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Determination of Decay Coefficient of Biomass through Endogenous Respiration

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ABSTRACT

Endogenous respiration processes of nitrifying/denitrifying bacteria under aerobic and anoxic conditions were investigated in a batch study using three identical bioreactors of size 5 L. The rate of digestion of microbial protoplasm was determined through the oxidation of cell tissue and the formation of new cellular materials using the Volatile Suspended Solids (VSS) method. Chemical Oxygen Demand (COD), ammonia, nitrate and VSS were monitored simultaneously at different sludge age. Three exponential phases of metabolism, starvation and death were observed during VSS reduction in both the aerobic and anoxic digestion. In the aerobic digestion, the average endogenous decay coefficient of volatile suspended solids was 0.055 L day^{-1} with a 95% confidence limit of 0.017 whereas 0.053 L day^{-1} was observed for anoxic digestion with a 95% confidence limit of 0.038 at 25±1°C. Results show that anoxic digestion has a slightly reduced decay rate than aerobic digestion. Therefore, the characterization of biomass and determination of stoichiometry coefficients is essential for understanding the activities of microbes in treatment processes.

Key words: Biomass decay, aerobic, anoxic, predation, metabolism, lysis

INTRODUCTION

Biological wastewater treatment is an effective method commonly used in various industries due to its configurational dynamism and reduced cost (Ezechi *et al*., 2014a). As a result of different wastewater composition from various sources, biological wastewater treatment systems are designed to meet the desired objective of the operators. For instance, produced water has a unique composition when compared with other wastewater sources and its treatment requires specifically designed systems (Ezechi *et al*., 2012a, b, 2014b, 2015a; Isa *et al*., 2014). The recycling potential of wastewater has been a subject of various studies in order to reduce water body deterioration, prevent eutrophication and supplement limited fresh water resources, especially in arid areas (Ezechi *et al*., 2015b, c). Therefore, it is pertinent to examine the design coefficients for any biological wastewater treatment system for optimum performance.

Quantitative measurement of endogenous respiration of biomass is essential to understanding the decay rate of bacteria in natural and engineering environments. Endogenous respiration of

biomass can be referred as the consumption of cell-internal substrate in the absence of external organic matter/substrate (Vanrolleghem, 2002). Bacteria decay rates affect nitrification performance of a wastewater treatment plant (Manser *et al*., 2006). Therefore the knowledge of the decay of nitrifying bacteria is essential for effective modeling of nitrification in biological wastewater systems.

The common techniques to examine the endogenous decay of biomass are anaerobic and aerobic processes depending on the purpose. Anaerobic digestion has wide industrial application especially in the production of biogas (Mata-Alvarez *et al*., 2000). However, it is costly in small wastewater treatment plants due to the energy required to maintain the corresponding temperature. Aerobic digestion is common and often used in the digestion of different sludge (Bernard and Grey, 2000). Its advantages include low capital cost, easy to operate and prevents nuisance odors (Ritter, 1970). The role of denitrifying bacteria is essential for complete denitrification in a wastewater treatment plant. Therefore, it is important to understand and determine the decay rate of biomass under anoxic conditions.

The endogenous decay rate of sludge could be quantified through the Volatile Suspended Solids (VSS) based and Oxygen Uptake Rate (OUR) based methods respectively. Both principles depend on the change of either the VSS or the OUR with time. The VSS based and the OUR based methods could be expressed by the following equations, respectively (Ramdani *et al*., 2010):

$$\text{VSS based method: } VSS(t) = VSS_u + (1 - f_H) \cdot X_H(0) \cdot e^{(-b_H \cdot t)} \quad (1)$$

$$\text{OUR based method: } OUR(t) = (1 - f) \cdot (1 + 4.57 \cdot f_N) \cdot b \cdot X_H(0) \cdot e^{-b \cdot t} \quad (2)$$

Where:

X_H (0) = VSS concentration of the active biomass at time zero (mg VSS L^{-1})
VSS (t) = VSS concentration at time t (mg VSS L^{-1})
VSS (0) = VSS concentration at time zero (mg VSS L^{-1})
VSS_U = VSS concentration at the end of the batch test (mg VSS L^{-1})
b_H = Endogenous decay rate (day^{-1})
f_H = Endogenous residue fraction (-)
f_{av} = Active fraction of the sludge (-)
OUR (t) = Total oxygen uptake rate (mg O_2 L^{-1} day^{-1})

