The Anaerobic Treatment of Low Strength Soluble Wastewaters

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Proefschrift

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Propositions

1. Aerobic organisms are essential for providing a proper environment for anaerobic organisms.

2. Mass transport inside anaerobic biofilms is not solely restricted to diffusion, as described by Pavlostathis and Giraldo-Gomez.
   Pavlostathis, S. G. and Giraldo-Gomez, E. 1991. Kinetics of anaerobic treatment. Wat. Sci. Technol. **24**: 35-59.

3. Contrary to the views of Sayles and Suidan, anaerobic bioreactors are not mere pre-treatment steps for high strength wastewaters.
   Sayles, G. D. and Suidan, M. T. 1993. Biological treatment of industrial and hazardous wastewaters. p. 245-267. *In*: M. A. Levin and M. A. Gealt (eds.), Biotreatment of industrial and Hazardous Waste. McGraw-Hill, New York.

4. For achieving a desired high treatment efficiency of low strength soluble wastewaters, the organic loading capacity of the anaerobic EGSB is higher compared with that of the aerobic activated sludge process.

5. Pollution, as a synonym of economical development and welfare for all, was one of the visible slogans used by Brazilian dictators. The present day reality is welfare for a minority and pollution for the majority.

6. According to the historian Gonsalves de Mello (*Tempo dos Flamengos*), during the time of the *Nederlands-Braziliё*, a measure taken in Recife in order to improve the urban practices, was that all the inhabitants should sweep the streets in front of their houses and not discharge the filth there, but onto the beach. The present practice of citizens keeping the beaches clean but leaving the streets filthy, is a backward step compared with that measure.

7. It is a contradiction that countries criticizing the lack of protection of rain forests offer the biggest market for the wood from those forests.

8. Both poor and rich people are hungry. The poor are hungry due to a lack of resources while the rich are hungry due to an excess of resources.

9. During peace or war time, the bankers always have their profits.

10. One may have the present burned and the future dark when one remains committed to the past.

11. The human mind and the diskette have higher capacities when they are empty.

12. In Wageningen, the bike thieves prefer expensive locks than old bikes.

Propositions belonging to the thesis of Mario Takayuki Kato entitled **"The Anaerobic Treatment of Low Strength Soluble Wastewaters"**. Wageningen, 10th June, 1994.

Voor Lourdinha, Mahita en João Henrique

Aan mijn ouders

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Abstract

Kato, M. T. (1994) The Anaerobic Treatment of Low Strength Soluble Wastewaters. Ph.D. Thesis. Wageningen Agricultural University. Wageningen, The Netherlands.

Low strength soluble wastewaters with chemical oxygen demand (COD) of less than 2000 mg/*l* are mostly from food processing industries. They commonly contain simple substrates such as short-chain fatty acids, alcohols and carbohydrates. The application of anaerobic technology has been mostly directed towards the treatment of medium and high strength wastewaters rather than those of low strength. Problems limiting the treatment of dilute wastewaters are related to the wastewater and the reactor design. This dissertation investigates the application of the conventional upflow anaerobic sludge blanket (UASB) and its modification, the expanded granular sludge bed (EGSB), for the treatment of low strength soluble wastewaters. The main topics studied concern the wastewater related problems. The effect of dissolved oxygen on the methanogenic activity of granular sludges and the effect of low substrate levels inside reactors on the treatment performance were evaluated. Moreover, some aspects of reactor design related problems such as the retention of biomass and wastewater-biomass contact were considered.

Methanogens located in granular sludge have a high tolerance to oxygen. The concentration of oxygen found to cause 50% inhibition to methanogenic activity was between 7% and 41% oxygen in the head space of flasks, which corresponded to 0.05 mg/*l* and 6 mg/*l* of dissolved oxygen prevailing in the media, respectively. The most important mechanism for the tolerance was the consumption of oxygen by facultative bacteria while metabolizing substrates. The most highly tolerant sludges had the highest respiration rates. The hypothesis considered is that anaerobic microenvironments are created inside granules protecting the methanogens. The absence of facultative substrate for respiring oxygen decreases the tolerance of methanognens to O_2. The coexistence of methanogenic and facultative bacteria competing for substrate in one single bioreactor was explored under highly aerobic conditions, in order to verify the possible application of anaerobic-aerobic co-cultures for the removal of recalcitrant pollutants. Simultaneous methane production and oxygen uptake occurred in an oxygen tolerant sludge while at least 2 mg/*l* of dissolved oxygen was present in the media. The healthy co-culture was evident even after longer periods of oxygen exposure, when methane oxidizing bacteria eventually also developed.

The feasibility of UASB and EGSB reactors at 30°C was demonstrated. In UASB reactors, COD removal efficiencies exceeded 95% at organic loading rates (OLR) up to 6.8 g COD/*l*.d and influent COD concentrations (COD_{in}) ranging from 422 to 943 mg/*l*, during the treatment of ethanol substrate. The efficiencies exceeded 86% at OLR up to 3.9 mg COD/*l*.d when whey was used as a substrate. Below 630 mg COD/*l*, acidification of whey instead of methanogenesis was the rate limiting step. The retention of biomass is not a problem in the UASB, but the mixing intensity is not high enough to decrease the biofilm diffusion limitation of substrate transport into granular biofilms. The EGSB was shown to have superior potentials compared with the UASB. COD removal efficiencies were above 80% at OLRs up to 12 g COD/*l*.d with COD_{in} as low as 100 to 200 mg/*l*. The effect of low substrate levels was not significant in the EGSB due to the intense turbulent mixing regime obtained by applying high hydraulic and organic loads. The very low apparent K_S value of 9.8 mg COD/*l* found for the biofilms in the reactor, was comparable to the intrinsic K_S values determined for the most predominant acetoclastic methanogen found in anaerobic bioreactors, *Methanothrix soehngenii*. This indicates that all transport limitations of substrate movement into the biofilms were overcome. Optimized operation without sludge washout is achieved when liquid upflow velocities (V_{up}) below 5.5 m/h are applied. The problem of sludge retention is also restricted when sludge flotation occurs due to the buoyancy forces of gas attached to biofilms. The required equilibrium between mixing intensity and sludge retention limits the operation of the EGSB to OLRs up to 7 g COD/*l*.d and V_{up} values ranging from 2.5 and 5.5 m/h. Both reactor studies confirmed that in practice dissolved oxygen does not constitute any detrimental effect on the treatment performance. Improved mixing intensity in the UASB and improved sludge retention in the EGSB will enable higher OLRs and lower COD_{in} which can be tolerated, compared with those of this study.

Contents

Chapter 1

The Anaerobic Treatment of Low Strength Soluble Wastewaters: General Introduction

1. Introduction

The decline of the quality of life in many developing countries can be attributed to a great extent to the environmental deterioration. One of the main reasons for the deterioration has been the rapid increase in the population density and industrial activities within urban areas. An adequate protection of the environment has not paralleled the rate of the population and industrial increase. Consequently, a common visible problem is the serious water pollution, since the wastewaters are being discharged into water bodies without the use of proper (if any) treatment facilities. A number of industrial wastewaters, in general from the food processing sectors, are frequently found with low strength since the water management is not always the most adequate in those countries.[11,13,30,55] Consequently, large volumes of dilute industrial wastewaters are produced, containing easily biodegradable matter, and contributing significantly to the water pollution.

In the attempt to revert the common dilemma of water pollution, an adequate low cost technology for wastewater treatment is essential. This signifies that a treatment system should be promoted that fulfills the following parameters: simple to design; the use of non-sophisticated equipment or installations; low energy consumption; and high treatment efficiency. The traditional aerobic activated sludge method can not be considered for the treatment of wastewaters in developing countries due to its high costs. In most of the urban areas the cost and availability of land are now obstacles for the advantageous use of the relatively simple stabilization ponds. A recent survey showed that the anaerobic technology offers great potentials in most developing countries, since it has successfully been applied for the treatment of a number of organic wastes.[92] The anaerobic processes can also be included for the treatment of low strength wastewaters containing easily degradable matter, since they are suitable economical up-to-date alternatives.[66]

However, the feasibility of anaerobic processes for the treatment of such wastewaters with low concentrations should be investigated more thoroughly in order to explore all their potentials and to possibly improve the present environmental situation in those countries. The anaerobic process alternative can also be considered for dilute wastewaters originating in industrialised countries. In order to investigate its applicability, the knowledge of the low strength wastewater characteristics, the available anaerobic technology for the treatment and the related possible problems should be considered.

2. Characteristics and Sources of Low Strength Wastewaters

In general, there is no rigorous classification of wastewaters with respect to the strength. However based on the wastewaters anaerobically treated, most of the effluents classified as medium and high strength are referred to those with chemical oxygen demand (COD) greater than 2000 mg/*l*.[71,81,99] Consequently, low strength wastewaters can be considered as those containing COD concentrations below 2000 mg/*l*, though many even contain concentrations of less than 1000 mg/*l*.

Soluble wastewaters commonly only contain easily biodegradable organic matter. These wastewaters are usually considered as those which do not contain significant amounts of suspended solids that are more difficult to anaerobically degrade, or toxic compounds at serious inhibitory concentrations for the anaerobic treatment. In general, they can contain some complex compounds like proteins and fats but at low concentrations. Such concentrations seldom result in severe problems such as intense foaming and scum layer formation during anaerobic treatment.[69] Thus, low strength soluble wastewaters can be defined as those dilute industrial effluents of less than 2000 mg COD/*l*, which may contain a variety of biodegradable compounds such as simple short-chain volatile fatty acids (VFA), alcohols and carbohydrates, as well proteins, fats and long-chain fatty acids (LCFA) in some cases.

Important examples of low strength soluble wastewaters are effluents from alcoholic and soft drink bottling industries, paper recycle and papermaking mills, fruit and vegetable canneries, and malting and brewing processes.[16,48,68,81] Some soluble wastewaters may have a broad concentration range since the COD of industrial effluents depends largely on the technological process. This is especially due to the use and recycling of water and the internal sources of wastewaters. Examples are the brewery and dairy industry wastewaters. Brewery industry wastewaters can have COD concentrations as low as 600-900 mg/*l* and up to 160,000 mg/*l*, since these effluents can consist or not of a mixture of process streams from the malting and brewing processes, spent grains and hops pressing liquor wastes; and many breweries have a soft drink bottling section which also discharges dilute wastewaters.[14,16,21,41,44,56,80] Effluents like whey or other wastewaters from the dairy industry have been reported as containing COD concentrations in the range between 500 and 2000 mg/*l*.[11,33,87,123] However, COD concentrations ranging from 4000 mg/*l* to 66,000 mg/*l* have also been reported in many cases.[5,119,139]

Thus, industrial processes may produce several streams with different characteristics either in flow or in COD concentration. Consequently, one may encounter dilute wastewater main- or sub-streams being discharged at a number of industrial processes.[8,33,48,122] Nevertheless in the case of low strength soluble wastewaters, attractive alternatives for the treatment are the anaerobic processes since they mostly contain simple substrates.[63,65,66,69]

3. The Anaerobic Technology

3.1. The high rate systems

The anaerobic treatment processes are well established methods for the elimination of easily biodegradable organic matter from wastewaters. Since the development of the high-rate and efficient anaerobic reactors, a large number of full-scale plants have been built.[50,69,73] The development of the anaerobic processes and the successful application for the direct treatment of such wastewaters can mainly be attributed to the high retention of active biomass, which is one of the most important characteristic of the various high rate systems developed.[50] In these systems, the bacterial adhesion resulting in biofilms is the major mechanism which enables a high retention of dense and active biomass.[43] Bacteria attach either to the surface of stationary or mobile inert carrier materials, or to each other by self-immobilization.

The most significant examples of reactor types where bacteria attachment to carriers is promoted are the anaerobic filter (AF), the downflow stationary fixed film (DSFF), the anaerobic attached film expanded bed (AAFEB) and the fluidized bed (FB). The most important examples of reactor which have no inert carrier support are the upflow anaerobic sludge bed (UASB) and the expanded granular sludge bed (EGSB). In these latter examples the biomass is immobilized as aggregates in the form of granules. A higher reactor biomass concentration (30-50 g VSS/*l*) can be reached or used with granular sludges compared with biofilms immobilized on stationary carriers (15-25 g VSS/*l*).[105] This signifies that higher maximum conversion rates can be expected in the UASB and EGSB processes. Since its earlier development, the UASB-like systems have been more widely applied in practice than systems using carrier materials.[65,69,73] The cost of various packing materials with large surface area for biomass attachment and difficulties with the control of the complex film build-up process resulting in segregation of aggregates according to density, are regarded as the main constraints of the systems like the FB. The presence of particles with different shapes, sizes and densities normally causes a sludge bed segregation where the heavier particles move down to the bottom forming a fixed bed, whereas the lighter are highly fluidized particles which move to the top, being even washed out of the reactor.[49] The good potentials of the AF for the treatment of soluble wastewaters is frequently limited by the non-vertical mixing and problems of clogging. Therefore, the choice of reactors using granular sludges, like the UASB or EGSB, are in principle favourable compared with the other currently existing types.

The UASB concept basically relies on the distinguished high aggregation of the anaerobic sludge that is typically observed to occur naturally under liquid upflow conditions.[72,75] The dense active sludge granules formed also have high settling characteristics and mechanical strength. Start-up of UASB reactors and granule formation have already been thoroughly investigated.[43] The start-up and granulation were even shown with domestic sewage as seed for the treatment of low strength brewery wastewaters.[41] An adequate retention of a high amount of sludge is enhanced in the UASB reactor by a simple GSL (gas-solid-liquid) device installed in the upper part of the reactor. The result is usually an effective separation of the gas from the sludge and liquid.

The UASB reactor concept represents a remarkable progress in the anaerobic treatment of wastewaters since besides being simple to design, it can accommodate very high volumetric organic loads and provide high treatment performance.[69,75,78] Compared with the aerobic processes, the UASB can tolerate organic loads which considerably exceed those of the activated sludge. Activated sludge plants are typically designed with organic loading rates (OLR) in the range of 0.3 to 2.4 g COD/*l*.d, while at least tenfold higher values can be applied in full-scale UASB reactors.[65,88,105] Consequently, several benefits can be derived from the UASB treatment of wastewaters compared with the activated sludge process. For comparable treatment requirements, less reactor volume and space for UASB plants are needed and high grade energy is produced from the biogas. The activated sludge process in contrast, consumes considerable energy to provide adequate aeration. Another advantage of the UASB is the relatively low cost of the technology requiring no sophisticated equipment. Additionally, the anaerobic granular sludge is generally well stabilized and significantly less excess sludge is produced compared with that of the aerobic systems. Also, when stored at low temperatures, activity and settleability are maintained even after being unfed for long periods.[66,73] Nevertheless, some modifications of the UASB reactor concept have been proposed in order to improve its applicability.[64] The conventional UASB reactor seldom utilizes effluent recirculation and a modification employing it resulted in the EGSB concept.

The characteristics of the EGSB reactor are very similar as in the UASB reactor. However in an EGSB reactor type, the granular sludge bed is expanded and the hydraulic mixing is intensified in order to improve the wastewater-biomass contact.[61,82] A higher superficial liquid velocity is achieved by applying effluent recirculation. In full-scale EGSB reactors, a sophisticated influent distribution system will be required. In the UASB reactor the sludge bed behaves more as a static bed because the liquid upflow velocity (V_{up}) is usually in the range of 0.5 to 1.5 m/h.[82] In contrast, the EGSB utilizes V_{up} exceeding 5 to 6 m/h by using high effluent recirculation ratios combined with taller reactors. In general, a relatively high height-diameter ratio of 20 or higher is used.[60,82,105] Nonetheless, shallow reactors can also be used. The EGSB reactor utilizes a partially or fully expanded bed of granule sludge. In the latter case, the liquid phase is completely mixed.[105] Granular sludges from UASB reactors should be used for the start-up of EGSB reactors. So far there are no reports of experiments about the type of biomass which is formed from a first start-up under EGSB operational conditions with digested sewage sludge. However, biomass aggregation was shown to develop in FB reactors utilizing inert carrier materials when they were operated in a similar regime as that of the EGSB.[51,61]

3.2. Past experience with the treatment of dilute wastewaters in UASB and EGSB reactors

Despite all the advances of the anaerobic processes, the UASB is still designed mostly for the medium and high strength wastewaters. It is considered as a mere pre-treatment step requiring further aerobic post-treatment to polish off the residual biodegradable fraction. Nonetheless, these conceptions about the applicability of the UASB are changing more and more. Recent attempts with full-scale UASB reactors indicate that the treatment of less concentrated domestic and industrial wastewaters can be feasible as well.[19,71,109] The practical application of the UASB for the treatment of dilute wastewaters has mostly been limited to domestic sewage. The results of full-scale reactors and of some investigations showed that satisfactory treatment performance was obtained at temperatures above 20°C (Table 1). For industrial effluents, research has mostly been conducted with lab- and pilot-scale reactors. The results in Table 2 reveal that in general good treatment performance can be achieved at high temperatures near 30°C, since COD removal efficiencies (T_{in} - S_{ef}) of 90% or higher were obtained at OLRs up to 15.3 g COD/*l*.d. A satisfactory COD removal efficiency of 70% was obtained in the treatment of acidified brewery wastewater in full-scale UASB reactor, even for temperature as low as 12°C.[119,122]

Tables 1 and 2 indicate that in general the more complex the wastewater (e.g. domestic sewage), the lower the organic loadings and the treatment efficiencies which are obtained with the UASB reactor. This can be attributed to several factors. Complex wastewaters by nature contain substrates more difficult to anaerobically degrade. Also, many of them are in the form of suspended solids (SS) which require hydrolysis and liquefaction previous to acidification and methanization steps. Those first steps can be responsible for the overall reaction rate limitation.[69] SS accumulate in the reactor due to the entrapment of the coarse inert fraction in the sludge bed, resulting in a dilution of the active biomass in the sludge. Also, adsorption of finely dispersed colloidal matter to the surface of the sludge may create a thick layer resulting in an increased hampering of substrate supply into the biofilm.[108] Consequently, a decreased organic load capacity can occur due to the decreased or even complete deterioration of the specific methanogenic activity in the sludge. Such complex substrates also require a better wastewater-biomass contact which can perhaps not be obtained in the conventional UASB reactor. This can be inferred by comparing the results of a conventional and a modified UASB, where recirculation was applied for the treatment of lipid containing wastewaters. The higher treatment efficiency obtained in the modified UASB (EGSB) was attributed to the higher mixing intensity levels achieved.[105] Since an intense mixing is also very important for the treatment of low strength soluble wastewaters, the EGSB does fulfill that requirement.

One of the first applications of the EGSB concept was using a tall reactor of 2 m and 9 cm diameter that was initially operated as a lab-scale UASB reactor.[67] However, the effect of increased upward velocity was not significant because the reactor was operated under

Table 1. Some results of the anaerobic treatment of low strength domestic sewage in UASB reactor.

Reactor volume	Sewage	COD_{in}	BOD_{in}	HRT	OLR	COD removal efficiency (%) [a]		Temperature	Reference
(m^3)		(mg/l)		(h)	(g COD/l.d)	$[T_{in} - T_{ef}]$	$[T_{in} - S_{ef}]$	(°C)	
0.12	raw	500	-	12	1.0	-	75	7-18	(77)
6	raw	472	-	8	1.4	30	55	10	(78)
6	raw	602	-	8	1.8	40	63	12	(78)
20	raw	900	-	13.5	1.4	16	-	15	(83)
0.055	UASB effluent	700	-	10	1.1	77	-	15	(83)
0.12	raw	431	-	5.5	1.88	54	-	17	(84)
0.24	raw	514	-	6	2.07	41	-	17	(84)
6	raw	628	-	11	1.37	44	59	17	(84)
20	raw	621	-	7	2.13	47	64	17	(84)
120	raw	285	130	4.4	1.6	34 (15)	65	18	(121)
120	raw	400	171	14.5	0.7	65 (55)	77	19	(121)
6	raw	555	-	8	1.7	48	73	19	(75)
67.5	raw	892	515	42	0.5	74 (80)	79	16-23	(122)
600	raw	563	214	6	2.3	68 (69)	-	20-30	(19)
300	raw	563	214	6	2.3	74 (75)	-	20-30	(19)
300	raw	563	214	6	2.3	70 (72)	-	20-30	(19)
0.12	raw	627	357	4	3.8	74 (78)	89 (91)	24	(2)
3300	raw	280	147	4.8	1.4	- (71)	-	25	(105,106,107)
120	raw	188	96	6.1	0.7	56 (56)	76	28	(121)

[a] : Values in parenthesis refer to BOD removal efficiency; $[T_{in} - T_{ef}]$ refers to COD removal efficiency based on the total COD in the influent and effluent; $[T_{in} - S_{ef}]$ is based on the total COD in the influent and soluble COD in the effluent.

Table 2. Some results of the anaerobic treatment of low strength industrial wastewaters in UASB reactor.

Reactor volume	Wastewater	COD_{in}	BOD_{in}	HRT	OLR [a]	COD removal efficiency (%) [a]		Temperature	Reference
(m^3)		(mg/l)		(h)	(g COD/l.d)	$[T_{in} - T_{ef}]$	$[T_{in} - S_{ef}]$	(°C)	
0.017	skimmed milk	1500	-	5.3	7.1	90	-	30	(72)
0.026	whey	501	-	5.1	2.34	89	-	35	(15)
0.026	whey	493	-	5	2.36	83	-	27.5	(15)
0.026	whey	532	-	4.9	2.6	46	-	20	(15)
0.001	cheese [b]	1339	-	3.1	10.4	88	-	35	(25)
0.001	cheese	1574	-	2.6	14.5	95	98	35	(25)
0.001	cheese	1651	-	2.6	15.3	96	97	35	(25)
0.009	synthetic [c]	428	-	6.5	1.6	79	-	30	(31)
0.009	synthetic	522	-	3.5	3.6	86	-	21.5	(31)
0.005	synthetic [d]	-	250	3	(2.0)	(78)	-	15	(57)
0.005	synthetic	-	250	3	(2.0)	(80)	-	25	(57)
0.005	synthetic	-	250	3	(2.0)	(83)	-	37	(57)
5	brewery [e]	1722	1000	6-8	5-13	81	90	35	(16)
1400	brewery [f]	1000-1500	700-1100	4	2-6	70-80	-	12-24	(118,122)
0.003	spoiled beer	1000	-	40	0.6	75	-	35	(12)
0.008	spoiled beer	1200	-	13	2.2	80	-	35	(12)
0.017	spoiled beer	1125	-	18	1.5	70	-	35	(12)
3.75	malting [g]	800-1600	-	6.0-13.7	1.7-3.4	52-75	-	12-20	(44)
88	cannery [h]	872	-	24	0.9	76	-	27	(30)
88	cannery	394	-	12	0.8	66	-	28.5	(30)
88	cannery	438	-	7.5	1.4	69	-	29	(30)
0.120	cannery [i]	960	-	5.5	4.2	-	84	29	(72)
2200	recycle paper	1000	500	5.5	4.4-5.0	70-72	75-83	28	(71)

[a] : Values in parenthesis refer to BOD; $[T_{in} - T_{ef}]$ refers to COD removal efficiency based on the total COD in the influent and effluent; $[T_{in} - S_{ef}]$ is based on the total COD in the influent and soluble COD in the effluent; [b] : Only cleansing water; [c] : Glucose + methanol; [d] : Glucose + corn-steep liquor; [e] : Brewery + soft drink wastewater; [f] : Brewery + malting + soft drink wastewater (after 1500 m^3 acidification tank); [g] : (After 4 m^3 acidification tank); [h] : Vegetable cannery; [i] : Fruit + vegetable cannery.

severe underloading and at a high influent COD concentration (COD_{in}). Further experiments with dilute vinasse at 8°C showed the importance of a high V_{up} for influents with a low COD_{in}. Even at a low SLR ranging from 0.025 to 0.040 g COD/g VSS.d, a COD removal efficiency of up to 75% was obtained at V_{up} higher than 5 m/h, compared with the 50% - 60% obtained when no recirculation was applied (V_{up} of 0.5 m/h).[82]

Low temperatures in anaerobic treatment have always been associated with low methanogenic sludge activity. However, this does not necessarily mean that psychrophilic wastewater treatment is infeasible. In an investigation with the treatment of dilute acidified wastewater in an EGSB reactor at 10°-13°C fed with COD_{in} ranging from 600 to 900 mg/*l*, COD removal efficiency of approximately 100% was observed after 100 days of operation.[76] The hydraulic retention time (HRT) applied was less than 2 h and the OLR was of 10 to 13 g COD/*l*.d. The very high treatment performance of low strength acidified wastewaters at low temperatures represents an important step forward in the potentials of the EGSB system.

Other investigations on the EGSB reactors were also carried out with complex wastewaters as lipid containing effluents[105] and pre-settled domestic sewage.[60,76]. A higher treatment performance was obtained in the EGSB compared with the UASB reactor for the degradation of sodium caprate and sodium laurate solutions at 30°C.[105] With the conventional UASB, the maximum OLR achieved was 4-5 g COD/*l*.d despite the use of a highly active dense granular sludge. Accumulation of precipitated LCFA salts either as lumps or adsorbed onto sludges occurred, causing local overloading, and heavy sludge flotation and washout. On the other hand, the EGSB could handle the LCFA without serious problems. An OLR up to 30 g COD/*l*.d with COD removal efficiencies between 83 to 91% were reported. This higher performance was attributed to the very high mixing intensity and efficient contact between the biomass and the substrate. Moreover, an efficient sludge retention was shown with the operation at an HRT of 2 h and a V_{up} of 7.2-7.7 m/h in the EGSB. Further research was conducted to evaluate the degradation of emulsified triglycerides in the EGSB. In this case however, a strong sludge washout occurred at a low OLR of 1-2 g COD/*l*.d and the granule aggregates showed very poor settleability. These facts were attributed mostly to gas bubbles adhering to the granules with adsorbed lipids.[105] Consequently, a modified GSL was required to retain the sludge at an acceptable level. The use of a floating layer of reticulate polyurethane foam held in place with a screen, or a sieve-drum separator placed above the expanded granule bed was investigated. With the latter GSL device, a better performance was shown, with COD removal efficiency of 80% at an OLR of 8 g COD/*l*.d and an HRT of 6 h, provided that the EGSB was inoculated with sludge of large granule diameter and using an easily degradable co-substrate.

The experiments with pre-settled domestic sewage were conducted at temperatures ranging from 8° to 20°C.[60] The total COD concentration (COD_{in}) ranged from 100 to 650 mg/*l* but with a soluble COD fraction (membrane filtered) of around 50%. The results showed that a removal efficiency of the soluble COD fraction was between 62% to 95% at HRT of 1.0 to 3.5 h, when the temperature was above 13°C and the COD_{in} was above 350 mg/*l*. Lower efficiencies were obtained at lower temperatures and values of COD_{in}. As anticipated, a post-treatment is needed for the COD removal of the SS fraction. Since the EGSB alone is not adequate for the removal of SS, a two-step system is also being considered for the treatment of domestic sewage. A hydrolysis upflow sludge bed reactor (HUSB) as a pre-treatment step for the EGSB reactor was evaluated.[60,128,129] Compared with the one-step system operating under the same conditions of HRT, a better result was obtained in relation to the removal of SS. Consequently, the HUSB pre-treatment resulted in an improvement in the overall COD removal because the SS were more effectively retained, hydrolysed and acidified prior to entering the EGSB reactor.

