euspen's 21st International Conference & Exhibition, Copenhagen, DK, June 2021
www.euspen.eu

Development of a mechanical, biocide-free method of disinfection for cathodic dip coating processes

E. Uhlmann[1,3], M. Hilt[2], C. Hein[1], M. Cudazzo[2], K. Huth-Herms[1], C. Jahnke[1], M. Quast[2]

[1]*Fraunhofer Institute for Production Systems and Design Technology IPK, Germany*
[2]*Fraunhofer Institute for Manufacturing Engineering and Automation IPA, Germany*
[3]*Institute for Machine Tools and Factory Management IWF, Technische Universität Berlin, Germany*

katrin.huth-herms@ipk.fraunhofer.de
markus.cudazzo@ipa.fraunhofer.de

Abstract

Technical fluids are often contaminated by bacteria as *Burkholderia cepacia* (*B. cepacia*), which is found in different industrial issues and affects manufacturing process chains by the formation of planktonic cell-aggregates and biofilms within the working fluids. *B. cepacia* is one of nine species of the *Burkholderia cepacia* complex (Bcc), a group of gram-negative, motile, non-spore-forming, and rod-shaped bacteria. Because of the opportunistic pathogenicity to plants, animals, humans, and the multi-drug and biocide resistance, *B. cepacia* is difficult to treat. This study aims to provide an application to reduce the germ numbers n_g in an eco-friendly and continuous process without the use of biocides. The approach to disinfect technical fluids is to apply high shear forces in a rotor-stator assembly to the fluid. A prototype of the rotor-stator assembly with a variably adjustable shear gap g_s and rotor speed s_{rot} was constructed. First experiments with a frequency f_{rot} 10 Hz ≤ f_{rot} ≤ 40 Hz a shear gap g_s = 83 µm and g_s = 166 µm showed a reduction of germ number n_{gr} = 99.6 %. It concluded that the disinfection of technical fluids by a rotor-stator assembly is a biocide-free alternative. In addition to defined process parameters such as shear gap g_S, temperature ϑ, frequency f_{rot} and time of machining process t_{mp}, also the peripheral speed v_p, rotational speed v_{rot}, flow rate f_r and shear stresses τ were used to assess the machining result and to develop an overall concept for disinfection of technical fluids.

Micro-manufacturing, microbiology

1. Introduction

Many industrial processes require the use of various technical fluids. A problem of water-based fluids is their susceptibility to bacterial contaminations, representing a risk for human health and a negative impact on the fluid's properties, independent of the cause of the contamination [1,2]. Bacterial contaminations in technical fluids may have different origins. The bacteria may descend from the working process itself, but it is also possible, that the contamination descends from one of the ingredients or the merging process, or even another source [3]. Cathodic dip coating systems run with water-based paint [4]. Contamination of the paint may result in a decrease in the quality, including among other offensive odor, viscosity changes, discoloration, or changes in adhesion [1].

Today's companies can rely on a wide range of disinfection methods to control microbial growth in industrial processes, including both, chemical and physical methods [5]. Despite the large selection of possibilities, most of the conventional methods show disadvantages or are unsuited for certain processes like cathodic dip coating. For example, the frequent use of biocides poses a high risk to human health and the environment [5,6]. Other disinfection agents like heavy metals, or oxidizing agents may negatively interact with the paint pigments [7]. Disinfection of large paint basins by heat sterilization or ultrasound is ecological unsuited due to high energy consumption [8]. Additionally, high temperatures would have a negative impact on the paint's properties [9].

Regarding the lack of suitable methods and the trend to use more environmentally friendly and biocide-free techniques to maintain the quality of technical fluids, it is important to detect more ecological methods of disinfection. One approach for a more environmentally friendly disinfection method is the killing of germs using high shear stresses τ [10]. For this approach, Fraunhofer IPK, Berlin and Fraunhofer IPA, Stuttgart developed a prototype of a rotor-stator assembly to produce high shear stresses τ.

Typical contaminants in cathodic dip coating systems are bacteria from the *Burkholdeeria cepacian* complex (Bcc). A frequent representative of Bcc is *Burkholderia cepacia* (*B. cepacia*), a gram-negative, motile, non-spore-forming, and rod-shaped bacteria. Besides potential damage of material and working processes, *B. cepacia* represents a health risk to human, animals and plants, regarding it opportunistic pathogenicity and multi-drug and biocide resistance [11,12].

