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# Modification of Activated Sludge Cohesion by Enzymatic Catalysis Process and Perspectives

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## Abstract

It is possible to transform the structure of organic compounds so as to modify some of their properties to make them more "elastic", for example. Many solid-liquid separation processes use the cohesion property of the solid in suspension to increase the performance of the separation system. In this work, we examine some beneficial aspects that enzyme catalysis may reveal when acting on the strength of cohesion of a suspension. The topic concerns the activated sludge (AS) produced by wastewater of urban (or industrial) treatment plants. We used urban sludge and industrial liquid enzymes produced by the firm NOVO. To accelerate the research process, we employed enzyme mixtures (we called "enzyme cocktails") that highlighted the active mixtures, but until now we have not characterized specifically active enzymes. Despite a significant number of experiences, it is clear that given the number of different enzymes available and the almost infinite possible ratios of cocktails, only a small number of conceivable methods have been tested (and even less optimized). Nevertheless, a few cocktails were active and promising enough to improve solids contact processes in various industrial processes. We have found a range of combinations that increases the sludge cohesion coefficient and considered this enzyme treatment to strengthen the separation by solid contact structures mainly to tackle important hydraulic shock.

**Keywords:** Cohesion coefficient; Activated sludge; Wastewater treatment plant; Settler; Clarifier; Enzymatic catalysis

## Introduction

The need to separate the non-biodegradable suspended solids from a liquid phase (water, for example) will become increasingly urgent over time. Not only because of solid waste, but also because of increased quantities of activated sludge from wastewater treatment plants. Among several methods, the "up flow" process involves passing a fluid stream containing suspended solids from the bottom to the top. Under good conditions, the solid tends to settle and the clarified water leaves the device, to be recovered (in a purification process) or to be released into the environment. Numerous methods based on the principle of an upstream liquid flow have emerged and are applied in various applications. In itself, the idea is simple (although the practical realization is not necessarily easy...). The basic principle of separation is shown in (Figure 1).

The "solid" part may be formed by a highly settling substrate (such as sand or slag) which helps in the suspended solids retention. In other cases, the retaining solid is absent and the "solid" (condensed) phase is only formed by the suspended particles (such as activated sludge of a wastewater treatment plant, for example). In a system like this, there are two flows in the opposite direction: an up flux liquid, flowing to the top and a downward solids settling flux. It is obvious that the process is applicable only if the considered compounds have particular characteristics, the most evident being the decant ability of the solid. But, that is not enough. The upstream liquid flow is characterized by an ascending velocity, $v_{asc}$, which is simply the ratio of the area of the container (the "cylinder" where clarification happens) to its section.

$$v_{asc} = Q(liquor\ volumic\ flux) / S(area\ of\ the\ clarifier)$$

The ascending rate has thus the dimensions of an area per unit time (m/h, for example). This up flow rate has a driving force on the suspended particles, from bottom to top. The less they are settleable, the faster they are driven upward. However, settleability is not the sole factor and they will also move upward more rapidly as they have less "solidarity". The ability of particles to "stick" to each other is called cohesion. This cohesion is quantified by the coefficient of cohesion, generally designated by K (in m/h, for example - ascending velocity units). The greater the interparticle cohesion, the greater the ratio of the lifting force that may be applied. Concurrently, the up flow clarifier performance increases as the ascending rate is higher (since the amount of clarified water is high).

The K factor of cohesion is therefore a crucial factor for clarifier performance since it increases the rate of climb (or overflow). For example, we give some useful overflow values (Table 1). Except for Densadeg, our device (see below) gives values close to systems used in the market. However, we still need to improve these values to improve the process and this is why we rely on an efficient enzymatic treatment. For reasons that will appear later, we focus here on activated sludge from urban wastewater treatment plants. The term "activated sludge" has an historical origin, and which is not really appropriate. This "mud" is in effect a rather compact biomass (several grams of dry matter per

| TYPE | MANUFACTURER | OVERFLOW RATE (m/h) |
|---|---|---|
| PULSATOR | DEGRÉMONT | 3-7 |
| AQUACYCLE | S.A.U.R | 3 |
| CIRCULATOR | DEGRÉMONT | 2 |
| DENSADEG | DEGRÉMONT | 10-20 |

