



Extent of endogenous decay and microbial activity in aerobic stabilization of biological sludge

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ABSTRACT

The study evaluated the role of endogenous decay and microbial activity in aerobic stabilization of biological sludge. Biomass acclimated to acetate in a fill and draw reactor operated at a sludge age of 8 d was subjected to aerobic stabilization with no external substrate for a period of 70 days. Achieved volatile suspended solids reduction remained limited to around 43% after 30 days. The magnitude of endogenous decay, presumably responsible for stabilization, was quite different with b_H values of 0.21/d and 0.06/d depending on the implemented respirometric procedure. Model profiles simulated with these values could not predict the observed VSS profile, mainly due to the accumulation of particulate metabolic products and residual organic cellular debris of disintegrated biomass during the process providing conclusive evidence that the total organic content of the stabilized biomass cannot be reduced below a critical level by means of biological processes.

Keywords: Activated sludge; Aerobic stabilization; Biological sludge; Endogenous decay; Respirometry

1. Introduction

Excess activated sludge generated in the course of biological treatment of wastewaters is a complex mixture of different particulate components. It embodies aside from active microbial community, inorganic matter either introduced with the wastewater or generated as cellular residues, inert particulate organics of influent origin, remaining fraction of the particulate slowly biodegradable substrate entrapped in biomass and

residual particulate microbial products generated in the course of metabolic reactions [1–3]. Consequently, the overall endogenous decay actually reflects different mechanisms such as maintenance energy requirements, decay of cells, endogenous respiration, grazing by higher trophic level microorganisms, inhibitory and toxic conditions affecting inactivation and lysis of cells [4]. Traditionally, activated sludge has been loosely defined in terms collective parameters such as total suspended solids (TSS) or volatile suspended solids (VSS); they could only be associated with an equally empirical rate coefficient, k_d , for endogenous decay,

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which merely indicated the observed overall decrease in the VSS content. A value of 0.05/d was commonly adopted for this coefficient [5].

Recent developments in the understanding of the activated sludge process have solved to a great extent the inconvenience and inability of the VSS parameter to differentiate between active biomass, particulate inert matter and particulate metabolic products presumably generated with endogenous decay, mainly because all these parameters were accounted for and included as components in activated sludge models [6–8]. These models defined a new endogenous decay coefficient, b_H , solely associated with active heterotrophic biomass and methods have been defined for its experimental assessment.

Sludge management is one of the most critical issues in environmental protection. Different protective measures and required characteristics are defined in detail, both by international and local authorities for safe disposal of the generated excess sludge in wastewater treatment plants [9,10]. Requirements include feasible stabilization conditioning processes before the disposal. One of the possible option is aerobic stabilization process [11,12]. It is implemented either as a separate process or as part of the biological treatment system operated as extended aeration scheme. While the fate of TSS is significant for assessing the achievable dry solids content, it is imperative to understand appropriate mechanisms during stabilization and this inevitably involves the undergoing endogenous decay process.

In this context, the main purpose of the study was to evaluate the extent of endogenous decay during aerobic stabilization of biological sludge, i.e. excess sludge generated in the biological part of the treatment scheme. The evaluation involved respirometric procedures and included two different procedures based on decrease of endogenous respiration and loss of activity in microbial biomass. The results obtained were evaluated in relation to the expected efficiency of the aerobic stabilization process.

2. Materials and methods

2.1. Sludge sampling and acclimation

Sludge sample was taken from a municipal wastewater treatment plant with a capacity of 4.550 m³/d and a sludge retention time of 14 days. The treatment plant includes physical and activated sludge treatment processes. The excess sludge of the plant is sent to the thickening unit and then sent to the belt filter press.

The collected sludge was cultivated in two laboratory-scale fill-and-draw reactors with a working

volume of 12 or 6 L. The reactors were operated in aerobic conditions with a sludge age of 8 days and a hydraulic retention time of one day. These reactors were fed with 400 mg COD/L sodium acetate acting as readily biodegradable organic matter. All other macro and micronutrients were also added in sufficient quantities for biological growth. The temperature of the systems was kept constant at 20 ± 0.5°C. The dissolved O₂ concentration in the reactors was kept above 2 mg/L using air stones. The pH of the reactors was maintained at 7 ± 0.5.

2.2. Sludge stabilization

Two glass cylinder reactors with a working volume of 3 and 6 L were used for aerobic stabilization of sludge at 20 ± 0.5°C and were mixed with a mechanical mixer. pH was kept at 7 ± 0.5. During the aerobic stabilization period, the dissolved O₂ concentration in the reactors was kept above 2 mg/L using air stones. Evaporation losses were made up with distilled water every day.

