Biomass Concentration by Density Measurement: Activated Sludge and Membrane Bioreactor

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ABSTRACT
In waste water treatment plant using biological treatment, the treatment efficiency strongly depends on the biomass concentration which controls the mass transfer in the bioreactor for activated sludge and the level of membrane separation for membrane bioreactor. This study shows the possibility of determining the total suspended solids concentration in a short time on activated sludge process or membrane bioreactor using density measurement for urban and industrial effluent. A linear relationship between density and total suspended solids concentration is obtained with a high value of $R^2$ (between 0.924 and 0.9996). The influence of the density-meter measurement modes (fast and slow equilibrium mode), clearly shows that the fast equilibrium mode is adjusted for biomass which can easily settled in the measuring cell. If a very good relation is obtained for each WWTP (Waste Water Treatment Plant), a general linear relation which is determined only for urban effluent and three WWTP is given to determine the total suspended solids concentration without calibration by measuring its density at 20 °C.

Keywords: Density; total suspended solids; activated sludge; waste water treatment plant; biomass concentration

1. INTRODUCTION
In biological Waste Water Treatment Plants (WWTP), polluted influents are mixed with aerobic micro-organisms consuming the pollution in an aeration tank. The resulting suspension composed of water, micro-organisms and exo-cellular polymeric substances coming from both polluted waters and micro-organisms metabolism is called activated sludge. The activated sludge total suspended solids (TSS) concentration is used to estimate the biomass concentration (the activated sludge TSS concentration is supposed to be proportional to the biomass concentration in WWTP). WWTP monitor the TSS concentration of activated sludge (AS) in order to control the water quality as it is a significant part of physical and aesthetic degradation but also a good indicator of other pollutants. Similar comments are summarized by Marrot et al. (2004) in a membrane bioreactor (MBR) review: “this treatment process strongly depends on the biomass concentration which controls the mass transfer in the bioreactor and the level of membrane separation”. The classical method for measuring TSS concentration (APHA, 1995) is time consuming; as consequence scientists are looking for new faster measuring methods with a quite good accuracy. In the past, many researches have been led to determine the biomass concentration instead. Gencer and Mutharasan (1979) introduced a new method to determine TSS concentration by capacitance measurement. A four-prong all-platinum electrode system was used in a modified fermenter vessel equipped with
temperature and pH measurements. This technique gave reliable and reproducible results when conductance of the fermentation broth is low. However, when the conductivity of the suspending medium is high or when an ionizable salt is present, the method fails to detect capacitance changes corresponding to changes in yeast cell concentration. A spectroscopic method for the determination of mycelial biomass was used by Banerjee et al. (1993). The optical density (450 nm) of samples of homogenized fungal biomass was correlated linearly with the dry weight of the biomass in the samples. However, the sensitivity of the measurements, defined as change in dry weight per unit change in optical density, increased with the homogenization. Some recent researches have been leaded to correlate TSS concentration to turbidity for combined sewer system (Hannouche et al., 2011) and rivers (Low et al., 2011; Packman, 1999; Petus, 2010) using a linear regression (with $R^2$ comprised between 0.7 and 0.8) or a log-linear model ($R^2 = 0.96$). Turbidity measurement was selected because it allows rapid determination and it can be used for online measurements. Although density measurement cannot be used for online measurement, it is a fast measurement method that can potentially be used for determining TSS concentration. The use of density measurement is clearly related to the recent developments on this analytic method and the generation of new apparatus.

In this context, the main objectives of this work are to study the ability of density measurement (at 20°C) to evaluate the TSS concentration of WWTP and MBR activated sludge by the development of a new measurement methodology.

2. MATERIALS AND METHODS

2.1 Activated sludge

Four activated sludge studied in this work come from two French urban WWTP using activated sludge (Aix en Provence and Gardanne), one from WWTP using MBR technology (Rousset) and finally one from a pilot scale MBR which treated industrial effluents. Hence, two of these installations use the MBR technology: immersed hollow fibers for Rousset WWTP and external ceramic for the pilot scale MBR. The samplings were conducted between May and October 2013.

2.1.1 Aix en Provence WWTP

Aix en Provence WWTP has a capacity of 165,000 inhabitant equivalent. It can treats up to 40,000 m$^3$ per day of urban waste water which is distributed on two channels of biological treatment. Each channel consists in two concentric tanks. The hydraulic retention time is about 20 hours; the activated sludge residence time is comprised between 15 and 20 days. The activated sludge was sampled in the recirculation loop between aeration basins and secondary settlers. The initial TSS concentrations ranged from 3 to 4 g/L.