**Table 1:** Sludge contact settlers in the market.

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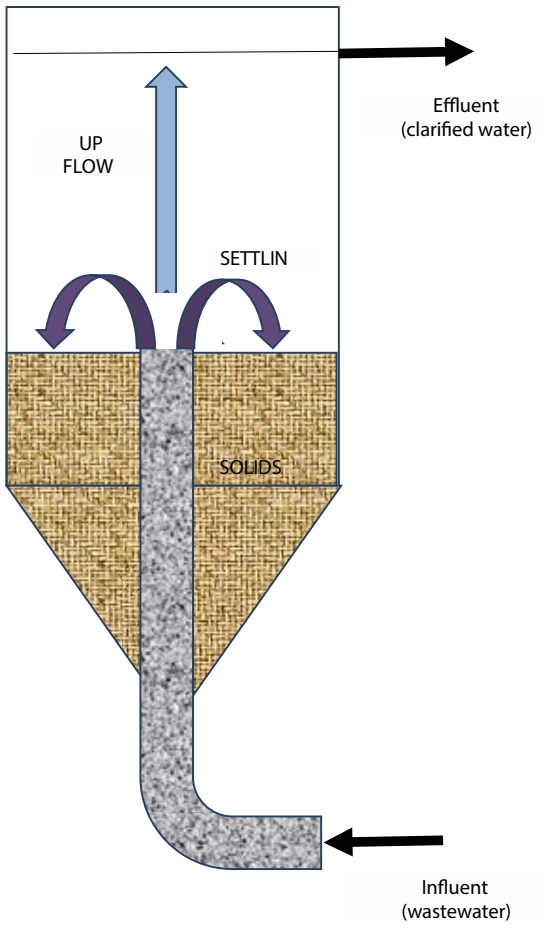



**Figure 1:** Schematic diagram of an up flow process.
The upwardly driving force of the liquor creates an ascendant velocity of the fluid; in the same time the solid particles settle. The volumic mass of the settling particles is generally very close to 1 g/cm$^3$, making the separation delicate.

liter), mainly composed of bacteria. These bacteria are embedded in flocs, a kind of "slime" structure of very complex composition which form a suspension in the mixed liquor. Just as for biofilms (which are fixed), the floc consists of microbial extracellular polymeric substances [2,3]. The chemical compositions thereof are very diverse, ranging from simple proteins, carbohydrates, polysaccharides, various nucleic acids, etc. The EPS turn out to be important, but play an only poorly understood role in floc cohesion, sludge grain and biofilms. The extraordinary diversity of chemical compounds and the relative misunderstanding of the phenomena dictated our strategy of using enzyme mixtures ("cocktails") to select more quickly effective mixtures although we will have to make it simpler later. Here we present our methodology, and some encouraging results. We are mindful of the immense work that continues to be answered.

## Materials and Methods

### Assembly

The apparatus (Figure 2) includes a large stand (2 m) in the top of which a graduated 2 L Squibb funnel is fixed (index: *amp*). A PTFE stopcock allows a continuous regulation of the flow from the funnel. A long silicone (or rubber) tube joined an ordinary glass tube that almost reached the bottom of a graduated cylinder (index: *GC*) 250 mL. The cylinder is placed in a tank which collects the excess effluent. On the course of the rubber tube a simple device (Figure 2a and 2b) allows to generate a discontinuous flow [1]. The dimensions of the graduated cylinder are: BRAND Din B (Silber BRAND 250 mL graduated 2 in 2 ml (error at 20°C: 1.5 mL) DURAN Germany External diameter: 3.86 cm internal diameter. 3.5 cm; Height: 31.5 cm; height to 250 mL: 25 cm. ΔH per 100 mL: 10 cm. (These dimensions are important for comparing data obtained elsewhere or to compare with the Degrémont method sometimes used for patents; Boutin, Combet, Communal) [4].

