A new technique for the performance evaluation of clean-in-place disinfection of biofilms

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A concentric cylinder reactor (CCR) is described that enables the steady-state kinetics of microbial biofilms to be evaluated under conditions of constant nutrient flow and variable shear-stress. The reactor has been used to evaluate the influence of fluid dynamic shear on the extent and mode of detachment of bacteria from biofilms. Using a food factory isolate of Pseudomonas aeruginosa, a general increase in the overall growth rate and detachment of the biofilms (cfu cm⁻² min⁻¹) with time was shown for each biofilm, regardless of the prevailing shear. As the shear rate was increased beyond 0.123 ms⁻¹, populations tended to a pseudo steady-state. Sudden changes in shear force, however, caused dramatic changes in the productivity of steady-state populations. The CCR provides an effective means of testing disinfectant activity, particularly for clean-in-place situations, and allows for an examination of the residual effects of a cleaning programme on a treated surface for three different chemical classes of disinfectant. Utilisation of the CCR would, therefore, provide enhanced ability to determine the efficacy and efficiency of chemical products for use in sanitation protocols. *Journal of Industrial Microbiology & Biotechnology* (2000) 25, 235–241.

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**Introduction**

The ability of bacteria that are attached to surfaces as biofilms to transfer to fresh surfaces and to their surrounding medium is not only imperative for long-term survival within a manufacturing plant, but is also central to the problems of product contamination. This is of particular importance to the food industry where processing environments have an abundance of exposed surfaces on which microorganisms may attach, grow and develop into biofilms [11,17,20]. Within product pipelines these biofilms are sometimes able to resist complete removal not only during the process flow but also by cleansing chemicals and disinfectants [16]. They are capable of rapid regeneration following such treatments and thereby provide a source of contamination for the next product-line [8]. Failure to eradicate biofilms is part of their notorious recalcitrance toward a panoply of antimicrobial agents and is associated with their ability to survive relatively harsh hygienic cleaning protocols [3,10,11,17]. Changes in food-hygiene legislation and increased public awareness of product quality makes cleanability testing an important facet of food manufacturing.

The hygienic condition of equipment and processing surfaces is controlled by the application of cleansing and disinfection systems that assist in the removal or control of biofilms [18]. Such sanitation processes are designed to reduce surface contamination initially through cleansing with detergent formulations and secondarily to reduce the viability of the residual attached population [12]. The overall aim of sanitation programmes is to prevent microbial growth from occurring during the interproduction period [23].

It is well established that biofilm populations are more resistant to biocides than are organisms growing in suspension [9]. Although previous studies have noted that both the adhesiveness of planktonic bacteria and the strength of attachment of biofilm populations to surfaces can be affected by treatment with disinfectants [8,15], such studies have not examined the effects of fluid shear on the adhesion of attached bacteria. Moreover, although a variety of annular biofilm reactors have been used to observe the formation and properties of biofilms [6], none allow the simultaneous generation of different shear rates on the same inoculating population. Indeed, few studies have examined the ease with which colonising bacteria may be removed or the extent and manner of their detachment from surfaces [21]. It is unlikely that cell attachment will be unaffected by fluid flow across a surface. Therefore, increased understanding of the impact of fluid flow dynamics associated with sanitation processes is required. In this paper a new experimental rig designed to model the effects of fluid flow in pipework is described. The device provides a utility model for testing and improving upon the effectiveness of product cleaning-in-place (CIP) procedures. The approach utilises a rig comprising concentric steel cylinders constructed from food-grade stainless-steel piping, which may be rotated at variable speeds, within a multichambered, continuously fed, culture vessel. This enables the steady-state kinetics of a microbial biofilm formed on the surfaces of each cylinder to be evaluated under conditions of constant nutrient flow and variable shear-stress. The potential use of the device to assess the effectiveness of different biocide formulations over a range of fluid dynamic shear rates is demonstrated.

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**Materials and methods**

**Organisms and culture maintenance**

*Pseudomonas aeruginosa* PaENV, isolated from the internal face of a stainless-steel pipe used to transfer raw milk within a dairy, was used throughout the study. This organism was collected post cleansing of the system. Cultures were identified using Vitek and maintained on nutrient agar (NA, Oxoid) slants, in the dark at 4°C, after overnight incubation at 30°C. Overnight cultures were prepared from the slants by inoculating volumes (50 ml) of dextrose peptone broth (10 g l⁻¹ dextrose; 20 g l⁻¹ peptone; 5 g l⁻¹ NaCl, pH 7.4), contained in Erlenmeyer flasks (250 ml), and incubating the flasks at 30°C for 16 h in an orbital incubator (200 rpm).

**Media and chemicals**

Dehydrated culture media were obtained from Oxoid (Basingstoke, UK). The biocide formulations were (i) a buffered (pH 10) tertiary alkyl amine formulation in an amphoteric surfactant (TAAS, 1.4% v/v), (ii) a hydrogen peroxide, peracetic acid, acetic acid (pH 1) formulation (HPPA, 1.0% v/v) and (iii) sodium hypochlorite/sodium hydroxide (pH 13, HYPO, 0.84% v/v) obtained from DiverseyLever (Turku, Finland). All other reagents were of the purest available grade and were obtained from BDH Ltd (London, UK) or the Sigma Chemical Co., (Poole, UK).

**Experimental rig**

Biofilms were produced in the experimental rig shown in Figure 1. The rig consists of four cylindrical sections constructed from food grade stainless steel that may be rotated, at variable speeds, within four concentric chambers. At any given angular velocity the fluid dynamic forces experienced by the stationary and rotating surfaces are proportional to their radius. The 101-mm cylinder is, therefore, subject to a greater shear force than are the smaller (76-, 50-, and 26-mm) cylinders. This allows shear forces in the range 0.024–0.53 ms⁻¹ to be generated. The assembled rig, termed the concentric cylinder reactor (CCR), can be sterilised by autoclaving before being mounted into a rigid support and aseptically connected to a medium reservoir. Each chamber of the CCR is independently supplied with fresh medium from its base at controlled flow rates via a peristaltic pump and maintained at a constant volume via pumped reservoir overflows. Separate sampling ports are also provided for each chamber.

**Attachment of microorganisms and development of biofilms**

To establish a biofilm population within the CCR, the four chambers were filled with fresh dextrose–peptone broth, equilibrated to 25°C, and each chamber was inoculated by syringe and needle with a stationary phase culture of *P. aeruginosa* PaENV to give ~10⁵ cfu ml⁻¹ in each chamber. Stationary-phase cultures

*Figure 1* The concentric cylinder reactor (CCR). Section A illustrates a cross-sectional view of the four cylinders interlocked within the collecting chambers. Sections B and C show the collection chambers and rotating cylinders, respectively.