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Research article

Surface properties of mycoparasitic *Pythium* species and their interaction with model materials

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A B S T R A C T

Pythium oligandrum, a soil-born oomycete, is an effective biological control agent exhibiting antagonistic and parasitic activity against pathogenic fungi. This study is the first attempt to characterize its surface properties and to apply models of physicochemical interactions (thermodynamic, DLVO and XDLVO) to quantify its adhesion properties to a model material, represented by magnetic beads (MB). The predictions of interaction models were based on experimental data (contact angles, zeta potentials, size). Adhesion intensities (AI) were determined experimentally taking advantage of MB with different surface properties. The role of weak physicochemical interactions was estimated by comparing experimental AI with model predictions. The results revealed that the surface properties of the three *Pythium* spp. studied were very similar and fell within the range for hydrophilic microorganisms ($\Delta G_{TOT} > 0$) with a predominantly negative surface charge. The most reliable description of AI was obtained using the DLVO model, including Lifshitz-van der Waals and electrostatic interactions. The highest AI between *Pythium* spp. and all three MB was observed at pH 3, which was supported by the DLVO prediction. The greater agreement between the sphere-sphere geometric version of the DLVO model and experiment suggests that the surface protrusions of the oospores increase the efficiency of adhesion. The surface properties of the pathogenic fungi, characterized in this work, fell within the range defined by MB and therefore it can be expected that their physicochemical interactions with *Pythium* spp. will also be favourable.

1. Introduction

The transition to a chemical fungicide-free environment is in line with the strategy for food security and sustainable food systems [1], and is minimizing the impact of pesticides [2]. In response to environmental and health concerns about the use of biocidal chemical compounds, there is considerable interest in finding alternative biological control agents (BCA) for use in integrated management strategies against fungal contamination of surfaces. *Pythium oligandrum* is a soil-born oomycete and it is a promising BCA exhibiting antagonistic and parasitic activity against many pathogenic fungi [3,4]. It has attracted attention due to being non-pathogenic but capable of acting against pathogens to directly and indirectly protect solid surfaces against fungi [5]. The antagonistic action of *P. oligandrum* is due to it secreting enzymes for nutrient acquisition from the host mycelium, the production of

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antimicrobial volatile compounds, coiling around the host hyphae, or a combination of these processes [6–8]. *P. oligandrum* has also been observed to act indirectly on fungal pathogens by triggering plant defence mechanisms mediated by microbe-associated molecular patterns and to stimulate growth through production of auxin precursors [5,8].

The usual method of application of *P. oligandrum* as a BCA is in the form of oospores, but also as mycelial fragments and zoospore cysts onto various solid surfaces, potentially colonized by the fungi. *P. oligandrum,* strain M1 has been approved as a biofungicide for plant leaves, seeds and roots [9]. Based on European Parliament and Council Regulations [10,11] the same strain was registered as a cosmetic preparation for human (skin, oral cavity), for veterinary hygiene and for preservation of construction materials.

To carry out its role as a BCA, *P. oligandrum* can interact with both hyphae of the host fungi and plant roots, completely enwrapping and colonising them [8]. Active growth of *P. oligandrum* towards *Phytophthora* host cells and their subsequent close contact was also observed [12]. Attachment is assisted by the release of peptides produced by *P. oligandrum* [6]. Although adhesion plays an apparent crucial role in the initial phase of these interactions with host surfaces, there is no information about surface properties and adhesive interactions of *P. oligandrum*. Only the zoospores of another oomycete, *Phytophthora parasitica,* have been shown to form highly organized and adherent biofilm communities on the surface of their hosts [13]. The motivation of this work was to fill the knowledge gap in surface properties/interactions of *P. oligandrum*.

The physicochemical aspects of microbial adhesion to surfaces can be evaluated using three theoretical approaches. A thermodynamic approach is based on the balance of interfacial free energies allowing to estimate the degree of probability with which the physicochemical surface properties of cells and solids would lead to adhesion [14]. The so called Derjaguin-Landau-Verwey-Overbeek (DLVO) theory includes electrostatic interactions and permits quantification of adhesion of cells to other particles (sphere-sphere interaction) or surfaces (sphere-flat plate interaction) [15]. Since neither the thermodynamic nor the DLVO theory can explain all the experimental observations of microbial adhesion, an extension of the DLVO (XDLVO) theory is used to interlink the two previous approaches to better grasp the phenomenon of microbial surface interactions [16].

It was hypothesized that the physicochemical properties of *Pythium* spp. are suitable for providing adhesion of this species to a wide range of surfaces. The aim of the work is to describe the surface properties of *Pythium oligandrum* strains M1 and CBS109982, and *Pythium* sp. strain X42, and to study their surface interactions using model materials (magnetic beads) with a broad spectrum of surface properties, which at the same time allow reproducible adhesion tests to be performed. By comparing experimental interaction intensities between oospore-enriched biomass and model materials, with predictions of colloidal interaction models, the driving force of the cell-surface interactions can be identified. Then the most suitable colloidal model can be used to predict the interaction of *Pythium* spp. with selected surfaces (pathogenic fungi, plants, skin etc.).

2. Materials and methods

2.1. *Pythium oligandrum* strains and cultivation

Pythium oligandrum strains M1 (corresponds to strain ATCC38472), CBS109982 (*Pythium oligandrum* Drechsler 1930) and *Pythium* sp. X42, were obtained from Biopreparaty, s.r.o. (Uherce, Czech Republic) culture collection and preserved on sterile malt extract agar (MEA, 50 g/L, pH 7). The strains were re-inoculated on MEA agar (Panreac AppliChem, Darmstadt, Germany) as 5 mm cubes every 14 days and kept in an incubator at 28 °C in the absence of light. *Pythium* spp. were grown in sugarcane molasses (SM, Rayner, UK) liquid medium (30 g/L, pH 7). Inoculation was with a 5 mm MEA agar cube from a 3 days old culture transferred to a flask containing 50 mL SM medium, which was kept at 28 °C for 10 days, under static conditions and in the absence of light. The growth was monitored with the OxiTop (Xylem, USA) control system for the automatic evaluation of biochemical oxygen demand (BOD). The quantification of oospores during cultivation was carried out using a haemocytometer (Assistent, Germany), while total biomass concentration was determined by a gravimetric method. Total biomass (TB) was separated from a 10-day old culture in 50 mL SM medium by centrifugation (2000×*g*, 10 min), washed twice with distilled water followed each time by centrifugation. TB was kept for 24 h at −80 °C and lyophilized (Heto Power Dry LL3000, Thermo Fisher, USA). Lyophilized TB was used for contact angle measurements.

