A Method for Exploring Suspended Particles Interactions by Means of Optical Density Measurements: Application to Bacterial Floc and Solid Nutrients

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Abstract

In experiments, we observed that, surprisingly, the optical density (OD) of a mixture made up of various particles could decrease when the number of particular particles is increased. Our aims were first to understand this unexpected result, and second to see if we can use these results for practical purposes. We derived a simple but realistic expression giving the optical density resulting from light scattering of mixed suspended particles. The equation we obtained is a linear relation of the product of the particles number by their cross section. We showed that the total OD (obtained by summing over the OD’s of the independent particles) is different from the OD of the same system when the particles are interacting when put together. Using a static model with two types of particles (“the binary model”), we showed which conditions are necessary to explain this apparently paradoxical phenomenon. This simple calculation already makes it possible to optimize strategies of flocculation/coagulation of solids. We then used this methodology to investigate the biodegradation kinetics of municipal wastewater (MWW) particles by activated sludge (AS) of a wastewater treatment plant. We obtained a good representation of the assimilation of the slowly biodegradable particulate nutrients.

Keywords: Suspended particles interactions; Activated sludge; Optical density; Urban waste water; Waste water treatment plan

Introduction

The study of natural or semi-natural systems (like wastewater treatment plants, for example) sets up large analytical chemistry difficulties. The snag arises as soon as it is question of quantifying compounds without fixed stoichiometry or which do not belong to a family quantifiable by a specific analytical test. In fact, we have to characterize extremely complex mixtures, whose composition varies both in space and in time. However, considering a natural phenomenon or a particular process, these mixtures have a simple “functional” meaning. For example, municipal wastewaters (MWW) are simultaneously the pollutant loading of a city or the nutrients for the microorganisms of a wastewater treatment plant. The relevant characterization of an effluent is of critical importance both for some ecological phenomena (like eutrophication, for example) and for the design and management of purification systems. These variables are not only chemically complex they are also very often heterogeneous. They present several physical phases: typically liquid and solid (in many cases, gas escapes the system). Although these properties are largely encountered, many natural phenomena representations make the economy of the heterogeneous character of these variables. It is true that it is often easier to deal with a homogeneous representation. Moreover, it should be noticed that, according to the description level considered, “the homogeneity approximation” is sometimes justified. Nevertheless, it is on occasion essential to discriminate between phenomena governed by homogeneous kinetics and those following heterogeneous mechanisms [1,2]. Thereafter, we will be concerned by adsorption or biodegradation phenomena only involving solid phases dispersed in an aqueous medium. We will especially deal with bacterial flocs of activated sludge (AS) wastewater treatment plant and with biodegradable “solid” particles of municipal wastewater (MWW). Understanding and managing these phenomena require quantifying such “complex” variables and this quantification make problems [3].

In this article, we will use light absorption and take advantage of its “defects” (due to interactions between particles) to study complex kinetics in unstable heterogeneous systems (cells and suspended particles). The same principles will also be utilized to the optimization of flocculation/coagulation processes corresponding to particular mechanisms (“binary model”).

After numerous studies relating to biodegradation kinetics of municipal wastewater by activated sludge WWTP, it appeared increasingly apparent to us that a great number of phenomena of this nature, both in semi-natural and in the natural environment, are controlled by heterogeneous kinetics. In particular, we were led to assume that depollution phenomena are strongly influenced by suspending particle interactions. This change of perspective in the usual purification analysis can result in a real enrichment in the way of apprehending the phenomena. Several innovative concepts remain to be improved or validated in a lot of different situations.

