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# Experimental characterization of the interactions between bacterial floc and suspended solids by means of optical density measurements.

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## Abstract:

Quantitative measurements of the properties of a natural multicomponent system, such as Municipal Wastewater (MWW) and Activated Sludge (AS), bring challenges in terms of analytical chemistry. In the present paper, we use the 'Binary Model' (BM), which is considering two kinds of particles of different nature able to absorb each other to form only one type of complex. This extremely simple modeling allows explaining non-intuitive measured optical properties behavior of MWW-AS mixtures. The experimental methodology used is very simple and relies on the measurements of the optical density of the mixtures by spectrophotometry. We derive a simple but realistic expression giving the optical density resulting from light scattering of mixed suspended particles. We report a series of experimental data obtained when characterizing samples of MWW and AS and illustrate the efficiency of the BM. This simple calculation already makes it possible to optimize strategies of flocculation/ coagulation of solids. We thus develop the bases of a simple and inexpensive methodology, able to provide information on complex particulate interactions which elucidation would otherwise require the use of very sophisticated analytical techniques.

**Keywords:** Suspended particles interactions; Activated sludge; Optical density; Urban wastewater; Wastewater treatment plant; multicomponent system.

## INTRODUCTION

Study of the Municipal Wastewater (MWW) biological treatment by Activated Sludge (AS) process requires dealing with the characterization of extremely complex multicomponent systems. These systems are not only chemically complex they are also very often heterogeneous. They present several physical phases: typically, liquid and solid. (With the exception of nitrogen and derivated compounds, other gases are quantitatively less important [1].) Although these properties are largely encountered, their heterogeneity is often neglected when modeling those natural processes. According to the level of description employed, "the homogeneity approximation" is sometimes justified. Nevertheless, it is sometimes essential to discriminate between phenomena governed by homogeneous kinetics and those following heterogeneous mechanisms [2].

In the present paper, we will be concerned by adsorption phenomena of MWW solid particles on AS flocs. In a very general way, MMW is composed of various fractions of soluble compounds and of suspended particles. Moreover, MMW involves many different particles, of different chemical nature, sizes or shapes. In the treatment plants, the mixed liquor is made of flocs grouping one (or more) bacterial consortium. Flocs are "structures" (it would be better to speak about "functional units" [3]), formed of polymers secreted by bacteria and embedding aerobic and/or anaerobic colonies of microorganisms. The structure of flocs has been widely studied and it was shown that they are essentially heterogeneous and characterized by a random dispersion of their components [4]. The polymer matrix, filled with water, is traversed by fine channels [5]. As a result, these flocs have a density barely higher than that of water (1.02-1.06 g/mL, [6]), they have an extremely slow settling rate and easily remain in suspension, even under very weak stirring.

Floc-forming bacteria mainly metabolize the biodegradable compounds of sewage. Flocs in suspension form a dispersed phase that is usually known as activated sludge (AS, [7]).

The complexity of these systems makes inefficient the use of common analytical chemistry methods. The snag arises as soon as it is a question of quantifying compounds without fixed stoichiometry or which do not belong to a family quantifiable by a specific analytical test.

However, considering a natural phenomenon or a particular process, these mixtures have a simple "functional" meaning. For example, MWW is simultaneously the pollutants load of a city or the nutrients for the microorganisms of a wastewater treatment plant. Relevant characterization of an effluent has a critical importance both for some ecological phenomena (like eutrophication, for example) and for design or management of water treatment systems, etc.The most natural approach to analyze these processes would probably consist in drawing up an inventory of the various types of particles and to select a specific method of quantification for each of them. This method would allow monitoring the evolution of each kind of particle over time. This approach seems natural but is in general completely inapplicable. Let us consider the measurement of suspended cells biomass. Flux cytometry, for example, allows counting free suspended cells with sufficient precision and the method applies in some case. But if it is a question of counting bacterial flocs, it is essential to eliminate the "slime" (mainly EPS: extra polymeric substances) surrounding the cells prior to carrying out the measurement. The particle is then denatured and does not fulfill its most important functions anymore [8]. As a consequence, one cannot ensure that the number of measured cells really corresponds to the "operational quantity" (the functional amount). As a matter of fact, in a lot of situations, the "slime" is precisely the matter we need to quantify. In a different way, some intracellular compounds are measured out to estimate the whole cellular weight. Macromolecules are mostly used and especially proteins are utilized to characterize the enzymatic cell content [9]. This method is however exact only during the steady state of a process because it is well known that the molecular composition of a microorganism varies according to its growth rate [10]. The method is thus inappropriate to monitor kinetics or, in general, when the specific growth rate is not constant in time.

