The method of contact angle measurements and estimation of work of adhesion in bioleaching of metals

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ABSTRACT

In this paper, we present our method for the measurement of contact angles on the surface of minerals during the bioleaching process because the standard deviation obtained in our measurements achieved unexpectedly low error. Construction of a goniometer connected with a specially prepared computer program allowed us to repeat measurements several times over a short time course, yielding excellent results.

After defining points on the outline of the image of a drop and its baseline as well of the first approximation of the outline of the drop, an iterative process is initiated that is aimed at fitting the model of the drop and baseline. In turn, after defining the medium for which measurements were made, the work of adhesion is determined according to Young-Dupré equation. Calculations were made with the use of two methods named the L-M and L-Q methods.

INTRODUCTION

The mechanism of bacterial leaching of sulphide minerals has been studied for fifty years, but in the last fifteen years investigators have paid attention to the interfacial aspects of the leaching process (1, 2).

Copper sulphides are hydrophobic, have a high surface energy and a negative surface charge. Some investigators claim that most organisms are hydrophilic and their adhesion to hydrophobic surface will be stronger than to a hydrophilic surface if short-range forces are involved (3). Others (4, 5) have found the opposite: greater hydrophobicity of cells and substrata results in greater attractive forces and a higher level of adhesion. Interfacial interactions between substrata and bacterial cells are more complicated in the case of ores or post-flotation wastes containing residues of copper sulphides and different origin ores (in our experiments limestone and sandstone are origin ores). In this case, such factors as surface texture and diversity of particles (copper sulphides and origin rock) may influence the adhesion process.

The aim of our earlier study (6) was to estimate how exopolymers produced by bacteria in the

bioleaching process may influence the relationship between cells and their substrate surface.

Bacterial adhesion to post-flotation particles (ore particles as well) is difficult to study directly due to the heterogenicity of the latter, because of, for example, variations in particle size, and ion exchange capacity. For biological purposes the Young-Dupré equation is particularly useful for obtaining the work of adhesion of a liquid to solid particles since the unknown surface tension (solid-vapour, solid-liquid) can be eliminated (7). The work of adhesion allows us to determine the wettability of substrata by the examined liquid (6). The method of measuring contact angles between liquid and non-homogenous solid surfaces was developed and is described below.

MATERIALS AND METHODS

Sample preparation

The contact angle of the exopolymer solution, control sample and redestilled water (MilliQ water) were measured. The exopolymer was isolated from a pure culture of *B.insolitus* strain no. 26 and from a mixed culture of strains naturally existing in post-flotation waste (6). The bacteria were isolated from post-flotation wastes in the course of copper bioleaching research. The strains were cultivated on mineral medium with post-flotation wastes as a substrate (thiosulphate in mineral Beijerinck's medium was replaced by 10 g post-flotation waste).

The control sample for the exopolymer was obtained from a control culture (sterile medium with post-flotation wastes). The isolation of the exopolymer was performed according to a modified procedure of Wrangstadh (8) as described earlier (6).

CONTACT ANGLE MEASUREMENTS

Contact angles were estimated with a goniometer equipped with a special optical system and a CCD camera (obtained from the Institute of Applied Optics in Warsaw) (6). A drop of liquid (5 µl) was placed on a specially prepared plate of substratum and the image was immediately sent via the CCD camera to the computer for analysis. For contact angle estimation, we used the L-M and L-Q methods (9). Contact angles were measured on dry glass (microscopic cover glass washed in ethyl alcohol), quartz plate and specially prepared plates of sulphur and copper ore. The sulphur plate was prepared by heating elementary sulphur and then making a thin smooth cast. The plate of ore was made from a thin section of crude ore by polishing. The surface of the ore plate was washed in ethyl alcohol and water. In the second experiment, all plates were covered by exopolymer film, and then dried at room temperature. The contact angle for water was estimated on such prepared surfaces. Temperature and moisture was constant during the experiment (23°C and 68% respectively).
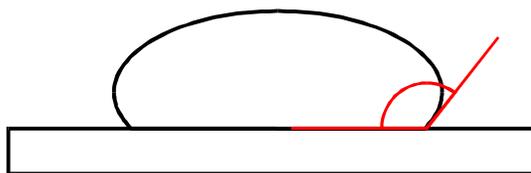
Fig. 1. The model of a drop on solid base

PROGRAM DROP—MEASUREMENT

CONTACT ANGLE BETWEEN DROP OF LIQUID AND SOLID BASE

The program created for analysis of images obtained with the goniometer coupled with the CCD camera allows recording of the image of a drop of liquid on a studied base. The program serves to calculate the angle of contact between the liquid and the solid base.