2.2. Separation of *Pythium* oospores

The *Pythium* total biomass (TB) pellet separated from SM medium after 10 days of cultivation was suspended in 10 mL of distilled water, and sonicated for 1 min at high intensity (75W, Labsonic L, B.Braun, Melsugen, Germany) in order to release the oospores from hyphae. The resulting suspension of oospores and mycelial fragments was then filtered through a 100 μm stainless steel fabric (Euro Sitex s.r.o., Příbram, Czech Republic) and washed with distilled water. This procedure of sonication and filtration through a sieve was repeated. Quantification of oospores was carried out using a Bürker chamber (Glaswarenfabrik Karl Hecht GmbH, Germany). The resulting biomass had in comparison to TB higher oospore concentration and therefore it will be termed as oospore-enriched biomass (OB). OB was used for adhesion tests and zeta potential measurements. A fraction of OB was kept for 24 h at −80 °C and lyophilized (Heto Power Dry LL3000, Thermo Fisher, USA); this was then used for contact angle measurements and adhesion tests.

2.3. Fungal strains, cultivation and biomass separation

The filamentous microscopic fungal strains *Alternaria alternata* DBM 4004, *Aspergillus niger* DBM 4054 and *Fusarium graminearum* DBM 4344 were provided by the Collection of Yeasts and Industrial Microorganisms (DBM, UCT Prague) and preserved on sterile potato dextrose agar at 4 °C (PDA, 42 g/L, VWR chemicals, Leuven, Belgium). The surface of the PDA agar in a Petri dish with the grown fungal culture was flooded with saline (0.9 % NaCl (w/v)) to release the spores. Subsequently, 2 mL of spore suspension was transferred to a 500 mL Erlenmeyer flask with 150 mL of sterile potato dextrose broth (PDB, 48 g/L, pH 5.2, VWR chemicals, Leuven, Belgium) and cultivated on a rotary shaker (150 rpm) at 30 °C for 7 days.

After cultivation, the biomass was separated by centrifugation (7 min, 9000×*g*), washed twice with saline, followed each time by centrifugation (7 min, 9000×*g*). The biomass so obtained was kept for 24 h at −80 °C and lyophilized (Heto Power Dry LL3000, Thermo Fisher, USA). The lyophilized fungal biomass was used for contact angle measurement. Prior to zeta potential measurement, the lyophilized fungal biomass (0.3 g in dry state) was triturated in a mortar and then suspended in 50 mL of 40 mM KCl. The suspension of biomass particles was filtered through a nylon mesh (Micron Nylon Filter Mesh, Macrokun Mesh Co., Hebei, China) with a mesh size of 10 μm.

2.4. Magnetic beads

Magnetic beads (MB, SiMAG-ionex, 1 μm diameter, Chemicell, Germany) carrying defined carboxyl and octyl functional groups were used as model surfaces. The third type of MB (1 μm diameter) with silanol functional groups (SiMAG-Silanol, Chemicell, Germany) were modified with diethylaminoethyl (DEAE) functional groups. The modification was carried out with 2-chloro-N,N-diethylamine hydrochloride as described by Brányik et al. (2001) [17]. Silanol MB (5 mL, 50 mg/mL) were immersed in a solution containing 2.14 g of Na_2SO_4 and 0.3 g of NaOH in 7.14 mL water and heated to 37 °C followed by gradual addition of 1.07 g of 50 % (w/v) 2-chloro-N,N-diethylamine hydrochloride (Sigma Aldrich, USA) over 2 h with continuous stirring. The reaction mixture was then heated to 60 °C for 30 min and washed with distilled water. MB were collected with a magnet, washed 4 times in distilled water, and stored at 4 °C.

2.5. Physicochemical surface characterization

Zeta potentials (ZP) of OB, MB and fungal biomass were measured in model environments (40 mM KCl, pH 3, 5 and 7) at 25 °C using the Zetasizer Nano-ZS (Malvern, United Kingdom) and calculated according to the Smoluchowski equation. Each sample was measured at least three times, and the results are presented as mean values ± standard deviation.

The surface properties of OB, MB and fungal biomass in the form of compressed pellets were characterized by contact angles (CA) on a CAM 200 goniometer (KSV Instruments, Finalnd) at 25 °C. Drops of water, formamide and 1-bromonaphthalene (volume ≈ 3 μL) were measured by capturing on camera the CA between the droplet and the surface at 10 ms, with a frame rate of 18 frames per second. Pellets were prepared from 0.05 g of OB, MB or fungal biomass by pressing (7 MPa, evacuable pellet press 13 mm, Pike Technologies, Fitchburg, Wisconsin, USA). The results are presented as mean values ± standard deviations obtained from at least 10 measurements with each test liquid. Both ZP and CA data were treated with post hoc Scheffe's test to support the statements on the significance (p-value <0.05) between results. Statistical parameters were obtained using MS Excel software. Pictures of oospores were taken using an Olympus BX51 microscope with Olympus C5050 digital camera. For cell size determination, ImageJ (NIH, USA) software was used.

2.6. Prediction models

Interactions of OB with MB were expressed through three physicochemical interaction models: thermodynamic, DLVO and XDLVO. For interactions of OB with fungi, only the thermodynamic model was used. The thermodynamic approach was used to calculate the total free energies of interaction from values of CA. The classical DLVO model included electrostatic double layer forces, while the XDLVO model combined the previous two approaches. The equations used to calculate the thermodynamic, DLVO, and XDLVO models are given in Refs. [14,15], and [16], respectively. The surface tension components of test liquids used in thermodynamic approach were adopted from Ref. [15]. For DLVO, the Hamaker constant was estimated from the literature for biological systems (0.8 kT), while for XDLVO, it was calculated from the ΔG^{LW} values. The characteristic decay length for acid-base (AB) interactions was 0.6 nm [18]. The models for interactions were made considering oospores as flat plates, as they were interacting with significantly smaller MB (sphere-plate geometry). However, it was observed that the protruding spines of oospores may be the points of adhesion and additional predictions in sphere-sphere geometry were carried out. (X)DLVO models were carried out for environmental conditions of pH 3, 5 and 7, and ionic strength 40 mM corresponding to SM medium.