The mechanism of endogenous respiration involves the decay and utilization of microbial cytoplasm by microorganism higher in the food chain in the absence of substrate. Representing activated sludge biomass with the empirical equation $C_5H_7NO_2$, aerobic digestion of the sludge can be represented as:

$$C_5H_7O_2N + 5O_2 \rightarrow 5CO_2 + 2H_2O + NH_3 \quad (3)$$

Endogenous respiration under anoxic conditions utilizes a carbon source in the form of nitrate. This can be represented as follows:

$$C_5H_7O_2N + 5NO_3 \rightarrow 5CO_2 + 3_{1/2}H_2O + NO + 4N_2 \quad (4)$$

The reduction of solid mass during endogenous respiration is assumed to take place only with biodegradable content of the sludge. However, some studies have indicated possible destruction of non-organics during endogenous respiration (Randall *et al*., 1975; Benefield and Randall, 1978). This study, therefore focuses only on the biodegradable content of the sludge represented by the first order biochemical reaction below:

$$\frac{dM}{dt} = -K_d M \quad (5)$$

where, $\frac{dM}{dt}$ is rate of change of biodegradable volatile solids per unit of time (mg L^{-1} day^{-1}), the k_d is the reaction rate constant (day^{-1}), M is concentration of biodegradable volatile solids at time t in the digester (mg L^{-1}) and t is time (d)

The objective of this study is to determine the endogenous decay rate of biomass under aerobic and anoxic conditions using the VSS method. The cellular degradation of microbes was monitored and determined through the measurement of VSS, COD, Ammonia and nitrate.

MATERIALS AND METHODS

Endogenous decay determination: In this study, the VSS method of endogenous decay determination was utilized.

Sludge sampling: Sludge was collected from a Sewage Treatment Plant (STP) 2 h prior to the experiment. The Mixed Liquor Suspended Solids (MLSS) and the Mixed Liquor Volatile Suspended Solids (MLVSS) of the biomass were 5200 and 4680 mg L^{-1}, respectively.

Process description: The bioreactors were made of perspex and has a volume of 5 L. Four liter of the biomass was introduced into the reactor for both aerobic and anoxic studies. The experiments were conducted at 25±1°C and supernatants were analyzed at different sludge age for a total period of 9 days. Hundred milliliter of the samples was withdrawn daily for analysis and supernatants from the aerobic tank of a Sewage Treatment Plant (STP) were added to the reactor to maintain constant volume. Sample temperature was controlled using HM-200 aquarium heater. After solid analysis were triplicated (MLSS and MLVSS), the withdrawn sample was centrifuged using Heraeus Bench centrifuge at 4000 rpm for 15 min. The pH of the sample was maintained at 7.0±0.2 by the addition of phosphate buffer (Stasinakis *et al*., 2002). The separated supernatant was used for COD, ammonia and nitrate duplicate analysis.

Endogenous respiration under aerobic condition: Biomass was placed in three identical batch bioreactors of size 5 L and was continuously aerated for a period of 8-9 days. The dissolved oxygen was maintained between 2-4 mg L^{-1} for effective biodegradation using aquarium pumps connected to ceramic plate diffusers placed at the bottom of the bioreactors. No substrate was added to the bioreactor. Samples were daily collected for analysis.

Endogenous respiration under anoxic condition: Biomass was placed in three identical batch bioreactors of size 5 L and was continuously stirred for a period of 8-9 days. Nitrate was dosed to the reactor in the form of potassium nitrate (KNO_3) in order to prevent anaerobic conditions

(Kaelin *et al*., 2009). Under anoxic conditions, autotrophic bacteria cannot gain adenosine triphosphate (ATP) because they are obligate aerobes. Therefore, the bioreactor was aerated daily for 5 min in order to prevent any shortage of energy in the form of ATP (Manser *et al*., 2006). No substrate was added to the reactor. Samples were daily collected for analysis.

Analytical procedure: Chemical Oxygen Demand (COD), Mixed Liquor Suspended Solids (MLSS) and Mixed Liquor Volatile Suspended Solids (MLVSS) were analyzed according to the 21st edition of standard methods for the examination of water and wastewater. Ammonia-nitrogen (NH_3-N) and nitrate (NO_3-N) were determined using spectrophotometric method with DR 2000. Dissolved oxygen was monitored using a DO meter equipped with a DO sensitive probe.