2.3. Respirometric assessment of endogenous decay

Respirometric assessment of endogenous decay rate, b_H was conducted in accordance with a procedure proposed by [13]. In this method, the endogenous respiration rate, b_H , is determined by monitoring changes in the oxygen uptake rate (OUR) profile of a continuously aerated sludge sample without any addition of external substrate. Relevant basis for evaluation may be defined as follows: The decrease in active heterotrophic biomass, X_H , with time is expressed as in Eq. (1):

$$X_H = X_{H0}e^{-b_H t} \quad (1)$$

On the other hand, OUR corresponding to endogenous decay monitored in the experiment may be assessed by Eq. (2):

$$\text{OUR} = (1 - f_E)b_H X_H \quad (2)$$

where f_E is the total endogenous residue, i.e. soluble and particulate microbial products released during endogenous decay. A relationship between $\ln \text{OUR}_t$ and b_H given in Eq. (3) may be obtained by rearranging expressions 1 and 2:

$$\ln \text{OUR}_t = \ln[(1 - f_E)b_H X_{H0}] - b_H t \quad (3)$$

Consequently, by plotting \ln OUR vs. time, the slope of the OUR curve yields the value of the endogenous decay coefficient, b_H .

2.4. Respirometric assessment of microbial activity

The study also utilized another respirometric procedure, which evaluates endogenous decay based on loss of activity and viability of heterogenic biomass in the sludge based on the method defined by [14]. The method relies on assessing change in the measured OUR values as a function of $\mu_H X_H$, which determines the magnitude of the initial OUR level. The method is designed to maintain a constant level for μ_H and this way, it allows calculating the variation of X_H with time during aerobic stabilization. Related evaluation is based on the assumption that the initial OUR in a batch reactor operated with sufficient/excess substrate sustains maximum growth conditions. A constant amount of readily biodegradable substrate is added in successive intervals and corresponding initial OUR plateaus are defined by Eq. (4):

$$\text{OUR}_t = \left[\frac{1 - y_H}{Y_H} \hat{\mu}_H + (1 - f_E) b_H \right] X_{H0} e^{-b_H t} \quad (4)$$

Linearized version of the above equation (see Eq. (5)), yields the corresponding b_H value, as the slope of the \ln OUR plot.

$$\ln \text{OUR}_t = \ln \left[\frac{1 - y_H}{Y_H} \hat{\mu}_H + (1 - f_E) b_H \right] X_{H0} - b_H t \quad (5)$$

2.5. Respirometric measurements

According to the method proposed by [14], 400 mg/L VSS sample were transferred from the aerobic stabilization reactor to respirometric reactor with a total volume of 2 L. OUR measurements were started prior to the feeding of the carbon source in order to obtain the OUR profile of the culture. After the constant endogenous decay rate level (b_H) in terms of respiration was maintained, 100 mg COD/L acetate was added into the respirometric reactor. The OUR data were collected with RA-Combo continuous respirometer (Applitek, NV, SA). For the assessment of b_H , according to the method proposed by [13], sludge samples were withdrawn at 5-min daily intervals from the aerobic stabilization reactor and the oxygen electrode was directly placed in the measurement flasks.

These measurements were conducted using WTW type oxygenmeter. Nitrification inhibitor (Formula 2533TM-Hach Company, USA) was added in order to prevent nitrification activity in the all respirometric experiments.

2.6. Analytical measurements

Liquid samples were periodically taken to monitor TSS, VSS, soluble chemical oxygen demand (COD) and pH. For soluble COD determination, samples were filtered through 0.45 μm membrane filters. All analyses were conducted as defined in Standard Methods [15].

3. Results and discussion

3.1. Acclimation experiments

The two fill and draw reactors used for the acclimation of biomass with acetate feeding as the sole organic carbon source were observed to reach steady-state conditions after 20 d of operation—a period of around three times the selected sludge age of 8 d. Following an initial decrease within the first 10 d, TSS and VSS concentration stabilized around average values of $1,698 \pm 85$ and $1,054 \pm 50$ mg/L, respectively, as plotted in Fig. 1. The reactors were quite effective as the daily COD removal efficiencies varied in the range of 92–95% suggesting that all the available substrate was utilized and generated residual soluble metabolic products [2].