3.3. Possible problems in the treatment of low strength soluble wastewaters in UASB and EGSB reactors

The previous UASB and EGSB experiments showed that some difficulties can be expected during the treatment of dilute wastewater. The possible problems are either wastewater or reactor design related. The wastewater related problems are as follows: (i) low substrate concentration occurring inside the reactor; (ii) possible presence of dissolved oxygen; and (iii) low temperature.

The reactor design related problems are concerned with the requirements for high retention of biomass and good wastewater-biomass contact. Both requirements are dependent on the mixing intensity of the bulk liquid phase. An adequate mixing can be achieved by a proper hydraulic turbulence and expansion of the sludge bed. A compromise between the retention of biomass at high levels and good wastewater-biomass contact should be established for the following reasons: (i) high mixing intensity enhances the penetration of substrate into the biofilm ensuring adequate wastewater-sludge contact; (ii) however, sludge washout may result due to excessive expansion of the sludge bed, and due to sludge erosion and deterioration by the high shear forces.

4. The Low Strength Wastewater Related Problems

4.1. The low influent COD concentration related problems

Low influent COD concentrations result in low substrate levels inside anaerobic bioreactors. In the case the bulk liquid phase is completely mixed, the concentration in the reactor effluent is very similar to that inside the reactor. However, the effective concentration inside the granules can even be lower than that of the bulk liquid phase when the consumption of substrate by bacteria of high activity is faster than the transport of substrate into the biofilms.

Low substrate levels

Low substrate levels inside the bioreactor and the granules result in low activity of the biomass. This can be explained due to Monod kinetics,[89] model which is generally utilized for describing the conversion rates during the anaerobic treatment of soluble substrates.[18,69,97] According to Monod kinetics, the specific growth rate (μ) and the specific sludge activity (V) depend on the substrate concentration (S). The saturation constant, K_S, defines the affinity of a microorganism for a limiting substrate. The higher the values of K_S, the lower the affinity. The K_S is the substrate concentration corresponding to the half of the maximum activity (μ_{max} or V_{max}). The dependency of μ or V on S is approximately linear for concentrations around K_S or below when the reactions may be considered as first-order; the reactions are half-order in the S range between K_S and saturation and zero-order in the saturation range. The expressions for μ and V as a function of S are given by the equations 1 and 2, respectively, as follows:

$$\mu = \frac{\mu_{max} \cdot S}{K_S + S} \qquad (1)$$

$$V = \frac{V_{max} \cdot S}{K_S + S} \tag{2}$$

The relationship between both rates is given by the cell yield (Y), as expressed by the equation 3:

$$\mu = Y \cdot V \tag{3}$$

Since it is desired to have the highest possible COD elimination in wastewater treatment, then it is inevitable that the reactor contents have a low substrate concentration. Therefore, an optimized treatment performance means that reactor sludge should have high specific activity and the K_S should be very low. The specific activity is high in the case of methanogenic granular sludge from UASB reactors. Values ranging from 0.6 to 2.2 g COD-CH_4/g VSS.d or higher have been reported.[9] The intrinsic (true) values of K_S refer to the transport of substrate into dispersed bacterial cells in perfect suspensions. The values of true K_S depend on the type of substrate utilized by specific microorganisms. Examples are hydrogen and acetate, the main precursors of methane formation.[40] In the case of methanogenesis from hydrogen, the values are in the range between 0.093 to 0.6 mg COD/*l*.[97] In the case of methanogenesis from acetate, two common acetoclastic methanogens in wastewater treatment are known from the genera *Methanothrix* and *Methanosarcina*. The values of K_S found are 18 to 30 mg COD/*l* for *Methanothrix* and 257 to 300 mg COD/*l* for *Methanosarcina*.[18,97,105] These values can also explain why there is predominance of a given methanogen. The substrate affinity in fact plays an important role in the competition for acetate among methanogenic bacteria. Although *Methanothrix* has a slower growth rate, its substrate affinity is higher due to the lower K_S. Consequently, *Methanothrix* is more commonly encountered since in general the substrate in the reactor effluent, being similar in concentration to the reactor contents, is maintained at low levels during the wastewater treatment. Therefore, *Methanosarcina* is the dominant bacteria only in the case of high content of acetate in the effluent.

In the case of sludge granules, there is substrate transport limitation. The possible existence of substrate gradients with less substrate available for the biomass inside the granules than in the bulk liquid phase, will result in a situation where not all of the biomass is being effectively utilized. The formation of substrate gradients is illustrated in Figure 1. Substrate gradient formation is more significant when the sludge has a high specific activity, since the substrate consumption will more likely be faster than the transport.[18] Therefore, perhaps the lowest COD_{in} possible for anaerobic treatment will be quite high.

Apparent K_S values of sludge granules are lower than true K_S values due to mass transfer problems affecting the actual substrate removal kinetics. Values of apparent K_S were found to be significantly higher than the intrinsic values in a study with different floc sizes.[91] Other experiments with granular sludge from UASB reactors showed that mass transfer limitation occurred due to the increased size of the granules.[18] No mass transfer limitation was detected for VFA when granules relatively small (< 2 mm) and with relatively low specific methanogenic activities (< 0.63 g COD-CH_4/g VSS.d) were utilized with VFA as substrate. Also, mass transfer was not significant when a high concentration of VFA in the bulk liquid phase was maintained, since the concentration was many fold greater than the apparent K_S range. Nevertheless, apparent K_S values found in those experiments with granular sludge using acetate as substrate were in the range between 60 and 228 mg COD/*l* which are significantly higher than those values of intrinsic K_S values for *Methanothrix.* Substrate diffusion limitation in granules was also justified for the apparent K_S value of 200 mg COD/*l* found for sludge from UASB reactor in an experiment with acetate.[105] Experiments with mixed cultures from digested sludge also using acetate as substrate resulted in values of apparent K_S varying from 152 to 927 mg COD/*l*.[62,141] Thus in terms of wastewater treatment, the mass transport limitation should be decreased in order to decrease apparent K_S values of sludge granules. Two transfer mechanisms are considered: diffusion and convective mass transport.

Diffusion is concerned with the transport of solutes in stagnant fluids and the driving force is the solute concentration gradient. Convective mass transfer refers to the transport of solutes in a moving fluid which is limited by the conductivity of the fluid in porous biofilm matrices.

Diffusion of substrates to the cell is expressed by Fick's law [34,42,97] which can be illustrated by the diffusivity or the diffusion coefficient. The values of the diffusion coefficient increase with temperature, decrease with size of the solute and are dependent on the solute concentration. Some values are given in Table 3.

The mass transport inside biological matrices is often considered to be due to diffusion only, and consequently is modelled by Fick's law.[18,42,97] However, it may be expected that the diffusion coefficients for solutes inside the methanogenic aggregates will be lower than those found in clean water. In this case, the diffusion coefficient values

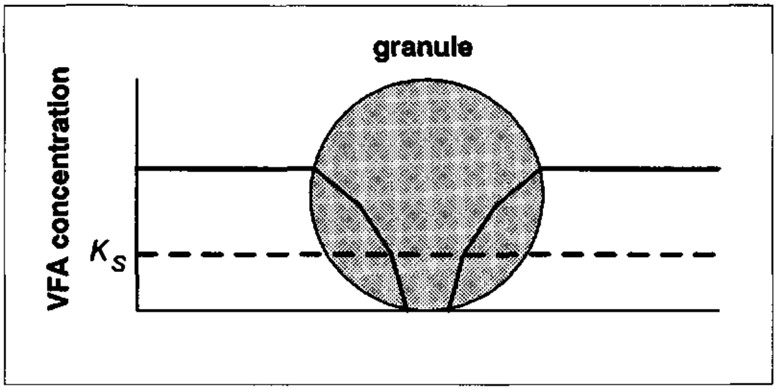

Figure 1. Substrate gradient in granule sludge

obtained for the solute in water are multiplied by an empirical factor determined in biofilms. Compared with those of water, values of diffusion coefficients found in anaerobic biological matrices at 35 °C were 12%, 12% and 27% for ethanol, acetate and hydrogen, respectively.[97] This is attributed to significant barriers to solute transport inside the aggregates by diffusion. Namely, solutes must move along a longer path in porous medium compared with water.

If a high COD removal rate is desired, then the substrate should be available at suitable concentrations. Therefore, it will have to be replenished faster by forced transport of convective mass transfer, since this rate is often higher than the rate of biofilm diffusion alone. Convective mass transport into the biofilm can be created by intensifying the hydraulic turbulence in the bulk liquid phase at increased V_{up}. Some evidences are reported which indicate that convective transport of substrate into anaerobic biofilms actually occurs.[97] A sixfold increase in the turnover of acetate was achieved in an anaerobic CSTR (continuous-flow stirred-tank reactor or complete-mix reactor) digester under vigorous agitation compared with normal agitation.[29] No significant external or internal mass transfer limitations were observed in the acetate consumption in a well mixed fluidized-bed methanogenic reactor.[130] From the study of Ngian *et al.*,[91] it can be concluded that sufficient agitation is essential in order to eliminate all mass transfer limitations in highly flocculating microbial cultures. High recirculation in a DSFF resulted in a rapid increase of the specific activity of the biofilms. This was inferred by the rapid increase in the maximum loading rate achieved at the same

Table 3. Diffusion coefficients for dilute water solutions.

Solute	Temperature (°C)	Diffusivity $(cm^2/s).10^{-5}$	Reference
acetic acid	12.5	0.82-0.91	(131)
acetic acid	25	1.26	(34)
ethanol	10	0.5-0.83	(131)
ethanol	25	1.24	(34)
ethanol	25	0.84	(17)
ammonia	15	1.77	(131)
ammonia	25	1.64	(17)
CO_2	20	1.77	(131)
CO_2	25	2.00	(34)
methanol	15	1.28	(131)
methanol	25	0.84	(17)
formic acid	25	1.52	(34)
propionic acid	25	1.01	(34)
H_2	25	4.8	(34)
O_2	25	2.41	(34)

COD removal efficiency, compared with that of previous periods, when a reactor was shifted from a recirculation ratio of 4 to 500. Thus, the increased specific activity can be attributed to the effect of recirculation on increasing the substrate transport into the biofilm by convective flow.[20] These experiments suggest that turbulence increases mass transport which cannot be explained by diffusion transport only. Thus, turbulence can overcome diffusion rate limitations by convective transport of fluids into the biofilm and consequently, a good contact between substrate and biomass can be obtained. A good contact signifies that the substrate is available to all populations of syntrophic microorganisms living in multicellular associations. Consequently, the diffusion distance inside the granules will be minimized for interspecies intermediates.[43,112]

Low natural mixing

During the anaerobic treatment of medium and high strength wastewaters, natural turbulence is provided by the high gas production. However, dilute wastewaters result in low gas production per volume of influent due to the low COD concentrations. Thus, during the anaerobic treatment of dilute wastewaters gas production cannot be relied upon to provide adequate mixing. Nevertheless, the reactor hydrodynamics affecting the process efficiency and stability can greatly be influenced by the gas.[121]

Gas bubbles progressively move upwards where the pressure is lower and, consequently, they increase in volume and their buoyancy forces. The increased buoyancy forces cause increased upward movement of the bubbles which displace liquid creating currents and eddies, and thus, turbulent flow is created. The turbulence of the sludge bed is enhanced due to collision of the bubbles with the granules. Thus, the mixing energy transferred by the gas to the bulk liquid phase increases from the bottom to the top of the reactor. This can be demonstrated from experiments conducted in order to verify the effect of gas flow on the sludge bed mixing.[121] At a gas flow velocity (V_{gas}) higher than 0.6 m/h, it was shown that no dead space or short circuiting occurred in the sludge bed. A higher dispersion was achieved resulting in limited plug-flow behaviour of the sludge bed. The dispersion was measured as energy transferred which was comparable with the energy applied for complete mixing in a CSTR (8-10 W/m^3). Under these conditions, 95% of the reactor volume conformed to a completely mixed hydraulic phase.

Low methane production

The expected low energy recovery due to the low biogas production per unit of wastewater would possibly reduce the economic benefits of anaerobic treatment. 70 to 80% of the biogas in general is composed of methane, a useful form of energy as fuel. However according to Henry's law, the solubility of methane for such a biogas composition would result in 65 to 75 mg COD/*l* of dissolved methane at 30°C (H_{CH_4} = 27.2) when in equilibrium. This signifies that dissolved methane can leave the reactor without being collected as biogas. Considering that anaerobic reactors are usually operated under mesophilic conditions, the treatment of low strength wastewaters would loose considerable amounts of possible useful energy. The lower the influent COD concentration, the higher the loss. Figure 2 shows that big losses would occur at low concentrations. The losses would only start to become small (< 10%) at influent COD concentrations higher than 750 mg/*l*.

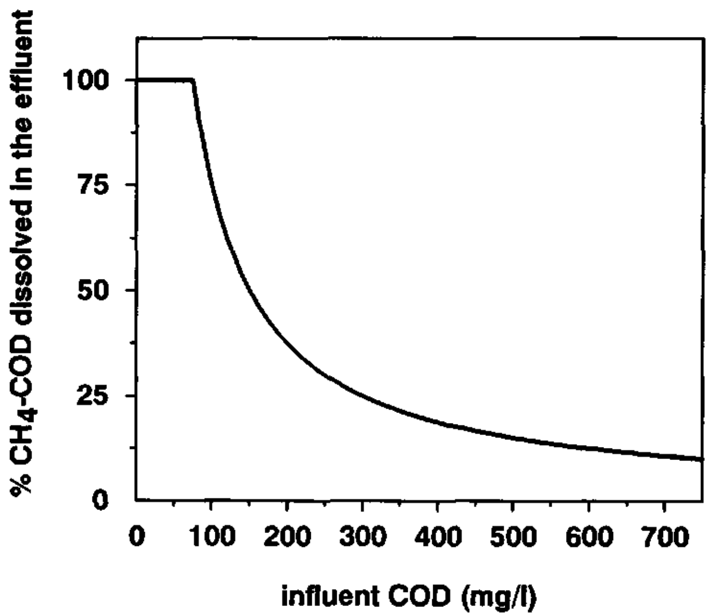

Figure 2. Fraction of methane production which is lost as dissolved in the effluent, as a function of the influent COD.

4.2. The oxygen related problems

The oxygen effect to anaerobes in pure cultures

The anaerobic treatment of dilute wastewaters can face a serious problem due to the possible presence of dissolved oxygen. Oxygen is considered as a suspect toxic compound since several investigations reported a detrimental effect, especially for the methanogens which are usually regarded as strict anaerobes.[45,132] Oxygen is a powerful reagent which generates potentially toxic radicals to all living cells, especially hydrogen peroxide and superoxide.[39,90,100] The toxic effect can damage the chromosomal DNA, as suggested for *Roseburia cecicola* which is a strict anaerobe considered as intolerant to oxygen.[85]

Obligate anaerobic bacteria, contrary to the aerobic and facultative bacteria, can be defined as those microorganisms unable to synthesize a respiratory chain with oxygen as terminal electron acceptor and oxidize organic substrates to carbon dioxide and water. They are considered to strictly live without oxygen.[39] Aerobic and facultative bacteria are regarded as possessing appropriate protective mechanisms against the oxygen radicals. The main hypotheses for the protective mechanisms is the ability to produce two enzymes, superoxide dismutase (SOD) and catalase. SOD seems to be indispensable to all the aerobes,[39,90] despite the claim that a few aerobes lack it.[23] The total lack of SOD has also been suggested as the reason for the oxygen intolerance among the strict obligate anaerobes.[90] Curiously enough, many obligate anaerobes do contain SOD (Table 4) and they can tolerate to some extent low levels of oxygen.[90,107] These findings reveal that the obligates anaerobes differ in their sensitivity to oxygen, varying from those with strict intolerance to others possessing some intrinsic tolerance.

Oxygen tension and redox potential were reported as affecting the sensitivity. The effect of oxygen on 8 different species of anaerobic bacteria was shown to be related to the oxygen tension (0.2 to 3 atm), resulting in different oxygen sensitivity patterns for the microorganisms.[32] From another experiment, microorganisms which did not exhibit growth even on reduced media at oxygen tensions not greater than 0.5% were grouped as strict anaerobes, e.g. *Clostridium haemolycum*; at oxygen tensions greater than 0.5% up to as high as 2 to 8% as moderate anaerobes, e.g. *Bacteroides fragilis*; and those which grew poorly in the absence of oxygen but maximally at intermediate levels of 5 to 10%, were best described as microaerophiles, e.g. *Vibrio sputorum* and *V. fetus*.[79] The exclusion of molecular oxygen together with an environment of very low redox potential are postulated as being essential for the anaerobic microorganisms, especially the methanogens.[39,45,46] However, some experiments showed the separate effects of oxygen and redox potential on anaerobic cultures.[94,127] The continuous anaerobic culture of *Bacteroides fragilis* submitted to different conditions of redox alone revealed that there was no detectable change in the viable cell density. However, the introduction of oxygen to 10-100% of atmospheric saturation during 6 h resulted in a steady decline in the viable cell density. The mechanism of oxygen-mediated inhibition was assumed to be due to the formed superoxide radicals. However, the effect was bacteriostatic rather than bactericidal. The explanation of the

Table 4. Oxygen sensitivity of microorganisms related to the levels of the enzymes superoxide dismutase (SOD) and catalase.

Microorganisms	Enzyme levels (units/mg proteins) SOD	catalase	Period of O_2 tolerance (h)	Reference
Aerobes and facultatives				
Lactobacillus plantarum	49.7	32.2	>72	(107)
Escherichia coli (aerobically grown)	33.4	46.0	>72	(107)
Escherichia coli (anaerobically grown)	15.3	11.0	>72	(107)
Pseudomonas aeruginosa	10.9	112.9	>72	(107)
Micrococcus radiodurans	7.0	289.0	n.r.[a]	(90)
Saccharomyces cerevisiae	3.7	13.5	n.r.	(90)
Mycobacterium sp.	2.9	2.7	n.r.	(90)
Rhizobium japonicum	2.6	0.7	n.r.	(90)
Halobacterium salinarium	2.1	3.4	n.r.	(90)
Pseudomonas sp.	2.0	22.5	n.r.	(90)
Escherichia coli	1.8	6.1	n.r.	(90)
Obligate anaerobes				
Clostridium perfringens (clinical)	1.4	0	>72	(107)
Clostridium perfringens	0.4	0	>72	(107)
Bacteriodes fragilis (clinical)	6.8	7.1	48	(107)
Bifidobacterium adolescentis	0.3	0	48	(107)
Bacteriodes vulgatus	12.5	0	8	(107)
Propionibacterium acnes (clinical)	0.9	103.2	8	(107)
Propionibacterium acnes	0.3	136.2	8	(107)
Bacteriodes fragilis	7.0	15.2	4	(107)
Bacteriodes melaninogenicus	0	0	2	(107)
Eubacterium lentum	3.6	0	1	(107)
Fusobacterium nucleatum	0	0	1	(107)
Clostridium aminovalericum	0	0	0.75	(107)
Peptostreptococcus anaerobius	0	0	0.75	(107)
Butyribacterium rettgeri	1.6	0	n.r.	(90)
Streptococcus lactis	1.4	0	n.r.	(90)
Streptococcus faecalis	0.8	0	n.r.	(90)
Zymobacterium oroticum	0.6	0	n.r.	(90)
Streptococcus mutans	0.5	0	n.r.	(90)
Streptococcus bavis	0.3	0	n.r.	(90)
Streptococcus mitis	0.2	0	n.r.	(90)
Veillonella alcalescens	0	0	n.r.	(90)
Clostridium pasteurianum, sticklandii, lentoputrescens, cellobioparum, barkeri	0	0	n.r.	(90)
Clostridium acetobutylicum	0	n.r.	n.r.	(90)
Clostridium sp. (strain M. C.)	0	0	n.r.	(90)
Butyrivibrio fibrisolvens	0	0.1	n.r.	(90)

[a] : n.r. = not reported.

bacteriostatic effect was that strains of *B. fragilis* contained significant concentrations of superoxide dismutase, as parallel investigations showed.[95]

The most noteworthy group of obligate anaerobic bacteria for the wastewater treatment processes are the methanogens. Since the works for the isolation of pure cultures of *Methanosarcina barkeri* and *Methanobacterium formicicum*, the widespread view is that the methanogens are fastidious microorganisms requiring strict anaerobic conditions for growth and methane production. Afterwards, the isolation of *Methanobacterium ruminantium* added significantly to this belief due to the Hungate-technique which is still employed today with some modifications.[141] Possible traces of oxygen were eliminated by the addition of reducing agents such as cysteine or sulphide, to poise the redox potential within the low range.[46] This is exemplified by *Methanococcus voltae* and *Mc. vannielii* which are considered highly sensitive to oxygen due to their lack of SOD.[58] Also, two thermophilic methanogens isolated from a 55°C anaerobic kelp digester were shown to immediately cease growth and methane production, after being exposed to only 0.1% oxygen in the head space.[24] *Methanobacterium ruminantium*, *M. mobile* and *Methanobacterium* strain AZ were found to be highly sensitivity to oxygen since their growth and methane production were completely prevented at 0.01 ppm dissolved oxygen.[142] However, it was also shown that the sensitivity of methanogens to oxygen does not necessarily mean that the effect is bactericidal. The resistance to oxygen contact is demonstrated in the case of *Methanobacterium* strain AZ. The presence of 7 ppm dissolved oxygen during four days did not result in the die-off, after the removal of all oxygen and restoration of the reducing conditions.[142]

In fact, further investigations also demonstrated that at least some methanogens do have intrinsic tolerance to oxygen. Only a slow decline was observed in the methane production of *Methanobacterium* strain M.o.H. when exposed to oxygen.[106] Also, the oxygen tolerance was observed in several bacteria belonging to the order *Methanomicrobiales* and *Methanobacterium bryantii*. This was attributed to the SOD, which was detected and correlated to the defense against oxygen toxicity.[58,59]

Ecosystems such as sludge digesters can be periodically subjected to stress by accidental entrance of air. This fact has been used as an argument in favour of the development of oxygen tolerant methanogens isolated in pure or enriched cultures from such ecosystems.[47,58] Examples are *Methanobacterium thermoautotrophicum*, *Methanobrevibacter arboriphilus* and *Methanosarcina barkeri* which showed ability to survive for hours in the presence of air without decrease in the number of colony forming units. These strains were originally isolated from sludge digesters. *Methanobacterium* strain AZ was also isolated in pure culture from sewage digested sludge.[142] In contrast, *Methanococcus voltae* and *Mc. vannielii*, which were killed without any lag phase upon contact with air, were originally isolated from sea and lake sediments where they were not exposed to oxygen. *Methanothrix soehngenii* could be enriched not only from several sludge digesters but also from aerobic samples of pretreated raw sewage. No lysed cell and similar rates of methane production compared with the controls were observed in experiments with pure oxygen, up to 48 h exposure.[47] *Methanobacterium* and *Methanosarcina* are also commonly isolated from dry and oxic paddy soil demonstrating that they can survive under aerobic conditions in-between the flooding periods. That methanogenesis starts within a few days after the flooding was demonstrated in research with the isolated pure cultures of *Methanosarcina barkeri* strain Fusaro, *Methanosarcina* strain MVF4 and *Methanobacterium* strain HVF5.[27]

It was also suggested that besides the intrinsic tolerance due to the SOD that some methanogenic species possess, other factors could be responsible for protection against the oxygen. *Methanosarcina barkeri* strain Fusaro was shown to have a number of redox carriers decreasing the redox potential when chemical oxidant agents were used. However, this reducing capacity was not enough to avoid the inhibition by oxygen at concentrations higher than 0.5% in the gas phase. Nonetheless, the capacity to adjust the redox potential in its own redox environment to a certain extent could also be one of the reasons to explain the good survival in dry and oxic soil.[26] The existence of cells in aggregates can also affect the oxygen tolerance. This seems to be the case for *Methanosarcina* that showed higher oxygen tolerance in cell aggregates compared with dispersed cells. The arrangement in cell aggregates is postulated to provide protection of the cells against oxygen, since individual cells showed higher sensitivity to oxygen.[58] *Methanosarcina mazei* S-6 is normally regarded to grow as large aggregates, allowing the sedimentation and permanence in a completely oxygen free environment of natural sediments and in anaerobic reactors.[138]

The oxygen toxicity to anaerobes in mixed cultures

In the case of natural mixed cultures of anaerobic sludges or sediments, oxygen can incidentally come into contact with methanogens. Oxygen was detected in the gas of a number of anaerobic digesters.[114] By measurements *in situ*, oxygen was found even in the rumen liquor and gas evolved from cattle, sheep and goats, and it is known that rumen microorganisms in pure cultures are killed by traces of oxygen.[113] Addition of oxygen at increased pressure in a sample of bovine rumen liquor showed that in fact, methane production decreased. However, some minutes later after each addition of oxygen methane producing activity was reversible although not totally. Dissolved oxygen could not be detected in the samples exposed to oxygen. This was attributed to the presence of facultative bacteria in the rumen. The facultative bacteria can rapidly consume oxygen and create anaerobic microenvironments and thus explain the survival of the methanogens in the bulk liquid phase.[113,114] Due to the role of the facultative bacteria, it is expected that each unit of dissolved oxygen introduced into the anaerobic mixed culture would be reflected in a unit of substrate oxidized. Since the oxygen dissolved in liquid is very low and the available substrate is relatively high, it would be unusual to detect oxygen in the gas phase of digesters, as reported by Scott *et al.*[114]

Natural mixed cultures represent in fact a complex consortium of microorganisms, where the consumption of oxygen in a single reactor can very likely occur due to the presence of facultative bacteria in natural anaerobic sludges. Regardless of the intrinsic tolerance that has been reported in some strict obligate anaerobes like the methanogens, the aerobic or facultative bacteria are probably the most important factor for the protection of methanogens in anaerobic sludges against O_2 exposure. In the presence of aerobic or facultative substrates, the oxygen can undoubtly be rapidly consumed. This has been demonstrated during ethanol production by a coimmobilized mixed culture of the aerobic fungus *Aspergillus awamori* and the anaerobic bacterium *Zymomonas mobilis*, under highly aerobic conditions.[120] Defined mixed cultures of an obligate aerobic *Pseudomonas testosteroni* and an anaerobic *Veillonella alcalescens* strains also grown in a microaerophilic chemostat, were shown to coexist and compete for common substrates.[37] The co-cultivation of the facultative anaerobes *Vibrio* sp. and the strictly anaerobic sulphate-reducing bacteria *Desulfovibrio HL21* was demonstrated in glucose chemostats under oxygen-limiting conditions.[38]

Marine sediments are generally reducing environments covered only by thin highly oxidized surface layers.[3,53,54] The presence of the obligate anaerobe *Desulfovibrio* spp. and the sulphide oxidizing bacteria *Beggiatoa* spp were found near the surface layers. The occurrence of pyrite (FeS_2) in the oxidized layer can only be explained on the one hand by the rapid oxidation of Fe^{+2}, and on the other hand by the formation of H_2S from the sulphate within reducing environments.[53,54]

Oxygen limitation within natural biofilms may also occur due to the biofilm itself. The biofilm thickness and architecture represent a physical barrier for the oxygen diffusion, enhancing the creation of segregated zones of lowered oxygen concentrations towards the centre. Since very complex systems can exist in separate layers or microniches, growth and competition occur between different groups of microorganisms. In this case, the oxygen limitation within biofilms depends on the available substrate and oxygen concentration in the bulk liquid phase. Nitrification and denitrification are examples of aerobic and anaerobic layer formation, respectively. Several investigators have used microsensors to determine the oxygen profiles within the biofilms, usually resulting in gradients from the oxic outlayers to the anoxic layers in the centre.[4,42,98,103,104,133,135] A general finding is that oxygen rarely penetrates more than few hundreds μm due to the oxygen uptake and diffusion limitation. Consequently, anaerobic microorganisms located inside the biofilms would be adequately protected against contact with oxygen, allowing the occurrence of denitrification, sulphate reduction or methanogenesis.[134] The occurrence of microniches of strict anaerobes in aerobic environments is best illustrated by aerobic sludge samples or by the well aerated flocs in activated sludge. The fact that methanogens are present in aerobic sludge was demonstrated when *Methanothrix soehngenii* could be enriched from such samples.[47] Activated sludge was used as seed for the start-up of two UASB reactors.[137] Very interesting was the granule formation in the UASB reactors. *Methanobacterium*, *Methanococcus* and *Methanosarcina* were observed in both the original aerobic activated sludge flocs and the granular sludges formed. According to fluorescent microscopic examination, several anaerobic nuclei may have existed deep inside the flocs. Granulation also occurred in a UASB reactor seeded with raw wastes from an activated sludge tank.[93] The start-up of an anaerobic filter with activated sludge has also been described.[11]

The practical application of co-cultures of anaerobic and facultative bacteria

The occurrence of methanogenesis in oxygen-limiting chemostats was also demonstrated in co-cultures. The combined activity of facultative and methanogens competing for the same substrate was evidenced by the simultaneous oxygen consumption and methane production. A gradual increase of oxygen supply did not significantly interfere on methanogenesis. Very interesting was that at low oxygen supply, the methane production was stimulated by 20%.[37] The increased methanogenesis in the presence of traces of oxygen was also reported for a reactor fed with domestic sewage or algal biomass, and containing sludges from anaerobic reactors.[102,114] This was explained by the possible stimulation of acetate production for the methanogens by the facultative bacteria. Further experiments with several mixed cultures of methanogenic and aerobic bacteria clearly indicated that they can share the same habitat under oxygen limited conditions. Co-cultures of *Methanobacterium formicicum* or *Methanosarcina barkeri* with the aerobic heterotrophic *Comamonas testosteroni* were evident in continuous chemostats fed with small amounts of oxygen.[36]

From these previous studies, it turns out that the coexistence of anaerobic and aerobic or facultative bacteria in a single reactor competing for the same substrates can be regulated through a balanced oxygen supply. Separate anaerobic and aerobic phases have commonly been used for removal of pollutants. However, new possibilities are the use of a single phase anaerobic-aerobic wastewater treatment system. In practice, these possibilities may include the polishing off the residual biochemical oxygen demand (BOD) and the biodegradation of polychlorinated hydrocarbons and adsorbable organic halogens (AOX).