### Filling

The filling liquid of the device is the effluent from the wastewater treatment plant where the sludge is coming from (to avoid differences in pH, osmotic strength, etc.). It is imperative to eliminate all bubbles in the unit (a small volume of effluent at the bottom of the graduated cylinder was often been useful). After completing the circuit, the Squibb stopcock is closed and the bulb carefully zeroed (always with the specific WWTP effluent of the studied sludge).

### Sludge re-concentration

It is sometimes more effective to work with re-concentrated sludge. A sample of stirred AS $V_{in}$=100 ml (for example) is placed in a graduated vessel. After settling, a fixed volume of supernatant ($V_{out}$) is carefully removed (by pumping, for instance) without disturbing the settled sludge. The final concentration of the sludge is then $M_{AS}/(V_{in}-V_{out})$ (instead of $M_{AS}/V_{in}$).

### Enzyme Cocktails

We used several enzyme mixtures of various formulas ("cocktails"). *NOVO* brand, they were used in liquid form and stored at less than 6°C. Proteases were added to the cocktail at the last moment. Unfortunately, the compositions of these cocktails are subject to a confidentiality agreement and cannot be disclosed here. (To quote the main: *NEUTRASE*: protease used to more or less deeply broken proteinaceous matter; *LIPOLASE*: the first fat-splitting enzyme; *VISCOZYME L*: cellulolytic enzyme mixture; *FLAVOURZYME:* an exo-protease; *AQUAZYM*: is used to remove starch, etc). The processed AS (index: *TR*) were mixed with the enzymes and agitated via magnetic stirrer for variable periods (from a few minutes to several hours), usually at room temperature (20-25°C).

## Procedure

We tested the action of enzyme catalysis using two methods, starting in the same manner. An initial volume of AS (reconcentrated or not), V0, is introduced into the bottom of the graduated cylinder. During a very short time, the Squibb funnel stopcock is opened to adjust a "reasonable" flow of the effluent (neither too fast nor too slow).

### Method 1 (M1)

This procedure consists in carrying out the following steps batch wise. The activated sludge being at its original volume (V0), discontinuous liquid volumes are added (thanks to device of Figure 3) to a desired volume AS ($V_{SA}$=75 mL, for example) in the graduated cylinder; the effluent is constantly discontinuously added to maintain the wanted volume for a fixed (long) time (two to three minutes). Stop the flow and reduce the AS back to the initial volume, V0; proceed as above for another value of $V_{AS}$ (100 mL, then 150, and so on starting each time from V0 again). This method is relevant, if we admit that the AS swelling/deflation process is reproducible (or sufficiently reproducible). This method can be called "maintenance of swelling" at