2.1.2 Gardanne WWTP

Gardanne WWTP has a capacity of 50,000 inhabitant equivalent. It can treats up to 10,000 m$^3$ per day, on average the WWTP receives 35% of its water capacity. The hydraulic retention time is about 89 hours; the activated sludge residence time is comprised between 31 hours and 4.5 days. The activated sludge was sampled from a nozzle positioned at the bottom edge of the clarifier. The initial TSS concentrations ranged from 5 to 6 g/L.

2.1.3 Rousset WWTP-MBR

The activated sludge was sampled after different pre-treatment in the external immersed MBR of Rousset WWTP. The external immersed MBR consists in 8 hollow fibers modules with a total filtration surface of 3,000 m$^2$. It can treats up to 1,800 m$^3$ per day.
of waste water. The WWTP contains three concentric tanks; the external one (with the higher diameter) contains the biomass used for seeding the MBR. In this tank, the aeration is syncopated in 10 min with air and 40 min without air. The middle tank contains the un-pre-treated effluent. The smaller tank contains the anaerobic phase membrane. The hydraulic retention time is about 25 hours and the activated sludge retention time is between 20 and 25 days. The initial TSS concentration is about 9 g/L. The MBR is used for the treatment of urban effluent.

2.1.4 Industrial effluent-pilot scale MBR

The pilot scale membrane bioreactor is an external MBR composed by an 18 L bioreactor equipped with a cooling coil in order to maintain the biomass at approximately 25°C. The membrane system used is a tubular ceramic membrane (ultrafiltration, Novasep-Orelis, France) characterized by a 150 kD cut off and a 0.2 m² filtration area. Cross filtration was operated with a centrifugal pump which recycled sludge back to the membrane. The influent was provided to the bioreactor with a fed pump. During the acclimation in the external MBR, TSS was stabilized around 11 g/L. The activated sludge was sampled when purging the 18 L bioreactor. The pilot scale MBR is used for the treatment of olive mill wastewaters (OMWW) generated by the oil extraction.

2.1.5 Samples preparation

The selection of these four types of waste allows to take into account the various existing processes (AS and MBR). Both types of urban and industrial effluent are tested. Samplings were carried out several times on all sites to take account of the seasonality and/or changes in processing conditions.

In order to obtain a large range of TSS concentrations, sludge was diluted with its own permeate obtained after soft (gravimetric) filtration using paper filters (average size of pores around 100 µm). This filtration process and more especially the concentrate was also used to concentrate the activated sludge.

2.2 Determination of activated sludge TSS concentration

The activated sludge TSS concentration was determined by centrifuging 30 mL of sampled sludge during 15 min at 13,500 rpm (Sigma, model 2-16, Germany). After centrifugation, the residue was collected in an aluminum cup and introduced in an oven (Nabertherm LE 21/11/R6) at 105°C during 24 h in order to evaporate all water contained in the sample. The activated sludge TSS concentration was then calculated using the weight of dried residue [Mettler Toledo, AK160].

2.3 Density-meter

2.3.1 Principle

The activated sludge density was measured with an Anton Paar DMA 5000 M density-meter. The sample is introduced into a U-shaped borosilicate glass tube of about 1 mL, shown in Fig. 1, which is being excited to vibrate at its characteristic frequency. The characteristic frequency changes depending on the density of the sample. Through a precise determination of the characteristic frequency and a mathematical conversion given directly by the apparatus, the density sample can be measured. The sample was introduced in the measuring cell using a 2 mL syringe. The unit displays the result with a precision equal to 10⁻⁶ g/cm³. The TSS concentrations studied ranged from about 2 to 28 g/L. This concentration range reflects the biomass concentration found in all WWTP using conventional or membrane technology.
2.3.2 Density-meter measuring modes and methods

The density-meter offers the possibility to work with different equilibrium measurement mode: predetermination, equilibrium fast and equilibrium slow. The equilibrium depends on the temperature settled, for example if predetermination mode is selected, the instrument finishes the measurement before temperature equilibrium was reached and calculates the density at the set temperature in advance. As a consequence, the slower the equilibrium, the more accurate are the results as the measuring cell will be equal to the set temperature. In this study, density was measured at 20°C ($d_{20}$), measurements were conducted at equilibrium fast and slow modes. Measurement with equilibrium fast and slow mode can take up to ten of seconds and five minutes respectively. This measuring time did not take into account the cleaning time of the measuring cell.

Steps 2 to 5 are repeated to plot the TSS concentration against the activated sludge density. Each measurement was at least repeated two times after cleaning and drying the measuring cell. The permeate density, filtration with a paper filter (100 microns) was also measured for each activated sludge as it corresponds to initial point of TSS concentration 0 g/L determined by the determination of activated sludge TSS concentration method (2.2).

