An approach for substrate mapping between ASM and ADM1 for sludge digestion

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Abstract Kinetic modelling of the hydrolysis stage of municipal activated sludge, which is presumed to be the rate-limiting step in the anaerobic sludge digestion process, was studied by measuring methane production rate (MPR) in anaerobic batch tests. The MPR curves revealed that the degradable organic components in municipal sludge could be classified into two fractions having different kinetics. The first fraction (X_{S1}) constituted about 55% of the sludge COD and degraded with first-order kinetics. The second fraction (X_{S2}), which degraded during the initial phase, accounted for about 21% of sludge COD. The degradation kinetics for X_{S2} was expressed by Contois-type equation with respect to concentration of substrate in the fed sludge and that of active biomass in the mixture. Simultaneous batch aerobic respirometric tests showed that the activated sludge was composed of 53% heterotrophic biomass ($X_{H\text{-}Aerobe}$) COD and 20% of slowly biodegradable COD (X_S), that had same kinetic expressions as observed in the batch anaerobic tests. The observed correlation between substrate fractions suggests X_{S1} and X_{S2} could be directly mapped to the aerobic state variables of $X_{H\text{-}Aerobe}$ and X_S respectively. The degradation of X_{S1} seems to be anaerobic decay of $X_{H\text{-}Aerobe}$ while X_{S2} is thought to be hydrolysis of X_S by microcosm of the sludge.

Keywords ADM1; ASM; hydrolysis; respirometry; sludge composition

Water Science & Technology **Vol 54 No 4 pp 83–92** © IWA Publishing 2006

Introduction

Anaerobic Digestion Model No.1 (ADM1) presented by the IWA task group is one of the structured mathematical models describing the anaerobic digestion process (Batstone *et al.*, 2002). ADM1 unifies the basis for anaerobic digestion modelling and provides a common platform for discussion of dynamic simulation for a variety of anaerobic processes. Therefore, the objectives of ADM1 are very similar to those of Activated Sludge Models (ASM) (Henze *et al.*, 2000). In the case of modelling of anaerobic sludge digestion, it would be desirable, if the two models could be integrated with a common set of state variables. Defining a common set of state variables will seamlessly integrate the well established ASM with ADM1 for sludge digestion. The fundamental idea and needs for plant-wide modelling were well pointed out by Copp *et al.* (2003) and Vanrolleghem *et al.* (2005). For such an integration to be possible, however, it would be required to develop a mapping between the ASM constituents of activated sludge (namely, heterotrophic biomass (X_H), slowly biodegradable organic solids (X_S) and biologically inert particles (X_I)) to the kinetically defined fractions in ADM1.

ADM1 assumes that the degradation of organic compounds proceeds in the order: (1) disintegration, (2) hydrolysis, (3) acidogenesis, (4) acetogenesis and (5) methanogenesis. In the municipal sludge treatment, disintegration and hydrolysis is the "entry and rate limiting stage" of anaerobic degradation and hence will most influence the characteristics

doi: 10.2166/wst.2006.529

of substrate mapping between ASM and ADM1. Until now, combination of steps of (1) and (2) have been modelled using simple empirical first-order kinetics with respect to total solid concentration. In fact, the first-order kinetic for the hydrolysis stage is adopted from the various reported datasets (Vavilin *et al.*, 1996; Blumensaat and Keller, 2005). However, to integrate ASM and ADM1, it would be necessary to identify individual hydrolysable organic fraction by using the concepts of substrate characterization based on hydrolysis kinetics and link them to ASM components. Based on this background, the main objectives of this study were: (1) to characterize activated sludge solid substrate based on hydrolysis kinetics throughout experimental procedure; and (2) establish a possible relationship between the particulate state variables of ASM and ADM1.

Material and methods

Sludge samples

Fresh concentrated activated sludge samples were taken from a gravity thickener of conventional activated sludge wastewater treatment plant operated at about 5 days of SRT. The samples were stored at 4 °C for 2 days until the batch tests were conducted. Anaerobically digested sludge for seed was collected from the pilot-scale anaerobic tank with HRT of 60 days in the plant. The digested sludge was stored at warm temperature of 30–35 °C so as not to lose much of the biological activity during the delivery period of about half-day to the laboratory. Before starting the test, the sludge was pre-incubated for about 1 day without addition of substrate at 35 °C, which was the same temperature as that of the source anaerobic digestion tank. Considering that the anaerobic digestion process at the plant occurs at long sludge retention time, it is expected that loss of biological activity due to substrate deficiency is minimal even though the preparation period for tests took about 1–2 days.

