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Mr. E.B. Nickols
Bartlesville Project Office
P.O. Box 1398
Bartlesville, OK 74005

Subject Contract: DE-AS19-81BC10508, Final Technical Report
Bacteria Transport Through Porous Material

Dear Mr. Nickols:

Enclosed is the revised copy of the final technical report.
We have made the necessary corrections and revisions.

Thank you.

Sincerely,

T.F. Yen
Professor of Civil and
Environmental Engineering

enclosure.

cc: Carroll A. Lambton, Contracting Officer
Michelle Dombrowski, Contract and Grant Administrator

MASTER

✓ P.S. I believe we have already sent you the actual photographs
for Figs. 4-6, pages 18-20.

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SCOPE OF WORK

In the third phase of the work we will improve bacterial transport by deriving cellular surface functional groups and/or by modifying the rock surface. We will also investigate the influence of different nutrients on the surface properties of bacteria, and hence on the transport in the geological core. Models will be set up to simulate the actual penetration of bacteria in an actual reservoir.

PERSONNEL INVOLVED

Principle Investigator: T.F. Yen

Research Associates: John Findley

Long-Kuan Jang

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OBJECTIVE

This research effort has the following objectives:

- a) Examination of the interaction between bacteria and geological porous media containing oil and brine.
- b) Study of transport phenomena of bacteria through porous media under applied pressure.
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INTRODUCTION

In recent years, several conventional enhanced oil recovery processes have been under various stages of development. Microbial enhanced oil recovery (MEOR) is perhaps the youngest of these processes and has only recently gained acceptance as being a technically viable alternative to the other EOR processes. With a large percentage of original oil in place (~70%) being left in the reservoir at the end of water flooding, a great deal of effort has gone into the development of EOR processes. The DOE-sponsored university research programs in MEOR which began in 1979 have been the focal point of many of the developments that have launched MEOR from its infancy to its current status as an acceptable and viable alternative process. The initial studies conducted here and at other universities have been very promising and demonstrate the great potential MEOR has for the future.

At present, however, numerous problems need to be resolved before MEOR can be successfully applied on a commercial scale. It is advisable to focus attention on a few of these problems and resolve them so that some of the basic questions in MEOR may be answered with some degree of certainty. Transport of bacteria in reservoirs is one such problem that has been studied in our laboratories for the past few years. The problem of the transport of bacteria is linked closely with the success or failure of a bacterial injection process and its ability to enhance oil recovery. The injection and penetration of bacteria into a reservoir is the most problematic and crucial of the steps in any MEOR process. Its study, therefore, is critically important to the implementation of any MEOR program.

During the first four years, the following significant progress has been made from our study of bacterial transport in porous media:

1. Spores, together with nutrients were found to penetrate deep into formations with rather low permeability [1].
2. Prediction of deposition rate based on known properties of rock and cells has been made [2,7,8].
3. Modification of the rock surface and the bacterial surface by chemical methods for

facilitating transport was demonstrated [3,10].

4. Bacterial transport through Berea sandstone by chemotaxis alone was first shown (without external pressure) [4].
5. Preliminary experiments of MEOR in the laboratory were successful. About 40% based on residual oil was recovered (better than caustic flooding) [5].
6. Surfactant-producing aerobe Bacillus subtilis and acid- and solvent-producing anaerobe Clostridium acetobutylicum were found to effectively displace residual heavy crude remaining after secondary waterflooding [6].
7. An approach of colloidal chemistry was adopted to predict the rate of attachment of cells suspended in a laminar flow [9].
8. Effect of a molasses-pyrophosphate mixture on bacterial transport and oil recovery efficiency has been studied [11].
9. Experiments were conducted to investigate the bacteria-phage-rock interaction for the lytic control of bacteria in MEOR processes [12].

In the last phase of our work, the research effort in this field was continued and more valuable information on bacterial transport in porous media was obtained. A great deal of progress was made to determine chemical bonding characteristics between adsorbed bacteria and the rock surfaces. Such information is extremely important in determining the extent of penetration of bacteria through a reservoir rock matrix. In order to investigate the bonding immediately adjacent to the solid surface, the ESCA (Electron Spectroscopy for Chemical Analysis) method was employed (section 2). We could successfully deposit a monolayer of B. Subtilis onto a 50 angstrom, silica-covered, 300 m-thick silicon wafer. Several micrographs of different magnifications were obtained using a scanning electron microscope, and for each spectra the nature of the bonding formed by cellular adhesion to the plate was carefully examined. Moreover, the effects of surface roughness and of other adsorbed materials such as inorganic ions or oil was also assessed.

In order to further enhance our knowledge of the effects of surface tensions on bacteria transport through porous media, a new approach was taken to illustrate the effect of liquid surface

tension on bacterial transport through a sandpack column (section 3). In order to explain the surface thermodynamics of bacterial adhesion, a theoretical thermodynamic model was employed to predict the extent of bacterial transport through porous media. The prediction was closely supported by the experimental results.

Since it is postulated that the pore walls within a reservoir rock are mostly comprised of mineral oxides, further work in surface charge characterization of reservoir rock as a composite oxide system was accomplished (section 4). Much experimental and theoretical work was performed in that regard and valuable information on surface charge behavior of a mixed oxide system was obtained. The investigation of inhibition of cell growth by indole was further progressed in this phase of our work (section 5). These studies are aimed at elucidating the cell changes which take place when a cell population becomes acclimated to grow on inhibitory carbon substrates. The results obtained from this part of the work would be very informative in molecular comparison studies between the wild type and indole metabolizing population of cells.

Finally, in the last section of this report (section 6) a mathematical model to simulate the simultaneous diffusion and growth of bacteria cells in a nutrient-enriched porous media is proposed. The purpose of this model is to predict the mobility of bacterial cells in the bio-"huff-and-puff" process as it was designed by our research group in the earlier MEOR experimental work. The development of this model could greatly assist in effective control of in situ MEOR processes where much detailed analysis is essential for such a model to be applied.

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DETERMINATION OF CHEMICAL BONDING BETWEEN BACTERIAL CELLS AND RESERVOIR ROCK SURFACES

It is generally believed that bacteria cells, when attached to a solid surface, will always leave some residue, even after being treated and sheared off by strong convective currents or by mechanical means. Some researchers attribute this to polymer bridging between bacteria and the solid surface. It has also been reported that gram-negative bacteria in contact with a solid surface can shrink and leave the solid surface if chelating agents or lysozymes are added to the system. For bacteria adsorbing water-soluble polymers on the cellular surface, the addition of polyvalent cations (such as calcium and magnesium) can trigger rapid deposition onto a solid surface. These examples vividly indicate that certain chemical bonds, although weaker than covalent bonds, exist between adsorbed bacteria and solid surfaces.

In order to investigate the bonding immediately adjacent to the solid surface, the ESCA (Electron Spectroscopy for Chemical Analysis) method is often used. ESCA is a powerful tool for studying surface structures of solids. The method involves the measurement of binding energies of electrons in molecules by determining the energies of electrons ejected by the interactions of a molecule with a monoenergetic beam of X-rays. The sample for measurement may be solid, liquid, or gas and the technique is essentially non-destructive. One advantage of this method is that the information obtained is directly related to the electronic structure of a molecule and the theoretical interpretation is relatively straight forward. ESCA provides a tool for monitoring the outermost (<100 angstroms) layer of a sample which yields information about the coating material or surface contaminating substances when compared to the bulk constituents. Contamination by an aluminum foil in the hot pressing technique of producing polytetrafluoroethylene films has been studied successfully by this method (1). The elements present in the materials responsible for the adhesion of bacteria and the elements of the molecules adsorbed onto rock grains may be analyzed in a similar manner.

It is projected at the present stage that the use of ESCA can yield important and useful results to determine if an actual chemical bonding exists between adsorbed bacteria and the rock surfaces. Such information is extremely important in determining the extent of penetration of bacteria through

a reservoir rock matrix.

Theoretical Considerations

Generally, when an atom is irradiated by a photon of energy ($E_{\text{ex}}=h\nu_1$), it is possible that one of the electrons in the atom will absorb the photon and be ejected with a kinetic energy E_{kin} . In order to escape from the atom, the electron must overcome the energy (E_b) by which it is restrained. This is the well-known photoelectric effect first explained by Einstein in 1905. The only difference is that, in this context, the atomic bonding energy (E_b) replaces Einstein's metallic work function. In the usual ESCA experiment, a beam of X-rays of known energy is used to irradiate a sample. The ejected photoelectrons have a variety of kinetic energies, depending on which level and which element they are originated from. The ESCA spectrometer makes an accurate measurement of the kinetic energies of the freed electrons. The binding energy (E_b) for each level is thus given by the difference between the excitation energy (E_{ex}) and the kinetic energy (E_{kin}) of a particular photoelectron as shown in Figure 1.

$$E_b = E_{\text{ex}} - E_{\text{kin}} = h\nu_1 - E_{\text{kin}}$$

where E_b is calculated, $h\nu_1$ is precisely known, and E_{kin} is measured with the instrument.

ESCA is a surface method of analysis in the sense that it yields information on the atomic and molecular structure to a depth of less than 100 angstroms. The sample is irradiated with a beam of X-rays of known energy and the energy of the emitted electrons is analyzed to give information about the elemental composition and chemical state of the atoms. In the Auger process, the electrons emitted have energies which are characteristic of each element. Also, a chemical bond which changes the electron density around the nucleus will cause a change in the energy of the emitted electrons. This change is known as the "chemical shift" and can be related to the chemical state of the atom. When bacteria-rock surface interactions are being studied, the chemical shift caused by nitrogen bonding to SiO_2 should be carefully considered.

Fig. 1. Excitation and kinetic energy levels for primary electrons.

Experimental Procedure

The theoretical studies suggest that Bacillus subtilis could be deposited on a SiO₂-covered Si wafer, and the resulting spectrum could be examined to investigate any presence of a chemical shift due to the chemical bonding of bacteria and the surface. To obtain the spectrum, Bacillus subtilis species were deposited onto a phosphorus-doped (0.04–0.1 Ω cm) Si(100) wafer which had been thermally oxidized to SiO₂ to a depth of 50 angstroms after initial preoxidation, stripping, and cleaning steps. Before deposition, the bacteria were rinsed with distilled water five consecutive times, followed by centrifugation (15 min. at high rpm) and decantation. A drop of the residuum was then placed onto the wafer each time for a total of three drops. The Si(100) wafer was put into a desiccator and left overnight to evaporate the excess moisture. 1500 angstroms of metal Al was then evaporated from a tungsten filament onto the bacteria-covered surface to provide mechanical strength. The evaporation step was performed in a cryopumped evaporation chamber of 10⁻⁶ Torr. Care was taken to avoid excessive exposure to atmosphere during the oxidation, inoculation and metallization step.