The following experiments will be performed with *B. cepacia*, isolated from a cathodic dip coating system and alternating designs of the experiment, combining different rotational speed v_{rot}, shear gap g_S and cell numbers n_c.

2. Materials and Methods

2.1. Test microorganisms and cultivation conditions

The analysis of the disinfectant potential of the rotor-stator assembly was performed with *B. cepacia* isolate *Burk_52* from a cathodic dip coating system. *Burk_52* was incubated in R2A media at a temperature ϑ = 31°C, a frequency f_r = 200 1/min

and a cultivation time t_c = 18 h until it reaches an optical density of OD_{595} = 0.7.

The efficiency of the disinfection was analyzed by cell counting after a cultivation time t_c = 40 h at temperature ϑ = 31°C on R2A agar. After incubation the cell number n_c in colony forming units per mL (cfu/mL) of the untreated and the homogenized samples were counted and a reduction of the germ number n_{gr} was determined. For this, two different technical fluids with heavily contaminated cell numbers $n_c \geq$ 1.00e09 cfu/ml (shown +++) and with less heavily contaminated cell numbers $n_c \leq$ 5.00e06 cfu/ml (shown ++) were analyzed.

2.2. Homogenizer with rotor-stator assembly

The homogenizer (Fig. 1) is operated by an electric motor, its rotational frequency f_{rot} is set via a frequency converter. A transmission with a gear ratio g_r = 2.592 is flanged to the electric motor. The torque to the rotor-stator assembly is transmitted via a clutch to compensate inconcentricities in the gearbox shaft.

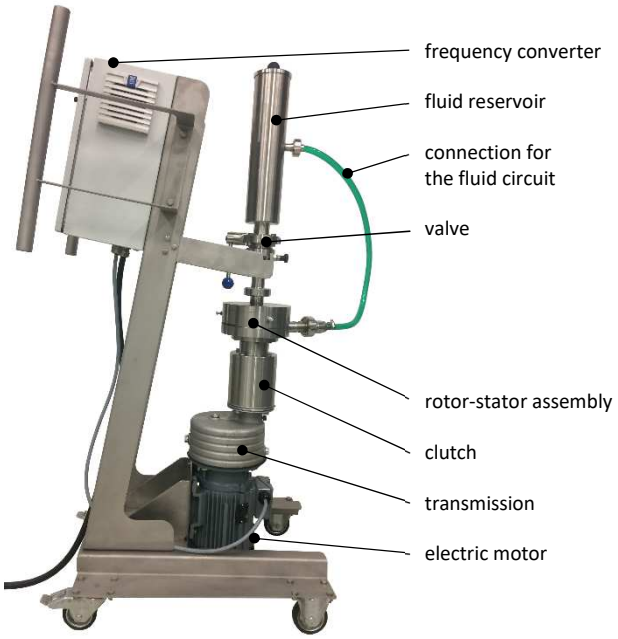

Figure 1. Structure of the homogenizer

Preculture of *B. cepacia* to be examined was diluted in phosphate buffered saline (PBS). In the tests, the medium is filled into the fluid reservoir. During operation the medium flows continuously through the rotor-stator assembly for specific machining process time t_{mp} in a loop. The fluid is returned to the reservoir via the green tube.

The rotor-stator assembly consists of a rotor and a tightly fitting stator unit which is screwed into the housing via a thread, this allows the adjustment and variation of the shear gap g_S. During operation, the medium flows from above through the shear gap to the lower side area in the assembly. Due to the pumping action of the rotor, the medium is pumped back into the reservoir.

2.3. Procedure of the disinfection process

During disinfection in techniqual fluids between rotor and stator, shear stresses τ are generated depending on the dynamic viscosity η of the medium, the peripheral speed v_p on the rotor and the shear gap g_S. Shear stresses τ are calculated using this formula:

$$\tau = \eta \cdot \frac{\partial v_p}{\partial g_S} = \eta \cdot \dot{\gamma}$$

By the variation of peripheral speed v_p and shear gap g_S, different shear stresses τ are tested. The differential in the formula describes the shear rate $\dot{\gamma}$. In a rotor-stator homogenizer, high shear rates $\dot{\gamma}$ are defined with $20^{\cdot}000 \leq \dot{\gamma} \leq 100^{\cdot}000$ 1/s according to Zhang et al. [13]. With a viscosity of the technical fluid η = 0.02205 kg/m/s, this corresponds to a shear stresses of 441 Pa ≤ τ ≤ 2205 Pa. According to Shirgaonkar et al. this high shear stresses τ cause cell disruption in technical fluids [10].