Batch respirometric test

Apparatus for respirometry. To identify organic fractions and kinetic parameters in the aerobic biological process, batch respirometric tests, in which oxygen uptake rate (OUR) is measured, are commonly used (Kappeler and Gujer, 1992; Copp *et al.* 2002). Since oxygen consumption is associated with biological substrate removal from the liquor and bacterial growth/decay in the system, the respirogram provides enough information to construct a reliable model structure. Unlike the conventional biochemical methane potential test that is based on integrated-curve analysis to obtain ultimate methane conversion and overall kinetics (Speece, 1996), the respirometric technique is based on differential-curve analysis that makes it possible for precise detection of kinetics and constituents of substrate. For developing kinetic expression and substrate characterization under anaerobic conditions in a similar manner to that in aerobic test, a batch *anaerobic* respirometric apparatus was used for the measurement of methane production rate (MPR) associated with anaerobic degradation of organic solids (Young *et al.*, 1991; TG ABAI, 2003).

Figure 1 illustrates the schematic diagram of the test apparatus and the major components. Many sets of anaerobic batch test were conducted using a respirometer supplied by Challenging Systems Inc., USA (AER-8). A single respirometer unit consisted of four components. The components were: (1) a gas-tight incubation vessel on the magnetic stirring base for mixing sample to provide contact between microorganism and substrate, (2) a cell device measuring methane gas production (or oxygen gas consumption in case of the aerobic test), (3) an interface module to convert gas production data to digital form, and (4) a computer for data acquisition. Temperature of the incubation vessel and the cell base were maintained at 35 ± 0.2 °C in a temperature-controlled incubator. A small scrubber consisting of a caustic material was set between the incubation vessel and

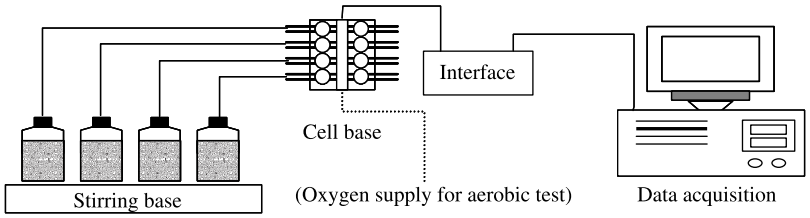

Figure 1 Illustration of schematic diagram of the test apparatus and the major components

the cell base to absorb CO_2 from the headspace gas. The data regarding methane gas production was logged at every four-hour interval in the computer. Based on the anaerobic respirogram, organic fraction in the sludge was characterized visually according to the shapes in the graph. Aerobic batch tests were also conducted at the same temperature of 35 °C with the same apparatus to measure oxygen uptake rate (OUR) for identification of different particulate organic fractions in activated sludge using model structure defined in ASM.

Test procedure for anaerobic respirometry. Activated sludge samples were placed in each of incubation vessels of 500 mL working volume. Buffer solution ($NaHCO_3$: 872 mg/L, K_2HPO_4: 80 mg/L, KH_2PO_4: 80 mg/L) which had comparable salt concentration as of the original liquor of anaerobically digested sludge was added to keep pH at about 7.5. After mixing seed sludge, buffer solution and the activated sludge, the headspace in the incubation vessel was replaced by nitrogen gas. The incubation vessels were then sealed and MPRs were measured. For collecting respirograms showing a variety of degradation pattern, the tests were carried out under various F/M ratios ranging from 0.00 to 0.31 (COD/COD). F/M ratio in the tests was varied by either fixing the substrate concentration while varying the microorganism concentration (Type I) or vice versa (Type II). The former makes it possible to know whether the degradation is affected by seed sludge concentration. On the other hand, the latter gives information with respect to the influence of substrate concentration on the degradation pattern. A control test was also conducted without addition of activated sludge in order to subtract the base line from the respirograms. The sludge concentrations and F/M ratio in the study are listed in Table 1. Furthermore, in order to evaluate contribution of heterotrophic activated sludge biomass in solubilization of organic solids, soluble COD concentration was monitored in tests, conducted without addition of seed sludge (F/M = infinity).

