

---

## Applications of two-phase anaerobic degradation in industrial wastewater treatment

---

Shuizhou Ke\* and Zhou Shi

Department of Water Engineering and Science, College of Civil Engineering, Hunan University, Changsha, Hunan 410082, PRC  
E-mail: kshuizhou@yahoo.com    E-mail: zhous@hnu.cn

\*Corresponding author

Herbert H.P. Fang

Department of Civil Engineering, Environmental Engineering Research Centre, The University of Hong Kong, Pokfulam Road, Hong Kong, PRC  
E-mail: hrechef@hkucc.hku.hk

**Abstract:** Prospects for the phased anaerobic treatment of wastewater are extremely promising. With the variety of reactor designs available and the amenability of reactors to modification, existing treatment systems may be replaced or upgraded as required to achieve increased stability, higher loading capacities and greater process efficiencies than single-stage systems. In recent time, various reactor configurations and substrates are applied to two-phase anaerobic process. This paper reviews applications and studies of two-phase anaerobic degradation for wastewater treatment, sums up the performance of application to treating waste from distillery, landfill leachate, coffee, cheese whey and dairy, food, pulp and paper, sludge and solid, etc., and summarises reactor configurations, environmental and operational conditions, and comparisons of two-phase anaerobic digestion with other anaerobic reactors.

**Keywords:** anaerobic; acidification; methanogenesis; industrial wastewater; two-phase.

**Reference** to this paper should be made as follows: Ke, S., Shi, Z. and Fang, H.H.P. (2005) 'Applications of two-phase anaerobic degradation in industrial wastewater treatment', *Int. J. Environment and Pollution*, Vol. 23, No. 1, pp.65–80.

**Biographical notes:** Shuizhou Ke received his BSc in Engineering in 1984; a MSc in 1989; both in Engineering at Hunan University (HNU) in China and is currently a doctoral candidate and Associate Professor of Water Engineering at HNU where he has taught since 1984. His present research interests include anaerobic degradation, advanced oxidation processes of wastewater, ultrafiltration, etc. He has published over 40 articles related to water and wastewater treatment in journals and international conference proceedings.

Zhou Shi is Professor of Water and Wastewater Engineering at the College of Civil Engineering, Hunan University. He received his PhD in Civil Engineering from the Department of Civil and Environmental Engineering at the University of Tennessee at Knoxville, USA. His current research interests are in the areas of modelling and remediation of recalcitrant environmental pollutants, theory and methods for water quality control with high efficiency and low cost.

He has published papers in many major prestigious environmental journals, including *Environ. Sci. Technol.*, *Wat. Sci. Technol.*, etc.

Herbert H.P. Fang received his PhD from University of Rochester in 1972, and is presently Chair Professor of Environmental Engineering at the University of Hong Kong where he has taught since 1987. He has published over 130 journal papers related to environmental and pollution, and held a US patent. His present research interests include biofilm, anaerobic degradation, bio-hydrogen production from wastewater, etc. He is the editorial board member of *Advances in Environmental Research* (Elsevier), *World Journal of Microbiology and Biotechnology* (Kluwer) and *Biofilms* (Cambridge), and also holds Visiting Professorship at eight universities in China.

---

## 1 Introduction

Over the past 30 years, anaerobic wastewater treatment process became more popular due to its advantages over aerobic processing, including low biomass production, low nutrient requirements, and the production of fuel gas, such as methane gas and hydrogen. Studies have shown that anaerobic treatment is a stable process under proper operation. But parameters such as process configuration, temperature, biomass, pH, nutrient, and substrate must be carefully scrutinised in order to make successful anaerobic treatment. Many process configurations have been investigated. An improvement in the efficiency of anaerobic digestion can be brought about by either digester design modification or advanced operating techniques.

Anaerobic degradation process can be separated into two phases. The first or 'acid fermentation' phase, leads to the production of intermediate products predominated by the volatile organic acids; and the second, or 'methane fermentation' phase, resulting in the conversion of these intermediates to stable end products, principally methane and carbon dioxide. The first phase is different from the second one in bacterial varieties, digestion rate, environmental demands, degradation process and products. Conventional anaerobic digestion is proceeded in a single reactor where acidogenesis and methanogenesis both occur. Acidogenesis and methanogenesis are respectively proceeded in two separate reactors and each phase is in the best environmental conditions. The introduction of an acidogenic phase should enable optimisation of the conditions required for many of complex organic (recalcitrant) chemicals present in a wastewater to be converted to short-chain volatile fatty acids (VFA) and other simple compounds. This, in turn, buffers the slow-growing methanogens, predominantly present in second phase reactor, from possible toxins or inhibitors and ensures a uniform feed stock for the methanogens.

Pohland and Ghosh (1971) have long advocated the merits of two-phase anaerobic degradation (TPAD). They investigated the acidogenesis of sewage sludge in order to achieve a significant improvement in sludge treatment efficiencies. Since this initial investigation into the TPAD, considerable research has been carried out with many high-strength industrial wastewaters to assess the advantages of this treatment over conventional single-phase systems.

Massey and Pohland (1978) suggested that the process could be applied to complicated as well as simple substrates, and equations were derived describing the growth of bacteria during substrate-utilisation in the dual-phased treatment system. Research undertaken to provide kinetic predictions for the phase separation of the anaerobic degradation process has been reported. Estimate kinetic parameters for the acidogenic and methanogenic phases were obtained by sequential reductions in hydraulic retention time (HRT) in a single-staged completely mixed reactor to the point at which the production of VFAs was the domination reaction (Ghosh and Pohland, 1974). The operation of a two-phase anaerobic process employing sludge as the primary substrate has also been recorded (Ghosh et al., 1975), although estimates of the kinetic parameters were retarded by problems in the measurement of the concentration of active bacteria, as recycle and biomass separation facilities had not been included in the reactor system or phases.

The advantages of two-phase operation have been extensively documented (Pohland and Ghosh, 1971; Ghosh et al., 1975; Cohen et al., 1980, 1982). Prospects for the phased anaerobic treatment of wastewater are promising. With the variety of reactor designs available and the amenability of reactors to modification, existing treatment systems may be replaced or upgraded as required to achieve increased stability, higher loading capacities and greater process efficiencies than are possible using single-stage systems. In recent time, various reactor configurations and substrates are being applied to two-phase anaerobic process. This paper reviews applications of TPAD.

