

Environment Canada

Water Science and
Technology Directorate

Direction générale des sciences
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Environnement Canada

Surface Thermodynamics and Hydrophobic
Properties of Microbial Flocs

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NWRI Contribution # 04-173

TD
226
N87
no.
04-173

National Water Research Institute - Publication Form

Database Locator No. 04-039
date assigned 3 June 2004

NWRI Contribution No. or Branch Approved No. (e.g., abstract, technical note) 04-173

A General Information

Date Submitted: 30 April, 2004

Branch: AEMRB

Project: UWMP

Senior NWRI Author: LEPPARD, G. G.

Additional NWRI Authors: Droppo, I. G.

Authors: Liao, B.Q, G.G. Leppard, D.G. Allen, I.G. Droppo & S.N. Liss

Title: Surface thermodynamics and hydrophobic properties of microbial flocs

Please specify the **program** or **EC Region** this work is in support of:

or General



Business Lines: Clean Environment ☒; Nature ☒; Weather and Environmental Predictions ☐.

B Scientific Manuscripts - To be Submitted to:

☐ **Journal**

Name

☒ **Book or Chapter**

Title Flocculation in Natural and Engineered Environmental Systems

Editor(s) Droppo, I.G., G.G. Leppard, T.G. Milligan & S.N. Liss

Publishers Lewis Publishers/CRC Press City, Country Boca Raton, FL, USA,

☐ **Full Paper - Conference/Workshop Proceedings** - Please indicate above if Proceedings will be in Journal or Book

Conference/Workshop Name:

Location:

Date

☐ **NWRI Printed Contribution**

of copies requested

C Other Types of Material

☐ **Branch Technical Note** (e.g., QA/QC report to clients, other technical reports for VNR clients)

☐ **Conference/Workshop Abstract** → Oral ☐ Poster ☐ Presenter's Name:

Conference/Workshop Name:

Location:

Date:

☐ **Other** (letter to editor, newspaper article, web page, etc.)

Provide full reference/details:

Additional Information:

D Review/Approval

Peer Reviewed by External multiple peer review system of the Editors

Date 27 April, 2004

Approved by Project Chief

J. Mayabeh

Date June 14/04

Approved by Branch Director

J. Lawrence

Date 16 June '04

Is a Briefing Note required (Branch Director to decide): ☐ Yes ☐ No

Does anyone else within the department need to be alerted? ☐ Yes ☐ No Names:

Does the report contain any confidential information? ☐ Yes ☐ No or sensitive information? ☐ Yes ☐ No

Action taken by Branch Director:

Comments and required changes

NOTE: After Branch Director's approval, Author to ensure Branch Coordinator has a final electronic copy of abstract, research summary and manuscript.

SURFACE THERMODYNAMICS AND HYDROPHOBIC PROPERTIES OF MICROBIAL FLOCS

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ABSTRACT

The level of effluent suspended solids in biologically treated wastewaters is a strong indicator of bioflocculation and is related to the physicochemical properties of microbial floc formed in the aeration tank of wastewater treatment systems. Increasing experimental evidence indicates hydrophobic interactions play an important role in bioflocculation. The interfacial free energy of bioflocculation ($\Delta G_{\text{flocculation}}$) for microbial flocs at different solids retention times (SRTs) in well-controlled laboratory-scale sequencing batch reactors was estimated by contact angle measurement and using Neumann's equation-of-state. Sludges from higher SRTs (>12 days) were more hydrophobic with larger contact angles of water and found to have a lower $\Delta G_{\text{flocculation}}$. The $\Delta G_{\text{flocculation}}$ values were found to strongly correlate to the level of effluent suspended solids (ESS) in the final treated effluent. The results demonstrate that bioflocculation can be interpreted in terms of system free energy and that the hydrophobic properties of flocs are a good indicator of microbial floc formation.

NWRI RESEARCH SUMMARY

Plain language title

The surface tension of sludge flocs is a predictor of how well they will aggregate and settle in a wastewater treatment tank.

What is the problem and what do scientists already know about it?