Result significance: The obtained data for the decay coefficient under aerobic and anoxic conditions was subjected to descriptive statistical analysis using Microsoft Excel 2007. The sample mean, 95% confidence limit, standard deviation and standard error were all obtained for both the aerobic and anoxic digestion processes.

RESULTS AND DISCUSSION

VSS reduction: Figure 1 shows the reduction of VSS concentration with time. Both the aerobic and anoxic VSS endogenous decay rate method followed similar pattern for VSS reduction. Three significant phases could be established from Fig. 1. Metabolism and utilization of internal storage products of biomass were more prominent in phase 1 from day 0 to day 1.5. During this period, the microorganisms utilized their internally stored products and only a little VSS reduction was observed due to negative net growth rate. In phase 2 (Days 1.5-3.0), starvation, exhaustion of essential nutrients and microorganism adaptation was observed with a little decrease of VSS. In phase 3 (Days 4 - 9), predation, accumulation of inhibitory products, depletion of essential nutrient and death was prominent with a high VSS reduction. The final VSS concentrations in all three reactors for the aerobic and anoxic digestion processes were less than 5% of the initial value. This observation is in agreement with the OUR pattern of Friedrich and Takacs (2013) and also in agreement with the report of Manser *et al*. (2006), who observed a sharp loss of autotrophic activity during the first two days of their experiment and about 60% reduction of both heterotrophic and autotrophic activities after 7 days. It has been reported that a fasting environment could induce a depletion of food storage (Chen *et al*., 2001). In both the aerobic and anoxic conditions under

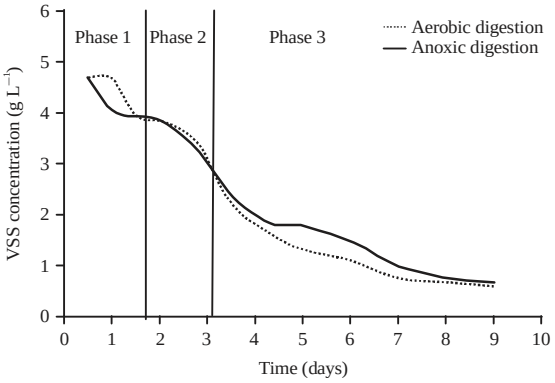

Fig. 1: Different phases for endogenous decay rate determination

fasting conditions of 2 h, Chen *et al*. (2001) observed a slight reduction of COD and Mixed Liquor Suspended Solids (MLSS). Therefore, a more significant depletion of both the storage products and the living cells would occur at prolonged fasting period as observed in this study.

The linearized form of the endogenous decay rate is presented in Eq. 1. In the VSS method, a non-linear regression could be applied to the obtained data according to Eq. 1. Accordingly, a plot of ln[VSS(t)-VSSu] vs t provides a straight line of slope b with an intercept of $\ln[(1-f)X_H(0)]$ from which the value of $X_H(0)$ can be estimated (Ramdani *et al*., 2010).

Aerobic decay rate: The plots of ln[VSS(t)-VSSu] vs t for the aerobic VSS digestion method is presented in Fig. 2. The endogenous decay rate (b) was estimated from the slope of ln[VSS(t)-VSSu] vs t. The endogenous decay rate (b) for the three bioreactors under aerobic digestion was 0.057, 0.055 and 0.054 L day^{-1} with correlation coefficients (R^2) greater than 0.980, respectively (Table 1). During the experiments, there was no external addition of substrate, which limits the activities of the microbes to decay and hydrolysis processes. The biomass concentration and respiration rate slowly decreased while inert particulate matter and biomass debris accumulated as the experiment proceeded.

