Global NEST Journal, Vol 17, No 2, pp 397-405, 2015

THE USE OF POTENTIOMETRIC MASS TITRATION (PMT) TECHNIQUE FOR DETERMINING THE ACID-BASE BEHAVIOR OF ACTIVATED SLUDGE

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Received: 06/01/2011
Accepted: 23/04/2015
Available online: 21/05/2015

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ABSTRACT

Activated sludge is a biological floc composed of microorganisms, organics, and inorganics and plays an important role in wastewater treatment. The pH of an activated sludge suspension determines the acid – base behavior and the charge of the microorganisms in water. In the present study, the determination of acid – base behavior of activated sludge was performed using microelectrophoresis and Potentiometric Mass Titrations (PMT). For activated sludge that is a complicated biological system, direct microelectrophoresis is not a suitable method for the determination of its ζ-potential. This method leads to a variety of isoelectric point (iep) values due to the presence of different microorganisms in activated sludge. On the contrary, the PMT method used for the first time for activated sludge can provide the overall point of zero charge (pzc) value that is 8.2. In addition, a simple methodology is proposed for the quantitative determination of the overall solid charge as a function of solution pH, which requires only (a) the titration curves of an electrolyte suspension and of the blank electrolyte solution and (b) the calculation of the H^+ ions consumed for the above titration curves.

Keywords: Activated sludge, wastewater treatment, microelectrophoresis, charge of microorganisms, potentiometric mass titration, point of zero charge (pzc)

1. Introduction

Protonation of microorganisms in water leads to the formation of pH dependent electrically charged surfaces. This surface charge governs many environmental processes including adhesion to interfaces, transport through porous media, biofilm formation, etc (Hong and Brown, 2006). The solution pH regulates the ionization of acidic and basic functional groups of bacterial cell surface such as carboxylic, phosphoric, hydroxyl, and amine groups (Claessens *et al.*, 2006 a,b; Hong and Brown, 2008). These functional groups are associated with peptidoglycan, teichoic acid, and teichuronic acid on the surfaces of Gram-positive bacteria and with lipopolysaccharides, phospholipids, and proteins on the surfaces of Gram-negative bacteria. A more complicated picture exists when we refer to activated sludge which is a mixture of organic and mineral suspended solids, including a plethora of microorganisms (Bitton, 2005).

The pH at which the net surface charge of a colloid is equal to zero, or in other words the charge of the positive surface sites is equal to that of the negative ones, is called point of zero charge (pzc). For the determination of the pzc the titration method is usually applied. The use of an indifferent electrolyte is required for the titrations. An electrolyte is termed indifferent (or inert) when the ions do not adsorb specifically.

The two common methods for the pzc determination are the classical potentiometric titration (Parks 1965) and the newest method 'Potentiometric Mass Titration" (PMT) (Bourikas *et al.*, 2003; Vakros *et al.*, 2002). In the classical potentiometric titration, the pzc is the common intersection point of three surface-charging curves obtained at various ionic strengths, whereas in the PMT, the pzc is determined by the common intersection point of titration curves with different mass or the section point of the titration of a suspension and the corresponding solution of the indifferent electrolyte.

The pzc term can be used for biocolloids such as bacteria, viruses, and proteins (Kosmulski, 2001), although one might argue that potentiometric titration includes not only the functional groups on the surface but functional groups within the particle that are accessible to the potential determining ions (H^+). For the activated sludge floc, these include the functional groups at the bacterial cell surface, in the bacterial cell wall and in and on polymers holding the floc together, along with functional groups associated with the protozoa, nematodes, rotifers, etc. that are present in activated sludge. So far, the determination of pzc using titration methods is rather rare, usually the researchers utilize microelectophoresis for determining the pzc of the biocolloid (Abudalo *et al.*, 2005; Kosmulski, 2009; Kulczycki *et al.*, 2005; Michen and Graule, 2010).

Microelectrophoresis is simple and requires extremely low quantities of microorganisms for each measurement. In this method, a dilute suspension is used and the ζ-potential of the material at electrolyte concentrations up to about 0.1 M is measured. The ζ-potential is related to the surface charge of the material and is the electric potential in the interfacial electric double layer (EDL) at the location of the slipping plane relative to a point in the bulk fluid away from the interface. The sign and magnitude of ζ-potential depends on the solution conditions and the nature of the solid. When the surface of a particle is charged then a "structure", called the electric double-layer (EDL) will be developed such that the particle surface charge is neutralized by an adjacent layer in solution containing an excess of ions of opposite charge to that of the surface. The pH at which the ζ-potential is equal to zero is the isoelectric point of the material.