3.2. Stabilization experiments

After acclimation, biomass in the reactors was transferred to stabilization reactors after thickening so

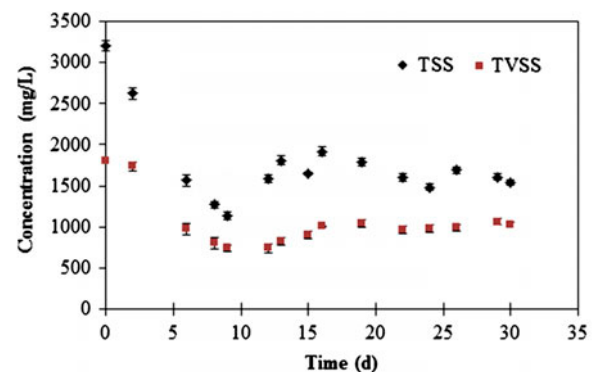


Fig. 1. TSS and VSS profiles during the acclimation period.

that the stabilization experiments were started with initial TSS and VSS concentrations of $3,800 \pm 78$ and $2,400 \pm 21$ mg/L, respectively. The corresponding

VSS/TSS ratio could be calculated as 0.63 mg VSS/mg TSS, quite in agreement with the level previously reported for the municipal sludge [16,17]. As shown in Fig. 2, the experiment continued for more than 70 d; however, effective decrease in TSS and VSS level could only be observed within the first 30 d. At the end of this period, TSS and VSS profiles exhibited a decrease down to 2,970 and 1,370 mg/L, respectively, yielding a final VSS/TSS ratio of 0.46 mg VSS/mg/TSS; the VSS removal efficiency was calculated as 43% on the thirtieth day of stabilization.

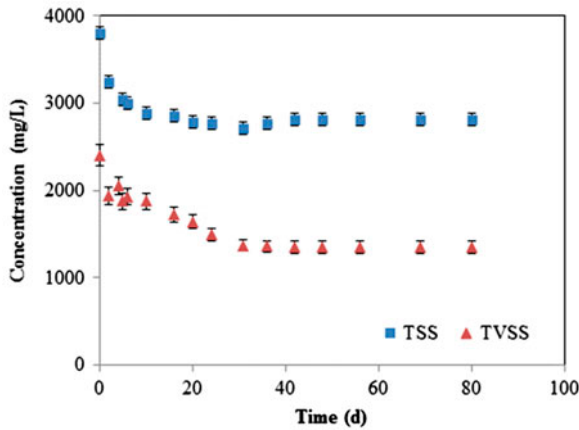


Fig. 2. TSS and VSS concentration profiles during aerobic stabilization.

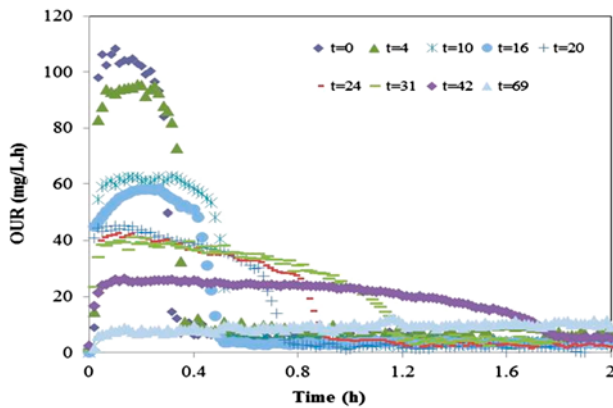


Fig. 3. OUR profiles obtained with pulse feeding of acetate along the stabilization period.

3.3. Respirometric experiments

Respirometric assessment of heterotrophic biomass activity in the course of stabilization yielded, as expected, variable OUR profiles as a function of time. As shown in Fig. 3, the value of the initial OUR plateaus starting from 105 mg O₂/L.h level at the beginning of the experiment, exhibited a gradual decrease to around 80 mg O₂/L.h after two days and finally to 40 mg/L.h at the end of 20 d. Manipulation of the OUR data as indicated in Eq. (5), produced a linear relationship with $R^2 = 0.9812$ with a slope which defined by a b_H value of 0.06/d (Fig. 4). This value, while lower than the commonly accepted b_H range of 0.15–0.24/d, is in agreement with the slightly higher b_H of 0.09/d determined similarly for acetate as the organic substrate and biomass acclimated to a sludge age of 15 d [14].