Additional batch tests. The hydrolysis step is supposed to be the rate-limiting step if the fed substrate is mainly in particulate form (Eastman and Ferguson, 1981). However, if the methane conversion rate in the tests is markedly slower than that of solubilization of organic solids, temporal elevation of soluble COD concentration is expected in the liquid phase. Under these circumstances, the MPR will not correspond to the solid degradation rate. Therefore, to accurately assess the solid degradation rate by means of the MPR, no

Table 1 Experimental condition of sludge concentrations and F/M ratios

F: Substrate (mgCOD/L) (activated sludge)	0	1,296	1,296	1,296	2,592	2,592
M: Seed sludge (mgCOD/L) (anaerobically digested sludge)	10,646	10,646	7,985	5,323	10,646	7,985
F/M ratio	0.00	0.12	0.16	0.24	0.24	0.31

accumulation of soluble COD in bulk needs to be confirmed. For such confirmation, another sets of anaerobic batch tests under identical experimental conditions were carried out and soluble COD concentration was measured. About 100 mg/L of soluble COD was observed to accumulate in first 12 hours in runs, which were conducted at high substrate concentration of 2,592 mg/L. Thus, in these periods methanogenesis rather than hydrolysis was the rate-limiting step and hence the data from these periods was not used for developing hydrolysis kinetics.

Results and discussion

Characteristics of activated sludge degradation under anaerobic and aerobic condition

Two kinds of biodegradable fraction (X_{S1}, X_{S2}) were visually distinguished in the composite sample of the activated sludge in the anaerobic respirometric tests as shown in Figure 2. The degradation of X_{S1} could be expressed by first-order reaction as it showed linear decline in semi-logarithm plot. On the other hand, fraction X_{S2} appearing on the graph until the second day in the incubation period had markedly higher degradation rate than that of X_{S1}. The degradation rate of X_{S2} was observed to be quite high for about 1 day and decreased rapidly afterwards. Based on the characteristic pattern, Contois-type or Monod-type equation may be applied for its rate expression. In the aerobic respirometric tests also, two kind of biodegradable fractions appeared as shown in Figure 3. These were heterotrophic biomass in the sludge ($X_{H\text{-}Aerobe}$) and slowly biodegradable substrates (hydrolysable) (X_S) respectively.

Kinetics and characterization of activated sludge under anaerobic and aerobic conditions

Correlation between X_{S1} and $X_{H\text{-}Aerobe}$. As shown in Figure 4, comparable MPR plots for degradation of X_{S1} were observed for the runs in which same substrate concentration

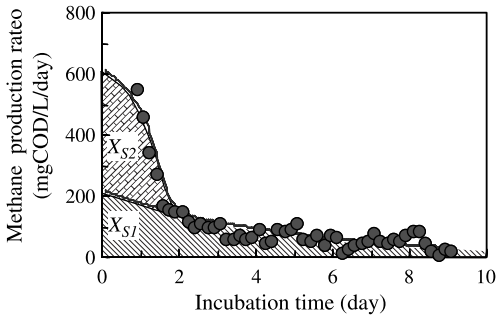

Figure 2 Respirogram in anaerobic condition, after subtracting base line, $F = 2{,}592$ mg/L, $M = 10{,}646$ mg/L