The model of a drop resting on a solid base involves the assumption that a drop is sufficiently small (light) to allow its shape to be taken as that of a fragment of an ellipsoid of revolution.

After reading the image of the drop into the program, the points lying along the outline of the drop (DROP) and baseline (BASE) should be defined. In turn the initial approximation of the ellipse should be drawn and, if necessary, its image modified with the use of existing markers to allow its position to best correspond to the real outline of the drop on the image.

After defining points on the outline of the image of the drop and baseline as well of the first approximation of the outline of the drop, an iterative process should be initiated aimed at fitting the model of drop and baseline.

In turn, after defining the medium for which measurements were made, the work of adhesion can be determined according to the formula:

$$W = \sigma \, [1.0 + \cos(\phi)]$$

where: W - work of adhesion (dependent on material of substratum, type of liquid and temperature);

ϕ - interior angle of contact between drop and base;

σ - energy (size defined by kind of liquid and temperature).

Calculations were made with the use of two methods named the L-M and LQ methods.

To record the correct image of the drop several conditions should be met:

Depth of focus

Since a drop on a base is a "deep", three-dimensional object, an image taken with the camera is out of focus along the baseline. The camera's depth of focus is usually too small in relation to the size of the drop and the base, the goal being to observe both the outline of drop and baseline. Because of the geometric configuration, the baseline is the outline of the sample that as a rule is in the area outside the plane of sharpness of lens.

Coherent plane

The configuration of the measurement should strive at such positioning of the sample that the optical axis is in the surface plane of the base. Otherwise, the baseline in the obtained image will be above its real position (situation shown in the figure 3).
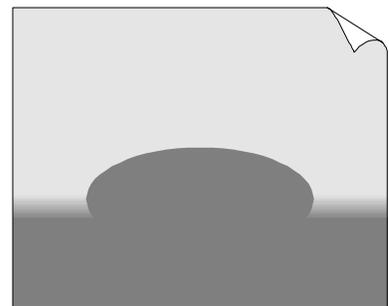
Fig. 2. The influence of depth of focus on the outline of drop and baseline
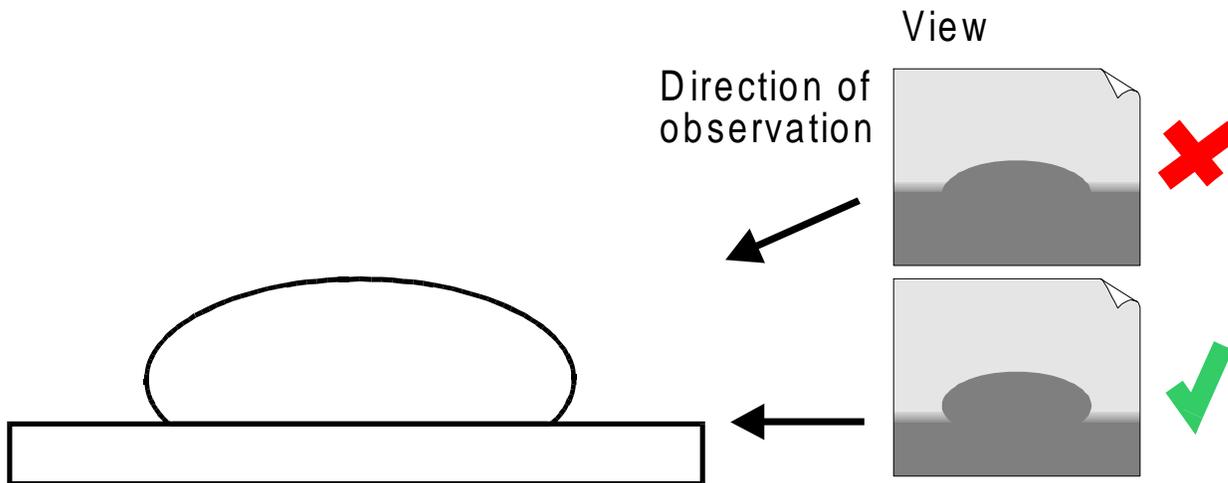
Fig. 3. The coherence of the optical axis and the plane of the surface of base

