Chapter 12

Development of Vortex Bioreactor Technology for Decentralised Water Treatment

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Additional information is available at the end of the chapter

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Abstract

The vortex bioreactor (VBR) is a simple decentralised water treatment system (DeWaTS) that sits at the interface between swirl flow, biotechnology and chemical engineering. The device utilises swirl flow and suspended activated beads to achieve downstream water processing and has been tested for applications including centrifugal-driven separation, pathogen neutralisation and metal absorption. The VBR was optimised for the treatment of faecally contaminated effluents in the developing world, and the design features related to the key challenges faced by the wastewater industry are highlighted here. The VBR has two aspects that can be modified to generate different reactor conditions: the impeller, where the swirl flow is modified through alterations of rotation speed, and impeller geometry and the suspended activated beads, which facilitate mixing and alter the reactor surface area. Data from testing for some of the different applications mentioned above are presented here, and future planned developments for the technology are discussed.

Keywords: DeWaTS, swirl flow, wastewater treatment, bioreactor, remediation

1. Introduction

1.1. Water and wastewater

Water covers 70% of the surface of the planet, and yet the world currently faces a water crisis. Of this hugely abundant resource, less than 1% is available for human consumption. Two-thirds of all fresh water is locked up in glaciers and ice caps where it is typically physically separated from humans and is, therefore, not widely available for use. The remaining 97% of

the global water is saline, present within the seas and oceans. This is inappropriate for agricultural uses, industrial cleansing or human consumption without significant energy inputs and desalination efforts, although it can be employed for some limited applications, such as certain types of cooling in industrial processes. These water sources have not changed in the last 100 years, but in that time the population has undergone rapid expansion. The majority of water used by humans is either as an energy carrier in thermo-electrical power generation; where it is used for both cooling and steam production to generate the driving force for the turbines or in agricultural irrigation and cleaning [1]. The United Nations Food and Agriculture Organisation (UNFAO) estimate that 11.8% of the 3918 $km^3\ yr^{-1}$ fresh water withdrawn annually is used for municipal purposes, where it makes its way to households for drinking, washing and recreational purposes [2].

Global water volumes remain constant in a system referred to as the water cycle, and so with the exception of deserts or very densely populated areas, physical limitations of water are not usually an issue. A more significant problem, however, is the limited supply of water that is either potable (suitable for human consumption) or at a sufficient quality for other municipal and industrial applications. After water has been used in an anthropogenic process, it is referred to as wastewater. Wastewater is classified as containing output of some combination of the sources given in **Table 1**. According to the UNFAO, in 2012, the world had access to 52,600 $km^3\ yr^{-1}$ fresh water resources, which is just over thirteen times higher than the amount drawn annually; however, this resource is not evenly distributed. Asia, for example, has access to around a quarter of available world water resources, but has almost 60% of the world population [2]. The majority of people are based in global urban centres, 80% of which are located on the coast or major waterways. Many cities around the world—even in Countries which have both high annual rainfall, and are members of the Organisation for Economic Cooperation and Development (OECD countries), such as London—are considered 'water stressed'. Being 'water stressed' occurs when an area requires access to more clean water than is available, or produces more wastewater than can be treated effectively. This results in a direct release of wastewater into waterways causing a reduction in water quality. This in turn has economic costs, through both work lost due to human illness and damage to the surrounding environmental resources—such as fishing stocks [3].

Name	Example contents	Risk factors
Blackwater	Excreta, urine and faecal sludge	**Pathogens**
Greywater	Bathing and washing water	*Volume increase*
Bluewater	Urban run-off and storm water	*Grit, debris*
Greenwater	Agricultural effluent*	**Eutrophication**, **pesticides**
Redwater	Industrial effluent	**Chemical and thermal hazards**

Each category has been assigned a colour to simplify reference within the text. For each, a brief summary of the category is given, and some associated risks with the wastewater are highlighted. Risks of untreated release to human health or the environment are in boldface, whilst risks that affect downstream processing are italicised.

**Agricultural effluent includes effluent from aquaculture and horticulture*.

Table 1. Wastewater can be broken down into five key categories, which pose individual risks to both human health and downstream processing methods.

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1.2. Water treatment

The core role of wastewater treatment is to remove waste additives from a water stream until it is at a level suitable for environmental release. These levels are set by governments in each country, for example, the Environmental Protection Agency sets recommendations for the USA that are considered safe for aquatic life [4]. Depending on the source of the wastewater, different treatment methods are required—for example, bluewater (urban run-off and storm water) can contain grit and large debris and so needs to be put through Stage 1 processing or 'screening'; as unless removed, this debris can cause blockages and serious wear/damage to downstream machinery. This is the stage where most centrifugal type separators are employed in wastewater treatment, as swirl flow is generally not employed for transport, separation or waste processing beyond the initial latrine and grit removal stages. A general outline of the stages of centralised wastewater treatment is presented in **Figure 1**, and a brief explanation of the workflow is given below (**Figure 1**). As mentioned in the example above, Stage 1 processing is the removal of grit and debris. This is usually done using a combination of grids and baffles, however, hydrocyclones have been employed for grit removal in some cases. Stage 2 processing is an important precursor to Stage 3 water treatment, as it removes the majority of the activated sludge from suspension, typically through natural settling or through the addition of a flocculating agent such as iron chloride. This sludge can then be collected and dried, or run through anaerobic digestion to generate useful products such as methane, which can be used to power other parts of the wastewater treatment or sold to mitigate operational costs. Stage 3 processing is used to reduce phosphate and nitrate levels in the final effluent, preventing harmful downstream effects like eutrophication. The residual bacteria remaining in solution after flocculation are aerated and encouraged to grow, and in the process, the nutrients in the liquid are depleted and more sludge is produced. This sludge is then flocculated and processed as in Stage 2. Finally, the nutrient and sludge-depleted liquid will still contain some organisms that did not flocculate, so needs to be sterilised before leaving the treatment plant. This is typically done with UV sterilisation, but can also be done

