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DEHYDROGENASE ACTIVITY AND QUANTITATIVE IMAGE ANALYSIS OF MORPHOLOGICAL CHARACTERISTICS OF ACTIVATED SLUDGE AT CERNAVODA WASTEWATER TREATMENT PLANT (ROMANIA)

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The purpose of paper was to study the dehydrogenase activity (DHA) in connection with a quantitative microscopic analysis of activated sludge aggregates. The study has been carried out during December 2009 – June 2010 at Cernavoda Wastewater Treatment Plant (WWT). The morphological characteristics analyzed were total number, perimeter and area of aggregates of activated sludge. These morphological parameters varied significantly during the study period. DHA ranged from 14.51 ug triphenylformazan (TPF)/mL mixed liquor suspended solids (MLSS) in December to 25.12 ug TPF/mL MLSS and correlated positively with average values of aggregates perimeter and area.

Keywords: Dehydrogenase activity, microscopic analysis, activated sludge aggregates.

1. Introduction

The biological activity of activated sludge relies upon the action of microorganisms grouped specifically as aggregates and flocs. Degradation and removal of organic wastes are carried out by the combined action of microbial enzymes [1,2]. A large number of enzymes have been found to exist in activated sludge, many of them being of biotechnological interest [3]. Environmental factors influencing the microbial enzymatic activities may affect wastes processing and the quality of final effluent. Dehydrogenases have been widely used to assess the metabolic state of microbial community found in different environments such as different types of soils [4, 5] and activated sludge systems [6, 7]. These enzymes are involved in many metabolic reactions by transferring an electron pair from some substrates to NAD^+ or $NADP^+$ and giving a measure of respiratory activity of living organisms [5]. In recent years, there is an increased

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interest regarding the automated analysis of activated sludge morphological properties. It is well known that the number, size and shape of activated sludge flocs can affect the transfer at the microscale level of oxygen and nutrients, thus influencing the efficiency of waste treatment [8]. Quantitative microscopic analysis of activated sludge flocs proved to be very useful in assessing the operating performance of wastewater treatment systems [9, 10, 11]. Different techniques have been used to estimate quantitatively total suspended solids in activated sludge systems, predict the bulking episodes and settling problems [12] as well as the effect of toxicants [6] on the microbial activities in treatment systems. The paper analyzes the relationship between some physico-chemical parameters (temperature, dissolved oxygen, sludge volume index and total suspended solids), morphological properties of activated sludge flocs and dehydrogenase activity (DHA) at Cernavoda Wastewater Treatment Plant in order to understand the complex factors that control the microbial activity in activated sludge.

2. Materials and methods

Studies have been carried out during December 2009 – June 2010 at Cernavoda Wastewater Treatment Plant. Samples were collected aseptically from five points along the aeration tank, mixed up and subsequently used for microscopic measurements and dehydrogenase activity (DHA). Determination of T^{o} C, dissolved oxygen (DO), total suspended solids (TSS), and sludge volume index (SVI) were done according to laboratory standards [13].

Mixed liquor suspended solids (MLSS) was used undiluted and 50 uL of sample were pipette on a sterile, clean microscope slide that was carefully covered with coverslip to ensure uniform dispersion and avoid air bubbles trapping. Axio Imager microscope (Carl Zeiss) equiped with AxioVision LE camera (AxioVision LE 4.6 software). Image capture was made with a calibrated 10x objective. Frames were converted to grayscale mode before applying autosegment function. Automated calculations included area, perimeter, and number of objects and were plotted as scatter diagrams using Microsoft Excel. Ten microscopic fields were taken and analyzed for each sample.