In order to evaluate the accuracy of measurements (density and TSS concentration), the uncertainty of the measurements and the standard deviation were determined:

$$U = \frac{S(x)}{\sqrt{N}}$$  \hspace{1cm} (1)

$$S(x) = \sqrt{\frac{1}{N-1} \sum_{i=1}^{N} (x_i - \bar{x})^2}$$  \hspace{1cm} (2)

with:

- $U$ uncertainty of measurement (density or TSS concentration)
- $S(x)$ standard deviation
- $N$ number of measurements
- $x_i$ measurement $i$
- $\bar{x}$ mean

The uncertainty on activated sludge TSS concentration determination was estimated to about $0.13 \times 10^{-3}$ g/cm$^3$, and the uncertainty on
activated sludge density measurement is ranged between $2 \times 10^{-6}$ g/cm$^3$ and $1 \times 10^{-4}$ g/cm$^3$ depending on the origin of activated sludge.

3. RESULTS AND DISCUSSION

This paper presents the biomass concentration given by the TSS concentration as a function of the density value to conclude that with this new method it is easy to determine the biomass concentration in a WWTP.

3.1 Cleaning protocol

The accuracy of the measurements depends on the cleanliness of the U-shaped borosilicate glass tube (measuring cell), as a consequence depending on the sample studied an appropriate cleaning method must be employed. Working with activated sludge leads to develop the following procedure for cleaning the measuring cell after each measurement:

i. Rinse the measuring cell with bleach
ii. Empty the measuring cell
iii. Rinse the measuring cell with distilled water
iv. Empty the measuring cell
v. Dry the measuring cell with the density-meter air pump and wait until the measuring cell is dry
vi. Rinse the measuring cell with acetone
vii. Empty the measuring cell
viii. Dry the measuring cell with the density-meter air pump and wait until the measuring cell is dry.

Steps i to v are accomplished in order to remove efficiently all organics compounds contain in activated sludge from the measuring cell. Steps vi to viii are accomplished in order to remove efficiently all water particles from the measuring cell. After cleaning, air density was checked in order to evaluate the efficiency of the cleaning and drying procedure. Each sample is injected after each cleaning. With this methodology a good reproducibility of the measurement is obtained.

3.2 Effects of dilutions

The correlation curves were plotted using the activated sludge permeate as diluent. It would have been possible to carry out the dilutions with another type of diluent. The effects of diluent for plotting the correlation curves using tap water and French mineral water (Mont-Roucous; pH = 5.85, dry residue at 180°C: 25 mg/L) were studied. This French mineral water was chosen because it is weakly mineralized and because its properties, given in Table 1, are well known.

The characteristics of the diluted activated sludge and the measurement mode used are reported on Table 2. The comparison between the dilutions operated with activated sludge permeates and tap water or Mont-Roucous mineral water is given in Fig. 2.

The results obtained clearly show that the slopes are slightly different. This result was expected as the diluent studied in this part did not have the same chemical properties than the activated sludge permeate. Hence, these results clearly shows that it is necessary to perform measurements with activated sludge diluted with its own permeate in order to plot the correlation curves. When the concentration increases the effect of the diluent type decreases and the initial difference of TSS concentration is due to the quality of the tap water, mineral water and permeate. With this methodology developed it is possible to make a difference between these types of water density equal to 0.998264, 0.998526, 0.998796, 0.998892 and 0.998906 g/cm$^3$ respectively for mineral water, tap water, AS Gardanne permeate, AS Aix-en-Provence permeate and AS Pilote scale MBR permeate.
3.3 Relation between biomass concentration and density ($d_{20}$)

Fig. 3 shows a linear relation of the TSS concentration as a function of the density at 20°C. The initial biomass concentrations in each WWTP were circled on Fig. 3. The correlation gives fairly good results with a regression coefficient ($R^2$) between 0.9240 and 0.9996 for both equilibrium fast and slow modes. Depending on the type of activated sludge studied, it can be observed different behaviors with respect to the measurement mode: the measurements made on Aix-en-Provence activated sludge are much better when using the equilibrium fast mode, the unit may indicates sometimes errors when using the equilibrium slow mode. This difference can be explained by the fact that using equilibrium slow mode for measuring density can take up to five minutes. Hence, sludge particles may settle in the measuring cell due to the time of measurement inducing errors during the determination the U-shaped tube oscillations. This effect may be due to the structure of this activated sludge, it was not observed with the other activated sludge studied here. However, the results obtained by using equilibrium fast mode are more satisfactory. Using equilibrium fast mode, no settling is observed whatever the activated sludge used. Hence, for a WWTP manager it is now possible to determine with high precision the biomass concentration in 10 seconds.