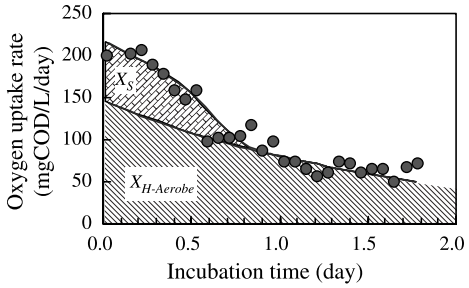

Figure 3 Respirogram in aerobic condition, activated sludge: 595 mg/L

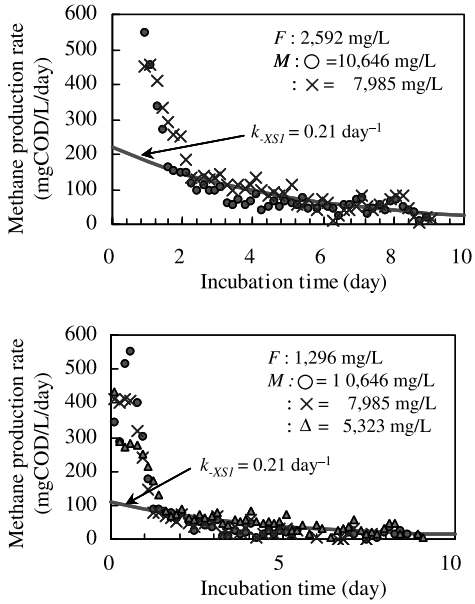

Figure 4 Kinetics of X_{S1} in the anaerobic condition, comparison based on Type I

with variable seed concentration was used (Type I). By applying first-order expression, a specific degradation rate (k_{-XSI}) of 0.21 day^{-1} was estimated. This value is consistent with the reported literature value (Vavilin *et al.*, 1996).

The extrapolated intersections (initial MPR) were found to be proportional to the fed substrate concentration. Based on this, the initial concentration of fraction of X_{SI} ($X_{SI(0)}$) in the composite activated sludge sample (X_C) was calculated to be 55% from Equation (1). In using Equation (1), the values of inert fraction (f_{XI}) and yield coefficient ($Y_{H\text{-}anaerobic}$) were assumed to be 0.20 gCOD/gCOD and 0.08 gCOD/gCOD respectively (Batstone *et al.*, 2002). Similarly, under aerobic conditions, specific decay rate ($b_{H\text{-}Aerobe}$)

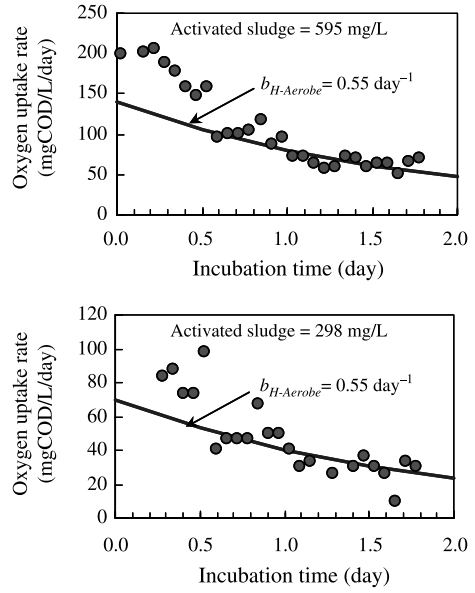

Figure 5 Decay of $X_{H\text{-}Aerobe}$ in the aerobic condition

of $X_{H\text{-}Aerobe}$ was calculated based on the data of OUR (Figure 5). Further, the $X_{H\text{-}Aerobe(0)}$ fraction in activated sludge was estimated by using Equation (2). The calculated $b_{H\text{-}Aerobe}$ and $X_{H\text{-}Aerobe(0)}$ fractions were 0.55 day^{-1} and 53% in the sludge COD respectively. In applying Equation (2), the value of f_{XI} was assumed to be the same as under anaerobic conditions.

$$\frac{X_{S1(0)}}{X_C} = \frac{\left(\frac{\text{Initial methane production rate}}{(1-Y_{H-Anaerobic})\cdot(1-f_{XI})\cdot k_{-XS1}}\right)}{X_C} \tag{1}$$

$$\frac{X_{H-Aerobe(0)}}{X_C} = \frac{\left(\frac{\text{Initial oxygen uptake rate}}{(1-f_{XI})\cdot b_{H-Aerobe}}\right)}{X_C} \tag{2}$$

