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Protein recovery from solubilized sludge by hydrothermal treatments

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A B S T R A C T

New alternatives for sludge management have been developed in recent years, with hydrothermal treatments being one of the most attractive ones. Even though many studies have been made on the application of hydrothermal treatments as pre-treatment or end-line technologies for sludge stabilisation and/or minimization, there is a lack of knowledge about the products generated during the process and its characteristics. This information is a crucial step for the assessment of the recovery of valuable products of the sludge, mainly proteins, humic acids and carbohydrates, which can considerably improve the economic balance of the hydrothermal treatment.

This work assesses, for the first time, the potential of hydrothermally hydrolysed sludge as renewable source for proteins recovery. For this purpose, firstly, the concentrations and properties of the main soluble biopolymers generated during the hydrothermal treatment, either in presence (wet oxidation, WO) or absence (thermal hydrolysis, TH) of oxygen, were measured, determining the reaction time necessary for a maximum solubilisation. Peak concentrations of 7.7 g/l (0.291 g/gVSSo) of proteins for WO and 7.2 g/l (0.272 g/gVSSo) for TH, were achieved at 87 min of experiment.

Afterwards, different separation methods, usually applied at industrial scale, were assessed for the separation of protein from the hydrolysed sludge, in terms of protein recovery and selectivity. Ammonium sulphate addition was found to be the best separation method, achieving 87% and 86% of protein recovery for TH and WO samples respectively, and the highest selectivity. Although further studies are required in order to achieve complete protein purification, a new perspective in sludge management is now open, by recovering valuable compounds.

1. Introduction

Every day, huge amounts of sludge are being generated in municipal wastewater treatment plants (WWTP). For instance, in the EU, more than 10 million tons of dry solids of sewage sludge were produced in 2008 (Comission, 2008). Traditionally, sludge has been majority recycled as fertilizer in agriculture, or has been disposal in landfill. However, sludge landfill disposal is highly regulated due to its hazardous characteristics, such as pathogens presence and toxic compounds that can be generated when the sludge is disposed in landfills. Most countries are focused in the recycling of the sludge, for example, using it to produce energy by incineration or anaerobic digestion (Kacprzak et al., 2017). Either way, the management of this sludge represents a big problem, due to their high organic load and low dewaterability (Neyens and Baeyens, 2003). Sludge dewatering by physical operations such as flotation, thickening, filtration or sedimentation are extended options, but they are too expensive (almost 50% of the total disposal cost) (Neyens et al., 2004) and the required operations are difficult since they depend on the sludge characteristics such as structure, which, in turn, is affected by its composition (Dursun and Dentel, 2009; Tsang and Vesilind, 1990). As mentioned before, anaerobic digestion processes are the most common solution in the sludge management. These techniques have the methane generation as main advantage, which considerably improves the economic balance of the WWTP, not only by using methane itself, but selling the energy produced. However, anaerobic digestion is a very slow process, requiring well controlled conditions, which supposes its main disadvantage.

Nowadays, new pre-treatment methods are being studied in order to speed up this process (Carrère et al., 2010), highlighting among others the hydrothermal techniques (Abe et al., 2013), either thermal hydrolysis (TH) or wet oxidation (WO). Both require high temperatures, usually over 200 °C, and high pressures, usually over 60 bar. The difference between them is that an oxidant agent is needed in WO, while it is not required for TH, so the final effluent characteristics and products will differ. WO allows the solubilisation and partial oxidation of the sludge, whereas there is no degradation, only solubilisation in TH, due to the absence of any oxidant.

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This fact means that TH is a good pre-treatment for anaerobic digestion, improving its methane yield up to 50% (Haug et al., 1978). Either TH or WO seem to be promising methods for sludge management, but they are in the optimization period yet, as can be observed in the existing bibliography. In fact, most of the studies related to these techniques are focused on the effect of the operation conditions on solubilisation or mineralization degrees and methane yields (Hii et al., 2014, 2013). Nevertheless, there is a lack of knowledge about the mechanisms involved and deeper characterizations of the products obtained than COD or TOC measurements are not available.