2.7. Adhesion tests

Adhesion between OB and MB was tested in defined model environments similar to a previously described procedure [18]. OB suspensions (2 mL) of a defined concentration (1.6×10^6 oospores/mL) in electrolyte (40 mM KCl, pH 3, 5 and 7) were mixed (15 rpm, orbital mode, Hulamixer, Invitrogen, USA) with specific amounts of MB for 10 min in plastic test tubes in order to create different MB-to-oospores ratios. Subsequently, the bottoms of the tubes were exposed to an NdFeB magnet (25× 10 mm, Neomag, Czech Republic) for 1 min followed by removal of the supernatant into empty test tubes. The NdFeB magnet was then reapplied from the side of the second tube for an additional 1 min for effective separation of MB. This was followed by measurement of supernatant absorbance (570 nm). Adhesion intensity (AI, %) was calculated according to the equation: $AI = [(A_0\text{-}A_1)/A_0] \times 100$, where A_0 is the absorbance of the initial OB suspension and A_1 is the absorbance of the supernatant after two magnetic separations of MB. Self-sedimentation of OB was evaluated but due to the speed of magnetic separation (1 min) was considered negligible. All experiments were performed in triplicate and results are presented as mean values ± standard deviations. A post hoc Scheffe's test was used to support the statements on significance (p-value <0.05) between results. Statistical parameters were obtained using MS Excel software.

3. Results

3.1. Growth and oospore formation by Pythium oligandrum

The growth of *P. oligandrum.* was quantified through measurement of biochemical oxygen demand (BOD). The maximum achieved BOD after 10 days of cultivation was very similar for strains *P. oligandrum* CBS109982 and *Pythium* sp. X42, while for *P. oligandrum* M1, it was 15 % lower (Fig. S1). The maximum BOD (1400 mg/L) corresponded to approximately 0.3 g/L of dry TB. The oospore concentration (number/g biomass) started to increase together with TB, and for strains CBS109982 and X42, it reached a maximum on the 10th day of cultivation (Fig. S1 A, C). In the case of *P. oligandrum* M1, the maximum oospore concentration was achieved after 7 days of cultivation (Fig. S1 B). The highest concentration of oospores was formed by strain *P. oligandrum* CBS109982 (Fig. S1 A). The oospore-enriched biomass (OB) for all three strains contained 1.7 ± 0.1 times more oospores as compared to their concentration in TB after 10 days of cultivation (Fig. S1).

The average diameters of oospores of strains *P. oligandrum* CBS109982 and M1, and *Pythium* sp. X42, as measured by image analysis, were 20.5 ± 2.3, 21.2 ± 1.5 and 19.6 ± 2.1 μm, respectively. The curvature of the protruding spines of the oospores, used as an input parameter in interaction models, was estimated based on microscopic images to be 0.5 μm in diameter, (Fig. S2).

3.2. Physicochemical surface properties of cells and magnetic beads

Hydrophobic surfaces are characterized by negative total free energies of interaction (ΔG_{TOT}), when considering surface-surface (SWS) interactions in water [14]. Calculations of ΔG_{TOT} revealed a hydrophobic character for only two MB (octyl and DEAE) and *Aspergillus niger* (Table 1). Besides positive ΔG_{TOT} (SWS) interactions in water [14], hydrophilic surfaces were characterized by $\gamma_{LW} \approx$ 40 mJ/m^2, $\gamma^+ \approx 0$ mJ/m^2, and $\gamma^- > 28$ mJ/m^2. Calculations of surface tensions revealed similar hydrophilic and prevailing electron donor characteristics ($\gamma_{LW} = 40$–43 mJ/m^2, $\gamma^- = 62$–76 mJ/m^2, $\gamma^+ = 0.8$–1.8 mJ/m^2) for TB of all *Pythium* strains. The hydrophilic character of *Pythium* strains can also be seen from positive ΔG_{TOT} values (Table 1). From the point of view of ΔG_{TOT}, enrichment of the biomass with oospores (OB) resulted in fewer hydrophilic surfaces (lower ΔG_{TOT}) for all strains (Table 1). The higher values obtained for γ^- as compared to γ^+ for all *Pythium* strains is in line with the ZP measurements, showing predominantly negative surface charges on both TB and OB (Table 2).

The free energies of interaction (ΔG_{TOT}) between fungal cells (SWS) indicate a range of wettability. While *F. graminearum* and *A. alternata* have hydrophilic surfaces ($\Delta G_{TOT} > 0$), *A. niger* is, on the contrary, hydrophobic ($\Delta G_{TOT} < 0$). The surface properties of *F. graminearum* and *A. alternata*, expressed through ΔG_{TOT}, were comparable to the least hydrophilic *P. oligandrum* strain CBS109982. The wettability of filamentous fungi was within the range of values defined by MB model surfaces (Table 1).

Table 1
Average contact angles, total surface tensions (γ_{TOT}) and their apolar (γ_{LW}, LW - Lifshitz-van der Waals) and polar (γ_{AB}, AB – acid-base) components and total free energies of interaction (ΔG_{TOT}) for the system surface-water-surface (SWS) of *Pythium oligandrum* strains CBS109982 and M1, and *Pythium* sp. X42 (total biomass or oospores enriched biomass), magnetic beads (DEAE, carboxyl or octyl) and filamentous fungi. Means of contact angles with at least one letter the same (x, y, z) are not significantly different (p > 0.05). Comparison of statistical significance is presented separately for each test liquid and each type of surfaces (TB, OB, Magnetic beads and Fungi).