The aerobic bacteria are usually used in a post-treatment step to polish off the residual BOD after the anaerobic treatment of wastewaters.[69] Since the occurrence of methanogenesis has been demonstrated under oxygen-limited conditions,[36,37,102,114] the residual BOD can be eliminated in the same reactor where anaerobic treatment is occurring. Also in some cases, the residual BOD is caused by toxic micropollutants which are not substrates for anaerobic bacteria. Examples are resin acids in the forest industry wastewaters.[28,74,115,116] Thus, the addition of small amounts of oxygen into an anaerobic reactor can eliminate them, too. Polychlorinated hydrocarbons are not known to be degraded by aerobic bacteria, or mineralized by anaerobic bacteria. These pollutants have only been eliminated in sequenced anaerobic-aerobic reactors, since reductive dechlorination is first necessary to yield hydrocarbons, which are then mineralized under aerobic conditions.[22] The use of sequenced anaerobic-aerobic reactors was also shown to be more efficient to remove AOX from chorolignins in bleachery wastewaters, compared with the use of aerobic or anaerobic treatment alone.[52] Also, the application of simultaneous anaerobic and aerobic degradation was demonstrated for the elimination of recalcitrant pollutants under limited oxygen conditions. Many recalcitrant pollutants cannot be fully mineralized under aerobic or anaerobic conditions, but require the sequenced activity of anaerobic and aerobic bacteria. Immobilized co-cultures of *Enterobacter cloacae* and *Alcaligenes* sp. were able to degrade 1,1,1-trichloro-2,2-bis-(4-chlorophenyl)-ethane (DDT) or 4-chloro-2-nitrophenol (CNP).[6,7] Mineralization of the herbicide 2,3,6-trichlorobenzoic acid was also demonstrated in a single microaerophilic chemostat. An anaerobic enrichment resulted due to the aerobic activity of *Pseudomonas aeruginosa* JB2 consuming oxygen.[35] These previous possibilities may, therefore, represent promising applications for the environmental technology. A biofilm type worth considering to be used in such single reactors could be the granular sludge. Anaerobic microenvironments would be better protected and maintained in granular sludge because oxygen would hardly penetrate deep into the biofilm, contrary to dispersed sludge. A model for the creation of anaerobic zones in granular sludges inside an aerated reactor is shown in Figure 3.

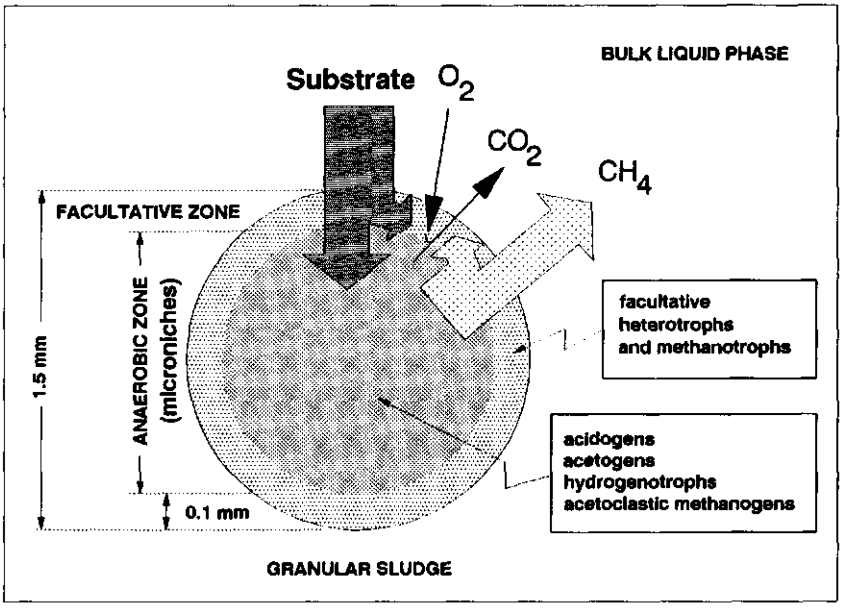

Figure 3. Model for the degradation of simple substrates by co-cultures of anaerobic and facultative bacteria in granular sludge inside an aerated reactor.

5. UASB and EGSB Reactor Related Problems

5.1. The problematic of granular sludge bed reactors

The proper design of a granular sludge reactor should fulfill the requirement of adequate wastewater-biomass contact, while at the same time guarantee the retention of the biomass in the reactor. In the EGSB reactor, high hydraulic loads are applied to create turbulence which enhances the wastewater-biomass contact in two ways. Firstly, by expanding the sludge bed which allows for the even distribution of the wastewater by preventing dead zones and short circuiting. Secondly, the turbulence enables convective transport of substrates from the bulk liquid phase into the biofilm; thereby increasing the total rate of substrate transport beyond that of diffusion alone. On the other hand if the hydraulic load is excessive, poor retention of active biomass will result. Care should be taken to prevent washout of granules due to the expansion of the sludge bed into the settling compartment of the GSL. Likewise if the turbulence is too severe, shear forces will cause erosion of the granules and subsequent washout of the fines. The hydraulic loading regime that is finally chosen must be carefully selected to compromise between maximizing mixing intensity and minimizing sludge washout.

The problem of excessive mixing

Excessive mixing intensity of the bulk liquid phase can result in a serious problem for the biomass retention due to the resulting sludge washout and sludge flotation. Sludge washout is hydraulically assisted and concerned with expansion of the sludge bed. Sludge flotation is gas assisted due to buoyancy forces.

The sludge washout has to be minimized during the treatment of low strength wastewaters. Intact granules are dense and large with settling velocities as high as 100 m/h,[43] allowing their retention inside reactor under a high hydraulic turbulence. However, the retention of dispersed and floccule sludge under a similar hydraulic regime, as that applied to intact granules, is obviously more difficult.

Hydraulically assisted sludge washout occurs due to excessive expansion of the sludge when increased V_{up} is applied. Increased V_{up} is obtained by applying effluent recirculation or designing tall reactors. Recirculation ratios (effluent recirculation flow/influent flow) can be as high as 20 or higher in order to maintain V_{up} values of 5-6 m/h or higher.[82]

The sludges with poor settleability will be the first fraction to be washed out when expansion of the bed starts to be excessive. However, a strong washout of high settleable intact granules can also result when too much expansion of the sludge bed occurs, since the sludge is forced out of the reactor.

The washout of granules can also occur under high mixing intensity due to the different sizes of particles in a granule sludge bed. The size of granule sludge is a result of the dynamic equilibrium between the biomass detachment by fluid shear forces and bacterial growth. High mixing intensity increases the shear forces that causes erosion of granules resulting in fines. The granules of lower mechanical strength can also break into pieces as a result of the shear forces. Restricted growth of biomass on the outer layers of biofilms, which occurs during diffusion limitation, can result in a hollow core due to bacterial decay, leading ultimately to a decline in granule strength.[1] Thus, eroded fines and chunks of broken granules can be washed out even in a hydraulic regime intended for intact granules.

The problem of sludge retention can be aggravated by the gas production. However, the role of the biogas on the expansion of the bed and mixing intensity is still not well understood. UASB reactors have been reported to be very dependent on the gas production.[75] Since such reactors normally operate at V_{up} below 1.5 m/h where expansion of the bed hardly occurs,[82] it can be inferred that the biogas production is very important for the natural mixing intensity of the bulk liquid phase. Thus, it seems that adequate expansion and mixing is guaranteed in the UASB, as long as medium and high strength wastewaters are applied at OLRs providing high gas productivity. However, the expansion of the bed due to liquid and gas has been regarded as a curious phenomenon. While an increase of the liquid velocity always causes an increased expansion, an increase of the gas velocity can result in a compaction of the bed expansion, as long as the gas is not attached in any way to the biomass.[75,96] Nevertheless, gas does tend to become attached to the biofilm when it is produced from within the granules. Thus, the most important effect is perhaps due to buoyancy forces of gas bubbles adhered inside or attached to granules causing sludge flotation. An attached gas bubble can cause the flotation of a granule up to 14 times its volume.[10] Therefore, the resulting buoyancy forces can drive the granules upwards, which is frequently the case when high amounts of biogas are produced.

Three causes of sludge flotation can be identified: (i) gas entrapped inside granules; (ii) gas adhered to granules; and (iii) gas entrapment under filamentous material. The first type of sludge flotation occurs due to gas entrapped inside thick highly active granules without sufficient release of the bubbles into the bulk liquid phase. In this case, the biofilm structure provides a high resistance to the gas flow. This problem can be solved by lowering the OLR to produce less biogas, and by increasing the hydraulic turbulence to provide higher shear forces in order to create smaller granules. The second type of flotation is due to small bubbles bound to the surface of the granules. This occurs when the granule surfaces are apolar as in the case of adsorbed fats, or when very fine bubbles are produced providing high surface areas for adsorptive interaction with the granules. This scenario is worsened in tall reactors since smaller bubbles will be formed under the high hydrostatic pressure in the deep portions of the reactor. Solutions to solve this problem include the use of shallow reactors and to ensure that fatty substances are fully degraded by underloading the reactor. Also, the use of a vibrator could ease the desorption of the bubbles and improve the natural separation due to the impact of colliding aggregates. The third type of flotation is due to the existence of fibres or filamentous bacteria in the sludge bed causing gas entrapment in a fibrous network holding granules. The entrapped gas bubbles cause the granules connected to the fibrous material to float. A pre-separation of fibres before the wastewater enters into the reactor, or wastewater pre-acidification in order to avoid the filamentous acidifying bacteria can be solutions for this problem.

The problem of poor mixing

A poor expansion of the bed results in sludges settling to the bottom of the reactor forming a static bed and causing non-ideal flow patterns such as dead zones, channelling and gas pockets. The occurrence of dead zones means that the active granules in certain portions of the reactor volume are not being fully utilized for substrate conversion. Channelling in the sludge bed signifies preferential flow of substrate leaving the reactor without adequate contact with biomass. The gas hold-up due to the accumulation in dense unexpanded sludge bed causes the formation of large gas pockets in the sludge bed. When the buoyancy of the accumulated gas is high enough the sludge bed can move upwards in a piston or the gas pockets can explode. Significant bypass occurs in reactors with low biogas production due to the periodical eruption of very large gas bubbles in the sludge bed.[121] Granules can be dragged up in the wake of the bubbles. Additionally, in dense beds there is the possibility of localized pH drops due to the accumulation of volatile fatty acids and poor distribution of bicarbonate alkalinity.

5.2. The technological solutions to counteract the problems

In order to minimize the problems of sludge retention such as washout and flotation, and poor wastewater-biomass contact, full-scale granule sludge reactors can be designed and operated with accessible technology. The main technological tools available for controlling the retention of biomass and contact are concerned with the effluent recirculation, the inlet distribution system, the GSL devices and the carrier support material (or lack of carrier) for the attachment of biomass.

Effluent recirculation

Since an optimum mixing intensity of the bulk liquid phase should be achieved, reactor operation will be dictated by a suitable V_{up}. Effluent recirculation is used to provide an increased V_{up}, adequate for the expansion of the sludge bed and turbulence. When the V_{up} increases, the bed is expanded and the granules are suspended. In this way, the distance between the granules increases as the expansion increases, resulting in a more homogeneous bed.[49] The bed does not have to be fully expanded like that of a FB reactor. Instead, a partial expansion which has less danger of causing sludge washout, may very well provide good wastewater-biomass contact. Adequate axial or vertical dispersion is obtained when turbulent flow is achieved at high hydraulic velocities.

The effect of increased V_{up} on the expansion of the sludge bed can be well illustrated by comparing the UASB and the EGSB reactors. In the case of a UASB reactor, it has been shown that the expansion of the bed does not significantly occur at V_{up} below 1.5 m/h. In this case, the basic mixing pattern determined by using lithium as a tracer was very similar to that prevailing in partially mixed reactors.[82] However, at values exceeding 1.5 m/h the sludge bed gradually expands and the mixing patterns approximated complete mixing conditions. This latter situation is that prevailing in EGSB reactors. V_{up} values ranging from 5 to 10 m/h have been applied in lab-scale EGSB reactors.[82,105] A full-scale EGSB reactor was designed to operate at a V_{up} of 8 to 10.5 m/h for the treatment of baker yeast and brewery wastewaters.[124] Increased V_{up} can also be applied by using tall and thin reactors with height-diameter ratios as high as 20.[82] A height of 6 m for full-scale UASB reactors has been recommended, although a reactor as high as 10 m has been built.[68]

Since high V_{up} can be applied resulting in high shear forces, it is important that granules have high settleability and mechanical strength. Values of sludge volume index (SVI) as low as 12 ml/g and settling velocities as high as 60 m/h have been reported for granules with diameters higher than 2 mm. The mechanical strength measurements showed that disintegration only occurred at high pressures ranging from $0.26 \cdot 10^{-5}$ to $1.51 \cdot 10^{-5}$ N/m^2. Typical size distribution of sludge granules is in the range between 0.2 to 7 mm.[43]

Influent distribution system

An even distribution system of the influent is required for a proper reactor operation since problems such as channelling and non-homogeneous distribution of wastewater should be prevented. In general, the inlet system is designed to have an adequate number of inlet lines and nozzles based on the applied OLR. High strength wastewaters can be fed into reactor at high OLR resulting in high gas production. In this case for UASB reactors, one distribution point for each 7 to 10 m^2 can suffice.[117] In the case of low strength wastewaters resulting in low OLRs, one distribution point should be used for each 1 to 2 m^2.[68,78,117] A more sophisticated inlet system, like that employed in FB reactors, may be necessary for the EGSB reactor since higher V_{up} is required.[68] Possible clogging of the nozzles can be avoided by applying intermittent influent supply to only some pipes, to increase the jet velocity.[68] The control of clogging at individual distribution points in UASB reactors can be done when the influent feeding is applied by gravitational flow from above the reactor surface. Regulated weirs located in channels can be used to control the flow rate to each distribution point and allow the visual control of clogging.[117]

Gas-solid-liquid separator

In order to maximize the retention of sludge under operational conditions, granule sludge reactors have to be equipped with a proper GSL device. The objective is to separate the gas from the bulk liquid phase and create a quiescent zone for sludge settling. However, specific measures to prevent sludge washout are perhaps necessary due to the high mixing intensity which can prevail in the reactor.

Incidental washout of intact granules can be prevented by improving the conventional GSL device utilized earlier in UASB reactors.[78] Some innovations are already applied in full-scale UASB reactors.[69] The installation of additional plates to force a downward flow in the bottom part of the settler is claimed to favour the return of sludge. Instead of one, three gas collectors placed above each other are claimed to increase the efficiency in collecting gas. A labyrinthic settler with various compartments was also proposed for UASB reactors.[86] Multiple laminar plates in the settler have been utilized in the design of full-scale EGSB reactors.[123]

The retention of fines originating from granule erosion can be achieved by using sieve-drums or sophisticated microscreens.[69,105,140] The sieve-drums were installed in lab-scale EGSB reactors operating at V_{up} up to 6.5 m/h. A concentrical screen column was installed in a UASB (30 l) with a brusher placed in the centre to prevent clogging of the screen, with the objective to avoid sludge washout at high recirculation ratios up to 35. Such measures however, may represent excessive additional costs in full-scale reactors.

Specific measures for preventing sludge flotation can be the use of a vibrator for the separation of gas bubbles from granules. Such a vibrator can also represent significant extra cost and require supplementary equipments. Granules reaching the reactor effluent weir by flotation can be retained by the installation of additional baffles. This measure has been applied in practice and does not represent excessive additional costs.[19,69]

Alternatives to granular sludge for biofilm attachment

Instead of granule sludge reactors, systems with biofilms fixed on inert stationary carriers but under high liquid down flow (V_{down}) could also be considered. These systems would imitate a natural occurrence of slime attached to stones existing in polluted rivers with rapid streams, where organic matter concentration can be comparable with low strength wastewaters. The application of this natural phenomenon can best be illustrated by the DSFF. A higher V_{down}, resulting from recirculation ratio up to 500, considerably enhanced the mixing intensity and the reactor treatment performance.[20] Needle punched polyester was used as the support material and COD removal efficiencies up to 88% were obtained at OLRs up to 17.3 g COD/*l*.d.

The immobilization of biomass conforming to the hydraulic regime as that prevailing in the EGSB reactor can also be a possibility. Instead of using granular sludge from existing UASB reactors for the start-up of EGSB, the natural biomass arising from the high hydrodynamic conditions is worth considering. This can be inferred from previous experiments in expanded bed (beet sugar wastewater) and FB (pre-settled domestic sewage) reactors, when bacterial aggregates developed that attached to inert carrier materials, under similar operational regimes as those in EGSB systems.[51,61] Granules of 2 to 3 mm were formed at V_{up} of 3.6 m/h during the treatment of acidified beet sugar wastewater. Though started-up with inert carrier particles of polyvinylchloride plastic (PVC), biomass formation gave rise to a rapid accumulation of both attached biomass and free granules at high OLRs. The relative amounts of each type of biomass observed in the sludge bed were 60% attached to PVC and 40% present as granules.[51]

6. Scope of this Dissertation

The scope of this thesis is to investigate the feasibility of anaerobic treatment of low strength soluble industrial wastewaters. The applicability of UASB or EGSB reactors for the treatment of such wastewaters is evaluated. This study specifically focussed on the wastewater related problems. The effect of dissolved oxygen present in dilute influent on methanogenesis and the effect of low substrate concentrations on reactor performance were studied.

Chapter 2 quantifies the effects of oxygen exposure on methanogenic activity and determines the main mechanisms of oxygen tolerance in several anaerobic granular sludges.

Chapter 3 explores the role of oxygen in the competition for substrates between methanogenic and facultative bacteria in anaerobic granular sludges under highly aerobic conditions.

Chapters 4 and 5 describe the investigations on determining the lowest influent COD concentrations which are feasible for the anaerobic treatment of dilute soluble wastewaters. The feasibility of UASB and EGSB reactors for the treatment of such wastewaters was studied.

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Chapter 2

High Tolerance of Methanogens in Granular Sludge to Oxygen

Mario T. Kato, Jim. A. Field and Gatze Lettinga

Summary

This research assessed the effect of oxygen exposure on the methanogenic activity of anaerobic granular sludges. The toxicity of oxygen to acetoclastic methanogens in five different anaerobic granular sludges was determined in serum flasks with effective gas-to-liquid volumes of 4.65 to 1. The amount of oxygen that caused 50% inhibition of the methanogenic activity after 3 days of exposure ranged from 7% to 41% oxygen in the head space. These results indicate that methanogens located in granular sludge have a high tolerance for oxygen. The most important factor contributing to the tolerance was the oxygen consumption by facultative bacteria metabolizing biodegradable substrates. Uptake of oxygen by these bacteria creates anaerobic microenvironments where the methanogenic bacteria are protected. The results also indicate that methanogens in sludge consortia still have some tolerance to oxygen even in the absence of facultative substrate for oxygen respiration.

Key words: oxygen toxicity · anaerobic biofilm · facultative and methanogenic bacteria wastewater treatment.

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1. Introduction

Oxygen is considered as a potential toxic compound during anaerobic treatment, especially for the end-of-food-chain microorganisms, the acetogens and principally the methanogens, which are usually regarded as strict anaerobes.[8,20] However, some previous studies on the oxygen tolerance of pure cultures have demonstrated that anaerobic bacteria differ in their ability to withstand exposure to oxygen.[10,13,14,17,21] Among microorganisms classified as strict anaerobes some even survive exposure periods up to 2 h with up to 0.5% oxygen in the head space.[10,17] One of the main hypotheses formulated for the intolerant anaerobes is that they do not possess superoxide dismutase. This oxygen defense enzyme of the aerobes neutralizes toxic oxygen radicals.[11,15,16]

In practice, when natural mixed cultures of methanogenic granular sludge are exposed to air for short periods of time, reactor upset has seldom been reported.[1,3] Perhaps this may be due to the rapid utilization of oxygen by facultative bacteria. Wastewaters contain a variety of substrates which the facultative bacteria can metabolize at the expense of oxygen respiration. The methanogens located deeper in the biofilm might be protected in this way from coming into contact with dissolved oxygen. Consequently, the facultative bacteria and the biofilm thickness can probably overshadow the oxygen effects in practice.

Few experiments have been conducted to assess the effects of oxygen on the natural mixed cultures in anaerobic reactors. The objectives of this study were to quantify the effects of oxygen exposure on methanogenic activity and determine mechanisms of oxygen tolerance in anaerobic sludges. Particularly, the role of facultative bacteria and the biofilm thickness on the oxygen tolerance were evaluated. The biological system chosen as a model was granular biofilms from anaerobic reactors treating domestic and industrial wastewaters.

2. Materials and Methods

2.1 Granular sludges

Five granular sludges were used in the oxygen toxicity experiments. One sludge was obtained from a pilot-plant expanded granular sludge bed (EGSB) reactor treating domestic sewage and the others from full-scale upflow anaerobic sludge blanket (UASB) reactors treating industrial effluents. The source and the period of previous storage (at 4°C) of each of the sludges are given in Table 1.

The granular sludges were elutriated to remove the fines and all the samples were again stored at 4°C until used. Nedalco sludge was also subjected to various treatments. When crushed, a hypodermic syringe was used for passing the granules through a needle of 0.51 mm diameter (gauge 21).

Table 1. Granular sludges used in oxygen toxicity experiments.

Sludge	Wastewater	Reactor	Storage (months)
Bennekom	Domestic sewage	EGSB 120 *l*	5
Nedalco	Alcohol distillery	UASB 700 m^3	1
Roermond	Paper	UASB 740 m^3	8
Latenstein	Wheat starch	UASB 450 m^3	10
Eerbeek	Recycled paper	UASB 2184 m^3	8

2.2. Basal medium

The inorganic macro- and micronutrients were prepared as a fivefold concentrated solution. After dilution, the basal medium used in the assays contained (mg/*l*): $NaHCO_3$ (5880), NH_4Cl (280), $K_2HPO_4 \cdot 3H_2O$ (327.4), $MgSO_4 \cdot 7H_2O$ (100), $CaCl_2 \cdot 2H_2O$ (10), yeast extract (100), H_3BO_3 (0.05), $FeCl_2 \cdot 4H_20$ (2), $ZnCl_2$ (0.05), $MnCl_2 \cdot 4H_2O$ (0.05), $(NH_4)6Mo_7O_{24} \cdot 4H_2O$ (0.05), $AlCl_3 \cdot 6H_2O$ (0.09), $CoCl_2 \cdot 6H_2O$ (2), $NiCl_2 \cdot 6H_2O$ (0.05), $CuCl_2 \cdot 2H_2O$ (0.03), $NaSeO_3 \cdot 5H_2O$ (0.1), EDTA (1), resazurin (0.2), and 36% HCl (0.001 m*l*/*l*).

2.3. Analyses

Ethanol and volatile fatty acids (VFA) were determined with an HP 5890 gas chromatograph (Palo Alto, CA). The 2 m × 2 mm glass column was packed with Supelcoport (100- to 200-mesh) coated with 10% Fluorad FC 431. The temperature of the column was 70° and 130°C, for ethanol and VFA, respectively. The temperatures of the injection port and flame ionization detector were 220° and 240°C, respectively. Nitrogen saturated with formic acid was used as carrier gas at a flow of 40 m*l*/min. Before use, the chromatograph was calibrated with standard solutions of ethanol or VFA.

The methane and oxygen concentrations in the head space of the serum flasks were determined by gas chromatography. For methane a 200-μl gas sample was injected in a Packard-Becker 438/S chromatograph (Delft, The Netherlands). The 2 m × 2 mm steel column was packed with Poropak Q (80- to 100-mesh). The temperatures of the column, injection port, and flame ionization detector were 60°, 200° and 220°C, respectively. Nitrogen was used as carrier gas at a flow rate of 20 m*l*/min. Oxygen was analyzed with a 500-μl gas sample in an HP 5890 chromatograph, equipped with a 1.5 m × 2 mm steel column packed with molecular sieve 5A (60- to 80-mesh). The temperatures of the column, injection port, and thermal conductivity detector were 40°, 110°, and 125°C, respectively. Argon was used as carrier gas at a flow of 20 m*l*/min. All gas sample analyses were conducted after calibration with standards of known amounts of the respective gases and always with the use of a Dynatech A-2 pressure-locked syringe (Baton Rouge, LA).

The pH was determined immediately after sampling with a Knick 510 pH/mV-meter (Berlin, Germany) and a Schott Nederland N61 double electrode (Tiel, The Netherlands). Volatile suspended solids (VSS) were determined according to the *Standard Methods*.[19] Wet sludge refers to solids before drying in oven at 100° to 103°C. Sulphur compounds of sludges were determined according to the method described by Novozamsky et al.[12]

The concentrations of ethanol and VFA are referred to in chemical oxygen demand (COD) units, commonly employed in the field of wastewater treatment. Also methane production is expressed as its equivalent in COD per liter of liquid. Conversion factors utilized were 2.087 g COD/g ethanol, 1.067 g COD/g acetate, and 2.577 g COD/*l* CH_4 at 30°C.