Our research work still in progress involves the etching and analysis steps for the above sample. First, the aluminum surface of the sample will be bonded with high-purity indium to a gold-plated sample platen, and the exposed bottom surface of the wafer will be stripped of oxide using HF:ETOH solution. The sample will then be transported under nitrogen to a gas-phase etching chamber, where XeF₂ is used to etch away the silicon to expose the SiO₂. The SiO₂-bacteria interface will then be analyzed. First, however, a blank sample without the deposited bacteria will be analyzed.

A schematic diagram of the etching procedure is shown in Figure 2. The initial step is a gas-phase etch with XeF₂. XeF₂ is a white solid with a vapor pressure of about 4.5 Torr at room temperature. It would aggressively attack elemental silicon, but is inert with respect to SiO₂ (2,3). A schematic diagram of the etching chamber is shown in Figure 3. A sorption pump reduces the background pressure to approximately 1m Torr. The XeF₂ will be allowed to sublime into the vacuum. Although the etch efficiency of the XeF₂ is on the order of 1% per molecular collision (4), the overall efficiency (atoms of Si removed per molecule of XeF₂) will be raised to as high as

Fig. 2. Schematic illustration of three-stage sample-etching procedure. (Modified after Hecht et al.(4))

Fig. 3. Diagram of XeF_2 etching system. The XeF_2 can be delivered either in a continuous flow, or else by filling the buffer volume and releasing a measured dose. Sample cooling capabilities also exist. (After Hecht et al.(4)).

40% by balancing the flow and partial pressure of the XeF_2 so that many collisions occur for each molecule injected into the system (5). In this way, the entire 280m-thick Si wafer will be etched away in a period of 6-10 hrs. without damage to the 50 angstroms SiO_2 film. No plasmas or external energy sources, which might induce damage to the SiO_2 , will be used in this step.

After the XeF_2 etch, the sample will be transferred in air to a liquid-phase etching station coupled to the X-ray photoelectron spectrometer (XPS). Results for unannealed samples have indicated that the interface is uniformly protected by the SiO_2 layer, and hence is unaffected by the atmospheric exposure (4). The etching station will be contained in a nitrogen flushed glove box to eliminate further atmospheric exposure. The sample at this state will consist of a 50 angstrom SiO_2 film protecting the bacteria and the 1500 angstrom aluminum layer, which will be supported by the gold platen. The integrity of the SiO_2 layer will be checked for complete absence of oxidation of the underlying bacteria and aluminum after air exposure. After initial spectra are recorded, the SiO_2 layer will be thinned with $\text{HF}:\text{ETOH}$, according to a procedure described by Grunthaner et al. (6,7), in order to increase spectroscopic sensitivity to interface. The spectrometer that will be used is a Hewlett-Packard 5950A XPS system which has been modified for high transmission and resolution.

Conclusion

To date, we have successfully deposited a monolayer of B. Subtilis onto a 50 angstrom silica-covered 300 μm -thick silicon wafer. Micrographs of different magnifications using a scanning electron microscope are shown in Figures 4, 5, and 6. The resulting three-dimensional images demonstrate that the cell membrane is in contact with the pure silica surface which mimics the outer layer of sandstone. In order not to damage or disintegrate the cell, the energetic beam for irradiation in conjunction with ESCA has to be from the backside of the cell. Therefore, the 300 μm thickness of silicon wafer must be etched off by gaseous xenon fluoride. In this manner, we can study the ESCA spectra of the bonding formed by cellular adhesion to the plate. Furthermore, the effects of surface roughness and of other adsorbed materials such as inorganic ions or oil can also be assessed by this method.

Fig.4. B. subtilis deposited onto an SiO₂ covered Si wafer. Magnification at 12,500x.

Fig.5. B. Subtilis deposited onto an SiO₂ covered Si wafer. Magnification at 25,000x.

Fig. 6. B. Subtilis deposited onto an SiO_2 covered Si wafer. Magnification at 50,000x.

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EFFECT OF LIQUID SURFACE TENSION ON BACTERIAL TRANSPORT THROUGH SANDPACK COLUMN

Introduction

The extent of bacterial transport in the reservoir is considered to be one of the most important factors which may affect the success of a MEOR process. However, the fundamental mechanisms governing bacterial transport are poorly understood and have not been well defined due to the complexities of the bacteria. As we noted in earlier reports, both the surface tension of bacteria and of the injected fluids affect the bacterial transport process in porous media. It is therefore essential to further study the interfacial tension characteristics among bacteria, sand, and liquid, theoretically as well as experimentally.

In recent studies of tissue (1) and bacterial adhesion (2) it was found that the extent of adhesion was related to the change in surface tension of the liquid media. A thermodynamic model was proposed which will be discussed in the following section. The concept of our study is based on this model and the purpose of our study is to determine the possible application of this model to MEOR processes.

Theoretical Considerations

The theoretical model developed by Neumann et al. (2) in order to explain the surface thermodynamics of bacterial adhesion, could be applied to predict the extent of bacterial transport through porous media. The prediction of bacterial adhesion is based on surface thermodynamics. This implies that a properly identified thermodynamic potential, i.e., the free energy, will be minimized at equilibrium. If bacterial adhesion causes the free energy to decrease, bacterial transport will be poor, thus yielding favorable adhesion. If bacterial adhesion causes the free energy to increase, allowing for easy penetration of bacteria, then it will prove unfavorable. For systems in which the effects of electric charges and biochemical interactions (e.g., receptor-ligand) may be neglected, the change in the free energy function (ΔF^{adh}), per unit surface area, is:

$$\Delta F^{\text{adh}} = \gamma_{\text{BS}} - \gamma_{\text{BL}} - \gamma_{\text{SL}} \quad (1)$$

where ΔF^{adh} is the free energy of adhesion, γ_{BS} is the bacterium-substratum interfacial tension, γ_{BL} is the bacterium-liquid interfacial tension, and γ_{SL} is the substratum-liquid interfacial tension. Through the use of Young's equation, it becomes possible to obtain experimental data for various interfacial tensions involving a solid surface. Young's equation is as follows:

$$\gamma_{\text{SV}} - \gamma_{\text{SL}} = \gamma_{\text{LV}} \cos\theta \quad (2)$$

where γ_{SV} , γ_{SL} , and γ_{LV} are, respectively, the interfacial tension between a solid substratum S and the vapor phase V, between S and the liquid L, and between L and V; θ represents the contact angle of the liquid on the solid. Only the liquid surface tension (γ_{LV}) and θ are readily determined experimentally. Using experimental contact angle data and liquid-vapor interfacial tensions, an equation of state has been formulated explicitly (3) as follows:

$$\gamma_{\text{SL}} = \frac{(\sqrt{\gamma_{\text{SV}}} - \sqrt{\gamma_{\text{LV}}})}{1 - 0.015 \sqrt{\gamma_{\text{SV}} \cdot \gamma_{\text{LV}}}} \quad (3)$$

In conjunction with Young's equation, this yields:

$$\cos\theta = \frac{(0.015\gamma_{\text{SV}} - 2.00) \cdot \sqrt{\gamma_{\text{SV}} \cdot \gamma_{\text{LV}}} + \gamma_{\text{LV}}}{\gamma_{\text{LV}} (0.015 \sqrt{\gamma_{\text{SV}} \cdot \gamma_{\text{LV}}} - 1)} \quad (4)$$

With the aid of equation (4) the unknown surface tension of the substratum, γ_{SV} , may be determined from the easily measurable quantities γ_{LV} and θ . If equation (3) is considered to be a generic equation used to calculate any interfacial tension γ_{12} from (given or predetermined) from interfacial tensions γ_{13} and γ_{23} , where the subscripts 1, 2 and 3 refer to different

phases, all required interfacial tensions in equation (1) may be computed; thus permitting explicit thermodynamic prediction of the relative extent of bacterial adhesion to various substrata. A theoretical calculation of free energy change (ΔF^{adh}) for the attachment of bacteria from suspension to various substrata as a function of γ_{SV} is illustrated in Fig. 1. We should note some important points in this figure:

- (1) for $\gamma_{\text{LV}} < \gamma_{\text{BV}}$, ΔF^{adh} decreases with increasing γ_{SV} ,
- (2) for $\gamma_{\text{LV}} > \gamma_{\text{BV}}$, ΔF^{adh} increases with increasing γ_{SV} and
- (3) for $\gamma_{\text{LV}} = \gamma_{\text{BV}}$, ΔF^{adh} becomes equal to zero independently of value of γ_{SV} .

As for a certain value of γ_{SV} , we may predict the extent of bacterial adhesion in a different liquid media by considering the differences in free energy. From this, we will be able to estimate the relative extent of bacterial transport through a sand packed column as a simplified model of a porous media.

Materials and Methods

Preparation of bacterial suspension: Staphylococcus epidermidis, provided by Dr. Goodman, was grown for 5 days at 36°C in nutrient broth (from Difco Laboratories). The bacteria was removed from the media and centrifuged and washed and then resuspended in the appropriate liquid. The suspension liquid media used in this experiment was 85% NaCl solution, with different concentrations of dimethyl sulfoxide (DMSO). The concentrations of DMSO ranged from 0% to 15% (Vol/Vol), resulting in surface tensions from 73 ergs/cm² to 63 ergs/cm² (Table 1).

Preparation of sandpack column: Standard Ottawa sand, SX0070-3, from MCB Manufacturing Chemicals, Inc. was used as the packing material for the column. The sand was thoroughly washed with distilled water to get rid of fines and then dried at 80°C for two days. Before each run the column was freshly packed with dried, cool, 20 to 40 mesh Ottawa sand previously prepared. The sandpack column was an acrylic cylinder 9.6 cm in length and 2.54 cm in diameter. The porosities of the sandpack column for each run are presented in Table 2.