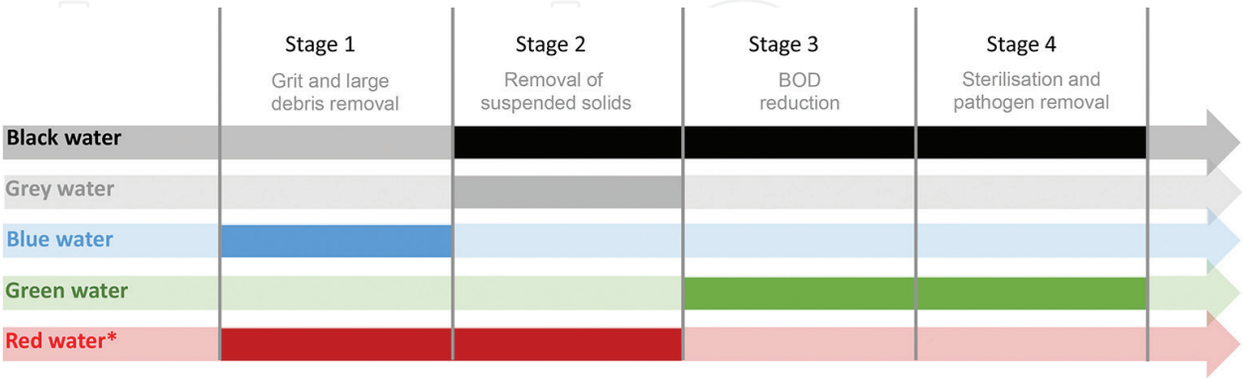

Figure 1. The different streams of wastewater described in **Table 1** as run through a classical wastewater treatment process. The stages of wastewater treatment have been grouped together into general stages to demonstrate the key aims of wastewater treatment, and shaded sections on the flow line indicate that the waste stream requires this stage of processing. These stages are (1) grit and large debris removal; (2) clarification/flocculation of suspended solids; (3) reduction of biological oxygen demand; and (4) sterilisation/pathogen removal. *Industrial effluent can vary significantly depending on the source as a result it is typically treated on-site to remove hazardous contaminants.

with chlorine or ozone dosing where the clarity of the effluent is an issue. The vortex bioreactor has applications for Stages 2–4 (clarification/flocculation of suspended solids through centrifugal-type separation, removal of nutrients by acting as a bioreactor and sterilisation/pathogen removal) of the water treatment processes. For further reading on the stages of wastewater treatment process, see Ref. [1], and for a comprehensive compendium of sanitation systems, see Ref. [5].

Large urban treatment plants are economic and highly effective at treatment of municipal wastewater, however, they are not always suitable [1]. This is particularly true in areas that lack established sewerage systems—sewerage systems have relatively high initial capital expenditure requirements and in rural environments the population density is simply too low to justify the cost. Centralised water treatment is also not a good solution for an area that suffers from intermittent power loss, as power is required for pumping the water to and from the central station and certain treatment processes. The power requirements for running wastewater treatment in the US in 1996 came to approximately 75 billion kilowatt hours (kWh), around 3% of the US annual electricity consumption that year [6]. Aside from the power costs, interruption of the electricity supply, such as from brownouts or blackouts, pose a significant risk to the fidelity of the wastewater treatment process. The large capital expenditure and operating costs involved in centralised water treatment results in public ownership or subsidies, which can be a major issue in countries experiencing political instability [3]. Finally, the water treatment industry in the developed world is incredibly resistant to innovation [7]. New large-scale technologies that could produce a step-change in processing techniques are slow to be implemented, a stance that is reinforced by effluent regulation requirements and possible fines resulting from a failure to meet water treatment standards.

Urban populations are rising faster than the average population growth rate, as more people move away from rural areas to cities [3]. Due to space limitations, growth of urban centres tends to occur in the outskirts of urban areas. These peri-urban areas, between the urban and rural zones, have a higher population density than the rural areas but, due to rapid growth, lack the key infrastructure of developed urban areas. As a result, wastewater management is a major issue for peri-urban areas; particularly municipal wastewater, which consists of grey (washing water), black (faecal contaminated) and blue (urban run-off) wastewaters. Due to the dynamic nature of these spaces, designing a suitable water treatment plan that is future-proofed, suitable and cost-effective is challenging. In these cases, decentralised treatment options are an ideal solution.

1.3. DeWaTS

Decentralised water treatment systems (DeWaTS) are small-scale water treatment systems ideally suited to operating in the urban, peri-urban and rural environments in developing countries—particularly in cases where pre-existing water infrastructure is either insufficient for requirements or unavailable [8]. A DeWaTS can be an individual unit, or a complete water treatment system, and can be utilised in either domestic or industrial water treatment. The amount of wastewater produced by an individual varies depending on environment, but typically an average person produces around 60 L of wastewater per day, with blackwater

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making up around 2 L on average and the remaining coming from greywater [5]. This implies that the average person requires access to 60 L clean water each day, and so any household level water purification system should be able to accommodate this requirement for all members of an individual home.

Typically, a DeWaTS will operate in the range of 1 m^3 day^{-1} (1000 L day^{-1}) for a household unit, to 1000 m^3 day^{-1} for a community treatment system. There are a number of defining characteristics that differentiate a DeWaTS from a model or an experimental water treatment system. The system should be reliable, built to last, tolerant to fluctuating inputs, cost-effective and above all have low control and maintenance requirements [9]. A DeWaTS designed to produce a profit should aim to return the initial cost of investment through sales of cleaned water or products produced from waste within the first 1–2 years of operation, to ensure uptake of the technology [3]. The vortex bioreactor was, therefore, designed with these vital features in mind.