Uniform illumination

Deterioration of the image of the drop will also occur if the "background" against which the drop is recorded is not uniform. Differences in illumination falsify the real position of the outlines (figure 4).
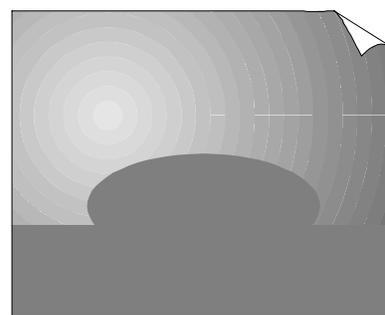
METHODS

The program for determining the best fit between the elliptic equation to the image of the drop involved the use of two methods: the L-M Method and the L-Q Method. Because of the different definition of the error function, the solutions obtained are different for each method.

Fig. 4. The possibility of the deterioration of drop image

Both methods implement equations describing the searched ellipse and consequently they can behave differently in the process of fitting.

Since the number of searched parameters of the ellipse is 5, iteration can be begun after defining at least 6 points along the outline of drop and at least two points along the baseline.

Fitting of elliptic equation to image of drop

L-M Methods

The L-M (Levenberg-Marquardt) method involves the iterative finding of the best fitting (non-linear model) function described by set of M parameters to the set of points. The number of M parameters is not greater than 5 (depending on the assumed strategy) and is equal to the number of coefficients describing the elliptic equation, i.e. x0, y0, A, B and Fi. This is an interactive method that demands the giving by the user of several parameters describing the strategy of fitting. The process is controlled by the size of parameter Lambda.

The user influences not only the degree and speed of sweeping of error space but can also determine the set of parameters that can be "corrected" in a given iteration.

The L-M method is easy to use in practice and frequently gives better results than for the standard least squares method. Initial fitting does not have to be as accurate as in the L-Q method, but the rate at which the solution converges to a minimum in the L-M method strongly depends on the character of the function and its use is recommended in the "final" stage of the process in order to optimize the solution.

In the DROP program, the fitted ellipse has a strongly non-linear character (equation in the rotated co-ordinate system), and hence convergence is not rapid and depends strongly on the strategy employed. The number of points defining the outline of the drop does not significantly affect the speed of calculations in this method.

LQ Method

This method involves the non-linear least squares method without an independent variable. Because of strong non-linear (confounded) elliptic equation in this method with respect to parameters x0, y0, A, B and Fi, the process of iteration may be unstable of the initial values of the parameters are "very distant" from the optimal solution. In the first iteration a message may appear that the obtained indirect values of the parameters do not describe an ellipse. If the message re-appears, iteration should be discontinued and the parameters of the ellipse corrected manually. In the absence of such a message the solution found in subsequent iterations is rapidly convergent and further iterations introduce only very slight improvements.

Fitting of linear equation to baseline

Fit BASE

The orientation of the baseline is determined by the linear least squares method. To determine the baseline equation it is necessary to define at least two points determining the line on the image.

The program involves the use of several classical methods for enhancing the quality and clarity of the image. They enable the improvement of the appearance of the analysed image to allow easier and more precise determination of the points defining the drop and the baseline.

It is also possible to fit the equation of a parallel ellipse to the base. Measurement of the angle should be on a levelled plane. The goniometer should be equipped with levels allowing levelling of the stage on which measurements are made. However, the base of the sample is not always flat or parallel and therefore the drop may not be flat, causing the calculated contact to be unequal.

The fitting of the ellipse is based on points indicated on the image. In the course of iteration there is no information about the "real" orientation on the plane. Consequently, this option should be used only when we assume that the base of the sample is flat. If we do not have this certainly, that is think that the plate is at an angle that significantly departs from a zero degrees, fitting should be made with the Fixed Fi option disabled.

RESULTS

Tables 1 and 2 present the results obtained during contact angle measurements with the use of a goniometer and the DROP computer program. These results were published earlier (6). In this paper, we want to present only the standard deviation of our measurements because the used methods prepared in our lab achieved unexpectedly low error.