Material and Methods

Preliminary remark

In the introduction, we evoked the concept of the optical measurement reproducibility. This concept of reproducibility, generally, is not enough considered and deserves a small comment. Let us consider the suspended cells system as a biphasic system comprising a liquid dispersing phase (the culture medium, for example) and a solid...
phase (the cells). From a thermodynamic point of view, such a system is unstable and will unavoidably tend towards a phase separation (unless no sufficient energy is provided by the system to maintain the suspension, as mechanical agitation, for example). This phase separation obeys a precise dynamics (often very complex), which can be slow or fast. How much the settling rate is fast or slow is determined by comparison with the time necessary to make a measure. If the settling is slow compared to the time of measurement, the system can be regarded as stable. No particular precautions have to be taken and measurements will generally be sufficiently reproducible. On the contrary, if the settling rate is fast or of the same order of magnitude as the measurement time, a protocol must be defined to carry out the measure. In particular, it must ensure a constant time interval between sampling and measures and define particular precautions to assure the homogenization of the sample. (The ideal solution would be agitation in the core of the sample cell, but this solution is generally inapplicable). (Another cause of the lack of reproducibility can be due to the interaction of the cells or the particles with a possible soluble reagent added to the sample. This case is rather unusual, but is sometimes used to distinguish living from dead cells.) We have taken the instability of these heterogeneous systems into account and defined a simple method to alleviate these inconveniences and to improve the measurements reproducibility.

Sampling

The activated sludge (AS) came from the Dyle’s wastewater treatment plant (Basse-Wavre, Belgium) and was harvested at the outlet of the secondary settler. The municipal wastewater (MWW) was taken at the entry of the same plant.

Static experiment

Measurements and harvesting were carried out the same day (Figure 1). The mixture was performed in test tubes. A volume VMWW of wastewater was mixed with increasing volumes of activated sludge VAS to constitute a constant total volume of 35 ml (VMWW+VAS=35 ml). 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 water volumes. VAS/VMWWx100 calculates the relative concentration, in percent (v/v). Several test tubes containing different relative concentrations are prepared as quickly as possible (in the following example, we used 5 test tubes, but this number is not critical and depends on what is under study (minimum of the curve, range for binary model validity). Once the mixture carried out, the test tubes were fixed perpendicularly to an axis and agitated by rotation (24 rpm) during a variable time. The various times of agitation were approximately 0, 5, 15 and 20 minutes (again, these times values are not very important, as long as the “decrease plateau” was reached). In this case, for time shorter than 15 min, the complication was found to be incomplete; beyond, we observed saturation (Figure 1). Time used was thus of 15 min. After 15 minutes of rotation, 3.5 ml of mixture were harvested and inserted in a disposable spectrophotometric sample cell (DispolabKartell 1941 PMMA; optical pathway of 1 cm). To avoid bias related to settling, a manual agitation method was standardized: the sample cell was closed by a piece of Parafilm, held between two fingers and was reversed five times consecutively with constant rate (approximately one per second). The reading was then carried out at 600 nm in a spectrophotometer (Pharmacia LKB Novaspec II). New agitations and new reading, etc., were done 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. OD measurement is problematic when settling materials are used, as it is the case. It is necessary to agitate the sample in the cuvette in a repeatable manner and to carry on the measure as soon as possible.

The “blank” solution normally consists of a filtrate of the MWW and AS mixture in suitable proportions. However, the OD of the MWW filtrate is generally so low that this fraction can systematically be neglected. In some circumstances, distilled water could even be used, without significantly biasing measurements. Nevertheless, tap water reduces the osmotic shock for live cells (mainly bacteria).

Note: It is interesting to note that the complexes formation showed a real kinetics, taking some time (about 15 min. The Figure 1 shows this phenomenon [1]). Hypothetically, this may be interpreted a modification of the particles surface. Of course, only a better understanding of biochemical and bacterial properties shall permit to enhance the phenomenon comprehension.

Results

Fundamental results

In the following experiment, increasingly large quantities of wastewater treatment plant mixed liquor were added to municipal wastewater (MWW). The MWW are formed by various fractions; so far as we are concerned, one can represent these fractions as follows:

- SOLUBLE COMPOUNDS
  - Easily biodegradable
  - Slowly biodegradable
  - Not biodegradable
- PARTICLES
  - Easily biodegradable
  - Slowly biodegradable
  - Not biodegradable

The not biodegradable particles are, essentially, mineral particles (sands, clays…) which settle very quickly and are almost a part of the system. 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”, [4] formed of polymers (EPS: extra polymeric substrate [5] secreted by bacteria and surrounding aerobic or anaerobic colonies of microorganisms. These flocs have a density barely higher than that of water (1.02-1.06 g/mL, [6]) and are traversed by fine channels. As a result, the flocs have an extremely slow settling rate and easily remain in suspension. 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).