1.4. Summary of the VBR

The vortex bioreactor system (VBR) is a highly versatile, modular DeWaTS, which utilises suspended activated beads within a recirculating swirling flow system to facilitate downstream liquid processing and multiphase reactions. The swirl flow and accompanying vortex, for which the device is named, are induced by an impeller, which can be driven by a variety of devices such as an electric drill motor or a 3D printable hand crank. Under certain operating conditions, the device can perform liquid-liquid separation and acts as a type of centrifugal separator, where a lighter liquid phase is entrained by the precessing vortex and is siphoned into a separate flow channel. Increasing the impeller speed increases turbulence in the system, resulting in better mixing, more interaction between the contents and as a result acceleration of chemical reaction rates. Notably, this effect is also modulated by the activated beads, which can enhance, but in some cases dampen, the turbulence effect. Altering the impeller design has been shown to change the vortex characteristics, although investigations into this with the VBR system are still ongoing. To date a hydrofoil type design, a rounded blade design and a lily design have been utilised for the impeller, but so far the effects of impeller geometry have not been systematically investigated. Finally, by altering the properties of the suspended activated beads, it is possible to run a variety of different reactions or separations. For example, reducing the density of the beads by introducing sealed air microbubbles during their creation causes them to move to the core region of the VBR for easy separation and recycling, whilst sponge-like porous beads with a high surface area can be used as both a heterogeneous catalyst for multi-phase reactions and an adsorbant surface for sequestration of toxic materials, such as heavy metals.

Due to the swirl generated by the impeller, the flow within the VBR is not uniform but rather characterised by low velocities in the core region due to the formed vortex and higher velocities outside. Fluid in the core moves far more slowly than outer part, and under some regimes, the suspended beads can be held almost completely stationary within the slow-moving core region [10]. A CAD image of a prototype variant of the VBR can be seen below (**Figure 2**). This prototype consists of a closed loop, built from widely available standard plumbing

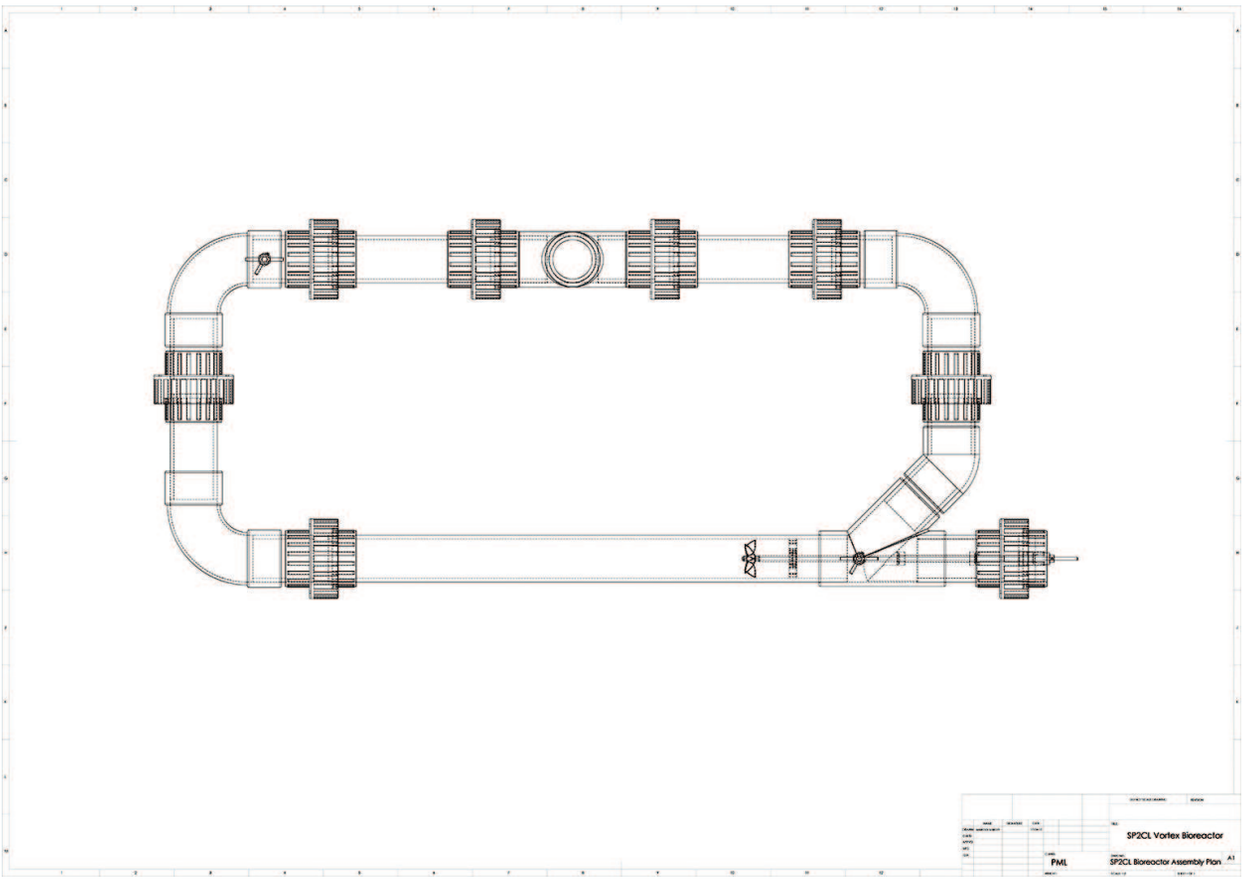

Figure 2. This CAD image shows one possible setup of the VBR system. The flow runs through a rounded quadrilateral pipe system, driven by an impeller. There is an inflow located at the top of the reactor. The prototype design also has a ball-valve controlled outflow (not shown) for emptying the system.

parts forming a rounded quadrilateral of recirculated flow. The design was constructed from 60.4 mm outside diameter and 57.4 mm inner diameter clear polycarbonate, joined with 2-acrylonitrile butadiene styrene (ABS) fittings. There is an inlet for filling the reactor at the top, and in the working prototype, a ball-valve controlled outlet was introduced at one corner for draining the VBR. Swirl flow is induced with an impeller on a shaft, which is driven by a 450 W variable speed drill motor (0–2400 RPM Bosch). This design is capable of generating a stable horizontal vortex, which entrains less dense materials, such as air or oil in water, and separates them from the carrier fluid [10].

