MECHANICAL CELL LYSIS DEVICE

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ABSTRACT

Sample-in result-out lab-on-a-chip approaches require the integration of all functionalities from lysis to detection. Mechanical lysis methods, and bead beating in particular, have been shown very versatile for difficult to lyse micro-organisms. A miniaturized beat beater creating high mechanical forces to lyse efficiently is intrinsically demanding to realize. Based on numerical methods, driving point and operational deflection shape analyses a miniaturized and flexible bead beater chamber is realized. Cell-wall rupture of diffult-to-lyse Saccharomyces cerevisiae cells has been demonstrated in a chamber of 100 microliter filled with 35% beads and actuated with 100 Hz at 20% of the chamber height).

KEYWORDS: lysis, bead beating, mechanical forces, cell rupture mechanism

INTRODUCTION

Cell lysis is the process of cell wall rupture to obtain intracellular components, e.g. proteins or nucleic acids, for analysis. In several sample preparation procedures lysis is one of the first sample preparation steps to the detection of a cellular component, e.g. of nucleic acid based pathogen detection in sputum. A large array of enzymatic, chemical and physical based lysis methods have been reported [1]. Enzymatic and chemical methods could be miniaturized, but these methods are associated with high costs, assay inhibition, storage issues and/or pathogen specificity. Therefore the more generic physical based lysis methods are preferred. Methods reported include electrical [2], mechanical [3], optical [4], thermal [5] and ultrasound [6] based methods. In common lab-practice mechanical methods and esp. bead beating is recognized as the golden standard method, especially for rather difficult to lyse microorganisms [3, 7]. Due to the abundant dynamical mechanical characteristics of bead beating, it is one of the most challenging methods to integrate into a small-size sample-in result-out system using disposable cartridges.

In this paper a miniaturized bead beater concept is presented. Numerical methods are complemented by dynamic characterization to get a basic understanding of the parameters determining the lysis conditions.

EXPERIMENTAL

The micro bead beater concept is based on a small chamber (typical volume = 100 microliter) made of a flexible material, which is relatively free-free mounted for optimal mechanical lysis. The bottom of the chamber is contacted to a vibrating mechanical actuator (figure 1a,1b). The chamber is filled with beads. The vibration and/or deformation of the chamber result in bead collisions and/or inter-bead shear-flow.

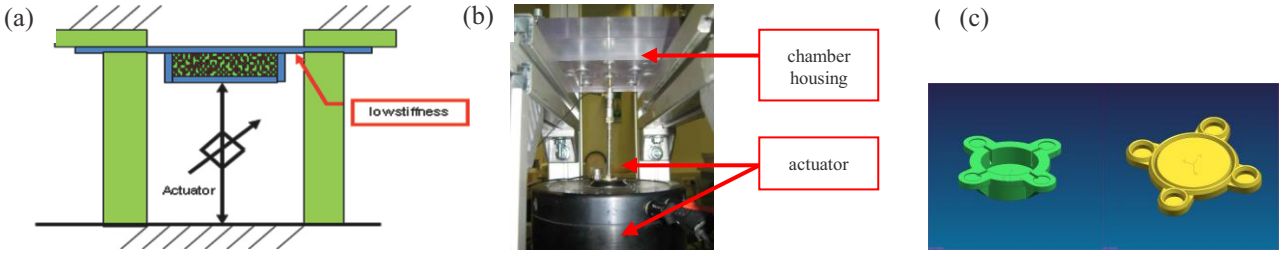


Figure 1: (a) Schematic set-up; (b) Experimental set-up; (c) Lysis chamber (green) and lid (yellow).

An Eulerian/Lagrangian modeling framework enhanced by stochastic analysis of glass bead collisions has been used to optimize the setup with respect to chamber geometry and properties, chamber deflection, bead filling and frequency.

Dynamic characterization has been performed to get insight in the chamber deformation and vibration by using an operational deflection shape (ODS) analysis. The final design objective is to use a low-power, small size actuator operating in one or more resonance frequencies. In figure 2 the analysis set-up is presented.

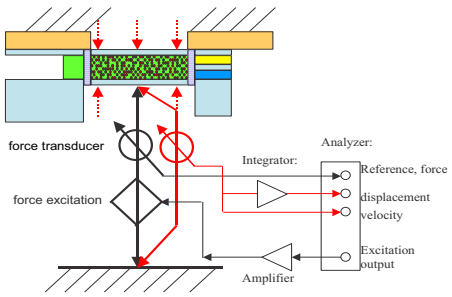


Figure 2: (a) Schematic set-up dynamic characterization.

Saccharomyces cerevisiae cells were taken as a model for difficult to lyse micro-organisms. Cells are dispersed in water or PBS before introduction into the lysis chamber.

RESULTS AND DISCUSSION

Cell rupture takes place, when the kinetic energy of the colliding beads exceeds the elastic energy stored in the cell. Inter-bead collision lysis requires relative speeds of ~ 0.3 m/s, which could only be achieved at large volumetric chamber vibrations. Inter-bead shear flow to achieve lysis for a typical (Gramm positive) bacteria should be in the range of 5-10 m/s [8]. Consequently inter-beads collisions is the primary mechanism of cell rupture during lysis.