Conversely, application of the method proposed by [13] based on successive measurements of descending OUR levels in the course of sludge stabilization yielded a significantly higher b_H value of 0.21/d, with an equally reliable statistical confidence reflected by $R^2 = 0.985$ as plotted in Fig. 5. This value is quite typical for the activated sludge treatment of domestic

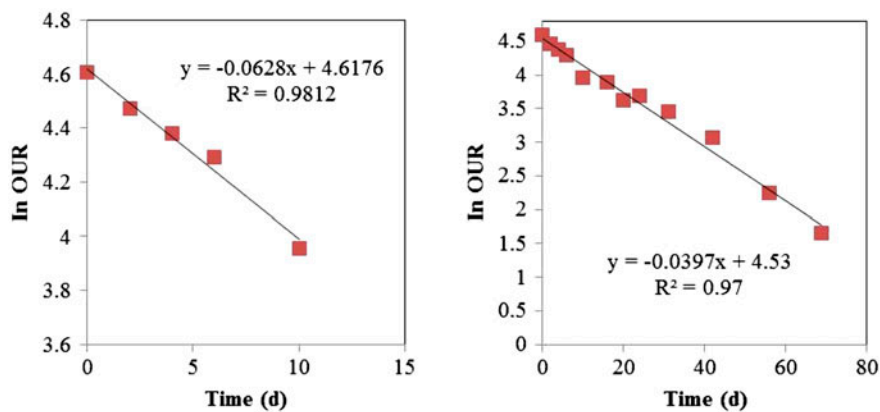


Fig. 4. Assessment of the endogenous decay coefficient based on biomass activity.

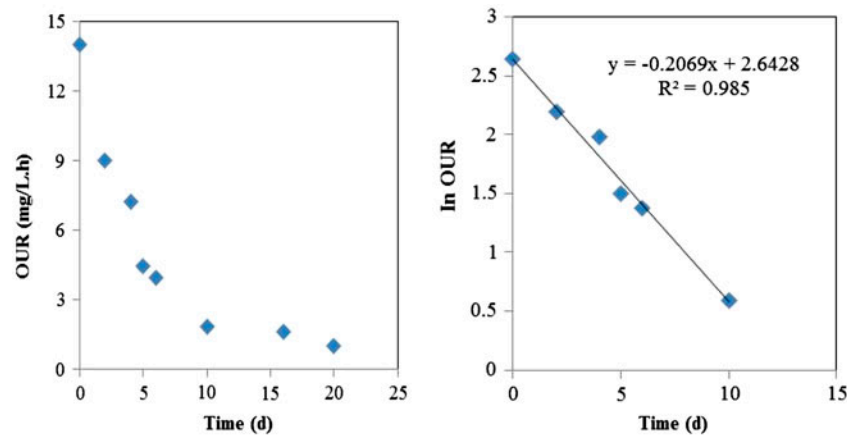


Fig. 5. Assessment of the endogenous decay coefficient based on successive OUR values.

Table 1

Reported values for the endogenous decay coefficient using the respirometric procedure of [5]

| Wastewater type | Temperature (°C) | Endogenous decay rate, b_H (day ⁻¹) | References |
|-----------------------------|------------------|---|------------|
| Acetate ($\theta_x = 8$ d) | 20 | 0.21 | This study |
| Domestic | 20 | 0.24 | [18] |
| Domestic (SRT = 10 d) | 22 | 0.40 | [19] |
| Domestic | 20 | 0.40 | [7] |
| Domestic | 15 | 0.11 | [20] |
| Domestic (SRT = 10 d) | 22 | 0.24 | [21] |
| Domestic | 20 | 0.18 | [22] |
| Domestic | 20 | 0.20 | [23] |
| Domestic (SRT = 17 d) | 20 | 0.10 | [12] |
| Domestic (SRT = 18 d) | 20 | 0.07 | [16] |
| Domestic (SRT = 18 d) | 20 | 0.20 | [17] |

sewage, close to the default value of 0.24/d suggested in activated sludge models [6] and within the range of values determined on the basis of similar studies (Table 1). It should be noted that monitoring could be pursued with the 30 days of stabilization; beyond this period, observed OUR values were too low to allow a reliable evaluation.