Although the technique provides the isoelectric point (iep) and not the pzc, these two points are the same if the electrolyte used is not specifically adsorbed on the surface of the microorganism. In any other case, there is a shift observed for pzc and iep values towards opposite directions to each other. However, even for the well-studied inorganic oxides there are some limitations concerning the application of microelectrophoresis.

The aim of the present study is to propose PMT as a simple method for the determination of the acid – base behavior and also the charge and properties of activated sludge. More specifically, the information provided by electrophoresis was compared with the PMT results and a methodology that will allow the extraction of all relevant information through data interpretation was described.

2. Experimental methods

2.1 Activated sludge

Mixed liquor samples were collected directly from the aeration tank of the wastewater treatment plant of the University campus of Patras (Rio, Patras, Greece). It is an oxidation ditch activated sludge system. The system achieves partial nitrification. For the analysis of protozoan community, aliquots of 200 μl were received from the sample.

Analysis was conducted for the identification of species in vivo using an optical microscope (Leica DMLB, Leica, Germany) with 10x/20 magnification on the eyepiece lens and with 10x/0.25, 40x/0.75, and 100x/1.25 magnification on the objective lens. Identification of protozoa was mainly based on their morphology and movement by comparison to morphological descriptions in Standard Methods (APHA, 1998). A fresh sample was collected and characterized immediately. Activated sludge samples were titrated either as a mixed liquor with no further process or as solid samples after mild centrifugation.

2.2 Microelectrophoresis

The method of microelectrophoresis was used in order to study the electrostatic properties of activated sludge cells. The measurements of the electrophoretic mobility of the samples were conducted at a constant temperature (25 °C) using a Zeta meter (Nano ZS, Malvern Instruments). Sufficiently diluted suspensions (1% w/v) of activated sludge used as it was collected from the aeration tank of the wastewater treatment, were prepared using NaCl 0.15 M as an electrolyte. The activate sludge was mixed with the solution on a magnetic stirrer. The pH of the suspensions was adjusted between 2 and 11 by adding small amounts of 0.1 M HCl or 0.1 M NaOH. The measurements were analyzed using Zetasizer Nano software and the ζ-potential was determined (with the use of Smoluchowski equation built in the software) according to the measurements of the electrophoretic mobilities of the cells.

2.3 Potentiometric mass titration

The study of the acid-base behavior of the activated sludge was performed using the method of PMT (Vakros *et al.*, 2002). The electrolyte solutions (0.1 M $NaNO_3$) were prepared using $NaNO_3$ (Merck, analytical grade) dissolved in triply distilled water (Jencons, Autostills).

The PMT were performed at a constant temperature (25±0.1 °C), under N_2 atmosphere. The suspensions were equilibrated for 12 h and then, a small amount of base (0.2 ml of 1 M NaOH) was added to deprotonate a significant part of the charged sites, rendering the surface negative, without exceeding pH 10 for the suspension of activated sludge. This was chosen because above pH 10, significant cell lysis occurs, and may interfere with the buffering measurements (Fein *et al.,* 2005).

After 3-5 min, the new equilibration pH value was recorded and noted as the initial pH. The suspension was then titrated by adding automatically small volumes of a certified volumetric standard of 0.1 M HNO_3 and the pH was recorded as a function of the volume of titrant added to the suspension. The overall time of the titration was less than 1 h. This was done to ensure short time duration of the sludge especially in high (>9.5) and low (<4.5) pH values.

3. Results and discussion

3.1 Activated sludge microfauna

The initial mixed liquor suspended solids (MLSS) concentration was 4000 mg/L and the pH ranged from 8.0 to 8.5. The presence of at least three trophic levels was identified: bacteria (essential to all activated sludge processes), protozoa (*Vorticella sp., Chilodonella sp., Euplotes sp., Litonotus sp.,* and *Paramecium sp.*), and metazoa (Rotifers: *Euchlanis sp., Philodina sp.,* and *Lecane sp.*) (Sfaelou *et al.*, 2015). This combination indicates the stability of the present activated sludge "ecosystem" (Gerardi, 2006; Papadimitriou *et al.*, 2013). All the species present are indicative of "well dominated microfauna with good biodegradation ability" (Madoni, 1994).