This result slightly differed from the result of Ramdani *et al*. (2012a), who obtained an endogenous decay rate of 0.24 L day^{-1} under aerobic condition. In their study, the biomass were fed with a soluble and completely biodegradable influent using sodium acetate as the sole carbon (feasting). In another study, Ramdani *et al*. (2012b) obtained an endogenous decay rate of 0.0075 L day^{-1} in a batch test using thickened sludge at 20°C for an intermittently aerated system which is in agreement with our study. The major variation in these experiment is the test method (OUR/VSS) and operation process (fasting/feasting). In the study of Manser *et al*. (2006) under fasting condition, an aerobic decay rate of about 0.15 L day^{-1} for Conventional Activated Sludge (CAS) and 0.14 L day^{-1} for membrane bioreactor (MBR) was observed which is in close agreement with our obtained aerobic decay rate of 0.055 L day^{-1}. Lee and Oleszkiewicz (2003) also obtained an aerobic endogenous decay rate of 0.153 L day^{-1} under fasting condition, whereas Siegrist *et al*. (1999) observed the reduction of decay rate of nitrification activity from about 0.2-0.03 L day^{-1} from aerobic to anaerobic conditions. Wen *et al*. (1999) reported an endogenous decay rate of about 0.08 L day^{-1} for a membrane bioreactor (MBR) treating urban wastewater.

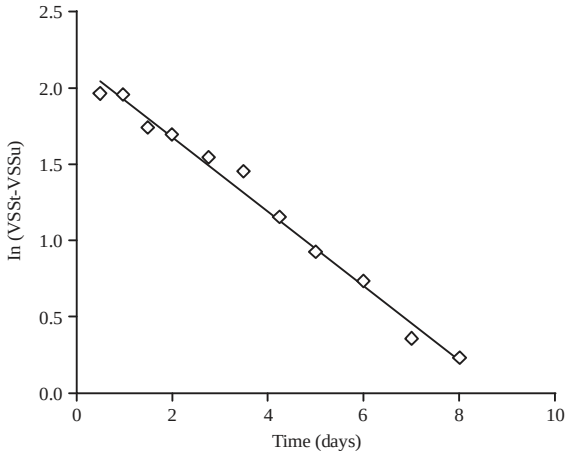

Fig. 2: Aerobic VSS digestion plot

It is reported that environmental parameters such as load, temperature and hydraulic parameters can influence the reduction of microbial activity (Martinage and Paul, 2000). Martinage and Paul (2000) observed some variation in decay rate under aerobic conditions from 0.08-0.16 L day^{-1} due to the changes in influent quality from the same source (industrial wastewater) at 30°C. Further variation was observed (0.36 L day^{-1}) when 30% of the influent load consists of urban wastewater. Therefore, the variance of the aerobic endogenous decay rate found in literatures could be summarily attributed to environmental factors (influent characteristics, influent load, temperature and hydraulic parameters) and microbial community composition.

Anoxic decay rate: The plots of ln[VSS(t)-VSSu] vs t for the anoxic VSS digestion method is presented in Fig. 3. The endogenous decay rate (b) was estimated from the slope of ln[VSS(t)-VSSu] vs t. In the anoxic digestion, the endogenous decay rate (b) was found as 0.051, 0.054 and 0.054 L day^{-1} for the three bioreactors with correlation coefficients (R^2) greater than 0.950, respectively (Table 1). The average endogenous decay rate (b) for the three bioreactors under aerobic and anoxic conditions were 0.055 and 0.053 L day^{-1}, respectively. The decay rate under anoxic conditions is in agreement with the study of Manser *et al*. (2006), who obtained a decay rate of 0.02 L day^{-1} or lower for both CAS and MBR, respectively. An anoxic endogenous decay rate of 0.097 L day^{-1} was obtained under fasting conditions for Sequential Batch Reactor (SBR) sludge (Lee and Oleszkiewicz, 2003). Martinage and Paul (2000) observed about 50% decrease of decay rate under anoxic conditions due to microfauna grazing of autotrophic bacteria. Wastewater characteristics, microbial community composition and operational parameters are significant factors that could influence microbial activity under anoxic conditions (Manser *et al*., 2006).

The decay rate for the heterotrophic organisms under anoxic conditions slightly decreased compared to the autotrophic organisms under aerobic conditions. Similar observations is reported in literature (Siegrist *et al*., 1999).