3.4. Discussion

Two observations seem to merit further emphasis in correlating experimentally assessed endogenous decay with sludge stabilization. The first one is the discrepancy between b_H values determined by means of two different experimental procedures. The methods originally prescribed by [5] rely solely on measured OURs, which cannot differentiate between endogenous respiration and biodegradation of particu-

late matter either adsorbed onto biomass or stored as intracellular biopolymers. Selection of acetate as a simple substrate avoided the interference of particulate matter adsorption but favours polyhydroxybutyrate storage, which may lead, at least in the early phases of the experiment assessment, to higher rates. Furthermore, stabilization is likely to result in cellular death and lysis yielding organic microbial residues which may serve as additional endogenous substrate for the remaining viable biomass. Consequently, this mechanism would be better interpreted in terms of a death/regeneration model recently used for this purpose [11]. In this context, the high b_H value of 0.21/d may be partly due to *regeneration*, i.e. growth on cellular organic residues. On the other hand, loss of viability as ascertained by the method of [14] would indeed reduce the level of endogenous respiration associated with the remaining fraction of viable biomass, but

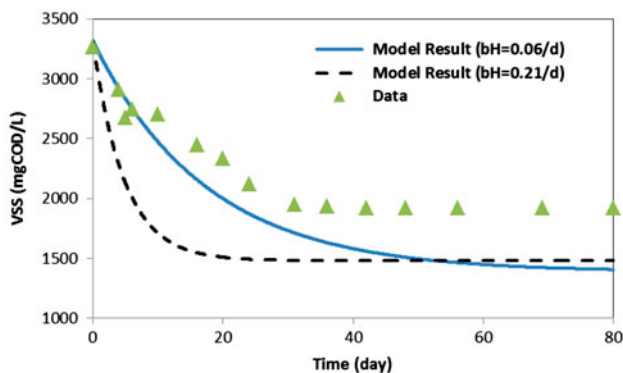


Fig. 6. Observed and simulated VSS profiles based on experimentally determined b_H values.

inactivation/death does not necessarily mean cellular decay and lysis and hence a lower b_H value.

The second significant observation is the failure of endogenous decay defined by both methods in predicting the efficiency of stabilization in terms of achievable VSS removal. As shown in Fig. 6, the observed VSS reduction during stabilization remained substantially lower than the levels predicted by the b_H values experimentally determined in the study; in other words, simulated VSS profiles based on b_H values of 0.21/d and 0.06/d indicate significantly higher reduction performances with respect to the evolution of the observed VSS profile. This is obviously due to the accumulation of particulate metabolic products and residual organic cellular debris of disintegrated biomass during the process providing conclusive evidence that the total organic content of the stabilized biomass cannot be reduced below a critical level by means of biological processes.

4. Conclusions

Stabilization of activated sludge acclimated to acetate, as the sole organic carbon source, provided a limited VSS reduction of around 43% after a period of 30 days confirming similar results reported for domestic sewage.

Different values were obtained for the endogenous respiration rate, obviously the major mechanism in aerobic sludge stabilization depended on the respirometric methodologies implemented for its assessment.

Experimental results suggested that endogenous respiration, while quite significant in biological systems for substrate removal, has no predictive value alone due to parallel biochemical reaction such as microbial death and lysis, growth on cellular residues, etc., which interfere with the experimental assessment.

Accumulation of residual particulate matter and metabolic product should be evaluated as decisive parameters for the efficiency of stabilization and the decrease of the total organic carbon, below the desired level.

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Nomenclature

| | |
|------------------|--|
| X_H | — active heterotrophic biomass concentration (M COD/L ³) |
| X_{H0} | — initial active heterotrophic biomass concentration (M COD/L ³) |
| Y_H | — heterotrophic yield coefficient (M COD/MCOD) |
| $\hat{\mu}_H$ | — maximum heterotrophic growth rate (1/T) |
| b_H | — heterotrophic endogenous decay rate coefficient (1/T) |
| f_E | — inert biomass fraction |
| OUR | — oxygen uptake rate (M O ₂ /L ³ T) |
| OUR _t | — initial OUR value after time t (M O ₂ /L ³ T) |

