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Understanding the break-up phenomena in an orifice-valve high pressure homogenizer using spherical bacterial cells (*Lactococcus lactis*) as a model disruption indicator

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ABSTRACT

The break-up phenomena occurring in a high pressure homogenizer equipped with an interchangeable orifice valve were investigated by measuring the inactivation of *Lactococcus lactis*. Data were collected at varying the orifice size (80, 100, and 150 μm), the operating pressure (100–200 MPa), the number of passes (1–10), and the fluid viscosity (2.5–7.9 mPa s, changed by adding 0–50 % wt PEG 200 to buffered peptone water) to identify the correlations of the fragmentation occurring in the valve with the main fluid dynamic phenomena (turbulence, elongational and shear stresses, and cavitation). In addition, also the effects of a purely shearing or ultrasound treatment on cell break-up were considered.

The results show that the most intense break-up phenomena occur for the smallest orifice size, highest pressure, and lowest viscosity. However, at low viscosity, turbulence, together with the elongational stresses appear to be the controlling factors of cell break-up, whereas, at higher viscosities, the shear stresses become increasingly important. The occurrence of cavitation is only slightly affected by viscosity, and mainly depends on the velocities reached in the homogenization valve.

1. Introduction

High-pressure homogenization (HPH) is an emerging technology, which has found wide application as a wet-milling, size-reduction process to produce fine suspensions of particles or droplets (Singh et al., 2016) or submicrometric emulsions (Gupta et al., 2016), as well as to disrupt cells, for the recovery of intracellular material or the reduction of the microbial load in liquid matrices (Diels and Michiels, 2006; Donsì et al., 2013, 2009a; 2009b; Maresca et al., 2011).

An HPH unit is a relatively simple continuous system, where the process fluid is compressed in one or more pressure intensifiers, and forced through a specifically designed homogenization chamber. In the homogenization chamber, the pressure energy accumulated in the fluid is released in the passage through a narrow gap, resulting in the development of fluid velocities up to 200–400 m/s (Donsì et al., 2013; Floury et al., 2004a), with the generation of significant fluid mechanical stresses, such as elongational and shear stresses, turbulence and cavitation, which cause particle or cell disruption (Donsì et al., 2013, 2009a; Floury et al., 2004b).

The disruption efficiency in HPH processes is mainly related to the operating pressure and the valve geometry, comprising the flow path and the gap size. Therefore, the research efforts in recent years have mainly focused on the development of new materials capable of withstanding pressure levels up to 350–400 MPa (Dumay et al., 2013), as well as on the proprietary design of valve geometries better finalized to optimizing the final product properties (Donsì et al., 2013, 2012; Lee and Norton, 2013).

More specifically, the microbial inactivation by HPH occurs through a purely mechanical process of disruption of the cell walls, whose efficiency depends on the balance between the disruptive forces generated in the homogenization chamber and the cell wall resistance (Donsì et al., 2013, 2009a). However, the fundamentals of the process need to be better clarified.

Previous studies from our research group, comparing the microbial inactivation in a piston-valve system with that achieved in an orifice-valve system, showed that the inactivation levels in the piston valve were always significantly higher than in the orifice valve for *Escherichia coli* (Gram-negative), *Lactobacillus delbrueckii* (Gram-positive) and *Saccharomyces cerevisiae* (yeast). Therefore, it was hypothesized that the fluid dynamics conditions establishing in the piston valve are extremely favorable to microbial inactivation, probably also because of the direct interaction of the valve surfaces with the cell walls, which reduced the

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List of symbols

a_j	kinetic constant of cell break-up [−]
b_j	kinetic constant of cell break-up [−]
BPW	buffered peptone water
Ca	capillary number [−]
d_{cell}	mean droplet size of the bacterial cells [m]
d_{valve}	orifice-valve diameter [m]
HPH	High Pressure Homogenization
j	treatment type (HPH, Sh, or US)
K	cavitation number [−]
k_j	kinetic constant of cell break-up [−]
L_c	orifice length [m]
n	HPH pass number [−]
N	bacterial population [cfu/mL]
N_0	initial bacterial population [cfu/mL]
P_2	absolute pressure downstream of the homogenizing valve [MPa]
P_v	vapor pressure of water downstream of the homogenizing valve, at local temperature conditions [MPa]
Ca	capillary number [−]
PEG 200	Poly(ethylene glycol) with an average molecular weight of 200 g/mol
Re	Reynolds number [−]
Sh	shearing treatment
T_{in}	inlet temperature to the homogenization valve [°C]
T_{out}	discharge temperature from the homogenization valve [°C]
US	ultrasound
u_{valve}	average fluid velocity in the valve orifice/gap [m/s]
We	Weber number [−]
W_{US}	volumetric power of the applied US treatment [W/mL]
ΔP_h	total pressure drop in the homogenization valve [MPa]

Greek symbols

α	fluid acceleration in the valve orifice, u_{valve}/L_c [s^{-1}]
γ	shear rate [s^{-1}]
η	dynamic viscosity of the process medium [Pa·s]
ϑ_j	treatment duration [as number of passes *n*, or s or min]
ϕ_j	intensity level of each treatment [Pa for HPH or Sh, or W/mL for US]
ρ	density of the process medium [kg/m^3]
σ	cell wall resistance [N/m]
τ	shear stress [Pa]

effect of the differences in cell shape and wall resistance (Donsì et al., 2013).