2.4. Assays

Sedimentation assay

The sedimentation assay was conducted to determine the mean granule diameter of the sludge samples. The assay used for the particle size distribution was described by Hulshoff Pol et al.[6] The method is based on relating sedimentation velocities to the size and density of the granules. For each granular sludge the assay was performed in triplicate.

Methanogenic toxicity assay

The methanogenic toxicity assay was conducted in triplicate during two distinct periods and under shaken conditions. Serum flasks closed with butyl rubber stoppers and screw caps were used, with effective head space and liquid volumes of 465 and 100 m*l*, respectively. The first period consisted of exposing the sludge to oxygen during 3 days with ethanol (unless otherwise stated) as substrate. The second period consisted of measuring the residual acetoclastic methanogenic activity of the sludge in anaerobic conditions.

In the first period, sludges were added to the flasks and completed to 100 m*l* with the basal medium solution containing 1000 mg COD/*l* of ethanol. The final amounts of sludge ranged from 1.55 to 2.16 g VSS/*l*. A N_2/CO_2 gas mixture (70/30) was used to flush the flasks and the excess was removed to maintain atmospheric pressure. Two needles were inserted in the stoppers, one connected to the gas cylinder and another to air. At the end of the flushing the second needle was connected to a flask containing water. Known amounts of oxygen were supplied to provide a concentration range from 0% to 90% in the head space, with the objective to obtain inhibition of methane production up to 100%. Subsequently, the flasks were incubated during 3 days in a temperature-controlled room at 30 $\pm$ 2°C. Monitoring consisted of periodic measurements of methane production and oxygen consumption.

In the second period, all serum flasks were provided with fresh sodium acetate (1000 mg COD/*l*) supplemented with basal mineral medium. N_2 was flushed to maintain an anaerobic atmosphere while exchanging the media. The final anaerobic atmosphere and pH buffer were prepared by flushing the head space with the N_2/CO_2 gas mixture. All the flasks were then again incubated at 30 ± 2 °C. Thereafter, methane production was periodically measured. At the end of the assay the residual substrate and pH were also measured. During both incubation periods all flasks were mechanically shaken in a Gerhardt RO 20 orbital motion shaker (Bonn, Germany) at 80 strokes/min with a rotary diameter of 4.5 cm.

The procedure was modified in some additional experiments conducted with Nedalco sludge. To evaluate the effect of substrate during the exposure period either no substrate or 1000 mg COD/*l* acetate was used instead of ethanol. In one experiment, 200 m*M* 2-bromoethanesulfonic acid (BES) was added during the exposure period, as a specific inhibitor of methanogenesis.

The effect of shaking during oxygen exposure was evaluated in additional experiments with the five granular sludges. The sludges were exposed to 0% and 18% oxygen in the head space and incubated with and without shaking.

The kinetic parameters for oxygen uptake by facultative bacteria during oxygen exposure were calculated from the rate of decreasing O_2 concentration in the head space. The V_{max} of O_2 uptake was calculated with a Lineweaver-Burk plot. The units are based on the total oxygen in the head space and liquid, initially supplied (mg O_2/l liq). The concentration range used for V_{max} calculation was from 0% to 30% oxygen supplied in the head space.

2.5. Chemicals

All chemicals were of analytical grade and purchased from Merck (Darmstad, Germany). Exceptions were the yeast extract from Gist-Brocades (Delft, The Netherlands); resazurin from Fluka (Buchs, Switzerland); BES from Janssen (Tilburg, The Netherlands); and the gases from Hoekloos (Schiedam, The Netherlands).

3. Results

3.1. Granule characteristics

The properties of the five granular sludges selected for this study are listed in Table 2. The results show that the highest methanogenic activity and aerobic heterotrophic activities were found for Nedalco sludge and the lowest for Bennekom sludge. This indicates that sludges highly concentrated with methanogens were also highly concentrated with oxygen consuming facultative bacteria. Low values of K_S, except for Nedalco sludge, show that the affinity for oxygen is apparently high. The mean particle diameters of the five sludges are in the range of 1 to 4 mm, which are typical values found for sludges from EGSB and UASB reactors.

Table 2. Granule characteristics.

Sludge	Maximum activity methanogenic bacteria [a]	Maximum O_2 uptake rate and K_S facultative bacteria		Mean diameter (mm)
		V_{max} [b]	K_S [c]	
Nedalco	867	10,813	254.4	1.28
Eerbeek	233	1340	13.1	2.25
Latenstein	170	1293	17.1	1.55
Roermond	157	1032	13.3	1.95
Bennekom	53	743	8.8	2.84

[a] Milligrams COD-CH_4/g VSS · d (acetate as substrate).
[b] Milligrams O_2/g VSS · d (total O_2 in head space + liquid volume per g VSS per day).
[c] Milligrams O_2/*l* liq (dissolved O_2 per *l* liquid).

3.2. Toxicity of oxygen for methanogens

The effect of oxygen concentration during the exposure period on methanogens was evaluated by measuring residual acetoclastic methanogenic activity of the sludges. Figure 1A plots methanogenic activity as a function of oxygen supplied in the head space at start of the exposure period. Figure 1B illustrates the dissolved oxygen levels present in the media at the end of the exposure period. The concentrations corresponding to 50% inhibition compared with the control activity (50% IC) are summarized in Table 3. Values ranged from 7.1% to 40.7% oxygen supplied in the head space at the start. In terms of final dissolved oxygen concentrations remaining at the end of the exposure period, the 50% IC ranged from 0.05 to 6.10 mg O_2/l liq. The results reveal that methanogens in granular sludge have a high tolerance to oxygen. The sludges can be classified in two main groups. Nedalco and Eerbeek have a high tolerance to oxygen and the three others have a lower tolerance. Considerable methanogenic activity was still present in cases where up to 12.4 mg O_2/l liq dissolved oxygen was present in the media of tolerant sludges. This corresponded to over 60% O_2 supplied in the head space. Under such conditions, no methane production was evident during the exposure period.

Table 3. Effect of oxygen toxicity on methanogenic activity: values of 50% inhibition compared with the control activity, as a function of oxygen concentration during the exposure period.

Sludge	Concentration of O_2 during exposure period causing 50% inhibition		
	Initial total (mg O_2/l liq)	Initial head space (%)	Final dissolved (mg O_2/l liq)
Nedalco	2447.0	40.7	6.00
Eerbeek	1990.1	33.1	6.10
Latenstein	426.9	7.1	0.05
Roermond	865.8	14.4	0.75
Bennekom	601.2	10.0	0.20

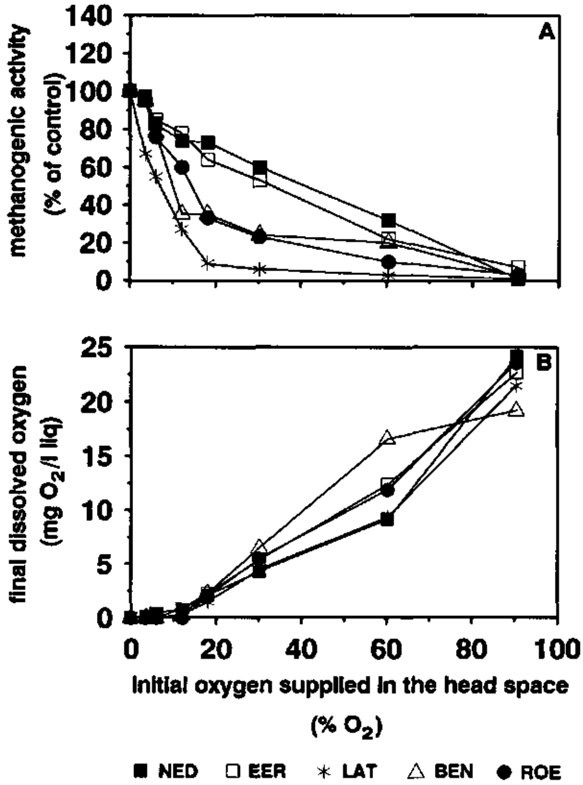

Figure 1. Toxicity of oxygen to methanogens of the five granular sludges. (A) Effect on methanogenic activity as a function of oxygen supplied at start of 3-day exposure period. (B) Dissolved oxygen levels present in media at end of exposure period. Mean values of triplicates.

3.3. Mechanisms of oxygen tolerance

A study of the effects of specific treatments on reducing the oxygen tolerance of methanogens was conducted with Nedalco sludge. Figures 2 to 4 show the effect of various treatments on altering the tolerance of methanogens to oxygen.

The substrate was the most important factor governing oxygen tolerance (Fig. 2). Absence of substrate during the exposure period drastically decreased the oxygen tolerance. However, some oxygen tolerance was still evident, even in the absence of substrate. Ethanol provided higher oxygen tolerance than acetate. The use of 200 m*M* BES, a selective inhibitor of methanogenesis, during the exposure period, increased the tolerance of the methanogens that survived the treatment. The maximum methanogenic activity of the BES-treated control however, was only 78 mg COD-CH_4/g VSS · d.

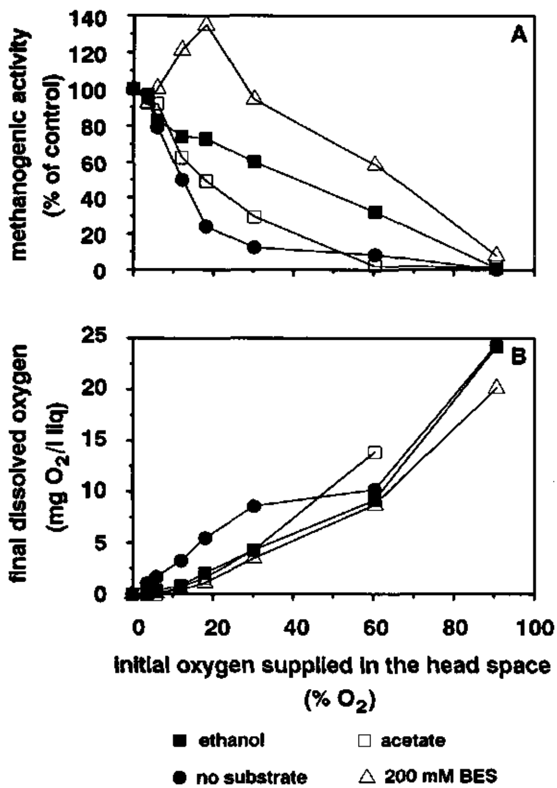

Figure 2. Toxicity of oxygen to methanogens of Nedalco sludge. Sludge treatment by substrate and BES. (A) Effect on methanogenic activity as a function of oxygen supplied at start of 3-day exposure period. (B) Dissolved oxygen levels present in media at end of exposure period. Mean values of triplicates.

Crushing the sludge only had a minor effect on the tolerance of Nedalco sludge (Fig. 3).

The effect of static conditions during the exposure period on oxygen tolerance showed that, for most of the sludges, oxygen tolerance was highly enhanced compared with shaken conditions. However, shaking was not detrimental for the highly tolerant Nedalco and Eerbeek sludges (Fig. 4).

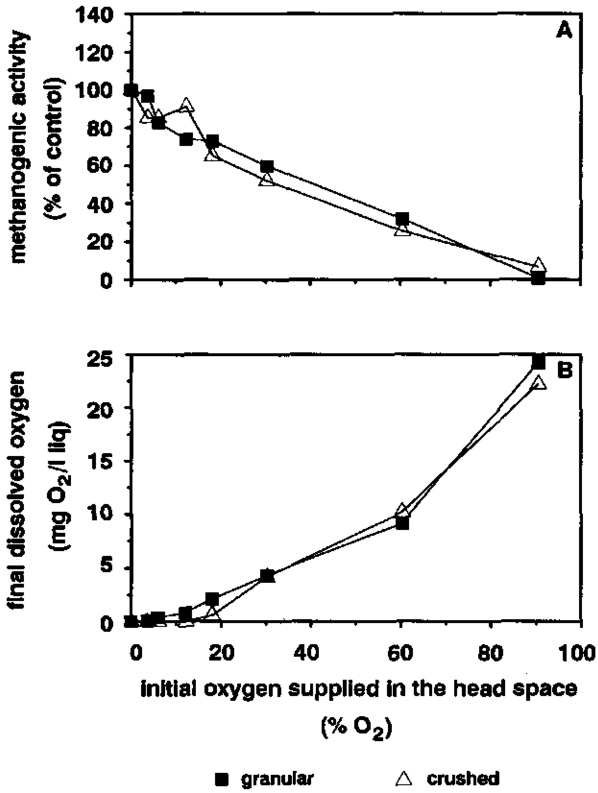

Figure 3. Toxicity of oxygen to methanogens of Nedalco sludge. Sludge crushed. (A) Effect on methanogenic activity as a function of oxygen supplied at start of 3-day exposure period. (B) Dissolved oxygen levels present in media at end of exposure period. Mean values of triplicates.

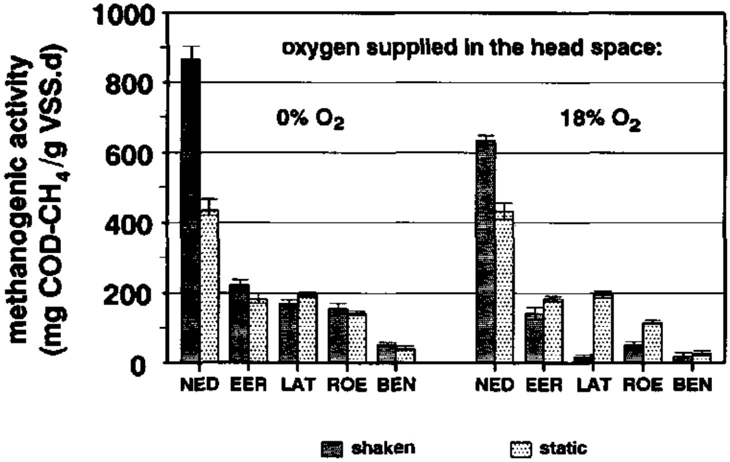

Figure 4. Toxicity of oxygen to methanogens of the five granular sludges. Effect of mixing on methanogenic activity as a function of 0% and 18% oxygen supplied at start of 3-day exposure period. Mean values of triplicates.

3.4. Correlations

The toxicity data were correlated to various characteristics of the sludges to evaluate possible mechanisms of oxygen tolerance.

Figure 5A shows the nonexistence of any correlation between oxygen tolerance and sludge granule diameter. Figure 5B shows that a higher correlation ($\rho < 0.10$) is obtained between the maximum oxygen uptake rate by facultative bacteria and oxygen tolerance. A very high correlation is obtained when considering only data from Nedalco sludge treatments ($\rho < 0.002$). The *Y*-axis intercept indicates that even when oxygen uptake is completely absent, methanogens in Nedalco sludge still have a significant level of oxygen tolerance.

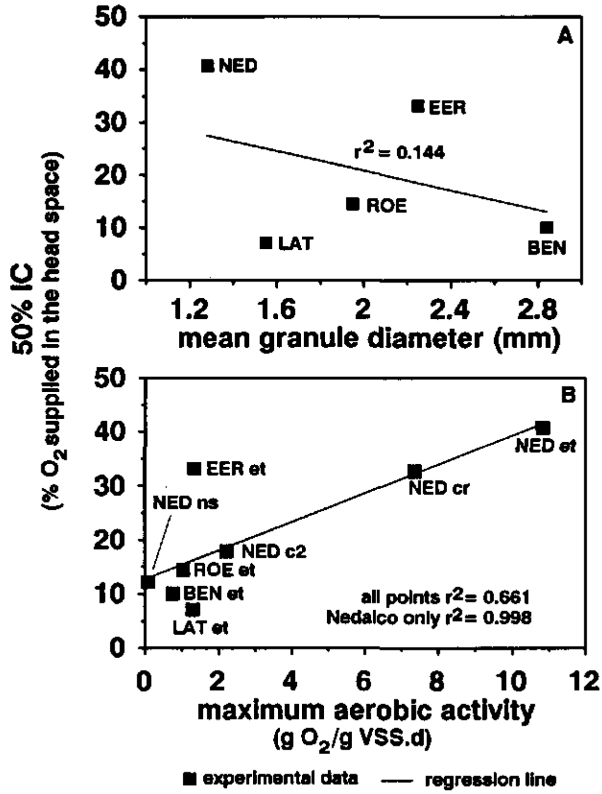

Figure 5. Correlation between sludge characteristics and oxygen tolerance. (A) Granule diameter. (B) Oxygen uptake rate. Mean values of triplicates (et = ethanol, c2 = acetate, ns = no substrate, cr = crushed + ethanol).

4. Discussion

The results of our study reveal that granular sludges from anaerobic wastewater treatment systems have a high tolerance for oxygen. The commonly held belief that methanogens are oxygen intolerant is reflected by the widely used practice of handling anaerobes under strict oxygen-free conditions.[8] Pure cultures of *Methanosarcina barkeri*, *Methanococcus vannielly*, and *Mc. voltae* indeed do not have any tolerance for oxygen exposure.[9] Likewise, methanogens lack superoxide dismutase, which is usually considered a prerequisite for oxygen tolerance.[11,15,16] However, in contrast to the paradigm, strains of *Methanobrevibacter thermoautotrophicum* and *arboriphilus* showed ability to survive for hours in the presence of air without any decrease in the number of colony-forming units.[9] Enrichment cultures of *Methanothrix* could be exposed up to 48 h to pure oxygen without causing a complete kill off.[5] These examples suggest that at least some methanogens can tolerate oxygen.

Regardless of the intrinsic oxygen tolerance of any given methanogenic bacteria, tolerance to oxygen in mixed cultures can first be explained by the role of facultative bacteria. Facultative bacteria will undoubtedly consume oxygen when present together with substrates. Thus, these microorganisms could potentially protect intolerant methanogens from exposure to oxygen.

The activity of facultative bacteria was shown in this research to be the most important mechanism of oxygen tolerance. The maximum oxygen-consuming activity of facultative bacteria in any given sludge or sludge treatment was highly correlated with oxygen tolerance of the methanogens.

The absence of facultative substrates during the whole exposure period dramatically decreased the oxygen tolerance. But, even without substrate there was tolerance either due to facultative substrates naturally present in sludges or due to chemical uptake of oxygen. Anaerobic environments contain chemical reducing components like sulphides (total sulphur of the sludges ranged from 11.3 to 27.2 mg S/g VSS), which could rapidly consume oxygen by abiotic means. Chemical uptake of oxygen, however, was only significant at very high oxygen tensions.

Intrinsic tolerance of methanogens might also explain the survival of methanogenic bacteria at low substrate levels. A significant level of intrinsic oxygen tolerance was evident for methanogens in Nedalco sludge. The *Y*-axis intercept of the correlation in Figure 5B indicates that there would have been methanogenic activity in the absence of oxygen consumption. Moreover, in cases where facultative substrates were present, there was no complete die-off of the methanogens, even when high concentrations of dissolved oxygen were present in the media after all substrates had already been consumed.

Biofilm architecture could also play a role in the oxygen tolerance mechanism, because methanogens located deep inside the biofilm would be in a highly protected microniche. Activated sludge contains methanogenic bacteria,[5] suggesting that anaerobic microniches exist even in highly aerated environments. Successful start-up of anaerobic UASB wastewater treatment reactors using activated sludge was also demonstrated.[22] The existence of oxygen-free microenvironments within biofilms has also been shown utilizing microsensors. In experiments with carrageenan gel particles containing immobilized *Escherichia coli B*, the oxygen penetration depth was 100 μm with glucose as substrate.[7] Microprofiles have also been measured in aggregates of nitrifying reactors seeded with activated sludge. The results showed that oxygen concentration decreased to zero at a depth of 300 μm. In biofilms and sediments receiving high levels of oxididable organic matter, oxygen penetration depth can be as small as 100 μm, and sometimes even almost 0 μm.[2]

The role of granule thickness was shown to be of minor importance. The absence of any correlation with biofilm diameter can probably be attributed to the fact that most of the granules were in large excess of the diameter required for anaerobic zones. Crushing had no effect on enhancing oxygen toxicity in Nedalco sludge probably because crushed granules were still thick enough compared with the oxygen penetration depth found in other research.[2,7,18,23] Other factors in oxygen tolerance are perhaps more important. Shaking generally decreased tolerance of most sludges, indicating that better transport of oxygen to the deeper positions of the biofilms was occurring. The highly tolerant Nedalco sludge was distinct and was not affected by shaking. This could be due to the fact that facultative bacteria were intricately attached to the fabric of the granules. Nedalco was the only sludge in which no turbidity increase was seen after 3 days of oxygen exposure, suggesting growth of the facultative bacteria within the granule. This is exactly the most strategic location for protecting neighboring methanogens in the biofilm.

In practice, anaerobic treatment of low strength wastewaters in high rate anaerobic reactors requires a high volumetric intake of influent. As dilute wastewaters contain dissolved oxygen, it may have been considered that the oxygen loads were excessive. The results of this study indicate that oxygen toxicity would never occur in anaerobic reactors under normal conditions, where only dissolved oxygen is entering the system: at least as long as the oxygen in the wastewater is lower than the stoichiometric requirement to oxidize the COD. Considering the low aqueous solubility of oxygen (<10 mg/*l*) found in practice, this level will never be so high, because even dilute wastewaters usually contain at least several hundred milligrams of COD per liter. The common practice of adding chemical reducing agents like sodium sulphide to the wastewater before the anaerobic treatment, is not necessary for preventing oxygen toxicity. On the contrary, the results indicate that anaerobic reactors can tolerate a modest level of aeration without any detrimental effect on the methanogenic population. Perhaps air can be added directly to anaerobic treatment systems to enhance complete mineralization of environmental pollutants.[4]

5. Acknowledgements

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Chapter 3

Methanogenesis in Granular Sludge Exposed to Oxygen

Mario T. Kato, Jim. A. Field and Gatze Lettinga

Summary

Substrate competition between methanogenic and facultative bacteria under highly aerobic conditions was investigated in batch experiments. Natural mixed cultures of anaerobic bacteria immobilized in granular sludge were able to concurrently utilize oxygen and produce methane when supplied with ethanol as substrate. The most oxygen tolerant sludge converted 3 to 25% of substrate chemical oxygen demand to methane after 3 days while 23 to 2 mg/*l* of dissolved oxygen were present in the media. The tolerance of methanogens to oxygen and their coexistence with facultative bacteria were evident even after long periods of oxygen exposure. Eventually, methane oxidizing bacteria developed in the co-culture. The consumption of oxygen by facultative bacteria, creating anaerobic microniches inside the granules, is hypothesized to protect the methanogens.

Key words: Anaerobic methanogenic and aerobic facultative bacteria; Co-culture; Substrate competition; Methanogens

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1. Introduction

Sequential anaerobic-aerobic processes are frequently used in the biological treatment of wastewater pollutants. Well known is aerobic post treatment, which is commonly used to polish off the residual biodegradable oxygen demand (BOD) from effluents after anaerobic wastewater treatment.[1] A succession of anaerobic and aerobic microorganisms is required for the complete biodegradation of polychlorinated hydrocarbons.[2] The biodegradation of adsorbable organic halogens in bleachery wastewaters is also enhanced by sequenced anaerobic-aerobic treatment.[3]

If microbial conversions in both anaerobic and aerobic environments are so desirable, why not encourage their coexistence in a single phase anaerobic-aerobic bioreactor. Previously, several research groups have shown that anaerobic consortia can be maintained in co-culture with aerobic bacteria in oxygen limited chemostats.[4,5,6] Under these microaerophilic conditions dissolved oxygen is usually lower than 0.003 mg/*l* for methanogenic consortia. However, perhaps methanogenic bacteria can even be maintained in an environment supplied with an excess of oxygen. Some strains of methanogens are in fact able to withstand long exposure to higher levels of oxygen,[7,8,9] contrary to the common belief that they are oxygen intolerant. The survival of anaerobic communities in aerobic environments also depends on their access to substrate. Excess competition for substrate by facultative bacteria should be regulated if a healthy anaerobic population is desired. In this study we will analyze the role of oxygen in the competition for substrate between anaerobic and aerobic bacteria. Anaerobic granular biofilms obtained from anaerobic wastewater treatment plants were chosen as a model consortium for the study.

2. Materials and Methods

2.1. Biomass and basal medium

The anaerobic granular sludges used in the experiments were obtained from a pilot-plant expanded granular sludge bed (EGSB) reactor treating domestic sewage (Bennekom), and from several full-scale upflow anaerobic sludge blanket (UASB) reactors treating industrial effluents. The source of each of the sludges from UASB reactors was alcohol distillery (Nedalco), paper (Roermond), wheat starch (Latenstein) and recycled paper (Eerbeek) wastewater. The sludges were elutriated to remove the fines and all the samples were stored at 4°C until used. The properties of the sludges, the mean granule diameter (in mm), the maximum methanogenic activity (in mg chemical oxygen demand (COD)-CH4/g VSS·d), the maximum oxygen uptake (in mg O_2/g VSS·d, total oxygen in the head space plus liquid volume) and K_S value (in mg O_2 dissolved per liter liquid volume) of the facultative bacteria are, respectively: Nedalco (1.28, 863, 10823 and 254.4), Eerbeek (2.25, 193, 1340 and 13.1), Latenstein (1.55, 209, 1293 and 17.1), Roermond (1.95, 115, 1032 and 13.3) and Bennekom (2.84, 81, 743 and 8.8). The properties were determined as described elsewhere.[10] The inorganic macro- and micronutrients used in the basal medium were also described before.[10]

2.2. Oxygen exposure assay

Oxygen exposure experiments were performed in 0.565 *l* glass serum flasks containing 100 m*l* of media providing a head space to liquid volume ratio of 4.65 to 1. The assay was conducted in triplicate under shaken conditions on a Gerhardt RO 20 shaking table (Bonn, Germany) of 4.5 cm rotary diameter at 80 strokes/min.

The sludges (1.65 to 2.16 g VSS/*l*) were added to the flasks and the volumes brought up to 100 m*l* with the basal mineral medium solution, containing approx. 1000 mg COD/*l* of ethanol as substrate. After flushing with a N_2/CO_2 gas mixture (70/30) the flasks were closed with butyl rubber stoppers (1.8 cm thick) and screw caps. Excess gas in the head space was removed to provide atmospheric pressure. Subsequently, all the flasks were supplied with known amounts of oxygen, after removing the same respective volumes of N_2/CO_2 gas. The removal and addition of gases were done by using a syringe. Table 1 shows the total amount of oxygen supplied per liter of liquid and the respective concentrations in the head space and dissolved in the liquid at the start of the experiment. All the flasks were then incubated in a temperature controlled room at 30 $\pm$ 2 °C. Afterwards, methane production and oxygen consumption were periodically measured. At the end of the assay the residual substrate, absorbance and dissolved oxygen were also measured.

The procedure was modified in some additional experiments conducted with Nedalco sludge. To evaluate if the sludge supplied itself substrate for anaerobic and aerobic microbial metabolism no substrate was added. To quantify the abiotic uptake of oxygen, 10 g/*l* of sodium azide was added to the sludge. Additionally, the coexistence between facultative and methanogenic bacteria was verified for longer periods. Two types of experiments were conducted. In one experiment 6 repeated feedings were carried out with 18% oxygen and 1000 mg COD/*l* ethanol as substrate. In a 7th feeding, the methanogenic activity of the sludge was checked under O_2 free gas phase. Subsequently, 3 repeated feedings were conducted with 18% oxygen and methane as substrate in order to verify the aerobic mineralization of methane.