For example, it is well known that hydrothermal treatments break cells, thus releasing intracellular compounds, mainly proteins, humic acids and carbohydrates. Nevertheless, the characterization and separation of these biopolymers is a topic scarcely studied (Urrea et al., 2016), even when it could provide valuable information to the development of the hydrothermal treatments by two main reasons. Firstly, a deep knowledge of the biopolymers formed during either the wet oxidation or thermal hydrolysis is key for identifying improvements in the subsequent treatment. Knowing these mechanisms and products is necessary, because they condition the design of the following operations, such as pumping, anaerobic digestion or membrane operations (Judd, 2010). This is due to the effect of the soluble biopolymers, mainly proteins, on the main physicochemical properties of the effluent, such as viscosity, biodegradability, settleability or dewaterability (Hoa et al., 2003; Ruiz-Hernando et al., 2015; Wang et al., 2014, 2009; Zhang et al., 2015). At the same time, information about the separation and purification of these products, as well as reducing the effluent organic load, also brings a different perspective in the sludge management, considering it as a new renewable source for not only energy, but also for resources recovery (Suárez-Iglesias et al., 2017; Tyagi and Lo, 2013). This fact could be the key point in the implementation of hydrothermal technologies for sludge management at full scale, due to its high costs. As previously explained, products obtained by hydrothermal treatments include proteins, humic acids and carbohydrates, which have high commercial value if can be isolated from the hydrolysed sludge stream. For example, predominant biopolymers, proteins, can be used as fertilizers, adhesives or animal feed (Hwang et al., 2008) and their separation considerably reduces the nitrogen content of the effluent. Humic acids, which usually exhibit a low biodegradability, thus reducing the methane yield of a subsequent anaerobic digestion, have already been used as biosurfactants and fertilizers (Salati et al., 2011). Finally, carbohydrates from sludge are a cheap substrate for fermentations (Tekin et al., 2014). Proteins generate more interest, owing to their high proportion in the sludge, which is a 61% (Chen et al., 2007). Hence, isolation and revaluing of these molecules should be studied firstly.

Taking into account the previous information, the aim of this work was to study and characterise the hydrothermal techniques (TH and WO), either as sludge management process or as revaluing treatments by soluble proteins recovery. In order to carry it out, this includes the analysis of the physical-chemical parameters of the sludge as well as the characterisation of the products generated and their purification by the application of several isolation methods.

2. Material and methods

2.1. Sludge characterisation

The experiments were performed using a secondary sludge thickened by flotation in a municipal WWTP (Asturias, Spain) with the characteristics showed in Table 1.

Table 1
Main parameters of the sludge used in the experiments.

Parameter	Units	Value
pH		6.54
TCOD	mg 0_2/l	37,200
SCOD	mg 0_2/l	210
TSS	g/l	31.92
VSS	g/l	26.47
SVI	ml/g	31
Proteins*	mg/l	181
Humic acids*	mg/l	281
Carbohydrates*	mg/l	82
Uronic acids*	mg/l	22.46
DNA*	mg/l	18.1
TOC*	mg C/l	373.20
IC*	mg C/l	126.80

* Indicates soluble concentrations.

2.2. Analytical methods

Total suspended solids (TSS), volatile suspended solids (VSS), fixed suspended solids (FSS), total and soluble chemical oxygen demand (TCOD and SCOD), sludge volume index (SVI) and pH measurements were carried out according to Standard Methods (APHA, 1998).

TOC and IC values were measured by means of a TOC analyser (Shimadzu TOC-VCSH, Japan). SCOD concentrations were determined by closed reflux, colorimetric method, using a DR2500 spectrophotometer (Hach Company, USA).

Protein and humic acid concentrations were simultaneously measured using the Lowry modified method (Lowry et al., 1951; Frølund et al., 1996) with BSA (Bovine Serum Albumin) and humic acid as standards, respectively. Carbohydrate concentration was estimated by the Dubois method (Dubois et al., 1956) using D-glucose as standard. DNA concentrations were measured according to Burton (1956) by using calf-thymus DNA as standard. Uronic acid concentration was measured using the method of Blumenkrantz (Blumenkrantz and Asboe-Hansen, 1973) with glucuronic acid as standard. All these methods are spectrophotometric, and absorbances were measured using a UV/vis spectrophotometer (Thermo Scientific, Heλios γ).

Soluble biopolymers molecular sizes were determined by HPLC (Agilent 1200, Agilent Technologies Inc., California, USA), employing a Yarra SEC-2000 (300 $\times$ 7.8 mm) column. The column preparation and operational terms were the same as in Urrea et al. (2016), but in the present study, a wavelength of 280 nm was selected. For the sake of having a better understanding of the molecular size evolution, the fingerprint area was divided into four regions. The first three ones were located in the zone of size exclusion, corresponding to low (0–35 kDa or 10.02 min to 11.8 min), medium (35–150 kDa or 7.6 min to 10.02 min) and high (>150 kDa or 5.45 min to 7.6 min) molecular weights. The last one was established in the final area of the column total volume (11.8 min). The peaks with a retention time higher than the one relating to the total column volume have been associated to molecules that interact with the filling material, being commonly observed this phenomenon with either extracellular or intracellular polymeric substances from sludge (Frølund et al., 1996; Urrea et al., 2016). Görner et al. (2003) reported that some of those peaks were retained for longer times due to hydrophobic interactions. Therefore, these facts suggest that the polymers which were effectively separated according to their size, that is to say, those eluted before of 11.8 min, had more hydrophilic characteristics.

In order to have a simpler view of the size distribution of the molecules and its hydrophilic or hydrophobic characteristic, the percentage of area for each size and the hydrophobic zone were calculated in relation to the total area of the analysis.