## **2 Applications and performances**

Two-phase anaerobic process have been applied to treat many kinds of wastewater and solid wastes from following sources: distillery, landfill leachate, coffee, cheese whey and dairy, starch, fruit and vegetable solid, food, pulp and paper, olive mill, abattoir, dye, primary and activated sludge and solid. Table 1 summarises available performance data of these applications.

### *2.1 Slaughterhouse waste*

Wastewater from a slaughterhouse arises from different steps of the slaughtering process such as washing of animals, bleeding out, skinning, cleaning of animal bodies, cleaning of rooms, etc. The wastewater contains blood, particles of skin and meat, excrements and other pollutants. Typical characteristics of wastewater from slaughterhouses are given in follows (Ruiz et al., 1997): pH = 6.8–7.8; COD = 5200–11400 mg/l; TSS = 570–1690 mg/l; Phosphorus = 7–28.3 mg/l;  $\text{NH}_3\text{-N}$  = 19–74 mg/l; Protein = 3250–7860 mg/l. The performance data of various reactors used for the treatment of slaughterhouse wastewater are given in Table 2.

Table 1 Performance data on two-phase anaerobic degradation

Feedstock	Reactor configuration	System HRT (days)	System organic loading rate (OLR) (kg COD/m <sup>3</sup> .d)	COD removal (%)	CH <sub>4</sub> yield (m <sup>3</sup> /kgCOD)	Reference
Distillery wastewater	UASB-UASB	-	16.5-44.0	80	16.5 <sup>a</sup>	Shin et al. (1992)
Cane-molasses alcohol stillage	TPAD (thermophilic-thermophilic)	5.6-32.7	4.65-20.0	85	0.168	Yeoh (1997)
Coffee waste	-	-	21 <sup>b</sup>	-	1.7 <sup>c</sup>	Ikbal et al. (1994)
Coffee waste	-	-	-	70	0.3 <sup>d</sup>	Kida et al. (1994)
Cheese whey and dairy waste	hybrid reactor	-	10	98	-	Malaspina et al. (1996)
Cheese whey and dairy waste	hybrid reactor	-	0.97-2.82	91-97	0.287-0.359	Strydom et al. (1997)
Dairy waste	CSTR <sup>1</sup> -UAF <sup>k</sup>	2.0	5	90	-	Ince (1998)
Cheese whey	CSTR-UAF	4-7	-	-	0.55	Yilmazer and Yenigum (1999)
Abattoir waste	-	4-12	1.4-7.0	-	0.3	Banks and Wang (1999)
Wheat starch wastewater	membrane reactor	-	20	99	0.33	Yanagi et al. (1999)
Pulp and paper	UASB-UASB	-	12	84	0.3	He et al. (1995)
Olive mill waste	-	-	2.3-2.4	-	0.36	Gharsallah (1994)
Landfill leachate	TPAD (mesophilic-mesophilic)	4.75-16	2.41-7.98	>90	-	Lin (1991)
Dye waste	CSTR-UASB	1.0	-	71 <sup>e</sup>	-	Chinwekitvanich (2000)
Dye waste	APBR <sup>1</sup> -APBR	3-5	0.25-1.0	90 <sup>e</sup>	-	Talarposhti et al. (2001)
Food waste	-	20	7.9 <sup>f</sup>	70 <sup>g</sup>	0.056 <sup>h</sup>	Lee et al. (1999)
Fruit and vegetable markets solid waste	TPAD (mesophilic-thermophilic)	12	-	-	0.6 <sup>i</sup>	Pavan et al. (2000)
Activated sludge	-	1	4.7 <sup>f</sup>	70 <sup>g</sup>	0.29 <sup>h</sup>	Ghosh et al. (1995)
Sewage sludge	UASB-UASB	3-3.7	19	-	-	Fongsatitkul et al. (1994,1995)
Primary and mixed primary-activated sludge	TPAD (mesophilic-mesophilic)	10	-	43 <sup>g</sup>	0.11 <sup>h</sup>	Ghosh and Taylor (1999)

<sup>a</sup>l/d; <sup>b</sup>kg TOC/m<sup>3</sup>.d; <sup>c</sup>m<sup>3</sup>/kg waste; <sup>d</sup>m<sup>3</sup>/kg waste; <sup>e</sup>colour removal; <sup>f</sup>kg VS/m<sup>3</sup>.d; <sup>g</sup>VS removal; <sup>h</sup>m<sup>3</sup>/kg VS; <sup>i</sup>m<sup>3</sup>/kg TVS;

<sup>1</sup>CSTR (continuous stirred tank reactor); <sup>2</sup>UAF (upflow anaerobic filter); <sup>3</sup>APBR (anaerobic packed bed reactor).

COD (chemical oxygen demand), TOC (total organic carbon), VS (volatile solid), TVS (total volatile solid).

**Table 2** Performance data of treatment system for slaughterhouse wastes

Reactor type	OLR (kg COD/m <sup>3</sup> .d)	COD removal (%)	Reference
UASB (granular)	11	85	Johns (1995)
UASB (flocculated)	5	80–89	Johns 1995
UASB	2.7	77	Zheng and Wu (1985)
UASB	7	85	Sayed et al. (1987)
UASB	6–10	87–91	Lettinga et al. (1982)
UASB	1–6.5	90	Ruiz et al. (1997)
AF	2.3	85	Johns (1995)
AF	1–6.5	<90	Ruiz et al. (1997)
Anaerobic contact	3	92.6	Johns (1995)
ABR	0.9–4.7	75	Polprasert et al. (1992)
TPAD	1.4–7	87	Banks and Wang (1999)

## 2.2 Cheese whey and dairy waste

The liquid waste in a dairy originates from manufacturing process, utilities and service section. The various sources of waste generation from a dairy are spilled milk, spoiled milk, skimmed milk, whey, washed water from milk cans, equipment, bottles and floor washing. Whey is the most difficult high strength waste product of cheese manufacture. This contains a proportion of milk protein, water-soluble vitamins and mineral salt (Rajeshwari et al., 2000). The performance data of different types of anaerobic reactors for the treatment of cheese whey and dairy wastewater are given in Table 3.