Table 1. Amounts of oxygen supplied at the start of the exposure period

O_2 concentration in the head space (% O_2)	Dissolved O_2 conc. in the liquid (mg O_2/*l* liq)	Total O_2 supplied (mg O_2/*l* liq)
0.0	0.0	0.0
3.6	1.3	217.9
6.0	2.2	363.1
12.1	4.5	726.2
18.1	6.7	1089.3
30.2	11.2	1815.5
60.4	22.4	3631.1
90.6	33.6	5446.6

2.3. Analyses and chemicals

Ethanol and volatile fatty acids (VFA) were determined gas chromatographically.[10] The methane and oxygen concentrations in the head space of the serum flasks were determined by gas chromatography with a 200 and 500 μl gas sample, respectively.[10] All gas sample analyses were conducted after calibration with standards of known amounts of the respective gases and always with the use of a Dynatech A-2 pressure locked syringe (Baton Rouge, USA).

The turbidity of the supernatant was measured by visible light absorbance at 660 nm (1cm cuvettes) in a Milton Roy Spectronic 601 equipment (Rochester, USA). Dissolved oxygen (DO) was measured directly in the flasks immediately after opening, with a WTW OXI-196 O_2-meter (Weilheim, FRG). Volatile suspended solids (VSS) were determined according to the *Standard Methods*.[11] Wet sludge is referred to the solids before drying overnight in an oven at 100-103 °C. The dense granule sludges contained from 0.060 to 0.142 g VSS/g wet sludge.

The concentrations of ethanol and VFA are referred to in chemical oxygen demand units, commonly employed in the field of wastewater treatment. Also methane production is expressed as its equivalent in COD per liter of liquid media. Conversion factors utilized were 2.087 g COD/g ethanol, 1.067 g COD/g acetate and 2.577 g COD/*l* CH_4 at 30 °C. Theoretical BOD used for ethanol was 0.444 g O_2/g COD-ethanol assuming $C_5H_7NO_2$ for aerobic cells and cell yield of 0.557 g COD-cell/g COD-ethanol.

All chemicals were of analytical grade and purchased from Merck, Darmstad, Germany. Exceptions were the yeast extract from Gist-Brocades, Delft, The Netherlands; resazurin from Fluka, Buchs, Switzerland; and the gases from Hoekloos, Schiedam, The Netherlands.

3. Results

3.1. Substrate competition

Substrate competition between facultative and methanogenic bacteria in the 5 granular sludges is shown in Fig. 1, after 3-day exposure to various concentrations of oxygen. The amount of methane produced and oxygen consumed reveal that both methanogens and facultative bacteria in oxygen tolerant Nedalco and Eerbeek sludges were competing for substrates with 3.6 to 90.6% O_2 in the head space corresponding to 23 to 2 mg/*l* of final dissolved oxygen in the media, respectively (Fig. 2). In the other sludges, methane

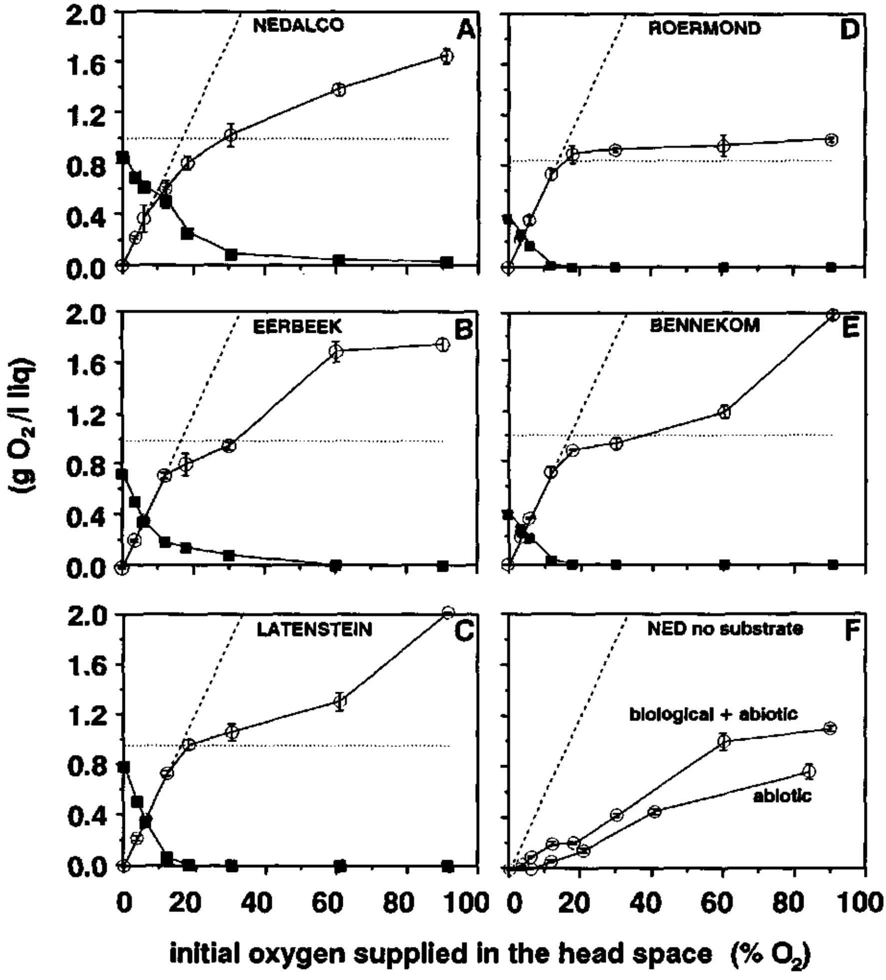

Figure 1. Competition between methanogenic and aerobic metabolism of the 5 granular sludges after 3-day exposure to different concentrations of O_2. ▪ methane production (g COD/*l* liq); o oxygen uptake (g O_2/*l* liq); --- oxygen supplied (g O_2/*l* liq); ... ethanol supplied (g COD/*l* liq); bars represent standard deviations.

production was only significant up to 6% O_2 in the head space when the dissolved oxygen was non-detectable. Beyond these concentrations of oxygen, ethanol was almost completely consumed by facultative bacteria.

Analyses of ethanol confirmed that supplied substrate was completely consumed since non-detectable amounts were left in the media over the entire range of oxygen supplied. In Roermond and Bennekom sludges, having the lowest methanogenic activities some VFA (95.8 to 289.1 mg COD/*l*) from acidified ethanol was present in the 3-day-old media with up to 6% oxygen, but VFA was not detected at higher O_2 concentrations nor in other sludges. Turbidity measurements (results not shown) indicate the growth of dispersed facultative cells when oxygen was supplied.

Fig. 1 also reveals that almost all of the oxygen was consumed, as long as the oxygen supplied was less than that of the COD of the substrate. This O_2 uptake represents substrate consumption by facultative bacteria that otherwise was destined for methanogenesis. This finding would be expected since the specific activity of oxygen uptake by the 5 sludges (see earlier) was in excess of the specific methanogenic activity by approximately 6.5- to 14-fold. Nonetheless, methanogens in the oxygen tolerant sludges were not completely outcompeted when large excesses of oxygen were supplied.

It is remarkable that in all cases the oxygen uptake exceeded the COD of ethanol supplied at high levels of oxygen supplied. Two explanations for this include the possibility that the sludge itself supplies substrate to aerobic facultative bacteria and the possibility that abiotic mechanisms of oxygen uptake were occurring. Fig. 1F illustrates uptake of oxygen by Nedalco sludge when no substrate was supplied indicating that the sludge itself contained substrates for aerobic bacteria. When sodium azide (10 g/*l*) was used to inhibit biological activity, O_2 uptake occurred at high O_2 concentrations. Most of the oxygen uptake at low oxygen supplied (< 12% O_2) was due to metabolism of the sludge, whereas at higher concentrations most of the uptake was due to chemical reactions.

3.2. Coexistence of methanogenic and facultative bacteria

The simultaneous coexistence of methanogenic and facultative bacteria is demonstrated by the concurrent uptake of oxygen with the production of methane (Fig. 3). This coexistence

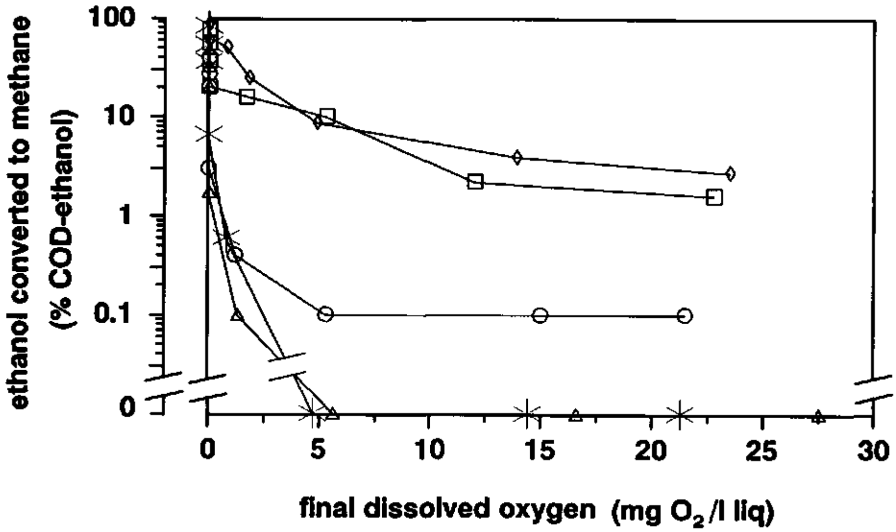

Figure 2. Ethanol converted to methane, as a function of dissolved oxygen present in the media at the end of the 3-day exposure period. Sludge types: ◇ Nedalco; □ Eerbeek; ○ Bennekom; △ Roermond; * Latenstein.

continued for longer periods when 6 repeated feedings with ethanol as substrate and 18% oxygen were supplied (Fig. 4). The occurrence of facultative methanotrophs was evident by the 4th feeding when the methane produced was significantly consumed. During the course of the repeated feedings, methane metabolizing bacteria were growing. In the 7th feeding, the methanogenic activity was checked (no O_2 supplied) and the methane production was similar to that of the 0% oxygen control. At the end of the experiment the methanotrophic activity was also checked by supplying only methane as a substrate together with oxygen. Methane consumption and oxygen uptake occurred during this feeding confirming the presence of methane oxidizers.

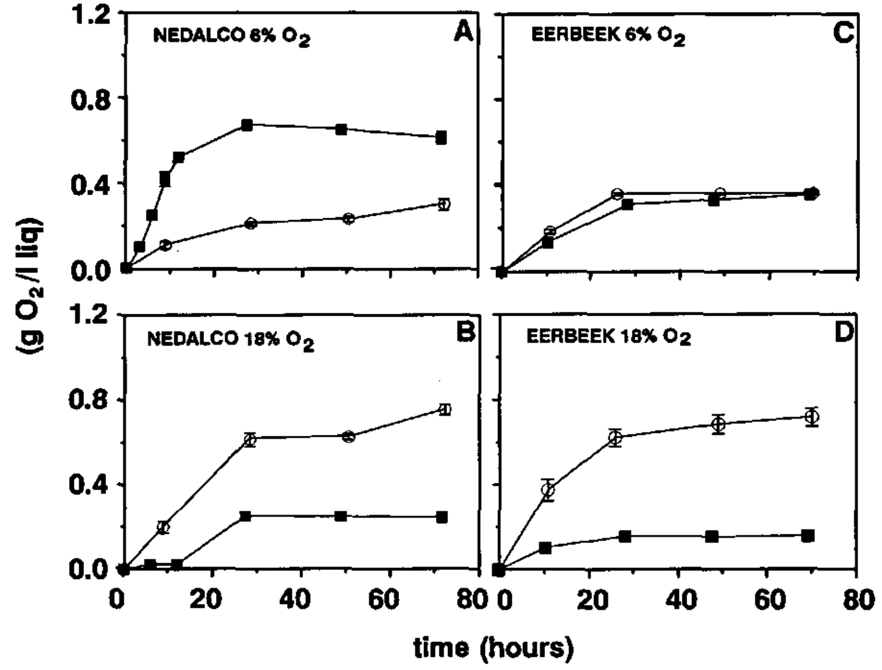

Figure 3. Simultaneous methane production and oxygen uptake of Nedalco and Eerbeek sludges, as a function of time. Percent O_2 is the initial concentration supplied in the head space. ▪ methane production (g COD/*l* liq); o oxygen uptake (g O_2/*l* liq); bars represent standard deviations.

4. Discussion

Previous studies have demonstrated that methanogens can occur in co-culture with aerobic bacteria, provided that microaerophilic conditions were prevailing. In this study, we demonstrate the occurrence of methanogenesis despite aggressive competition for substrate by facultative bacteria with excess O_2 supply. Therefore, we can conclude that methanogenesis is occurring under truly aerobic conditions. Ethanol substrate COD was converted to CH_4 while up to 23 mg/*l* of dissolved oxygen was present in the liquid media.

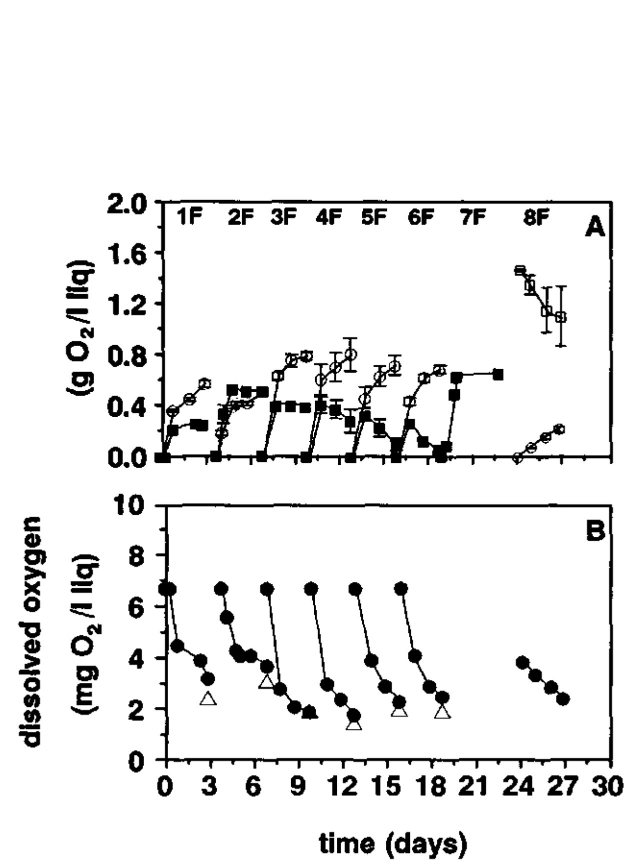

Figure 4. Coexistence of methanogenic, facultative heterotrophic and methanotrophic bacteria for extended periods (Nedalco sludge). A. Methane production or consumption and oxygen uptake (1st to 6th feedings supplied with ethanol and 18% oxygen in the head space; no oxygen supplied in the 7th feeding; 8th feeding supplied with methane and 12% oxygen in the head space). ▪ methane production (g COD/*l* liq); ○ oxygen uptake (g O_2/*l* liq); □ methane consumption (g COD/*l* liq). B. Dissolved oxygen present in the media. • calculated from oxygen measurement in the head space and Henry's law; △ directly measured. Bars represent standard deviations.

The occurrence of methanogenesis in excess oxygen suggests that methanogens have a high tolerance for oxygen or are present in highly protected microniches inside sludge granules. Highly tolerant methanogenic granular sludges were only inhibited by 50% when 6 mg/*l* dissolved oxygen was prevailing in the media.[10] Facultative substrates enhance the oxygen tolerance. Presumably, the rapid consumption of O_2 resulting from their metabolism generates anaerobic zones in the biofilms.[12,13,14,15] However, in the absence of facultative substrates, anaerobic zones cannot be created, yet the methanogens were not completely inhibited by oxygen.[10] This indicates an intrinsic tolerance of some methanogens. An enrichment culture of *Methanothrix soehngenii*, a common acetoclastic methanogen found in anaerobic wastewater treatment systems, was shown to withstand 48 hours of exposure to pure oxygen.[8]

Aerobic microorganisms can metabolize substrates more efficiently than anaerobes as is reflected by an order of magnitude higher specific activities of O_2 respiration compared to methanogenesis in the granular sludges tested. Consequently, we would expect that each unit of oxygen added would be reflected in a unit of available substrate stolen from anaerobic microorganisms. This was indeed the case, for the three oxygen intolerant sludges, Roermond, Latenstein and Bennekom. Methanogenesis was no longer evident once the oxygen supply exceeded the BOD of the ethanol substrate. On the other hand, some methanogenesis was still occurring in Nedalco and Eerbeek sludges even when the oxygen supply exceeded the BOD of ethanol by a factor of 11. This finding can only be explained by the occurrence of microaerophilic or anaerobic microniches deep inside the biofilm where the O_2 content is so low that the aerobic respiration rate is limited by the low oxygen flux or by Monod kinetics. Typically, oxygen penetrates biofilms of metabolically active aerobic bacteria by only 100 to 300 μm in well aerated bioreactors,[13,14,16,17] while in this study granules having diameters of 1.28 to 2.84 mm were used. Ethanol and VFA with higher aqueous solubilities would consequently be expected to have higher mass transport into the biofilms than oxygen. Thus, these substrates could potentially be present in the microniches at suitable concentrations. Paradoxically, sludges with the highest specific respiration rates would provide the lowest penetration of oxygen, and thus be the most suited for supporting anaerobic consortia which could compete for substrates.

Our results show the evidence of an anaerobic-aerobic co-culture maintained even in a prolonged oxygen exposure assay. A healthy methanogenic population was still evident even after 18 days of exposure to oxygen. Aerobic methanotrophs developed which eventually started to consume most of the methane generated by the anaerobic population. Therefore, this study reveals a co-culture composed of three trophic groups, methanogenic, facultative heterotrophic and methanotrophic bacteria.

The possibility that anaerobic-aerobic co-cultures occur naturally in sludges of wastewater treatment plants can be inferred from several studies. The presence of methanogenic bacteria in activated sludges has already been documented.[8,12] The existence of methane oxidizing bacteria in anaerobic sludges was demonstrated when a pure culture of a methanotroph, strain 81Z, was isolated from a methanogenic reactor.[18]

Anaerobic and aerobic bacteria co-existing in one single bioreactor represents many new possibilities for environmental technology. A particularly promising application is the biodegradation of recalcitrant environmental pollutants which require sequenced anaerobic-aerobic activity such as polychlorinated hydrocarbons.[2] The superior potential of anaerobic-aerobic co-cultures to degrade DDT,[19] 2,3,6-trichlorobenzoic acid [5] and 4-chloro-2-nitrophenol [20] has already been demonstrated in oxygen limited conditions. The mineralization of these and other environmental pollutants still needs to be tested in oxygen unlimited anaerobic-aerobic co-cultures.

Moreover the presence of methanotrophs in oxygen unlimited conditions adds to the arsenal of biodegradative capacities. Methane oxidizing bacteria have been applied for the destruction of hazardous organics, especially chlorinated solvents.[21,22] The concept lies upon cometabolic transformation by the enzyme methane monooxygenase. The specific contaminants of interest for this application include common halogenated aliphatics.

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Chapter 4

Treatment of Low Strength Wastewaters in Upflow Anaerobic Sludge Blanket (UASB) Reactors

Mario T. Kato, Jim. A. Field, Robbert Kleerebezem and Gatze Lettinga

Summary

Low strength wastewaters can be those with chemical oxygen demand (COD) below 2000 mg/*l*. The anaerobic treatment of such wastewaters has not been fully explored so far. The suboptimal reaction rates with low substrate concentrations, and the presence of dissolved oxygen in the influent are regarded as possible constraints. In this study, the treatment of low strength soluble wastewaters containing ethanol or whey was studied in lab-scale upflow anaerobic sludge bed (UASB) reactors at 30°C. The high treatment performance obtained demonstrates that UASB reactors are viable for treating both types of wastewaters at low COD concentrations. The treatment of the ethanol containing wastewater resulted in COD removal efficiencies exceeding 95% at organic loading rates (OLR) between 0.3 to 6.8 g COD/*l*·d with influent concentrations in the range of 422 to 943 mg COD/*l*. In the case of the more complex whey containing wastewater, COD removal efficiencies exceeded 86% at OLRs up to 3.9 g COD/*l*·d, as long as the COD influent was above 630 mg/*l*. Lowering the COD influent resulted in decreased efficiency with sharper decrease at values below 200 mg/*l*. Acidification instead of methanogenesis was found to be the rate limiting step in the COD removal at low concentrations, which was not the case when treating ethanol. The effect of dissolved oxygen in the influent as a potential danger in anaerobic treatment was investigated in reactors fed with and without dissolved oxygen. Compared with the control reactor, the reactor receiving oxygen showed no detrimental effects in the treatment performance. Thus, the presence of dissolved oxygen in dilute wastewaters is expected to be of minor importance in practice.

Key words: low strength soluble wastewater - UASB reactor - presence of dissolved oxygen in the influent - anaerobic treatment of ethanol or whey containing wastewater

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1. Introduction

The upflow anaerobic sludge blanket (UASB) concept is a simple design which has been demonstrated as a very efficient process for wastewater treatment. The success of the UASB can be attributed to the adequate retention of dense granular sludge with high specific activity, enabling good treatment performance at high organic loading rates (OLR).[1,2,3] Mixing of the bulk liquid phase, which is considered essential for optimal wastewater-biomass contact, is obtained in the UASB reactor by the natural turbulence. This turbulence is firstly achieved due to the high biogas production at high OLR and secondly due to the hydraulic load resulting from the influent flow. An even influent distribution system is also required.[1,4] The UASB was originally developed for the treatment of industrial wastewaters of medium to high strength.

However, the potentials of the UASB is not necessarily restricted to medium and high strength wastewaters. Its application is being explored more and more for the less concentrated wastewaters with chemical oxygen demand (COD) below 2000 mg/*l*.[5,6,7] Attempts have been made towards the treatment of low strength domestic sewage with full-scale UASB plants. Several research groups are reporting satisfactory performance at temperatures above 20°C.[2,8,9,10,11,12,13,14] Treatment performance efficiencies of 70% to 89% were achieved at influent concentrations in the range of 267 to 660 mg COD/*l*, at OLRs up to 3.8 g COD/*l*·d. Nonetheless, the use of UASB for treating low strength soluble industrial wastewaters has not yet been fully explored. Research with other high rate systems like the anaerobic attached expanded bed (AAFEB) or fluidized bed (FB) in lab- and bench-scale reactors has been conducted. Researchers claim that these system may be more effective for low strength wastewaters due to the superior mass transfer at high hydraulic loads in these reactor types compared with that of the UASB.[15,16,17,18,19] Nonetheless, some investigations with the UASB indicate that its application can be valid as well for low strength wastewaters. When treating synthetic wastewater consisting of cheese whey, a COD removal efficiency of up to 89% was obtained at OLR of 2.6 g COD/*l*·d and at influent COD concentration (COD_{in}) of 500 mg/*l*.[20] The treatment of paper recycle industry wastewater of less than 1000 mg COD/*l* in a full-scale UASB reactor resulted in an efficiency higher than 70% at OLRs ranging from 4.4 to 5.0 g COD/*l*·d.[5]

A number of factors can be enumerated that could explain the limited application of the UASB reactor for low strength wastewaters. The low concentration and the possible presence of dissolved oxygen in some dilute wastewaters are possibly the main constraints.

The low COD concentration in the influent results in low substrate levels inside the UASB reactor. Consequently, due to Monod kinetics suboptimal reaction rates will prevail, which may possibly result in a poor treatment performance. Granular sludges from UASB reactors have been shown to have apparent K_S values for acetoclastic methanogens of 30 to 200 mg COD/*l*.[21,22,23] Additionally, the expected lower gas production from dilute wastewaters would reduce the natural mixing of the bulk liquid phase and the economic benefits derived from energy production. In order to verify the limits of the UASB application, the lowest COD concentration that can efficiently be treated should be determined. Oxygen is considered as a potential danger in the anaerobic treatment, especially for the syntrophic acetogens and methanogens, which are usually regarded as strict anaerobes.[24,25] Consequently, dissolved oxygen in the influent of the UASB may result in reactor upset. If this is really the case in practice, it should also be determined.

In view of limited data available, this study was conducted to assess the applicability of UASB reactors for treating low strength soluble wastewaters. The treatment of such effluents consisting of a simple and complex substrate was assessed with ethanol and whey solutions, respectively. Two UASB reactors of 13.8 *l* (ethanol) and two of 2.15 *l* (whey) were used under several conditions of OLR and COD_{in}. The effect of dissolved oxygen was also assessed by running the two ethanol-fed reactors in parallel with and without oxygen in the influent.

2. Materials and Methods

2.1. Biomass

Anaerobic granular sludge used in all the experiments was obtained from a full-scale UASB reactor treating alcohol distillery wastewater of Nedalco (Bergen op Zoom, The Netherlands). The sludge was collected and stored at 4°C until used. Before starting the experiments the sludges were elutriated to remove the fines. In the UASB experiments with ethanol as substrate, the sludge was already stored for 12 months, while in the case of whey the sludge was collected one month before the start of the reactor experiments.

2.2. Basal medium and chemicals

For the batch methanogenic activity assay essential inorganic macro- and micronutrients were used as described elsewhere.[26] The concentration of $NaHCO_3$ buffer solution varied as a function of the assay substrate (ethanol, acetate or volatile fat acids mixture) used. After dilution, the bicarbonate concentration in the basal medium solution used in that assay was (mg $NaHCO_3/l$): 2100 and 400 for ethanol and the two acidified solutions as substrates, respectively. For the continuous experiments the standard solutions of macro- and micronutrients were prepared according to Rinzema.[21] These solutions were supplied to the influent of the reactors at a dilution of 6 m*l* and 0.1 m*l* per litre influent for the macro- and micronutrients, respectively. Alkalinity was also provided as sodium bicarbonate in the influent and the amount varied depending on the type and concentration of the substrate-fed (ethanol or whey) utilized. When ethanol was used as feed, 0.5 g $NaHCO_3$ was added for each g COD in the influent. This ratio was varied between 1.0 and 0.5 g $NaHCO_3$/g COD in the case of whey as feed.

All chemicals utilized were of analytical grade, with exception of the sodium bicarbonate (99.5% by Boom, Meppel, The Netherlands) added to the influent of the reactors. They were supplied by Merck (Darmstad, Germany). Exceptions were the yeast extract by Gist-Brocades (Delft, The Netherlands); resazurin by Fluka (Buchs, Switzerland); and the gases by Hoekloos (Schiedam, The Netherlands).

2.3. Analyses

Ethanol and volatile fatty acids (VFA) were determined by gas chromatography, as well the biogas composition (CH_4, CO_2 and H_2S) of reactors. The pH and redox potential were determined immediately after sampling with a Knick 510 pH/mV-meter (Berlin, Germany). The latter measurement was conducted *in situ* at the influent and effluent lines of two parallel oxygen experiment reactors. Other specifications of those analyses were described elsewhere.[27,28] COD and volatile suspended solids (VSS) were determined according to the *Standard Methods*.[29]

The concentrations of ethanol, VFA and the methane production are referred to in COD units. Conversion factors utilized were (g/g): 2.087, 1.067, 1.515 and 1.820, for ethanol, acetate (C_2), propionate (C_3) and butyrate (C_4), respectively. For methane, a factor of 2.577 g COD/*l* CH_4 at 30°C was utilized. COD, ethanol and VFA of reactor effluents are referred to samples centrifuged at 17 000 × *g* for 4 min.