The following apparatus: A schematic diagram of the flow apparatus is shown in Figure 2. The bacterial suspension was kept at a higher position in order to minimize the bacterial settling in the

Figure 1. The Free Energy of Adhesion (F^{adh}) as a Function of Substrate Surface Tension for Three Different Conditions. (Ref. 2)

TABLE 1
Surface tension of suspending medium

Experiment (number)	Conc. of DMSO (%; Vol/Vol)	Surface tensions ⁽⁴⁾ (ergs/cm ²)
1	0	73
2	5	70
3	10	67
4	15	63

TABLE 2
Experimental parameters used in each run of study

Experiment (number)	Porosities of sandpack (%)	Flow rates (ml/min)
1	36.8	1.30
2	38.6	1.31
3	37.9	1.25
4	38.3	1.24

Figure 2. A Schematic Diagram of Flow Apparatus

inlet flow lines. A peristaltic pump was employed to pump the bacterial suspension from a source flask that was continuously stirred with a magnetic stirrer. A constant flow was maintained throughout each run. The exact flow rates of each run are presented in Table 2. The pressure drop across the sandpack column was monitored and recorded throughout the experiment by a transducer and demodulator connected to a chart recorder. Before the bacterial suspension in each run was pumped, the appropriate liquid was passed through the whole system for one hour and the effluent was checked for any impurities. The initial permeability of the sandpack was determined at that time.

The effluent was collected in a test tube, and the number of bacteria was determined based on turbidity by using a UV/Visible spectrophotometer, Beckman Model 25. The absorbance was measured at a wavelength of 240 nm. A calibration curve (Figure 3) was then prepared for estimating the number of bacteria.

Four runs of experimentals were conducted. The differences among them were the surface tensions of liquid suspension media. Other conditions in each run were maintained the same throughout the experiment.

Results and Discussion

In order to use the predictive model mentioned above, it is necessary to know the surface tension of the suspension liquids (γ_{LV}), as well as the surface tensions of the bacteria (γ_{BV}) and the sand (γ_{SV}).

The surface tension of S. epidermis has been determined by Absolom et al. as 66.9 ergs/cm² in his recent study (2). The surface tension of the sand is estimated to be 78 ergs/cm², which is basically the surface tension of the silica (5). The liquid surface tension values are summarized in Table 1.

With the aid of a computer program (Table 3), the interfacial tensions between bacteria B and Liquid L, between B and sand S, and between S and L, could be readily obtained. A theoretical plot for our system was created through the input of these interfacial tensions and is shown in Figure 4. From

Figure 3. A Calibration Curve for estimating Number of Bacteria.

Figure 4. Free Energy of Adhesion as a Function of Substrate Surface Tension (γ_{sv}) for Various Liquid Surface Tensions (γ_{lv}).

Table 3 A Computer Program for Determining Interfacial Tension. (Ref. 6)

C
C
C

PROGRAM TENSION

```

REAL G1V,G2V,G12
WRITE(*,1)
1 FORMAT('1',3X,'G1V',7X,'G2V',7X,'G12')
2 READ(*,3) G1V,G2V
3 FORMAT(2F10.4)
  IF (G2V.LE.0.0) GO TO 5
  CALL EOS(G1V,G2V,G12)
  WRITE(*,4) G1V,G2V,G12
4 FORMAT(3F10.4)
  GO TO 2
5 CONTINUE
STOP
END

SUBROUTINE EOS(MSV,MLV,MSL)
REAL MSV,MLV,MEL
A=0.015
CHEK=0.0001
GAMSV=MSV
GAMLV=MLV
  IF (GAMSV.LE.GAMLV) GO TO 993
  GAMSV=MLV
  GAMLV=MSV
993 IF (GAMLV.GT.30.0) GO TO 995
994 GAMSL=((SQRT(GAMLV)-SQRT(GAMSV))*2)/((1-A*SQRT(GAMSV*GAMLV))
  GO TO 999
995 IF (GAMLV.GT.50.0) GO TO 997
  GAMSL=((SQRT(GAMLV)-SQRT(GAMSV))*2)/((1-A*SQRT(GAMSV*GAMLV))
  IF ((GAMSL-GAMSV).GT.0.0) GO TO 999
996 GAMSL=((((2-A*GAMSV)*SQRT(GAMLV)-SQRT((2-A*GAMSV)**2*GAMLV
  #-4*(GAMLV-GAMSV))**2)/4.0
  GO TO 999
997 GAMS1=10.0
  B=SQRT(GAMLV)
998 GAMS=GAMS1
  C=SQRT(GAMS)
  SLOPE=((1-A*B*C)*(1-B/C)-(GAMS-2*B*C+GAMLV)*((-A/2)*B/C))
  #/(1-A*B*C)**2
  SLOPE1=SLOPE+1.0
  U=1-A*B*C/2.0+(A*(GAMLV**1.5)/2.0-B)/C
  V=(1-A*B*C)**2
  DU=-((A*B)/(4*C)-(A*(GAMLV**1.5)/2.0-B)/(2*(GAMS**1.5))
  DV=-A*B/C+(A*B)**2
  SLOPE2=(V*DU-U*DV)/V**2
  GAMS1=GAMS-SLOPE1/SLOPE2
  IF (ABS(GAMS1-GAMS).GT.CHEK) GO TO 998
  GAMS2=((SQRT(GAMS1)-SQRT(GAMLV))*2)/((1-A*SQRT(GAMS1*GAMLV))
  IF (GAMSV.LE.GAMS1) GO TO 994
  IF (GAMSV.GE.GAMS2) GO TO 996
  GAMSL=GAMS1+GAMS2-GAMSV
999 MEL=GAMSL
RETURN
END

```

this figure it could be seen that, at a substrate surface tension (γ_{SV}) of 78 ergs/cm², the free energy of adhesion (ΔF^{adh}) increases as DMSO concentration in liquid decreases. This may imply that the bacterial attachment to sand decreases with decreasing liquid DMSO content. Thus, better bacterial transport can be expected at a lower DMSO concentration with the corresponding higher liquid surface tension.

The prediction was closely supported by the experimental results. The ratio of effluent bacterial concentration (C_e) to influent bacterial concentration (C_o) at various times is plotted in Figure 5. At higher γ_{LV} (lower DMSO concentration in liquid), the C_e/C_o ratio is higher due to the lesser adhesion of bacteria to the sand surface. The curves for two higher γ_{LV} (0% DMSO and 5% DMSO) follow a similar trend. The C_e/C_o ratio increases sharply up to two pore volume injection of fluids, and increases steadily thereafter. On the other hand, the C_e/C_o ratios for two relatively lower γ_{LV} (10% DMSO and 15% DMSO) reach a maximum at PV = 3.5, and then drop significantly.

A comparison on the permeability changes for the three separate runs is presented in Figure 6. The relative permeability was measured against various times (presented as pore volumes). For the case of injection of liquid without DMSO, up to injection of four pore volume, only the slight permeability reduction is observed. There is a big drop in permeability after that, reaching a steady value of 40% after fourteen pore volumes of fluid are injected. For the case of 5% DMSO liquid, the reduction of permeability is more significant at the beginning, but the total reduction is much less than in the previous case. This phenomenon may imply that, after running through four or five pore volumes, straining instead of surface adsorption becomes the governing mechanism. For the last case with 15% DMSO liquid, the permeability of the column is greatly reduced to a level of 30% after seven pore volumes of liquid have been injected. This observation is in good agreement with our modeling prediction.

In order to investigate the influence of substrate surface tension on the extent of bacterial transport, a plot of free energy of adhesion versus liquid surface tension is constructed based on the data from Figure 1 and is shown in Figure 7. It is very interesting to note that the trend of

Figure 5. A Comparison of Effluent Concentration Profiles of Injection Of s. epidermis using Different Fluids

Figure 6. A Comparison of Permeability Reduction for Different Injected Fluids.

Figure 7. Free Energy of Adhesion as a Function of Liquid Surface Tension (γ_{sv}) for two different γ_{sv} .

the change in free adhesion energy is dependent upon the surface tension of substrate for a specific bacteria. As illustrated in the figure, if polyethylene, which has a surface energy of 35 ergs/cm (5) is used as substrate, the opposite trend of free adhesion energy change is demonstrated when compared to that for the sand. Thus, the increase in liquid surface tension will decrease the free adhesion energy and consequently inhibits the transport of bacteria in this specific case.

From the discussion above we might conclude that the thermodynamic model for predicting the adhesion of bacteria onto substrate serves well in the prediction of bacterial transport through a sandpack column. However, we must bear in mind that there are some other factors which might influence the transport of bacteria. These factors include electrostatic interaction and the presence of cations in the liquid. In order to better understand this subject, we plan to perform more studies in the future. However, these experiments have shown that the general trend can be obtained if the required information is available. This prediction may be very helpful in bacterial transport studies through porous media where the surface thermodynamics of bacteria adhesion need to be accurately modeled by experimental as well as theoretical means.

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FURTHER INVESTIGATION OF SURFACE CHARGE CHARACTERIZATION OF RESERVOIR ROCK AS A COMPOSITE OXIDE SYSTEM

Introcuccion

Surface charge characterization of the pore walls within a reservoir rock requires a detailed analysis of its major components, mostly of mineral oxides, in their interfacial electrochemical reactions with the existing cations and anions in the aqueous media. Most of the studies in the past have been devoted to the study of simple oxides such as SiO_2 , Fe_2O_3 , and Al_2O_3 , that resulted in much valuable information regarding the formation of surface charge, double layer structure, etc. In those studies purity of the oxide materials used is of great concern, perhaps, for the consistency of experimental results and the easiness of interpretation by thermodynamic analysis as well as for having a well characterized system. However, mineral oxides in the natural systems such as an oil reservoir are rarely pure. Instead, there are usually some impact materials present and the structures of the oxides are quite complex. In recent publications several attempts have been made to correlate the point of zero charge (ZPC) of a mixed oxide system with those of its component oxides. As a first approximation, Parks (1) suggested that the ZPC of a binary system could be estimated by the appropriately weighted average of the ZPCs of the component oxides. The model is based on an assumption that there is no interaction between different types of sites. The total charge attributed by each site group depends on the relative number of each type of site, which is reflected in the fraction of the total surface area occupied by each site group. Therefore, the weighted average is likely to be based on the surface area of each component. In studying the ZPC-composition relationship of mechanical mixtures of silica and alumina, Tschapek et al. (2) found that the ZPC of the mixture is not additive and the alumina is the dominant component. They stated that such a behavior is comprehensible because the alumina is more sensitive to pH changes. Later on Pyman et al. (3) made some justification and proposed that the predicted relationship can only be

based on actual titration curves expressed on a charge per gram basis and not on any straight line approximations. They stated that Parks' predictions are only valid for the case where the two surface areas are equal because he did not convert the titration curves from a charge/cm² basis to a charge/g basis. More recently, Schwarz et al. (4) also studied the ZPC of silica-alumina oxide suspensions of varying compositions. They found that the ZPC of the mixed oxide varies linearly with the weight fractions of the component oxides. They concluded that the experimental results are in direct agreement with a "mass" weighting model suggested by Parks. It seems that some discrepancies exist. It is the purpose of this part of our work to give plausible explanations in order to clarify the differences among these publications.