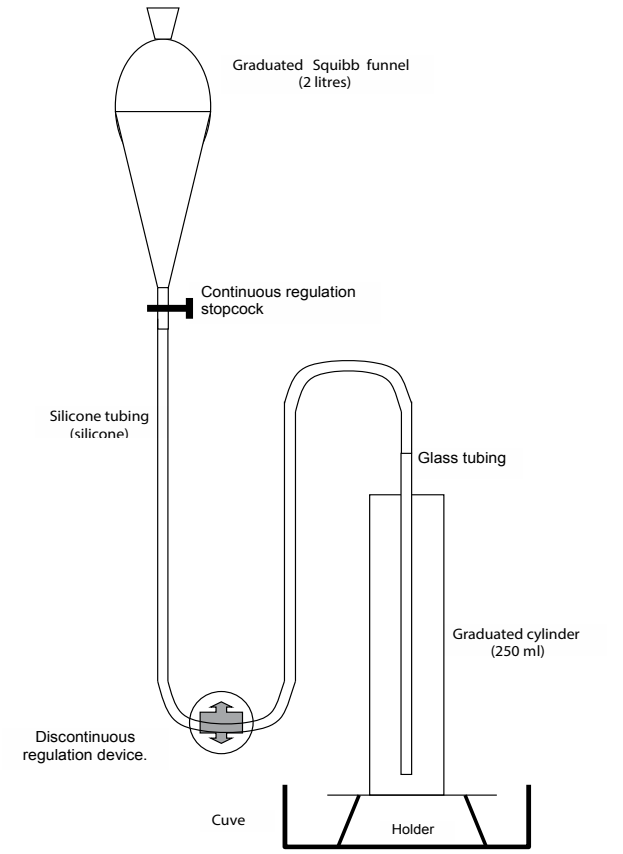



**Figure 2:** Scheme of the general assembly for determining the cohesion coefficient K.

a given value.

## Method 2 (M2)

The previous procedure (M1) includes two factors: first, the activated sludge is brought to a desired volume; secondly, it is maintained in this volume. The flow time was short compared to the total time. In the second method (M2) conversely, the aim was to bring the sludge from V0 to $V_{AS}$ as fast as possible, the principle being to keep a front sludge (sludge blanket) as demarcated as possible. The procedure was the same as in M1, but the measurement is interrupted when $V_{AS}$ is reached. Here we speak about a "propelling" flow of AS rather than a maintenance flux.

In both cases, it is necessary to simultaneously measure:

- The flow volume in the Squibb funnel ($V_{AMP}$);
- The corresponding time ($t_{AMP}$). This time corresponds to the duration of the flow admitted through the discontinuous device of Figure 3.
- The sludge volume in the graduated cylinder ($V_{AS}$).

Both methods give results which obviously depend on the cohesion of the sludge that is to say their ability to "stretch" and to maintain their consistency.

## Results

We applied successively both methods (M1 and M2) on the same enzyme-treated (T) and untreated (NT) sludge. Treated or not, the AS have always been mechanically stirred in the same manner, in order to have identical manipulations in both cases (with and without treatment). We showed that this could be important for sticking phenomena and flocculation sludge [5] we performed a dozen experiments using different enzyme cocktails (differing both qualitatively and quantitatively). Each experiment included 4 results as well, according to the diagram below (Table 2).

Here are the synthetic results of a representative experiment (enzymatic cocktail: CKTL01). The sludge was taken at the wastewater treatment plant of Nivelles (Intercommunale du Brabant Wallon - IBW). The dry matter was 4.29 ± 0.13 gDW/L. The **T** sludge was treated by the CKTL01 enzyme mixture for 1 hour at 17.7°C, with magnetic stirring at 250 RPM. In all four cases, 50 mL of sludge were reconcentrated to 30 to 40 mL (V0) by careful aspiration of the supernatant. The dry matter was of course modified (Table 3a-3d).

The values of the sludge cohesion coefficient were obtained by linear regression using the graphs

$$v_{asc} = K\left(\frac{V}{V_0} - 1\right)$$

that is to say, straight lines [1] relating K and the ascending rate in the graduated cylinder. K has thus the units of an ascending velocity. The cohesive "force" of K is inversely proportional to its value as shown in Figure 4. (It is easily seen that the sludge volume in the graduated cylinder ($V_{AS}$) decreases rapidly with K. In our specific example, it is calculated that the sludge comes out of the cylinder for K ≈0.5 m/h). Figure 5a shows tests of treated sludge using method 1, keeping sludge level for 2-3 minutes (and EXP1 EXP3). The K values are very similar (2.5 and 2.62 m/h respectively). The enzymatic treatment doesn't seem to be efficient for the maintenance of sludge in the cylinder. On the contrary, Figure 5b shows a significant difference, with cohesion coefficients of 2.5 for treated sludge and 3.53 m/h for untreated sludge, so they are less cohesive.