3.2 Microelectrophoresis of activated sludge

During ζ-potential measurements of activated sludge, the peaks obtained were complicated, as it was expected due to the existence of multiple microorganisms. Usually, two or three peaks were obtained at each pH value. Also, every peak having high width value, probably describes more than one type of microorganisms since activated sludge microorganisms are heterogeneous. A typical example of a potential distribution with such peaks is presented in Figure 1.

The ζ-potential values of these peaks as a function of solution pH are shown in Figure 2. As it can be seen there are three curves describing possible microorganism groups due to the fact that there was more than one peak in each measurement. The values of the isoelectric points for the microorganisms are at pH < 5. These low values are common in the literature (Gélabert *et al.,* 2004; Bundeleva et al., 2011) and imply that activated sludge is negatively charged at pH commonly observed for municipal wastewater On the other hand, detailed experiments have shown that these low values describe dead

microorganisms and the real values are higher (Abudalo *et al.*, 2005; Martinez *et al.*, 2008; Michen and Graule, 2010).

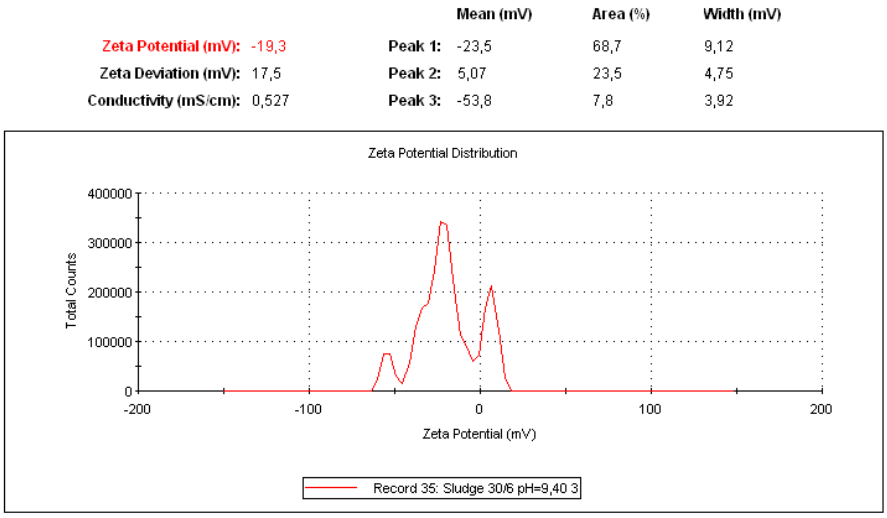

Figure 1. A typical example of a ζ- potential distribution

The Smoluchowski equation can provide good results only if the ζ-potential is not too high and when applied to large colloidal particles and high ionic strengths (Kosmulski and Rosenholm, 2004). Other studies have pointed out that it can only be applied to rigid particles having no polymers (Ohsima, 1995). On the contrary, bacterial cell surface is a soft surface which contains polyelectrolytic polymeric layers that extend out from the cell wall, such as lipopolysaccharides and teichoic acids (Poortinga *et al.*, 2002; Silhavy *et al.*, 2010). It was argued in the literature (Hong and Brown, 2008) that the soft particle model does not accurately represent the bacterial cell surface electrostatic properties. This is due to variations in the charges within the polyelectrolytic layer (de Kerchove and Elimelech, 2005) or due to variations in the length of polyelectrolytic layers (Rodriguez et al., 2002).

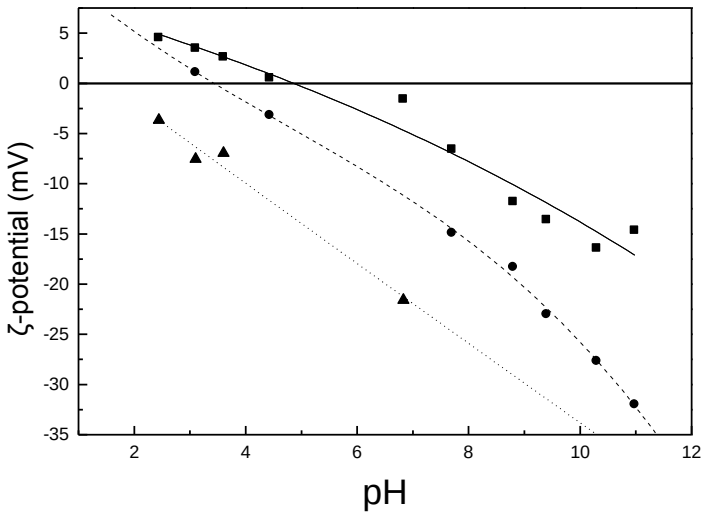

Figure 2. ζ-potential of activated sludge as a function of the pH of the solution at constant ionic strength (0.15M NaCl)

Finally, the direct microelectrophoresis of the activated sludge is not suitable for ζ-potential measurements because inherently the method requires the presence of a colloidal particle. Activated sludge is a mixture of bacteria and polymeric materials.