Surfaces	Contact angle (°)			Surface tension[b]			ΔG_{TOT}[b]
	W[a]	F[a]	B[a]	γ_{LW}	γ_{AB}	γ_{TOT}	
TB[c]							
CBS109982	28.6^x ±3.4	49.3^x ±3.7	14.3^x ±0.5	43.0	15.5	58.5	48.1
M1	31.0^x ± 1.0	55.9^y ± 1.3	19.5^y ± 2.4	41.9	23.3	65.2	48.3
X42	38.7^y ±1.1	54.7^y ± 1.5	24.6^z ±1.2	40.5	14.8	55.3	41.1
OB[d]							
CBS109982	58.3^x ±1.0	67.2^x ±2.9	18.9^x ±1.2	42.0	21.9	63.9	15.2
M1	48.5^y ± 1.5	54.3^y ± 1.3	25.6^y ± 0.9	40.1	8.3	48.4	24.5
X42	42.2^z ±2.3	49.9^z ±0.9	29.4^z ±1.7	38.8	4.5	43.4	34.1
Magnetic beads							
DEAE	58.3^x ±1.1	25.9^x ±0.9	14.5^x ±0.5	43.0	9.9	52.9	−29.2
Carboxyl[e]	31.9^y ± 1.2	20.6^y ± 0.5	11.5^y ± 0.1	43.5	10.7	54.2	15.3
Octyl[e]	80.2^z ±1.9	33.0^z ±1.6	15.2^x ±0.2	42.9	2.0	44.9	−64.8
Fungi							
A. alternata	41.3^x ±0.7	31.6^x ±1.9	20.2^x ±2.3	39.5	10.3	49.8	9.9
A. niger	53.4^y ± 2.8	33.2^x ±3.4	5.1^y ± 0.1	44.2	7.0	51.2	−15.5
F. graminearum	41.8^x ±2.7	32.1^x ±1.9	21.3^x ±1.0	41.4	7.6	49.1	10.5

[a] W - water, F - formamide, B - 1-bromonaphthalene.
[b] units in mJ/m.^{2}.
[c] TB – total biomass.
[d] OB – oospores enriched biomass.
[e] Data from Strejc et al., 2019.

Table 2
Zeta potential for oospore enriched biomass (OB) of *Pythium* spp. strains CBS109982, M1 and X42, magnetic beads (DEAE, Carboxyl or Octyl) and filamentous fungi in 40 mM KCl at different pHs. Means of zeta potentials with at least one letter the same (a, b, c) are not significantly different ($p > 0.05$). Comparison of statistical significance is presented separately for each pH and each type of surfaces (OB, Magnetic beads and Fungi).

Surfaces		Zeta potential (mV)		
		pH 3	pH 5	pH 7
OB	M1	$1.6^{a} \pm 0.8$	$-10.1^{a} \pm 1.9$	$-11^{a} \pm 1.3$
	CBS109982	$2.1^{a} \pm 0.7$	$-12.4^{a} \pm 1.2$	$-15.1^{b} \pm 1.7$
	X42	$4.2^{b} \pm 0.3$	$-18.6^{b} \pm 1.8$	$-22.3^{c} \pm 3.4$
Magnetic beads	Carboxyl	$-11.6^{a} \pm 1.6$	$-27.4^{a} \pm 1.8$	$-32.4^{a} \pm 1.5$
	Octyl	$8.8^{b} \pm 0.8$	$-9.8^{b} \pm 0.7$	$-17.4^{b} \pm 0.2$
	DEAE	$14.2^{c} \pm 1.6$	$-14.6^{c} \pm 2.3$	$-11.5^{c} \pm 2$
Fungi	*A. alternata*	$4.7^{a} \pm 0.5$	$-13.1^{a} \pm 1.6$	$-17.6^{a} \pm 1.2$
	A. niger	$-1.1^{b} \pm 0.3$	$-3.0^{b} \pm 0.8$	$-2.8^{b} \pm 0.5$
	F. graminearum	$-5.9^{c} \pm 0.6$	$-18.4^{c} \pm 2.1$	$-17.4^{a} \pm 1.7$

Octyl and DEAE MB, according to ΔG_{TOT}, were hydrophobic, whereas carboxyl MB had a hydrophilic character. The surface hydrophobicity of MB increased in the order carboxyl < DEAE < octyl (Table 1).

The average ZP values of *Pythium* spp., as a function of pH in a symmetrical model electrolyte (40 mM KCl), indicate that OB was electropositive at pH 3 and electronegative at pH 5 and 7 (Table 2). The isoelectric points (pI) of *Pythium* spp. OB was around pH 3.5.

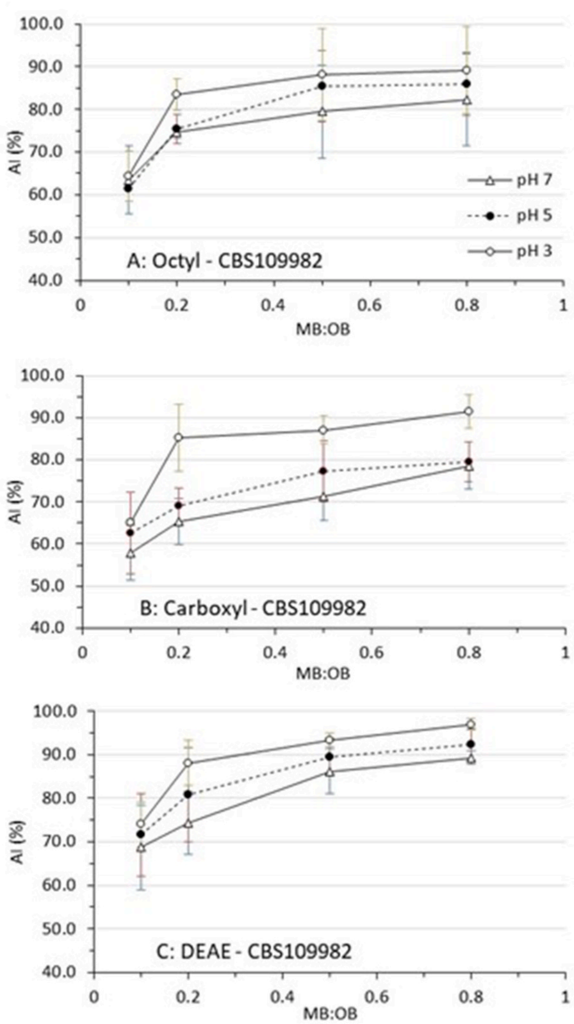

Fig. 1. Adhesion intensity (AI) of oospore-enriched biomass (OB) of *Pythium oligandrum* strain CBS109982 to octyl (A), carboxyl (B) and DEAE (C) magnetic beads (MB) in electrolyte (40 mM KCl) at a different pH and different MB-to-OB ratios (g/g).

The ZP of MB functionalized with DEAE and octyl groups showed an ionex character dependent on the pH of the environment. At pH 3 the DEAE and octyl MB carried a positive charge up until their isoelectric points (pI) in the range of 3.8–4.0. The same surfaces above pI had negative ZPs, including the carboxyl MB over the whole pH range studied (Table 2). The surface charge of *A. niger* and *F. graminearum* was electronegative and did not show pI in the pH range studied, while the ZP of *A. alternata* was positive at pH 3 with a pI at approximately 3.4 (Table 2). The surface charge of fungal strains was within the range of ZP values obtained for MB (Table 2).