## Discussion

We have described a promising method for the modification of the cohesion coefficient of activated sludge. Earlier results specifically apply to resistance to hydrodynamic shocks (sludge displacement, not constant level change). Without wishing to insist any more on the issue, resistance to hydraulic shock is also an important advantage, particularly for more common "gravity" settlers, which likewise present an ascending rate, unavoidable for the flood. The problem is broader than our previous work suggests. (We have concentrated on the cohesion measurement methodology rather than on the usable practical results). In a critical discussion on the enzymes uses for improved treatment processes, it is not possible to ignore the high cost of enzymes usages. First, we tested at this point some industrial extracted enzymes, sometimes roughly purified, generally in a liquid form (sometimes solid, making them more stable). Once the studies performed with known products, it is obviously possible to use the whole microorganisms rather than expensive extracts. The downstream processing could obviously greatly be reduced. It is even conceivable to grow these producing microorganisms directly on the site of the plants, directly on unchanged, or only slightly amended wastewater, in parallel with the purification processes. (One can expect to get good results with fungi, robust and very rich in various enzymes). We believe then that the process we proposed is a preliminary step to an integrated work on site deployment. Our way of proceeding as described above, is expected to greatly facilitate the screening of enzymes and producing

| | | |
|---|---|---|
| Method 1 Maintenance flow | Without enzymatic treatment NT-EXP1 | With enzymatic treatment T-EXP3 |
| Method 2 Propelling flow | Without enzymatic treatment NT-EXP2 | With enzymatic treatment T-EXP4 |

**Table 2:** The EXPz label designates method and the presence or absence of enzymes.

A

| EXP 1 | $V_{AS}$ (mL) | t (s) | $V_{AMP}$ (mL) | $V_{asc}$ (m/h) | F(V) (nu) |
|---|---|---|---|---|---|
| | 75 | 153 | 100 | 2.48 | 0.94 |
| V0=39.6 mL | 100 | 193 | 215 | 4.22 | 1.59 |
| K (m/h)=2.50 ± 0.15 | 125 | 185 | 250 | 5.12 | 2.24 |
| | 150 | 182 | 350 | 7.29 | 2.89 |
| r=0.99 | 175 | 124 | 275 | 8.40 | 3.53 |
| | 200 | 96 | 275 | 10.86 | 4.18 |

B

| EXP 2 | $V_{AS}$ (mL) | t (s) | $V_{AMP}$ (mL) | $V_{asc}$ (m/h) | F(V) (nu) |
|---|---|---|---|---|---|
| | 75 | 19 | 30 | 5.98 | 1.63 |
| V0=28.5 mL | 100 | 32 | 75 | 9.02 | 2.51 |
| K (m/h)=3.53±0.45 | 125 | 35 | 100 | 10.92 | 3.39 |
| | 150 | 36 | 150 | 15.66 | 2.89 |
| r=0.97 | 175 | - | - | | - |
| | 200 | 77 | 240 | 11.89 | 7.18 |

C

| EXP 3 | $V_{AS}$ (mL) | t (s) | $V_{AMP}$ (mL) | $V_{asc}$ (m/h) | F(V) (nu) |
|---|---|---|---|---|---|
| | 75 | 183 | 100 | 2.07 | 0.94 |
| V0=38.6 mL | 100 | 177 | 210 | 4.50 | 1.59 |
| 3.53 ± 0.45 | 125 | 141 | 225 | 6.05 | 2.24 |
| | 150 | 137 | 275 | 7.60 | 2.89 |
| r=0.99 | 175 | - | - | - | - |
| | 200 | 123 | 350 | 10.78 | 4.18 |

D

| EXP 4 | $V_{AS}$ (mL) | t (s) | $V_{AMP}$ (mL) | $V_{asc}$ (m/h) | F(V) (nu) |
|---|---|---|---|---|---|
| | 75 | 18 | 50 | 10.58 | 1.50 |
| V0=30 mL | 100 | 20 | 70 | 13.07 | 2.33 |
| K (m/h)=2.5 ± 0.28 | 125 | 26 | 90 | 13.22 | 3.17 |
| | 150 | 28 | 125 | 16.90 | 4.00 |
| r=0.98 | 175 | - | - | - | - |
| | 200 | 37 | 190 | 19.25 | 5.67 |