2. Development of the VBR and case studies

2.1. Development of the VBR

The first prototype was designed and built by the Allen research group at Plymouth Marine Laboratory in 2010. It was intended for the separation of high-value oils produced by micro-algae from an oil-water suspension, using centrifugal flow technology. A low-cost variant of this technique is widely employed by the oil industry in the form of hydrocyclones, but swirl

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flow is not generally utilised in biological applications. Successful oil-water separation tests were performed on the device (see below), and during testing, the question of completing the whole process 'in one box' arose, and so investigation into upstream processing was carried out to see if the VBR could also be used for cell growth, oil production, cell rupturing for extraction of the biochemical products and ultimately separation of the functional product from the liquid media.

Microalgae are microscopic, photosynthetic organisms that are found in both marine and fresh water environments. They can be an environmental and health hazard when they bloom in waterways and are responsible for causing discolouration of standing water, but are a promising set of organisms for photosynthetic biotechnological production [11]. One of these high-value products is speciality oils, such as the omega 3 oils Eicosapentaenoic acid (EPA) and docosahexeanoic acid (DHA), which are valuable 'nutraceuticals' [12]. To extract these oils from microalgae, first the cells must be broken open, a process also referred to as cellular lysis. It has been documented previously that shear forces, when applied at high enough levels, can literally tear cells apart through an unevenly distributed pressure gradient against the cell membrane, or if cells are at a high enough density trigger cellular damage and death through violent collisions [13]. It is important to note that this threshold varies with different organisms and is dependent on the presence of air bubbles; when air is absent from the system, cells are much more tolerant to mechanical shear forces [13]. The large shear forces introduced into the flow system during turbulent flow were found to trigger cell death in *Escherichia coli* (a laboratory 'model organism') at high power inputs; however, at lower power, the shear forces actually contributed to cellular growth due to enhanced mixing and mass transfer characteristics [14]. This was when the 'activated beads' aspect was first introduced to the design, to encourage cell lysis at lower impeller speeds, and also where the design was first altered to be a DeWaTS, rather than just a downstream processing and separation system.

The activated beads were found to modify the VBR operation significantly. Not only did they improve the shear and mixing effects within the system, but they also opened it up to a variety of other modifications and applications far beyond the humble swirl-flow system origins. They appear to hold three key functions: they increase the reactor surface area, they appear to decrease the impeller speed needed to attain turbulent flow within the VBR in the conditions tested so far and they transform the device into a different class of reactor—from a chemical engineering point of view, it acts as a fluidised bed reactor and from a bioengineering point of view, it acts as a immobilised microcapsule perfusion bioreactor, where organisms can adhere to or be embedded within the activated beads. To estimate the increased surface area and determine how to control for it, a simple model was prepared, considering the beads as solid spheres in suspension.

$$\frac{4}{3}\pi r^3 = \text{vol of sphere} \; ; \frac{\pi}{3\sqrt{2}} = \text{dense sphere packing} \; ; 4\pi r^2 = \text{area of sphere} \tag{1}$$

$$\frac{x}{4r^3\sqrt{2}} = \text{number of beads} \tag{2}$$

$$4\pi r^2\left(\frac{x}{4r^3\sqrt{2}}\right) = x\frac{\pi}{r\sqrt{2}} = \text{bead surface area} \quad (3)$$

where x is the volume of beads added to the VBR, and r is the radius of each beads. The formula indicates a linear increase in external surface area with respect to both bead radius and the volume of beads added, so if double the surface area is required, then either the volume of the beads should be doubled, or the radii of the beads halved. Here, the surface area is approximated based on the volume of beads added, an assumption of dense sphere packing – such as face centred cubic packing or hexagonal close packing, and the radius of each individual bead, as it is impractical to physically count the beads and an estimation based on volume is much more convenient.

The internal surface area of the VBR can be calculated by taking the pipe circumference and multiplying it by the length of the reactor. In the case of the 9 L VBR prototype system described above, the internal pipe circumference is 18 cm and the length is 318 cm, resulting in an internal surface area of 5.7×10^3 cm^2. Using the model above, adding 1 L beads results in an addition of 2.22×10^4 cm^2, raising the overall surface area to 2.79×10^4 cm^2.

$$1000\ \text{cm}^3\frac{\pi}{0.1\ \text{cm}\sqrt{2}} = 2.22 \times 10^4\ \text{cm}^2 \quad (4)$$

Whilst this model only accounts for the outer surface of the beads, which is a conservative estimate for increased surface area in the 9 L system, it shows that adding 1 L of 1 mm radius beads to the prototype reactor design increases the internal surface area by approximately five-fold.

2.2. Case study: oil-water separation

To characterise the ability of the system to separate oil and water effectively, a series of experiments were conducted on a model system. It was not practical to directly test algal oils throughout the experiment due to the expense and the volumes needed, so a model oil consisting of vegetable oil dyed with Nile Red so it could be observed in water was used instead. Dyed vegetable oil is a good model for algal oil, as it is cheap, available in large quantities, and has a similar density and viscosity. Algal oil has a density of 0.864 kg L^{-1}, whilst the model oil had a density of 0.93 kg L^{-1}. The dynamic viscosities are shown in **Table 2**.

Test liquid	Dynamic Viscosity at 25 °C
Test oil and dye	μ = 0.0562 Ns m−2
Test oil	μ = 0.0625 Ns m−2
Algal oil	μ = 0.0233 Ns m−2

Table 2. The dynamic viscosities for the dyed vegetable oil was used as an affordable substitute for algal oil, the vegetable oil without the addition of the dye, and the algal oil that was being modelled.