Theoretical Background

Most mineral oxide surfaces show amphoteric properties. The surface ionization reactions of an amphoteric colloid with AH surface groups in a 1:1 electrolyte solution are given by (5):

and

where $[\text{H}_{\text{S,A}}]$ is the proton concentration at the surface of oxide A, K_{A1} and K_{A2} are the intrinsic surface acidity constants. The surface charge density of oxide A may then be expressed as:

$$\sigma_{\text{O,A}} = \frac{e N_{\text{S,A}} ([\text{AH}_2^+] - [\text{A}^-])}{([\text{AH}_2^+] + [\text{AH}] + [\text{A}^-])} \quad [3]$$

where $N_{s,A}$ is the total number of sites per unit area. Operationally, the proton concentration can only be measured in the bulk solution, it is then assumed that the effective surface proton concentration is given by the Boltzmann relationship:

$$[H^+]_{s,A} = [H^+] \exp\left(-\frac{F \psi_{o,A}}{RT}\right) \quad [4]$$

where $\psi_{o,A}$ is the surface potential of oxide A relative to the bulk solution, F is the Faraday constant, and R is the universal gas constant. The combination of Eqs. [1] to [4] yields:

$$\sigma_{o,A} = \frac{e N_{s,A} \left\{ \frac{[H^+]}{K_{A1}} \exp\left(-\frac{F \psi_{o,A}}{RT}\right) - \frac{K_{A2}}{[H^+]} \exp\left(\frac{F \psi_{o,A}}{RT}\right) \right\}}{\left\{ 1 + \frac{[H^+]}{K_{A1}} \exp\left(-\frac{F \psi_{o,A}}{RT}\right) + \frac{K_{A2}}{[H^+]} \exp\left(\frac{F \psi_{o,A}}{RT}\right) \right\}} \quad [5]$$

By the same token, a similar equation can be derived for oxide B:

$$\sigma_{o,B} = \frac{e N_{s,B} \left\{ \frac{[H^+]}{K_{B1}} \exp\left(-\frac{F \psi_{o,B}}{RT}\right) - \frac{K_{B2}}{[H^+]} \exp\left(\frac{F \psi_{o,B}}{RT}\right) \right\}}{\left\{ 1 + \frac{[H^+]}{K_{B1}} \exp\left(-\frac{F \psi_{o,B}}{RT}\right) + \frac{K_{B2}}{[H^+]} \exp\left(\frac{F \psi_{o,B}}{RT}\right) \right\}} \quad [6]$$

The unit for $\sigma_{o,A}$ and $\sigma_{o,B}$ is charges/unit area, e.g. C/cm². Assuming that the two types of oxides behave independently, the surface charge density of the whole system may then be expressed as:

$$\sigma_{o,total} = f_A \sigma_{o,A} + (1 - f_A) \sigma_{o,B} \quad [7]$$

where f_A is the fraction of the total surface area contributed by the oxide A. The surface charge densities of the system at various pH could be obtained from Eq. [7] provided the information regarding the surface potential-pH relationship of component oxides, if available, and numerical values of intrinsic surface acidity constants, fraction of surface area, and site densities are known. The ZPC of the system is the pH when the value of $\sigma_{o,total}$ is zero. Eq. [7] would also be valid if all the surface charge densities are expressed in charge/unit mass and f_A is

correspondingly the mass weight fraction of the oxide A. Although the above relationships were originally derived for mechanical structure of oxides A and B, similar equations also hold for a complex oxide with different types of sites.

It is important to note here that the physical meanings of ZPC for a simple oxide and a mixed oxide are not quite the same. By definition, the net uptake of proton and hydroxide ions for a system is zero at ZPC. In the case of one component system the surface potential is zero and hence the surface proton concentration is equal to the proton concentration of the bulk solution under the ZPC condition. However, these two conditions do not hold for a mixed oxide system. The surface potentials for the individual component are not zero and the proton concentrations at the oxide surface are different from that of the solution. This is part of the reason that a simple relationship does not exist between the intrinsic surface ionization constants and pH_{ZPC} . For a simple oxide the following relationship is true:

$$\text{pH}_{\text{ZPC}} = 1/2 (\text{p}K_1 + \text{p}K_2) \quad [8]$$

Experimental

In addition to discussing the available data in the literature, a set of experiments was conducted in our laboratory for the purpose of comparison. The silica and alumina samples were from Thiokol Company, the BET surface areas were determined to be 0.21 and 0.14 m^2/g and the particle sizes were -300 mesh and 100 x 200 mesh, respectively. All the samples used were pretreated with hot hydrochloric acid and then washed thoroughly with double distilled water and stored dry.

Potentiometric titrations were carried out to determine the surface charge densities of the samples. The method used was essentially the same as that used by Huang (6). Detailed experimental procedures are given elsewhere (7). Briefly described, two titration cells, each containing 5 g of the sample and 20 ml of a sodium chloride solution of desired concentration, were shaken in a wrist

action shaker (Burrell Corp., Model 75) under a nitrogen atmosphere for about 20 min. The solids were allowed to settle and the supernatant liquid in one of the cells was decanted into Teflon centrifuge tubes and centrifuged for 15 min at 12,000 rpm. The clear supernatant was then pipetted back into a titration cell without any solid sample, to be used as a blank run. The titrations were performed by adding small equal aliquots of 0.1 N HCl or NaOH solutions to the sample cell and the blank and recording the pH after allowing for an equilibration time of 10 min, during which time the mixture was continuously shaken. All pH measurements were made in clear supernatant after the particles had settled. The surface charge densities of silica, alumina and a mechanical mixture of 50% by wt. of silica at various pH with 0.1 M or 1.0 M NaCl as supporting electrolyte were determined.

Results and Discussion

If the surface charge densities vs. pH for each component oxide are available, the surface charge behavior of a mixed oxide system can be easily determined by a graphic method. The basic assumption for the graphic method is the irrelevancy between the component oxides, therefore, Eq. [7] applies. Parks used the graphic method to illustrate the ZPC for complex oxide, AB_x (Figure 5 in Ref. 1). In his simplified approach, it was assumed that the surface charge densities of the component oxides vary linearly with pH, $pH - pH_{ZPC}$, and the slopes for the two straight lines are equal. The second assumption, equal slope, could be the source of the discrepancy between Parks' approach and the experimental results by Tschapek et al.

A review of some of the available literature (8,9,10) reveals that the sensitivities of surface charge densities to the changes of pH vary among simple oxides. Effect of the sensitivity on the ZPC value for a mixed oxide system is illustrated in Fig. 1. In the figure, System 1 is made of a mixture of oxide A and B and System 2 is composed of A and B' where A, B, and B' possess equal total surface area in each system. Oxides A and B are assumed to have similar sensitivities for the

Figure 1: An illustrative diagram showing the effect of sensitivities charge densities to pH changes on the ZPC value of mixed oxides.

charge density to the pH change; the ZPC of System 1 is then found to be right at the mid-point of the ZPC's of the two component oxides. On the other hand, the oxide B' which is twice as sensitive to the changes of pH in the bulk solution, compared to the oxide A, causes the ZPC value of System 2 closer to that of oxide B'.

Recently, Sprycha (11) used the graphic method to project a surface density vs. pH curve for Zn_2SiO_4 using that data curves of ZnO and SiO_2 from other researchers. Since the model curve did not fit the experimental results well, he suggested that interactions between $-\text{SiOH}$ and $-\text{ZnOH}$ groups at the zinc orthosilicate surface caused the disagreement. However, one factor he failed to mention is that the data for simple oxides generated by different researchers may not be the same due to various reasons such as the origin or preparation procedures of the sample. For example, in the review by Park (12), the ZPC of alumina varies from batch to batch, sample to sample. Thus, uncertainties could arise with regard to the selection of the best pair of literature data sets of simple oxides. The other point that might be worth mentioning is that in his graphical determination the following relationship was used:

$$\sigma_{\text{Zn}_2\text{SiO}_4} = 2 \sigma_{\text{ZnO}} + \sigma_{\text{SiO}_2} \quad (\text{C/cm}^2) \quad [9]$$

However, according to Eq. [7] it is more appropriate to estimate the charge densities of zinc orthosilicate by:

$$\sigma_{\text{Zn}_2\text{SiO}_4} = 2/3 \sigma_{\text{ZnO}} + 1/3 \sigma_{\text{SiO}_2} \quad (\text{C/cm}^2) \quad [10]$$

In studying the ZPC of mechanical mixtures of silica with hydrous alumina or ferric hydroxide, Pyman et al. (3) found that the ZPC of the mixtures varies with composition in a manner expected from titration curves of the component oxides. It is also stated in their conclusions that "the predicted relationship can only be based on actual titration curves expressed on a charge/g basis and not on any straight line approximation." We agree with most of the above valuable findings except that the

titration curves used in predicting the behavior of the mixture do not necessarily need to be expressed on a charge/g basis. As mentioned in the theoretical background section, Eq. [7] holds for both cases when the surface charge densities are expressed either on a charge/g or a charge/cm² basis, depending on the corresponding weighting factor, f_A , chosen. As the charge densities of the oxides are a surface phenomenon, it seems more convenient to express them on a charge/unit area basis. Recent research has proven that the charge/unit area basis is more widely used than the charge/unit mass unit. Pyman et al. commented that Parks' prediction seems to be ambiguous, and is only valid for cases where the two surface areas are equal because he did not convert the titration curves from a charge per cm² basis to charge/g. Actually, the fallacy of Parks' model is in the assumption that different sites or oxides have similar H⁺ adsorption isotherms, either linear or non-linear (see Figures 5 and 6 in Ref. 1).