# EXP=Experiment number
V0=AS initial volume in the graduated cylinder (t=0 sec) in mL
K=AS cohesion coefficient (m/h)
r=Fitting correlation coefficient
$V_{AS}$=Sludge volume to obtain (or to maintain) in the graduated cylinder (mL)
t=Time (in sec) necessary to attain (or to maintain) $V_{AS}$
$V_{AMP}$=Elapsed volume from the Squibb funnel during the above time
$V_{ASC}$=Ascending velocity in the graduated cylinder $F(V)=K\left(\frac{V}{V_0}-1\right)$ where A is the section of the graduated cylinder minus the section of the glass tubing supplying the effluent; m/h)
F(V)= $F(V)=K\left(\frac{V}{V_0}-1\right)$ to keep the Degrémont [1] representation (nu=no units)

**Table 3:** Typical results of an experiment.

microorganisms. There is yet another aspect to consider about the cost price of the process. Following an estimate of Leonard [6] from the University of Liège, there may be an annual sludge production of around 13 $10^6$ tons (dry weight, DW) in 2020 in Europe (via Eurostat). According to an ADEME and CEMAGREF [7] study, the costs per ton of DW activated sludge could spread from 300 to 555 €; based on these partial data, we would spend around 4 to 6,500,000,000 euros merely to treat sewage sludge. Even approximately, these amounts are huge! Two approaches are possible to mitigate such costs: reducing the production and recovery of such waste. Clearly these two ways of remediation are

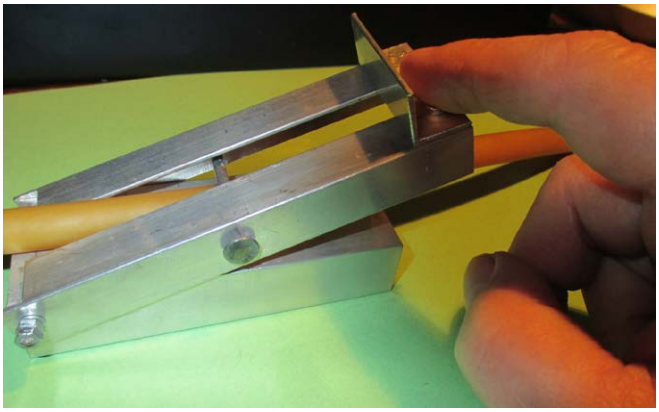


**Figure 3a:** The elasticity of the rubber tube maintains a high position of the lever. This lever comprises a clip that reposes on the tube but does not crush it: the fluid flows.

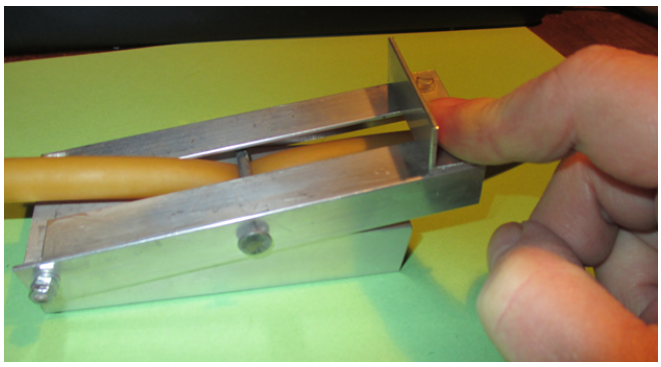


**Figure 3b:** A push of the lever compresses the tube with the clip: the fluid doesn't flow anymore. The device is made of aluminum profiles, screwed and steel axes. The set is very easy to construct and has proven very effective.