**Table 3** Performance data of different types of anaerobic reactors for the treatment of cheese whey and dairy wastewater

Reactor type	Inlet COD (g/l)	HRT (days)	OLR (kg COD/m <sup>3</sup> .d)	COD removal (%)	Reference
UASB	5–77	2.3–11.6	1–28.5	95–99	Kalyuzhnyi et al. (1997)
UASB	47–55	5.4–6.8	7–9.5	90–94	Kalyuzhnyi et al. (1997)
UASB	16–50	3.3–12.8	1–6.7	90–95	Kalyuzhnyi et al. (1997)
UASB (dairy)	2.05	1.7 h	31	90	Gutierrez et al. (1991)
UASB	11	1.5	7.1	94	Schorder et al. (1989)
UASB	5–28.7	5	0.9–6	97–99	Yan et al. (1989)
UFFLR	79	5	14	95	Wildenauer et al. (1985)
DSFFR	13	5	2.6	88	de Haast et al. (1985)
FBR	7	0.4	7.7	90	Boening et al. (1982)
FBR	0.8–10	0.1–0.4	6–40	63–87	Denac et al. (1988)
AAFEB	5–15	0.6–0.7	8.2–22	61–92	Switzenbaum et al. (1982)
AnRBC	64	5	10.2	76	Lo et al. (1986)
SDFA	69.8	–	16.1	99	Barford et al. (1986)

**Table 3** Performance data of different types of anaerobic reactors for the treatment of cheese whey and dairy wastewater (continued)

<i>Reactor type</i>	<i>Inlet COD (g/l)</i>	<i>HRT (days)</i>	<i>OLR (kg COD/m<sup>3</sup>.d)</i>	<i>COD removal (%)</i>	<i>Reference</i>
DUHR (TPAD)	68	7	10	97	Malaspina et al. (1995)
TPAD	–	–	10	98	Malaspina et al. (1996)
TPAD	–	–	0.97–2.82	91–97	Strydom et al. (1997)
TPAD	–	2	5	90	Ince (1998)

UFFLR: upflow fixed film loop reactor; DSFFR: downflow stationary fixed film reactor; FBR: fluidised bed reactor; AAFEB: anaerobic attached – film expanded – bed reactor; AnRBC: anaerobic rotating biological contact reactor; SDFA: semicontinuous digester with flocculant addition; DUHR: downflow upflow hybrid reactor; TPAD: two-phase anaerobic digester.

### 2.3 Pulp and paper waste

In the pulp and paper industry, there are various points of wastewater generation. Some wastewater results from leaks and spills from digester. Pulp washing and bleaching gives wastewaters of various characteristics depending on the bleaching sequence. Bleaching section results in wastewater and chlorolignins. Wastewater is also generated from paper machine section, caustic chlorine manufacture and black liquor recovery. There are variations in the COD, inhibitors and the degradability depending upon the source of the wastewater (Rintala et al., 1994). Table 4 lists the characteristics of wastewater generated from pulp and paper industry. Comparison of performance data of various reactors for wastewaters from different streams of pulp and paper is given in Table 5.

**Table 4** Characteristics of wastewater generated from pulp and paper industry (Rintala et al., 1994)

<i>Wastewater</i>	<i>Inlet COD (g/l)</i>	<i>COD removal (%)</i>	<i>Inhibitors</i>
Wet debarking	1.3–4.1	44–78	Tannins, resin acids
Pulping thermomechanical	1.0–5.6	60–80	Resin acids
Chemithermomechanical	2.5–13	40–60	Resin acids, fatty acids, sulphur
Sulphite condensate	7	–	Sulphur, ammonia
Chlorine bleaching	0.9–2	30–50	Chlorinated phenols, resin acids
Sulphite spent liquor	120–220	–	
Craft condensate	1–33.6	83–92	Sulphur, resin acids, fatty acids, terpenes
Sulphite condensate	7.5–50	50–90	Sulphur, organic sulphur

**Table 5** Comparison of performance data of various reactors for wastewaters from different streams of pulp and paper

Reactor type	Wastewater	OLR (kg COD/m <sup>3</sup> .d)	COD removal (%)	Reference
Fluidised bed	Debarking	0.66 <sup>a</sup>	50 <sup>b</sup>	Rajeshwari et al. (2000)
UASB	Debarking	40	40	Rajeshwari et al. (2000)
UASB	Mechanic pulping	12–31	60–70	Rajeshwari et al. (2000)
UASB	Mesophilic	80 and 13	60	Rajeshwari et al. (2000)
UASB	Thermomechanical (55–70°C)	4 and 20	60	Rajeshwari et al. (2000)
UASB	Chemithermomechanical	4.7–22	35–55	Rajeshwari et al. (2000)
Contact process	Sulphite condensate	5	30–50	Rajeshwari et al. (2000)
Two-phase UASB	Mechanical pulping	12	84	He et al. (1995)

<sup>a</sup>m<sup>3</sup>/m<sup>3</sup>.d; <sup>b</sup>BOD removal.

### 3 Factors affect TPAD

#### 3.1 Reactor configurations

Acidogenesis and methanogenesis of two-phase anaerobic digestion process are respectively proceeded in two separate reactors. To accelerate acidogenesis and methanogenesis process respectively, the two reactors may be applied in various high rate anaerobic reactors such as UASB – UASB system, continuous stirred tank reactor (CSTR) – upflow anaerobic filter (UAF) system, hybrid reactor, CSTR – AFBR system, two-phase plug-flow reactor (TPPFR), and anaerobic packed bed reactor (APBR).

#### 3.2 Operational parameters

The anaerobic digestion process is affected significantly by the environmental and operating conditions. Two-phase anaerobic digestion made it possible that acidogenesis and methanogenesis process are all in the best environmental conditions and operation parameters. Each stage of two-phase anaerobic degradation not only applied above various reactor configurations, but also used different temperature, pH, HRT, organic loading rate (OLR) etc., so as to get optimum results.

Anaerobic digestion is strongly influenced by temperature and can be grouped based on the temperature (Pol, 1995): psychrophilic (0–20°C), mesophilic (20–42°C) and thermophilic (42–75°C). Anaerobic bacteria well resist temperature changes. In the mesophilic range, the bacterial activity and growth decreases by 50% for each 10°C drop. Thus, for a given degradation degree, the lower the temperature, the longer is the degradation time. The effect of temperature on the acidogenesis process is not very significant, as among the mixed population there are always some bacteria that have their optimum within the range concerned. However, the methanogenesis process can only be taken by a few microorganisms, thus, are much more sensitive to temperature shift

(Mudrak and Kunst, 1986). Anaerobic treatment of high strength wastewater or sludge was usually applied to mesophilic or thermophilic range. Each stage of two-phase anaerobic degradation process was also used in either mesophilic or thermophilic range. According to the temperature range of anaerobic digestion, two-phase anaerobic digestion included in: mesophilic–mesophilic system, thermophilic–thermophilic system, mesophilic–thermophilic system, and thermophilic–mesophilic system.