Table 1. Contact angle (degrees) of distilled water, control sample and exopolymer solutions - concentrated (a) and diluted 5-fold (b) on different substrates, " - " no data, ±- standard deviation. Each measurement was repeated 20x by the same researcher who knew the number of the sample but not the contents.(6)

Liquid	Substrate plate				
	Glass	Quartz	Sulphur	Origin ore	Sulphides
H_2O	51.05±0.84	32.79±1.12	81.08±0.35	57.42±0.33	63.08±1.13
Exopolymer from *B. insolitus* strain no. 26					
a	59.45±0.37	30.20±0.44	87.41±0.11	-	-
b	51.44±0.73	32.23±1.06	91.05±0.13	71.20 ±0.56	77.71±0.90
Exopolymer from mixed culture					
a	46.16±1.15	20.20±0.44	79.48±0.44	54.53±0.83	67.39±0.98
b	49.76±0.18	27.78±0.72	83.79±0.57	59.44±1.33	73.50±1.09
Control solution					
a	54.89±0.64	14.07±1.57	77.90±0.61	59.34±0.33	68.09±0.15
b	52.69±0.41	31.05±0,46	79.80±0.05	57.50±0.52	69.66±0.94

Table 2. Work of adhesion (mN/m) of distilled water, control sample and exopolymer solutions - concentrated (a) and diluted 5-fold (b) on different substrates, " - " no data, ±- standard deviation (6)

Liquid	Substrate plate				
	Glass	Quartz	Sulphur	Origin ore	Sulphides
H_2O	118.13±0.84	133.33±0.81	83.87±0.48	111.71±0.36	105.31±1.29
Exopolymer from culture of *B.insolitus* strain no.26					
a	108.63±0.41	134.27±0.29	75.27±0.15	-	-
b	116.82±0.72	132.64±0.73	70.70±0.17	95.21±0.67	87.31±1.11
Exopolymer from mixed culture					
a	122.29±1.02	140.26±0.19	85.59±0.55	114.26±0.86	100.07±1.14
b	119.14±0.18	136.28±0.44	80.19±0.72	108.87±1.41	92.82±1.30
Control solution					
a	113.80±0.67	141.99±0.32	87.34±0.76	109.06±0.37	99.18±0.18
b	115.98±0.41	134.06±0.31	85.02±0.07	111.01±0.55	97.23±1.10