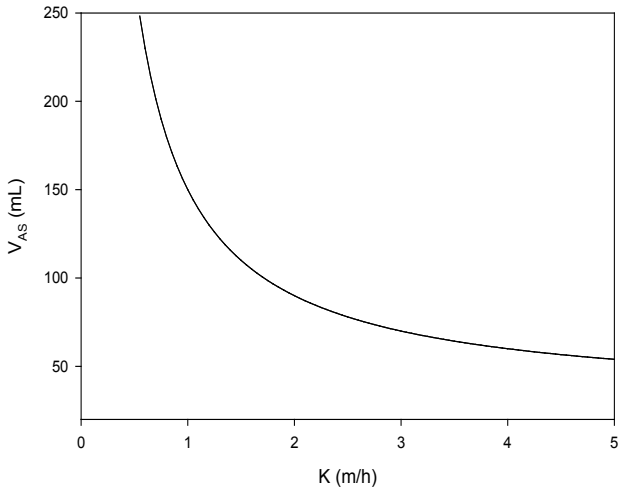
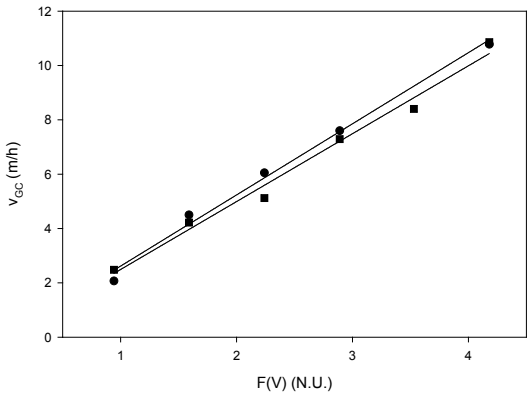



**Figure 4:** Volume of activated sludge (VAS) in the cylinder as a function of cohesion coefficient according to the "classical" relation (constants: V0=30 mL; Vasc upload velocity=4 m/h).
The figure shows that the sludge volume (and thus its height) in the cylinder is higher when K is small. The low cohesion leads to greater sludge expansion for (Vasc) equal ascending rate.




Ascending rate as defined by the Degrémont (1989) relationship.

$v_{asc} = K\left(\frac{V_{asc}}{V0} - 1\right)$

This relationship is a straight line allowing to determine the value of the cohesion

**Figure 5a:** This figure represents the result of the method maintaining the sludge height constant in the graduated cylinder at different ascending rate ($V_{asc}$=$V_{GD}$). One sees that the straight slopes for AS processed (●) and untreated sludge (■) are almost identical. K(●)=2.50 ± 0.15 m/h ; r=0.99 / K(■)=2.62 ± 0.13 m/h ; r=0.99. coefficient K by linear regression (NU=no units).

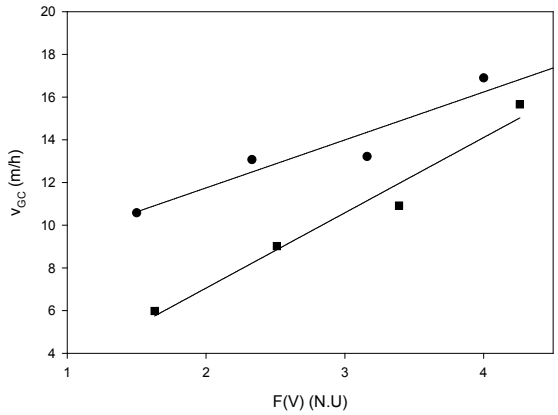



**Figure 5b:** This figure represents the result of the method bringing the height of the sludge in the graduated cylinder as quickly as possible to different volumes values ($V_{asc}$=$V_{GD}$). One sees that the straight slopes for treated AS (●) and untreated sludge (■) are now distinct. K(●)=3.53 ± 0.45 m/h ; r=0.97 / K(■)=2.50 ± 0.28 m/h ; r=0.98.

(or may be) related to sludge cohesion. Some chemical [4] or physical [8] processes could be aligned in this concept and do not necessarily use cheaper or safer processes for the environment (as some flocculants). The use of enzymes as clarifier's stabilizers against hydraulic shocks but also as cofactors in sludge treatment appears as a realistic perspective, after an optimization period of the process both in the hydrodynamic field and from the perspective of treatment and recovery of sewage sludge application [9-12].

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