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

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ABSTRACT

Despite the increasing use of Decentralised Waste Water Systems (DEWATS) in the developing world, which effectively dewater sludge, the problem of preventing the pathogen-laden water produced by these systems from re-entering the food chain constitutes a continuing burden on developing countries, which hinders subsequent advancements. We report on a swirl flow reactor generating high mixing areas which in conjunction with Cu/alginate beads effectively reduces *Escherichia coli* numbers by five orders of magnitude in 10 min. The system is simple, low cost, portable and modular; it can be assembled with simple plastic plumbing parts available in most areas and, once further developed, may represent a useful adjunct for both existing and new DEWATS facilities.

1. Introduction

The rapid expansion of mankind's endeavours and prosperity seen during the industrial revolution was fuelled by the discovery of how to effectively separate sewerage and potable water. The result of effective waste water treatment was the ability for large numbers of people to co-exist in urban areas, due to the prevention of the massive mortality and morbidity caused by pandemics of diseases such as Cholera and Typhoid. This also resulted in a reduction in infant mortality, further fuelling growth and prosperity, through an expanding and healthy workforce [12].

Unfortunately, there are still vast areas of the planet where humans are denied even rudimentary toilet and waste water treatment facilities. For example, in Sub-Saharan Africa an estimated 59% of the rural population has no access to clean drinking water [21], and even in the African economic powerhouse of South Africa, an estimated 5 million people, over 5% of the population, have no direct access to clean drinking water [35].

Despite a worldwide reduction in infant mortality during the last 30 years, the number of premature infant deaths in Africa has reduced at a slower rate, with an estimated mortality rate of 101 per 1000 live births reported in Sub-Saharan Africa during 2010 [2]. Two thirds of these deaths were due to infectious diseases, and although the increase in mortality due to Acquired Immunodeficiency Syndrome (AIDS) accounts for many of these deaths [19,16], diarrheal diseases still account for up to a third of mortalities, and up to 10.6 million infant deaths worldwide per annum [19].

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 [35], the cost of associated morbidity is vast, amounting to an estimated $6.24 per household for every episode [3]. 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 [20,27,31], *Escherichia coli* and the closely related *Shigella* sp. are the major causative agents of diarrheal diseases in Africa (Adjuik et al. [2]). The increasing prevalence of enterotoxigenic (ETEC), enteropathogenic (EPEC) and enterohemorrhagic (EHEC) serotypes which are associated with higher infant mortali-

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ties 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 [6,23,24].

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 [23,24], 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 [30]. Infection is often by the faecal–oral route, and although infection in developed countries is often due to contaminated food, contaminated water is still a major reservoir of infection in regions such as Africa, where an estimated 345 million people are still unable to access clean drinking water [2,21].

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

There is much ongoing research into cheap and robust methods of sewage treatment in rural and densely populated urban areas of developing countries where infrastructure and practicalities mitigate against traditional waste water treatment methods. In areas such as Africa and the Indian subcontinent, DEWATS are increasingly used as part of the solution for sewerage remediation [28,21]. These modular systems use anaerobic digestion coupled with the dewatering of the resultant sludge, followed by the subsequent disposal of the solid content [28]. Unfortunately, there remain large volumes of pathogen-laden water, and the release of this effluent into the water course can constitute a considerable public health hazard [21]. Water treatment technologies suitable for use in developing countries have been reviewed previously by Loo et al. [17], and the technologies typically utilised in developed countries, such as ozonation, chlorination and UV treatment, are neither cost effective nor efficient for this particular waste stream, due to factors such as high solid loading, high colour content, remoteness of location or high capital and running costs [34]. 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 [18]. 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 [13]. 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 [22]. 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. [33] to reduce the minimum inhibitory concentration of dendritic copper to *E. 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 recirculation resulting in extensive contact between particles) with those obtained from a 6 L fermenter (equipped with two Rushton-type impellers with each of them generating two counter rotating vortices in its vicinity) as a baseline for scale up from bench scale tests, and determine the effect of temperature, dissolved oxygen and bacterial loading on the efficacy of the system.

2. Methods

2.1. Swirl flow reactor

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

2.2. Flow measurement

The flow in the bioreactor was characterised by means of Particle Image Velocimetry (PIV) which is an established optical diagnostic method to measure fluid flow [25]. A schematic of the experimental set up is shown in Fig. 2a. The 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% [9,15]. 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 Fig. 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 220 mm 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.