Anaerobic reactions are also highly pH dependent. The optimal pH range for methane producing bacteria is 6.8–7.2 while for acid-forming bacteria, a more acid pH is desirable (Mudrak and Kunst, 1986). The pH of conventional anaerobic system is typically maintained between methanogenic limits to prevent the predominance of the acid-forming bacteria, which may cause VFA accumulation. It is essential that the reactor contents provide enough buffer capacity to neutralise any eventual VFA accumulation, and thus prevent build-up of localised acid zones in the conventional anaerobic digestion. While in two-phase anaerobic degradation system, each stage can use different pH value so that acidogenesis and methanogenesis processes proceed in optimal conditions, respectively. pH controlling is very important, especially to the methanogenic stage.

The overall performance of a two-phase anaerobic wastewater treatment system was determined as a function of the extent of recycle of the effluent from the methanogenic reactor to the acidification reactor (Romli et al., 1994). The system consisted of a well-mixed continuous reactor as the acidification stage, which was controlled at pH six by automatic addition of caustic soda, and a fluidised sand bed reactor for the methanogenic stage, which was left uncontrolled. The results showed that the introduction of recycle could minimise the total operational costs of treatment due mainly to a considerable saving in alkali addition. The results confirmed earlier model predictions. The introduction of recycle acidified the methanogenic reactor, thereby removing dissolved carbon dioxide to the gas phase; this reduction in the concentration of weak acids was the principal cause of the decrease in the caustic consumption. Shin et al. (1992) applied a two-phase UASB–UASB system to treat distillery wastewater. After the formation of granular sludge in both reactors, it was possible to maintain the appropriate pH in the first phase only by recirculating the effluent from the methanogenic phase without the addition of alkaline chemicals. In a pilot scale two-phase anaerobic digester of Korean food wastes in any city near Seoul (Lee et al., 1999), the effluent from the second reactor was recycled to the first reactor to provide alkalinities.

### 3.3 *Effect of sulphate on TPAD*

Sulphate is another industry pollutant and inhibitor of anaerobic process. The inhibition of anaerobic bacteria by methanogenesis by hydrogen sulphide, a major end product of sulphate reduction which is produced when wastewaters containing high levels of sulphates are subjected to anaerobic digestion has been widely reported in literature.

OchiengOtieno (1996) investigated a system of TPAD and demonstrated that when the operating parameters especially pH are carefully controlled, sulphate could be reduced in the acidogenic reactor without adversely affecting the normal process of acidogenesis. Phase separation could thus be said to be capable of successfully removing or reducing bacteria and methanogens. It is hoped that this process could enable wider application of anaerobic digestion to the treatment of industrial effluents especially those



with high levels of sulphates, which were previously considered unsuitable for anaerobic treatment.

As compared with anaerobic toxicity assays (ATA) for industrial toxicants focused on the acetoclastic methanogens, two phase-separated ATAs for sulphate and sulphide have been conducted by means of acidogenic and methanogenic culture (Shin et al., 1995). Seed sludge was obtained from a two-phase upflow anaerobic sludge blanket (UASB) reactor which operated on glucose as a substrate. The obtained results were compared with those of mixed culture. In the acidogenic ATA, the glucose utilisation rate could be expressed in a first-order reaction kinetic. Gas production and substrate utilisation were affected slightly when sulphate concentration was up to 6000 mg SO<sub>4</sub>-S/l. However, the substrate conversion to acetate was retarded by sulphate addition. When sulphide over 800 mgS/l was added in test bottles, glucose utilisation rate was retarded and the first-order kinetic constant decreased. From the results of the methanogenic ATA, it was found that methanogens were more sensitive than acidogens as the sulphate and sulphide concentration increased. Although methane production was slightly retarded by added sulphate, methane was production up to 60% of control when the sulphate was 6000 mg SO<sub>4</sub>-S/l. The methane production was not inhibited up to 200 mgS/l; however, it was severely retarded at 400 mgS/l. The methane production was completely inhibited in excess of 800 mgS/l. In mixed culture, the inhibiting effects of sulphate and sulphide on mixed culture differed from those of phase-separated anaerobes. Methane production was only slightly inhibited when sulphate and sulphide concentration reached up to 4000 mg SO<sub>4</sub>-S/l and 1200 mg S/l, respectively.

Anaerobic treatment of distillery wastewaters containing high sulphate concentrations was carried out on a two-phase process (Reis et al., 1991). The acidogenic phase was operated so as to produce the more favourable intermediates for methanogenic bacteria coupled with maximum sulphate removal. Sulphate removal was directly affected by pH and dilution rate (D). The maximum sulphate removal and acid production was achieved at pH 6.6 and D = 0.035 h<sup>-1</sup>. A linear relationship between acetic acid produced and sulphate removal was observed, indicating that acetic acid was mainly produced by sulphate reducing bacteria with important operational advantages. Higher concentrations of butyric acid were obtained at low pH values and high dilution rates.

The performance data of TPAD of pulp and paper wastewater containing high concentration of sulphate is given in Table 5. Treatment efficiencies for various reactor configurations are given in Table 6.

**Table 6** Treatment efficiencies of wastewater containing sulphate for various reactor configurations

Reactor type	Inlet COD (g/l)	COD:SO <sub>4</sub> ratio	OLR (kg COD/m <sup>3</sup> .d)	COD removal (%)	Reference
ABR	20	8:1	20	50	Fox and Venkatasubbiah (1996)
UASB	0.7–2	2:1–0.5:1	~5	90–95	Visser et al. (1992)
AF	49.8	8:1–4:1	11–18.6	29–36	Hilton and Archer (1988)
TPAD	45.2	10:1	–	85	Reis et al. (1995)

#### 4 Comparison of TPAD with conventional single-phase digestion in parallel

As compared with conventional single-phase (or single-stage) degradation, TPAD exhibited more advantages.

Jeyaseelan and Matsuo (1995) investigated the treatment characteristics of two different substrates by two-phase anaerobic digestion at 20°C. One substrate contained 87% carbohydrates and proteins and the other 94% carbohydrates and lipids. The anaerobic system consisted of a completely mixed reactor for hydrolysis and acidogenesis reactions, and an upflow filter for methanogeneous conversions. The experiments showed that the best phase separation occurs from four to eight hours of detention times within the acid reactor. A single anaerobic system with upflow anaerobic filter alone for the same total detention times at the same substrate concentrations was operated for comparison. The results proved that two-phase digestion systems have higher digestion efficiencies than that of corresponding single-phase digestion systems. For substrate with more lipids the digestion efficiencies were very much greater.