2.4. Batch methanogenic activity assay

The maximum specific methanogenic activity of the sludges was determined using 0.6 *l* glass serum flasks sealed with a rubber septum and a screw cap. The sludge was added to the flasks and then the liquid volume completed to 0.5 *l* with the basal mineral medium solution, bicarbonate and tap water. This volume contained 4 g COD/*l* of ethanol, acetate or VFA-mixture solution as assay substrate. The final concentration of the sludge was 1.5 g VSS/*l*. The pH of acetate and VFA stock solutions was 7.0. The COD ratio of the VFA stock was 24.3 : 34.4 : 41.3% of the total COD for C_2, C_3 and C_4, respectively. After flushing the medium with nitrogen gas, the flasks were sealed and incubated in a temperature-controlled room at 30 ± 2°C. The flasks were provided with a second feeding when more than 80% of the substrate COD supplied in the first feeding was converted to methane. Monitoring consisted of periodic measurements of methane production by modified Mariotte flasks. These flasks contained a 3% (w/v) NaOH solution to remove the carbon dioxide from the biogas. The maximum specific methanogenic activity was calculated from the slope of the methane production versus time curve. All assays were conducted in duplicate under static conditions.

2.5. Continuous experiments in UASB reactors

In this study, two experiments were conducted in a temperature-controlled room at 30 ± 2°C with synthetic wastewaters containing ethanol or whey. The anaerobic treatment of the ethanol containing wastewater with and without oxygen in the influent was studied in a first experiment. Two 13.8 *l* plexiglass reactors (R1 and R2) with a height of 2.17 m and interne diameter (ID) of 9.0 cm were used. The treatment of the whey containing wastewater was evaluated in a second experiment. Two 2.15 *l* glass reactors (R3 and R4) with a height of 40 cm and ID of 9.5 cm were used. Figure 1 shows the schematic diagram of the used UASB reactor systems.

In the first experiment, reactors R1 and R2 were operated under different operational conditions of hydraulic and organic loads, and substrate concentrations to evaluate the treatment performance of the ethanol containing wastewater. In this experiment, reactors were operated in parallel in order to assess the influence of dissolved oxygen in the influent. Reactor R1 (-O_2) was operated without oxygen supply in the influent. Sodium sulphide was supplied to the dilution water to obtain a concentration of 50 mg S^{-2}/*l* in the influent in order to remove all oxygen. In the case of reactor R2 (+O_2), the dilution water was aerated to obtain a relatively high concentration of dissolved oxygen (average of 5 mg O_2/*l* influent). The different conditions in terms of imposed OLR resulted in 8 experimental periods for both reactors. The reactors were initially inoculated with granular sludge and periodically adjusted to a sludge concentration of 20 g VSS/*l*. At the beginning of the period 7 the concentration was adjusted to 10 g VSS/*l* in order to assess the maximum capacity of the reactor sludges.

In the second experiment, reactors R3 and R4 were operated to evaluate the treatment performance of the more complex whey substrate and to assess the lowest possible COD_{in} that could efficiently be treated. Different operational conditions of hydraulic and organic loads, and substrate concentrations were also applied, resulting in six experimental periods. Both reactors started up with only 5 g VSS/*l* of granular sludge and this sludge concentration was periodically adjusted. With relatively low biomass concentration, it was intended to imitate the non-ideal conditions normally encountered during the first start-up of UASB reactors.[20,30]

The performance of the UASB reactors was evaluated using the parameters: A = % conversion COD_{in} to VFA (A = M_{total} + VFA_{ef}); CELLS = % conversion COD_{in} to cells (estimation of cells = E - M_{total}); COD_{ef} = effluent COD (mg COD/*l*); COD_{in} = influent COD (mg COD/*l*); E = % COD_{in} removed based on the COD_{ef}; EOH_{ef} = effluent ethanol concentration as % of COD_{in}; $M_{dissolved}$ = methane dissolved in the effluent (estimated according to Henry's law); M_{total} = % conversion COD_{in} to methane (methane measured with gas meter plus estimated methane dissolved in the effluent); OLR = space organic loading rate (g COD/*l*·d); O_2LR = oxygen loading rate (g O_2/*l*·d). SLR = sludge loading rate (g COD/g VSS·d); VFA_{ef} = effluent VFA concentration as % of COD_{in}. At least 10 HRTs were allowed to pass after each change in OLR or COD_{in} range before analyses and data were taken.

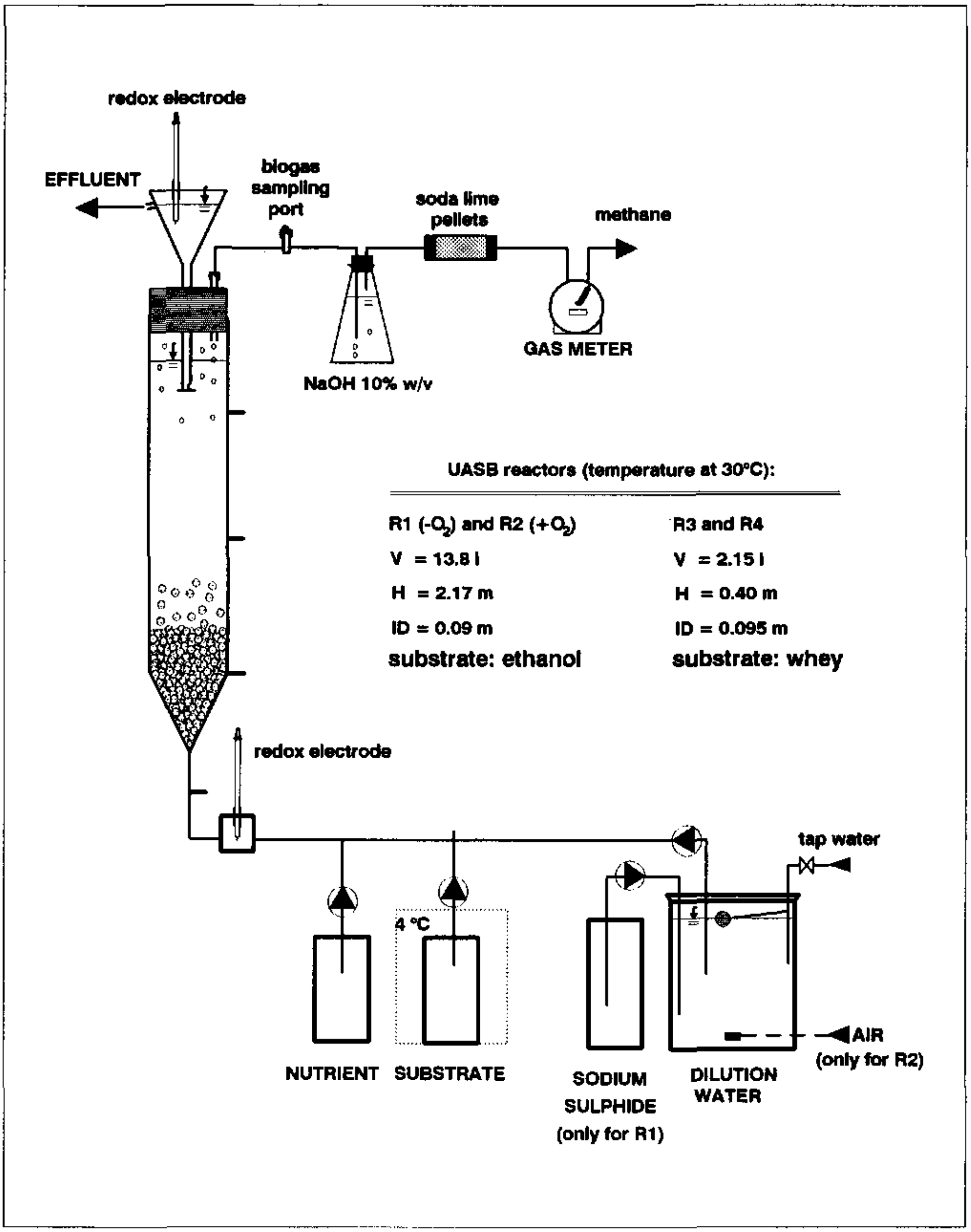

Figure 1. Schematic diagram of the UASB systems used in this study. 13.8 *l* ethanol reactors (R1:-O_2 and R2:+O_2) and 2.15 *l* whey reactors (R3 and R4).

3. Results

3.1. The Anaerobic treatability of the ethanol containing wastewater

The effect of dissolved oxygen on the treatment performance was studied in reactors fed with ethanol containing wastewater. The treatment performance and the measured redox potential of two parallel reactors, R1 ($-O_2$) and R2 ($+O_2$), were compared. The average treatment performance (E, M_{total}, $M_{dissolved}$, A) during periods of distinct operational conditions are shown in Tables 1 and 2 for reactor R1 and R2, respectively.

Comparing the results of both reactors, it is observed that reactor R2 receiving dissolved oxygen in the influent did not show any difference in treatment performance as compared with that of reactor R1. The effluent redox potential was the same in both reactors (around -410 mV), indicating that dissolved oxygen was consumed in reactor R2.

A discernible difference was observed in the treatment performance between periods of organic underloading and overloading. The reactors were underloaded (SLR $<$ 0.30 g COD/g VSS·d) during periods 1 to 7 and overloaded (SLR $>$ 2.06 g COD/g VSS·d) during period 8. A very high performance was obtained when both reactors were underloaded with COD removal efficiency above 95%. During the first 6 periods reactor sludges were maintained at 20 g VSS/*l* and the range of COD_{in} applied was between 422 and 943 mg/*l*. OLRs up to 6.8 g COD/*l*·d were achieved by decreasing the HRT to 2.6 h (period 6, Table 1). In the seventh period, the sludge concentration was adjusted to 10 g VSS/*l* but a high efficiency (99%) was still obtained since the condition of underloading was maintained. Underloading resulted in only minor amounts of acetate in the effluent.

A decrease in COD removal efficiency was observed only when the OLR was increased to above 20 g COD/*l*·d in the 8th period. In this case, the SLR was clearly greater than the maximum COD removal capacity of the reactor sludges. Aside from decreased treatment performance, overloading also resulted in a distinct effluent composition. Propanol and propionate were detected together with ethanol and acetate. Similar observation occurred in the batch activity test using ethanol as substrate. The occurrence of reduced compounds like propanol in the effluent reflects the excess of reducing equivalents occurring in the overloaded reactors. Reduction of propionate concomitantly with the ethanol oxidation to

Table 1. Operational conditions and treatment efficiency of the lab-scale UASB reactor fed low strength ethanol wastewater without oxygen supply (R1) at 30°C. Inoculation with 20 g VSS per liter reactor. Average values.

Parameter [a]		Experimental periods [b]							
		1	2	3	4	5	6	7 [c]	8
Operational:									
COD_{in}	(mg COD/l)	637	943	670	637	822	722	690	2402
HRT	(h)	28.3	16.2	10.2	4.1	10.0	2.6	10.3	2.6
OLR	(g COD/l·d)	0.5	1.3	1.6	3.7	2.0	6.8	1.6	22.7
SLR	(g COD/g VSS·d)	0.03	0.07	0.08	0.19	0.10	0.34	0.16	2.27
O_2LR	(g O_2/l·d)	0	0	0	0	0	0	0	0
Efficiency (% COD_{in}):									
E		99	99	99	99	99	97	99	77
CELLS		24	32	22	25	13	25	19	37
M_{total}		75	67	77	74	86	72	80	40
$M_{dissolved}$		12	8	11	12	9	10	11	3
A		76	68	78	75	87	75	81	53

a Abbreviations are defined in Materials and Methods

b Periods: 1 (day 0-9), 2 (day 10-24), 3 (day 25-44), 4 (day 45-47), 5 (day 48-51), 6 (day 52-54), 7 (day 78-85), 8 (day 85-88)

c Sludge concentration adjusted to 10 g VSS/*l*

Table 2. Operational conditions and treatment efficiency of the lab-scale UASB reactor fed low strength ethanol wastewater with oxygen supply (R2) at 30 °C. Inoculation with 20 g VSS per liter reactor. Average values.

Parameter [a]		Experimental periods [b]							
		1	2	3	4	5	6	7 [c]	8
Operational:									
COD_{in}	(mg COD/l)	422	673	720	693	914	671	846	2102
HRT	(h)	29.0	15.0	10.6	3.9	9.9	2.7	11.1	2.4
OLR	(g COD/l·d)	0.3	1.1	1.6	4.2	2.2	5.9	1.8	20.6
SLR	(g COD/g VSS·d)	0.02	0.05	0.08	0.21	0.11	0.30	0.17	2.06
O_2LR	(g O_2/l·d)	0.004	0.008	0.011	0.031	0.012	0.044	0.011	0.050
Efficiency (% COD_{in}):									
E		99	99	99	97	99	95	99	66
CELLS		12	11	18	26	15	24	23	26
M_{total}		87	88	81	71	84	71	76	40
$M_{dissolved}$		18	11	10	11	8	11	9	4
A		88	89	82	74	85	76	77	57

a Abbreviations are defined in Materials and Methods

b Periods: 1 (day 0-9), 2 (day 10-24), 3 (day 25-44), 4 (day 45-47), 5 (day 48-51), 6 (day 52-54), 7 (day 78-85), 8 (day 85-88)

c Sludge concentration adjusted to 10 g VSS/*l* acetate was also observed previously.[31,32]

In this experiment, we visually observed a gradual increase in the size of granular sludge. Stereomicroscopic examination of cross sectioned granules also revealed an alteration in the granule structure (results not shown). Cavities were observed in those granules that had increased in size. Specific methanogenic activity of reactor sludges was periodically measured and the results are shown in Table 3. A 20% increase in the activity was observed by the end of this experiment (day 88) when using ethanol or acetate as the assay substrate. However when the VFA mixture was used as the assay substrate, the activity decreased by 35% probably because propionate and butyrate are not intermediates during ethanol degradation under normal OLR conditions.

3.2. The Anaerobic treatability of the whey containing wastewater

The effect of low COD_{in} coupled with low OLR was also evaluated in an experiment utilizing a slightly more complex wastewater, whey. During all the experimental periods, the two reactors R3 and R4 were underloaded (SLR < 0.78 g COD/g VSS·d) and the only operational difference consisted in the COD_{in} applied during period 2. In reactor R3, the COD_{in} was maintained constant and a sudden decrease was imposed at the end to achieve a COD_{in} of only 113 mg COD/*l* in the subsequent period. On the other hand, in reactor R4 the COD_{in} was gradually decreased to almost the same value. Tables 4 and 5 show the average results of the treatment efficiency, under the various periods of operation for reactors R3 and R4, respectively. Figures 2 and 3 also show the daily data for reactors R3 and R4, respectively.

Decreasing the COD_{in} during period 2 in different ways did not result in any difference of the treatment performance in period 3. Both reactors had a low COD removal efficiency of less than 37% when the COD_{in} was dropped below 200 mg/*l*. When the COD_{in} in both reactors was again increased in period 4 to the previous levels, a high treatment performance was reestablished. The treatment performance also deteriorated again in the last two periods when COD_{in} levels were reduced below 500 mg/*l* (Table 5). The effect of low substrate concentrations can also be observed even when comparing periods with similar OLRs. In the range of 2.0-2.4 g COD/*l*·d, the efficiency was above 91% (periods 2B and 4, Tables 4 and 5) with COD_{in} > 1342 mg/*l*, while the efficiency was only 77% with COD_{in}

Table 3. The specific methanogenic activity of the sludge in the reactors R1 and R2 during continuous operation of UASB fed ethanol low strength wastewater (determined in batch assays).

Time	Specific methanogenic activity (g COD-CH_4/g VSS·d)					
	Reactor R1 (-O_2)			Reactor R2 (+O_2)		
	ethanol	acetate	VFA-mixture	ethanol	acetate	VFA-mixture
day 0 [a]	1.07	0.43	0.53	1.07	0.43	0.53
day 44 [b]	1.29	n.d.	n.d.	1.11	n.d.	n.d.
day 88 [c]	1.21	0.49	0.35	1.23	0.51	0.46

a Start period 1
b End period 3
c End period 8
n.d. Not determined

Table 4. Operational conditions and treatment efficiency of the lab-scale UASB reactor fed low strength whey wastewater (R3) at 30°C. Inoculation with 5 g VSS per liter reactor. Average values.

Parameter [a]		Experimental periods [b]					
		1 [c]	2	3	4	5	6
Operational:							
COD_{in}	(mg COD/l)	1876	1941	113	1435	1165	630
HRT	(h)	16.8	17.0	16.4	17.4	9.3	10.1
OLR	(g COD/l·d)	2.7	2.9	0.2	2.0	3.9	1.5
SLR	(g COD/g VSS·d)	0.54	0.58	0.04	0.40	0.78	0.30
Efficiency (% COD_{in}):							
E		79	89	30	91	91	86
CELLS		3	14	-28 [d]	7	15	13
M_{total}		76	75	58	84	76	73
$M_{dissolved}$		3	3	58	5	6	10
A		97	76	58	84	76	77

a Abbreviations are defined in Materials and Methods

b Periods: 1 (day 0-12), 2 (day 13-58), 3 (day 59-65), 4 (day 66-80), 5 (day 111-155), 6 (day 155-181)

c Feeding with VFA-mixture

d Due to the poor accuracy in the measurement of the CH_4 production at low COD_{in}, the CELLS estimate was apparently negative

Table 5. Operational conditions and treatment efficiency of the lab-scale UASB reactor fed low strength whey wastewater (R4) at 30°C. Inoculation with 5 g VSS per liter reactor. Average values.

Parameter [a]		Experimental periods [b]								
		1 [c]	2				3	4	5	6
			A	B	C	D				
Operational:										
COD_{in}	(mg COD/l)	1715	1884	1342	1010	548	182	1434	491	339
HRT	(h)	17.6	14.4	15.0	15.1	15.0	19.0	16.2	5.0	5.1
OLR	(g COD/l·d)	2.4	3.2	2.1	1.6	0.9	0.2	2.2	2.4	1.6
SLR	(g COD/g VSS·d)	0.48	0.64	0.42	0.32	0.18	0.04	0.44	0.48	0.32
Efficiency (% COD_{in}):										
E		89	91	91	85	77	37	92	77	73
CELLS		16	12	4	10	6	1	5	23	28
M_{total}		73	79	87	75	71	36	87	54	45
$M_{dissolved}$		4	4	5	6	12	36	5	13	19
A		84	79	87	76	71	36	87	54	45

a Abbreviations are defined in Materials and Methods

b Periods: 1 (day 0-12), 2A (day 13-37), 2B (day 38-44), 2C (day 45-51), 2D (day 52-58), 3 (day 59-65), 4 (day 66-80), 5 (day 111-155), 6 (day 155-181)

c Feeding with VFA-mixture

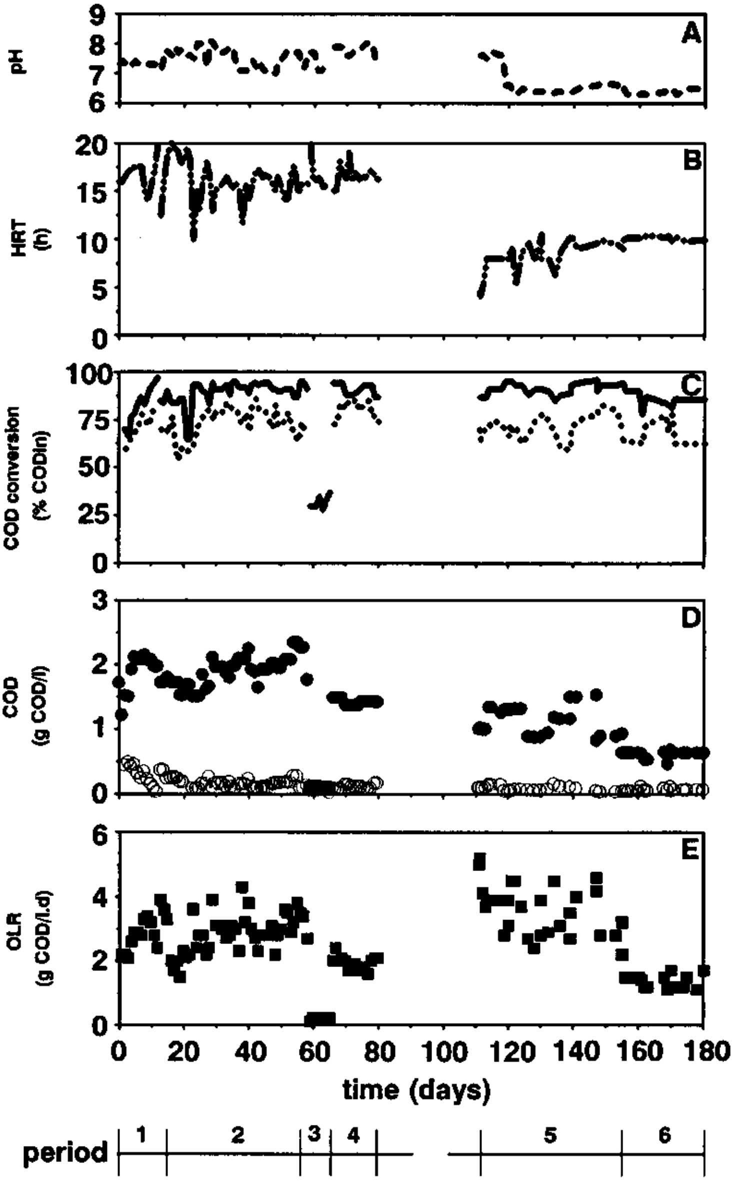

Figure 2. Operation and treatment efficiency of low strength whey containing wastewater in UASB reactor R3. A. pH (——). B. HRT (—••—) C. COD conversion (—— COD removal efficiency, ••• CH_4-COD removal). D. COD concentration (● influent, ○ effluent). E. OLR (■).

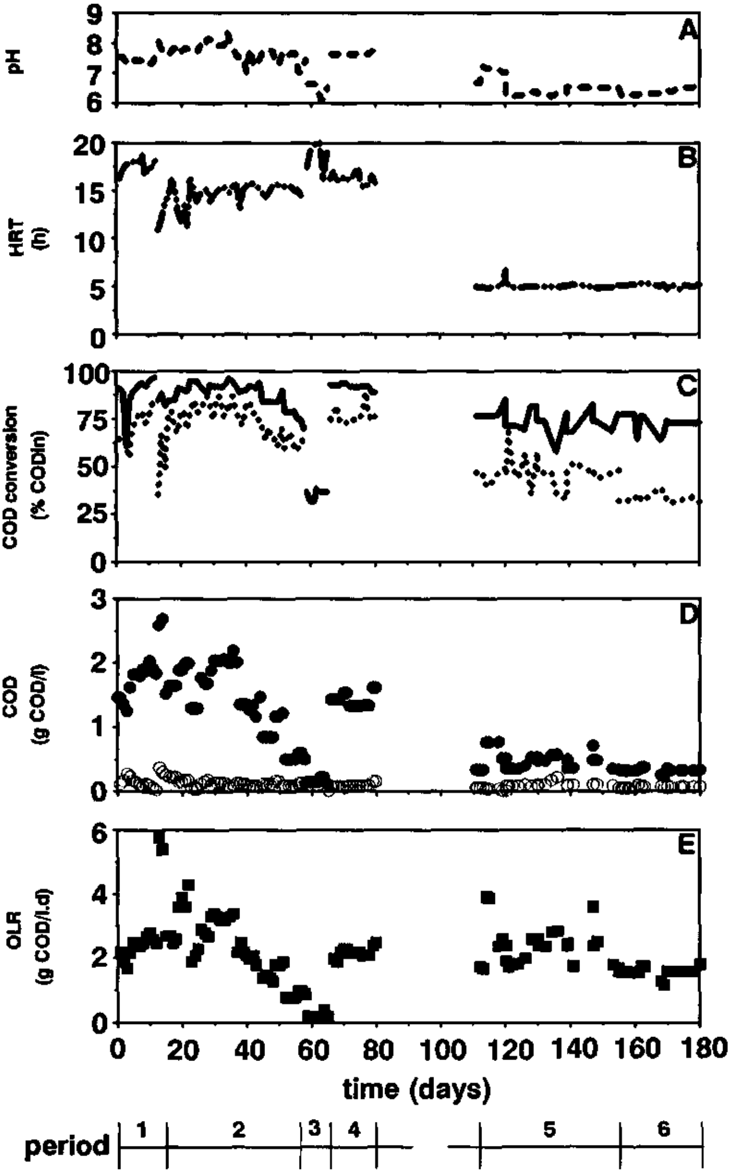

Figure 3. Operation and treatment efficiency of low strength whey containing wastewater in UASB reactor R4. Symbols: see Fig. 2.

at 491 mg/*l* (period 5, Table 5). Similar observation occurred at OLR in the range of 1.5-1.6 g COD/*l*·d (periods 2C and 6) where a decreased efficiency for the lower COD_{in} can again be observed. Additionally, VFA could almost not be detected in the effluent COD even when the treatment efficiency was low due to the low COD_{in}. This indicates that the acidification of whey was not complete in the reactor experiments. However, the whey anaerobic biodegradability test [26] conducted with an initial concentration of 2000 mg COD/*l* and pH between 6 and 7, showed that more than 95% could be converted to methane and cells after 1 week (results not shown). Methane production occurring at pH values around 6 [33] was shown to be in the same levels as at pH 7.

As in the reactor experiment with ethanol, a similar change in the size and structure of the reactor granular sludges was also observed. However in both experiments, no clear evidence was shown in terms of deterioration which could negatively affect the sludge hold-up. Batch tests were also conducted to verify possible changes in the specific methanogenic activity of the sludges using VFA-mixture as the assay substrate. The results of this test are shown in Table 6. Compared with the values at day 0, a significant increase (50%) in the specific methanogenic activity of the sludges was observed by the end of the experiment (day 181).

4. Discussion

The results of this study reveal that the presence of dissolved oxygen in UASB influents has no detrimental effect on the anaerobic treatment of low strength wastewaters. The potential danger of dissolved oxygen present in the influent of dilute wastewaters was evaluated, since the acetogens and methanogens are generally considered to be highly sensitive to oxygen toxicity.[24,25] However in practice, they are adequately protected by facultative bacteria in the granular sludge consortium. The important role of facultative bacteria present in mixed cultures on protecting strict anaerobes was demonstrated in previous research.[27,28,34] The rapid consumption of oxygen by facultative bacteria and the creation of anaerobic microniches inside the granules are regarded as the main factors of such protection. These findings were confirmed in this study with parallel reactors fed with oxygenated and deaired influents containing ethanol as a substrate. The same values of redox potentials that were found in the effluents of both reactors and the comparable treatment performance demonstrate the rapid consumption of oxygen. Therefore, we can conclude that

Table 6. The specific methanogenic activity of the sludge in the reactors R3 and R4 during continuous operation of UASB fed whey low strength wastewater (determined in batch assays).

Time	Specific methanogenic activity (g COD-CH_4/g VSS·d)	
	Reactor R3	Reactor R4
	VFA-mixture	VFA-mixture
day 0 [a]	1.01	1.01
day 80 [b]	1.35	1.41
day 181 [c]	1.44	1.55

[a] Start period 1
[b] End period 4
[c] End period 6

in practice the presence of dissolved oxygen will be of minor importance since no detrimental effect was found.

