare the results obtained within the swirl reactor (referred to in house as a vortex bioreactor, which generates a central vortex core with a relatively long

128 recirculation resulting in extensive contact between particles) with those obtained
129 from a 6 litre fermenter (equipped with two Rushton-type impellers with each of them
130 generating two counter rotating vortices in its vicinity) as a baseline for scale up from
131 bench scale tests, and determine the effect of temperature, dissolved oxygen and
132 bacterial loading on the efficacy of the system.

133

134 2. *Methods*

135 2.1 *Swirl flow reactor*

136 The flow reactor comprised a recirculating design and was manufactured from 60.4
137 mm outside diameter (OD) and 57.4 mm internal diameter (ID) clear polycarbonate
138 tube with 2" acrylonitrile butadiene styrene (ABS) fittings. The system was of a total
139 length of 1300 mm, a width of 55 cm and a working volume of 6.5 litres. The
140 drive/pump system consisted of a four blade brass Kort nozzle type propeller (Figure
141 1, item 1 [316s, 58mm diameter; pitch 63 mm]). The impeller velocity was controlled
142 by a 450 watt variable speed motor (0-2400 rpm [Bosch]). The propeller induced flow
143 in a clockwise direction and, by adjusting its rotational speed, the flow rate and the
144 strength of the swirl motion could be determined. Air was added to the system via a
145 400 mm length of 3 mm ID stainless steel tube, sealed at one end, with 0.5 mm holes
146 drilled at 10 mm intervals along the length. Dissolved Oxygen (DO) concentration in
147 the test media was controlled by the flow rate of air into the system through a bespoke
148 316S stainless steel diffuser, via a 240 volt solenoid (GHL Profilux, Germany)
149 connected to a Profilux 3 controller (GHL Profilux, Germany), which also controlled
150 temperature via a 300 watt stainless steel tube heater (Hydor, Wiltshire, UK) inserted
151 through the sampling tube.

152 2.2 *Flow Measurement*

153 The flow in the bioreactor was characterised by means of Particle Image Velocimetry
154 (PIV) which is an established optical diagnostic method to measure fluid flow (Raffel
155 *et al.*, 2007). A schematic of the experimental set up is shown in Figure 2a. The
156 impeller was driven by a stepper motor (SmartDrive Ltd, Cambridge, UK) controlled

by a 52,000 microstep/revolution controller (SmartDrive Ltd, Cambridge, UK). The speed of rotation was varied between 0 and 1000 rpm and was accurate to within 1% (Dusting and Balabani, 2009; Imomoh *et al.*, 2010). The reactor was filled with pure water, and then seeded with silver-coated hollow glass spheres (Dantec Dynamics, Bristol, UK) with an average diameter of 10 μm and a specific gravity of 1.1 g/cm, which were used as tracers for the PIV measurements. A vertical slice of the pipe (parallel to the pipe axis, as shown in Figure 2a) was illuminated using a continuous laser of wavelength 532 nm. Images of the flow (containing the tracers) were then captured using a high speed camera (IDT X-3 CMOS). Measurements were taken at four locations along the pipe; 40, 90, 160 and 220mm downstream of the impeller. At each location, 1000 images were acquired. These were then processed using the JPIV software package and standard cross-correlation methods, which resulted in 999 instantaneous velocity fields at each of the four locations.

Four rotational speeds were studied: 239, 477, 716 and 954 rpm. These corresponded to 25 - 100 rad/s, and were the highest speeds which could be examined before the fluid velocity became too large to be accurately measured. The corresponding

Reynolds numbers, defined as $Re = \frac{ND^2}{\nu}$ where N is the rotational speed in rev/s, D

the impeller diameter and ν the kinematic viscosity of water, ranged from 1.34×10^4 to 5.35×10^4 indicating that the flow was fully turbulent. The impeller tip velocity, V_{tip} , defined as πND , is a measure of the tangential velocity imparted to the flow by the impeller and provides an indication of the strength of the swirl. Tip velocities in the present study varied from 0.73 m/s (lowest impeller speed) to 2.9 m/s (highest rotational speed).