The ecological importance of this type of measurements is such that many searches are undertaken in this field [11]. To avoid the difficulty related to specific determinations, many nonspecific analytical methods were developed since many years. They have become so important that several efforts have been made to standardize [12] or to normalize (ISO (International Organization for Standardization), for example) the protocols. Some are very well known, like the dry weight for the estimate of the biomass, the cells counting [13] or the COD (chemical oxygen demand) for the estimation of the carbon nutrients and the oxidizable matter in wastewater [12]. These techniques have advantages and inconveniences. As global nonspecific measurements, they often present an empirical and approximate aspect compared to more traditional and (perhaps) more rigorous methods. This unavoidable situation justifies investing important efforts in the field. However, it is necessary to recognize that in many cases these methods are the sole currently known which are able to bring quantitatively (or semi-quantitatively, if one prefers) information about a lot of phenomena whose complexity defies the use of more specific or more rigorous methodologies.

It is because we encountered some problems of this kind (to quantify bacterial flocs and solid nutrients interactions) that we tried to develop a global, nonspecific method based on some easily measurable optical properties.

Optical Density (OD) measurement is a well-known method for characterizing the biomass of MWW or AS samples and is a common experimental quantity to define the concentration of a bacterial sample [14-16]. As long as some precautions are taken [17], it is indeed possible to establish a reliable correlation between OD and the total suspended solids of a sample. In general, the precautions consist in experimentally establishing a calibration curve for the system under study [18] and respecting the bounded concentration domain of the obtained correlations [19]. For historical reasons, linked to the use of optical filters to obtain monochromatic illumination, the most commonly used wavelength for OD measurements is 600 nm. In general, the wavelength is selected according to experimental constraints and objectives. For many applications, such as bacterial cultures developed in a colored medium, or in the study of systems containing some colored microorganism, the measurement of the overall number of bacteria is obtained by measuring the OD outside of the absorption bands. A typical example is the study of algae chlorophyll: $OD_{540}$, avoiding interferences with the absorption band of chlorophyll, is used to monitor cell numbers during growth phase [20-21]. However, $OD_{678}$, corresponding to a chlorophyll absorption peak, is used to monitor the chlorophyll content [22]. Thus, depending on the system under study, different wavelengths (visible, UV or (near) infrared) can be used [23]. On the other hand, in the context of biological samples, ultraviolet should be avoided, because of their damaging properties [24-25] on living cells or organelles.

In a previous paper [26], Thierie intended to measure light absorption to investigate the interactions between MWW particles and AS flocs. Measurements were performed with a colorimeter or in a spectrophotometer and OD or transmittance are the turbidity units. He proposed a very simple model, the "Binary Model" (BM), to interpret the decrease in OD when mixing WWA and AS samples as the experimental evidence of interactions between the flocs and the particles. The purpose of the present paper is to test the BM through a series of experiments using MWW and AS samples. In particular, we aim to investigate the validity of the concentrations range of the BM, and to test the use of the critical point of the model to define a strategy for the optimization of the absorption process of particles of MWW on AS flocs. For the sake of clarity, we provide a definition of the OD and a description of the BM in the first section of the paper. The experimental protocol is described in detail in the experimental section. Experimental data are then reported and discussed.

## *Representation of the optical density due to several particles.*

At first, we introduce the following basic comments in order to define the Optical Density that we use in experiments. Light/matter interaction is a very complex phenomenon and is not yet fully understood [27]. It is obvious that the equations we are using here are approximations of a finer representation of the phenomenon. Nevertheless, we show that use of very simple relations allows taking advantage of light scattering concepts to study complex particles interactions.

The relationship between the intensity of transmitted and incident light through an optical path of length *h* (thickness) is given by

$$\frac{I_t}{I_i}=\exp\left(-\alpha_{ext}.h\right) \quad (1)$$

where $I_i$ is the incident light, $I_t$, the transmitted light and $\alpha_{ext}$ , the extinction (or attenuation) coefficient.

For *M* categories of different particles, the extinction coefficient is given by

$$\alpha_{ext}=\sum_i^M N_i\sigma_i^T \quad (2)$$

where $N_i$ is the number of particles and $\sigma_i^T$ , the total cross section of class *i.*

While injecting this value in (1), it comes that

$$\frac{I_t}{I_i}=\exp\left(-h.\sum_i^M N_i\sigma_i^T\right) \quad (3)$$

Defining the optical density (OD) by the logarithm of the ratio of transmitted to incident light one finally has that

$$OD=h\sum_i^M N_i\sigma_i^T \quad (4)$$

The general relation (4) expresses that the OD is simply proportional to the sum (on all the classes) of the products of the particles number by the total scattering cross-section.

