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Estimation of effluent quality parameters from an activated sludge system using quantitative image analysis

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HIGHLIGHTS

- Quantitative image analysis was used to evaluate an activated sludge system.
- COD, ammonia, and nitrate effluent concentrations were estimated by partial least squares.
- Using morphological and physiological data provided the best predicting abilities.
- Quantitative image analysis has the ability to be used in process monitoring.

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ABSTRACT

The efficiency of an activated sludge system is generally evaluated by determining several key parameters related to organic matter removal, nitrification and/or denitrification processes. Off-line methods for the determination of these parameters are commonly labor, time consuming, and environmentally harmful. In contrast, quantitative image analysis (QIA) has been recognized as a prompt method for assessing activated sludge contents and structure. In the present study an activated sludge system was operated under different experimental conditions leading to a variety of operational data. Key parameters such as chemical oxygen demand (COD) and ammonium ($N\text{-}NH_4^+$), and nitrate ($N\text{-}NO_3^-$) concentrations, throughout the experimental periods, were measured by classical analytical techniques. QIA was further used for the microbial community characterization. Partial least squares (PLS) models were used to correlate QIA information and the aforementioned key parameters. It was found that the use of the morphological and physiological data allowed predicting, at some extent, the effluent COD, $N\text{-}NH_4^+$, and $N\text{-}NO_3^-$ concentrations based on chemometric techniques.

1. Introduction

In environmental systems, processes and reactions are often dynamic, irreversible, and occurring in systems which are difficult to define, and unviable to describe using deterministic models [1]. Disturbances detection is an important task for process performance enhancement in wastewater treatment plants (WWTPs), due to strict regulations regarding effluent quality. Among the operational parameters most commonly used for assessing wastewater quality, the chemical oxygen demand (COD), as an indirect measure of the organic matter concentration, is of the utmost importance. Ammonium (NH_4^+) and nitrate (NO_3^-) concentrations are also widely assessed, reflecting the extent of nitrification/denitrification processes. However, traditional methodologies for determining these parameters are costly, labor and time-consuming, and may even present environmental risks associated to end products. Therefore, alternative methodologies that could predict these parameters, in a time and labor effective manner are of great interest.

In recent years, a great deal of attention has been given to different monitoring strategies to help clarifying the behavior of biological processes, such as fluorescent microscopy techniques and/or mathematical modeling. It has been previously found that Fluorescence *in situ* Hybridization (FISH) and epifluorescence microscopy (as an alternative to the use of confocal laser scanning microscopy) are among the best strategies to quantify nitrifying bacteria in activated sludge [2]. However, other microorganisms found in those systems also contribute to the behavior of the biological process. Recently, mathematical models were applied to

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activated sludge systems and it has been deduced from many full-scale observations that improper sludge age, food/biomass ratio (fed and removed), and feed composition imbalance could result in bulking, foaming, and other malfunctions. These relations have been recently summarized in attempts to model a WWTP risk to suffer from solids separation problems [3–5]. Furthermore, another model was developed [6] showing the bacteria community predators significance on the performance of a nitrifying system. Indeed, these models have been shown to allow for the characterization of the effluent quality in activated sludge bioreactors. Furthermore, it is nowadays considered that biomass characterization provides important information about the biological processes leading to a number of different bulking events. In this sense, microscopy techniques coupled to quantitative image analysis (QIA), characterizing the system as a whole, may present an advantage for prediction purposes, alongside mathematical modeling.

In order to assess activated sludge operating parameters, QIA applications have been employed for aggregated and filamentous bacteria contents determination, biomass structure and physiology (Gram-positive/Gram-negative and viable/damaged bacteria ratios) assessment, and even intracellular storage polymers (PHA, glycogen, poly-phosphates) quantification [7–16]. All of the above demonstrate that image processing and analysis methodologies are able to provide valuable information about activated sludge systems, and are nowadays recognized as valid monitoring tools. Indeed, the possibility of accurately monitoring the filamentous bacteria contents (responsible for bulking phenomena), or to characterize and quantify the aggregated biomass contents, size stratification, and structure (related to pin point or viscous bulking phenomena), by this simple, quantitative, automated and near real time methodology, compares favorably to the conventional monitoring methods.

As recent advances in computer and instrumentation techniques lead to the collection of large amounts of data from different processes, the integration of mathematical and statistical methods is crucial. Furthermore, this increased number of monitored variables in WWTPs, due to the use of computerized measurement devices, hinders data interpretation. Therefore, a systematic method to handle and analyze data is needed to effectively extract relevant information for process monitoring and supervision [17]. In this sense, chemometric techniques are increasingly used for a wide variety of tasks, including data evaluation and interpretation, optimization and development of predictive models in processes, and extraction of a maximum of information from experimental data [1,18]. Partial least squares (PLS) is a widely used statistical technique able to model a given predicted variable and detect deviations [19]. Furthermore, PLS models have been recently used for the estimation of COD, nitrate (NO_3^-), total organic carbon (TOC), and mixed liquor suspended solids (MLSS) using ultraviolet–visible and near-infrared spectroscopy on wastewater treatment systems [20–22]. Despite the prior use of image analysis data, coupled to PLS models, to estimate sludge volume index (SVI) and MLSS [11,12,15], QIA has not yet been applied, to our knowledge, neither to estimate the COD removal ability nor nitrification/denitrification occurrence.