To correct the sporadic poor settling of flocs in water treatment tanks costs the North American economy about one billion dollars per year. We know that floc-specific properties determine the extent of bioflocculation and resultant settling. Surface tension is likely to be one of these properties, and its exploitation in minimizing the frequency of sporadic poor settling is explored here.

Why did NWRI do this study?

NWRI has a mandate to develop new technology and explore new concepts for improving the cost-effectiveness of water treatment.

What were the results?

Bioflocculation can be interpreted in terms of system free energy, and the hydrophobic properties of flocs are a good indicator of microbial floc formation.

How will these results be used?

These results suggest that manipulation of sludge retention time might be a means to reduce the frequency of sporadic poor settling in treatment tanks.

Who were our main partners in the study?

NWRI, AEMRB, WTC, DFO-Bedford Institute of Oceanography, Brockhouse Institute for Materials Research, Ryerson University, International Association for Sediment Water Science

THERMODYNAMIQUE DE SURFACE ET PROPRIÉTÉS HYDROPHOBES DES FLOCS MICROBIENS

B. Q. Liao, G. G. Leppard, D. G. Allen, I. G. Droppo et S. N. Liss

RÉSUMÉ

La concentration de matières en suspension dans les eaux usées épurées de façon biologique est un bon indicateur de la biofloculation et est liée aux propriétés physicochimiques des floes microbiens formés dans le bassin d'aération des systèmes d'épuration. De plus en plus de données expérimentales indiquent que les interactions hydrophobes jouent un rôle important dans la biofloculation. On a estimé, à l'aide de l'angle de contact et de l'équation d'état de Neumann, l'énergie libre de la biofloculation ($\Delta G_{\text{floculation}}$) à l'interface de floes microbiens pour divers temps de rétention des matières solides (TRS) en conditions bien contrôlées dans des réacteurs discontinus séquentiels expérimentaux. Les boues dont le TRS était plus long (> 12 jours) étaient plus hydrophobes, leur angle de contact avec l'eau étant plus grand, et possédaient une valeur plus faible de $\Delta G_{\text{floculation}}$. On a constaté que les valeurs de $\Delta G_{\text{floculation}}$ sont en forte corrélation avec la concentration de matières en suspension dans l'effluent épuré terminal. Les résultats démontrent que la biofloculation peut être représentée en termes d'énergie libre d'un système et que les propriétés hydrophobes des floes sont un bon indicateur de la formation de floes microbiens.

Sommaire des recherches de l'INRE

Titre en langage clair

La tension de surface des floccs de boue permet de prévoir dans quelle mesure ces floccs vont s'agréger et se décantier dans un bassin d'épuration des eaux usées.

Quel est le problème et que savent les chercheurs à ce sujet?

Pour corriger les problèmes sporadiques de décantation des floccs dans les bassins d'épuration, il en coûte environ un milliard de dollars par année à l'économie nord-américaine. Nous savons que des propriétés propres aux floccs déterminent l'ampleur de la bioflocculation et de la décantation résultante. La tension de surface est probablement l'une de ces propriétés, et son exploitation dans la diminution de la fréquence des problèmes sporadiques de décantation fait l'objet de la présente étude.

Pourquoi l'INRE a-t-il effectué cette étude?

L'INRE a comme mandat de mettre au point de nouvelles méthodes et d'étudier les nouveaux concepts qui permettraient d'améliorer le rapport coût-efficacité de l'épuration des eaux.

Quels sont les résultats?

La bioflocculation peut être représentée en termes d'énergie libre d'un système, et les propriétés hydrophobes des floccs sont un bon indicateur de la formation de floccs microbiens.

Comment ces résultats seront-ils utilisés?

Les résultats semblent indiquer qu'une modification des temps de rétention des boues peut constituer un moyen pour diminuer la fréquence des problèmes de décantation sporadiques dans les bassins d'épuration.

Quels étaient nos principaux partenaires dans cette étude?

L'INRE, la Direction de la recherche sur la gestion des écosystèmes aquatiques (DRGEA), le Centre Technique des Eaux Usées (CTEU), l'Institut océanographique de Bedford du ministère des Pêches et des Océans (MPO), le Brockhouse Institute for Materials Research, l'Université Ryerson et l'International Association for Sediment Water Science.