2.4. DoE trials and process parameters

In design of experiment (DoE) trials the shear gap g_S is adjusted between 83.34 µm ≤ g_S ≤ 166.68 µm and frequencies f_{rot} are varied 10 Hz ≤ f_{rot} ≤ 40 Hz. This results in rotational speeds of 1555.2 1/min ≤ v_{rot} ≤ 6220.8 1/min. The mean peripheral speeds are 5.05 m/s ≤ v_p ≤ 20.19 m/s. Measured flow rates f_r from 0.0263 l/s ≤ f_l ≤ 0.1412 l/s occure in the rotor-stator assembly. In this study, shear stresses τ of 667.88 Pa ≤ Ts ≤ 5343.08 Pa are applied on two different contaminated technical fluids in order to demonstrate the reduction of germ numbers n_g.

Various process parameters are set according to the DoE method for measuring the germ numbers n_g. Four test matrices result from the variation of the individual parameters (tables 1, 2, 3, 4).

Table 1. Process parameters at frequency f_{rot} = 10 Hz

process parameters	A	B	C	D
shear gap g_s [µm]	83.34	83.34	166.68	166.68
peripheral speed v_p [m/s]	5.05	5.05	5.05	5.05
rotational speed v_{rot} [1/min]	1555.20	1555.20	1555.20	1555.20
flow rate f_r [mL/s]	26.27	26.27	53.05	53.05
shear stresses τ [Pa]	1335.77	1335.77	667.88	667.88
cell numbers n_c [cfu/ml]	1.56e09	4.40e06	2.30e11	9.50e05

Table 2. Process parameters at frequency f_{rot} = 20 Hz

process parameters	E	F	G	H
shear gap g_s [µm]	83.34	83.34	166.68	166.68
peripheral speed v_p [m/s]	10.10	10.10	10.10	10.10
rotational speed v_{rot} [1/min]	3110.40	3110.40	3110.40	3110.40
flow rate f_r [mL/s]	50.94	50.94	76.75	76.75
shear stresses τ [Pa]	2671.54	2671.54	1335.77	1335.77
cell numbers n_c [cfu/ml]	1.86e12	7.15e05	1.61e11	3.30e05

Table 3. Process parameters at frequency f_{rot} = 30 Hz

process parameters	I	J	K	L
shear gap g_s [µm]	83.34	83.34	166.68	166.68
peripheral speed v_p [m/s]	15.15	15.15	15.15	15.15
rotational speed v_{rot} [1/min]	4665.60	4665.60	4665.60	4665.60
flow rate f_r [mL/s]	75.70	75.70	107.53	107.53
shear stresses τ [Pa]	4007.31	4007.31	2003.65	2003.65
cell numbers n_c [cfu/ml]	2.43e10	1.11e06	2.62e10	3.16e06

Table 4. Process parameters at frequency f_{rot} = 40 Hz

process parameters	M	N	O	P
shear gap g_s [µm]	83.34	83.34	166.68	166.68
peripheral speed v_p [m/s]	20.19	20.19	20.19	20.19
rotational speed v_{rot} [1/min]	6220.80	6220.80	6220.80	6220.80
flow rate f_r [mL/s]	92.94	92.94	141.24	141.24
shear stresses τ [Pa]	5343.08	5343.08	2671.54	2671.54
cell numbers n_c [cfu/ml]	8.70e11	7.15e05	5.41e11	3.63e06

After a machining process time t_{mp} of 0.5, 1, 3 and 5 minutes, samples of the medium are taken from which the germ numbers n_{gr} are determined. From these, the percentage reduction of germ number n_{gr} is calculated based on the cell numbers n_c.

3. Results

The following figures show the reduction of germ number n_{gr} and thus efficiency of disinfection according to the respective machining process times t_{mp} for each trial. The corresponding test parameters are shown in tables 1, 2, 3 and 4.

Figure 2 (frequency f_{rot} = 10 Hz) shows that there is no disinfection in trial A using shear gap g_s = 83.34 µm with heavily contaminated media (+++) and in trial B with less heavily contaminated media (++).

The same result was found in trail D using less heavily contaminated media (++) and the shear gap g_s = 166.68 µm.

Whereas in trial C using g_s = 166.68 µm and (+++) the reduction of germ numbers n_{gr} increased to $n_{gr} \geq 94.7$ % over the machining process time t_{mp} = 5 min. After t_{mp} = 0.5 min there is no significant disinfection. In the further course in machining process times t_{mp} = 1 min to t_{mp} = 3 min there is an increase disinfection from n_{gr}= 31.3 % to n_{gr}= 83.7 %.