Considering similar fractional magnitude of $X_{S1(0)}$ and $X_{H\text{-}Aerobe(0)}$ in activated sludge and their first-order degradation under anaerobic and aerobic conditions respectively, it could be argued that that the anaerobic fraction of Xs_1 directly originates from the heterotrophic microorganism ($X_{H\text{-}Aerobe}$) in the activated sludge. This consideration strongly suggests that a substrate mapping between the ASMs state variable of $X_{H\text{-}Aerobe}$ to ADM1 particulate components of X_{S1} could be possible. Another interesting observation is that the k_{-XS1} (anaerobic decay of $X_{S1} = X_{H\text{-}Aerobe}$) was significantly lower (about 40%) than $b_{H\text{-}Aerobe}$ (aerobic decay of $X_{H\text{-}Aerobe}$). Siegrist *et al.* (1999) have also reported similar reduction in decay rates of nitrifiers under anoxic and anaerobic conditions. As the decay of the heterotrophic microorganism is a description for many complex processes of cell maintenance, lysis and predation (van Loosdrecht and Henze, 1999), it is difficult to point out exact reasons for the decrease in the decay rate of $X_{H\text{-}Aerobe}$ under anaerobic conditions.

Comparison of fraction X_{S2} and X_S. It was assumed that both X_{S2} *and* X_S fractions could be hydrolyzed through surface reactions by active microorganism in the system and thus the Contois-type equation was applied. By considering the individual contribution of $X_{H\text{-}Anaerobe}$ and $X_{H\text{-}Aerobe}$ in the hydrolysing X_{S2} fraction, the measured degradation pattern of X_{S2} could be reasonably simulated for different runs (Figure 6). The aerobic degradation of X_S is also shown in the same figure. The applied kinetic parameters and state variables are as listed in Table 2. It is notable that X_{S2} and X_S are present in comparable percentage of 20–21% in X_C. Based on this similarity, it can be deduced that X_{S2} is the compounds originated from X_S although the kinetic parameters ($k_{H\text{-}max}$, K_X) under anaerobic conditions were slightly different from that under aerobic condition. These variations may be due to the differences in compositions of bacterial species, which arise due to addition of anaerobic microorganism under anaerobic conditions.

$$X_{H-Anaerobe} \cong Y_{H-Anaerobic}\cdot\left\{(1-f_X)\cdot\left(\frac{k_{-XS1}\cdot SRT}{1+k_{-XS1}\cdot SRT}\right)\cdot X_{S1(0)}\cdot + X_{S2(0)}\right\} \times \frac{1}{1+b_{H-Anaerobe}\cdot SRT} \tag{3}$$

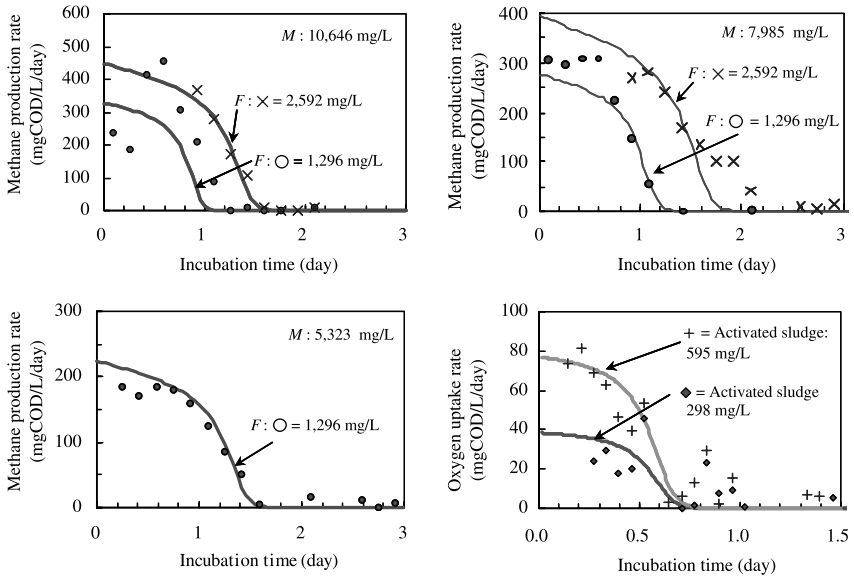

Figure 6 Respirograms for the degradation of X_{S2} and X_S under various F/M ratio conditions, upper left, lower left and upper right: anaerobic respirogram for X_{S2}, comparison based on type II; lower right: aerobic respirograms for X_S (activated sludge only)