Also in this study, the treatability of low strength soluble wastewaters in UASB reactors is demonstrated by the high COD removal efficiencies obtained. This finding confirms earlier reports from full-scale UASB reactors satisfactorily treating low strength domestic sewage and recycle paper industry wastewater.[5,13,14] Therefore, the potentials of the UASB can no longer be regarded as restricted to medium and high strength wastewaters. Wastewaters containing simple or complex substrates such as ethanol and whey were treated efficiently at COD_{in} below 1000 mg/*l*.

A distinction was observed in this study between the treatment of the two wastewaters. The treatment performance of the ethanol-fed reactors was always better than that of the whey-fed reactors. This is demonstrated by the higher COD removal efficiencies at similar COD_{in} values. The acidification of whey at low COD_{in} concentrations was slower than that of ethanol. Whey, which contains some proteins and fat,[16,20,35] requires hydrolysis and acidification steps to form intermediates like propionate and acetate. At COD_{in} higher than 630 mg/*l* these steps were not a problem, since the COD converted to VFA, methane and cells was higher than 90%. Similar results were also obtained in the anaerobic biodegradability test which revealed that 95% of the COD was biodegradable. However, at COD_{in} less than 630 mg/*l*, a significant fraction of the COD was not converted. The fact that the unconverted COD in the effluent could not be accounted for by VFA, indicates that at the low concentrations mainly hydrolysis and acidification were the rate limiting steps. A gradual decrease in the COD removal efficiency was found to be associated with decreasing COD_{in} below 1000 mg/*l*. A sharper decrease occurred when the COD_{in} dropped below 200 mg/*l*. The effect of lowering the substrate level inside the UASB reactors on the treatment performance can be explained by Monod kinetics. Apparent K_S values of granular sludge from UASB reactors for the overall COD conversion rate found in previous research with spoiled beer waste were in the range of 90.5 to 429.7 mg COD/*l*.[36] In the whey-fed reactors of this study effluent values of 92 to 166 mg COD/*l* were found when COD_{in} values ranging from 182 to 1844 mg/*l* were applied (Table 5). Therefore, a relatively high apparent K_S value can also be presumed to have prevailed in our experiments. Consequently, the resulting COD removal efficiency is lower for the lower COD_{in}. The overall COD removal rate of ethanol was dependent on the rate of acetoclastic methanogenesis. The higher COD removal efficiency in the ethanol-fed reactors compared with the whey-fed reactors at low COD_{in} can be explained by the lower values of apparent K_S for acetoclastic methanogenesis compared with whey acidification. Values of apparent K_S for acetoclastic methanogenesis ranging from 30 to 200 mg COD/*l* were determined in previous research using granular sludge from UASB reactors.[21,22,23] The value of apparent K_S strongly depends on the mixing intensity inside the reactor. The mixing in the UASB reactor in turn is very dependent on the gas production.[37]

A high gas production in the UASB reactor is very important for obtaining an optimal wastewater-biomass contact due to the improved natural mixing of the bulk liquid phase, and also for energy recovery due to the methane production. To obtain high gas production, high OLRs should be applied. In this study, the SLRs were far below the maximum capacity of the reactor sludges. Additionally, the amount of biomass utilized in the reactor experiments was relatively low. Consequently in practice, it is expected that higher OLRs can be accommodated in the UASB reactor treating dilute wastewaters, since higher concentrations of active VSS can be utilized resulting in higher gas production. In this way, it is expected that the mixing intensity can be increased which may result in lowering the apparent K_S value. Previous experiments demonstrated the importance of high mixing levels obtained by high effluent recirculation.[28] An extremely low apparent K_S of less than 10 mg COD/*l* was observed for acetoclastic methanogenesis, resulting in a high treatment efficiency for levels as low as 100 to 200 mg COD/*l* influent.

Energy recovery from biogas can also be expected to be of importance in UASB reactors treating low strength wastewaters. This study showed that at COD_{in} below 1000 mg/*l*, in general 60 % and 80% of the COD was recovered as total methane from whey and ethanol, respectively. Considering that only 15% of the total methane was lost due to dissolved CH_4 in the effluent, the economic benefits due to the biogas production are quite considerable.

Based on the results of this study, recommendations for the lowest acceptable COD_{in} for the UASB treatment of soluble wastewaters were determined. In order to obtain COD treatment efficiencies of 85% or more, COD_{in} should be greater than 422 and 630 mg/*l* for ethanol and whey substrates, respectively. However, it is anticipated that even lower concentrations could be tolerated if higher OLRs were applied, since natural mixing from the extra biogas production would be expected to lower the apparent K_S.

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Chapter 5

Feasibility of Expanded Granular Sludge Bed Reactors for the Anaerobic Treatment of Low Strength Soluble Wastewaters

Mario T. Kato, Jim. A. Field, Paul Versteeg and Gatze Lettinga

Summary

The application of the expanded granular sludge bed (EGSB) reactor for the anaerobic treatment of low-strength soluble wastewaters using ethanol as a model substrate was investigated in laboratory-scale reactors at 30°C. Chemical oxygen demand (COD) removal efficiency was above 80% at organic loading rates up to 12 g COD/$l \cdot$d with influent concentrations as low as 100 to 200 mg COD/l. These results demonstrate the suitability of the EGSB reactor for the anaerobic treatment of low-strength wastewaters. The high treatment performance can be attributed to the intense mixing regime obtained by high hydraulic and organic loads. Good mixing of the bulk liquid phase for the substrate-biomass contact and adequate expansion of the sludge bed for the degassing were obtained when the liquid upflow velocity (V_{up}) was greater than 2.5 m/h. Under such conditions, an extremely low apparent K_S value for acetoclastic methanogenesis of 9.8 mg COD/l was observed. The presence of dissolved oxygen in the wastewater had no detrimental effect on the treatment performance. Sludge piston flotation from pockets of biogas accumulating under the sludge bed occurred at V_{up} lower than 2.5 m/h due to poor bed expansion. This problem is expected only in small diameter laboratory-scale reactors. A more important restriction of the EGSB reactor was the sludge washout occurring at V_{up} higher than 5.5 m/h and which was intensified at organic loads higher than 7 g COD/$l \cdot$d due to buoyancy forces from the gas production. To achieve an equilibrium between the mixing intensity and the sludge hold-up, the operation should be limited to an organic loading rate of 7 g COD/$l \cdot$d and to a liquid upflow velocity between 2.5 and 5.5 m/h.

Key words: granular sludge bed • wastewater, low-strength soluble • dissolved oxygen • sludge hold-up • anaerobic treatment

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1. Introduction

Anaerobic treatment processes are utilized for the elimination of readily biodegradable organic matter from wastewaters. The number of full-scale plants has increased rapidly since the development of highly efficient anaerobic bioreactors. In these reactors, active biomass is immobilized as biofilms on carriers, such as in the anaerobic filter [26] or as sludge aggregates in the form of dense granules, as in the upflow anaerobic sludge blanket (UASB).[18,19] Consequently, very high volumetric organic loading rates can be applied, which considerably exceed those of the aerobic activated sludge process. However, to date, most anaerobic reactors are designed for the treatment of medium- to high-strength industrial wastewaters only. In recent years, attempts have also been made toward the anaerobic treatment of lower strength industrial wastewaters of less than 1 g chemical oxygen demand (COD)/*l*. Such effluents are exemplified by wastewaters from alcoholic and soft drink bottling, paper manufacturing, fruit and vegetable canneries, malting, and brewing.[4,10,15,20] However, there are a number of problems associated with the anaerobic treatment of low-strength wastewaters.[3,6,16,23]

These problems can be attributed to the low substrate concentration and possibly in some cases to the presence of oxygen. The low COD concentrations of the influent result in very low substrate levels inside the reactor. Due to Monod kinetics, the actual sludge activity would be far from optimal. Apparent K_S values reported for acetoclastic methanogenesis in sludge granules range from 30 to 200 mg COD/*l*.[5,22] The low substrate concentration will also result in less biogas production, and consequently, a lower mixing intensity and a poorer substrate-biomass contact will be the result. Additionally, the suboptimal biological activity prevailing in the reactors under these conditions means that perhaps the loading rate limitation will no longer be the organic loading, as is the case with medium- to high-strength wastewaters. Instead, the loading rate limiting parameter is the maximum hydraulic load which can be tolerated prior to biomass washout due to excessive sludge bed expansion. Low-strength wastewaters often also contain dissolved oxygen. Since the methanogens are regarded as strict anaerobes,[8,25] oxygen might represent a potential danger in the anaerobic treatment.

A modification of the UASB reactor is the expanded granular sludge bed (EGSB), which has primarily been developed in order to improve the wastewater-biomass contact during anaerobic treatment by expanding the sludge bed and intensifying the hydraulic mixing.[14,21] In this system, higher superficial liquid velocity can be achieved by applying recirculation. This reactor type is typically characterized by a relatively high height-diameter ratio, but shallow reactors can also be used. With the use of effluent recirculation, liquid upward velocities exceeding 5 to 6 m/h can be achieved, which is significantly higher than the 0.5 to 1.5 m/h range generally applied for UASB reactors.[21]

An EGSB reactor uses a fully or partially expanded bed of granules, and the system stability relies on the high settleability and mechanical stability of these granules. While on the one hand a high liquid upflow velocity is used to improve the mixing regime, on the other hand a higher sludge washout may result. Additionally, sludge erosion may also be enhanced. Therefore, a decrease of the maximum sludge hold-up is expected, which is one of the major factors dictating the maximum potential organic load. Consequently, a balance must be found with respect to the liquid upflow velocity needed for sufficient mixing and that for maintaining the sludge hold-up at a high level. So far, few data are available on the application of EGSB reactors for the anaerobic treatment of very-low-strength soluble wastewaters. The objective of this study was to study their feasibility for such applications. An optimal range of liquid upflow velocity should be established for the process performance, with respect to the treatment efficiency and system stability. For this purpose, the equilibrium between good mixing and minimum sludge washout was evaluated at a variety of relevant operational conditions, such as substrate concentrations, and organic and hydraulic loading rates. Additionally, the potential danger of high levels of oxygen intake was investigated by operating reactors in parallel with and without dissolved oxygen in the influent. These experiments were conducted in EGSB reactors of 2.5 *l* fed with ethanol.

2. Materials and Methods

2.1. Biomass and basal medium

The anaerobic granular sludges used in all the experiments were obtained from a full-scale UASB reactor treating alcohol distillery wastewater (Nedalco, Bergen op Zoom, The Netherlands). The sludges were elutriated to remove the fines and again stored at 4°C until used. The physical characteristics of these sludges were determined in a previous experiment.[12] The total suspended solids (TSS) and volatile suspended solids (VSS) content were 8.0% and 7.5%, respectively. The density, mean settling velocity, and granule diameter were 1019 g/l sludge, 35 m/h, and 1.3 mm, respectively. The granule size distribution test revealed that 28% of the sludge weight was in the diameter range between 0.6 and 1.0 mm, 42% between 1.0 and 1.5 mm, and 30% between 1.5 and 1.9 mm.

For the batch methanogenic activity assay a concentrated stock solution of essential inorganic macro- and micronutrients was prepared. After dilution, the basal medium used in this assay contained (in mg/l): NH_4Cl, 280; $K_2HPO_4 \cdot 3H_2O$, 327.4; $MgSO_4 \cdot 7H_2O$, 100; $CaCl_2 \cdot 2H_2O$, 10; yeast extract, 100; H_3BO_3, 0.05; $FeCl_2 \cdot 4H_20$, 2; $ZnCl_2$, 0.05; $MnCl_2 \cdot 4H_2O$, 0.05; $(NH_4)6Mo_7O_{24} \cdot 4H_2O$, 0.05; $AlCl_3 \cdot 6H_2O$, 0.09; $CoCl_2 \cdot 6H_2O$, 2; $NiCl_2 \cdot 6H_2O$, 0.05; $CuCl_2 \cdot 2H_2O$, 0.03; $NaSeO_3 \cdot 5H_2O$, 0.1; ethylenediaminetetraacetic acid (EDTA), 1; resazurin, 0.2; and 36% HCl, 0.001 ml/l. For the continuous experiments other similar standard solutions of macro- and micronutrients were prepared, as described elsewhere.[22] These solutions were supplied to the influent at a dilution of 6 and 0.1 ml/l influent for the macro- and micronutrients, respectively. Alkalinity in the batch and continuous experiments was provided as sodium bicarbonate in an amount depending on the type and concentration of the substrate utilized.

2.2. Analyses and chemicals

Ethanol and volatile fatty acids (VFA) were determined with a Hewlett Packard 5890 gas chromatograph (Palo Alto, CA). The 2-m × 2-mm glass column was packed with Supelcoport (100 to 200 mesh) coated with 10% Fluorad FC 431. The temperature of the column was set at 70° and 130°C, for ethanol and VFA, respectively. The temperatures of the injection port and flame ionization detector were 220° and 240°C, respectively. Nitrogen saturated with formic acid was used as carrier gas at a flow of 40 ml/min. Before use, the chromatograph was calibrated with standard solutions of ethanol or VFA.

The pH and redox potential were determined with a Knick 510 pH/mV-meter (Berlin, Germany). Measurements of pH were conducted immediately after sampling with a Schott Nederland N61 double electrode (Tiel, The Netherlands). Redox potential was measured in situ at the influent and effluent lines of two parallel oxygen experiment reactors. The measurements were conducted with combined platinum indicating and silver chloride reference electrodes (Schott Nederland PT 6180). The COD, TSS, and VSS were determined according to the Standard Methods.[2]

The biogas composition of the continuous reactors was determined immediately after sampling with a syringe at a gas sampling port. The CH_4, CO_2, and H_2S were analyzed with the same sample of 100 μl injected into a Packard Becker 433 chromatograph (Delft, The Netherlands), equipped with two columns connected in parallel (split 1 : 1). One column of 1.5 m × 1/8 in. Teflon was packed with chromosorb 108 (60 to 80 mesh) and the other of 1.2 m × 1/8 in. steel with molecular sieve 5A (60 to 80 mesh). The oven, injection port, and detector temperatures were 40°, 110°, and 125°C, respectively. Helium was used as carrier gas (45 m*l*/min). The H_2 was analyzed with a 1000-μl gas sample in an HP 5890 chromatograph equipped with a 1.5-m × 2-mm steel column packed with molecular sieve 5A. The temperatures of the column, injection port, and thermal conductivity detector were 40°, 110°, and 125°C, respectively. Argon was used as carrier gas at a flow of 20 m*l*/min. All gas sample analyses were conducted after calibration with standards of known amounts of the respective gases.

The concentrations of ethanol and VFA as well as the methane production are referred to in COD units. Conversion factors utilized were 2.087 g COD/g ethanol, 1.067 g COD/g acetate (C_2), 1.515 g COD/g propionate (C_3), and 1.820 g COD/g butyrate (C_4). For methane, a factor of 2.577 g COD/*l* CH_4 at 30°C was utilized. When needed, a factor of 1.42 g COD/g VSS was used. The COD, ethanol, and VFA of reactor effluents are referred to samples centrifuged at 17,000 *g* for 4 min.

Except for the sodium bicarbonate added to the influent of the reactors, all other chemicals were of analytical grade and purchased from Merck (Darmstad, Germany). Exceptions were the yeast extract from Gist-Brocades (Delft, The Netherlands); resazurin from Fluka (Buchs, Switzerland); and the gases from Hoekloos (Schiedam, The Netherlands).

2.3. Batch methanogenic activity assay

The specific methanogenic activity of the sludges was determined using 0.6-*l* glass serum flasks sealed with a rubber septum and a screw cap under static conditions and in duplicate. The sludge was added to the flasks and then the liquid volume was completed to 0.5 *l* with the basal mineral medium solution, bicarbonate, and tap water containing 4 g COD/*l* of ethanol, acetate, or VFA-mixture solution as substrate. The final concentration of the sludges was 1.5 g VSS/*l*. The used acetate and VFA solutions were neutralized. The composition of the VFA-mixture solution was 24(C_2) : 34(C_3) : 41(C_4) on a COD basis. After flushing the medium with nitrogen gas, the flasks were sealed and incubated in a temperature-controlled room at 30 ± 2°C. The assays were provided with a second feeding when more than 80% of the substrate COD supplied in the first feeding was converted to methane. Monitoring consisted of periodic measurements of methane production by modified Mariotte flasks. These flasks contained a 3% (w/v) NaOH solution to remove the carbon dioxide from the biogas. The maximum specific methanogenic activity was calculated from the slope of the methane production versus time curve.

2.4. Sludge bed expansion test

Before starting the continuous experiments in EGSB reactors, the expansion of the sludge bed in relation to the upflow liquid and gas velocities was assessed in short term tests of 10 min for each flow condition. In these tests conducted at 30°C, we used three different concentrations of biomass (10, 20, and 30 g VSS/*l*) and operated a reactor under several conditions of hydraulic and gas flow by applying dilution water alone and with air injection, respectively. The hydraulic flow was adjusted to reach the maximum possible upflow velocity which could be tolerated without sludge washout. To assess the effect of the biogas production, we injected small air bubbles into the reactor; a gas flow up to 15 *l*/d corresponding to a V_{gas} of 0.32 m/h was adjusted based on that anticipated for the EGSB experiments. Monitoring consisted of measuring the sludge washout and the expansion of the sludge bed, which is referred to in percentage in relation to its height without any flow. All the used sludge samples were later refused.

2.5. Continuous experiments in EGSB reactors

In this study, EGSB experiments were conducted with low-strength ethanol-containing wastewater with and without oxygen in the influent. Two 2.5-*l* glass reactors (R1 and R2) with a height of 1.0 m and internal diameter of 5 cm were used in a temperature-controlled room at 30 ± 2°C . Figure 1 shows the schematic diagram of the used EGSB reactor systems.

The reactor R1 was operated under different operational conditions of hydraulic load and substrate concentrations and without oxygen supply. Sodium sulfide was supplied to the influent at a rate of 10 mg S^{-2}/l in order to remove all oxygen. Reactor R2 was operated in parallel under very similar conditions, except that the dilution water was aerated to ensure a significant concentration of oxygen (average of 3.8 mg O_2/l influent). The seed sludge for both reactors (10 g VSS/*l*) had already been used in an experiment with laboratory-scale UASB reactors fed low-strength ethanol solution during 4 months. The two reactors were run using two different main ranges of influent concentrations: the first at concentrations between 500 and 700 mg COD/*l* and the second at concentrations between 100 and 200 mg COD/*l*. In each influent concentration range, different hydraulic and organic loads were imposed to the systems, resulting in nine separate experimental periods. The applied hydraulic retention time (HRT) was varied from 0.09 to 2.1 h, resulting in organic loading rates (OLR) up to around 39 g COD/*l*·d.

The performance of the EGSB reactors was followed and evaluated using the following parameters: COD_{in} = influent COD (mg COD/*l*); COD_{ef} = effluent COD (mg COD/*l*); EOH_{ef} = effluent ethanol concentration as % of COD_{in}; VFA_{ef} = effluent VFA concentration as % of COD_{in}; M_{total} = % conversion COD_{in} to methane (methane measured with gas meter plus methane dissolved in the effluent); $M_{dissolved}$ = methane dissolved in the effluent in % of COD_{in} (maximum solubility of methane estimated according to Henry's law); A = % conversion COD_{in} to VFA ($A = M_{total} + VFA_{ef}$); E = % COD_{in} removed based on the COD_{ef}; CELLS = % conversion COD_{in} to cells (estimation of cells = $E - M_{total}$); OLR = space organic loading rate (g COD/*l*·d); SLR = sludge loading rate (g COD/g VSS·d); O_2LR = oxygen loading rate (g O_2/l·d).

At least 10 hydraulic retention times were allowed to pass after each change in the COD_{in} range, hydraulic retention time, and liquid upflow velocity (V_{up}) before analyses and data were taken. Data obtained during the first day after the changes were only considered in the cases when the results typically indicated steady state.

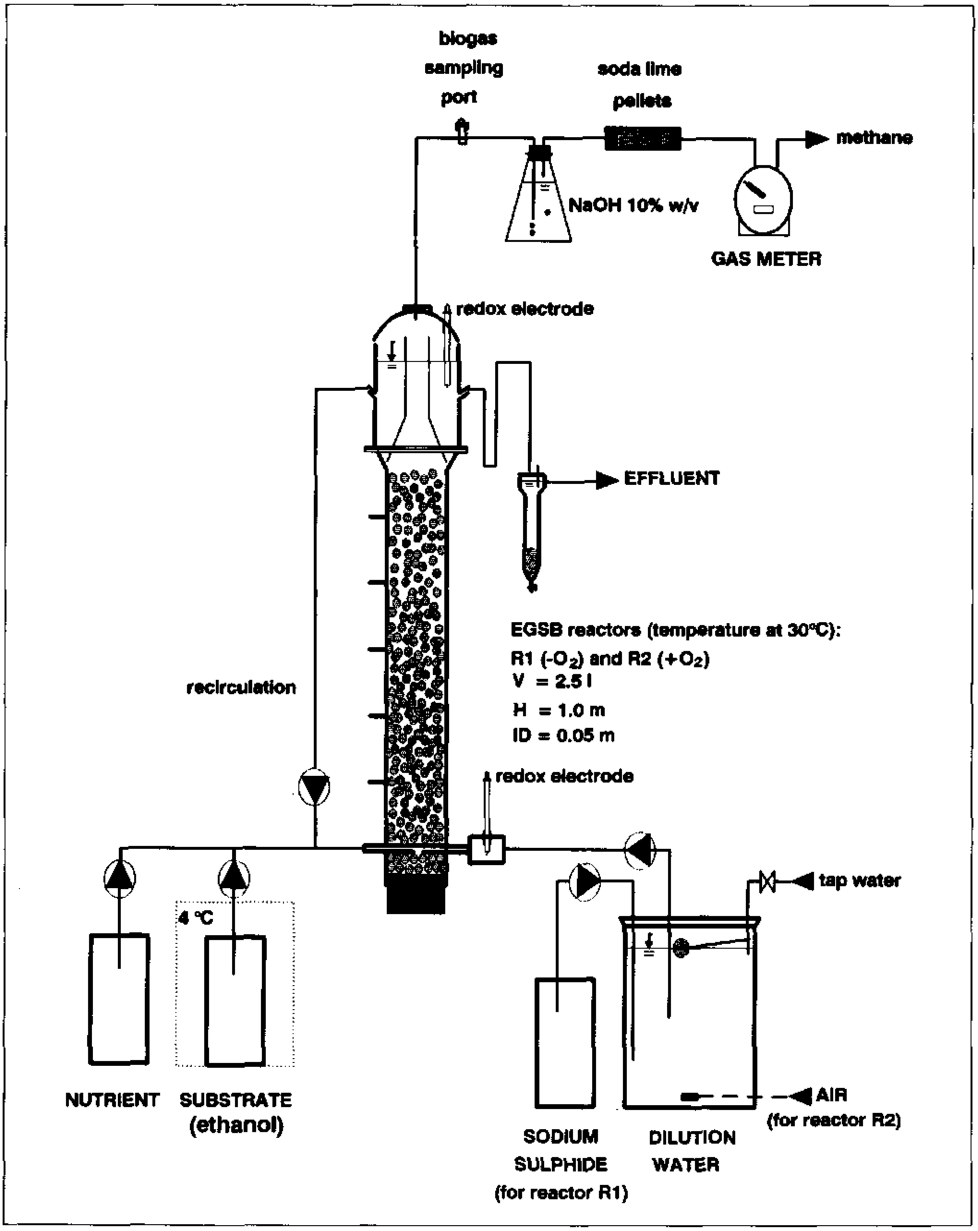

Figure 1. Schematic diagram of 2.5-*l* EGSB reactors: reactors R1 (without oxygen supply: -O_2) and R2 (with oxygen supply: +O_2).

3. Results

3.1. The treatment efficiency in the EGSB reactor

The treatment efficiency has been studied in relation to the hydraulic and organic loading rate. Two laboratory-scale EGSB reactors R1 and R2 were operated without and with dissolved oxygen, respectively. They were run in parallel with the objective of establishing the continuous anaerobic treatment of low-strength ethanol-containing wastewater. The applied average values of COD_{in}, HRT, OLR, SLR, and O_2LR, and the assessed treatment efficiencies (E, M_{total}, $M_{dissolved}$, A) in the various periods of the experiments are listed in Tables 1 and 2 for reactors R1 and R2; respectively. The operational conditions and treatment performance are also shown in Figures 2 and 3 for reactors R1 and R2, respectively.

During the first three periods of the experiments (days 0 to 43) the reactors were fed at COD_{in} levels ranging from 500 to 700 mg COD/*l* (Tables 1 and 2). Each period corresponded to one range of OLR at HRTs varying from 2.1 to 0.5 h. A high treatment performance with COD removal efficiency exceeding 90% was obtained at OLRs up to 7 g COD/*l*·d and exceeding 80% at OLRs up to 12 g COD/*l*·d. Even when the reactors were operated up to loading rates of 32 g COD/*l*·d, the efficiency still remained above 55%. During periods 4 to 9 (days 43 to 91), the reactors were fed at COD_{in} values ranging from 100 to 200 mg COD/*l* at HRTs varying from 1 to 0.09 h. As in the previous periods almost the same pattern of treatment performance was obtained at OLRs up to 12 g COD/*l*·d, with COD removal efficiency above 80%. However, at higher OLRs, the efficiency decreased more sharply compared with the previous periods. This was particularly the case at loads near 30 g COD/*l*·d.

The occurrence of high levels of oxygen intake in EGSB reactors treating low-strength wastewaters could potentially affect the methanogens detrimentally because they are usually regarded as strict anaerobes. We evaluated the potential toxicity of dissolved oxygen by comparing the treatment performance and from the measured redox potential as well. Comparing Figures 2 and 3, the results show the lack of any significant difference in the treatment performance and effluent redox (around -400 mV) of the parallel reactors. This fact indicates that dissolved oxygen was rapidly consumed and did not affect the treatment performance.

Table 1. Operational conditions and treatment efficiency of the laborarory-scale EGSB reactor fed low-strength ethanol wastewater without oxygen supply (R1) at 30°C. [a]

Parameter [b]		Experimental periods [c]								
		1 [d]	2	3	4 [e]	5	6	7 [e]	8	9
Operational										
COD_{in}	(mg COD/l)	641	613	675	196	154	223	133	146	147
HRT	(h)	1.9	1.0	0.5	1.0	0.5	0.2	0.5	0.2	0.09
OLR	(g COD/l·d)	8.1	14.7	32.4	4.7	7.4	26.8	6.4	17.5	39.2
SLR	(g COD/g VSS·d)	0.81	1.47	3.24	0.47	0.74	2.68	0.64	1.75	3.92
O_2LR	(g O_2/l·d)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Efficiency (% COD_{in})										
E		92.3	76.2	72.6	96.9	88.7	28.1	89.2	66.6	26.2
CELLS		30.0	19.3	36.4	30.1	17.0	-5.6	17.1	4.9	-25.8
M_{total}		62.3	56.9	36.2	66.8	71.7	33.7	72.1	71.5	52.0
$M_{dissolved}$		11.7	12.2	11.1	38.3	48.7	33.6	56.4	51.4	51.0
A		70.0	80.7	63.6	69.9	83.0	94.6	82.9	98.2	96.3

a Inoculation with 10 g VSS/*l* reactor. Average values.
b Abbreviations are defined in Materials and Methods.
c Periods: 1 (days 0-18), 2 (18-26), 3 (26-43), 4 (58-67), 5 (67-76), 6 (76-77), 7 (77-87), 8 (87-89), 9 (89-91).
d Inoculation with Nedalco sludge from 4-month operation of a laboratory-scale UASB reactor treating low-strength ethanol-containing wastewater.
e New inoculation with stored Nedalco sludge from full-scale UASB reactor.