3.3 Potentiometric mass titration of activated sludge

Figure 3 shows that there is a common intersection point for the potentiometric titration curves of two different volumes of activated sludge mixed liquor in 0.1 M $NaNO_3$ solutions and the titration curve of a blank 0.1 M $NaNO_3$ solution.

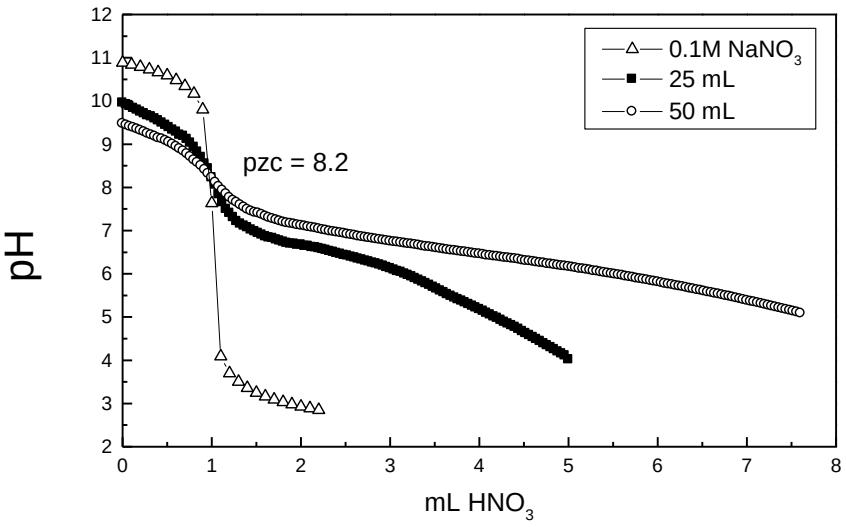

Figure 3. Potentiometric mass titration curves of mixed liquor activated sludge samples using 0.1 M HNO_3

The pH of this common intersection point corresponds to pzc value and it was found to be 8.2. Noteworthy differences are observed between the activated sludge and the blank solution in their acid-base properties. These differences clearly show that the cells neutralize significant amounts of acid or base solution. This buffering effect is due to the functional groups of the activated sludge, which consume added acid. Also, the suspension of activated sludge displays relatively weak inflection points. This clearly shows that there are so many multiply bonding sites with an overlapping range of pK that they became indistinguishable on the titration curve (Ohsima, 1995). Finally, the observed value of pzc is within the range of the operation pH of the aeration tank of the municipal wastewater treatment plant (pH 8.0 to 8.5). This means that the cells are more stable and/or the supernatant of the mixed liquor can regulate the behaviour of the sludge.

The pzc for the solid sludge was found to be 7.3 as can be seen in Figure 4. This is almost one unit of pH lower than the pzc value determined for the mixed liquor. This difference between the pzc value of the solid and MLSS can be explained by the fact that centrifugation possibly destroys the bacterial cell wall and releases acid groups from the inner cell to the solution (Liermann *et al.*, 2000). This is in accordance with the fact that dead cells exhibit lower values of iep, and thus pzc.

The most important finding from the presented results is that, although the activated sludge has a large number of microorganism species, it is possible to measure one pzc value and thus, understand the apparent charge of the suspension at different pH values.

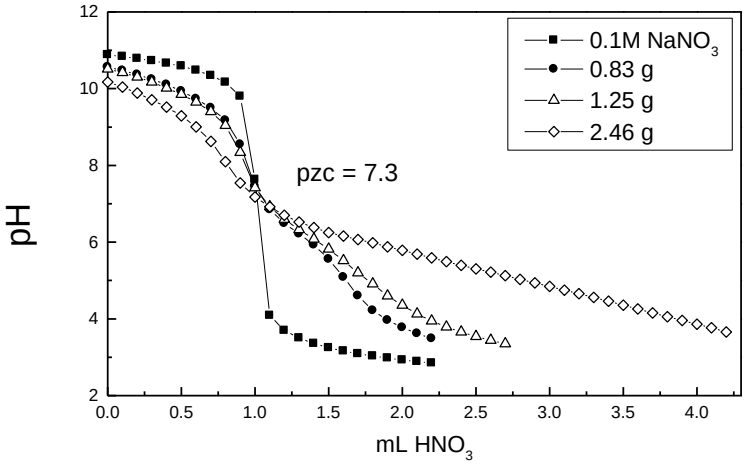

Figure 4. Potentiometric mass titration curves of solid activated sludge samples using 0.1 M HNO3