Pure oxides or mixed oxides of silica and alumina are widely used as support materials in the catalyst industry. Recently, Schwartz et al. (4) determined the ZPC of a series of seven silica-alumina supports by potentiometric titrations. They found that the ZPC of the homologous series varied linearly with the weight fraction of the pure components and claimed that their results are in direct agreement with a mass weighting model suggested by Parks. Doubtlessly their experimental results and the linear correlation between the ZPC and the weight fraction of the specific samples must be good. However, we would like to make two arguments. First, the weighted average used in Parks' approach is more likely to be based on the surface area of each component instead of on the mass. Second, as mentioned, surface charge density is a kind of surface behavior, thus correlation based on the surface area would be more reasonable. For example, if equal weight of non-porous silica and porous alumina with similar particle sizes are present, undoubtedly the apparent surface charge densities of the mixture would be dominantly controlled by the alumina due to its much higher surface area. The authors gave no information on the surface composition of the silica-alumina supports, hence, further discussions on the results are not feasible. It is supposed that a certain relationship exists between the surface composition and the weight composition of the supports to yield the linear correlation of the ZPC.

Figure 2. Surface charge densities of silica as a function of pH and ionic strength from potentiometric titrations using NaCl as supporting electrolyte.

Figure 3. Surface charge densities of alumina as a function of pH and ionic strength from potentiometric titrations using NaCl as supporting electrolyte.

Figure 4. Surface charge densities of a mechanical mixture of 50% (by wt.) silica + 50% alumina as a function of pH and ionic strength from potentiometric titrations using NaCl as supporting electrolyte.

Figure 5. Comparison of charge densities of silica, 50% (by wt.) silica + 50% alumina with 0.1 M NaCl as supporting electrolyte. The broken line shows the calculated charge densities of the mixture by using Eq. (7).

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INHIBITION OF CELL GROWTH BY INDOLE

Introduction

Earlier experiments have indicated that the growth of P. putida 12633 is inhibited by the presence of indole in the growth medium. This phenomenon will be examined in the following procedure which is part of a larger study aimed at elucidating the cell changes which take place when a cell population becomes acclimated to grow on inhibitory carbon substrates. Bacterial titers and UV spectrophotometry are done on samples from test tubes containing bacteria cells with varying amounts of indole in fluid thioglycollate medium.

Materials and Methods

Bacteria. Pseudomonas putida 12633 biotype A was obtained from the American Type Culture Collection (Rockville, MD). The cells were grown in 10 ml sodium thioglycollate medium at 30°C in loosely covered 18 x 150 mm test tubes.

Acclimation Media. The first acclimation medium was prepared by rehydrating sodium thioglycollate medium (STM) in a series of test tubes with indole to yield a concentration of indole ranging from 0 to 0.20% (w/v). Due to possible inaccuracies arising from nonhomogeneous solutions of indole, each sample tube was loaded with the indole individually and then 10 ml STM was pipetted in. Autoclave sterilization for 20 minutes at 121°C then followed. Subsequent acclimation media were made similarly with appropriate concentrations of sample and STM. A summary of the different acclimation media is listed in Table 1.

Acclimation Procedure. Prior to inoculation, samples of the acclimation media were benzene extracted and analyzed for indole. The acclimation media was then inoculated with 0.1 ml *P. putida* grown in STM overnight. The inoculated tubes were then analyzed for the indole concentration and bacterial titer on a regular basis. An aliquot of cells which were successfully grown in an acclimation series was then transferred to the next series. Generally, the following series contained a lower concentration of STM. Eventually the cells were transferred to an indole only medium. Samples of cells from each series were to be stored at 4°C in anticipation of future comparative analysis, however during the course of the experiments a cold room malfunction destroyed the samples.

UV Analysis. Sample volumes of 0.1 ml were added to 18 x 150 mm test tubes followed by 5 ml of benzene. Each tube was then briefly vortexed and then allowed to settle for 3 minutes. A spectrum from 310 to 275 nm was made and the digital display was recorded at 287 nm.

Results and Discussion

Because no information was available concerning how well the cells would grow in the presence of fairly high amounts of indole, the first acclimation series contained 0 to 0.20% (w/v) indole. After inoculating the first acclimation series with 10^6 cell/ml of *P. putida*, titers were done after incubating at 30°C for 1, 3, 6, 8, and 14 days. The data from these titers comprise Tables 2 to 6. The growth inhibitory effect of the indole is clearly seen from the Day 8 and Day 14 titers. On the Day 8 titer, Table 5, strong cell growth can be seen in the tubes with 0.04% indole or less. In the 0.06% indole tube, the viable cell density was 10^5 less. By the second week, the 0.04% indole tube still had 10^9 cells/ml and the 0.06 tube had less than $10^{2.6}$ cells/ml. Two other points of interest can be seen in Tables 3 and 4. In Table 3, the concentration of cells in the 0.04% tube was about 10^6 cells/ml which was the initial inoculum concentration. The next higher indole-containing tube had a viable cell concentration of 10^5 indicative of cell attrition. In

both Tables 3 and 4, an extended lag phase can be seen in the cell populations cultured with increasing amounts of indole. For the cases of indole concentrations 0.06% or higher, this lag led to a drop in population counts.

The conclusions from the bacterial titers are roughly corroborated by the UV absorbance values at 287 nm corresponding to the disappearance of indole. Table 7 summarizes those values. By the second week the indole concentrations were less than 30% of the original in sample tubes with 0.04% or less indole. In tubes with 0.06% or higher initial indole concentrations, greater than 60% of the indole remained. Table indicate that some reduction of indole occurred up to the 0.15% indole-containing tube. It is difficult to say whether actual bacterial metabolism of indole had occurred in the cases where more than 0.1% indole was involved. These results strongly demonstrate the bacteriocidal effect of indole on wildtype cells in concentrations higher than 0.04%.

After two weeks, 0.1 ml aliquots from the first acclimation series tube containing 0.04 indole were transferred to a new set of tubes with indole ranging from 0 to 0.04%. The growth medium in this new series was the same as the one before it. The objective here was to see if the lag period, the titer or the rate of indole degradation was any different. According to the data presented in Tables 8 to 12, none of those characteristics differed significantly from the previous series. After one month, the titer was approximately 10^8 to 10^{10} cells/ml, Table 13, and the indole peaks were 20 - 30% their original height, Table 15.

The third acclimation series was inoculated from the second series after a one-month incubation. Comparing the peak heights observed after one week from Tables 14 and 15, it appears that the percent original peak height is lower for the third acclimation series. This result seemed fairly promising especially considering that this series contained a lower concentration of STM.

Encouraged by the observations from the last series, the fourth acclimation set was begun from cells incubated in the third series for one week. The fourth series contained STM (1/4x) with 0 to 0.04% indole. Table 15 lists the indole peak heights vs. time for the fourth acclimation. The amount of degradation by Day 11 of the fourth acclimation series, Table 16, is very similar to the results presented in Table 14 for the 15th day of the second acclimation. By the 19th day of

incubation, 10–40% of the indole was still present. After 11 days, an aliquot from this series was transferred to an indole only tube series. This new series did not change after 11 days, thus the results from part B of this chapter were not duplicated. Foreseeing this possibility and assuming that the cells needed a longer adjustment period, the previous acclimation series had been allowed to incubate concurrently with the indole only series. After four months the fourth acclimation series was analyzed again. Surprisingly, less than 10% of the indole still remained, Table 16.

The final acclimation series containing STM (1/10x) and 0, 0.02, 0.04 and 0.06% indole was inoculated from the fourth series after four months. Interestingly, the tolerance of the cells increased to allow degradation of indole at the 0.06% level – Tables 17–19. Comparing these results to the first acclimation series, Table 7, we see that the breakdown of indole was similar for concentrations below 0.06%. After four months, all the tubes which had 0.06% indole or less had below 10% of the original indole remaining. The indole peaks in control tests with unacclimated cells did not change over a four-month period.

The conclusions that can be drawn from this section are:

- 1) Wildtype P. putida 12633 biotype A cannot grow in environments containing indole in excess of 0.04%,
- 2) Wildtype P. putida is not able to utilize indole as a sole source of carbon and energy,
- 3) Derivatives of Pseudomonas putida capable of co-metabolizing up to 0.06% indole in the presence of STM (1/10x) can arise after growing the cells in media containing successively lower amounts of STM.

The results from this part of the work would be very informative in molecular comparison studies between the wildtype and indole metabolizing population of cells.

Table 1. A summary of the acclimation media used.

Media #	STM ¹	Indole (%)
1	(1x)	0 - 0.20
2	(1x)	0 - 0.04
3	($\frac{1}{2}$ x)	0 - 0.03
4	($\frac{1}{4}$ x)	0 - 0.04
5	0	0 - 0.03
6	($\frac{1}{10}$ x)	0 - 0.06

¹Sodium thioglycollate medium.

Table 2. 1st acclimation series bacteria titer of tubes containing indole in sodium thioglycollate medium, 1-day incubation.

Indole(%)	Dilution	Counts	Titer	Log ₁₀ Titer
0	5 x 10 ⁻⁶	CD	CD	---
	5 x 10 ⁻⁷	135,185,211 ¹	1.4 x 10 ⁹	9.2
0.01	5 x 10 ⁻⁶	CD	CD	---
	5 x 10 ⁻⁷	96,125,131 ¹	9.4 x 10 ⁸	9.0
0.02	5 x 10 ⁻⁶	CD	CD	---
	5 x 10 ⁻⁷	111,141,160 ¹	1.1 x 10 ⁹	9.0
0.03	5 x 10 ⁻⁵	194,211,213	4.1 x 10 ⁶	6.6
	5 x 10 ⁻⁶	17,21,27	4.3 x 10 ⁶	6.6
	5 x 10 ⁻⁷	1,3,6	6.7 x 10 ⁶	6.8
0.15	5 x 10 ⁻⁵	0,1,2	2.0 x 10 ⁴	4.3
	5 x 10 ⁻⁶	1,1,1	2.0 x 10 ⁵	5.3
	5 x 10 ⁻⁷	1,1,5	4.7 x 10 ⁶	6.7

¹ CD, confluent discrete.
¹ one-quarter plate counted.

Table 3. 1st acclimation series bacteria titer of tubes containing indole in sodium thioglycollate medium, 3-day incubation.