In a general way, the extinction is due to two phenomena: light absorption and light scattering. In our experiments, we concluded that absorption is negligible with respect to scattering (See Figure 3), and we thus consider that scattering was the main contribution to OD.

## The binary model

The model is called "binary" because, as a first approximation, it only uses two types of particles ($P_1$ and $P_2$), each species being able to absorb to each other. In other words, the sole interactions taken into account concerned, say, the adsorption of $P_1$ on $P_2$. In spite of considering that all the particles are identical, we can interpret the data as average values. Thus, we can imagine that the $P_2$ particles, larger than particles 1, are able to fix, on average, *x* of these particles, and form "complexes" according to the scheme

$$xP_1+P_2\rightarrow P_2\left[P_1\right]_x \quad (5)$$

Without changing the final conclusions (and for reasons which will appear further) we can assume that there is a not-complexing (not reactive) fraction of $P_2$ particles, which does not adsorb any particle of type 1:

$$P_2\rightarrow P_2 \quad (6)$$

Let us designate by $\theta$ this not-complexing fraction. For constant particles number, the mass balance imposes that the complexing fraction is $(1-\theta)$ , the sum of the two fractions being equal to 1.

The "reactive mixture" of the particles can be depicted as in Figure 1.

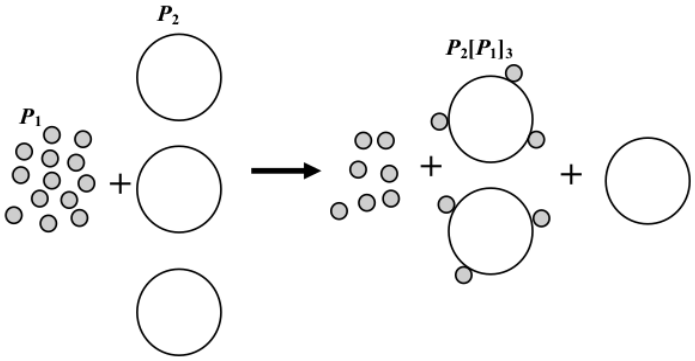


**Figure 1: Sketch of the "binary model".** Two different kinds of particles, $P_1$ and $P_2$ can adsorb to each other to form one type of complex. As an example, we used *x=3* in the figure.

A detailed discussion of the hypothesis used in the BM model was provided in [14]. In a system where only weak interactions between the particles are considered (typically, physicochemical interactions not involving dramatic particles modifications nor important color change), we stated that the OD of the mixture, $OD_M$, is simply decomposable in additive terms. In the binary model, described in (5), we consider only three significant species: the particles $P_1$ and $P_2$ and, the complex C; the OD can, therefore, be written as:

$$OD_M = hN_{1,M}\sigma_{1,M}^T + hN_{2,M}\sigma_{2,M}^T + hN_{C,M}\sigma_{C,M}^T \quad (7)$$

This relation is general and applies and only if particles $P_1$, $P_2$, and complex *C* have significant contributions. In the presence of interactions only (without chemical reactions, for example), there is no phase change, all the matter remains contained in the sample cell and the mass balance must apply. It should be noted, on the other hand, that the number of particles before mixing, *Ni*, and after mixing, $N_{i,M}$, may change (and must change, if complexes formation occurs). In the same way, there is no reason for the cross-section of separate/isolated particles to be identical to the cross-section of particles in the mixture.

Introducing to the mass balance in (7), as long as particles $P_1$ are in excess (i.e. some $P_1$ remain free after mixture), the OD of the mixture is:

$$OD_M = h\{N_1 - x(1-\theta)N_2\}\sigma_{1,M}^T + h\theta\sigma_{2,M}^T h + h(1-\theta)N_2\sigma_{C,M}^T h \quad (8)$$

This relation clearly reveals a possible negative contribution of the particles $P_2$ to the OD of the mixture. Under the condition:

$$x(1-\theta)\sigma_{1,M}^T > \theta\sigma_{2,M}^T + (1-\theta)\sigma_{3,M}^T \quad (9)$$

all the other values being constant, the optical density of the mixture decreases linearly with $N_2$.