Figure 2 shows the optical density of a mixture of activated sludge and municipal wastewater for increasing AS concentrations.

Filtration with a 0.22 µm porosity filter (eliminating flocs, dust and the majority of the particles) showed that the optical density of the soluble compounds was negligible for all the AS concentrations (OD600 <0.02). Clearly, the decrease in optical density is due to light diffusion by particles and not to color (absorption).

Figure 1 show that the OD decreased in the weak AS concentrations domain, passed by a minimum and increased again. How to explain this OD drop? We often noticed that wastewater treatment plants flocs have coagulating properties and are able to “stick” particles. Thus, when the wastewater particles are in small quantity (<1%), they are adsorbed on the flocs and the OD decreases. For a particular AS concentration (towards CREL=1.5%), the flocs are saturated with wastewater particles and the OD is minimal. Thereafter, any AS addition initiates the OD increase.

Remark on statistical analysis: The experiment described here was repeated several dozens of times, by different operators. The results of some experiments were statistically processed ([1] and others). We do not reproduce these statistics in this article because it is relatively insignificant in a WWTP where sampling can never be reproduced in situ. We can characterize the performance of measurements by a large variability, however, the results are fairly reproducible on conditions that samplings were performed on the same WTTP and with short intervals of time (1-2 days) and as far as possible, under the same conditions (day-time, aeration phase, etc.)

Representation of the optical density due to several particles: Note: Light/matter interaction [7,8] is a very complex phenomenon and is not yet fully understood [9,10]. It is obvious that the equations we are using here are rough 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 intensity of transmitted and incident light through an optical path of length h (thickness) is given by

$$\frac{I_t}{I_0} = \exp\left(-\alpha_{ext} h\right)$$

where $I_0$ 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

$$\alpha_{ext} = \sum N_i \sigma_i^T$$

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

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

$$\frac{I_t}{I_0} = \exp\left(-h \sum N_i \sigma_i^T\right)$$

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

$$OD = h \sum N_i \sigma_i^T$$

The general relation (4) expresses that the OD is simply proportional to the sum (on all the categories) 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. One thus has that

$$\sigma_i^T = \sigma_i^{abs} + \sigma_i^{dif}$$

from where

$$\alpha_{ext} = \sum N_i (\sigma_i^{abs} + \sigma_i^{dif})$$

which expresses the extinction coefficient according to absorption and scattering cross sections.

When the absorption phenomena are negligible compared to scattering, as it is the case in our observations, the extinction coefficient can be approximated by

$$\alpha_{ext} = \sum N_i \sigma_i^{dif}$$

The optical density then reduced to

$$DO = h \sum N_i \sigma_i^{dif}$$

We think that general and particular relations (4) and (8) can be cautiously generalized. Indeed, the formalism remains the same, but the meaning of the terms changes according to whether particles are interacting or not. To clarify that, we will reason on a simple small model:

The binary model: The model known as “binary” uses only two types of particles (P1 and P2), each species adsorbing to each other’s. In other words, the sole depicted interactions concern, say, the adsorption...
of P1 on P2. In spite of considering that all the particles are identical, we can interpret the data as average values. Thus, we can imagine that the P2 particles, larger than particles 1, are able to fix, on average, x of these particles, and form “complexes” according to the scheme

$$xP_1 + P_2 \rightarrow P_1[xP_2]$$

(9)

Without anything to change with the final conclusions (and for reasons which will appear further) we can assume that there is a not complexing (not reactive) fraction of P2 particles, which does not adsorb any particle of type 1:

$$P_2 \rightarrow P_2$$

(10)

Let us designate by this not complexing fraction. The mass balance (the number of particles is presumed constant), imposes that the complexing fraction is then, the sum of the two fractions being equal to 1.

The “reactive mixture” of the particles can be depicted as in Figure 3.

### Optical density of separate systems and mixture without interactions

Let us now express the optical density of the separate systems. The separate systems consist of only one type of particle (1 or 2), with concentrations and in a dispersing medium identical to that of the mixture.