Moreover, the comparison of the microbial inactivations achieved by different homogenization valve assemblies, even when equipping the same HPH machine, is hindered by the variation in the temperature reached by the processing medium due to the different heat dissipation rates occurring in each assembly (Cavender and Kerr, 2011). In fact, a significant part (40–60%) of the pressure energy is dissipated as frictional heat (Cortés-Muñoz et al., 2009), causing an instantaneous temperature increase in the product of 0.15–0.20 °C/MPa (Donsì et al., 2013), which might significantly affect the extent of microbial inactivation, if not rapidly removed by heat exchange devices, not only through the direct thermal inactivation, but also because of the changes occurring in fluid viscosity and vapor pressure, which influence the intensity of the fluid-mechanical stresses and cavitation.

In addition, in the case of the emulsification process, it was reported that the drop fragmentation is strongly correlated to the valve geometry and in particular to the volume over which the energy dissipates (Lee et al., 2014), which is, often, in the discharge region where a jet forms (Innings and Trägårdh, 2007). However, in the discharge region also recoalescence phenomena take place, which are controlled by the extent of adsorption of the emulsifier molecules at the newly formed interfaces (Håkansson et al., 2009a), making the comparison of different valve geometries even more complex.

Previous results have shown that the emulsification process is more efficient in the orifice valve than in the piston valve (Donsì et al., 2012; Stang et al., 2001), and that the differences between the two valves diminish when using fast-adsorbing emulsifiers, supporting the hypothesis that the recoalescence phenomena, in the case of food oil-in-water emulsions, play a dominating role (Donsì et al., 2012). More recently, it was shown that the performance of a Microfluidizer chamber (impinging jets microchannels) is better than a piston valve system for the production of O/W emulsions (Lee and Norton, 2013), whereas for the production of W/O emulsions, characterized by a significantly higher viscosity of the continuous phase, the performances of the two homogenization valves are comparable (Lee et al., 2014), suggesting a strong role of the viscosities of the continuous and dispersed phases.

This work aims at investigating the fundamentals of the break-up phenomena occurring in an HPH disruption chamber, by using a simple fixed geometry, based on a disruption chamber with interchangeable orifices. Orifice valves are becoming of increasing interest in food processing, because of their capability to sustain ultra-high operating pressures (Mohan et al., 2016). Moreover, the proposed setup enables to minimize the alteration of the fluid distribution especially in the discharge region, as well as to use the same pressure intensification device and to apply similar rates of heat removal. As a model on-off disruption indicator, we propose the use of cocci bacteria (*Lactococcus lactis*), to enable the quantification of the occurrence of disruption on simply enumerating the survivor bacteria. In previous studies, it was shown that HPH treatment causes an on-off microbial disruption, without any measurable sub-lethal damage in *Listeria innocua* (Briñez et al., 2006c) and *Escherichia coli* (Briñez et al., 2006a): apparently, once the cell membrane is torn apart by the fluid-mechanical stresses generated in the homogenization valve, the microbial cells are not able to recover. In addition, *L. lactis* exhibit two additional advantages in the planned experiments: they are nearly spherical microorganisms, enabling to rule out the shape factors from the break-up process, and have an average size of 1 μm (Nomura et al., 2009; Yeung et al., 2016), which is significantly lower than the size of the smallest orifice valve used (80 μm), reducing the extent of interaction with the valve surfaces.

2. Materials and methods

2.1. Bacterial viability tests

2.1.1. Bacterial suspension

Lactococcus lactis is a bacterial species of nearly spherical shape, as resulting from several observations by scanning electron microscopy (Nomura et al., 2009; Yeung et al., 2016), with an average size comprised between 1 and 2 μm (Nomura et al., 2009; Yeung et al., 2016).

The *Lactococcus lactis* subsp. *cremoris* (ATCC® 19257™) bacterial culture was grown in brain heart infusion broth (BHIB, Oxoid, UK) to the stationary phase (approximately 10^8 CFU/mL) in an aerated incubator (Function Line 7000 incubator (Heraeus Instruments, Germany)) for 24 h at 30 °C.

Subsequently, the culture was diluted with buffered peptone water (BPW, Oxoid, UK) to obtain a working suspension at a final concentration of 10^4 CFU/mL.

The viscosity of the working suspension (buffered peptone water