Relation (8) is valid only if $N_1$ is in excess. If not, $N_{1,M}$ would become negative, which is impossible. When particles 2 are in excess (i.e., when all particles 1 are adsorbed), the OD of the mixture is:

$$OD_M = h\theta N_2 \sigma_{2,M}^T h + h\frac{N_1}{x}\sigma_{C,M}^T \quad (10)$$

This time, all values being constant, the optical density of the mixture varies linearly with $N_2$ and is positively correlated. This form is identical to the usual Beer-Lambert's law.

For a system with all parameters constant and for which (7) is satisfied, the increase in particles 2 results first in a linear decrease followed by a linear increase of the optical density of the mixture. There is thus a critical value in $N_2$ corresponding to a minimal value of $OD_M$. This value exactly corresponds to the conditions $N_{1,M}=0$ and is easily calculated as:

$$N_2^{crit} = \frac{N_1}{x(1-\theta)} \quad (11)$$

## MATERIALS AND METHODS

In the following experiments, samples of MWW were mixed with large quantities of wastewater treatment plants mixed liquors (activated sludge) and the OD of the mixtures were measured. We provide in this section all the information about the experimental protocol.

### Sampling

The activated sludge (AS) came from the Thines's wastewater treatment plant (Nivelles-Monstreux, Belgium). This wastewater treatment plant uses a conventional biological AS process and performs the water epuration of carbonaceous, nitrogenous and phosphorus materials. It is a weak-load station and it doesn't use primary settler. It performs tertiary treatment and uses sludge of about 20 days age. The MWW was harvested at the entrance of the station, immediately following the physicochemical stage (screening, grit removing, de-oiling …). An automated sampler collects one liter per hour during 24 hours. On arrival at the station, the 24 liters were mixed and a representative sample is used in the experiments. AS were directly taken from the biological tank. Samples were kept in cold room at 7°C and used within 72 hours after sampling. All experiments were performed at room temperature (20 ± 2 °C). The dilutions were carried out using a graduated 10 ml pipette whose end was enlarged to approximately 2 mm, allowing the passageway of all the particles of interest; after widening of the end, the pipette was recalibrated by weighing known water volumes.

### Concentration measurements

Concentrations were measured in dry weight per unit of solution volume (DW/L), according to the standard methods [28]. We used previously dried Sartorius cellulose acetate filters, with pore size 0.45 µm. After filtration, filters and cakes were dried and weighed to constant weight (±0.0001g). For each sample, the measurement was repeated three times and the average value was used.

### Optical Density Measurements

OD was measured on a Pharmacia LKB Novaspec II spectrophotometer. We used disposable spectrophotometric sample cell (DispolabKartell 1941 PMMA with an optical pathway of 1 cm).

To avoid bias related to settling of sludge in the cuvette, a manual agitation method was standardized: 3.5 ml were harvested and the sample cell was closed by a piece of Parafilm, held between two fingers and reversed five times consecutively with a constant rate (approximately one per second). New agitations and new reading-were made several times (from min 5 to max 10 measurements, according to the rate of settling observed, with at least two measurements of the blank solution). The value used was the average of these measurements. It was then possible to calculate the standard deviation and standard error of a series of measures. The "blank" solution consisted of a filtrate of the MWW and AS mixture in suitable proportions. However, the OD of the MWW filtrate was generally so low that this fraction can be neglected. In some circumstances, distilled water could even be used, without significantly skewing measurements.

### Sample mixing

Mixing the sample can influence the adsorption kinetics of the MWW particles on the AS flocs. A standardized procedure was used for all measurements. The mixture was prepared in glass test tubes. A volume $V_{MWW}$ of wastewater was mixed with increasing volumes of activated sludge $V_{AS}$ to constitute a constant total volume of 35 ml ($V_{MWW}+V_{AS}=35$ ml). The test tubes were fixed perpendicularly to an axis and agitated by rotation (24 rpm) during the required stirring time.

## RESULTS AND DISCUSSIONS

### Selected wavelength

Preliminary experiments were performed in order to observe the influence of the wavelength. We measured the spectra of AS and MWW samples, diluted in distilled water. The blank used for those measurements was distilled water. Results are reported in Figure 2.