A preliminary computational study was performed to visualise the helical nature of the swirling flow generated by the impeller using ANSYS CFX as shown in Figure 2b. The simulation model replicated the geometry, fluid properties and flow conditions used in the experiments. The flow domain comprised two parts: a 40 mm rotating domain surrounding the impeller with the impeller at its centre, and a stationary domain, which covered the following 200 mm length of pipe, downstream of the impeller. In this manner, the effects of the impeller rotation on the flow could be examined for various rotational speeds. The accurate simulation of turbulent swirling flows remains a significant challenge in fluid mechanics, and is computationally expensive. However, Figure 2b indicates that the relatively simple computational model presented here captures the primary features of the flow (i.e. the swirl and the axial flow generated by the rotating impeller). Although the simulation data is less reliable than the physical experiments, they allow the full flow field to be examined, including the 3D swirling nature of the flow, which was not feasible to measure experimentally.

2.3 Bead formation

Beads were prepared according to a modification of the method described by Albarghouthi *et al.* (2000). Briefly, 4% (w:v) sodium alginate (Sigma Aldrich, Poole, UK) was dissolved in deionised water by heating to 60°C whilst stirring on a magnetic stirrer at 100 rpm. Once dissolved, the alginate solution was autoclaved at 15 psi for 15 minutes. Following cooling to approximately 20°C, dendritic copper microparticles (specific surface 1600 cm²/g, < 63 µm average particle size [GMCA Ltd, Oldham, UK]) were added at concentrations between 1-4% (w:v). The particles were dispersed

by sonication using a VibraCell sonic probe (Sonics and materials, Dansbury, Ct, USA) for 60 minutes. The mixture was stored at 4°C until further processing.

Beads were formed by the dropwise addition of alginate/copper mixture via a 0.8 mm gauge needle into a 4°C solution of CaCl₂ (2.5% w:v; Sigma Aldrich, Poole). Once formed, beads were washed in deionised water three times and stored in deionised water at 4°C until used.

2.4 Growth of E.coli ATCC 11775

E. coli ATCC 11775 from -80°C stored stocks was inoculated into 50 ml of sterilised Luria-Bertani (LB) broth (Sigma Aldrich, Poole, UK) and incubated overnight at 37°C in a shaking incubator (Bibby scientific, Staffordshire, UK) at 150 rpm; 1 ml of this stock was then added to 250 ml of LB broth, which was incubated overnight under the same conditions. The bacterial cell numbers were then determined using the Gram staining method (Svec 2009) and subsequent enumeration using an improved Neubauer hemocytometer.

2.5 Toxicity assay procedure

All experiments were performed in the swirl flow reactor described above, whilst baseline values were obtained using a 6 litre scale Infors Minifor fermenter (Reigate, UK) which represents an established bench-scale, well mixed reactor. All assays were performed in a media containing 0.5 g/l Bacto peptone and 0.2 g/l Yeast extract (both Sigma Aldrich, Poole, UK). Briefly, overnight *E. coli* culture was added at the appropriate amount to give the desired concentration, dependent on initial culture

density (typically 30-100 ml), to 6.5 litres of assay media and then added to the swirl flow reactor. The speed of rotation was set to 956 rpm (+/- 20 rpm) and the temperature was maintained at 37°C for 30 minutes to allow acclimation. Alginate beads (650 g) or Cu-Alginate composite beads (650 g) containing copper particles at concentrations between 0.25 and 1 % (w:v) were then aseptically added giving a final copper concentration of between 0 and 1 mg/ml in the 6.5 litre swirl flow reactor. Suspended solids were added, where appropriate, as insoluble cellulose (Sigma Aldrich, Poole, UK). 1 ml of sample was then removed at 15 minute intervals for microbial enumeration. Each sample was serially diluted at 4°C in 1/8 strength Ringers solution (Fisher, Leicester, UK) and plated onto 90 mm Petri dishes containing MacConkey media number 2 (Sigma Aldrich, Poole, UK). The Petri dishes were then incubated overnight at 37°C and the distinctive pink colonies (indicative of *E. coli*) counted. All treatments were prepared in triplicate, and technical triplicate replicates also performed. The effects of dissolved oxygen (DO) on copper-induced microbial toxicity were assessed using the same methods as above, in the swirl reactor, and at 6 litre scale in an Infors Minifor fermenter (Reigate, UK). DO was measured via the manufacturer's proprietary probe and adjusted by air injection and variable stirring rate via the equipment's software.

3. Results

3.1 Flow characteristics

Figure 2b indicates that the tangential flow and the swirl are strongest close to the impeller and tend to get weaker downstream (which is apparent from both the direction of the streamlines and their colour; i.e. the velocity magnitude). This

reduction in the magnitude of the swirl is likely to be caused by the friction due to the pipe walls and the effects of the viscosity of the fluid.