For the separate (= isolated) system 1 one can write that:

$$DO_1 = N_1\sigma^T_1 h$$

(11)

where is the cross section of particle 1 (for the selected wavelength and including the necessary constants, etc.). (The index T indicates the total cross section, and not that of diffusion alone. This is done to ensure a general reasoning, but it is obvious that, in the real systems that we study, the diffusion is the governing phenomenon).

N1 is the number of particles 1; h is the optical path (constant).

In the same way, for the separate system 2:

$$DO_2 = N_2\sigma^T_2 h$$

(12)

Let us now define a quantity, DOT, representing the sum of the optical densities of the two separate systems

$$DO_T = DO_1 + DO_2$$

(13)

It is clear that

$$DO_T = h\left(N_1\sigma^T_1 + N_2\sigma^T_2\right)$$

(14)

This quantity doesn’t have, a priori, any particular meaning, because it represents the sum of two measurements taken on separate (independent) systems. However, two physically separate systems (in different spectrophotometric sample cells, for example) can be compared to systems physically in contact but not interacting. If this is the case, by really carrying out the mixture of the two systems, one obtains a resulting optical density, DOM such as

$$DO_M = DO_T$$

(15)

In conclusion, concerning the optical density, the mixed systems do not interact, since they behave like separate systems. (It should be noted that DOM, contrary to DOT, has a well-defined physical meaning: the optical density of the mixture.)

The result obtained can seem commonplace, however relation (15) permits the following conclusion: In a system without interaction, the optical density of the mixture is equal to the sum of the optical densities of the separate systems; it follows that the optical density of the mixture is higher than that of each system (DO=0 being excluded) and thus

$$DO_M > DO_i \forall i$$

(16)

### Optical density of a mixture in general

Let us consider the optical density of the two following separate systems:

a) H2O + NaOH (aqueous solution of sodium hydroxide);

b) alcoholic solution of phenolphthalein.

Measured separately at a “red” wavelength and with water as blank, the OD of each system is very low (if not zero). When the mixture is achieved, phenolphthalein solution will develop a deep red color and the optical density will be high.

This simple example shows that the relation (15) is a particular case that is far from being always satisfied. In fact, any interaction between the two systems will involve that

$$DO_M \neq DO_T$$

(17)

The example of the phenolphthalein shows that the optical density of a mixture can be higher than the sum of the OD’s of the separate systems. We observed a phenomenon where, on the contrary, the OD of the mixture is lower than the sum of the OD’s of the separate systems.

Note: Relation (17) necessarily implies an interaction, whereas (15) does not absolutely imply the absence of interactions. One can imagine compensation phenomena satisfying (15) in spite of modifications which have taken place at the time of the mixture.

In a completely general way, the optical density of a mixture will be represented by

$$DO_M = N_M\sigma^T_M h$$

(18)

and no correlation can a priori be established between optical densities of the separate systems and that of the mixture.

We make the assumption that for weak interactions (typically, physicochemical interactions not involving dramatic particles modifications or important color change), the optical density of the mixture is very nearly decomposable in additive terms and that
\[ DO_M = \sum_i N_{i,M} \sigma_{i,M}^T h + \phi(.) \]  
(19)

where CM is the number of possible combinations between “the different objects” (particles) forming the system and Ni, M the number of particles of species i in the mixture. The term is a corrective term, assumed to be negligible in the binary model. Using (19), the optical density of the mixture can be represented by the following approximation:

\[ DO_M \approx \sum_i N_{i,M} \sigma_{i,M}^T h \]  
(20)

In the binary model, described in (9), we consider only three significant combinations, P1, P2 and (P1P2), the complex. Thus CM=3 (see (19)) and relation (20) becomes

\[ DO_M = N_{1,M} \sigma_{1,M}^T h + N_{2,M} \sigma_{2,M}^T h + N_{3,M} \sigma_{3,M}^T h \]  
(21)

This relation is general and applies if relation (20) applies and if contributions of only particles 1, 2 and of the complex are significant. When only weak interactions exist (without chemical reactions, for example), there is no phase change and all the matter remains confined in the sample cell. The mass balance must thus apply. It should be noted, on the other hand, that the number of particles before and after mixing may change (and must change, if complexes formation occurs). In the same way, there is no reason for the cross section of separate particles to be identical to the cross section of particles in the mixture. We will now search for the expression of the OD of a mixture using the mass balance. To keep the definition of the optical density used up to now, we will establish the balances in term of number of particles.