References

- [1] P.L. Dold, G.A. Ekama, G.v.R. Marais, A general model for the activated sludge process, *Prog. Water Technol.* 12 (1980) 47–77.
- [2] F. Germirli, D. Orhon, N. Artan, Assessment of the initial inert soluble COD in industrial wastewaters, *Water Sci. Technol.* 23(4–6) (1991) 1077–1086.
- [3] D. Orhon, D. Okutman, Respirometric assessment of residual organic matter for domestic sewage, *Enzyme Microb. Technol.* 32 (2003) 560–566.
- [4] M.C.M van Loosdrecht, M. Henze, Maintenance, endogenous respiration, lysis, decay and predation, *Water Sci. Technol.* 39 (1) (1999) 107–117.
- [5] G.v.R. Marais, G.A. Ekama, The activated sludge process part 1—steady state behaviour, *Water SA.* 2(4) (1976) 165–199.
- [6] M. Henze, C.P.L. Grady Jr., W. Gujer, G.v.R. Marais, T. Matsuo, Activated Sludge Model No.1. IAWPRC Sci. Technol. Report No. 1. IAWPRC, London, (1987).
- [7] M. Henze, W. Gujer, T. Mino, T. Matsuo, M.C. Wentzel, G.v.R. Marais, Activated Sludge Model No. 2, IAWPRC Task Group on Mathematical Modelling for Design and Operation of Biological Wastewater Treatment, IAWQ, London, 1995.
- [8] F.G. Babuna, D. Orhon, E.U. Cokgor, G. Insel, B. Yaprakli, Modelling of activated sludge for textile wastewaters, *Water Sci. Technol.* 38(4–5) (1998) 9–17.
- [9] D. Fyitili, A. Zabaniotou, Utilization of sewage sludge in EU application of old and new methods, *Renewable Sustainable Energy Rev.* 12 (2008) 116–140.

- [10] G. Insel, E. Kendir, A. Ayol, A. Erdinçler, O. Arıkan, İ. İmamoğlu, B. A. Alagöz, E. B. Gençsoy, F. D. Sanin, N. Büyükkamacı, Ö. Karataş, G. Saygili, G. Sevim, E. U. Çokgör, A. Filibeli, Current situation and future perspectives in municipal wastewater treatment and sludge management in Turkey, *J. Residuals Sci. Tech.* (2013).
- [11] A. Ramdani, P. Dold, S. Déléris, D. Lamarre, A. Gadbois, Y. Comeau, Biodegradation of the endogenous residue of activated sludge, *Water Res.* 44 (2010) 2179–2188.
- [12] E. Pehlivanoglu-Mantas, D.O. Tas, G. Insel, E. Aydin, D.C. Ozturk, T. Olmez, E. Gorgun, E.U. Cokgor, D. Orhon, Evaluation of municipal and industrial wastewater treatment sludge stabilization in Istanbul, *Clean-Soil Air Water* 35 (2007) 558–564.
- [13] G.A. Ekama, P.L. Dold, G.v.R. Marais, Procedures for determining influent COD fractions and the maximum specific growth rate of heterotrophs in activated sludge systems. *Water Sci. Technol.* 18(6) (1986) 91–114.
- [14] E. Avcioglu, D. Orhon, S. Sozen, A new method for the assessment of heterotrophic endogenous respiration rate under aerobic and anoxic conditions, *Water Sci. Technol.* 38 (8–9) (1998) 95–103.
- [15] APHA, AWWA, WEF. Standard Methods for the examination of water and wastewater, 21st ed., American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, 2005.
- [16] D. Okutman Tas, Respirometric assessment of aerobic sludge stabilization, *Biores. Technol.* 101 (2010) 2592–2599.
- [17] E. Ubay Cokgor, D. Okutman Tas, G.E. Zengin, G. Insel, Effect of stabilization on biomass activity, *J. Biotechnol.* 157 (2012) 547–533.
- [18] U. Solfrank, W. Gujer, Characterization of the domestic wastewater for mathematical modelling of the activated sludge process, *Water Sci. Technol.* 23(4–6) (1991) 1057–1066.
- [19] J. Kappeler, W. Gujer, Estimation of kinetic parameters of heterotrophic biomass under aerobic conditions and characterization of wastewater for activated sludge modelling, *Water Sci. Technol.* 25(6) (1992) 125–139.
- [20] H.Z. Sarıkaya, D. Orhon, H.A. San, S. Sozen, I. Demir, B. Inanc, E. Yuksel, E. Cokgor, E. Avcioglu, O. Arıkan, Wastewater Characterization in Tuzla Wastewater Treatment Plant Catchment Basin, Final Report Department of Environment Engineering Istanbul Technical University, 1997.
- [21] E. Cokgor Ubay, Respirometric evaluation of process kinetic and stoichiometry for aerobic systems, PhD Thesis, Istanbul Technical University, Turkey, 1997.
- [22] D. Orhon, O. Hanhan, E. Gorgun, S. Sozen, A unified basis for the design of nitrogen removal activated sludge process-The Braunschweig exercise, *Water Sci. Technol.* 38(1) (1998) 227–236.
- [23] D. Orhon, I. Kabdasi, E. Ubay Cokgor, O. Tunay, N. Artan, Experimental assessment of optimum operation strategy for large industrial wastewater treatment plants – A case study, *Environ. Eng. Sci.* 19(1) (2002) 47–58.