Indole(%)	Dilution	Counts	Titer	Log ₁₀ Titer
0	10 ⁻⁶	<10 ³ - 10 ⁴ >	---	---
	10 ⁻⁷	69,72,95	7.9 x 10 ⁸	8.9
0.01	10 ⁻⁶	117,136,158 ¹	5.5 x 10 ⁸	8.7
	10 ⁻⁷	41,76,83	6.7 x 10 ⁸	8.8
0.02	10 ⁻⁶	144,164,>300 ¹	---	---
	10 ⁻⁷	71,73,106	8.3 x 10 ⁸	8.9
0.03	10 ⁻⁵	<10 ⁵ >	---	---
	10 ⁻⁶	171,254,288 ¹	9.5 x 10 ⁸	9.0
	10 ⁻⁷	130,131,167	1.4 x 10 ⁹	9.2
0.04	10 ⁻²	<10 ⁴ - 10 ⁵ >	---	---
	10 ⁻⁴	171,171,200	1.8 x 10 ⁶	6.3
	10 ⁻⁶	1,2,2	1.7 x 10 ⁶	6.2
0.06	10 ⁻²	<10 ³ - 10 ⁴ >	---	---
	10 ⁻⁴	12,19,28	2.0 x 10 ⁵	5.3
	10 ⁻⁶	0,0,1	3 x 10 ⁵	5.5
*	*	*	*	*

¹one-quarter plate counted.

*Titers are not available for tubes containing >0.06% indole.

Table 4. 1st acclimation series bacterial titer of tubes containing indole in sodium thioglycollate medium, 6-day incubation.

Indole(%)	Dilution	Counts	Titer	Log ₁₀ Titer
0	10 ⁻⁷	47,50,54	5.0 x 10 ⁸	8.7
0.01	10 ⁻⁷	2,5,6	4.3 x 10 ⁷	7.6
0.02	10 ⁻⁷	5,5,10	6.7 x 10 ⁷	7.8
0.03	10 ⁻⁷	38,38,41	3.9 x 10 ⁸	8.6
0.04	10 ⁻⁷	20,24,25	2.3 x 10 ⁸	8.4
0.06	10 ⁻⁴	1,1,4	<2 x 10 ⁴	<4.3

Table 5. 1st acclimation series bacterial titer of tubes containing indole in sodium thioglycollate medium, 8-day incubation.

Indole(%)	Dilution	Counts	Titer	Log ₁₀ Titer
0	10 ⁻⁶	78,93,112 ¹	3.8 x 10 ⁸	8.6
	10 ⁻⁷	25,44,44	3.8 x 10 ⁸	8.6
0.01	5 x 10 ⁻⁶	<10 ⁴ >	2 x 10 ⁹	9.3
0.02	5 x 10 ⁻⁶	<10 ³ - 10 ⁴ >	---	8 - 9
0.03	10 ⁻⁶	<10 ³ >	10 ⁹	9
	10 ⁻⁷	62,82,94	7.9 x 10 ⁸	8.9
0.04	10 ⁻⁶	<10 ³ >	10 ⁹	9
	10 ⁻⁷	27,45,67	4.6 x 10 ⁸	8.7
0.06	10 ⁻²	14,20,95	4.3 x 10 ³	3.6

¹one-quarter plate counted.

Table 6. 1st acclimation series bacterial titer of tubes containing indole in sodium thioglycollate medium, 14-day incubation.

Indole(%)	Dilution	Counts	Titer	Log ₁₀ Titer
0	10 ⁻⁶	20,21,31	2.4 x 10 ⁷	7.4
0.01	10 ⁻⁶	101,113,115	1.1 x 10 ⁸	8.1
0.02	10 ⁻⁶	31,41,65	4.6 x 10 ⁷	7.6
0.03	10 ⁻⁶	128,140,163	1.4 x 10 ⁸	8.2
0.04	10 ⁻⁶	165,167,197 ¹	7.1 x 10 ⁸	8.9
0.06	5 x 10 ⁻³	1,3	---	<2.6
0.08	10 ⁻²	1,2,2	---	<2
0.10	10 ⁻²	0,0,1	---	<2
0.15	10 ⁻²	0,2,4	---	<2
0.20	10 ⁻²	0,0,3	---	<2
AGAR ONLY, 13 ml -		3		

¹one-quarter plate counted.

**Table 7. 1st acclimation series benzene extraction,
indole peak heights vs. incubation time.**

Indole (%)	Digital Display @ 287nm		
	0day	8day	14day
0	21	26	19
0.01	56	46	31
0.02	137	81	42
0.03	158	76	46
0.04	222	99	38
0.06	328	291	204
0.08	459		255
0.10	543		398
0.15	778		556
0.20	829		851

Table 8. 2nd acclimation series bacterial titer in tubes containing indole in sodium thioglycollate medium, 1-day incubation.

Indole (%)	Dilution	Counts	Titer	Log ₁₀ Titer
0	10 ⁻⁷	16,31,35	2.7 x 10 ⁸	8.4
0.01	10 ⁻⁷	19,20,23	2.1 x 10 ⁸	8.3
0.02	10 ⁻⁷	4,11,13	9.3 x 10 ⁷	8.0
0.03	10 ⁻⁵	10,17,19	1.5 x 10 ⁶	6.2
0.04	10 ⁻⁵	1,10,17	9.3 x 10 ⁵	6.0

Table 9. 2nd acclimation series bacterial titer in tubes containing indole in sodium thioglycollate medium, 3-day incubation.

Indole (%)	Dilution	Counts	Titer	Log ₁₀ Titer
0	10 ⁻⁷	92,120,129	1.1 x 10 ⁹	9.1
0.01	10 ⁻⁷	85,97,122	1.0 x 10 ⁹	9.0
0.02	10 ⁻⁷	61,62,91	7.1 x 10 ⁸	8.9
0.03	10 ⁻⁷	65,79,99	8.1 x 10 ⁸	8.9
0.04	10 ⁻⁴	321,322,364	3.4 x 10 ⁶	6.5

Table 10. 2nd acclimation series bacterial titer in tubes containing indole in sodium thioglycollate medium, 6-day incubation.

Indole(%)	Dilution	Counts	Titer	Log₁₀Titer
0	10 ⁻⁷	36,67,74	5.9 x 10 ⁸	8.8
0.01	10 ⁻⁷	41,71,74	6.2 x 10 ⁸	8.8
0.02	10 ⁻⁷	101,114,192	1.4 x 10 ⁹	9.1
0.03	10 ⁻⁷	63,64,70	6.6 x 10 ⁸	8.8
0.04	10 ⁻⁷	48,61,64	5.8 x 10 ⁸	8.8

Table 11. 2nd acclimation series bacterial titer in tubes containing indole in sodium thioglycollate medium, 8-day incubation.

Indole(%)	Dilution	Counts	Titer	Log ₁₀ Titer
0	10 ⁻⁷	26,31,43	3.3 x 10 ⁸	8.5
0.01	10 ⁻⁷	45,60,72	5.9 x 10 ⁸	8.8
0.02	10 ⁻⁷	73,93,100	8.9 x 10 ⁸	9.0
0.03	10 ⁻⁷	45,58,78	6.0 x 10 ⁸	8.8
0.04	10 ⁻⁶	<10 ³ >	10 ⁹	9
"	10 ⁻⁷	98,102,108	1.0 x 10 ⁹	9.0

Table 12. 2nd acclimation series bacterial titer in tubes containing indole in sodium thioglycollate medium, 14-day incubation.

Indole(%)	Dilution	Counts	Titer	Log ₁₀ Titer
0	5×10^{-6}	$<10^4 - 10^5>$	---	---
0.01	10^{-6}	184,253,271	2.4×10^8	8.4
0.02	10^6	167,192,250	2.0×10^8	8.3
0.03	10^6	219,226,308	2.5×10^8	8.4
0.04	5×10^{-7}	274,314,322	6.1×10^8	8.8

Table 13. 2nd acclimation series bacterial titer in tubes containing indole in sodium thioglycollate medium, 30-day incubation.

Indole(%)	Dilution	Counts	Titer	Log ₁₀ Titer
0	10 ⁻²	C	---	---
	10 ⁻⁴	CD	10 ⁶	10
0.01	10 ⁻²	C	---	---
	10 ⁻⁴	10 ⁴ - 10 ⁵	10 ⁹	9
0.02	10 ⁻²	C	---	---
	10 ⁻⁴	10 ⁶	10 ¹⁰	10
0.03	10 ⁻²	C	---	---
	10 ⁻⁴	10 ⁶	10 ¹⁰	10
0.04	10 ⁻²	C	---	---
	10 ⁻⁴	10 ⁶	10 ¹⁰	10

C, confluent colonies on plates.

CD, confluent but discrete colonies on plates.

**Table 14. 2nd acclimation series benzene extraction,
indole peak height vs. incubation time.**

Digital Display @ 287nm				
Indole (%)	0day	8day	15day	30day
0	23	26	18	16
0.01	67	56	37	32
0.02	117	74	47	30
0.03	188	83	57	35
0.04	251	157	105	55

**Table 15. 3rd acclimation series benzene extraction,
indole peak heights vs. incubation time.**

Indole(%)	Digital Display @ 287nm	
	0day	7day
0	16	17
0.01	79	45
0.02	112	43
0.03	144	51

**Table 16 4th acclimation series benzene extraction,
indole peak heights vs. incubation time.**

Indole (%)	Digital Display @ 287nm						
	0day	2day	4day	5day	11day	19day	3.5mo
0	21	18	15	15	20	21	24
0.01	70	57	50	42	35	28	27
0.02	97	84	80	68	51	50	31
0.03	174	144	136	106	61	68	35
0.04	255	213	180	162	110	85	38

Table 17 **5th acclimation series benzene extraction,**
indole peak height vs. incubation time.

Indole (%)	Digital Display @ 287nm			
	0day	5day	7day	11day
0	20	20	20	20
0.01	64	74	67	61
0.02	98	98	101	108
0.03	144	142	135	144

**Table 18 6th acclimation series benzene extraction,
indole peak heights vs. incubation time.**

Indole (%)	Digital Display @ 287nm					
	0day	7day	14day	28day	2mo	4mo
0	25	26	31	24	20	23
0.02	111	63	64	56	45	26
0.04	195	105	98	87	65	30
0.06	331	130	132	127	116	43

FURTHER ANALYSIS OF ELECTROCHEMICAL BEHAVIORS OF MIXED MINERAL OXIDE SYSTEMS

Introduction

Numerous experimental and model studies on the interfaces of mineral oxides have been conducted in the past three decades. Most of the effort was devoted to the study of simple oxides such as SiO_2 , Fe_2O_3 , and Al_2O_3 , and produced much valuable information regarding the formation of surface charge, double layer structure, etc. In those studies purity of the oxide materials used is of great concern because of its effect on the consistency of experimental results and the ease of interpretation by thermodynamic analysis.

