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Effect of moisture equilibration time and medium on contact angles of bacterial spores

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ABSTRACT

Contact angle measurement of microorganisms is often described in literature, either to investigate their hydrophobic characteristic or the adhesion behavior of cells. However, in some key aspects the preparation methods differ. Thus, it is difficult to compare results and to choose a procedure for repetition of measurements. The aim of this paper is to point out some critical points during microorganism film preparation that can alter the resulting contact angles. Depending on the moisturizing medium and equilibration time, contact angles differ significantly.

1. Introduction

Microbial adhesion is of major importance for processes in the environmental and medical area, but also in the food processing sector. Settlement of microorganisms in the early phase of biofilm formation can lead to biofouling in pipes of water supply and in food industry as foulants in filtration membranes (Characklis, 1981; Zottola and Sasahara, 1994). However, the ability to adhere provides an advantage for probiotics during the colonization of the gut (Amund, 2016) and is an important criteria for the immobilization of biofilms in bioreactor systems (Characklis, 1981). To estimate the predisposition for adhesion of bacterial spores, it is crucial to study their surface characteristics.

Van Oss et al. (1975) were the first authors to describe the measurement of contact angles on biological material. Their target was to investigate the opsonization behavior of phagocytes. The hypothesis was that phagocytosis depends on the surface hydrophobicity of the phagocytes and the particles or bacteria to be opsonized. To characterize the cells, they chose the contact angle measurement due to the fact that this method is suitable for fragile cells such as leucocytes (van Oss et al., 1975). Since then, many authors applied and modified the contact angle measurement to characterize the surface of cells and microorganisms, as shown in Table 1. Depending on the work group, the exact procedure for this method is different, but the basic approach, consisting of four steps, remains. Fig. 1 gives an overview on steps and variables of the procedure.

To prepare the microorganisms as smooth, homogeneous layer, the cells are filtered and the moisture content on top of the filter is subsequently adjusted by two steps: First, the equilibration time, where the cell covered filter is put on a moist medium to unify the moisture content throughout the filtercake. Second, the drying time, during which the filter is placed on a firm, dry surface to desiccate until a plateau in moisture is reached. The measurement takes place during the plateau time, whereat a drop of measurement liquid is deposited on the surface of the filter. By drawing a tangent along the drop contour through the three phase point, the contact angle can be determined.

Table 1 shows water contact angles of different vegetative microorganisms and bacterial spores. Clearly, the results differ depending on the species, but also depending on the strains (Chen et al., 2010; Ahimou et al., 2001). *Bacillus subtilis* spores show contact angles of 10,3° (Pruß et al., 2012) to 59° (Ahimou et al., 2001). Grasso et al. (1996) showed that contact angles also depend on the physiological state of the cells. However, there are some key aspects in the procedure that differ depending on the method applied by each of the work groups.

Regarding the equilibration time, some authors do not apply an equilibration time at all (van Loosdrecht et al., 1987; Chen et al., 2010) whereas other authors use glycerin-agar to unify the moisture content throughout the microorganism lawn (Ahimou et al., 2001; Seale et al., 2008; Pruß et al., 2012). In the majority of articles, no duration of the equilibration time is mentioned. To our knowledge, there is no literature available concerning the background of the equilibration media. Most likely the equilibration time is applied to attain a uniform moisture content throughout all the microorganism layers on the filter. Hence a

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Table 1
Summary of different references for contact angle measurements of microorganisms and their differences.