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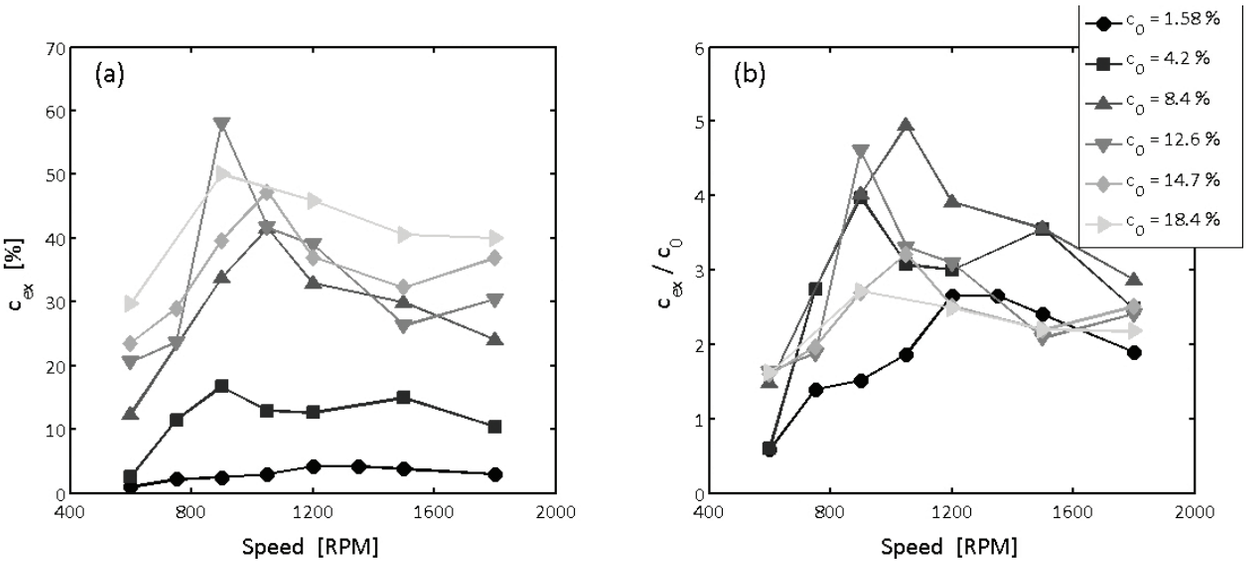

Figure 3. Variation in the oil concentration extracted from the VBR for a range of speeds and for a range of initial oil concentrations (a). The data in (b) is presented with respect to the initial oil concentration to show the efficiency of the extraction process.

An experimental series was run, beginning at an oil:water ratio of 1.58%. This ratio was modelled on a true extraction of algal oil from the growth media solution, based on the growth density of the algae and the relative quantities of oil they produce. The concentration of oil was gradually increased in the series, based on the assumption that the concentrated oil–water mix could be re-run through the system until a desired concentration had been attained or the volume limit was reached. This system was run at a number of impeller-tip speeds in order to optimise the vortex characteristics for maximum oil separation levels (**Figure 3**).

A series of experiments were run, in which the ability of the VBR to extract oil from a mixture of oil and water was tested for a range of impeller speeds and oil:water volume ratios; beginning with the expected initial value of 1.58%. The concentration of oil was gradually increased in the series, based on the assumption that the concentrated oil-water mix could be re-run through the system until a desired concentration had been attained, or the volume limit was reached. This system was run at a number of impeller-tip speeds in order to optimise the vortex characteristics for maximum oil separation levels (**Figure 3**). **Figure 3(a)** shows the concentration of oil in the mixture extracted from the syphon (cex) for a range of impeller speeds (600–1800 RPM) and a number of different initial concentrations of oil in the system (c0 = 1.58–18.4%). The same data is presented in **Figure 3(b)**, normalised with respect to the initial concentration to show the relative increase in the oil concentration at each step, i.e. the 'efficiency' of the system.

For most initial concentrations, the maximum concentration of oil extracted tends to occur in the range of 800–1200 RPM. At low speeds (<800 RPM), the swirling motion was too weak to entrain the oil droplets into the vortex core, and the oil remained at the top of the pipe. In contrast, when the impeller speed was high (>1200 RPM), the flow became strongly turbulent

and the oil broke into small droplets (a process known as 'emulsification') that tended to disperse throughout the fluid. This meant that fewer droplets remained in the vortex core, and the efficiency of the VBR was reduced. The data also suggest that the same efficiency may be achieved from a low initial concentration by sequential processing. For example, from an initial concentration of 1.5%, it is possible to increase the oil concentration to 4%; from 4% it is possible to increase to 12%, and so on. The maximum concentration extracted was close to 60%, although it is possible that the VBR could produce higher concentrations if the initial concentration of oil was greater.

A series of power consumption measurements were also made on the system, to determine the energy requirements and financial costs associated with operating the device at a range of rotational speeds. The measurements were initially performed when the device was filled with water, and subsequently, when it was filled with an oil-water mixture (**Figure 4**).

The measured power consumption is shown in **Figure 3**. When the motor was not moving, the controller drew approximately 4 W from the mains. As the motor speed increased, the power consumption also increased in a linear fashion. The power consumption is approximately given by