1. INTRODUCTION

The flocculating ability of activated sludge and adhesion of dispersed cell or fine flocs to large floc surfaces influence the level of effluent suspended solids (ESS), or non-settleable fine particles, in the final effluent of biologically treated wastewaters [1-8]. Proposed mechanisms for floc formation, including charge neutralization, and polymer- and salt-bridging, emphasize the importance of surface properties in floc interactions [9,2,3,10,4,5,11,12]. Increasing attention has been given to the hydrophobic nature of sludge floc and its role in bioflocculation. A more hydrophobic surface has been related to a lower level of ESS [13,14,6,15,16]. The composition and properties of extracellular polymeric substances (EPS), particularly proteins, have been shown to be major determinants of the physicochemical properties of flocs, including the hydrophobicity [17,18,12,15].

Microbial flocs are naturally hydrated, due to the presence of large numbers of hydroxyl, carboxyl and phosphate groups. Side chains in amino acids, the methyl groups in polysaccharides, and the long-chain carbon groups in lipids all contribute to the hydrophobic properties of sludge flocs. Flocs are negatively charged under neutral pH conditions. The presence of ionizable groups such as carboxyl, phosphate and amino groups, in the EPS and cell surfaces is responsible for the density of surface charge. The zeta potential of sludge flocs is usually in the range of -10 to -30 mv [19,2,13]. Simple measures of the physicochemical properties, including hydrophobicity and surface charge, may be reliable indicators for predicting bioflocculation in the operation of biological wastewater treatment processes [15].

The purposes of this study were to evaluate the surface tension of sludge flocs by using contact angle measurements and Neumann's equation-of-state, to investigate the influence of sludge

retention time (SRT) on the surface tension of sludge flocs, and to test the feasibility of using surface thermodynamic concept to predict bioflocculation.

2. EXPERIMENTS

2.1 Activated Sludge Samples Activated sludge samples were taken from the laboratory-controlled sequencing batch reactors (SBRs) fed a synthetic wastewater containing glucose and inorganic salts. The SBRs were operated at different SRTs (4 to 20 days). Details of the SBR system are given by Liao *et al.* [15].

2.2 Contact Angle Measurement Sludge samples collected from the SBRs were first washed with deionized distilled water twice using a centrifuge at 2,000x *g* for 5 minutes each time, then the washed sludge sample was dispersed by a Vortex mixer and deposited on a membrane filter (Black MSI Microsep*, 0.45 μm) under 400 mmHg vacuum. The sludge cake was filtered until moist and there were no signs of excess water that could be sucked. Contact angle of deionized distilled water on sludge cakes was measured on a partially hydrated sludge cake using the axisymmetric drop shape analysis- contact diameter (ADSA-CD) technique [20,21].

2.3 Liquid Surface Tension Surface tension of the treated effluent was determined by a CENCO tension-meter (Sigma Chemical Co., MO) equipped with a 6 cm diameter platinum ring at ambient temperature ($21 \pm 2^{\circ}\text{C}$). Prior to surface tension measurement, effluent was centrifuged at 15,000x *g* for 15 minutes at 4°C to remove colloidal particles.

2.4 Effluent Suspended Solids The flocculating ability of activated sludge was evaluated by determining the ESS after 40 min settling of mixed liquor in the SBRs. The mixed liquor suspended solids at different SRTs was maintained at the same level ($2000 \pm 150 \text{ mg/L}$). The measurement of ESS was in accordance with Standard Methods [22].

3. SURFACE THERMODYNAMIC MODEL

The surface thermodynamic model predicts that the system free energy is minimized at equilibrium and adhesion between two surfaces will occur [23,24]. Consequently, bioflocculation will be thermodynamically favored if the process itself causes the system free energy to decrease. Ignoring electrostatic interactions and other specific binding, two hydrophobic surfaces approaching at short distances will result in the surrounding bound water layers to overlap with the eventual displacement of the bound water into the bulk water. This would lead to a decrease in the interfacial free energy and thus bioflocculation.