**Balance 1**

Let us imagine, according to schemes (9) and (10) that particles 1 is in excess, i.e. some P1, remain free after mixture. Under these conditions, the following relation between the number of free particles 1 before (N1) and after (N1,M) mixing exists:

\[ N_{1,M} = N_1 - (1-\theta)N_2 \]  
(22.a)

This relation expresses that the number of free particles 1 in the mixture is equal to the number of particles 1 before mixture, minus the number of particles 1 allotted to the formation of complexes. This last value is equal to the complexing fraction (\(\theta\)) of particles 2 multiplied by x (the number of particles 1 fixed by particle 2).

In the same manner:

\[ N_{2,M} = \theta N_2 \]  
(22.b)

who expresses that the no complexing particles 2 are in equal number before and after mixture.

About the complex, it comes that

\[ N_{3,M} = (1-\theta)N_2 \]  
(22.c)

Particles 1 being in excess, the number of complexes is equal to the number of complexing particles 2.

Using the relations (22), the optical density of the mixture can be expressed according to the number of particles existing before the mixture. Injecting (22) in (21) leads to

\[ DO_M = h \left[ N_1(1-\theta)\sigma_{1,M}^T + \theta N_2 \sigma_{2,M}^T + (1-\theta)N_3 \sigma_{3,M}^T \right] \]  
(23)

This relation clearly reveals a negative contribution in the right hand side of (23). Under the condition that

\[ x(1-\theta)\sigma_{1,M}^T > \theta \sigma_{2,M}^T + (1-\theta)\sigma_{3,M}^T \]  
(24)

the N2 dependent factor becomes negative. Hence, if all the other values are constant, the optical density of the mixture decreases linearly with N2.

**Conclusions**

In spite of its simplicity, the binary model, by means of only two types of particles and a complex, is completely capable to account for the optical density diminution of a particulate mixture when the number of the complexing particles is increased.

**Particular case**

If all the particles would be complexing, then and (24) becomes:

\[ x \sigma^T_{1,M} > \sigma^T_{3,M} \]

or:

\[ x > \frac{\sigma^T_{3,M}}{\sigma^T_{1,M}} \]  
(25)

This shows that the condition is satisfied when the number of sites on the complexing particle is higher than the ratio of the complexes cross section over the particles to adsorb cross section.

**Balance 2**

Relation (22.a) clearly shows that the above analysis is true only if N1 is in excess. If not, N1,M would become negative, which is impossible. When particles 2 are in excess (i.e., when all particles 1 are adsorbed), the relations before and after mixing are as following:

\[ N_{1,M} = 0 \]  
(26.a)

expressing that all particles 1 are complexed.

\[ N_{2,M} = \theta N_2 \]  
(26.b)

meaning that the only not complexing particles 2 remain in the mixture.

\[ N_{3,M} = N_1/x \]  
(26.c)

The number of complexes is equal to the number of particles 1 divided by the average number of sites, x. (Note: this relation is approximate, because the number of complexes is an integer only if x is a multiple of N1. The approximation is however sufficient within the framework of this work, where the number of particles is large.)

While injecting these values in (21), it comes that

\[ OD_M = \theta N_x \sigma^T_{1,M} + \frac{N}{x} \sigma^T_{3,M} \]  
(27)

This time, for all values being constant, the optical density of the mixture varies linearly with N2 and is positively correlated. This form is identical to the usual Beer-Lambert’s law.

**Critical case**

For a system where all the parameters are constant and for which (24) 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

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is thus a critical value in N2 corresponding to a minimal value of DOM. This value exactly corresponds to the condition. The critical value is easily calculated from (22.a):

\[ N^* = \frac{N_1}{x(1-\theta)} \]  

(28)

An abridgment of the results is schematized on Figure 4.