Variability of the surface properties (ΔG_{TOT} and ZP) of the *Pythium* strains tested was not very large, therefore, the OB of *P. oligandrum* CBS109982 was chosen as a representative material for adhesion tests with MB and for a prediction of surface interactions with filamentous fungi.

3.3. Adhesion tests between magnetic beads and oospore-enriched biomass

Adhesion intensity (AI) to MB was dependent on the ratio of interacting entities and the pH of the environment. In all situations tested, adhesion increased with increasing MB-to-OB ratio, reaching a plateau (Fig. 1). When comparing AI at an MB-to-OB ratio of 0.2 g/g, a significantly higher AI (p-value < 0.05) was observed at the most acidic pH 3 for octyl and carboxyl MB (Fig. 1 A, B). Conversely, the interaction between MB and OB was the weakest at pH 7 (Fig. 1). The difference between AI at pH 5 and 7 was statistically insignificant (p-value > 0.05). In some cases, AI reached high levels (94–96 %), such as for interaction of DEAE MB with OB (*P. oligandrum* CBS109982) at pH 3 and an MB-to-OB ratio of 0.8 g/g (Fig. 1 C).

3.4. Comparison of adhesion tests with the thermodynamic model

For some combinations of TB/OB with MB a favourable adhesion energy balance of $\Delta G_{TOT} < 0$ was obtained, whereas for other combinations, the balance was unfavourable ($\Delta G_{TOT} > 0$) (Table 3). Comparing the ΔG_{TOT} of OB (*P. oligandrum* CBS109982) to octyl, carboxyl and DEAE MB (Table 3) with real adhesion experiments (Fig. 1), the thermodynamic approach was not able to identify sufficiently real adhesion. The thermodynamic model predicted the probability of favourable adhesion only between OB and octyl and DEAE MB, while in reality the adhesion of OB was observed to all MB. The thermodynamic model predicted an unfavourable balance of interaction energies ($\Delta G_{TOT} > 0$) for adhesion between OB of *Pythium* spp. and three filamentous fungi (Table 3). Since the thermodynamic model was not able to provide a generalized description of adhesion to model materials, the DLVO and extended DLVO (XDLVO) theories were also tested.

3.5. Comparison of adhesion tests with DLVO model

A clear relationship was seen between the highest AI at pH 3 (Fig. 1) and the absence of energy barriers, as predicted by the DLVO model (Fig. 2 A, B, C). Under conditions allowing stronger interactions (absence of an energy barrier), the MB-to-OB ratio resulting in

Table 3

Total free energies of interaction (ΔG_{TOT}) and their apolar (ΔG_{LW}, LW - Lifshitz-van der Waals) and polar (ΔG_{AB}, AB – acid-base) components, as calculated according to the thermodynamic balance of interaction energies for the system, surface1-water-surface2, consisting of *Pythium* spp. strains CBS109982 and M1, and *Pythium* sp. X42 (total biomass or oospores enriched biomass) either with one type of magnetic bead (DEAE, Carboxyl or Octyl) or filamentous fungi.

Interaction system surface1-water-surface2	Free energy of interaction (mJ/m^2)					
	TB[a]			OB[b]		
	ΔG_{LW}	ΔG_{AB}	ΔG_{TOT}	ΔG_{LW}	ΔG_{AB}	ΔG_{TOT}
CBS109982–W-DEAE	−7.1	12.0	4.9	−6.9	1.4	−5.5
M1-W-DEAE	−6.8	15.8	9.0	−6.3	−1.0	−7.3
X42-W-DEAE	−6.4	8.5	2.1	−5.9	0.8	−5.1
CBS109982–W-Carboxyl	−7.3	39.3	32.0	−7.0	22.6	15.6
M1-W-Carboxyl	−6.9	41.1	34.2	−6.4	26.2	19.8
X42-W-Carboxyl	−6.5	35.1	28.6	−6.0	30.1	24.1
CBS109982–W-Octyl	−7.1	−19.9	−23.0	−6.8	−20.6	−27.4
M1-W-Octyl	−6.8	−9.8	−16.6	−6.2	−29.2	−35.4
X42-W-Octyl	−6.3	−18.8	−25.1	−5.9	−29.5	−35.4
CBS109982–W-*A. alternata*	–	–	–	−5.9	19.5	13.7
M1-W-*A. alternata*	–	–	–	−5.4	22.2	16.8
X42-W-*A. alternata*	–	–	–	−5.1	25.8	20.7
CBS109982–W-*A. niger*	–	–	–	−7.2	10.7	3.5
M1-W- *A. niger*	–	–	–	−6.6	10.6	4.0
X42-W- *A. niger*	–	–	–	−6.2	13.5	7.3
CBS109982–W–*F. graminearum*	–	–	–	−6.4	20.5	14.1
M1-W- *F. graminearum*	–	–	–	−5.9	23.4	17.5
X42-W- *F. graminearum*	–	–	–	−5.5	27.1	21.6

[a] TB – total biomass.

[b] OB – oospores enriched biomass.

high AI was lower. For example, to remove almost 85 % of OB (*P. oligandrum* CBS109982) at pH 3 by adhesion to carboxyl MB, it was necessary to achieve a MB-to-OB ratio of 0.2 g/g (Fig. 1 B). In this situation, the DLVO theory predicts the absence of an energy barrier (Fig. 2 A). Similarly, carboxyl MB-to-OB ratio of 0.2 g/g resulted in only 65 % AI at pH 7 (Fig. 1 B). Accordingly, under these conditions, the DLVO theory predicted a potential energy barrier preventing the carboxyl MB interaction with OB of *P. oligandrum* CBS109982 (Fig. 2 G). The height of the energy barrier depended on the chosen geometry of the model. The sphere-sphere geometry predicted less pronounced energy barriers (Fig. 2 D, G, H) or their absence (Fig. 2 E, F, I), which was mirrored in relatively high AI under all conditions studied. For instance, the sphere-sphere geometry of the DLVO model (considering the interaction of oospores through the protruding spines) predicted the absence of energy barriers for DEAE MB vs. OB interaction at all three pH values (Fig. 2 C, F, I); and indeed, the differences between AI (Fig. 1 C) were statistically not significant. The predictions according to sphere-plate geometry were significantly different (Fig. 2 C, F, I) and were not reflected in similar differences during adhesion tests (Fig. 1 C).