A typical flow field measured using PIV (acquired at 716rpm) is shown as contours of the mean axial velocity in Figure 3. Negative flow velocities can be seen along the axis of the pipe (red colour) indicating flow reversal and high velocities near the pipe wall in the vicinity of the impeller due to the strong swirl produced.

The central recirculation flow region was observed for all rotational speeds examined (see also Figure 4); it is an inherent feature of confined swirling flows, characterised by fluid moving upstream towards the impeller in the centre of the pipe, whereas the surrounding region is characterised by forward moving fluid. Hence the flow comprises two regions: a central vortex core region and an annular region. This is clearly demonstrated in Figure 4 that compares the axial mean velocity profiles in two axial locations, 90 and 220 mm downstream of the impeller for all measured rotational speeds. The velocities are normalised by the impeller tip velocity; negative velocities can be seen in the centre of the pipe and high positive velocities near the wall. The negative velocities reach higher magnitude further downstream of the impeller, whereas the positive velocities are higher near the impeller. The width of the recirculation region (i.e. the region of the pipe diameter where $\bar{u} < 0$) does not seem to change between the two locations and is around 0.4 d. The present configuration constitutes a fairly large length- to- diameter swirling flow and interaction between the two flow zones identified as well as with the pipe wall will result in swirl decay as the flow moves downstream the impeller.

Figure 5 shows the instantaneous radial velocity, measured along the pipe centreline, 90 mm downstream of the impeller at N=716 rpm. The signal is highly unsteady and

274 shows large fluctuations indicating that the flow is highly turbulent in this region and
275 the mixing is efficient. Some periodicity is also evident in the signal which is most
276 likely due to the rotation of the impeller.

277 It is common to quantify the turbulence and mixing of the flow using the Reynolds
278 stresses which are defined as the time-averaged product of the unsteady velocity
279 signals. Normal and shear stresses can be defined in this manner considering the
280 fluctuations in the axial, radial and tangential velocity signals. The normal Reynolds
281 stresses in the radial direction are defined as $\overline{u'_r u'_r}$, where u'_r is the fluctuating (i.e.
282 zero mean) component of the radial velocity signal, and the overbar indicates the
283 product of the two signals are averaged. Various components of the Reynolds stresses
284 can be calculated; however, the component $\overline{u'_r u'_r}$, is sufficient to (a) quantify the
285 magnitude of the turbulence, and (b) observe how it varies along the pipe for different
286 impeller speed. In order to account for the changes in the impeller speed, the stresses
287 are normalised by the square of the tip velocity of the impeller, V_{tip}^2 .

Figure 6 shows the variation in the Reynolds stresses across the pipe diameter at various impeller speeds at two positions along the pipe. The profiles in Figure 6 a, acquired 90 mm downstream of the impeller, indicate that the turbulence and mixing are approximately uniform across the pipe width in this region. The fact that the magnitude of the normalised stresses is roughly the same for each impeller speed indicates that the absolute magnitude of the turbulence is proportional to V_{tip}^2 . This means that the magnitude of the mixing can be controlled by the impeller speed; for example, if required, the mixing intensity can be increased by a factor of four, by doubling the impeller speed.

Further downstream (Fig. 6b), a change in the distribution of stresses can be observed for high rotational speeds, with peak values exhibited on the pipe centreline. The magnitude of the stresses are similar to those observed closer to the impeller (Figure 6a), which indicates that although the swirl becomes weaker further from the impeller, this does not result in a significant reduction in the turbulence or mixing along the pipe. The flow in the fermenter with the dual Rushton-type impellers, is also turbulent ($Re = \sim 9000$) with a V_{tip} of 0.314; however due to the spacing of the impellers they operate independently producing four stable vortices with no interaction between the impeller streams as described by Rutherford *et al.* (1996). This results in mixing to be localised in the vicinity of the impellers.

3.2 System efficacy.

The fermenter was designed to promote bacterial growth, through efficient mixing and mass transfer, and, in order to maintain DO levels above 8 mg/l, the stirrer rotational speed was above 150 rpm with the consequent initiation of a weak vortex.