Discussion

The ideal optical measure should be of nephelometric nature, i.e., a measure of particles light scattering in various directions (various scattering angles with optical axis). In some precise applications, turbidity is the appropriate method (which is a 90° nephelometric measurement with). These measurements are characterized by their proper units (NTU, for example). However, in numerous cases, the optical data measured is the light absorption carried out in a colorimeter or in a spectrophotometer (what could be considered as "a 180° nephelometric measurement"). Optical density (OD) or transmittance is the turbidity units. A standardization curve is drawn, generally using the dry weight as biomass estimate. The linear part of this curve allows establishing the correlation between dry weight and optical density. The precision of this method is generally rather poor and strongly depends on the system under study. This drawback however is acceptable if the method is proven to be sufficiently reproducible.

Although the purpose of this article was to describe a general methodology using simple optical instrumentation to study complex particulate interactions, we think that the obtained results make it possible to carry out interesting applications. Thus, relation (28) is valid to define some strategy for using and optimizing complexing particles. Indeed is the number of particles 2 corresponding exactly to the adsorption of all particles 1. In the development of a process aiming at carrying out this mixture under the most advantageous conditions, this problem consists in minimizing this critical value. Once N1 is specified, there are two manners of minimizing (28):

a) acting on \( \theta \).

b) acting on x.

The nature of the adsorbent (or complexing, chelating, etc.) sites is not always clear. It is likely that numerous types of active sites are involved in the process of “binding” wastewater particles to the flocs. In any case, if one considers specific active sites of protein nature, for example, those often amount per thousand on only one cellular envelope. One can expect that the active sites on the flocs are in great number or potentially in great number. It is consequently clear that a significant increase in x must make it possible to strongly decrease the critical number of adsorbent particles.

In short, the binary model enables us to test a strategy for the development of an adsorbent medium:

I) to enrich the medium in complexing particles, if necessary.

II) “to create” the greatest number of active sites. This step is by far the most interesting in increasing the process yield and efficiency.

However, a remark is essential: Figure 4 shows that the binary model predicts linear variations of the OD_M on both sides of the critical value. Figure 2 shows clearly that such is not the case when the N_2 concentration becomes high: a saturation effect obviously appeared. The applicability of the binary model must thus be cautiously examined before being used. In this example, the model seems to apply when relative concentration ranges from 0.5 to 2.5%. Beyond this value, other interactions can intervene (like “complex-floc”, “complex-complex”, etc.). More complicated models can thus be built and assorted optimization strategies can result.

We showed that previous considerations permit to construct dynamic mathematical modeling, but, depending on the phenomenon, the validity of the results strongly depends on parametric estimation and systems rapidly become undetermined.

Conclusions

To conclude, we would like to highlight the fact that the method described in this paper allows to easily and rapidly obtain information that differently would require considerable efforts and money. This is indeed the “power” of the method and the good reason to improve it. But the method alone probably suffers also some “weaknesses”. In particular, the parametric estimation based on the sole OD’s measures is often “underdetermined” and it is difficult to guarantee that the results obtained during the minimization algorithm represent the global minimum. A straightforward way to reduce or circumvent this drawback consists in completing the data by others and unrelated measurements. In this perspective, the best way to improve the method without excessively lessening its capacity consists to optimize the “information gain” versus the "experimental and/or mathematical price". However, in view of our current experience, the convergence of the results on several examples suggests that the results yet obtained by the present method are probably significant (if not absolutely exact).
Acknowledgment

This article is signed by me, because it is my original idea and I was the sole to have written this manuscript. However, we must do justice to a multitude of people who have more or less participated in the experiments.

First, the society REALCO and his Manager, Mr. Blackman has made a major contribution to the sponsoring of this project (embedded in another). The first part was held in the lab of Prof. Agathos, at the GEBI (UCL-BIOPUR Convention; agreement with the Walloon Region); another in the service of Prof. Penninckx at the UPEM (ULB-FLOZYM Convention, No. 4069 in the Walloon Region) [11,12].

Dr. Jaouani was tasked to study some aspect about ultrafiltration at the University of Tunis (unfortunately without result).

The largest contribution is probably that of Quentin GALAND (Galand, 2005) during his final work for civil engineering chemist. Unfortunately, he wanted to make his cytometry measurements at 400 nm (instead of 600 usually), which makes their measurement not comparable to other results.

Some students accomplished experiments on the equipment that I had designed and built, and have validated a series of results.

I never managed to gather all the persons to write a collective work, so I took the initiative to write a (very simplified manuscript) and bring justice and thank those who have participated.

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