Dehydrogenase activity (DHA) was assayed as described by Casida [4] with slight modifications adapted to activated sludge particularities and is based on the ability of microorganisms to reduce 2,3,5 triphenyltetrazolium chloride (TTC) to red derivative triphenylformazan (TPF). One mL of MLSS was centrifuged at 8,000 rpm for 5 min and the supernatant was discarded. Subsequently, 1 mL of phosphate buffer (pH=7.4) was added to each vial as follows: 2 tubes were amended only with 10 uL of TTC 1% (2.3, tryphenylformazan) to estimate the actual activity, two tubes were amended with

10 uL of TTC and 50 uL of glucose 5% to estimate the potential activity, one tube received only phosphate buffer, TTC and glucose without activated sludge and served as blank. Samples were incubated at 28^{o}C for 4 h on a rotatory shaker. At the end of incubation period, samples were centrifuged, the supernatant discarded and enzyme action stopped by adding 1 mL of pure methanol. Extraction was made on a rotatory shaker for 10-15 min at 28^o C. Extracted TPFwas read at 485 nm on a Spectronic 20 spectrophotometer. Quantitative estimation of produced TPF was made by using a calibrated curve constructed in 15 points ranging from 2 to 50 ug/ml TPF. Statistical analysis (analysis of variance, correlations) of data was done with Statistica software package v.5.

3. Results and discussion

The temperature of wastewaters varied largely during the period of study ranging from 13.7 ^{o}C (December) to 24.9 ^{o}C (June) (Fig. 1a) while dissolved oxygen concentration decreased gradually from winter to summer from 5.2 mg/L to 3.02 mg/L (Fig. 1b). Low oscillations of SVI were observed and ranged between a maximum value of 40.09 mg/L (April) and a minimum of 29.16 mg/L (June) (Fig. 1c). TSS followed more or less the same seasonal trend as SVI, fact supported by a strong positive correlation (r=0.90). Instead, the relation between DO and temperature was negative (r= -0.94), situation that could be explained by variable solubility of O_2 between winter and summer.

The abundance of two size-class microscopic objects was analized: a) with size over 1,000 um and b) with size ranging from 100 to 1,000 um. Maximal values of flocs' perimeter varied between 3.248 um (May) and 4.200 um (April) (Fig. 2b and 2c). The number of objects over 1,000 um varied from a minimal value of 9 (December) to 22 objects (May). Particles of class-size between 100-1.000 um ranged from a minimal value of 61 (December) to a maximal value of 92 (May) (Fig. 1a and 1c).

In case of *aggregates'*area, it was analyzed the abundance of two size-class microscopic objects: a) with size over 10,000 um^2 and b) with size ranging from 1,000 to 10,000 um^2. Maximum area of objects was recorded on April and had the value of 106,767 um^2 while the minimum value of area was observed in June (47,525 um^2) (Fig. 3b and 3d). Objects over 10,000 um^2 varied between 13 (December) and 19 (May). Within 1,000-10,000 class-size, the number of objects varied between 34-36 (on December, respectively June) and 48-49 (April-May) (Fig. 3a, 3b, 3c, and 3d).

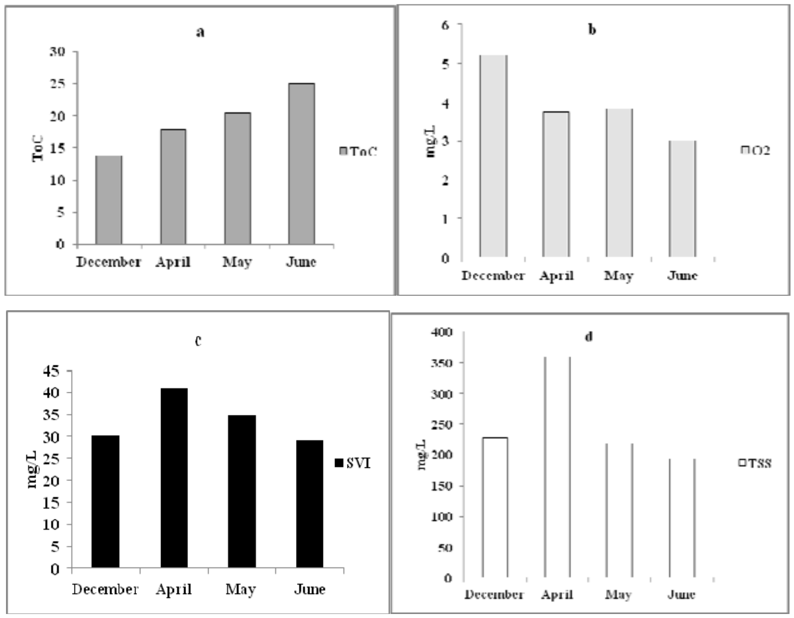

Fig. 1. Dynamics of physico-chemical parameters at Cernavoda Wastewater Treatment Plant during December 2009 – June 2010: a – temperature (T^{o}C); b – dissolved oxygen (O_2); c – sludge volume index (SVI); d – mixed liquor suspended solids (MLSS)