$$P = (0.064\,N + 4.1)\,\frac{V}{9.5} \tag{5}$$

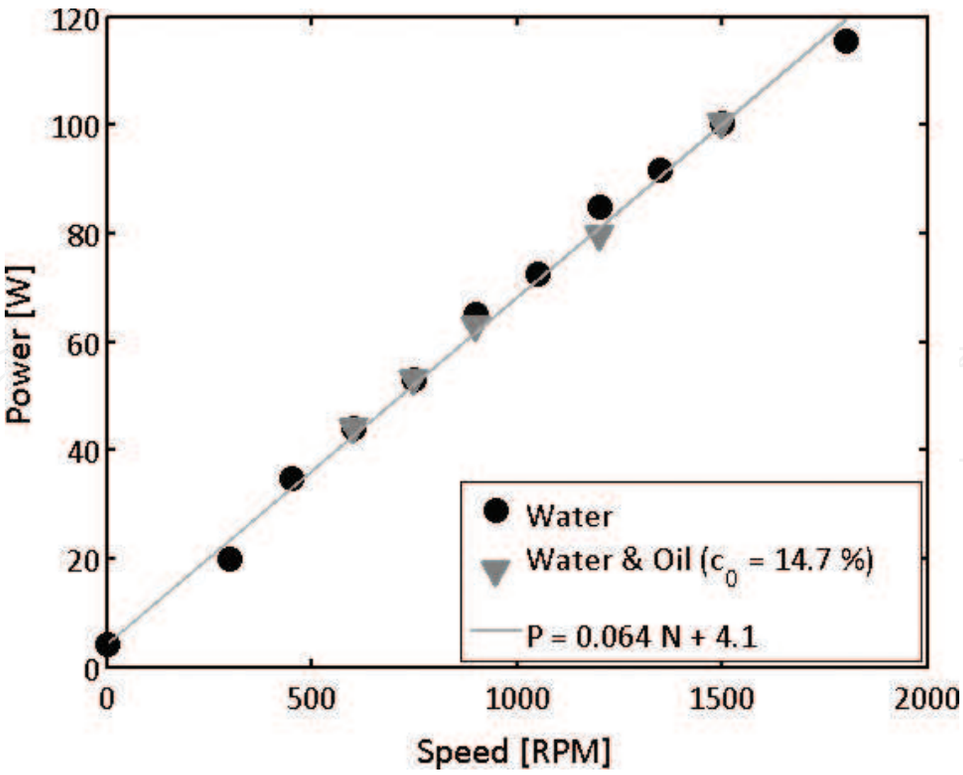

Figure 4. The variation in power consumption with increasing motor speed, measured when the SP-2 contained pure water (black circles) and a mixture of 14.7% oil (grey triangles).

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where N is the motor speed, (in RPM) and V is the volume of water in the VBR (V = 9.5 L in the current experiments).

The presence of the oil did not affect the total power consumption of the system. This indicates that to process 1000 L of oil-water mixture at optimal motor speeds (approximately 900 RPM), using a motor with a comparable efficiency, would require 6.5 kW. Over the course of an hour, this would cost £0.65, assuming an electricity cost of approximately £0.10 per kilowatt hour based on typical rates for domestic use in the UK [15]. This indicates that (i) the VBR represents a cost-efficient method of refining the concentration of oil in an oil-water mixture, and (ii) the use of multiple steps to achieve a given concentration is not associated with a significant increase in operating costs; however, these values may change, depending on the volume being processed and the efficiency of the motor being used.

2.3. Case study: application of VBR as a pathogen-removal system

Biological disease-causing agents, or pathogens, are a major concern in wastewater treatment. Human faecal effluent is a major health risk, as the organisms living within it are already attuned to living within humans. A number of diseases have been linked to human-derived pathogens, including dysentery, typhoid and gastroenteritis [16]. In wastewater treatment, the biological quality of the water is determined by quantifying 'coliforms'. Coliforms are a subset of bacteria that are easy to culture and are present in large numbers in warm-blooded animal faeces—making them a good test for faecal contamination in wastewater [17]. The organisms are quantified by colony forming units (CFUs), referring to the number of living cells within a fixed volume. Guidelines state that to minimise risks to human health, wastewater containing blackwater should contain no measurable CFUs per 100 ml, and waste water from a known non-faecal source, such as redwater (industrial effluent), should contain no more than 10 CFUs per 100 [18].

Copper has long been known as an antimicrobial substance, given its employment in hospital door handles and work surfaces [19]. To trigger this effect within the reactor, dendritic copper micro particles were added to the system as a powder. A copper pipe was also considered; however, due to the comparably limited surface area and relatively high cost of copper within the otherwise cheap final design and the risk of corrosion over time under continuous exposure to water, it was deemed to be unsuitable [14]. The copper powder was found to be effective at triggering cell lysis; however, separating the copper from the system afterwards was considered too complicated for the design, a vital step in this case, as copper is toxic to aquatic ecosystems—and humans [20]. To aid in terminal removal from the system after processing, the copper powder was embedded within spherical, alginate matrix, hydrogel particles—referred to as the activated beads herein. A test was carried out to see if embedding copper within alginate would still trigger cell lysis; intriguingly, not only did the cells still lyse, but they did so at a more rapid rate than a comparable study with the powdered copper [20].

When the activated beads were run in the VBR system, they were found to be more effective at triggering cell lysis than the equivalent amount of free copper microparticles. Death of all the cells in the sample (initially at a concentration of 1×10^8 CFU ml^{-1}) occurred within

15 min, a rate approximately 6× faster than had previously been reported in flask experiments. In addition, this effect was observed repeatedly when additional organisms were added into the system after all the previous cells were dead. Whilst the structural integrity had been affected by the turbulent flow at particularly high speeds, the activated bead fragments could still be extracted from the system safely with minimal copper leached into the system [10]. Development of the device subsequently received financial support from the Bill and Melinda Gates Foundation, for development as a low powered, affordable alternative to UV light sterilisation of water. This resulted in testing the device on wastewater streams in a number of facilities located around the world [21]. The device demonstrated clear bactericidal effects on coliforms within seconds of exposure to the VBR—activated bead combination, and even destroyed the radiation hardy *Deinococcus radiodurans* in a direct comparison with UV treatment. To finalise the device for widespread distribution, the main feedback received from the world-wide test was that the activated beads would need to be further developed; both to increase their longevity, and to include some additional method that could sterilise the copper-resistant and shear-resistant helminth eggs—as parasitic worm infections stemming from wastewater are a major health concern in the developing world [21].

2.4. Case study: VBR as a metal-sequestering device

Embedding the copper within an alginate matrix proved extremely effective as an immobilisation technique. The low levels of measurable copper in solution—despite fragmentation of the beads during operation of the reactor—and the greater than expected rate of bactericidal action within the VBR doped with copper-alginate beads, instigated an investigation on the metal adsorption qualities of alginate in the VBR. The theory behind this application is based on alginate being a polyionic polymer that forms a hydrogel stabilised by calcium ions; it was thought that copper—or some other metal ion—could displace these calcium ions and become sequestered in the material [22]. The advantage of using alginate beads for this application is that because the bulk of the beads are composed of liquid, and as a result can be dried after use to concentrate any extracted materials for recycling and reduce volume. This is a particularly useful application when considering the VBR as a device for redwater treatment, as many industrial processes involve the use of metals and metal salts that need to be extracted from the final product due to their value, toxicity and environmental impact.