Yeoh (1997) studied anaerobic treatment of cane-molasses stillage in a thermophilic two-phase system in comparison with the single-phase process. The experiments were conducted with HRT ranging from 36.0 to 9.0 days for single-phase and from 32.7 to 5.6 days for two-phase, corresponding to OLR of 3.452 to 14.487 and 4.646 to 20.022 kg COD/m<sup>3</sup>.d respectively. The methanogenic culture pH of both systems was maintained in a range 7.4–7.8 through self-regulation. The methane content of the biogas generated from the two-phase process was significantly higher by about 17% than that from the single-phase process, both decreasing with increasing substrate loading and shorter HRT. The mean overall methane yield was found to be 0.168 m<sup>3</sup> CH<sub>4</sub>/kg COD (added) or 0.292 m<sup>3</sup> CH<sub>4</sub>/kg VS (added) from two-phase methanogenesis, compared to 0.055 m<sup>3</sup> CH<sub>4</sub>/kg COD (added) or 0.082 m<sup>3</sup> CH<sub>4</sub>/kg VS (added) from single-phase fermentation. Treating the municipal solid waste coming mainly from fruit and vegetable markets, a comparison with one-phase system was carried out, showing that a two-phase system was much more appropriate for the digestion of this kind of highly biodegradable substrate in thermophilic conditions.

Ghosh et al. (2000) studied bioconversion of municipal solid waste-sludge blend by conventional high-rate and two-phase anaerobic digestion. RDF (refuse-derived fuel)-quality feed produced in a Madison, Wisconsin, USA, MRF (material-recovery facility) was used. High-rate digestion experiments were conducted with bench-scale digesters under target operating conditions developed from an economic feasibility study. The effects of digestion temperature, RDF content of digester feed, HRT, loading rate, RDF particle size, and RDF pretreatment with cellulase or dilute solutions of NaOH or lime on digester performance were studied. A pilot-scale two-phase digestion plant was operated with 80:20 (weight ratio) RDF-sludge blends to show that this process exhibited a higher methane yield, and produced a higher methane-content digester gas than those obtained by single-stage, high-rate anaerobic digestion. The two-temperature, two-phase anaerobic biofilter systems (Kaiser et al., 1995) were observed to outperform single-stage anaerobic filters operated at equivalent HRTs (24, 36, and 48 hours) and OLR (from 2 to 16 kg COD/m<sup>3</sup>.d) for synthetic milk substrate.

Azbar and Speece (2001) studied three anaerobic process configurations – namely, two-phase duel sludge (TPDS), two-stage single sludge (TSSS), and single-stage for effluent COD concentration. The same temperature, solid retention time (SRT), and

glucose substrate were used in all experiment. In every case, TPDS and TSSS configurations significantly outperformed the single stage. All experiments were carried out at a temperature of 35°C, and all reactors were operated as daily fill-and-draw with HRT = SRT. The following ranges for each design parameter were studied: pH (4.5, 5.5, and 6.5); first stage HRT (3, 8, and 24 hours); and floc load (3, 9, and 27 g COD/g VSS). The overall HRT/SRT of all systems was 30 days, and a pH-Stat system was used to control the pH in the acidification reactors at the desired value. Statistical evaluation of the results indicated that a floc load of three in the first reactor of TPDS yielded the lowest effluent COD concentrations under the studied range of parameters, while for the TSSS reactor configuration the staging of the system itself was the controlling phenomena responsible for reduced effluent COD.

Five substrates (glucose, propionate, butyrate, ethanol, and lactate) plus a mixed waste (60% carbohydrate, 34% protein, and 6% lipids) were studied under five reactor configurations: batch-fed single-stage CSTR, continuously fed single-stage CSTR, two-phase CSTR, two-stage CSTR, and single-stage UASB. The substrate feed concentration was 20000 mg/l as COD. The SRT and HRT in the CSTR reactors were 20 d, while HRT in the UASB was 2 d. All experiments were performed at 35 ± 1°C in a temperature-controlled room. All reactors were operated for at least 60 d. Two-phase CSTR, two-stage CSTR, and single-stage UASB configurations yielded the lowest effluent COD (130–550, 60–700, and 50–250 mg/l, respectively). The highest effluent COD were detected when feeding glucose, propionate, and lactate to continuously fed single-stage CSTRs (10400, 9900, and 4700 mg/l COD, respectively) and to batch-fed single-stage CSTRs (11200, 2500, and 2700 mg/l COD, respectively). The effluent COD was exhibited in Table 7 (Azbar et al., 2001).

**Table 7** Effluent COD (mg/l) concentrations for various substrates and process configurations ( $S_0 = 20,000$  mg COD/l)

<i>Substrate</i>	<i>Batch-fed single-stage CSTR</i>	<i>Continuously fed single-stage CSTR</i>	<i>Two-phase CSTR</i>	<i>Two-stage CSTR</i>	<i>Single-stage UASB</i>
Glucose	11,200 (1,250)	10,400 (1,600)	280 (280)	340 (20)	250 (60)
Propionate	2,500 (50)	9,900 (1350)	140 (40)	680 (50)	160 (60)
Butyrate	70 (100)	760 (300)	130 (80)	60 (90)	50 (10)
Lactate	2,700 (250)	4,700 (170)	230 (10)	240 (70)	240 (120)
Ethanol	500 (150)	400 (30)	300 (20)	400 (50)	50 (15)
Mixed waste	1,000 (300)	–	550 (100)	700 (170)	–

<sup>a</sup>Number in parenthesis two standard deviation above and below the mean.

## 5 Discussions and summaries

Massey and Pohland (1978), Ghosh and Klass (1978), Cohen et al. (1980, 1982), and Anderson et al. (1994) have shown improved performance with the implementation of phasing (two-phase configuration) when compared to a single-stage process. Applications and investigations of above have also exhibited the advantages of two-phase anaerobic

degradation processes. Each anaerobic bacteria species has an optimum microbiological environment that differs substantially from the other. Consequently, optimum anaerobic performance cannot be achieved in a single-phase anaerobic digester or reactor. Using both species of bacteria in the same reactor vessel significantly retards the efficiency of one or the other. This reality largely has been ignored or misunderstood by anaerobic treatment researchers and anaerobic treatment system manufactures. As a result, almost all existing anaerobic digesters are highly inefficient single-phase reactors. The benefits of two-phase treatment are substantial. Because each phase is maintained at its optimum pH and oxidation–reduction potential, it is able to perform at a high efficiency without an adverse environmental influence from the other. Plant startup can be achieved in weeks instead of months, and the required equipment can be reduced in size by at least a factor of three. In addition, segregation of each phase's gas generation increases the purity of the second-phase methane substantially, because it is not diluted by first-phase carbon dioxide. Such purity is an important factor if the biogas is to be used beneficially as a primary fuel and/or to generate electricity.