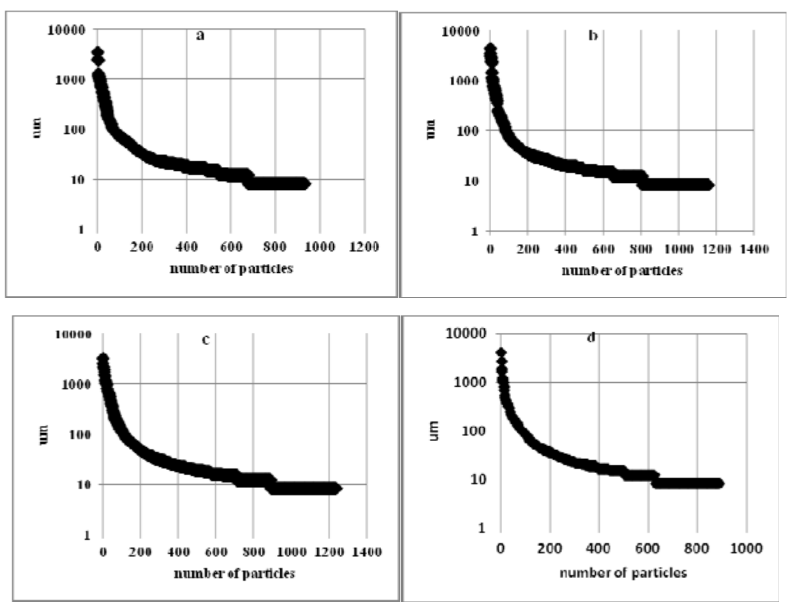

Fig. 2. Aggregates perimeter distribution of activated sludge during 2009-2010: a – December; b – April; c – May; d – June (number of points on diagrame correponds to ten microcopic fields)

On average, the highest number of objects was observed during December (142), number that progressively decreased to a minimal value recorded in June (89) (Fig. 3a). The average perimeter was lower in December and (50.43 um) and increased in springtime to a maximal value of 68.87 um (May) (Fig. 4d).

Average area of objects was also low in December (328 um^2) increased to a maximal value in April (687 um^2), then decreased again in June (463 um^2) (Fig. 4d). Total % area presented a minimal value in June (7.15%) and a maximal one in April (15.84%) (Fig. 4c).