There was a more rapid loss in *E. coli* viability using Cu-alginate beads in the swirl flow reactor at 10^6 viable bacteria/ ml, than in the fermenter, with 95.6% of bacteria non-viable after 10 minutes in the former system compared to 46.4 % in the fermenter performed assay (Figure 7). After 15 minutes, no viable cells were found in the swirl flow reactor sample, whereas the chemostat samples retained between 11.4 and 1.8% viability of *E. coli* until the conclusion of the assay (Figure 7). However, the efficiency of the swirl reactor was dependent on the dissolved oxygen concentration of the media, and, when using the Cu/alginate beads in the swirl reactor, increases in CFUs from 6×10^6 ml to 8.75×10^6 ml were observed when DO concentrations were maintained below 0.1 mg/l after 25 minutes (Figure 8). When DO concentrations were maintained above 8 mg/l, the number of CFUs of *E. coli* was reduced to zero after 10 minutes and remained so throughout the study. The low DO treatment did result in a reduction in CFUs to 6.48×10^6 per ml after 30 minutes, continuing to 7.5×10^4 per ml after 45 minutes, and, at the end of the study, 1.8×10^4 viable *E. coli* remained per ml (Figure 8), representing a mean loss of viability of 96.4%.

To try and assess the baseline effect of the swirl flow reactor and the durability of this technique, 1×10^6 per ml of *E. coli* was added to the reactor and sampled at ten minute intervals, for 30 minutes without beads. No loss in viability was observed during this time; in fact some growth of the culture was seen (Figure 9). However, upon addition of the Cu/alginate beads, a reduction in CFU/ml to 0 was observed after 15 minutes, and this reduction replicated upon repeat doses of *E. coli* every 30 minutes, until the end of the study at 150 minutes (Figure 9).

Bacterial loadings ranging from 10^3 /ml to 10^6 /ml produced a 0.85% loss in system efficacy (Figure 10). However, above this value there was a negative correlation between cell number and loss of viability ($r^2 = 0.950$). Even so, at a bacterial loading

337 of 10^9 /ml, 94% of bacteria were non-viable after 15 minutes (Figure 10) and 99.997%
338 after 30 minutes (data not shown).

339 Using the swirl flow reactor, there was a 99.7% reduction in *E. coli* viability at 19°C
340 after 35 minutes; this figure reduced to 95% at 13°C and 79% at 9°C. Interestingly,
341 the efficacy of the beads was reduced at the lower temperatures in the fermenter
342 system, with 99.7 % viability at 28°C falling to 76.1% at 25°C and 39% at 21°C
343 (Figure 11). The copper/alginate beads retained integrity during the trial, and still
344 retained effectiveness after 5 repeated trials.

345

346 4. Discussion.

347 The original remit of this research was to develop a cheap and effective means of
348 destroying pathogens such as *E. coli* from secondary effluent produced from
349 DEWATS in developing countries. The device tested herein, referred to in our
350 laboratory as the vortex bioreactor, had to be simple to build, with parts easily
351 available in remote locales. The device described above was manufactured from
352 plastic pipes and fittings commonly found in any plumbing supplies store, and were
353 powered by a simple motor; in the case of our bacterial exposure tests this was an
354 electric drill. The only part that may be difficult to obtain was the impeller, however
355 any centrifugal pump-type impeller could be adapted, and we have already
356 manufactured and tested impellers produced on a Makerbot 3D printer.

357 In order to generate anti-bacterial properties within the reactor we combined the
358 system with a Cu-alginate bead previously shown to be bactericidal (Thomas *et al.*,
359 2014). Indeed, when the beads were absent, *E. coli* was shown to not only remain
360 viable but actively grow in the running system. The introduction of beads to the swirl
361 reactor provided rapid and repeatable reduction in *E. coli* viability. We have
362 previously described the use of Cu alginate beads as an antimicrobial agent (Thomas
363 *et al.*, 2014), However, the lysis was more rapid in the swirl flow reactor than that
364 previously observed in shaken flask cultures and in a well-mixed fermenter system
365 (Figure 7). During this study, the flow parameters were measured and it was apparent
366 that the particular characteristics of the swirl flow served to increase bead/media
367 contact. The dispersion of particulate matter in turbulent vortical flows depends on
368 both particle parameters, such as size and density, as well as the fluid properties. Very

small particles like the *E. coli* will follow the flow faithfully; this means that they are more likely to be entrained by the large scale vortex formed along the pipe; the beads however are larger than *E. coli* and slightly heavier and tend to accumulate in the periphery of the central vortex and are not entrained by the vortex. The interaction of particles with vortices is expressed using the Stokes number, defined as the ratio of the particle response time to the flow characteristic time. When the Stokes number is much lower than unity ($Stk \ll 1$), as is the case for *E. coli*, the particles follow the flow streamlines and are trapped by vortices, but when the Stokes is around 1 (intermediate particle sizes), as is the case with the beads, they migrate from vortex cores and concentrate preferentially at the edges of the vortices (Crowe *et al.*, 1995). This compares to the fermenter with the Rushton impellers, which generates four stable vortices, with no interaction between the two streams from the impellers producing more localised mixing characteristics (Rutherford *et al.*, 1996).