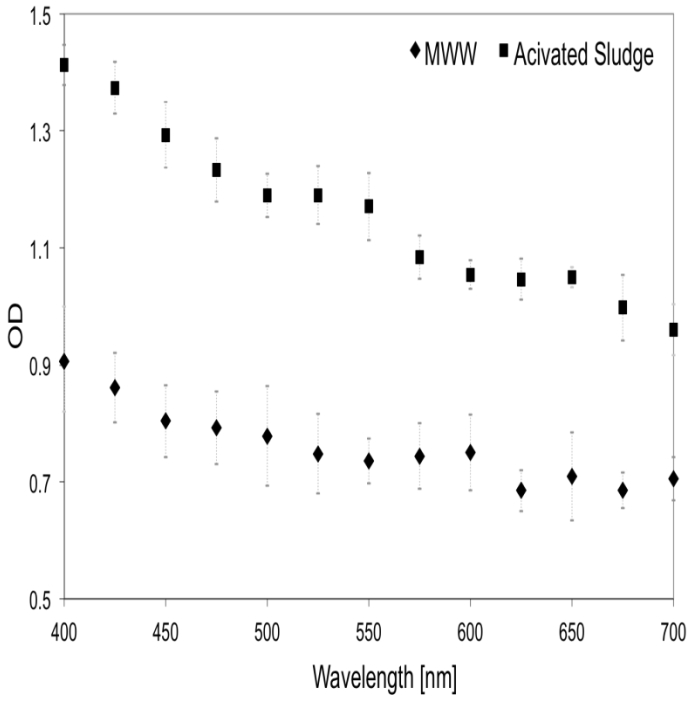


**Figure 2.: Spectra of Activated Sludges and Municipal Wastewater samples.** We measured spectra of typical AS and MWW samples used in this study. The optical density was measured with respect to distilled water. The optical density monotonously decreases with wavelength.

We observe a monotonous decrease of OD as a function of wavelength. For our samples, MWW and AS spectra do not show any absorption bands in the visible and we can freely select the working wavelength. The OD is significantly larger at 400 nm than at 700 nm. To confirm this result, we carried an additional experiment, measuring OD of several AS/MWW mixtures at 2 different wavelengths (400 and 600 nm). $OD_{400}$, $OD_{600}$ and their ratio are showed in Figure (Fig. 3). Note that concentrations are expressed in AS volume fraction.

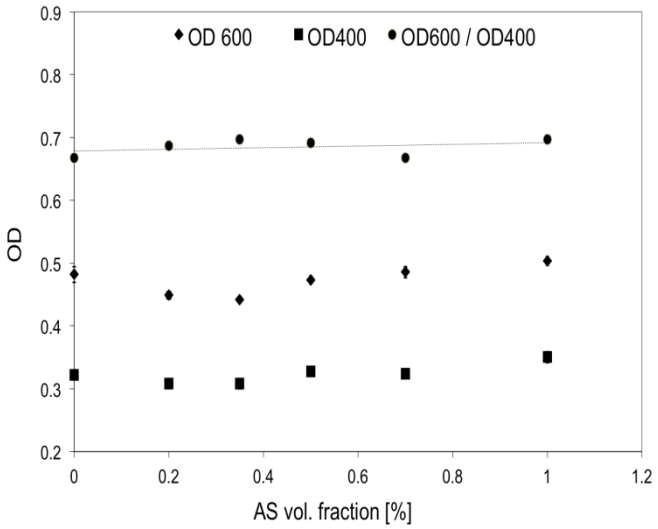


**Figure 3: The optical densities of a series of AS/MWW mixtures at two different wavelengths.** We measured $OD_{400}$ and $OD_{600}$ for a series of AS/MWW mixtures. No significant change occurred and the ratio $OD_{600}/OD_{400}$ was constant, indicating that working conditions were largely independent of wavelength.

The measured OD at both wavelengths were similar, as shown by the constant ratio $OD_{400}/OD_{600}$. This indicates very clearly that the measured behavior of OD of WSS and AS mixtures were not wavelength dependent. The OD is significantly larger at 400 nm than at 600 nm. Based on these results, it is obvious that measurements at lowest wavelength maximize the resolution. This determined us to work at the shortest possible visible wavelength available on our spectrometer. All subsequent measurements were performed at 400 nm.

## Stirring time

When mixing MWW samples with AS, we observed that some time was required for the interactions between the particles to become apparent through OD measurements. In other words, the formation of the "$P_1P_2$ complexes" in the binary model is not instantaneous and obeys to a well-defined kinetics. This kinetics can be seen as the time required for the MWW particles to be "stuck" onto the AS flocs. Given the rather complex structure of the bacterial flocs, it is likely that many parameters strongly influence the kinetics, in a way that we are not yet able to describe in details. One can, for instance, imagine that the MWW particles require some time to enter into contact with the complexation sites on the flocs. Additional complex phenomena, such as diffusion of the particles through the flocs structure are probably also influencing the characteristic time. We performed a series of experiments with the aim of defining the suitable experimental stirring time of the mixed samples prior to measurements in order to obtain reproducible OD values.