Table 1: Endogenous decay constants

Aerobic digestion				Anoxic digestion			
VSS (mg L^{-1})	b_T (day^{-1})	T (°C)	R^2	VSS (mg L^{-1})	b_T (day^{-1})	T (°C)	R^2
302	0.057	25±1	0.987	328	0.051	25±1	0.960
281	0.055	25±1	0.982	354	0.054	25±1	0.956
269	0.054	25±1	0.988	335	0.054	25±1	0.963

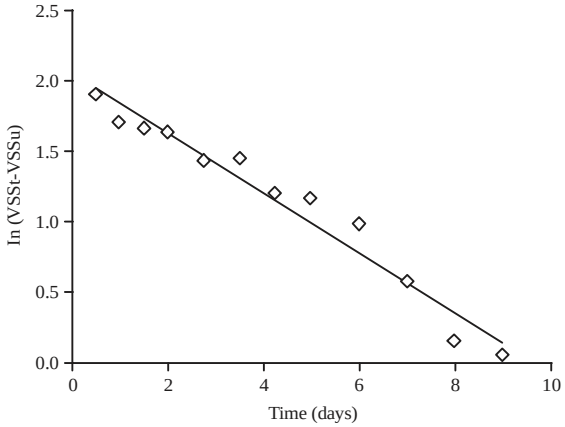

Fig. 3: Anoxic VSS digestion plot

It was observed that from time 0-t, a significant amount of active biomass disappeared from both the aerobic and anoxic digestion methods. After an extended digestion time of 9 days, the concentration of the biomass becomes near negligible (<5% of the initial value). Thus, the VSS concentration of both the aerobic and anoxic digested sludge reached an ultimate value.

During the initial stage for both reactors, the volatile suspended solids had a sharp increase and maintained a decreasing trend afterwards. The increase during the initial stage may be attributed to the utilization of internal storage products by the biomass in the absence of substrate. The breakdown of this product can sustain the biomass for a small period of time. At complete utilization of this product, the microorganisms begin to feed on its cytoplasm. The decay coefficients obtained in this study are similar to the ones reported in literature for ceramic membrane side-stream aerobic bioreactor treating raw wastewater (0.08 L day^{-1}) (Wen *et al*., 1999) and (0.05 L day^{-1}) for an MBR (Fan *et al*., 1996) at 30°C, respectively.

COD, ammonia and nitrate production: The decrease of total COD was monitored for both reactors and presented in Fig. 4. Data points from t = 0-1 was omitted in Fig. 4 because they represent the period of utilization of the internal storage products of the sludge. For both reactors, COD accumulated at the initial stage of the experiment, but slowly decreased with time. This indicates that the microorganisms could have used the organic nutrients produced within the bioreactors for survival. Similar observation is reported elsewhere (Ramdani *et al*., 2012b).

Ammonia and nitrate were produced from both reactors as shown in Fig. 5 and 6, respectively. Because nitrifying bacteria require oxygen as an electron acceptor in the aeration tank, they produce ammonia during cell lysis. However, the ammonia was effectively used up during the decay process. The ammonia produced under anoxic condition was slowly used up because denitrifying bacteria have a low rate of ammonia assimilation.