However, mineral oxides in a porous reservoir rock are rarely pure. Instead, there are usually some impure materials present and the structures of the oxides are quite complex. Adequate methods of predicting electrochemical properties of complex systems based on the well developed knowledge of simpler systems are useful in the study of the mixed oxide systems. In this phase of work, electrokinetic potentials of two mixed oxide systems, one consisting of silica and alumina and the other of Ottawa sand and activated alumina, were determined by streaming potential measurements. Discussions of the electrokinetic properties such as isoelectric points and zeta potentials between a mixed oxide system and its component oxides took place as well.

Background Review

The solids encountered in aqueous systems, whether natural or manmade colloids, sand grains, silt, or rock matrix, are electrically charged. The charge is instrumental in determining much of their behavior, e.g., the state of dispersion and the rheology of the suspensions, the extent to which the solids function as adsorbents or ion exchangers, and the species adsorbed. Through adsorption, the charged solids can modify the composition of the aqueous phase, perhaps controlling

the pH and concentrations of the trace elements [1,2].

Hydrous metal oxides are those solids that have as their sole constituents one or more metallic cations combined with the elements of water, hydrogen and oxygen. This includes the metal hydroxides, oxyhydroxides and also the metal oxides because in aqueous solution their surface chemistry invariably reveals that they are at least partially hydrated [3]. Hydrous metal oxides and many natural minerals, especially the clay minerals, exhibit ion exchange properties. The ion exchange capacity of the simpler oxides arises from the existence of a pH-dependent surface charge. The oxides are cation exchangers in a basic environment when the surface charge is negative. In an acidic environment the surface charge is positive, and they are anion exchangers. When the surface is uncharged, they have a small capacity for both cation and anion exchange. While surface charge is by no means the only factor responsible for electrolyte adsorption, particularly organic electrolytes, the extent of adsorption of the less specifically adsorbed species, such as the simple aquo ions and, for example, primary amines and alkyl sulfonates, decreases rapidly when the sign of the oxide's surface changed to that of the sorbing species [1].

Reactions of oxides in aqueous systems with cations and anions are of importance (1) in regulating the concentration of certain components in natural waters; (2) in geochemical processes (congruent and incongruent dissolution processes, weathering, nucleation and precipitation reactions); and (3) in characterizing the colloid chemistry, especially the colloid stability of dispersed oxide. Naturally occurring oxides, like those of Si, Al, and Fe(III), have large specific surface areas and have been shown to adsorb significant quantities of cations and anions. Thus they are believed to play a role in the transport of heavy metals and of anions like phosphate into the sediments.

Interfacial electrochemical behavior of mineral oxides also plays an important role in many industrial processes. The deposition of particulates to solid surfaces depends greatly on the surface charges. In nuclear power plants the deposition of particulate uranium oxide with its associated radioactivity, which is released from a defective bundle, to the system surfaces is of great concern [5]. Silica and alumina oxide are widely used as support materials for metallic

particles in the catalyst industry [6]. Performance of filter systems for either aqueous solutions or organic liquids, e.g. from coal or liquefaction processes, is closely related to the system's surface characteristics [7]. Surface property of rock matrix is an important parameter in determining the extent of bacterial transport through oil reservoirs and deciding if a microbial enhanced oil recovery process is successful [8].

Studies on the chemistry of hydrous metal oxides have appeared in a large number of scientific journals covering a wide range of disciplines. This interest usually arises from purely practical considerations, such as the need to scavenge trace amounts of an element from solution or for the improvement of the flotation of a mineral. It is for this reason that many of the studies have involved complex, and sometimes poorly defined, chemical systems. Interest has frequently been focused on defining the behavior of the particular ion-oxide systems, rather than understanding the mechanisms underlying the behavior. Studies utilizing natural mineral materials as experimental media are subject to many problems because the solid phase is a heterogeneous mixture of mineral and organic adsorbent surfaces and the aqueous phase contains a similarly complex mixture of soluble organic and inorganic species. Adsorption is readily estimated in such mixtures. However, because of difficulties in separating the reactions, it is difficult to interpret mechanisms from the data.

In the last three decades there has been considerable interest in the acid-base properties of the hydrous metal oxides, which has simulated interest in models of electrical double layer at the oxide-aqueous electrolyte interface. Success in interpreting mechanisms of adsorption has been achieved using suspensions of simple crystalline oxides, such as goethite and gibbsite, in aqueous electrolyte solutions containing adsorbate as experimental systems. In these systems, adsorbent characteristics, surface area and charge can be estimated, and the composition of the aqueous phase can easily be controlled. Ionic interactions in the interfacial region between aqueous solution and oxide lattices can be interpreted from the detailed mathematical treatments available from electrochemical theory. There is now a wealth of information on the adsorption of ions by particular hydrous oxides under a wide range of conditions.

Zero point of charge (ZPC) is a convenient reference for predicting the charge-dependent behavior

of oxide minerals and their surrounding solutions. The ZPC is the pH at which the solid surface charge from all sources is zero. pH_{ZPC} is different for different metal oxides: it is a measure of the acidity and basicity of the hydrated surface oxide group. Because H^+ and OH^- are presumably the p.d.i.s. for hydrous oxides, it is convenient to determine the pH_{ZPC} by potentiometric titration method [9]. Essentially, the method consists of measuring the proton or hydroxide ion consumption by the solid phase, by comparing the titration curve of a suspension of the inorganic oxide with that of the medium alone. Ideally, pH_{ZPC} can be obtained as pH at zero net adsorption of protons and hydroxide ions. Many studies have been reported on the titration of simple hydrous solids, such as Fe_2O_3 , FeOOH , SiO_2 , Al_2O_3 , TiO_2 , ZrO_2 , CaCO_3 . . . etc.

Recently, several attempts have been made to predict the ZPC of a complex system. Parks [1] suggested that, as a first approximation, the different types of sites for a simple oxide or a complex oxide are considered independent. The ZPC of a binary system could be approximated by the appropriately weighted average of the component oxides. The weighted average is based on the fraction of the total surface area occupied by each type of site or each type of component oxide. In the study of the ZPC-composition relationship of the mechanical mixture of SiO_2 and Al_2O_3 , Tshapek et al. [10] found that the ZPC of the mixture is not additive and Al_2O_3 is the dominant component. They stated that such behavior is comprehensible because the Al_2O_3 is more sensitive to pH changes. Schwarz et al. [6] also studied the ZPC of silica-alumina oxide suspensions of varying composition. They found that the ZPC of the mixed oxide varied linearly with the weight fraction of the pure component. They concluded that the experimental results are in direct agreement with a "mass" weighting model suggested by Parks. However, the model that Parks proposed is more likely based on the fraction of surface area instead of mass fraction. The three approaches yield different conclusions for estimating the ZPC of mixed oxides from the relevant data of the component oxides. In an attempt to explain the discrepancies, the authors recently performed a study of potentiometric titration on mixed oxide suspensions [11]. It was suggested that (1) the surface area-weighted average between the ZPCs of the component oxides is more suitable than the mass weighted average to predict the ZPC of the mixtures, and (2) it is advisable to take the individual

sensitivities of the surface charge to the solution pH into consideration if they differ sizably.

In addition to the potentiometric titration method, electrokinetic measurement plays an important role in the characterization of surface properties of solids. The electrokinetic phenomena arise from the interaction between a flow of electricity and a flow of liquid in the double layer.

Electrokinetic phenomena is the appearance of electrical potential differences during motions relative to each other of substances existing in different phases, and the motion of the phases relative to each other under influence of an electrical field. Zeta-potential, the potential at the shear plane when solid and solution move relatively to each other, can be determined by electrokinetic measurements. Detailed mathematical treatment has been given by Overbeek in 1952 [12].

The two most common electrokinetic techniques that have been used to investigate the charge characterization of the mineral-aqueous solution interface are the streaming potential method for fairly coarse particles and electrophoresis for particles in the micron size range [13]. In this study the streaming potential method was used to study the electrokinetic behaviors of mixed oxides because of the following advantages over the electrophoresis method:

1. It is easier to make precise streaming potential measurements than to determine reliable electrophoretic mobilities [14].
2. If there are intact core samples available, it is not necessary to crush the samples to perform the streaming potential measurement.
3. It is not feasible to have small particles of several controlled surface compositions made up of pure simple oxides for the electrophoresis experiment. Instead, the packed column can be packed with a mixture of component simple oxides of any composition for the streaming potential measurement.

The development of a streaming potential when an electrolyte is forced through a porous plug is, in fact, a complex process with charge and mass transfer occurring simultaneously by a number of mechanisms. The liquid in the plug carries a net charge (that of the mobile part of the electric double layer) and its flow gives rise to a streaming current and, consequently, a potential

difference. This potential opposes the mechanical transfer of charge by causing back conduction by ion diffusion and, to a much lesser extent, by electroosmosis. The transfer of charge due to these two effects is called leak current, and the measured streaming potential relates to an equilibrium condition when streaming current and leak current cancel each other.

Briefly, when the streaming potential technique is used, the zeta potential is evaluated by means of Helmholtz-Smoluchowski equation

$$\text{Zeta potential} = \frac{4\pi\eta\lambda E}{\epsilon P} \quad (1)$$

where η = viscosity of the streaming liquid.

λ = specific conductivity of the liquid within the pores of the plug

ϵ = dielectric constant of the solution

E = difference of potential (streaming potential) between ends of the plug

P = pressure drop between ends of the plug

Materials and Methods

An apparatus for the streaming potential measurement was previously set up in our laboratory. The schematic diagram of the apparatus and detailed experimental procedures were described earlier [4].

Two sets of oxides were used in this study. The first set was silica and alumina, and the other set was Ottawa sand and activated alumina. Silica and alumina were from Thiokol Company, the BET surface areas were 0.21 and 0.14 m²/gm, and the particle sizes were -300 mesh and 100 x 200 mesh,

respectively. Ottawa sand was from MCB Company, the particles were sifted to 40 x 50 mesh size range. Activated alumina (chromatographic grade) was from Matheson Coleman & Bell Company, and the particles were sifted to 80 x 100 mesh size range. All samples used, except activated alumina, were pretreated by hydrochloric acid, washed thoroughly with double distilled water to remove all traces of acid, and stored dry.