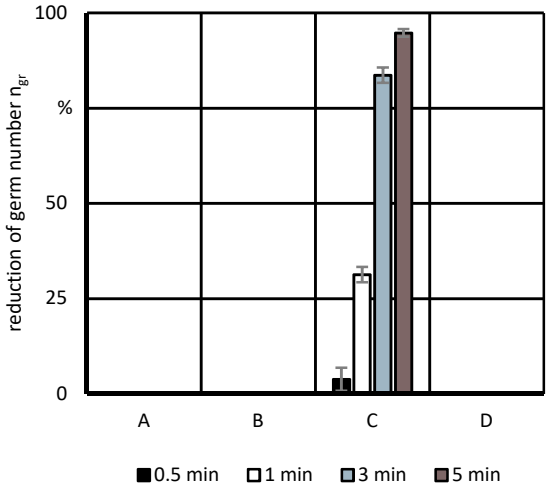

Figure 2. Reduction of germ number n_{gr} at frequency f_{rot} = 10 Hz. Error bars represent standard deviation of n = 3 experiments.

In trial E using g_s = 83.34 µm and (+++), shown in Figure 3 (frequency f_{rot} = 20 Hz), the disinfection is constant n_{gr} > 90.6 % at the different machining process times t_{mp} and reaches its maximum n_{gr} = 98.3 % after t_{mp} = 5 min.

Trial G using g_s = 166.68 µm and (+++) shows an increasing disinfection from n_{gr} = 76.3 % after t_{mp} = 0.5 min to n_{gr} = 85.7 % after t_{mp} = 1 min. After t_{mp} = 3 min and t_{mp} = 5 min there is no longer any increase in disinfection.

The trials F and H with less heavily contaminated media (++) show no disinfection.

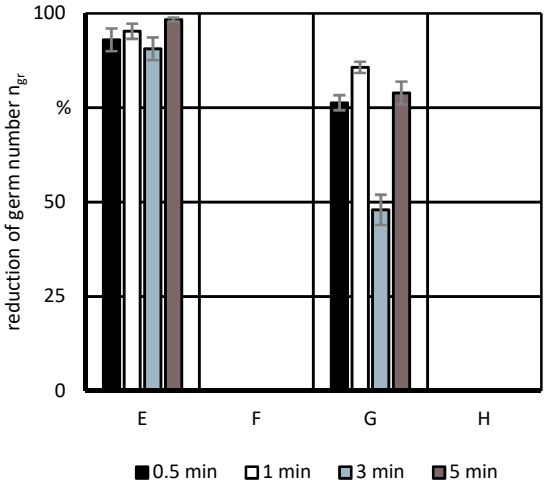

Figure 3. Reduction of germ number n_{gr} at frequency f_{rot} = 20 Hz. Error bars represent standard deviation of n = 3 experiments.

Figure 4 (frequency f_{rot} = 30 Hz) shows in trial I using g_s = 83.34 µm and (+++), nearly constant disinfection in the machining process time t_{mp} with 74.4 % ≤ n_{gr} ≤ 76.6 %.

Comparatively low disinfection takes place in trial J using g_s = 83.34 µm and (++) starting with no disinfection after t_{mp} = 0.5 min, increasing from n_{gr} = 3.2 % after t_{mp} = 1 min to n_{gr} = 16.3 % after t_{mp} = 3 min with a maximum of n_{gr} = 22.2% after t_{mp} = 5 min.

Trial K using g_s = 166.68 µm and (+++), the disinfection increases from n_{gr} = 65.7 % after t_{mp} = 0.5 min over n_{gr} = 90.2 % after t_{mp} = 1 min to n_{gr} = 98.0 % after t_{mp} = 3 min.

The results of trial L using g_s = 166.68 µm and (++) show a disinfection starting from n_{gr} = 52.9 % after t_{mp} = 0.5 min and n_{gr} = 54.2 % after t_{mp} = 1 min, which increases to n_{gr} = 65.9 % after t_{mp} = 3 min and n_{gr} = 86.7 % after t_{mp} = 5 min.