Validation of kinetic parameters and state variables

$$\text{Observed sludge digestion efficiency in the plant} \cong 1 - \left(\frac{X_{S1} + X_{H-Anaerobe} + X_I}{X_C}\right)$$

$$\cong 1 - \left[\begin{array}{c} \left(\frac{1}{1+k_{-XS1}\cdot SRT}\cdot X_{S1(0)}\right) \\ +\left[Y_{H-Anaerobic}\cdot\left\{(1-f_X)\cdot\left(\frac{k_{-XS1}\cdot SRT}{1+k_{-XS1}\cdot SRT}\right)\cdot X_{S1(0)}\cdot + X_{S2(0)}\right\}\cdot\frac{1}{1+b_{H-Anaerobe}\cdot SRT}\right] \\ +\left\{\begin{array}{c}(X_C - X_{S1(0)} - X_{S2(0)}) + f_X\cdot\left(1-\frac{1}{1+k_{-XS1}\cdot SRT}\right)\cdot X_{S1(0)} \\ +f_X\cdot b_{H-Anaerobe}\cdot SRT\cdot X_{H-Anaerobe}\end{array}\right\} \end{array}\right] \quad (4)$$

$$\div X_C$$

Although most of the kinetic parameters and state variables can be calculated through curve fitting of the measured respirogram, it was necessary to validate the values of kinetic parameters by applying the model to a full-scale anaerobic digester. Considering the low yield of methanogenesis and relatively rapid degradation of X_{S2}, the digested sludge under steady-state condition can be assumed to consist mainly of X_{S1}, $X_{H\text{-}Anaerobe}$ and X_I. Thus, the digestion efficiency during anaerobic digestion can be approximated by using Equation (4). The observed digestion efficiency at the plant and digestion efficiency using Equation (4) are compared in Figure 7. A good agreement was observed between calculated and observed sludge digestion efficiency.

Substrate mapping between ASM and ADM1 for sludge digestion

Based on the above results, a modified scheme of COD flux during anaerobic degradation of activated sludge is proposed (Figure 8). Based on the characterization scheme of ASM, three particulate state variables of $X_{H\text{-}Aerobe}$, X_S and X_I are considered to constitute the activated sludge solids. Under anaerobic conditions, these will represent X_{S1}, X_{S2} and X_I respectively. Two of these fractions namely, X_{S1}, X_{S2} are biologically degraded into

Table 2 Kinetic parameters and state variables for fraction of X_{S2} and X_S in the activated sludge

	Anaerobic condition	Aerobic condition
Maximum specific degradation rate ($k_{H\text{-}max}$)	1.5 day^{-1} [(a)], 0.18 day^{-1} [(b)]	0.83 day^{-1} [(c)]
Dimensionless half-saturation coefficient (K_X)	0.035 - [(a)], 0.035 - [(b)]	0.035 - [(c)]
Initial slowly degradable substrate in the activated sludge	$X_{S2(0)}$ of 21% in the activated sludge[(d)]	$X_{S(0)}$ of 20% in the activated sludge[(d)]
Biomass in the sludge ($X_{H\text{-}Anaerobe(0)}$, $X_{H\text{-}Aerobe(0)}$)	$X_{H\text{-}Anaerobe}$ of 1.4% in the seed sludge[(e)]	$X_{H\text{-}Aerobe}$ of 53% in the activated sludge[(f)]