A series of samples containing a fixed amount of MWW and increasing AS yield were prepared as quickly as possible. The samples were stored in test tubes and agitated by rotation. The initial time ($t=0$) corresponded to the beginning of the stirring, the first DO value being measured immediately after. The results are shown in Figure 4. For readability of the plot, we did not report confidence intervals of the OD values, and we used marked lines for each sample.

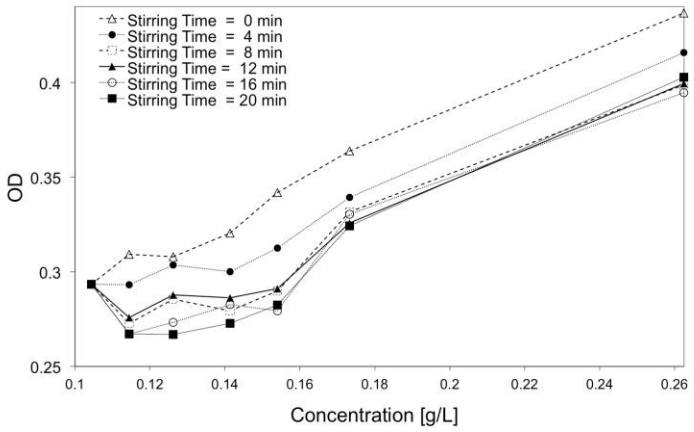


**Figure 4 : Influence of the stirring time on the optical density.** Some time is required for the interactions to become perceptible through OD measurements. In the absence of stirring time, the OD varies almost linearly with concentration. For longer stirring times, the interactions between the particles become apparent.

The results show that, in absence of stirring, the OD varies almost linearly with concentration. This meant that the samples were sufficiently diluted and obeyed a typical Beer-Lambert behavior. Increasing the stirring time for intermediate AS concentrations, a deviation from linearity appeared. We interpreted this effect as the indication of interactions between the MWW particles and the AS flocs. We also observed that significant differences arose in the curves corresponding to times ranging from 0 to 8 mins, while the curves corresponding to stirring times between 16 and 20 minutes are very close. In other words, the influence of the stirring time is more efficient at shorts times and very small for times greater than 16 minutes. An equivalent way of presenting the same result is to plot the OD of one sample over time. We observed the "sticking" kinetics of the particles on the flocs. The kinetics of the second sample of Figure 4 is shown in Figure 5:

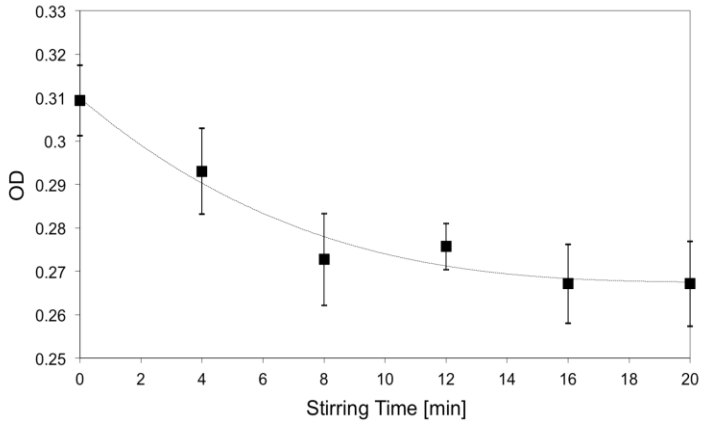


**Figure 5: Sticking kinetics of the MWW particles on the AS flocs.** Over time, the interactions between the particles, as described by the binary model, become apparent through optical density measurements. For stirring times longer than 16 mins, the sticking kinetics reaches a steady state and no further OD variations occur.

The stirring time significantly influenced the measured OD values. For times shorter than 16 min, the complexation is incomplete, while after 16 min, the kinetics reaches a steady state and no further significant OD variations occurred. Similar results were obtained in several experiments, validating a minimal characteristic time of about 16 min. For all subsequent experiments, we used 16 min stirring time.