3.4 Acid-base behavior and determination of suspension charge

Valuable information can be obtained from the titration curves of a suspension and the corresponding blank solution (Figure 5). The first information one can obtain from PMT curves is the pzc value from the common intersection point of the titration curves. These curves can also help us to determine the charge of the sludge as a function of the suspension pH. In order to achieve this, the titration curves of the solution and of the suspension need to be elaborated following specific steps.

When a particle is immersed in a polar medium (i.e. electrolyte solution) a surface electrical charge will be developed. There are many different charging mechanisms but for metal oxides and materials that contain carboxyl and/or amino groups (i.e., proteins, biocolloids, ionic polymers, polyelectrolytes), the most important is the ionization of surface groups. The ionization and/or dissociation of these groups release in the solution or consume from the solution H^+ and the net charge of the surface (and thus sign, either positive or negative) depends strongly on the pH of the dispersion media.

The titration curves of a blank solution and suspension presented in Fig. 5, intersect at the pzc value. This pzc value separates the diagram in two major pH regions. In the first region, where the pH is higher than the pzc, the activated sludge releases H^+ to the solution resulting in lower pH for the suspension. The sites are deprotonated and the activated sludge is negatively charged. In the other region, the solution releases H^+ into the activated sludge, resulting to higher pH values for the suspension when compared to the solution. These H^+ ions adsorbed onto the activated sludge, protonate the sites resulting to a positive charge.

For a given volume of acid added, there is a difference in the titration curves (e.g. see the A – B or A' – B' in Figure 5). From this difference, the H^+ ions consumed by the activated sludge can be calculated using the pH definition, the K_w value, and the volume of the suspension (solution). Then, the charge can be determined.

The reaction in the titration is the well known reaction

$$H^+_{(aq)} + OH^-_{(aq)} \rightarrow H_2O_{(l)} \quad (1)$$

The concentration of the H^+ ions in the bulk solution, $[H^+]_{(aq)}$, determines the concentration of the H^+ ions in the interface, $[H^+]_{(int)}$, through the following equilibrium

$$H^+_{(aq)} \rightleftharpoons H^+_{(int)} \quad (2)$$

and then protonation or deprotonation of the existing sites can occur depending on their relative strength and H^+ concentration in the interfacial region

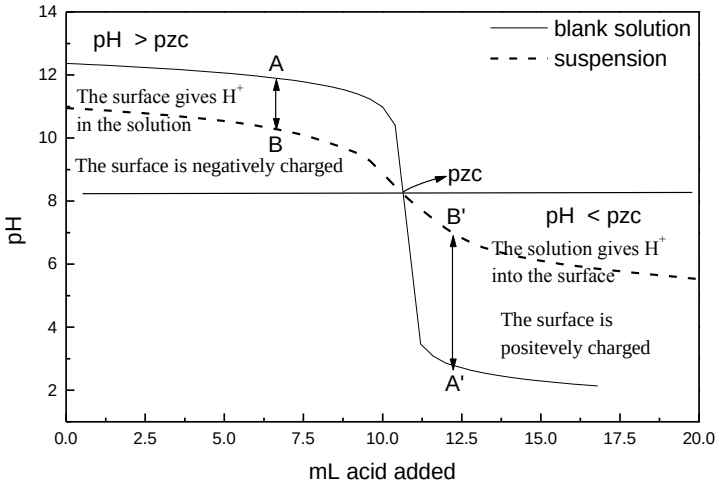

Figure 5. Titration curves of a suspension and the blank solution

The amount of H^+ ions is calculated by the following expression for both regions of the graph (Bourikas *et al.*, 2003):

$$H^+_{cons} = (H^+_b - H^+_s) + (OH^-_b - OH^-_s) \quad (3)$$

where: H^+_{cons} is the number of H^+ ions consumed by the surface (mol), H^+_b, OH^-_b, are the H^+ and OH^- ions in the blank solution (mol) and H^+_s, OH^-_s, are the H^+ and OH^- ions in the suspension (mol). The H^+_{cons} is negative when the pH of the suspension is higher than the pzc and positive in the opposite case.