3.6. Comparison of adhesion tests with the extended DLVO model

The relationship between predictions of the extended DLVO (XDLVO) model and AI between MB and OB of *P. oligandrum* CBS109982 were not clearly interpretable. Energy barriers were missing in the predictions for octyl MB for all pH values (Fig. S3 B, E, H), while the AI at pH 3 was significantly higher (p-value < 0.05) than at pH 5 and 7 (Fig. 1 A). Another discrepancy between the XDLVO prediction and experimentation appeared in the case of OB adhesion to carboxyl MB (Fig. 1 B). In this case, the XDLVO model predicted a similar secondary minimum (−26 to −47 kT at 5–7 nm) for pH 3, 5 and 7, followed by an energy barrier at a shorter separation distance (Fig. S3 A, D, G). Simultaneously, the AI of real adhesion tests was significantly higher at pH 3 compared to higher pH values (Fig. 1 B).

4. Discussion

P. oligandrum can serve as a BCA, directly protecting plants against fungal parasites and also triggering plant defense mechanisms against other pathogens, as well as improving plant growth and fitness [8]. It has been shown to have efficacy against different fungal species in both natural [19–21] and industrial environments [22,23]. Although the mechanism of action involves the surface interaction of *P. oligandrum* with the host fungi and/or plant, no information is available either on the surface properties of *P. oligandrum* or the physicochemical character (driving force) of this interaction.

The concept of this work is based on confronting the theoretical prediction of interaction with experimental adhesion of three mycoparasitic *Pythium* strains to model solid materials. The model materials, represented by different MB, were selected to cover a wide range of surface properties [18], as *P. oligandrum* must be able to interact with very different surfaces (different species of

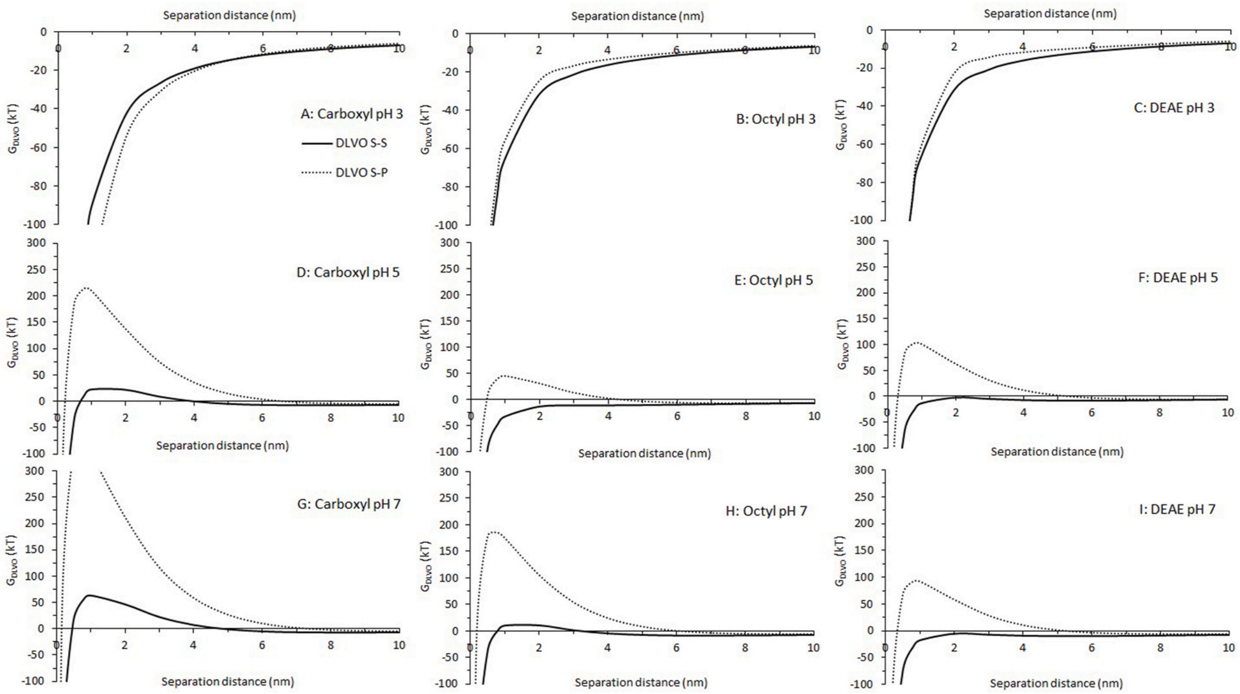

Fig. 2. Total interaction energy (G_{DLVO}) as a function of the separation distance between oospore-enriched biomass of *Pythium* spp. CBS109982 and carboxyl (A, D, G), octyl (B, E, H) and DEAE (C, F, I) magnetic beads in electrolyte (40 mM KCl) at different pHs according to the DLVO theory in sphere-sphere (S–S) and sphere-plate (S–P) geometry.

filamentous fungi, plant roots, leaves, human and animal skin). Carboxyl MB were selected to represent hydrophilic surfaces with negative ZP, such as some plant fibres [24]. DEAE MB were selected to imitate moderately hydrophobic surfaces with a positive ZP in an acidic environment. The modification of MB with octyl groups was selected in order to include an even more hydrophobic model surface representing plant cuticles [25] or waxy surfaces of grains [26]. The remaining experimental conditions for this study (ionic strength 40 mM, pH 5) simulated the growth conditions of *Pythium* strains, while pH 3 was chosen to mimic acidic conditions used in the food industry. A neutral pH 7 was chosen to mimic the rinsing of surfaces with water.

The filamentous fungi used in this work were chosen as representatives of the natural targets of mycoparasitic activity of *Pythium oligandrum*. The wettability of fungal species characterized in this work were also in the range from hydrophilic (*A. alternata* and *F. graminearum*) to moderately hydrophobic (*A. niger*). The water contact angles of *F. graminearum* were higher than those of *F. oxysporum,* as taken from the literature [27]. The free energy of interaction (ΔG_{TOT}) between *A. niger* spores was somewhat different (23.72 mJ/m^2) from the values obtained for the whole biomass in this study (−15.5 mJ/m^2). However, the authors El Abed et al. (2010) [28] do not provide methodological details of contact angle measurements of *A. niger* spores.