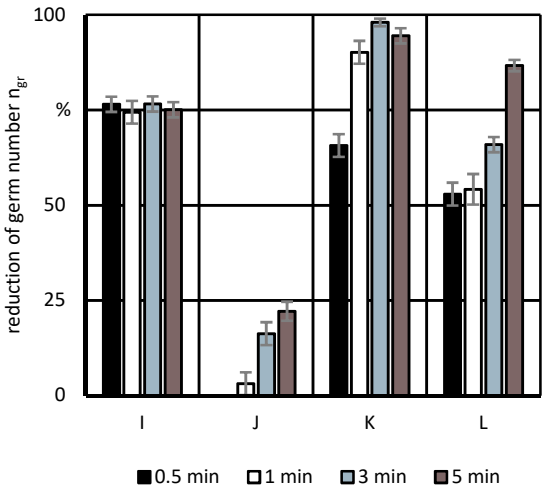

Figure 4. Reduction of germ number n_{gr} at frequency f_{rot} = 30 Hz. Error bars represent standard deviation of n = 3 experiments.

Trial M using g_s = 83.34 µm and (+++), shown in Figure 5 (frequency f_{rot} = 40 Hz), results in high disinfection in the machining process time t_{mp} with 93.1 % ≤ n_{gr} ≤ 99.2 %.

On trial N (g_s = 83.34 µm and (++)) disinfection starts at n_{gr} = 26.6 % after t_{mp} = 0.5 min, increasing to n_{gr} = 33.8 % after t_{mp} = 1 min. After t_{mp} = 3 min and t_{mp} = 5 min there is no longer any increase in disinfection.

In all trials the maximum disinfection can be found in trial O (g_s = 166.68 µm and (+++)). After t_{mp} = 3 min and t_{mp} = 5 min, the disinfection is n_{gr} = 99,6% and n_{gr} = 99.1 %.

Trial P (g_s = 166.68 µm and (++)) shows no disinfection.

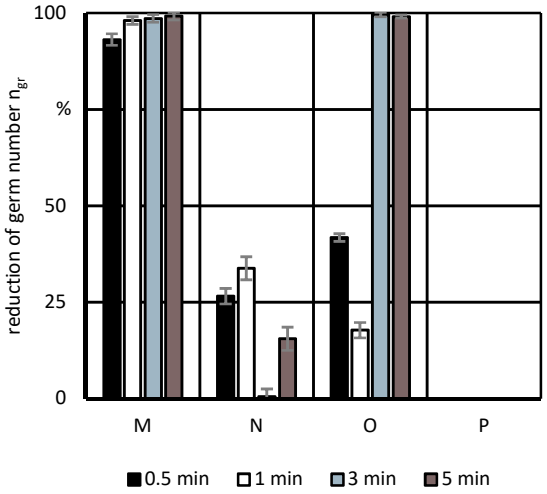

Figure 5. Reduction of germ number n_{gr} at frequency f_{rot} = 40 Hz. Error bars represent standard deviation of n = 3 experiments.

In the test series, also two usable test results were achieved with f_{rot} = 60 Hz and g_s = 83.34 µm with both heavily and less heavily contaminated cells numbers n_c (not shown). This corresponds to rotational speeds v_{rot} = 9331.2 1/min , which are, however, to be regarded as critical speeds for the operation of the homogenizer, as these lead to high vibrations in the machine structure. This led to disinfections for less heavily contaminated media n_{gr} = 91.4 % after t_{mp} = 3 min. With heavily contaminated media, disinfections n_{gr} = 99.2 % after t_{mp} = 3 min were achieved.

4. Discussion

Summarizing the results, it is recognizable, that the measured disinfection of the fluids is more effective when it is more heavily contaminated fluids and higher germ counts are used. Disinfection of low cell numbers of $n_c \approx$ 1.00e6 is mostly rather ineffective, with a reduction of germ numbers $n_{gr} \approx$ 25 % (Fig. 4 J, Fig 5 N) or not detectable at all (Fig. 2 B, D, Fig. 3 F, H, Fig. 5 P). A reason for the bad disinfection might be the limitations of the method of detection concerning lower cell concentrations in the media. On the contrary, disinfection of higher cell numbers of $n_c \approx$ 1.00e11 shows a reduction of germ numbers n_{gr} > 90 % with the only exception of Fig. 2 A, where no disinfection was detectable at all. The highest degree of disinfection is detectable by an applied rotor frequency f_{rot} = 40 Hz (Fig. 5 M, O) where the reduction of germ numbers n_{gr} reaches values of more than 99 % independent from the present shear gap g_s. Comparing the reduction of germ numbers n_{gr} with a shear gap g_s = 83.34 µm to the reduction with a shear gap g_s = 166.68 µm, it can be concluded, that there is no difference regarding the effectivity after a machining process time of t_{mp} = 5 min. But with a focus on shorter machining process times, the smaller shear gap g_s is better suited. The maximum reduction of germ numbers n_{gr} with a shear gap g_s = 83.34 µm is achieved after t_{mp} < 1 min (Fig. 3 E, Fig. 4 I, Fig. 5 M). However, using the wider shear gap g_s = 166.68 µm reaches its maximum of reduction n_{gr} after a process time of 3 min < t_{mp} < 5 min (Fig. 2 C, Fig. 4 K, L, Fig. 5 O). Besides, the used cell numbers n_c and the different shear gaps g_s the reduction of germ numbers n_{gr} is influenced by the applied rotor frequency f_{rot}. A higher rotor frequency f_{rot} results in a stronger reduction of germ numbers n_{gr}.