In this sense, the present work aims at assessing the performance of an activated sludge system in four different operational conditions. Microscopic images were obtained throughout each experiment and processed by a QIA procedure to obtain the image analysis data (QIA data) regarding the activated sludge biomass morphological and physiological characterization. PLS models were further applied by integrating QIA data alongside operational data. The final goal of this research was to establish the most appropriate data and methodology for the prediction of COD, ammonia ($N\text{-}NH_4^+$), and nitrate ($N\text{-}NO_3^-$) within the activated sludge system.

2. Materials and methods

2.1. Experimental setup

A lab-scale activated sludge system fed with synthetic wastewater containing acetate as the main carbon source was used. The reactor had a working volume of 17 L (aeration tank), followed by a 2.5 L clarifier. The system was equipped with two feeding pumps for influent dilution, air supply at the bottom allowing efficient agitation, and sensor apparatus such as a dissolved oxygen probe and a pH meter with associated control pump. Sludge recirculation from the clarifier to the aerated tank was also performed.

2.2. Experimental conditions

During the present work, four different experimental conditions (EC) were conducted by changing the organic loading rate (OLR) and the food to microorganism's ratio (F/M). The operated time of each EC was 75 (EC1), 85 (EC2), 118 (EC3), and 49 (EC4) days. The sludge residence time (SRT) was set to 20 d for the first experimental condition (EC1), 15 d for the second (EC2), 20 d for the third (EC3) and 6 d for the fourth (EC4). These conditions were defined based on the typical SRT range in conventional activated sludge systems [23]. During the EC1 a sharp decrease on the OLR and F/M from 2.7 to 0.67 $kg_{COD}\,m^{-3}\,d^{-1}$ and 0.88 to 0.23 $kg_{COD}\,kg_{MLSS}^{-1}\,d^{-1}$ was performed. EC2 was conducted decreasing gradually the OLR and F/M from 0.6 to 0.09 $kg_{COD}\,m^{-3}\,d^{-1}$ and 0.16 to 0.07 $kg_{COD}\,kg_{MLSS}^{-1}\,d^{-1}$, respectively. For EC3 the OLR was gradually increased from 0.27 to 0.87 $kg_{COD}\,m^{-3}\,d^{-1}$ and the F/M from 0.07 to 0.35 $kg_{COD}\,kg_{MLSS}^{-1}\,d^{-1}$. Finally, EC4 was performed with an OLR around 0.59 $kg_{COD}\,m^{-3}\,d^{-1}$ and an F/M of 0.15 $kg_{COD}\,kg_{MLSS}^{-1}\,d^{-1}$ with some minor fluctuations. These four strategies were conducted with the purpose of obtaining a variety of operational conditions, leading to different sludge morphological and physiological properties.

2.3. Synthetic wastewater composition

Beyond acetate and $(NH_4)_2SO_4$, the concentration of the other nutrients added in the synthetic feed is listed below (per L): 0.025 g $MgSO_4{\cdot}7H_2O$; 0.044 g KH_2PO_4; 0.059 g $K_2HPO_4{\cdot}2H_2O$; 0.03 g $CaCl_2{\cdot}2H_2O$; 0.019 g $FeCl_3{\cdot}6H_2O$; 0.105 g $NaHCO_3$, and 2 mL of a trace metals solution for biomass maintenance. The trace metals consisted of ($g\,L^{-1}$): H_3BO_3, 0.05; $ZnCl_2$, 0.05; $CuI_2{\cdot}H_2O$, 0.04; $MnCl_2$, 0.02; $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0.055; $AlCl_3$, 0.05; $NiCl{\cdot}6H_2O$, 0.11 (adapted from [24]).

2.4. Analytical procedures

The system was monitored for SVI and MLSS, COD, $N\text{-}NH_4^+$, and $N\text{-}NO_3^-$ concentrations. SVI was determined based on the sludge height variation monitored for 30 min and combined with MLSS results. MLSS were measured in accordance with the procedures described in Standard Methods [25]. Samples for COD, $N\text{-}NH_4^+$, and $N\text{-}NO_3^-$ were collected, centrifuged, and filtered, from the synthetic wastewater and aeration tank. COD was measured with Hach Lange COD cell tests (LCK 414 and LCK 514) on a spectrophotometer (Hach Lange DR 5000). $N\text{-}NH_4^+$ was determined according to Nessler's method [25]. $N\text{-}NO_3^-$ was determined by high-performance liquid chromatography (HPLC) according to Sarraguça et al. [20].