## The binary model

A series of experiments were performed to study the reproducibility of the BM. A series of 7 MWW and AS samples were investigated. All samples were harvested at the same treatment plant using a standardized sampling protocol, over a period of about three months. For each sample, we measured the concentration, both for MWW and AS. For all MWW samples, the suspended solid was found to be reasonably constant, although concentration changed of the order of about 20%. This was, of course, inevitable, especially due to climatic conditions, the moment of sampling, the day of the week etc. On the other hand, the AS samples showed rather large concentration variations, indicating that the age and the concentration of the sludges changed much over the study period. The age and concentration of sludges in the tanks depend on the operating conditions of the treatment plant.

For each sample pair, a series of experiments are carried out. The data are reported in Figure 6, where we determined the two straight lines of the BM. The left-hand line corresponds to the situation when the MWW is in excess (see Eq. 8), while the right-hand line corresponds to AS in excess (see Eq. 10). The slopes of those lines, $S_1$ and $S_2$, are summarized in Table 1. The intersection of the two lines corresponds to the minimum of the BM, as described by Eq. 11. In the graphs of Figure 6, the abscissa of the minimum, $C_{min}$, corresponds to the overall concentration of the mixture. It includes the sum of the suspended solids from the MWW and the AS:

$$C_{min} = C_{MWW,min} + C_{AS,min} \quad (12)$$

The "minimum" point can be considered as the point for which the quantity of AS in the mixture is optimal in for adsorbing the MWW particles. At this point, we compute a "Sludge Flocculation Index" $i_{floc}$ defined as:

$$i_{floc} = \frac{C_{MWW,\min}}{C_{AS,\min}} \quad (13)$$

$i_{floc}$ is the quantity of MWW particles stuck per unit of mass of sludge and can be seen as an indicator of the ability of the sludge to stick particles.

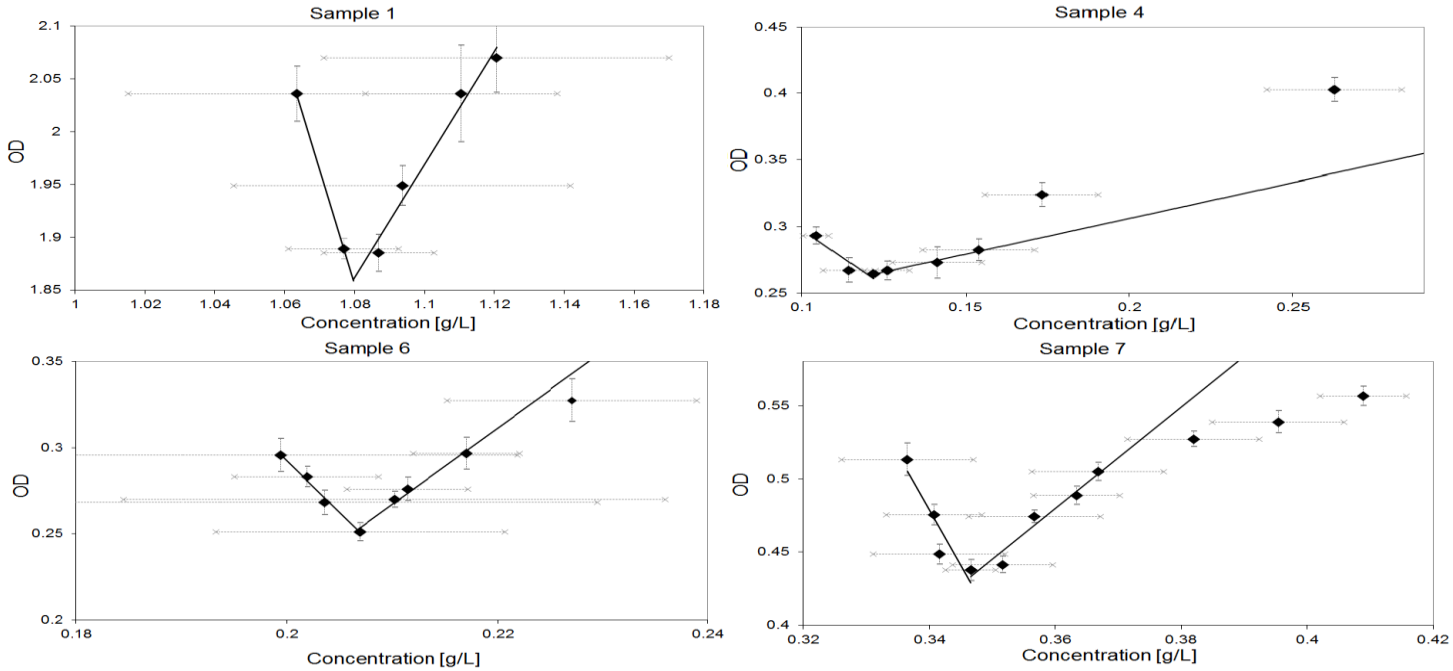


**Figure 6: Binary Model for a series of MWW/AS samples.** Optical density profiles were measured for a series of MWW/AS samples. The points in the vicinity of the minimum of OD were used to determine the two straight lines of the Binary Model (see Equations 8 and 10).