The interfacial free energy of the interaction between two identical bacterial cells (B), immersed in liquid (L) can be described as follows:

$$\Delta G_{\text{flocculation}} = -2 \gamma_{\text{BL}} , \quad (1)$$

where $\Delta G_{\text{flocculation}}$ is the interfacial free energy of floc formation and γ_{BL} is the interfacial tension for the bacteria (B)-liquid (L) interface. If the total free energy of a system is reduced ($\Delta G_{\text{flocculation}} < 0$) by cell interactions, then bioflocculation will be thermodynamically favored [25,26,23].

Neumann *et al.* [26] have demonstrated that γ_{BL} is a function of γ_{BV} and γ_{LV} (where γ_{BV} and γ_{LV} stand for the interfacial tension of bacteria-vapor and liquid-vapor, respectively), and developed an equation-of-state to describe the relationship among γ_{BL} , γ_{BV} and γ_{LV} :

$$\gamma_{\text{BL}} = (\sqrt{\gamma_{\text{BV}}} - \sqrt{\gamma_{\text{LV}}})^2 / (1 - 0.015 \sqrt{\gamma_{\text{BV}}} \sqrt{\gamma_{\text{LV}}}) \quad (2)$$

In conjunction with Young's equation:

$$\gamma_{BV} - \gamma_{BL} = \gamma_{LV} \cos(\theta) \quad (3)$$

a third equation is yielded as:

$$\cos(\theta) = [(0.015 \gamma_{BV} - 2.00) \sqrt{(\gamma_{BV} + \gamma_{LV}) + \gamma_{LV}}] / [\gamma_{LV} (0.015 \sqrt{\gamma_{BV}} \sqrt{\gamma_{LV}} - 1)] \quad (4)$$

Based on equation (4), the surface tension (γ_{BV}) of sludge flocs is determined by measuring the contact angle of a liquid with known surface tension (γ_{LV}). The change in the interfacial free energy of the system, $\Delta G_{\text{flocculation}}$, is then calculated from equations (1) to (4) [27].

4. RESULTS AND DISCUSSION

Table 1 shows the changes in surface tensions of sludge flocs and effluent, and the $\Delta G_{\text{flocculation}}$ with respect to SRTs. The surface tension of the effluent at different SRTs was similar and the values were quite close to the theoretical value (72 ± 1 ergs/cm²) of deionized distilled water at ambient temperature. This is perhaps not surprising, as the effluent, after centrifugation, contained only water and a small amount of soluble chemical oxygen demand (COD) and inorganic salts with a lower ionic strength (1.5×10^{-4} mol/L) (15). The presence of small amount of soluble COD [28] and inorganic salts [29] has only limited influence on the surface tension of water.

On the other hand, surface tensions of sludge flocs were significantly different with respect to SRTs. A lower surface tension was associated with sludge flocs at the higher SRTs (16 and 20 days), as compared to that at the lower SRTs (4 and 9 days) (Analysis of Variances (ANOVA), $p < 0.05$). It appeared that a transit range of SRT (about 12 days) existed for a significant change in

surface tension of sludge surfaces. This was the same for the interfacial tension between sludge surfaces and treated effluent, as shown in Table 1. These results suggest that the surface tension of sludge flocs and the interfacial tension between sludge surfaces and effluent can be biologically manipulated through the control of physiological status at a microbial community level.

A plot of ESS against the $\Delta G_{\text{floculation}}$ is shown in Figure 1. There is a strong positive correlation between the $\Delta G_{\text{floculation}}$ values and the effluent suspended solids (ESS) (Spearman's coefficient, $r_s = 0.85$, $p < 0.01$). A higher ESS is associated with a higher level of $\Delta G_{\text{floculation}}$, which is close to 0, particularly for sludge at lower SRTs. This is consistent with the prediction of the surface thermodynamic approach in that dispersed cells and fine flocs will aggregate to form settleable large flocs driven by the decrease in interfacial free energy (more negative). The large variation of the ESS for a given value of $\Delta G_{\text{floculation}}$, particularly for the higher level of $\Delta G_{\text{floculation}}$, indicates electrostatic interactions might also be involved in governing biofloculation except for hydrophobic interactions.