Physicochemical surface properties of two *Pythium oligandrum* (M1 and CBS 109982), and one *Pythium* sp. (strain X42) are described for the first time in this work. They were selected for this study because the strain M1 is already produced and commercialized (https://biopreparaty.eu/), strain CBS109982 is publicly available from a collection of microorganisms (https://catalog.mirri.org/) and strain X42 was recently isolated and characterized, showing promising mycoparasite properties [29]. The surface properties of TB for all three strains are rather similar and can be characterized as hydrophilic and an electron donor, while the enrichment of biomass with oospores (OB) preserved the overall character but resulted in reduced hydrophilicity. The shift towards a less hydrophilic character of oospores is relevant for their adhesion, as works by Liu et al. (2004) [30] found that microbes and spores with hydrophobic surfaces generally adhere easier to both hydrophilic and hydrophobic surfaces. Although there are significant limitations in cross-comparisons of literature data, very similar surface properties were found for *Chlorella vulgaris* (γ_{LW} = 35.7–38.2 mJ/m^2, γ_{AB} = 2.2–21.3 mJ/m^2, γ^- = 70.1–83.8 mJ/m^2, γ_{TOT} = 40.4–58.4 mJ/m^2), a hydrophilic microalga with strong electron donor surface properties [31]. When comparing the contact angles, the OB of *Pythium* spp. showed similarity with three strains of Streptococci and Lactobacilli [32]. In general, it can be said that the surface properties of *Pythium* spp. coincide with different taxa and are not found in extremes.

The surface of OB (strain CBS109982) readily interacted with all MB evaluated. The comparison of model predictions with adhesion experiments showed the DLVO model to be the qualitatively most reliable in predicting OB adhesion to model materials represented by MB. This means that the van der Waals and electrostatic interactions are the most important for the adhesion of MB to the OB surface. The electrostatic attraction/repulsion between surfaces (arising from the presence of carboxyl and hydroxyl functional groups) was often found to govern microbial adhesion, especially at low ionic strengths [31,33]. At the same time, the inclusion of acid-base interactions into the XDLVO model did not increase the agreement between model predictions and AI results. Electrostatic interactions can be most influenced by the ionic strength (I) of the environment, in such a way that lower I expands the double layer and the significance of electrostatic forces [34]. The attraction of electrostatic forces can also be enhanced by a suitable choice of pH, in which the interacting surfaces either display lower repulsion or even attraction, when oppositely charged. This was well-observed with an increased degree of adhesion at pH 3, which was consistent with the prediction of the DLVO model. An interesting finding is that the DLVO model better described the adhesion behaviour in sphere-sphere geometry, considering the curvature of the protruding spines of oospores. A similar phenomenon was observed for hematite particles, when their mutual interaction was found to be governed not by the overall particle radius but by the curvature radius for surface protrusions [35].

The filamentous fungi used in this work have surface properties (CA and ZP) in the range of MB model surfaces. Although the thermodynamic model does not predict a favourable energy balance ($\Delta G_{TOT} > 0$) for the adhesion of *P. oligandrum* to fungi, it can be expected that the interaction will be governed by the same forces as was the case with MB.

5. Conclusions

By extrapolating *Pythium* spp. surface properties and their adhesion (theory and experiment) to model materials (MB), or to the surface of natural prey and/or host plants, it can be concluded that *Pythium* spp. has surface properties and structures that allow interactions with a wide range of biotic surfaces. No significant repulsion was predicted and observed, which seems to be advantageous for a species, the survival of which is fundamentally linked with adhesion to prey and/or host. The most favourable conditions found for the adhesion of *Pythium* spp. to model surfaces can also have new applications. For example, the adhesion of *Pythium* spp. to man-made target surfaces can be facilitated by choosing a lower pH and/or ionic strength, or by modifying the surfaces in order to increase the attractive electrostatic interactions.

Data availability

Data will be made available on request.

CRediT authorship contribution statement

Katarina Majtan: Writing – review & editing, Methodology, Investigation. **Maja Klimentić:** Writing – review & editing, Investigation, Data curation. **Jan Martinik:** Methodology, Investigation. **Marketa Kulisova:** Methodology, Investigation. **Irena Jarosova:** Supervision, Funding acquisition. **Tomas Potocar:** Software, Formal analysis. **Tomas Branyik:** Writing – original draft, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Tomas Branyik reports financial support was provided by Grant Agency of the Czech Republic. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