Phase separation of anaerobic process has following major advantages: isolate and optimise potential rate-limiting steps (hydrolysis encouraged during first-phase, methanogenesis encouraged during second-phase); improve reaction kinetics and stability (pH control in each phase, improved reactor stability to shock loads, select for faster-growing microbes); potential for detoxification in first-phase. In the other hand, phase separation has the following disadvantages: disruption of syntrophic relationships; more difficult to implement, engineer and operate; lack of process experience and applicability to variety of wastes; uncertainty of linkage between substrate type and reactor configuration.

In general, substrate type and reactor configuration would determine whether or not the wastewater was amenable to two-phase digestion. There are many applications and investigations of two-phase anaerobic digestion. However, there are not enough process experience and applicability to variety of wastes. There will be much work to do.

## Acknowledgements

The authors wish to thank The Hong Kong University Development Fund for the partial financial support of this project. KE Shui-zhou wishes to thank The Hong Kong University for providing him the Visitorship.

## References

- Anderson, G.K., Kasapgil, B., and Ince, O. (1994) 'Microbiological study of two stage anaerobic digestion start-up', *Water Res.*, Vol. 28, pp.2383–2392.
- Azbar, N. and Speece, R.E. (2001) 'Two-phase, two-stage, and single-stage anaerobic process comparison', *J. Environmental Engineering*, Vol. 127, pp.240–248.
- Azbar, N., Ursillo, P. and Speece, R.E. (2001) 'Effect of process configuration and substrate complexity on the performance of anaerobic processes', *Wat. Res.*, Vol. 35, pp.817–829.
- Banks, C.J. and Wang, Z. (1999) 'Development of a two-phase anaerobic digester for the treatment of mixed abattoir wastes', *Wat. Sci. Tech.*, Vol. 40, No. 1, pp.69–76.

- Barford, J.P., Cali, R.G., Callander, I.J. and Floyd, E.J. (1986) 'Anaerobic digestion of high-strength cheese whey utilising semicontinuous digesters and chemical flocculant addition', *Biotechnol. Bioeng.*, Vol. 28, No. 11, pp.1601–1607.
- Boening, P.H. and Larsen, V.F. (1982) 'Anaerobic fluidized bed whey treatment', *Biotechnol. Bioeng.*, Vol. 14, pp.2539–2556.
- Chinwekitvanich, S., Tuntoolvest, M. and Panswad, T. (2000) 'Anaerobic decolorization of reactive dyebath effluents by a two-stage UASB system with tapioca as a co-substrate', *Wat. Res.*, Vol. 34, pp.2223–2232.
- Cohen, A., Breure, A.M., van Andel, J.G. and van Deursen, A. (1980) 'Influence of phase separation on the anaerobic digestion of glucose, 1. Maximum COD turnover rate during continuous operation', *Wat. Res.*, Vol. 14, pp.1439–1448.
- Cohen, A., Breure, A.M., van Andel, J.G. and van Deursen, A. (1982) 'Influence of phase separation on the anaerobic digestion of glucose, 2. Stability and kinetic responses to shock loadings', *Wat. Res.*, Vol. 16, pp.449–455.
- de Haast, J., Britz, T.J., Novello, J.C. and Verwey, E.W. (1985) 'Anaerobic digestion of deproteinated cheese whey', *J. Dairy Res.*, Vol. 52, pp.457–467.
- Denac, M. and Dunn, I.J. (1988) 'Packed and fluidized-bed biofilm reactor performance for anaerobic wastewater treatment', *Biotechnol. Bioeng.*, Vol. 32, No. 2, pp.159–173.
- Fongastitkul, P., Mavinic, D.S. and Lo, K.V. (1994) 'Two-phased anaerobic digestion process. Concept, process failure and maximum system loading rate', *Wat. Environ. Res.*, Vol. 66, pp.243–254.
- Fongastitkul, P., Mavinic, D.S. and Lo, K.V. (1995) 'A 2-phase anaerobic-digestion (UASB-UASB) process-design criteria and optimal system loading capacity', *Can. J. Civil Engineering.*, Vol. 22, pp.551–565.
- Fox, P. and Venkatasubbiah, V. (1996) 'Coupled anaerobic/aerobic treatment of high-sulphide wastewater with sulphate reduction and biological sulphide oxidation', *Wat. Sci. Technol.*, Vol. 34, Nos. 5–6, pp.359–366.
- Gharsallah, N. (1994) 'Influence of dilution and phase-separation on the anaerobic-digestion of olive mill wastewaters', *Bioprocess Engineering*, Vol. 10, pp.29–34.
- Ghosh, S. and Pohland, F.G. (1974) 'Kinetics of substrate assimilation and product formation in anaerobic digestion', *J. WPCF*, Vol. 46, No. 4, pp.748–759.
- Ghosh, S. and Klass, D.L. (1978) 'Two-phase anaerobic digestion', *Proc. Biochem.*, Vol. 15, p.2.
- Ghosh, S. and Taylor, D.C. (1999) 'Kraft-mill biosolids treatment by conventional and biphasic fermentation', *Wat. Sci. Tech.*, Vol. 40, Nos. 11–12, pp.169–177.
- Ghosh, S., Buoy, K., Dressel, L., Miller, T., Wilcox, G. and Loos, D. (1995) 'Pilot- and full-scale two-phase anaerobic digestion of municipal sludge', *Wat. Environ. Res.*, Vol. 67, pp.206–214.
- Ghosh, S., Conrad, J.R. and Klass, D.L. (1975) 'Anaerobic acidogenesis of sewage sludge', *J.WPCF*, Vol. 47, pp.30–45.
- Ghosh, S., Henry, M.P., Sajjad, A., Mensinger, M.C. and Arora, J.L. (2000) 'Pilot-scale gasification of municipal solid wastes by high-rate and two-phase anaerobic digestion (TPAD)', *Wat. Sci. Tech.*, Vol. 41, No. 3, pp.101–110.
- Gutierrez, J.L.R., Encina, P.A.G. and Polanco, F.F. (1991) 'Anaerobic treatment of cheese production wastewater using a UASB reactor', *Bioresource Technology*, Vol. 37, pp.271–276.
- He, Y.L., Zhang, A.L. and Yang, S.H. (1995) 'Anaerobic treatment of kenaf stem wood APMP waste-water', *Environ. Tech.*, Vol. 16, pp.467–476.
- Hilton, M.G. and Archer, D.B. (1988) 'Anaerobic digestion of a sulphate-rich molasses wastewater: inhibition of hydrogen sulphide production', *Biotechnol. Bioeng.*, Vol. 31, pp.885–888.
- Pol, H. (1995) *Waste Characteristics and Factors Affecting Reactor Performance*, Wageningen Agriculture University, The Delft, Netherlands.