Numerical output examples are presented in figure 3 and table 1. Both at low and high frequencies lysis inducing bead collisions could be achieved. As high amplitudes at low frequency are more practical to achieve, this mode is preferred. From the data it has been concluded that the amplitude of the vibration should be at least 60 % of the chamber height (= 20% = 0.6mm) at low frequency to exceed the minimal relative colliding speed of 0.3 m/s. Additional modeling c

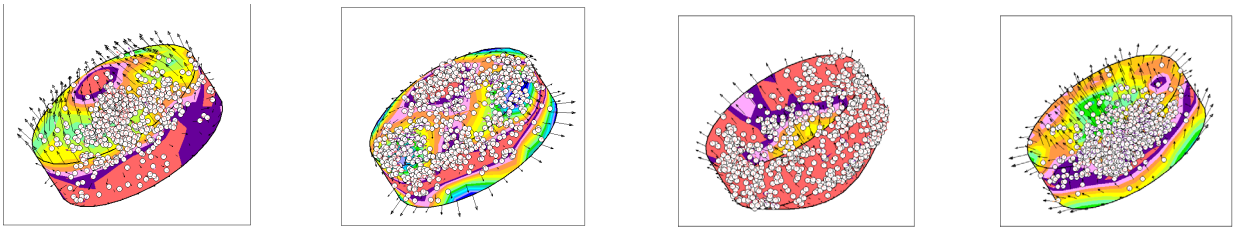


Figure 3: Velocity contours of particles in a vibrating bead-bed (50 Hz frequency and amplitude 80% of the chamber height, a chamber diameter of 10mm, a height of 3mm, a bead filling of 40%, and beads with a diameter between 250 to 500µm and a specific density of ρ = 2.5 – 3 g/cm^3).

Table 1. Summary of modeling data on collision velocity and probability

Topic	Amplitude ~ 40% f=50Hz	Amplitude ~ 80% f=50Hz	Amplitude ~ 80% f=25Hz
Max collision velocity (average in time)	0.25m/s	1.17m/s	0.85m/s
Collision probability within one time step (the time step is the same for all 3 calculations)	1%	4%	3%

Frequency dependent driving point measurements for three configurations are depicted in figure 4. Is shows that the stiffness and frequency response only changes with bead filling. At frequencies > ~ 1 kHz the stiffness of the system increases very significantly, consequently the amplitude required to achieve the discussed colliding particle speed could not be reached. Therefore, it has been chosen to continue lysis experiments in the constant force region. In practice the frequency is limited to about 100 Hz, because at higher frequencies the presently used rubber material starts deteriorating.

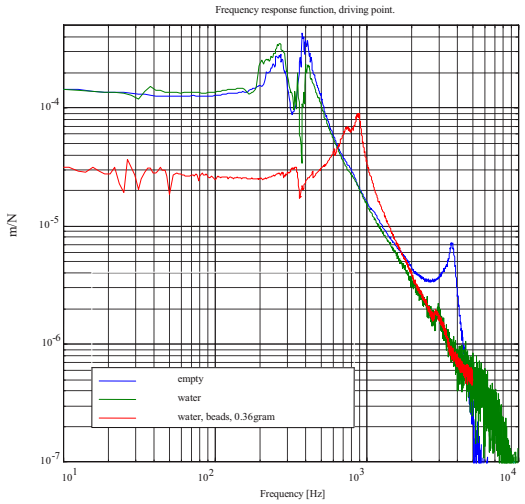


Figure 4: Measurement results frequency response functions.

Operation deflection shapes are determined to get insight in the relative volume displacement (preferred mode to achieve high inter bead collision speeds) and volume deformation of the chamber (figure 5). Measurements disclosed that in the constant force region for all three configurations the deformation is low. Displacements are relatively high for the empty

and water filled chamber and moderate for the bead filled chamber. Further optimizations should include chamber material properties and geometries.

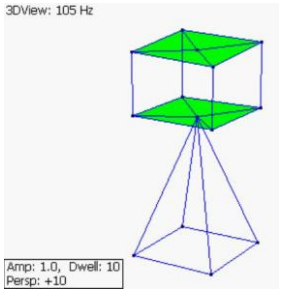
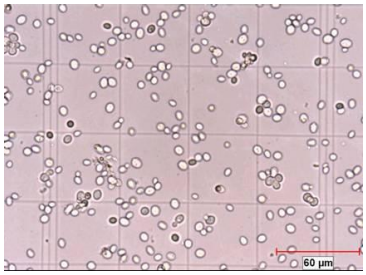


Figure 5: Operational deflection shape of a bead filled chamber at 105Hz shows moderate volume displacement and low chamber deformation.

Saccharomyces cerevisiae cells were taken as a model for difficult to lyse micro-organisms. It has been shown that a treatment of 5 minutes is sufficient to disintegrate a very large fraction of the yeast cell (figure 6). From the results is concluded that even the non-optimized design has high potential for difficult-to-lyse micro organisms.

(a) 

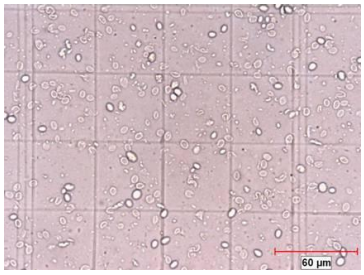
(b) 

Figure 6: Optical microscope photographs of: (a) Untreated Saccharomyces cerevisiae cells; (b) Treated Saccharomyces cerevisiae cells (5 minutes, 100 Hz, amplitude of 0.6mm, chamber 35% filling, zirconia beads of 300 and 400 micron).

CONCLUSIONS

From numerical simulations it is concluded that the vibration amplitude of the chamber should at least be 60 % of the chamber height at low frequency to achieve inter-bead collision speeds > 0.3 m/s. For the present chamber geometry the optimum bead size and chamber volume filling fraction are 300-500 µm and 30-40 %, respectively. Dynamical characterizations and optical deflection shape analyses have disclosed the system conditions suggested by simulations are experimental accessible, but are not yet optimal. Optimizations should include chamber material properties and geometries. Finally it has been shown that the micro bead beater could effectively lyse Saccharomyces cerevisiae cells a difficult-to-lyse microorganism.

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