If the H^+_{cons} is divided by the mass or the specific surface area (SSA) of the solid, then H^+_{cons} is expressed per g or per m^2. Then, the H^+_{cons} is converted into charge by multiplying H^+_{cons} with Faraday constant (F= 9.6485 10^4 C mol^{-1}).

In the present study, this methodology is used in order to determine the acid – base behavior and the charge of the activated sludge. The results are presented in Figures 6 and 7.

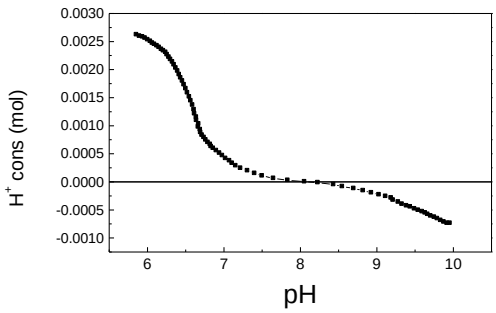

Figure 6a. H^+ consumed by the mixed liquor activated sludge

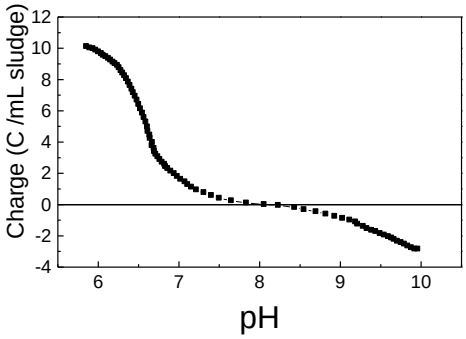

Figure 6b. Charge of the mixed liquor activated sludge

The results presented in Figures 6 and 7 show that the consumption rate of H^+ by the sludge is higher when the pH of the solution is low (<6). This means that the groups of the cell can be protonated easily and these groups can neutralize a relatively high volume of acid. This may not be relevant for the design of environmental applications of the activated sludge for municipal wastewater treatment, because in

most cases pH ranges from 7 to 8. However, the high cell capacity for H^+ neutralization can be important in the case of acidic effluents such as those from olive oil production units, dairy industries, or in other special cases.

At pH between 6 and 7, the activated sludge presents buffer capacity. The sludge can neutralize quite large amounts of acid without significant changes in the suspension pH (see also Figures 3 and 4). This amount is much higher in the case of mixed liquor sludge (Figure 6), although the quantity of the sludge solids was lower compared to the case of solid sludge experiment (Figure 7). This corroborates the explanation given above that the centrifugation possibly destroys the bacterial cell wall and releases acid groups from the inner cells of the sludge to the solution (Liermann *et al.*, 2000).

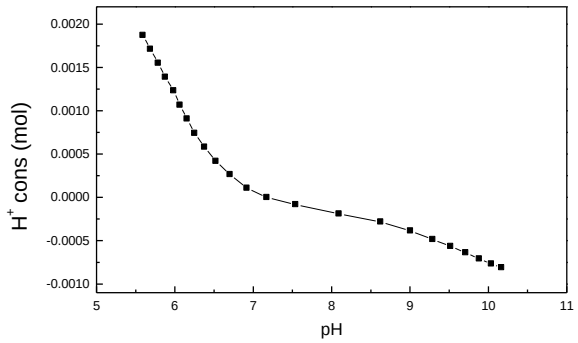

Figure 7a. H^+ consumed by solid activated sludge

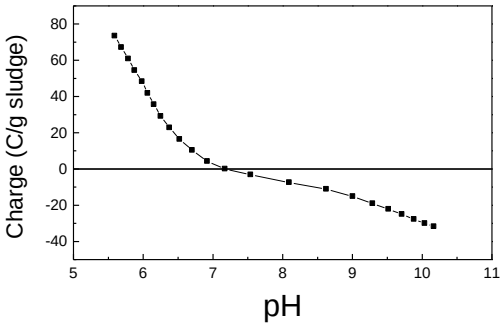

Figure 7b. Charge of solid activated sludge

4. Conclusions

The main conclusions from this study are the following:

1. For activated sludge, direct microelectrophoresis is not a suitable method for determining the iep and pzc values.

2. The determination of activated sludge apparent charge can be achieved using the PMT technique.

3. Although the activated sludge is a very heterogeneous system, there is a common intersection point in the titration curves, resulting to the apparent pzc of the sludge, showing that the PMT technique can be applied in complex systems.

4. The elaboration of the titration curves can reveal valuable information concerning the overall acid – base behavior and the charge of the activated sludge.

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