To generate hand drawn alginate beads, sodium alginate solution was added in a drop-wise fashion by hand from a syringe to cold calcium carbonate solution. The beads created by this process were approximately 2 mm diameter on average, although beads created in this fashion did show some size variation. An automated variant of this process was conducted with a syringe pump to generate a more repeatable size distribution. Features of alginate beads can be altered by changing the reagents used in their generation—for example, increasing sodium alginate concentration will create more rigid beads; whilst mixing copper-powder into the solution will create beads embedded with copper and air entrainment can create lighter beads for simpler separation from liquids such as water, as such beads are relatively more buoyant than the liquid.

The metal-absorbing properties of alginate beads were tested with metal salt solutions containing copper sulphate, nickel sulphate and chromium chloride at a high (100 mM) and low

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(30 μM) concentration. The alginate beads were added to make up 50% by volume of the total volume and were tested in shaking flasks containing each of the metal salt solutions. The beads were found to have metal-absorbing properties in shake flasks, with 30–40% of the metal by concentration being extracted from solution over a period of 30 min. The samples were left for a further 60 min, although a negligible amount of additional absorption was observed. The volumes of metal being extracted were consistent with partial volume removal of a fully diffused solution. This suggests that the liquid initially sequestered in the hydrogel gradually dilutes the sample as the metals permeate the beads. The experiment was repeated with stationary flasks, producing largely similar results, suggesting that fluid motion had a limited effect on diffusion within the beads. To verify this observation, beads that had already absorbed metals were extracted and recycled into fresh metal salt solution at the initial concentration. Each additional round of extraction resulted in a diminishing rate of return, as the beads became saturated with metal approaching the initial concentration.

When the beads were tested in the VBR, the same final rate of absorption was observed, however, an interesting phenomenon was observed repeatedly through all experiments. Within the first minute of extraction, the rate at which metal salt was extracted was far more rapid in the VBR than in either shaken flasks or static flasks. The rate of absorption equalised with other experimental set ups after around 8 min; however, these data suggested that the swirl flow within the VBR has a beneficial effect on the rate of metal absorbance, at least with the outer part of the beads. This observation needs experimental verification by running the system for 1 min with a coloured metal salt and then extracting and dissecting the beads to see if the effect is limited to the outer shell or actually uniform through the bead; however, the data suggests that this is the case as all three systems eventually reach the same point. Understanding this effect has broader-reaching consequences, as alginate is a major constituent of certain medically relevant biofilms. Biofilms are a biologically derived fouling effect that utilise polymeric substances and act as a protective layer to organisms within them, conferring resistance to antibiotics [23]. If this effect could be isolated, replicated and controlled, it could provide beneficial techniques for the removal of biofilms in industrial settings—where a cleaning agent, or a medical antibiotic, could be delivered directly into the biofilm, rather than just relying on diffusion. Whilst the metal capturing hypothesis was not found to be valid with alginate, making the beads from a polymeric substance that has specific metal-binding ability—such as polyacrylamide—could provide a more effective metal absorption process, although this has not yet been tested. The alginate was tested in this case as it followed on from previous work being conducted at the time and had demonstrated a good ability to hold metallic copper in situ. It is likely that the initially observed effects are a result of the metal particles being unable to escape from the bead due to steric hindrance, rather than through a specific affinity to the alginate.

3. Future developments

As a relatively new and highly versatile piece of technology, there are a number of features of the VBR that remain to be optimised and tailored to the application to more fully understand the range of future applications it could be implemented with. The two main aspects that

affect operation are the swirl-flow regime—which is controlled by speed and impeller design, and the active beads. These are discussed in the context of remaining issues for the device as a DeWaTS and the potential applications the device has as a bioreactor. There are several other potential avenues for investigation with this technology; however, they are beyond the scope of this chapter.

3.1. Remaining challenges for the VBR as a water treatment system

There are three main potential issues with the VBR as a widespread functional DeWaTS device for water purification. The first, as mentioned earlier in this chapter, is the stability of the beads. During the process, shearing from the impeller results in the beads becoming fragmented. This is an issue, as whilst the dendritic copper powder used in the beads is relatively cheap, and can be recycled in the system, it is still the most expensive part of the fully operational system. One possible avenue for solving this is through modification of the beads. They could be modified in a number of ways, such as using the minimal amount of copper that still produces a lytic effect on pathogens to reduce the cost or by increasing the resistance of the beads to impeller shear by modifying the type or concentration of polymer. It is also possible that a different flow regime and less dense beads could be used in tandem, holding the beads static within the reactor whilst the wastewater passed around them. This would protect the beads from the impeller shear; however, it may reduce pathogen destruction efficacy, as the beads would no longer be present at the interface between the core and the turbulent outer part of the flow, and as a result may not encounter denser material or particulates within the reactor [10]. In addition, the location within the reactor where the beads have the strongest pathogen neutralising effect is not known and is also difficult to measure—if the strongest effect occurs at the site of the impeller, then this solution of keeping the beads separated from the impeller would likely be infeasible due to diminished antimicrobial activity.

The second major issue is power consumption; as highlighted earlier in this chapter, interruption of power poses a major risk to wastewater processing. The prototype VBR operating at 956 RPM has been shown to take 15 min to sterilise 10 L volume, and so needs to run for 90 min to clean 60 L pathogen-laden waste water—the average amount of waste water from all sources produced per person per day. With the energy consumption figures shown above (**Figure 4**), this equates to approximately 65 W. It can, therefore, be considered to have an annual power footprint of around 36 $kW{\cdot}h^{-1}\ yr^{-1}$ per person, which at standard UK prices [15] comes to an average cost of £3.60 ($4.70) per person annually. An alternative impeller design could reduce power requirements here by as much as 30% [24], and the device could be made even more efficient by selecting a motor of the minimum power required to remove pathogens. Both of these are efficiency measures, however, and do not preclude the requirement of electricity to keep the device operational.