Table 1  French Mont Roucous mineral water composition

<table>
<thead>
<tr>
<th>Elements</th>
<th>Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca$^{2+}$</td>
<td>2.4</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>0.5</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>3.1</td>
</tr>
<tr>
<td>K$^+$</td>
<td>0.4</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>3</td>
</tr>
<tr>
<td>SiO$_2$</td>
<td>8.2</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>2</td>
</tr>
<tr>
<td>HCO$_3^-$</td>
<td>6.3</td>
</tr>
<tr>
<td>F$^-$</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2  Characteristics of the diluted activated sludge and measurement mode

<table>
<thead>
<tr>
<th></th>
<th>Type of diluent</th>
<th>Measurement mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pilot scale MBR</td>
<td>Tap water</td>
<td>Slow</td>
</tr>
<tr>
<td>Gardanne</td>
<td>Tap water</td>
<td>Fast</td>
</tr>
<tr>
<td>Aix-en-Provence</td>
<td>Mont-Roucous</td>
<td>Slow</td>
</tr>
</tbody>
</table>
Figure 2  Variation of biomass concentration as a function of the density: Effects of diluent on activated sludge density measurement
Figure 3 (to be continued)
Figure 3 (to be continued)
It is noticed that the values of all the curves’ slopes are similar around 3 000 for urban effluent (Fig. 3). The data were collected for different kinds of activated sludge sampled from various types of treatment processes (activated sludge WWTP and WWTP using MBR technology). As a consequence, the possibility of using a general correlation for determining TSS concentration for all activated sludge using density measurement was considered. The data collected were plotted on Fig. 4, in order to obtain a general correlation with slow and fast equilibrium mode. The correlations give fairly good results with a regression coefficient of 0.9580 and 0.9926 for equilibrium slow and fast mode respectively. The use of the
correlation obtained with the fast equilibrium mode provides a good estimation of the biomass concentration without need for prior calibration. However, it may be noted that the TSS concentration calculated with the correlation on Fig. 4 is less accurate than those on Fig. 3. The correlations obtained in this study are summarized in Table 3.

Generally, measuring TSS concentration by classical method comes together with measuring total volatile suspended solid (TVSS), the method presented in this paper is not able to determine directly TVSS. Nevertheless if the ratio TVSS/TSS is well-known (Heran et al., 2008; Villain and Marrot, 2013), this density method gives the possibility to estimate TVSS.

Figure 4  TSS concentrations as a function of the density \(d_{20}\) whatever the urban WWTP using equilibrium slow mode (a) and equilibrium fast mode (b) for three different WWTPs.
Table 3  Correlations of activated sludge TSS concentration to its density

<table>
<thead>
<tr>
<th>WWTP</th>
<th>Effluent</th>
<th>Process</th>
<th>Equilibrium</th>
<th>R² (slow/fast)</th>
</tr>
</thead>
</table>
| Aix-en-Provence | Urban    | Activated sludge | TSS = 4036×d₂₀ − 4031/20  
TSS = 3364×d₂₀ − 3360       | 0.9240/0.9742 |
| Gardanne      | Urban    | Activated sludge | TSS = 3181×d₂₀ − 3178/20  
TSS = 3220×d₂₀ − 3216       | 0.9972/0.9953 |
| Rousset       | Urban    | MBR       | TSS = 3192×d₂₀ − 3189/20  
TSS = 3298×d₂₀ − 3294       | 0.9902/0.9956 |
| Pilot scale   | Industrial | MBR      | TSS = 2512×d₂₀ − 2510/20  
TSS = 2440×d₂₀ − 2438       | 0.9930/0.9996 |
| Urban         | All      | All       | TSS = 3138×d₂₀ − 3133/20  
TSS = 3248×d₂₀ − 3244       | 0.958/0.9926 |

CONCLUSIONS

The results of this short communication indicate that density is a good surrogate measurement for determining activated sludge TSS concentration from WWTP. With this new methodology it is possible to determine the biomass concentration in 10 seconds thanks to density measurement regardless of the type of effluent or the type of the process. A new cleaning protocol was developed to take into account the properties of the active sludge and the specificity of the apparatus. The equilibrium fast mode appears to be the appropriate mode as settling effects can occur. For this mode, the data collected from 3 WWTP and pilot scale MBR show a good linear correlation (R² between 0.9974 and 0.9996) between density and TSS concentration. If more data collections from other WWTP are required to better establish a general correlation between activated sludge TSS concentration and its density, a general equation is provided for urban effluent. Overall, the results obtained can be used to improve efficiency in predicting TSS solids concentration in WWTP and MBR.

REFERENCES