Authors	Cells/microorganisms and water contact angle	Purpose of the study	Amount of cells per filter, moisturizing medium, equilibration time and drying time
van Oss et al. (1975)	Phagocytes *S. aureus* (17°) *E. coli* (19°)	Investigate opsonization behavior of phagocytes	- Layer cells on thin, flat glycerin agar (2% agar, 10 vol% glycerin) - Dry at least 3 h till plateau
Absolom et al. (1983)	*E. coli 055* (16,7°) *S. aureus 049* (18,5°) *E. coli 2627* (21,2°) *S. epidermidis* (23,4°) *L. monocytogenes* (26,1 °C)	Study the adhesion of five strains to polymeric surfaces	- Drying suspension on 1 cm^{-2} of glycerin agar (1% agar, 10 vol% glycerin) - Drying time not specified
Busscher et al. (1984)	Streptococci: *S. salivarius* (20°) *S. sanguis* (42°) *S. mitior* (26°) *Veillonella alcalescens* (55°)	Describe a technique to measure contact angles on bacterial layers	- Equilibration time on glycerin agar (1% agar, 10 vol% glycerin) - "… water contact angles were measured as a function of drying time."
Mozes and Rouxhet (1987)	*Enterobacter aerogenes* (62°) *Klebsiella fragilis* (53°) *Acetobacter aceti* (28°) *Moniliella pollinis* (Fungi, >90°) *Saccharomyces cerevisiae* (yeast, 26°) *Kluyveromyces oxytoca* (yeast, 50°)	Comparison of five methods to measure hydrophobicity of microorganisms, one being contact angle measurement	- Equilibration time on glycerin agar (1% agar, 10 vol% glycerin) - "… and contact angles were measured as a function of time."
Van Loosdrecht et al. (1987)	*Pseudomonas fluorescens* (21,2°) *Pseudomonas aeruginosa* (25,7°) *Pseudomonas putida* (38,5°) *Pseudomonas* sp. *strain 26–3* (20,1°) *Pseudomonas* sp. *strain 52* (19,0°) *Pseudomonas* sp. *strain 80* (29,5°) *Escherichia coli NCTC 9002* (15,7°) *Escherichia coli K-12* (24,7°) *Arthrobacter globiformis* (23,1°) *Arthrobacter simplex* (37,0°) *Arthrobacter* sp. *strain 177* (60,0°) *Arthrobacter* sp. *strain 127* (38,0°) *Micrococcus luteus* (44,7°) *Acinetobacter* sp. *strain 210 A* (32,6°) *Thiobacillus versutus* (26,8°) *Alcaligenes* sp. *strain 175* (24,4°) *Agrobacterium radiobacter* (44,*1°)* *Bacillus licheniformis* (32,4°) *Azotobacter vinelandii* (43,8°) *Corynebacter* sp. *strain 125* (70,0°) *Mycobacterium phlei* (70,0°) *Rhizobium leguminosarum* (31,0°) *Rodopseudomonas palustris* (34,3°)	Investigate the role of bacterial cell wall hydrophobicity in adhesion	- No equilibration time - Drying for 0,5–3 h
Rijnaarts et al. (1993)	*Arthrobacter* sp. *strain DSM 6687 (15°)* *Coryneform strain DSM 6685 (29°)* *Rhodococcus* sp. *strain C125 (70°)* *Rhodococcus erythropolis A177 (87°)* *Corynebacterium* sp. *strain DSM 6688 (89°)* *Corynebacterium* sp. *strain DSM 44016 (103°)* *Gordon asp. strain 1775/15 (115°)* *Gordona* sp. *strain DSM 44015 (117°)* *Pseudomonas oleovorans ATCC 29347 (17°)* *Pseudomonas fluorescens p62 (25°)* *Pseudomonas* sp. *strain B13 (32°)* *Pseudomonas putida mt2 (40°)*	Study the deposition of microorganisms with different hydrophobicities on Teflon and glass surfaces	- Not specified, refers to van Loosdrecht et al. (1987)
Grasso et al. (1996)	*Pseudomonas aeruginosa* - *Logarithmic state (33,5°)* - *Stationary state (49,8°)* - *Decay state (37,7°)*	Impact of physiological state on adhesion of *Pseudomonas aeruginosa*	- No equilibration time - Drying for 0,5 h
Garry et al. (1998)	*Bacillus subtilis* - Vegetative form (20 to 24,3°, depending on growth temperature) - Spores (34°) *Bacillus cereus* ATCC 14579 - Vegetative form (20,1 to 31,1°, depending on growth temperature) - Spores (36°)	Investigation of growth temperature on adhesion of *Bacillus cereus* and *Bacillus subtilis* vegetative bacteria and spores	- Equilibration time and conditions not specified - Drying time not specified
Ahimou et al. (2001)	*Bacillus subtilis ATCC 7058* - Vegetative form (31°) - Spores (59°) *Bacillus subtilis ATCC 12432* - Vegetative form (31°) - Spores (55°)	Comparison of hydrophobicity of *Bacillus subtilis* strains (vegetative cells, spores) with different methods including contact angle measurement	- Equilibration time on glycerin agar (1% agar, 10 vol% glycerin) for 2 h - Drying on air for 60 min