A hand-crank was designed and 3D printed for the prototype, as a fail-safe option for this when access to electricity was limited or cut off. Whilst this option could be useful in an emergency it has a few issues. First, the obvious sociological issue—the 90 min per person per day time requirement for sterilisation is not an issue for a motor; however, an individual operating the hand-crank may have serious reservations about the technology, particularly if

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they are providing water for more than one individual. In addition, whilst a motor can be run at a fixed speed for a fixed amount of time, this same regularity is not ensured with a human operator. The effects of modulating the rate of impeller rotation on the pathogen removal will need to be thoroughly investigated if this avenue is to be considered in more depth. Designs that control the rate of rotation could be implemented, but they also increase the complexity of the device, which may cause problems for operation and repair. An advantage of the mechanism of pathogen destruction using the copper-laden beads within the swirl-flow system is that due to the mechanism of action, it practically only has a minimum threshold for pathogen removal—although high speeds will cause premature degradation of the beads. The antimicrobial effect occurs as a result of extensive cell membrane damage, where the shear forces, presence of oxygen and copper all contribute towards this effect. The device will still remove pathogens passively, however, the rate at which this happens is around six-fold slower.

There is a strong argument that having a powered device, even a hand-powered device, is a less suitable solution than designing a passive swirl-flow system, such as a hydrocyclone. A gravity-driven device may be able to achieve similar effects without the need for an external power source. Whilst such a design would be favourable, it is important to keep in mind the key principals of a DeWaTS during its design. It is also important to ensure that not only is the operation of the device simple, which a passive device should be, but also that any repairs should be possible with locally available parts. If the device is too complicated for the end user to repair themselves, or if the repairs are infeasible due to the expense or scarcity of the materials used, then when an issue occurs and the device fails, it will simply no longer be used. This sociological angle needs to be considered when designing global challenge-type technologies, particularly those that will be maintained by individuals rather than by a dedicated authority.

The final key issue that needs to be considered for the VBR as a DeWaTS is a simple efficacy or a failure test. As this device is intended for use by individuals, it should also come with some form of simple, reliable and cheap test to verify that the device has performed its function. Growth assays commonly used to determine the presence of CFUs are accurate and the materials needed are cheap, but the test requires sterile conditions, as well as specific training in aseptic technique. Without these, the test will produce false positive results. Some form of this test is essential for an operator, especially in cases where the design has been modified or repaired by an individual. An attempted but failed repair could result in a seemingly operational device, which is outputting harmful pathogen-laden water that is presumed to be clean. The test would need to follow the same principals as the rest of the DeWaTS design, and be free of expensive or difficult to obtain reagents. The field of synthetic biology may be particularly useful for this. Synthetic biology is a branch of genetic engineering that differentiates itself from the rest of the field by being founded in core engineering principals, namely, those of characterised standard parts and rational design. Initiatives such as the international genetically engineered machines (iGEM) foundation have created a repository of standard parts, most of which are freely available [25]. The repository is boosted by an annual iGEM competition, where numerous teams have created parts based around wastewater treatment. The advantage of a genetically engineered 'biomarker' for this task is that once designed it can be propagated indefinitely for very low costs. Furthermore, a design like this could be utilised in ensuring functional operation in a variety of different water treatment devices beyond the VBR.

3.2. Opportunities for the VBR as a bioreactor system

The VBR was named for the cell growth aspects that were observed during operation [14]. When microalgae grow photo-autotrophically—with light as their sole source of energy—the ensuing photosynthesis results in toxic levels of oxygen being produced. One of the main advantages with the swirl flow in the VBR design is the high levels of gas exchange which occurs between the liquid and any gas present in the system. This high level of exchange results in the dissolved oxygen levels being kept at a level closer to that of ambient air, providing a free air exchange that is enabled with the outside environment. Oxygen toxicity resulting from limited gas exchange is a significant issue for impeller-driven photo-bioreactor systems, hence the widespread use of energy intensive gas-mixed systems, such as air lift reactors or bubble columns. Typically, if a gas is not used for mixing the liquid, then a dedicated degasser compartment will need to be added to the design of any photobioreactor. A vortex degasser could have wide-ranging functionality in this field, as vortex flow is both an effective gas exchange method and a scalable technology, however, tuning the shear forces to avoid killing organisms from each individual species whilst maintaining maximum oxygen exchange, likely precludes a 'one size fits all' passive design.

Modulating the shear forces on the VBR can provide a user controlled growth-lysis switch; however, it is also possible to grow the microalgae embedded within the beads, which provides protection during recirculation. This is already done with alginate beads in industrial bioreactors with Chinese Hamster Ovary (CHO) cells for high-value pharmaceutical production, as these mammalian cells are highly susceptible to shear-derived lysis and grow much more readily when affixed to a surface; however, it is important to note that recent research suggests that alginate is not the most suitable encapsulation polymer [26].

Within the VBR, there are three key benefits to using this growth regime. First, the mixing rate could be increased to a level that encourages maximum gas exchange but does not damage the cells, reduce growth rates or negatively impact the final product. Second, introducing a hostile, high-shear environment within the reactor reduces the movement of biological organisms between the environment and the bioreactor system. This is true in both the inward (contaminant) and outward (containment) directions. It is accepted that during microbial scale-up a certain amount of external contamination will occur, however, under a high-shear regime in the VBR, contaminating organisms will either be destroyed outright, or will have their growth diminished so that the chances of them outcompeting the encapsulated species in the reactor is lower. When working with genetically modified (GM) organisms, there is extensive concern about a GM release. The same principles mentioned above that keep the culture axenic (free from contamination) will also limit GM release. Finally, the process could be run completely continuously rather than in batch like the majority of other systems. This removes the need for expensive turnaround between cultures. With the culture confined to the beads, it also becomes trivial to separate them from the media and to recycle the media by replenishing only the nutrients that have become depleted rather than needing to replace the entire liquid volume.

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