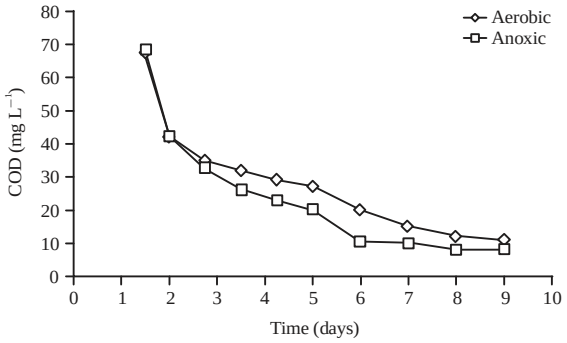

Fig. 4: Decrease of COD under aerobic and anoxic conditions

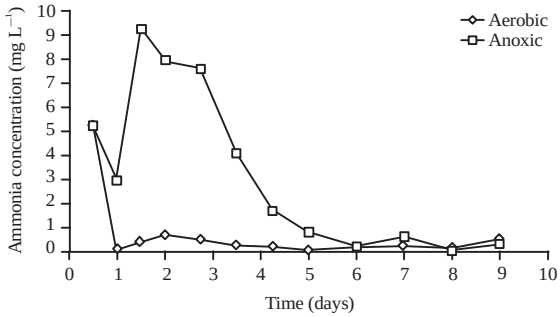

Fig. 5: Production and utilization of ammonia

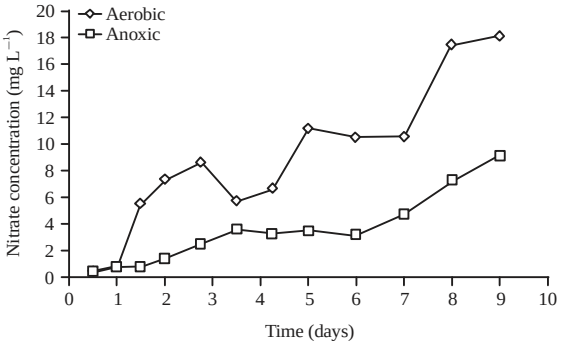

Fig. 6: Production and accumulation of nitrate

Table 2: Statistical analysis of endogenous decay rate at 25°C

	Aerobic digestion				Anoxic digestion			
Parameters	Mean	95% confidence limit	Std. dev.	Std. error	Mean	95% confidence limit	Std. dev.	Std. error
Bioreactor 1	0.845	0.0171	0.616	0.185	0.761	0.038	0.611	0.176
Bioreactor 2	0.833	0.0193	0.621	0.177	0.759	0.035	0.609	0.177
Bioreactor 3	0.849	0.0181	0.619	0.179	0.756	0.036	0.612	0.179

Std. dev: Standard deviation, Std. error: Standard error

Table 3: Comparison of previous studies

Test method	b (day^{-1})	Temperature (°C)	Test-time	R^2	References
OUR_{max}	0.075	18	n.i.	0.965	Avcioglu *et al*. (1998)
DNA	0.059	20	10	0.890	Liebeskind *et al*. (1996)
OURe, VSS, NO_3, Alk	0.246	20	6	0.970	Van Haandel *et al*. (1998)
VSS aerobic	0.055	25	9	0.982	Present work
VSS anoxic	0.053	25	9	0.956	Present work

The nitrate produced under aerobic condition accumulated in the reactor (Fig. 6). This can be explained thus; nitrate as the end product of nitrification cannot be effectively utilized by nitrifying bacteria and so accumulates in the reactor. For the anoxic condition, nitrate was continuously and slowly used up because denitrifying bacteria require nitrate as a carbon source for denitrification. Similar observation is reported in literature (Manser *et al*., 2006).

Significance of results: Table 2 shows the obtained values for the descriptive statistical analysis on the obtained VSS data.

From Table 2, it was found that mean value, 95% confidence limit, standard deviation and standard error were all statistically significant for the three bioreactors. The model coefficient for the aerobic digestion method was 0.057, 0.055 and 0.054 L day^{-1} with corresponding mean values of 0.845, 0.833 and 0.849 L day^{-1}. The standard deviation and standard error were all low. The anoxic digestion method also presented similar trend. The model coefficient for the three bioreactors was found to be 0.051, 0.054 and 0.054 L day^{-1} with corresponding mean values of 0.761, 0.759 and 0.756 L day^{-1}. A low standard deviation and standard error was observed.

On the basis of experimental validity, the obtained results from this study was compared with other reported results and methods as presented in Table 3. It was found that the obtained data and method have close similarity with respect to endogenous decay coefficient and duration of digestion of other reported studies.

CONCLUSION

This study has successfully determined the endogenous decay rate for the biomass of a sewage treatment plant through the VSS method. Its findings are critical and important when designing

a sewage treatment plant. The temperature investigated compliments the Malaysian weather in humid and hot seasons. The observation made during the study are as follows:

- Three exponential phases were observed for microorganism reduction in both the aerobic and anoxic digestion. The first phase consists of the metabolism and utilization of the internal storage products of microbes. The VSS reduction was very low in this phase. The second phase consists of hunger, exhaustion of essential nutrients and microorganism adaptation to starvation. The VSS reduction, increased in this phase. The third phase consists of predation, accumulation of inhibitory products, depletion of essential nutrient and death. The VSS reduction was high in this phase
- The average endogenous decay coefficient for the aerobic and anoxic digestion processes was found to be 0.055 and 0.053 L day^{-1}, respectively and was statistically justified at a 95% confidence limit
- Production of essential and inhibitory nutrients through cell lysis was monitored. The COD and ammonia was found to accumulate at initial stage but gradually decreased with time in both bioreactors. However, nitrate accumulated in both bioreactors throughout the investigation

The obtained results are therefore useful for the design of a sewage treatment plant. It is highly recommended to measure biomass decay coefficient for each wastewater treatment situation under consideration.

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