[1] HLPE, Agroecological and Other Innovative Approaches for Sustainable Agriculture and Food Systems that Enhance Food Security and Nutrition, 2019.
[2] E. Commission, On the Experience Gained by Member States on the Implementation of National Targets Established in Their National Action Plans and on Progress in the Implementation of Directive 2009/128/EC on the Sustainable Use of Pesticides, COM, 2020, p. 204, final, 2020.
[3] J. Brožová, Exploitation of the mycoparasitic fungus *Pythium oligandrum* in plant protection - a review, Plant Protect. Sci. 38 (2002) 30–35.
[4] N. Benhamou, G. le Floch, J. Vallance, J. Gerbore, D. Grizard, P. Rey, *Pythium oligandrum*: an example of opportunistic success, Microbiology (Read.) 158 (2012) 2679–2694.
[5] S. Takenaka, Studies on biological control mechanisms of *Pythium oligandrum*, J. Gen. Plant Pathol. 81 (2015) 466–469.
[6] N. Benhamou, P. Rey, K. Picard, Y. Tirilly, Ultrastructural and cytochemical aspects of the interaction between the mycoparasite *Pythium oligandrum* and soilborne plant pathogens, Phytopathology 89 (1999) 506–517.
[7] S. Ikeda, A. Shimizu, M. Shimizu, H. Takahashi, S. Takenaka, Biocontrol of black scurf on potato by seed tuber treatment with *Pythium oligandrum*, Biol. Control 60 (2012) 297–304.
[8] K. Bělonožníková, V. Hýsková, J. Chmelík, D. Kavan, N. Čeřovská, H. Ryšlavá, *Pythium oligandrum* in plant protection and growth promotion: secretion of hydrolytic enzymes, elicitors and tryptamine as auxin precursor, Microbiol. Res. 258 (2022) 126976.
[9] E. Commission, in: C.I.R. (EU) (Ed.), Commission Implementing Regulation (EU) No 540/2011 of 25 May 2011 Implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as Regards the List of Approved Active Substances Text with EEA Relevance, 2011. No 540/2011.
[10] E. Commission, Regulation (EU) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products, 2009. No 1223/2009.
[11] E. Commission, in: E. Parliament (Ed.), Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 Concerning the Making Available on the Market and Use of Biocidal Products, 2012. No 528/2012European Parliament.
[12] K. Picard, Y. Tirilly, N. Benhamou, Cytological effects of cellulases in the parasitism of *Phytophthora parasitica* by *Pythium oligandrum*, Appl. Environ. Microbiol. 66 (2000) 4305–4314.
[13] E. Galiana, S. Fourré, G. Engler, *Phytophthora parasitica* biofilm formation: installation and organization of microcolonies on the surface of a host plant, Environ. Microbiol. 10 (2008) 2164–2171.
[14] C.J. van Oss, Hydrophobicity of biosurfaces – origin, quantitative determination and interaction energies, Colloids Surf. B Biointerfaces 5 (1995) 91–110.
[15] R. Bos, H.C. van der Mei, H.J. Busscher, Physico-chemistry of initial microbial adhesive interactions – its mechanisms and methods for study, FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Rev. 23 (1999) 179–230.
[16] C.J. van Oss, Long-range and short-range mechanisms of hydrophobic attraction and hydrophilic repulsion in specific and aspecific interactions, J. Mol. Recogn. 16 (2003) 177–190.
[17] T. Brányik, A. Vicente, J.M. Cruz, J. Teixeira, Spent grains - a new support for brewing yeast immobilisation, Biotechnol. Lett. 23 (2001) 1073–1078.
[18] J. Strejc, L. Kyselova, A. Cadkova, T. Potocar, T. Branyik, Physico-chemical approach to adhesion of *Alicyclobacillus* cells and spores to model solid materials, Extremophiles 23 (2019) 219–227.
[19] K. Bělonožníková, V. Hýsková, M. Vašková, T. Křížek, K. Čokrtová, T. Vaněk, L. Halířová, M. Chudý, A. Žufić, H. Ryšlavá, Seed protection of *Solanum lycopersicum* with *Pythium oligandrum* against *Alternaria brassicicola* and *Verticillium albo-atrum*, Microorganisms 10 (2022) 1348.
[20] Z. Haghi, R. Mostowfizadeh-Ghalamfarsa, C. Steinberg, The efficacy of Iranian *Pythium oligandrum* isolates in biocontrol of soil-borne fungal pathogens of tomato, J. Plant Pathol. 105 (2023) 185–196.
[21] A. Klimek-Kopyra, J. Dłużniewska, A. Sikora, Influence of biofungicides containing microorganisms such as *Pythium oligandrum* and *Bacillus subtilis* on yield, morphological parameters, and pathogen suppression in six winter pea cultivars, Agriculture 13 (2023) 1170.
[22] C.A. Ng, M. Pernica, J. Yap, S. Běláková, K. Vaculová, T. Brányik, Biocontrol effect of *Pythium oligandrum* on artificial *Fusarium culmorum* infection during malting of wheat, J. Cereal. Sci. 100 (2021) 103258.
[23] C.A. Ng, M. Pernica, K. Litvanova, I. Kolouchova, T. Branyik, Biocontrol using *Pythium oligandrum* during malting of *Fusarium*-contaminated barley, Fermentation 9 (2023) 257.
[24] S. Kalia, K. Thakur, A. Celli, M.A. Kiechel, C.L. Schauer, Surface modification of plant fibers using environment friendly methods for their application in polymer composites, textile industry and antimicrobial activities: a review, J. Environ. Chem. Eng. 1 (2013) 97–112.
[25] V. Fernández, H.A. Bahamonde, J. Javier Peguero-Pina, E. Gil-Pelegrín, D. Sancho-Knapik, L. Gil, H.E. Goldbach, T. Eichert, Physico-chemical properties of plant cuticles and their functional and ecological significance, J. Exp. Bot. 68 (2017) 5293–5306.
[26] T. Brányik, A. Vicente, R. Oliveira, J. Teixeira, Physicochemical surface properties of brewing yeast influencing their immobilization onto spent grains in a continuous reactor, Biotechnol. Bioeng. 88 (2004) 84–93.
[27] T.H.M. Smits, L.Y. Wick, H. Harms, C. Keel, Characterization of the surface hydrophobicity of filamentous fungi, Environ. Microbiol. 5 (2003) 85–91.
[28] S. El Abed, F. Hamadi, H. Latrache, H.M. Iraqui, K.S. Ibnsouda, Adhesion of *Aspergillus niger* and *Penicillium expansumspores* on Fez cedar wood substrata, Ann. Microbiol. 60 (2010) 377–382.

[29] K. Bělonožníková, K. Vaverová, T. Vaněk, M. Kolařík, V. Hýsková, R. Vaňková, P. Dobrev, T. Křížek, O. Hodek, K. Čokrtová, A. Štípek, H. Ryšlavá, Novel insights into the effect of *Pythium* strains on rapeseed metabolism, Microorganisms 8 (2020) 1472.
[30] Y. Liu, S.F. Yang, Y. Li, H. Xu, L. Qin, J.H. Tay, The influence of cell and substratum surface hydrophobicities on microbial attachment, J. Biotechnol. 110 (2004) 251–256.
[31] M. Sirmerova, G. Prochazkova, L. Siristova, Z. Kolska, T. Branyik, Adhesion of Chlorella vulgaris to solid surfaces, as mediated by physicochemical interactions, J. Appl. Phycol. 25 (2013) 1687–1695.
[32] H.C. van der Mei, R. Bos, H.J. Busscher, A reference guide to microbial cell surface hydrophobicity based on contact angles, Colloids Surf. B Biointerfaces 11 (1998) 213–221.
[33] D. Yongabi, S. Jooken, S. Givanoudi, M. Khorshid, O. Deschaume, C. Bartic, P. Losada-Pérez, M. Wübbenhorst, P. Wagner, Ionic strength controls long-term cell-surface interactions – a QCM-D study of *S. cerevisiae* adhesion, retention and detachment, J. Colloid Interface Sci. 585 (2021) 583–595.
[34] M. Hermansson, The DLVO theory in microbial adhesion, Colloids Surf. B Biointerfaces 14 (1999) 105–119.
[35] A. Sayano, H. Ohshima, T. Hoshina, T. Tsurumi, What in particle morphology determines the DLVO interaction energy between hematite particles in electrolyte solutions? Colloids Surf. A Physicochem. Eng. Asp. 664 (2023) 131144.