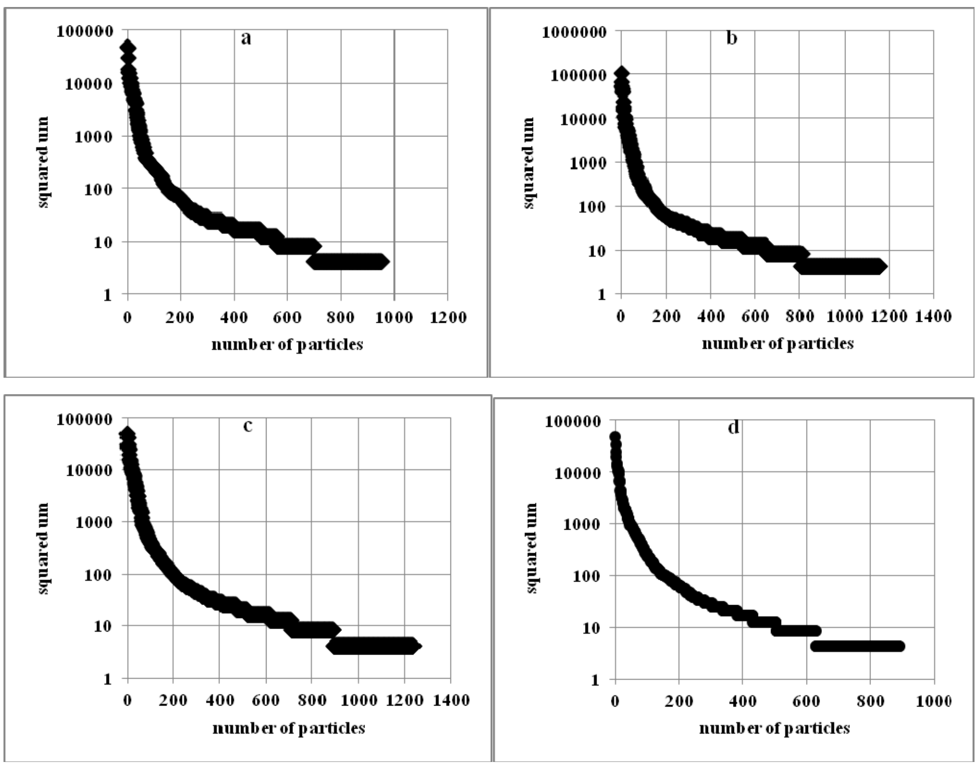

Fig. 3. Aggregates area distribution of activated sludge during 2009-2010: a – December; b – April; c – May; d – June (number of points on diagrame corresponds to ten microcopic fields)

The DHA in activated sludge was assessed by two different approaches: in unamended (actual DHA) and amended samples with glucose (potential DHA). First approach estimates DHA based on pre-existing substrata in MLSS while the second one evaluates the potential DHA in the presence of glucose.

The dehydrogenase activity was lowest in December (14.51 ug TPF/ml MLSS), increased in May (24.73 ug TPF/ml MLSS) and decreased again to 17.33 ug TPF/ml MLSS in June (Fig. 5a). Potential dehydrogenase activity followed a very similar pattern over time. Lowest value was recorded in December (15.65 ug TPF/mL MLSS), increased to highest values on April (24.97 ug TPF/mL MLSS) and May (25.12 ug TPF/mL MLSS), and decreased again in June to 20.96 ug TPF/mL MLSS (Fig. 5b).

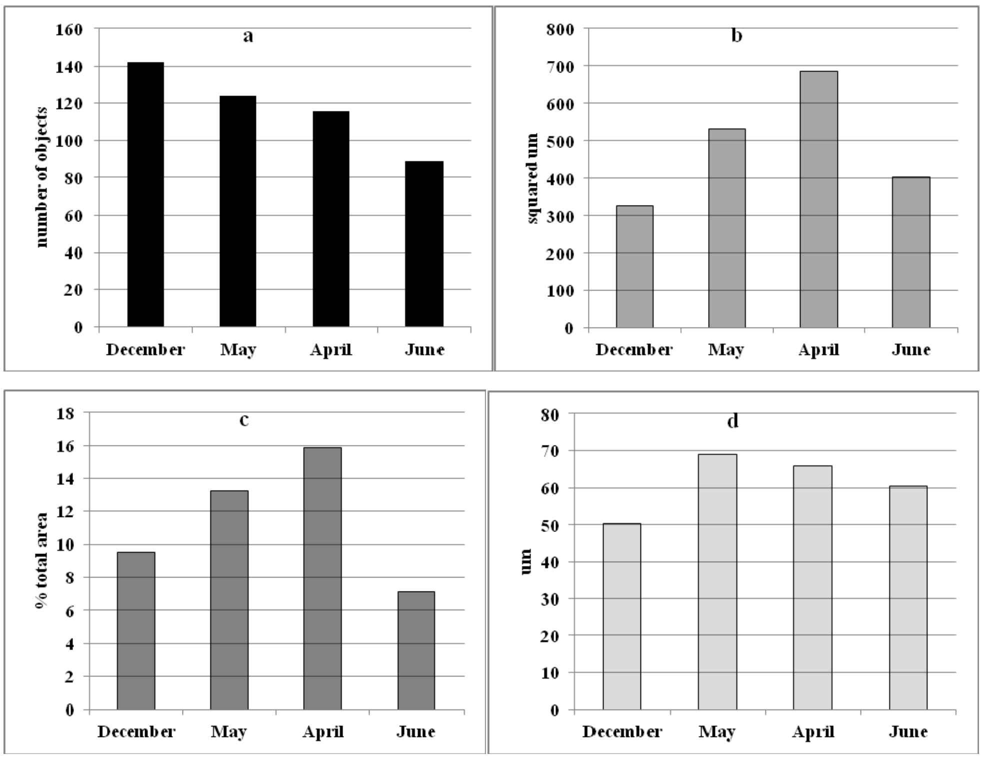

Fig. 4. Average values of morphometric propertiesduring 2009-2010: a – number of objects; b – average area; c – % of total area; d – average perimeter