Figure 6 shows that, in the vicinity of the minimum, the measured optical density of the mixture is remarkably well described by the linear dependence of the concentration, as formalized by the BM. At the right of the minimum, when the excess in AS increases, the measured OD deviate from the straight line and the linear dependence is no longer verified. A rigorous explanation of this behavior is probably very complex and many parameters should be taken into account. A first element is that the concentration of the samples increases and that the OD can no longer be represented by a simple Beer-Lambert law.

However, other parameters probably also come into play: activated sludge flocs are a particularly complex system. It is obviously not realistic to model them in the simple form of identical particles. Their size, bacterial composition, sludge age and related metabolism is much more complex. Consequently, more realistic modeling should take into account a population of bacterial flocs, with variable properties. A first conclusion of the present study is however that the BM reasonably describes the measured variations of OD of complex natural systems in a limited concentration domain.

Additional interesting comments can be formulated regarding the data of Table 1.

| Sample | $S_1$ | $S_2$ | $C_{min}$ | $OD_{min}$ | $i_{floc}$ |
|---|---|---|---|---|---|
| 1 | -10.91 | 1.079 | 1.079 | 1.858 | 0.015 |
| 2 | -1.68 | 0.54 | 0.121 | 0.263 | 0.163 |
| 3 | -17.42 | 3.08 | 0.217 | 0.466 | 0.010 |
| 4 | -2.03 | 6.37 | 0.296 | 0.667 | 0.092 |
| 5 | -1.8 | 9.19 | 0.241 | 0.511 | 0.193 |
| 6 | -6.01 | 3.64 | 0.207 | 0.256 | 0.04 |
| 7 | -7.52 | 1.91 | 0.344 | 0.445 | 0.024 |

**Table 1: Experimental parameters of the Binary Model for a series of MWW/AS samples.** $S_1$ and $S_2$ are the slopes of the lines of the Binary Model. $C_{min}$ and $OD_{min}$ are the coordinates of the minimum of the model. $i_{floc}$, the "Sludge Flocculation Index", defined in equation 13, is an indicator of the ability of the sludges to stick particles.

A quantitative analysis of these data shows that, concerning the samples that we have investigated, it is not realistic to try to determine reproducible parameters of the BM. From one sample to another, the minimum of DO varies from one order of magnitude. This observation is the consequence of the large variability of the characteristics of the samples used, especially of the properties of the sludges. Similarly, the slopes of the lines of the models vary in some cases of factor 20. The concentrations are measured in grams of dry weight per liter of AS liquor, showing that the variability of the parameters of the BM was not only influenced by the concentration of the AS samples but also, and mainly by the properties of the bacterial flocs themselves. In other words, different AS samples are characterized by very different particles sizes (i.e. by various particles cross sections in terms of light interaction) and structures. Moreover, we observed that it was not possible to obtain reproducible sludge flocculation index. This means that different AS samples present very different potentials in trapping capacities. For the samples that we investigated, the Binary Model is not capable, at this stage, to model quantitatively and reproducibly the interactions between MWW particles and AS flocs. In the future, a more developed version of the model has to be developed and validated in order to expand our understanding of the process involved in the interactions between MWW particles and bacterial flocs.

## CONCLUSIONS

We performed a detailed experimental study of the interactions of Municipal Wastewater particles and bacterial flocs of Activated Sludges. We used a simple method, based on Optical Density through spectrophotometric measurements, to investigate the interactions between the particles and the bacterial flocs. These measurements ascertain interactions between the particles and the flocs. The use of the Binary Model, a remarkably simple model, allows interpreting the linear dependence of the Optical Density of the sticking of particles on the bacterial flocs. The Binary Model very satisfactorily accounts, in a limited concentration range, for linear variations of optical density with concentration. The AS showed very variable properties. As a result of the complexity of the AS, we obtained very different parameters of the model for the different samples, and we showed that each AS sample is characterized by a specific sludge flocculation index. The encouraging results we presented in this work are to be considered as illustrations of the methodology rather than as a final interpretation of the phenomena.

We believe that the experimental methodology is of interest and could be used in a variety of applications while avoiding unexpected pitfalls.

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