The results from this study provide first hand information on the surface tension of sludge flocs and its relationship to biofloculation in a well-controlled laboratory activated sludge system. A higher SRT produces a sludge surface with a lower surface tension. A strong positive correlation was found between the interfacial free energy of biofloculation and the level of ESS, indicating the importance of surface thermodynamics in explaining sludge floc formation. To date the characterization of the physicochemical properties of microbial floc, particularly hydrophobicity and surface charge, has been an academic activity. Given the relative ease of measuring hydrophobic properties (e.g. microbial adhesion to hydrocarbon and contact angle measurement) and surface charge (e.g. colloidal titration) of flocs, properties that can be correlated to biofloculation and floc structure [15,30,16], such determinations may be of greater value in

assessing sludge separation properties than the emphasis given to the role of filamentous bacteria [31].

5. CONCLUSIONS

The surface tension of sludge flocs could be manipulated through the control of SRT. A higher SRT produces a sludge surface with a lower surface tension.

A strongly positive correlation was found between the $\Delta G_{\text{flocculation}}$ and the level of ESS, implying the importance of surface thermodynamic approach in explaining sludge floc formation.

6. ACKNOWLEDGEMENTS

The project was funded by the Natural Sciences and Engineering Research Council (NSERC) of Canada through the strategic grants to SNL, IGD and GGL (STR0167324) and SNL, DGA, GGL and IGD (STPGP201976-97). The assistance of Z. Policova, Applied Surface Thermodynamics Laboratory at the University of Toronto, in using the ADSA-CA technique for contact angle measurement is highly appreciated.

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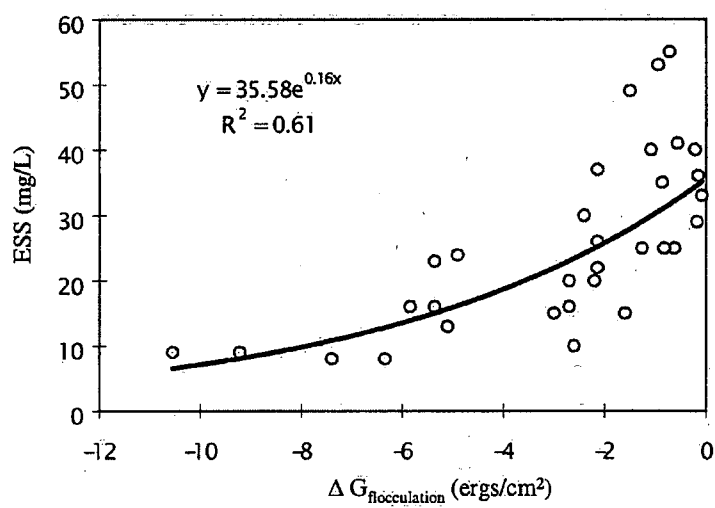
Table 1 Contact angles, surface tensions, interfacial tensions associated with bioflocculation of sludges at different solids retention times (SRTs)

SRT (days)	Contact angle ¹ (degrees)	γ_{BV} (ergs/cm ²)	γ_{LV} (ergs/cm ²)	γ_{BL} (ergs/cm ²)	$\Delta G_{\text{bioflocculation}}$ (ergs/cm ²)
4	20-29 (25 ± 3)	64.50 - 68.40	72 ± 1	0.28 - 1.07	-0.56 - -2.14
9	15-23 (17 ± 4)	67.20 - 70.12	72 ± 1	0.47 - 0.09	-0.18 - -0.94
12	29-31 (30 ± 1)	63.50 - 64.50	72 ± 1	1.07 - 1.35	-2.14 - -2.70
16	30-47 (36 ± 7)	54.70 - 64.00	72 ± 1	1.2 - 5.27	-2.40 - -10.54
20	31-45 (37 ± 6)	55.90 - 63.50	72 ± 1	1.35 - 4.61	-2.70 - -9.22

¹6-8 independent contact angle measurements were conducted at different experimental times at each SRT.

List of Figures

Figure 1 **Figure 1 Relationship between the surface free energy of bioflocculation**
($\Delta G_{\text{flocculation}}$ (ergs/cm²) and the level of ESS (mg/L)



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