The samples were packed into the streaming cell and then subjected to the streaming potential measurements. Sodium chloride was used as the supporting electrolyte, and the pH of the streaming solution was adjusted by using 0.1 M HCl and 0.1 M NaOH solutions. For each sample the streaming potentials were measured under various ionic strengths and pH conditions. Experiments were also carried out for the mixed oxide systems by mechanically packing 50% by wt. of activated alumina and 50% Ottawa sand into the streaming cell for one case, as well as 50% by wt. of silica and 50% alumina for the other.

Results and Discussion

Zeta potential derived from the streaming potential measurements for silica, 50% silica + 50% alumina, and alumina are shown in Figs. 1, 2, and 3, respectively. The isoelectric points (i.e.p.) are approximately at pH = 2.4 for the silica, pH = 2.5 for the mixture, and pH = 8.3 for the alumina. For comparison, Fig. 4 shows the zeta potential of the three samples at various pH conditions with 0.001 M NaCl used as the supporting electrolyte. From the figure, it appears that the 50% by wt. of silica behaves very much like the pure silica oxide. It is interesting to note that in the potentiometric titrations for the same weight ratio of the silica and alumina mixture suspensions, its apparent surface charges are close to those calculated values based on the surface area-weighted average of the two component oxides, neither the silica nor the alumina is the dominant species [11]. The specific area of the silica is $0.21 \text{ m}^2/\text{gm}$, only 50% higher than that of the alumina. A plausible explanation for silica being the dominant species for the electrokinetic properties of the mixture in the streaming cell is that the particle sizes of the

Figure 1. Zeta potentials of silica vs. pH

Figure 2. Zeta potentials of 50% silica + 50% alumina vs. pH

Figure 3. Zeta potentials of alumina vs. pH

Figure 4. Comparison of zeta potentials of silica, 50% silica + 50% alumina, and alumina

silica are much smaller than those of the alumina. The magnitude of the streaming potentials arising from the fluid flow through the packed materials depends on the surface composition of those pores invaded by the flowing fluid. The alumina is much larger in size than the silica, thus suggesting that the alumina is somewhat porous from the relative comparable specific surface areas of the two oxides. Some pores, which may contribute a lot for the measured BET surface area of the alumina, may not be accessible to the fluid flow. Being less porous and smaller in size, the silica may occupy the major portion of the surface exposed to the flowing fluid. By the way, the oxide samples chosen in this part of the work are originally designed for a comparison study of experimental results on the interfacial electrochemical properties of mixed oxide systems between using potentiometric titration measurements and streaming potential measurements. The criteria for choosing the samples are to have a large enough specific surface area for the titration and not to be porous for the electrokinetic measurements. To meet both requirements, the particle size would be quite small, causing a large pressure drop across the streaming cell and creating certain difficulty for the streaming potential experiments. From the experience gained in this study, we believe that it is instructive to use narrower size distribution of particles to pack the streaming cell in order to prevent possible fines migration, which might result in clogging of the cell. Also, to determine quantitative correlation of the electrokinetic properties between the mixed oxide system and its component oxides, it is advisable to use those non-porous component oxides with similar size range. Then, the projected surface composition of the mixed oxide systems from the individual specific area of the component oxides would be the same as, or at least close to, the actual average surface composition of the pores exposed to the fluid flow in the streaming potential measurements.

As far as we know, no information has been reported on the electrokinetic properties of activated alumina. Since the surface properties of activated alumina are closely related to its performance, it might prove worthwhile to have more information about it. Zeta potentials derived from the streaming potential measurements for the Ottawa sand, 50% Ottawa sand + 50% activated alumina mixture, and the activated alumina are shown in Figs. 5, 6, and 7. The i.e.p.s are approximately at $\text{pH} = 2$ for the Ottawa sand, $\text{pH} = 7.5$ for the mixture, and $\text{pH} = 7.8$ for the activated alumina. Again,

Figure 5. Zeta potentials of Ottawa sand vs. pH

Figure 6. Zeta potentials of 50% Ottawa sand + 50% activated alumina

Figure 7. Zeta potentials of activated alumina vs. pH

for comparison the zeta potentials for the three samples at various pH conditions using 0.001 M NaCl as the supporting electrolyte are shown in Fig. 8. In this case, the activated alumina is the dominant species for the electrokinetic potentials of the mixture, probably because of its much larger surface area than that of the Ottawa sand.

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Figure 8. Comparison of zeta potentials of Ottawa sand, 50% Ottawa sand + 50% activated alumina, and activated alumina

MODELLING THE DIFFUSION OF BACTERIA THROUGH NUTRIENT-ENRICHED POROUS MEDIA

In our previous work on bacterial transport through nutrient-enriched sandstone core using a double flask apparatus, different bacterial species were found to penetrate the core at different speeds. In order to achieve a better description of bacteria transport through porous media, a mathematical model is formulated here to simulate the simultaneous diffusion and growth of cells in a nutrient-enriched porous medium. The objective of this analysis is to predict the mobility of bacterial cells in the first stage of the MEOR process designed by our research group to the bio-"huff-and-puff" process.

The flooding differential equations are used to describe the conservation of mass as a bacterial inoculum grows and penetrates deeper into the nutrient-enriched porous medium with inlet and outlet valves closed:

Material Balance

(A) Bacteria in the Aqueous Phase

$$D_{B,EFF} \frac{\partial^2 C_{BL}}{\partial x^2} + \gamma_{BL} - \gamma_{AD,B} + \gamma_{DS,B} = \frac{\partial C_{BL}}{\partial T} \quad [1]$$

(B) Bacteria Attaching on Solid Surfaces

$$\gamma_{AD,B} - \gamma_{DS,B} + \frac{\rho_p}{\phi} \gamma_{BS} = \frac{\rho_p}{\phi} \frac{\partial C_{BS}}{\partial T} \quad [2]$$

(C) Surfactant

$$D_{F,EFF} \frac{\partial^2 C_F}{\partial x^2} - \gamma_{AD,F} + \frac{\gamma_{F/N}}{\gamma_{B/N}} \gamma_{B,TOTAL} = \frac{\partial C_F}{\partial T} \quad [3]$$

(D) Nutrient

$$D_{N,EFF} \frac{\partial^2 C_N}{\partial x^2} - \frac{\gamma_B}{Y_{B/N}} = \frac{\partial C_N}{\partial t} \quad [4]$$

where:

$$Y_{F/N} = \frac{\text{g Surfactant Produced}}{\text{g Nutrient Consumed}} \quad [5]$$

$$Y_{G/N} = \frac{\text{g CO}_2 \text{ Produced}}{\text{g Nutrient Consumed}} \quad [6]$$

$$Y_{B/N} = \frac{\text{No. of Cells Produced}}{\text{g Nutrient Consumed}} \quad [7]$$

$$Y_{M/N} = \frac{\text{g Misc. Metabolic Products}}{\text{g Nutrient Consumed}} \quad [8]$$

γ_{BS} = rate of bacterial growth on solid surfaces [No. cells/g sand grains]

ρ_P = bulk density of porous media (not containing water) [g/cm³ porous media].
= porosity [cm³ pore vol/cm³ porous media].

C_{BS} = density of cells on solid surfaces [No. cells/g sand grains].

$D_{N,EFF}$ = effective diffusivity of nutrient molecules.

γ_N = rate of nutrient consumption [$\gamma_{B,total} Y_{B/N}$].

$D_{F,EFF}$ = effective diffusivity of surfactant [cm²/s].

$\gamma_{AD,F}$ = rate of surfactant adsorption.

$\gamma_{B,TOTAL}$ = total rate of bacterial growth [No. cells/s cm³ pore]. [$\gamma_{BL} + \frac{\rho_P}{\phi} \gamma_{BS}$]

C_F = Conc. of surfactants in aqueous phase [g/cm³]

In the above equations, the growth of bacteria in the aqueous phase and on solid surfaces, the diffusion of cells through tortuous passages, the attachment of cells onto and detachment from solid surfaces, the consumption of nutrient, and the generation of metabolites and biogas are taken into account. As biogas is generated, the system pressure is increased. If the yield factors $Y_{B/N}$ and $Y_{M/N}$ are known, the rate of gas production at any instant of time can be calculated for any location in the core:

$$Y_G = Y_{B,TOTAL} \frac{Y_{G/N}}{Y_{B/N}} \quad [9]$$

Integrating throughout the core over entire period of incubation yields the expression for the total amount of gas generated can be expressed as:

$$G(T) = \left\{ \int_0^T \int_0^L Y_{B,TOTAL} \cdot (\pi R^2) \cdot dx \cdot dT \right\} \cdot \phi \cdot \frac{Y_{G/N}}{Y_{B/N}} \quad [10]$$

The biogas generated is partially soluble in water. Also, the pressure derived from biogas compresses the aqueous phase, making the biogas occupy a certain fraction (S_g) of the pore space:

$$S_L = \exp(-\beta(P - P_0)) \quad [11]$$

$$S_G = 1 - S_L = 1 - \exp(-\beta(P - P_0)) \quad [12]$$

where β is the compressibility of the aqueous phase, S_L the saturation of the aqueous phase, S_g the saturation of gas phase in the porous space, and P and P_0 are final and initial pressure, respectively.

Assuming equilibrium exists between the gas-phase biogas and dissolved biogas, an expression for pressure is obtained as follows using Henry's correlation:

$$P = \left\{ \frac{G(T) - P \cdot H \cdot S_L (\pi R^2 L) \cdot \phi}{(\pi R^2 L) (\phi) S_G} \right\} \cdot \frac{RT}{M.W.} \quad [13]$$

where H is Henry's constant. The numerator of Eq. 13 is the total amount of biogas dissolved in the aqueous phase and the denominator the total volume of the porous media occupied by the gas-phase biogas.

While all the important factors having been taken into account in the above analysis, much work remains to be done. The above mentioned equations contain terms of growth kinetics and yield factors. Although kinetic data of many bacterial strains are known, they were nevertheless obtained in experiments under normal conditions and with ample supplies of all essential nutrients. Such may not be the case in MEOR applications. Also, the yield factors can be greatly influenced by the nutrients available. For instance, ammonia-limited cultures produced large amounts of extracellular polysaccharides and resulted in over production of protease in B. licheniformis. The amount of lactate and ethanol produced by Clostridium thermohydrosulfuricum depended upon whether the culture was carbon or ammonia-limited (1). These studies promise to have great implications for the control of in situ MEOR processes.

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