[(a)]Parameters for $k_{H\text{-}max}$ and K_X of $X_{H\text{-}Anaerobe}$: determined by curve fitting in the anaerobic respirograms
[(b)]Parameters for $k_{H\text{-}max}$ and K_X of $X_{H\text{-}Aerobe}$ in the anaerobic condition: determined by curve fitting from the batch test without addition of seed sludge and anaerobic respirograms
[(c)]Parameters for $k_{H\text{-}max}$ and K_X of $X_{H\text{-}Aerobe}$ in the aerobic condition: determined by curve fitting in the aerobic respirograms
[(d)]Initial substrate concentration ($X_{S2(0)}$ and $X_{S(0)}$): calculated by corresponding area in the graph region, assuming yield coefficient of 0.08 mgCOD/mgCOD in the anaerobic condition and 0.67 mgCOD/mgCOD in the aerobic condition (Batstone *et al.*, 2002; Henze *et al.*, 2000)
[(e)]Anaerobic biomass in the seed sludge ($X_{H\text{-}Anaerobe(0)}$): calculated from the operating condition of the plant (SRT = 60 days) where the seed sludge was collected, by applying Equation (3) with assuming yield coefficient ($Y_{H\text{-}Anerobic}$) of 0.08 mgCOD/mgCOD, f_{XI}, of 0.2 mgCOD/mgCOD and decay ($b_{H\text{-}Anaerobe}$) of 0.04 day^{-1} in the anaerobic condition (Batstone *et al.*, 2002). In the equation, it was approximated that most of X_{S2} had been consumed due to long sludge retention time of 60 days
[(f)]Aerobic biomass in the activated sludge ($X_{H\text{-}Aerobe(0)}$): calculated from the area of X_H in the graphs of respirogram (Henze *et al.*, 2000)

soluble fermentable compounds (S_F). This S_F is further metabolized to lower molecule compounds such as sugars, amino acids and lipids for the source of aciditic and methane fermentation that can be modelled by ADM1. For fraction X_{S1}, it is assumed that the degradation is like a self-disintegration of $X_{H\text{-}Aerobe}$ under anaerobic conditions and may be termed as "anaerobic decay". Part of X_{S1} is then converted to inert fraction of X_I with a factor of f_{XI}. For fraction X_{S2}, the degradation is governed by the surface mediated hydrolysis by the active biomass in the system. A terminology of anaerobic hydrolysis could be used for defining this process. A small amount of soluble inert fraction (S_I) may be produced here, but its quantification was not conducted in this study.

The proposed concept can build a bridge between ASM and ADM1 because state variables in the activated sludge defined by ASM can be used for the anaerobic digestion process with minimum modifications. This mapping makes it possible to conduct precise estimation of sludge mass to be disposed from the system and quantity of biogas for utilization.

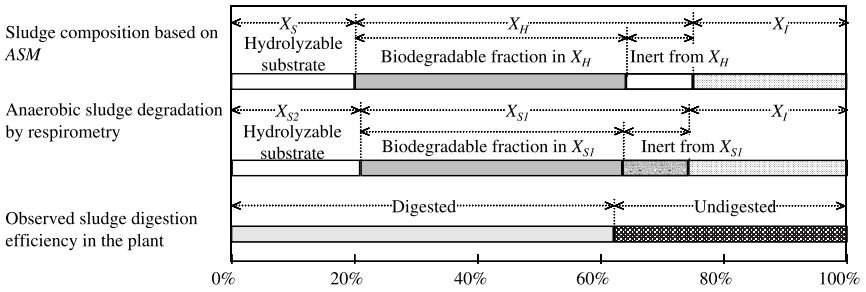

Figure 7 Comparison of state variables of the activated sludge and observed sludge digestion efficiency in the plant

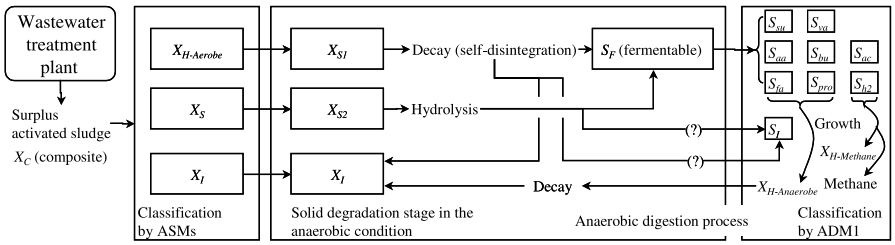

Figure 8 A bridge to meet ASM with ADM1 for solid degradation stage in the anaerobic condition