5. Summary

Searching for more ecological methods of disinfection, suited for a wide range of technical fluids, the reduction of germ numbers by a rotor-stator assembly seems to be a proper method. Alternating designs of the experiment, combining different rotational speed v_{rot}, shear gap g_s and cell numbers n_c showed, that the highest degree of disinfection is achieved by an applied rotor frequency f_{rot} = 40 Hz, using high cell numbers n_c. This experimental set up reaches a reduction of germ numbers n_{gr} > 99 % independent from the present shear gap g_s. Higher cell concentrations in the media and higher rotor frequencies up to f_{rot} = 40 Hz, the reduction of germ numbers n_{gr} is more effective. The variation of the shear gap g_s does not effect the long term disinfection. A smaller shear gap g_s reduces the time needed to reach the maximum reduction of germ numbers n_{gr}. Using a smaller shear gap g_s can reduce the necessary machining process time, but if the assembly is used either way in a continuous process, using a wider shear gap, the shear stresses τ working on the fluid can be halved without a negative impact on the disinfection.

The reduction of germ numbers n_{gr} using the described rotor-stator assembly is an effective, ecological alternative to existing disinfection methods of technical fluids. However, it remains to be seen whether the effectiveness can also be achieved with dip paints. The disinfection process must be adjusted so that the properties of paints are retained. In addition, the reliable reduction in the number of germs n_{gr} could be improved at lower cell numbers n_c. This is to be achieved by installing a cooling system, a continuous machining process time t_{mp} of several hours can be generated. As a result, a disinfection effect would be possible with less heavily contaminated fluids with cell numbers $n_c \leq$ 5.00e06 cfu/ml.

References

[1] Passman F J 2009 Microbial problems in metalworking fluid *Tribology and Lubrican Technology* **27** 14-17

[2] Cyprowski M, Piotrowska M, Zakowska Z and Szadkowska-Stanczyk I 2007 Microbial and Endotoxin Contamination of Water-Soluble Metalworking Fluids *International Journal of Occupational Medicine and Environmental Health* **20** 365-71

[3] Paint and Coatings industry 2002 Controlling Microbial Contamination

[4] NETZSCH-Feinmahltechnik GmbH 2020 Cathodic Dip Coating

[5] Helmers T 2020 Wasser-Wissen

[6] Schweer C 2010 Biocides – Risks and alternatives PAN Germany

[7] Ramasamy M and Lee J 2016 Recent Nanotechnology Approaches for Prevention and Treatment of Biofilm-Associated Infections on Medical Devices *BioMed Research International* **46** 1-17

[8] Earnshaw R G, Appleyard J and Hurst R M 1995 Understanding physical inactivation processes: combined preservation opportunities using heat, ultrasound and pressure *International Journal of Food Microbiology* **28** 197-219

[9] Ven C, Courvoisier C and Matser A 2007 High pressure versus heat treatments for pasteurization and sterilization of model emulsions *Innovative Food Sciences & Emerging Technologies* **8** 230-236

[10] Shirgaonkar I Z, Lothe R R and Pandit A B 1998 Comments on the mechanism of microbial cell disruption in high-pressure and high-speed devices *Biotechnology Progress* **14** 657–660

[11] Mahenthiralingam, E., Urban, T. A., Goldberg, J.B. 2005 The multifarious, multireplicon Burkholderia cepacia complex, Nat Rev Microbiol. **3** 144-156

[12] Research Occupational Health Program Boston University 2014 Burkholderia cepacia Agent information sheet

[13] Zhang J L, Xu S and, Li W 2012 High shear mixers: a review of typical applications and studies on power draw, flow pattern, energy dissipation and transfer properties *Chem Eng Process* **57–58** 25–41