It can therefore be speculated that the different particle dispersion characteristics of the beads and bacteria will cause them to follow different paths through the system. This will enhance the probability of contact between the beads and the bacteria, and thus increase the rate at which the *E.coli* are lysed.

The rate of loss in bacterial viability was also influenced by the dissolved oxygen (DO) concentration in the fluid. Even when no bacteria were present, the DO content of the system diminished rapidly due to pressure differences induced by the impeller (Camacho-Martínez *et al.*, 2010) and the flow restriction provided from the upstream 90° bend causing cavitation and consequent loss of O₂. Under anoxic (DO < 0.1mg/l) conditions the destruction of *E. coli* by copper alginate took longer than observed under oxic conditions (Figure 8): this is consistent with the findings of many, that the initial Cu-induced damage to bacterial cells is oxidative in nature and initially cell-

394 membrane targeted (Grass *et al.*, 2011; Gulya *et al.*, 2013; Mathews *et al.*, 2013;
395 Ochoa-Herrera *et al.*, 2011). In the experiment described above, the DO levels were
396 maintained above 8 mg/l by the addition of compressed air; however, the use of a
397 Venturi-type system may be more appropriate for further field trials, due to the
398 reduced energy demand. Additionally, the copper alginate beads performed across a
399 wider temperature range in the swirl flow reactor than in a fermenter (Figure 11). The
400 reasons for the positive effects of the increased turbulence on loss of bacterial
401 viability are not immediately apparent and require further investigation. It may be that
402 the increased shear forces or cavitation imparted by the reactor impeller imparts a
403 synergistic effect with the Cu/alginate beads, and that the cell membrane of *E. coli*
404 receives sub-lethal damage, which enables more efficient Cu²⁺ binding. Indeed a
405 similar synergistic effect has been observed in an Ag nanoparticle-cavitation system
406 (Dhermendra K. Tiwari, 2009), chlorination systems (Duckhouse *et al.*, 2004) and the
407 effect of high shear forces on bacterial viability is well known (Thomas *et al.*, 2011).
408 The system provided effective reduction in *E. coli* viability up to a density of 10⁶
409 cells/ml, but the effectiveness of the system was negatively correlated with cell
410 numbers above this threshold. This is probably due to saturation of the copper
411 substrate in respect to the bacterium in the typical fashion of a dose response curve.

412 However, the system still requires optimisation; swirling flows are particularly
413 complex, three dimensional and depend on a number of parameters, such as inlet and
414 outlet conditions. Further work is required to resolve the effects of the inlet
415 conditions, swirl generation (i.e. impeller geometry), length to diameter ratio and exit
416 conditions. Due to optical access limitations, the planar PIV flow measurements
417 reported in this study could not resolve the tangential velocities of the swirling flow;
418 future work with stereoscopic PIV measurements could address this issue. All flow

measurements were taken in the absence of the solid phase. However, particle laden flows are known to modulate turbulence: they can either increase or decrease turbulence and hence future work should take into consideration the particulate phase. Such work could also examine the effect of bead size and density in order to vary the dispersion characteristics of beads relative to *E. coli* and further optimise system efficiency. Although the beads retained integrity and efficacy during this study, there is the need to improve their mechanical strength before this system could be used on a commercial basis.

5. Conclusion

This early work indicates the potential for this system for other processes that require high mixing and shear forces. Swirl flow separators, such as hydrocyclones and voraxial separators, are well known in both waste water and oil water separators, and are claimed to be a low energy solution to liquid/liquid and liquid solid separation at very large volumes (Faram *et al.*, 2004). However, although hydrocyclones have been used to effectively separate cells (Elsayed *et al.*, 2006) to this group's knowledge, such systems have never been used to effectively lyse bacteria. Such systems should be scalable to local and municipal scales and be able to enhance such current treatment methods as chlorination or novel techniques such as metal ion addition. It could also be imagined that with further development and optimisation mixing and separation can be combined, to further enhance the utilisation of this method.

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