Conclusions

Kinetic modelling of the degradation (hydrolysis) stage of municipal activated sludge was studied by applying anaerobic respirometric analysis. The composition of activated sludge with respect to biological degradability was also measured and the kinetic expressions were compared with those reported for the structured model. The following results were obtained in this study. First, batch anaerobic respirometric tests were effectively used for characterization of degradable organic fractions showing different degradation kinetics in anaerobic digestion process. Secondly, the activated sludge degrading under anaerobic conditions has two organic fractions that show different kinetic behaviour. The first fraction degraded with first-order kinetics, while the second fraction degraded relatively rapidly in the initial phase could be expressed by Contois-type equation. The first fraction showing first-order reaction is likely to be arising from the heterotrophic biomass in the sludge and the other expressed as a Contois-type equation is supposed to be slowly biodegradable compounds remaining in the activated sludge. Finally, the results of this study suggest that a direct mapping of particulate substrate state variable between ASM and ADM1 could be possible, thus yielding a integrated platform for modelling both the water and sludge treatment processes.

Acknowledgements

This study was conducted by Kurita Water Industries and Tohoku University as the research activities in NEDO (New Energy and Industrial Technology Development Organization, Japan).

References

Batstone, D.J., Keller, J., Angelidaki, I., Kalyuzhnyi, S.V., Pavlostathis, S.G., Rozzi, A., Sanders, W.T.M., Siegrist, H. and Vavilin, V.A. (2002). *Anaerobic Digestion Model No.1 (ADM1)*, IWA Scientific and Technical report No.13. IWA, London, UK.

Blumensaat, F. and Keller, J. (2005). Modelling of two-stage anaerobic digestion using the IWA Anaerobic Digestion Model No.1 (ADM1). *Water Res.*, **39**, 171–183.

Copp, J.B., Jeppsson, U. and Rosen, C. (2003). Towards an ASM1 – ADM1 state variable interface for plant-wide wastewater treatment modelling. Session 58 in *Water Environment Federation Conference*, *WEFTEC 2003*, Los Angeles, CA, USA.

Copp, J.B., Spanjers, H. and Vanrolleghem, P.A. (2002). *Respirometry in Control of the Activated Sludge Process: Benchmarking Control Strategies*, IWA Scientific and Technical report No.11. IWA, London, UK.

Eastman, J.A. and Ferguson, J.F. (1981). Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. *J. Wat. Pollut. Control Fed.*, **53**, 352.

Henze, M., Gujer, W., Mino, T. and Loosdrecht, M. (2000). *Activated Sludge Models ASM1, ASM2, ASM2D, ASM3*, IWA Scientific and Technical report No.9. IWA, London, UK.

IWA-AD Task Group on Harmonization of Anaerobic Biodegradation, Activity and Inhibition Assay (TG ABAI) (2003). www.ftns.wau.nl/mt/abai/.

Kappeler, J. and Gujer, W. (1992). Estimation of kinetic parameters of heterotorophic biomass under aerobic conditions and characterization of wastewater for activated sludge modelling. *Wat. Sci. Tech.*, **25**(6), 125–139.

Siegrist, H., Brunner, I., Koch, G., Phan, L.C. and Chieu V.L. (1999). Reduction of biomass decay rate under anoxic and anaerobic conditions. *Wat. Sci. Tech.*, **39**(1), 129–137.

Speece, R.E. (1996). *Anaerobic Biotechnology for Industrial Wastewaters*. Archae Press, Nashville, TN, USA.

Van Loosdercht, M.C.M. and Henze, M. (1999). Maintenance, endogenous respiration, lysis, decay and predation. *Wat. Sci. Tech.*, **39**(1), 107–117.

Vanrolleghem, P.A., Rosen, C., Zaher, U., Copp, J., Benedetti, L., Ayesa, E. and Jeppsson, U. (2005). Continuity-based interfacing models for wastewater systems described by Peterson matrices. *Wat. Sci. Tech*., **52**(1–2), 493–500.

Vavilin, V.A., Rytov, S.V. and Lokshina, L.Ya. (1996). A description of hydrolysis kinetics in anaerobic degradation of particulate organic matter. *Biores Tech.*, **56**, 229–237.

Young, J.C., Kuss, M.L. and Nelson M.A. (1991). Use of anaerobic resipirometers for measuring gas production in toxicity and treatability tests. *Proc. 84th Annual Meeting of Air and Waste Management Association*, Vancouver, BC, Canada.