A significant difference between actual and potential dehydrogenase activities was recorded in April and suggested that microbial community from the activated sludge could be growth limited. Limiting factors could be either DO concentration or one or more essential nutrients (such as phosphorous).

During the study period we observed that a weak connection existed between the actual and potential DHA (r=0.63). As regard the actual activity, it correlated weakly with total number of microscopic objects (r=0.63), with % of total area (r=0.68) and with average perimeter (r=0.50). Instead, we recorded a better positive correlation between actual DHA and average value of area (r= 0.87). Potential DHA correlated also weakly moderate with % of total area (r=0.69), moderate with average value of area (r=0.88) and very well with average value of perimeter (r=0.98).

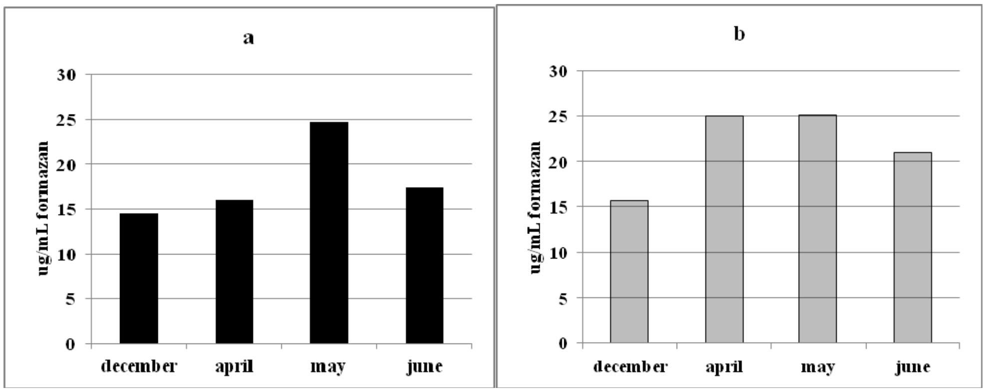

Fig. 5. Dehydrogenase activity in activated sludge samples during December 2009-June 2010: a – actual dehydrogenase activity; b - potential dehydrogenase activity

DHA has been used to assess the effect of specific environmental conditions on the activated sludge activity such as starvation [14] and toxicants [15, 16]. Along with quantitative microscopic analysis, DHA might be used successfully to monitor the performance of activated sludge systems and in specific technical conditions the method could be able to detect and unveil the metabolic status of microbial community involved in degradation and removal of biodegradable wastes in processes such as composting [17], bio-drying [18], bio-stabilization [19] and anaerobic digestion [20].

6. Conclusions

During the study period, average aggregates area ranged from 327.97 um^2 to 686.88 um^2. The perimeter showed less variability as size and had values between 50.43 um and 68.87 um. Actual DHA showed variations from 14.51 ug TPF/mL MLSS (December) to 24.73 ug TPF/mL MLSS (May). Potential DHA was a little higher and exhibited a similar seasonal pattern with a minimal value of 15.65 ug TPF/mL MLSS in December and a maximal value of 25.12 ug TPF/mL MLSS in May. Actual DHA correlated weakly positive with average number of aggregates/microscopic field (r=0.63) and % of total area (r=0.68). Potential DHA did not show any statistical connections with average number of microscopic objects. Instead, this parameter correlated moderate with average value of area (r=0.88) and well with average value of perimeter (r= 0.98).We believe that the approach to combine microscopic analysis of activated sludge with DHA is useful to understand the relationship between morphological characteristics of activated sludge and its metabolic properties.

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