- Ikbal, K.K., Teshima, M., Sonoda, Y. and Tanemura, K. (1994) 'Anaerobic-digestion of coffee waste by 2-phase methane fermentation with slurry-state liquefaction', *J. Fermentation and Bioengineering*, Vol. 77, pp.335–338.
- Ince, O. (1998) 'Performance of a two-phase anaerobic digestion system when treating dairy wastewater', *Wat. Res.*, Vol. 32, pp.2707–2713.
- Jeyaseelan, S. and Matsuo, T. (1995) 'Effects of phase-separation in anaerobic-digestion on different substrates', *Wat. Sci. Tech.*, Vol. 31, No. 9, pp.153–162.
- Johns, M.R. (1995) Development in wastewater treatment in the meat processing industry: a review', *Bioresource Technology*, Vol. 54, pp.203–216.
- Kaiser, S.K., Dague, R.R. and Haris, W.L. (1995) 'Initial studies on the temperature-phased anaerobic biofilter process', *Wat. Environ. Res.*, Vol. 67, pp.1095–1103.
- Kalyuzhnyi, S.V., Martinex, E.P. and Maptinez, J.R. (1997) 'Anaerobic treatment of high strength cheese whey wastewaters in laboratory and pilot UASB-reactors', *Bioresource Technology*, Vol. 60, pp.59–65.
- Kida, K., Ikbal and Sonoda, Y. (1994) 'Liquefaction and gasification during anaerobic-digestion of coffee waste by 2-phase methane fermentation with slurry-state liquefaction', *J. Fermentation and Bioengineering*, Vol. 77, pp.85–89.
- Lee, J.P., Lee, J.S. and Park, S.C. (1999) 'Two-phase methanization of food wastes in pilot scale', *Appl. Biochem. Biotech.*, Vol. 7, Nos. 7–9, pp.585–593.
- Lettinga, G., Hobma, S.W., Pol, H.L.W. and de Zeeuw, W. (1982) 'Design operation and economy of anaerobic treatment', *Wat. Sci. Tech.*, Vol. 15, No. 8, pp.175–195.
- Lin, C.Y. (1991) 'Anaerobic-digestion of landfill leachate', *Water SA.*, Vol. 17, pp.301–306.
- Lo, K.V. and Liao, P.H. (1986) 'Digestion of cheese whey with anaerobic rotating biological contact reactor', *Biomass*, Vol. 10, pp.243–252.
- Malaspina, F., Cellamare, C.M., Stante, L. and Tilche, A. (1996) 'Anaerobic treatment of cheese whey with a downflow-upflow hybrid reactor', *Bioresource Technology*, Vol. 55, pp.131–139.
- Malaspina, F., Stante, L., Cellamare, C.M. and Tilche, A. (1995) 'Cheese whey and cheese factory wastewater treatment with a combined biological anaerobic–aerobic plant', *Proceedings of the 3rd International Symposium on Waste Management Problems in Agro-industries*, Mexico, pp.63–76.
- Massey, M.L. and Pohland, F.G. (1978) 'Phase separation of anaerobic stabilization by kinetic control', *J. WPCF*, pp.2204–2221.
- Mudrak, K. and Kunst (1986) *Biology of Sewage Treatment and Water Pollution Control*, Ellis Horwood Ltd., England, p.193.
- OchiengOtieno, F.A. (1996) 'Anaerobic digestion of wastewaters with high strength sulphates', *Discovery and Innovation*, Vol. 8, pp.143–150.
- Pavan, P., Battistoni, P., Cecchi, F. and Mata-Alvarez, J. (2000) 'Two-phase anaerobic digestion of source sorted OFMSW (organic fraction of municipal solid waste): performance and kinetic study', *Wat. Sci. Tech.*, Vol. 41, No. 3, pp.111–118.
- Pohland, F.G. and Ghosh, S. (1971) 'Developments in anaerobic treatment processes', in Canale, R.P. (Ed.): *Biological Waste Treatment*, Interscience, New York, pp.85–106.
- Polprasert, C., Kemmadamrong, P. and Tran, F.T. (1992) 'Anaerobic baffled reactor (ABR) process for treating a slaughterhouse wastewater', *Environ. Technol.*, Vol. 13, pp.857–865.
- Rajeshwari, K.V., Balakrishnan, M., Kansal, A., Kusum, L. and Kishore, V.V.N. (2000) 'State-of-the-art of anaerobic digestion technology for industrial wastewater treatment', *Renewable and Sustainable Energy Reviews*, Vol. 4, pp.135–156.
- Reis, M.A.M., Lemos, P.C. and Carrondo, M.J.T. (1995) 'Biological sulphate removal of industrial effluents using the anaerobic digestion', *9th Forum for Applied Biotechnology*, Med. Fac. Landbouww. Univ. Gent, 60/4b, pp.2701–2707.