Fig.5 and Fig.6 present the scatter of the obtained results. It is clearly visible that the error was lower when the surface was smooth and homogenous (glass, sulphur).
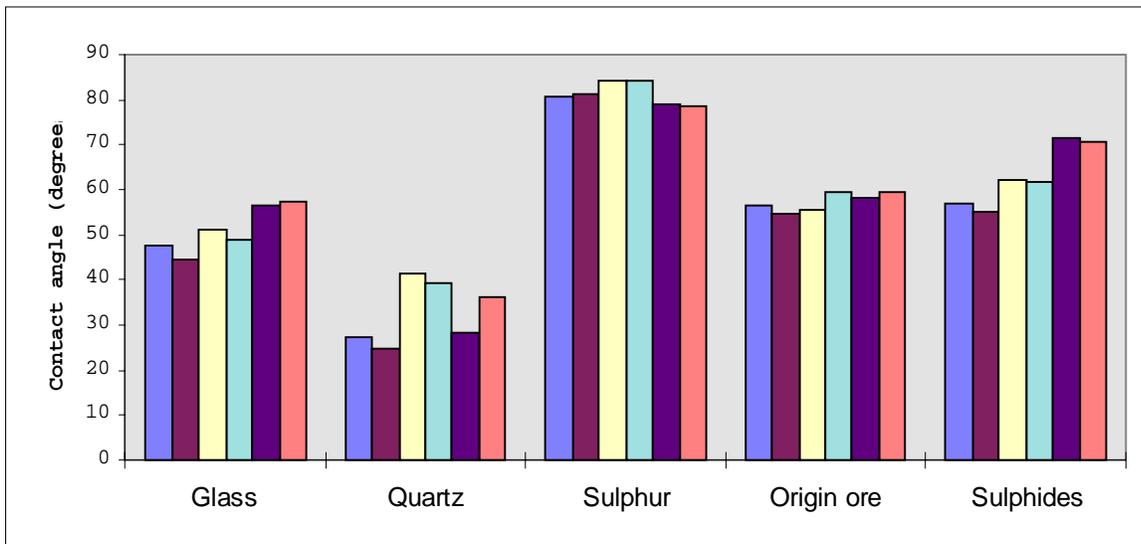
Fig. 5. Contact angle for water on different surfaces (six measurements for each surface)
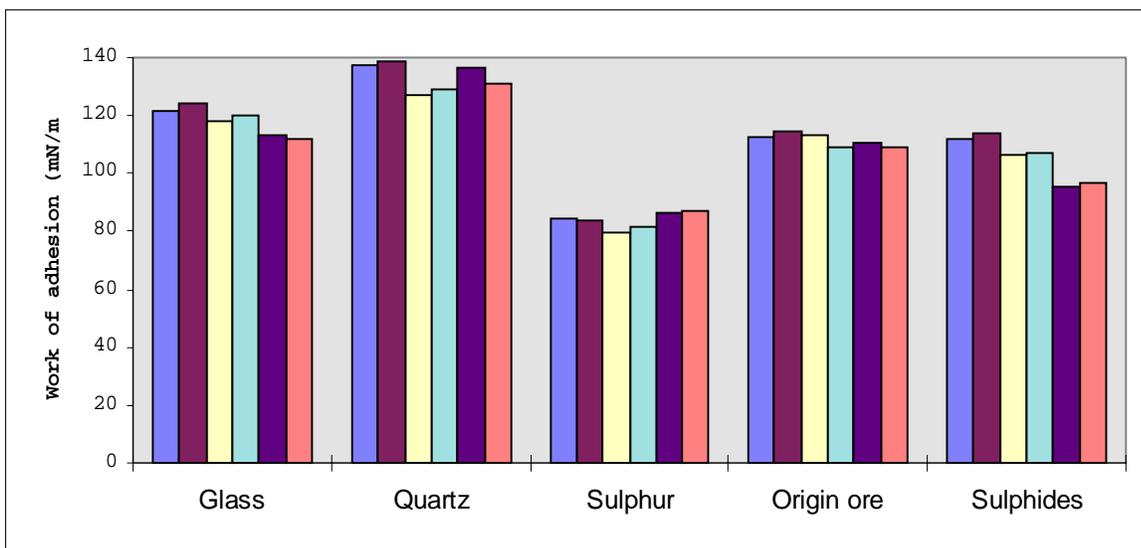

Fig. 6. The results of work of adhesion obtained for water on different surfaces (calculated from six measurements for each surface)

DISCUSSION

The plate of quartz was not ideally smooth. The plate of ore showed, as mentioned in the introduction, different surface texture and diversity of particles (copper sulphides and origin rock), but the surface of the origin rock prevailed. Copper sulphides occurred as a minor insertion in the origin rock. The error of measurements on homogenous surfaces was generally lower, but the maximal standard deviation in one case reached 1.57 for a contact angle of 14.07^{o} (Table 1) which was 11.15%. The surface in this case was homogenous but not ideally smooth as mentioned above and due to difference, the error for the quartz surface was slightly higher.

All the presented data have a comparative value due to the character of measurements (absolute measurements are impossible in such a complex system). Experimentally, it is impossible to determine the surface tension of solid-liquid and solid-vapour interfaces independently.

Construction of the goniometer and the specially prepared computer program allowed us to repeat measurements several times in short time, which resulted in excellent results with low statistical error. The small volume of the examined drop and the precision of Hamilton's syringe allowed us to place the drop on a strictly specified surface on the plate. This placement was very important, particularly in the case of measurments performed on the plate of ore containing origin ore (hydrophilic) as well as a grain of copper sulphides (hydrophobic). Additionaly, the image was immediately sent to computer via the CCD camera to eliminate the possible error connected with evaporation, however the time required for preparation of camera (10-15 s) was sufficient for drop stabilization to occur on the examined surface. All of these conditions and the number of replications of the same measurement (20x) had an influence on the error. We had expected error about 5-7% and we obtained the error between 0.5-2% with one exception described above.

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