- Reis, M.A.M., Lemos, P.C., Martins, M.J., Costa, P.C., Goncalves, L.M.D. and Carrondo, M.J.T. (1991) 'Influence of sulphates and operational parameters on volatile fatty-acid concentration profile in acidogenic phase', *Bioprocess Engineering*, Vol. 6, pp.145-151.
- Rintala, J.A. and Puhakka, J.A. (1994) 'Anaerobic treatment in pulp and paper mill waste management: a review', *Bioresource Technology*, Vol. 47, pp.1-18.
- Romli, M., Greenfield, P.F. and Lee, P.L. (1994) 'Effect of recycle on a two-phase high-rate anaerobic wastewater treatment system', *Wat. Res.*, Vol. 28, pp.475-482.
- Ruiz, I., Veiga, M.C., de Santiago, P. and Blazquez, R. (1997) 'Treatment of slaughterhouse wastewater in a UASB reactor and an anaerobic filter', *Bioresource Technology*, Vol. 60, No. 3, pp.251-258.
- Sayed, S., Campen, L. and Lettinga, G. (1987) 'Anaerobic treatment of slaughterhouse waste using a flocculant sludge UASB reactor', *Biol. Wastes*, Vol. 21, pp.11-28.
- Schorder, E.W. and de Haast, J. (1989) 'Anaerobic digestion of deproteinated cheese whey in an upflow sludge blanket reactor', *J. Dairy Res.*, Vol. 56, pp.129-139.
- Shin, H.S., Bae, B.U., Lee, J.J. and Paik, B.C. (1992) 'Anaerobic digestion of distillery waste-water in a 2-phase UASB system', *Wat. Sci. Tech.*, Vol. 25, No. 7, pp.361-371.
- Shin, H.S., Jung, J.Y., Bae, B.U. and Paik, B.C. (1995) 'Phase-separated anaerobic toxicity assays for sulfate and sulfide', *Wat. Environ. Res.*, Vol. 67, pp.802-806.
- Strydom, J.P., Britz, T.J. and Mostert, J.F. (1997) 'Two-phase anaerobic digestion of three different dairy effluents using a hybrid bioreactor', *Water SA*, Vol. 23, pp.151-156.
- Switzenbaum, M.S. and Danskin, S.C. (1982) 'Anaerobic expanded bed treatment of whey', *Agric. Waste*, Vol. 4, pp.411-426.
- Talarposhti, A.M., Donnelly, T. and Anderson, G.K. (2001) 'Colour removal from a simulated dye wastewater using a two-phase anaerobic packed bed reactor', *Water Res.*, Vol. 35, pp.425-432.
- Visser, A., Gao, Y. and Lettinga, G. (1992) 'Anaerobic treatment of synthetic sulphate-containing wastewater under thermophilic conditions', *Wat. Sci. Technol.*, Vol. 25, No. 7, pp.193-202.
- Wildenauer, F.X. and Winter, J. (1985) 'Anaerobic digestion of high strength acidic whey in a pH-controlled upflow fixed-film loop reactor', *Appl. Microbiol. Biotechnol.*, Vol. 22, pp.367-372.
- Yan, J.Q., Lo, K.V. and Liao, P.H. (1989) 'Anaerobic digestion of cheese whey using upflow anaerobic sludge blanket reactor', *Biol. Waste*, Vol. 27, pp.289-305.
- Yanagi, C., Sato, M. and Takahara, Y. (1994) 'Treatment of wheat-starch waste-water by a membrane combined 2-phase methane fermentation system', *Desalination*, Vol. 98, pp.161-170.
- Yeoh, B.G. (1997) 'Two-phase anaerobic treatment of cane-molasses alcohol stillage', *Wat. Sci. Tech.*, Vol. 36, Nos. 6-7, pp.441-448.
- Yilmazer, G. and Yenigun, O. (1999) 'Two-phase anaerobic treatment of cheese whey', *Wat. Sci. Tech.*, Vol. 40, No. 1, pp.289-295.
- Zheng, Y.J. and Wu, W.N. (1985) 'A study of meat packing plant wastewater treatment with upflow anaerobic sludge blanket process', *Proceedings of 4th Int. Symp. on Anaerobic Digestion*, China, pp.327-337.

## Bibliography

- Clarke, T.A. and Bruce, M.E. (1995) 'The effects of total sulphur concentration and reactor pH on the anaerobic co-treatment of CTMP waste-water and domestic sewage', *Appita Journal*, Vol. 48, pp.30-36.
- Ghaly, A.E. (1996) 'A comparative study of anaerobic digestion of acid cheese whey and dairy manure in a two-stage reactor', *Bioresource Technology*, Vol. 58, pp.61-72.

- Huyard, A., Ferran, B. and Audic, J.M. (2000) 'The two phase anaerobic digestion process: sludge stabilization and pathogens reduction', *Wat. Sci. Tech.*, Vol. 42, No. 9, pp.41–47.
- Ikbal, K.K and Sonoda, Y. (1992) 'Treatment of coffee waste by slurry-state anaerobic-digestion', *J. Fermentation and Bioengineering*, Vol. 73, pp.390–395.
- Ikbal, K.K and Sonoda, Y. (1994) 'Liquefaction and gasification during anaerobic-digestion of coffee waste by 2-phase methane fermentation with slurry-state liquefaction', *J. Fermentation and Bioengineering*, Vol. 77, pp.85–89.
- Lettinga, G., van Velsen, A.F.M., Hobma, S.W., de Zeeuw, W. and Klapwijk, A. (1980) 'Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment especially for anaerobic treatment', *Biotechnol. Bioeng.*, Vol. 22, pp.699–734.
- Liu, T. (1998) 'Anaerobic digestion of solid substrates in an innovative two-phase plug-flow reactor (TPPFR) and a conventional single-phase continuously stirred-tank reactor', *Wat. Sci. Tech.*, Vol. 38, Nos. 8–9, pp.453–461.
- McCarty, P.L. (1981) 'One hundred years of anaerobic treatment in anaerobic digestion 1981', Hughes *et al.* (Eds.): *Anaerobic Digestion 1981*, Elsevier Biomedical Press B, Vol. V, pp.3–21.
- Stephenson, R.J., Branion, R.M.R. and Pinder, K.L. (1994) 'Anaerobic 35°C and 55°C treatment of a BCTMP/TMP effluent – sulfur management strategies', *Wat. Sci. Tech.*, Vol. 29, Nos. 5–6, pp.433–445.
- Switzenbaum, M.S. and Jewell, W.J. (1980) 'Anaerobic attached-film expanded-bed reactor treatment', *J. WPCF*, Vol. 52, No. 7, pp.1953–1965.
- van der Last, A.R.M. and Lettinga, G. (1992) 'Anaerobic treatment of domestic sewage under moderate climatic (Dutch) conditions using upflow reactors at increased superficial velocities', *Wat. Sci. Technol.*, Vol. 25, No. 7, pp.167–178.
- Yoda, M., Hattori, M. and Miyaji, Y. (1985) 'Treatment of municipal wastewater by anaerobic fluidized bed: behavior of organic suspended solids in anaerobic reactor', in Switzenbaum, M.S. (Ed.): *Proceedings of the Seminar/Workshop on Anaerobic Treatment of Sewage*, Amgerst, USA, pp.161–196.
- Young, J.C. and McCarty, P.L. (1969) 'The anaerobic filter for wastewater treatment', *J. WPCF*, Vol. 4, pp.160–173.