Table 2. Operational conditions and treatment efficiency of the laboratory-scale EGSB reactor fed low-strength ethanol wastewater with oxygen supply (R2) at 30°C. [a]

Parameter [b]		Experimental periods [c]								
		1 [d]	2	3	4 [e]	5	6	7 [e]	8	9
Operational:										
COD_{in}	(mg COD/l)	560	504	579	163	127	127	127	148	148
HRT	(h)	2.1	1.0	0.5	1.0	0.5	0.2	0.5	0.2	0.12
OLR	(g COD/l·d)	6.4	12.1	27.8	3.9	6.1	15.2	6.1	17.8	29.6
SLR	(g COD/g VSS·d)	0.64	1.21	2.78	0.39	0.61	1.52	0.61	1.78	2.96
O_2LR	(g O_2/l·d)	0.04	0.09	0.19	0.09	0.19	0.47	0.19	0.47	0.78
Efficiency (% COD_{in}):										
E		94.4	82.9	55.8	96.8	92.2	69.0	80.4	69.1	45.1
CELLS		23.8	28.9	21.0	18.4	23.8	7.3	7.8	14.7	-6.3
M_{total}		70.6	54.0	34.8	78.4	68.4	61.7	72.6	54.4	51.4
$M_{dissolved}$		13.4	14.9	13.0	46.0	59.1	59.1	59.1	50.7	50.7
A		76.2	71.1	79.0	81.6	66.9	92.7	91.1	82.5	88.9

[a] Inoculation with 10 g VSS per liter reactor. Average values.

[b] Abbreviations are defined in Materials and Methods.

[c] Periods: 1 (days 0-18), 2 (18-26), 3 (26-43), 4 (58-67), 5 (67-76), 6 (76-77), 7 (77-87), 8 (87-89), 9 (89-91).

[d] Inoculation with Nedalco sludge from 4-month operation of a laboratory-scale UASB reactor treating low-strength ethanol-containing wastewater.

[e] New inoculation with stored Nedalco sludge from full-scale UASB reactor.

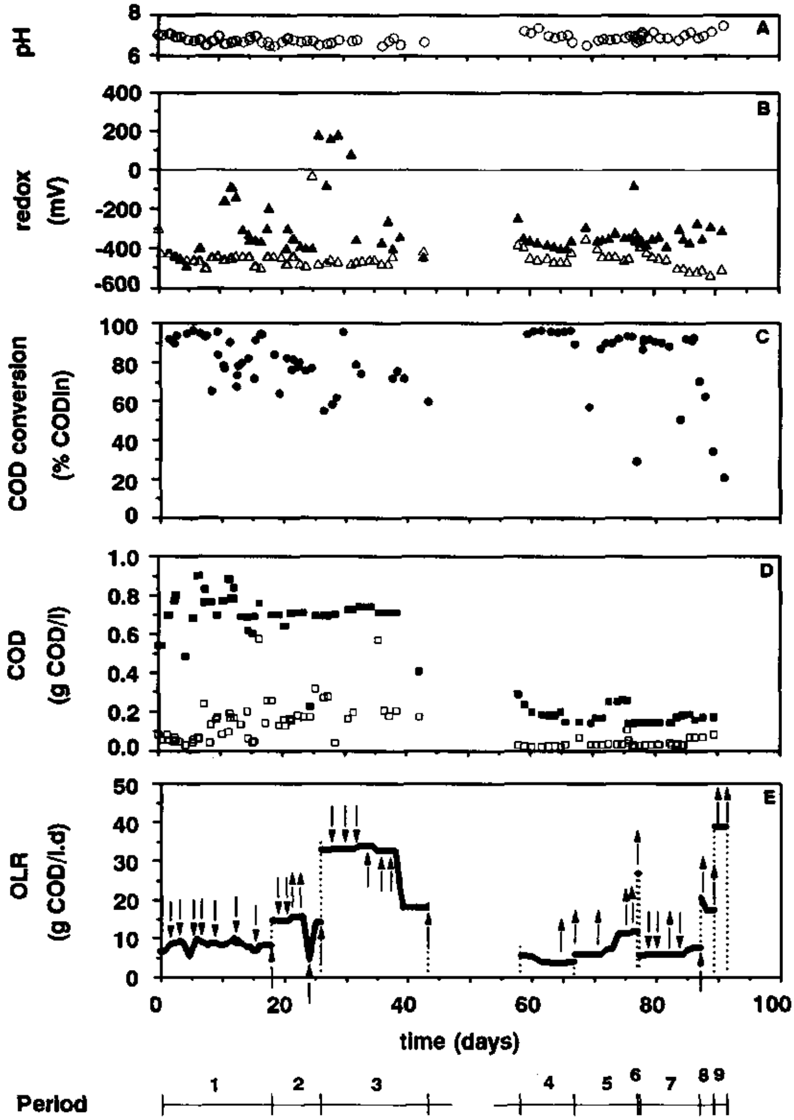

Figure 2. Operation and efficiency of low-strength ethanol-containing wastewater in EGSB reactor R1 ($-O_2$): A. pH (○); B. potential redox (▲ influent, △ effluent); C. COD conversion (●); D. COD concentration (■ influent, □ effluent); E. Organic loading rate (——). Up arrow means occurrence of sludge washout; down arrow means occurrence of sludge flotation.

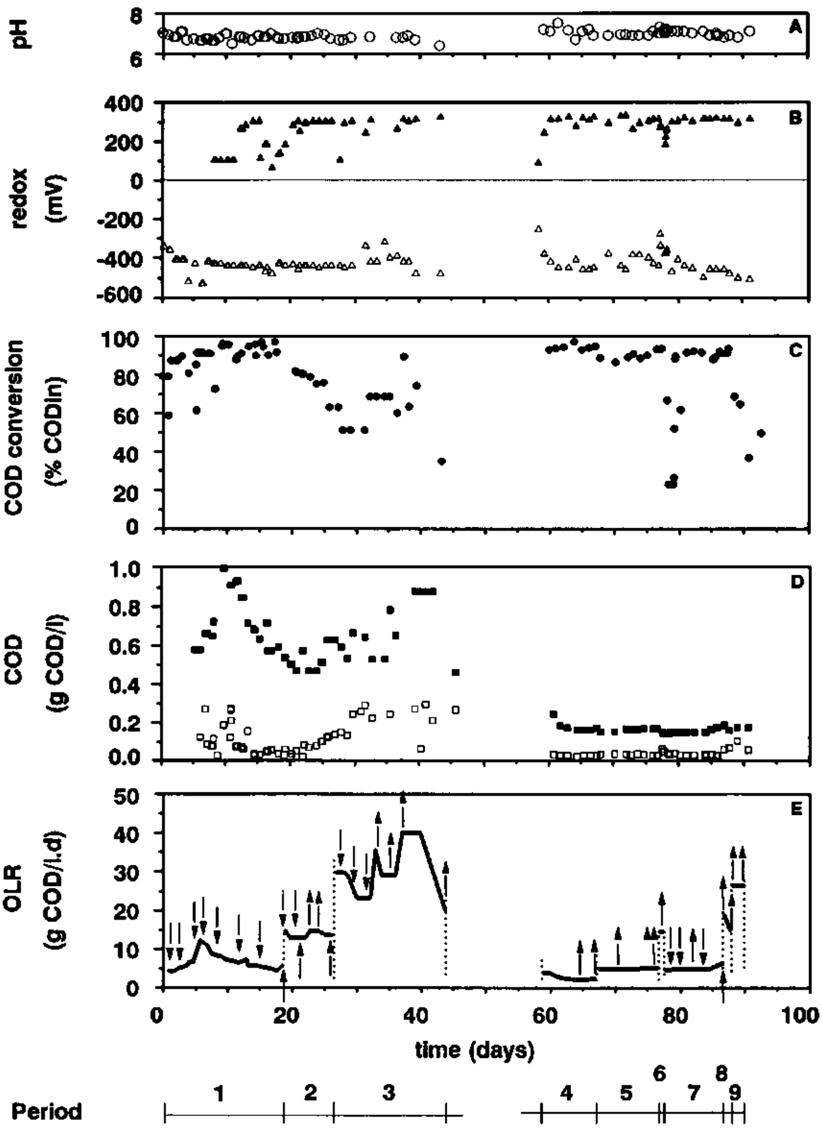

Figure 3. Operation and efficiency of low-strength ethanol-containing wastewater in EGSB reactor R2 ($+O_2$). See Fig. 2 for definitions of symbols.

During each experimental period, different liquid upflow velocities were applied by using effluent recirculation. Figure 4 shows the assessed correlation between efficiency and liquid upward velocity for each range of OLR. The results for both reactors reveal that a better efficiency is obtained at V_{up} exceeding 2.5 m/h. This is an indication of better wastewater-biomass contact as a result of the sludge bed expansion and improved mixing conditions in the reactors.

The effect of OLR on the efficiency for different COD_{in} is manifested in results shown in Figure 5. For comparable values of OLRs there is no clear difference in the efficiency at values up to 7 g COD/$l\cdot$d . Under these conditions, the system was apparently underloaded. However, at OLR exceeding 12 g COD/$l\cdot$d, the efficiency decreased more sharply for COD_{in} values in the range of 100 to 200 mg COD/l than for the range of 500 to 700 mg COD/l. This indicates a clear effect of influent COD concentration on the efficiency. This fact could be expected on the basis of Monod kinetics.

According to Monod kinetics, the specific substrate utilization rate depends on the prevailing reactor substrate concentration. For methanogens this would best be represented by the VFA effluent, assuming an intense mixing regime in which the reactor contents would have a composition similar to the effluent. In our experiments, the VFA in the effluent mainly consisted of acetate (results not shown). The assessed specific COD removal capacity in relation to the VFA effluent is shown in Figure 6. In order to estimate the kinetic parameters, we classified all available data in two ranges of V_{up} using a Lineweaver-Burk plot. In this way, for $V_{up} \leq 2.5$ m/h we found a maximum specific COD removal rate and apparent K_S values of 1.28 g COD/g VSS$\cdot$d and 28.3 mg COD/l, respectively. For $V_{up} >$ 2.5 m/h, the kinetic parameters were 1.16 g COD/g VSS$\cdot$d and 9.8 mg COD/l, respectively. It is clear that values found for apparent K_S are exceptionally low. This also indicates the positive effect of sufficient bed expansion and mixing intensity levels, since the K_S value is distinctly lower at higher V_{up}.

3.2. Sludge bed expansion and sludge washout

Aside from the treatment efficiency, the feasibility of the EGSB system depends on the retention of sludge at the highest possible level under all relevant operational conditions. For this requirement, the expansion of the sludge bed in relation to the liquid and gas upflow

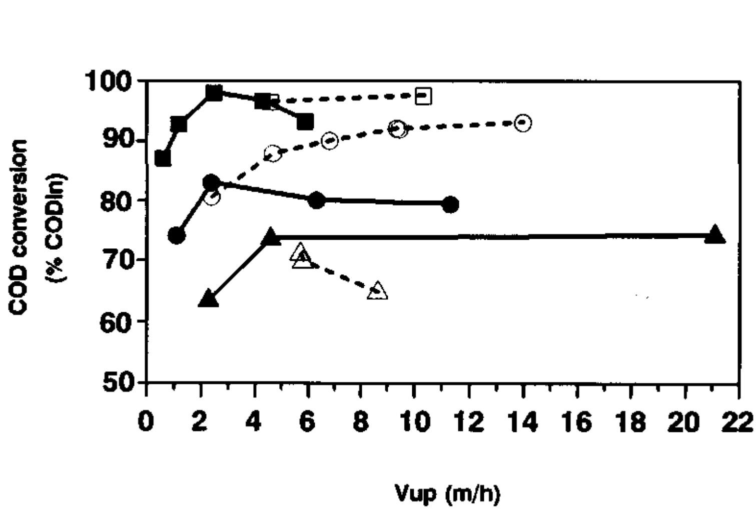

Figure 4. COD removal efficiency as a function of the liquid upflow velocity V_{up}: reactors R1 and R2. Solid and dashed lines refer to COD_{in} of 500 to 700 mg COD/*l* and 100 to 200 mg COD/*l*, respectively; values of organic loading rate (in g COD/*l*·d) are 6.9 (■), 12.1 (●), 32.1 (▲), 4.3 (□), 6.4 (○), and 18.7 (△).

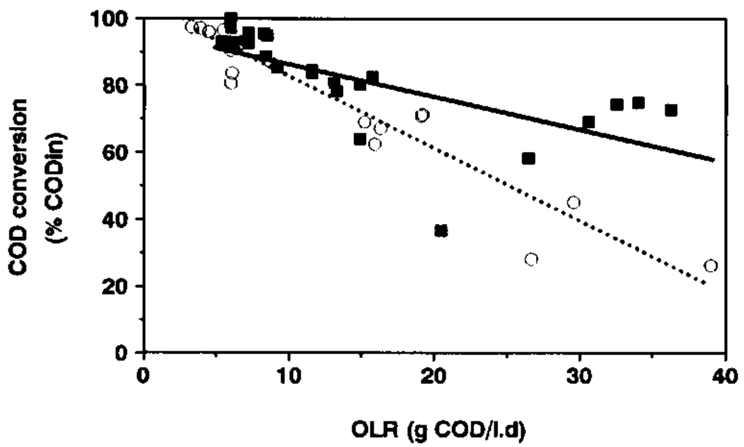

Figure 5. COD removal efficiency as a function of the organic loading rate: reactors R1 and R2. Symbols represent experimental data and lines the regression. Solid line (r^2 = 0.432) refers to COD_{in} of 500 to 700 mg COD/*l* (■); Dotted line (r^2 = 0.916) refers to COD_{in} of 100 to 200 mg COD/*l* (○).

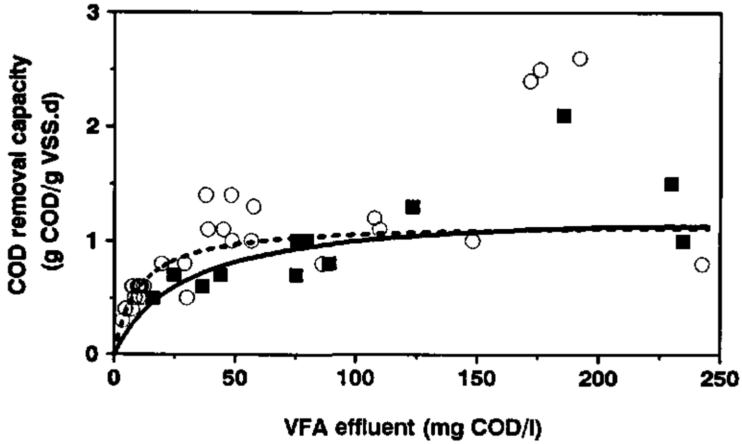

Figure 6. Role of effluent VFA concentration on the specific COD removal capacity of the reactor sludge. Data were fit linearly to a Lineweaver-Burk plot to determine the K_S values. Regression represented by the solid line was applied to data when $V_{up} \leq 2.5$ m/h (■); $r^2 = 0.714$; $K_S = 28.3$ mg COD/*l*; $V_{max} = 1.28$ g COD/g VSS·d; regression represented by the dashed line was applied to data when $V_{up} > 2.5$ m/h (○); $r^2 = 0.768$; $K_S = 9.8$ mg COD/*l*; $V_{max} = 1.16$ g COD/g VSS·d.

velocities are of primary importance for the granular sludge present or developing in the reactor. In this study, we decided to study the bed expansion under defined conditions utilizing the seed sludges. During the reactor operation, many other factors besides V_{up} and V_{gas} would be expected to affect the sludge bed dynamics, making it difficult to interpret the data.

In a first sludge bed expansion test, only the influence of the hydraulic flow was tested. We used Nedalco sludge samples after 4 months of operation of a laboratory-scale UASB reactor treating low-strength ethanol solution. An expansion of the sludge bed up to 400% (height 0.60 m) was obtained at 28 m/h with a concentration of 10 g VSS/*l*. At concentrations of 20 and 30 g VSS/*l*, a significant washout resulted at V_{up} of 25.5 and 22.9 m/h, respectively. Since we intended to apply higher values of V_{up}, we considered the use of 10 g VSS/*l* as the initial concentration of biomass in the EGSB experiments.

In a second sludge bed expansion test, considering the influence of both hydraulic and gas flow, we used stored Nedalco sludge since the previous sludge was no longer available. The results, shown in Figure 7, indicate that external gas additions at levels similar to those anticipated during normal OLR had a minimal effect in changing the expansion dynamics. The only noticeable effect was that the gas enhanced the washout of the fines from the sludge bed resulting in an apparent decrease in bed expansion.

After we started the EGSB experiments, in fact the reactors were difficult to operate during specific days of the experimental periods. Two problems with the sludge hold-up were encountered, namely, the sludge washout and piston formation (Figs. 2 and 3). A significantly high sludge washout occurred at liquid upflow velocities of 5.5 m/h due to the higher expansion of the sludge bed in the operating reactors compared with that of the sludge bed expansion tests. The upward velocity due to the gas production was always less than 0.32 m/h. Since the sludge bed expansion tests confirmed that the contribution of external gas flow on sludge washout was minimal, the effect of gas production on increasing sludge washout in the operating reactors must be due to buoyancy forces, caused by gas adsorbed or

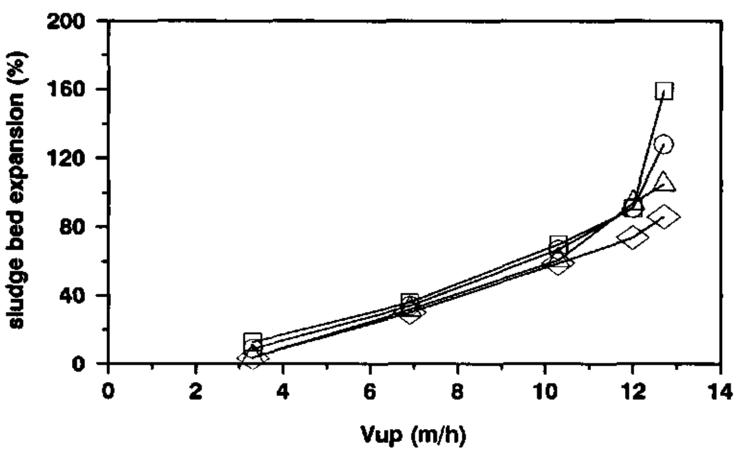

Figure 7. Sludge bed expansion test with stored Nedalco sludge from full-scale UASB reactor. The initial sludge bed height is 23 cm and the maximum expansion prior to washout would be equal to 335% under the conditions applied in this test. Values of V_{gas} applied (in m/h) are 0 (□), 0.08 (○), 0.24 (▵), and 0.32 (◇).

entrapped on or in the granules. During the days when the washout occurred, the sludge was separated and collected in a small external settler. When a significant amount accumulated, the sludge was returned to the reactors. During periods when the systems were operated under very high hydraulic and organic loads (HRT $\leq$ 0.5 h and OLR $\geq$ 12 g COD/$l\cdot$d), new start-ups (periods 4 and 7) were necessary because then too much sludge was lost (end of periods 3, 6, and 9).

Sludge piston formation occurred when the reactors were operated at low liquid upflow velocities ($\leq$2.5 m/h). Under this condition of low load, gas entrapment was observed generally starting at the bottom of reactors. This caused flotation of the sludge, lifting part or even the whole bed as a piston upward through the column. When this problem occurred, we temporarily stopped the reactor operation. We used a bar to break up and degas the floating piston.

3.3. Changes in sludge characteristics

In the experiments, we observed some gradual changes in properties of the reactor granule sludge. The main changes observed were in granule size and specific methanogenic activity. At the end of period 3 (day 43, COD_{in} 500 to 700 mg/l) and 9 (day 91, COD_{in} 100 to 200 mg/l) we compared the values of these characteristics with those found at the start of the concerning periods (days 0 and 77, respectively). The mean diameter of the granular sludges used at start of those periods was ca. 1.3 mm and increased to up to 5 mm during the course of the runs for both ranges of COD_{in}. Moreover, a higher mean diameter was found for the granules at the bottom (4 to 5 mm) of the reactors compared with those of the top (2 to 3 mm). Apparently some granule segregation occurred in the sludge bed. The settleability of the sludge remained excellent.

Values assessed for the specific methanogenic activity of the sludge samples are shown in Table 3. When the reactors were operated with COD_{in} in the range of 500 to 700 mg COD/l (days 0 to 43) the sludge activity increased more than twofold (using ethanol as assay substrate). On the other hand, when the reactors were operated at COD_{in} of 100 to 200 mg COD/l the specific activity decreased by 45%, 24% and 62% with ethanol, acetate, and VFA-mixture as assay substrates, respectively. Comparing the results with ethanol as assay substrate, they indicate that the specific methanogenic activity of the sludges is negatively

Table 3. Specific methanogenic activity of sludge in reactors R1 and R2 during continuous operation of EGSB fed ethanol low-strength wastewater (determined in batch assays).

Time	Specific methanogenic activity [a] (g COD-CH_4/g VSS·d)					
	Reactor R1 (-O_2)			Reactor R2 (+O_2)		
	Ethanol	Acetate	VFA-mixture	Ethanol	Acetate	VFA-mixture
day 0 [b]	1.2	n.d.	n.d.	1.2	n.d.	n.d.
day 43 [c]	2.3	n.d.	n.d.	1.8 (2.6) [d]	n.d.	n.d.
day 77 [e]	1.8	1.7	1.3	1.8	1.7	1.3
day 91 [f]	1.2	0.6	n.d.	0.9	0.4	0.5

a n.d. = not determined.

b Start of period 1 (COD_{in} 500-700 mg COD/*l*) with sludge Nedalco from 4-month operation of a laboratory-scale UASB reactor fed with ethanol low-strength wastewater.

c End of period 3 (COD_{in} 500-700 mg COD/*l*).

d Elutriated sludge.

e Start of period 7 (COD_{in} 100-200 mg COD/*l*) with stored Nedalco sludge from full-scale UASB reactor.

f End of period 9 (COD_{in} 100-200 mg COD/*l*).

influenced by the lower COD_{in}. The only difference observed between the two reactors was the presence of a fluffy layer covering the granules in reactor R2, not evident in reactor R1. An activity test was also conducted where the fluffy layer was removed after elutriation. Compared with the nonelutriated sludge, the activity was 44% higher, indicating that the fines covering the granules were less active.

Additionally, we also observed some granule erosion and changes in mechanical strength. Granule erosion occurred only during the initial stages of the periods when reactors were started up with new sludge (periods 1, 4, and 7). This was the case especially due to high shear forces when high liquid upflow velocities were applied. The fines were removed by washout, and based on visual observation, the granules were apparently more polished. Afterward, the granule diameter gradually increased. At the end of period 3, we measured the granule strength[24] of sludges from both reactors. A value of $3.2 \cdot 10^{-4}$ N/m^2 was found for the granules of both reactors, indicating no influence of the fluffy layer in the granule strength. Compared with the value of the initial sludge ($4 \cdot 10^{-4}$ N/m^2), the granule strength decreased by 20%. This indicates that high mechanical stability still remains under operational conditions of high liquid upflow velocity and low substrate concentration.

4. Discussion

4.1. Feasibility of the EGSB reactor

In this study, COD removal efficiencies of 80% to 97% at organic loading rates up to 12 kg COD/m$^3\cdot$d reveal that the EGSB reactor concept is in principle feasible for treating very-low-strength ethanol-containing wastewater (COD_{in} values of 100 to 700 mg COD/*l*). The fact that such high organic loads could be accommodated at liquid retention times even lower than 1 h confirms the potentials of the anaerobic treatment, since so far the systems were generally considered to be applicable merely for medium- to high-strength wastewaters. Our results confirm that several conditions must be met in order to obtain a high biological performance. Three main control parameters have been identified, namely, the liquid upflow velocity, the organic loading rate, and the substrate concentration.

The present results indicate that a high COD removal efficiency in EGSB reactors can be achieved at V_{up} ranging from 2.5 to 5.5 m/h. A very distinct decrease in efficiency is found at values of V_{up} lower than 2.5 m/h, resulting from poor mixing of the bulk liquid phase due to low hydraulic turbulence, poor wastewater-biomass contact, and inadequate expansion of the sludge bed. Consequently, sufficient diffusion of substrate is not achieved for adequate mass transport to positions deep inside the biofilm. Therefore, based on these results, we conclude that a minimum liquid upflow velocity of 2.5 m/h has to be applied in EGSB reactors for obtaining a good substrate-biomass contact. In full-scale EGSB reactors, a sufficient mixing will also depend on the evenness and density of the feed inlet distribution. Values of V_{up} exceeding 5.5 m/h did not increase the efficiency, but on the contrary the danger of sludge washout increased.

The organic loading rate turned out to be a very important parameter controlling the performance of the EGSB reactor. Above a certain value, the OLR becomes the major factor controlling the performance of the reactor. In our experiments, this value was found at OLR of 12 g COD/$l\cdot$d even for COD_{in} as low as 100 to 200 mg COD/l. The very high value of OLR can be explained by the maximum biological capacity of the reactor. Approximately 10 g VSS/l was present in the reactor with a maximum specific activity of 1.2 g COD/g VSS$\cdot$d. This indicates that the available methanogenic activity was efficiently used even though extremely low substrate levels were prevailing in the reactors. To achieve a given treatment efficiency, higher organic loads could be accommodated in periods when the COD_{in} was 500 to 700 mg COD/l than in periods when it was 100 to 200 mg COD/l. This also corresponds to the higher values of specific methanogenic activity of the sludges, as assessed in periods of higher COD_{in} (Table 3).

The effect of COD_{in} on the COD removal efficiency can be observed at various OLRs. The difference in COD removal efficiency between both ranges of COD_{in} can be explained by the maximum specific activity of the reactor sludge (Fig. 6), as long as the effluent VFA was greater than 50 mg COD/l (i.e. $V \approx V_{max}$). Since this critical effluent VFA concentration appears to be the same irrespective of the COD_{in}, the resulting efficiency will necessarily be lower for the lower COD_{in}. The interesting observation that a high COD removal efficiency was still achieved at very low substrate levels can be attributed to the extremely low apparent K_S value found (9.8 mg COD/l). This finding represents a very important step forward in the application of anaerobic treatment systems. So far significantly higher values of apparent K_S were usually presumed to prevail in granular sludge beds. Our present results demonstrate how adequate hydraulic mixing is essential for lowering the apparent K_S. Apparent K_S values (30 to 200 mg COD/l) determined in the previous research[5,22] were obtained in batch assays, which probably did not adequately imitate the hydraulic regime in a well-mixed reactor, as is the case of the EGSB reactor.

In this study, we also demonstrated the feasibility of EGSB reactors even in the case in which 3.8 mg/*l* dissolved oxygen was present in the influent. The effect of dissolved oxygen was negligible because no significant difference could be detected in the COD removal efficiency between the two reactors, one receiving and the other not receiving dissolved oxygen. This finding shows the minor importance of dissolved oxygen present in the influent of anaerobic treatment systems, especially regarding the commonly held belief that the methanogens are oxygen intolerant.[8,25] Full-scale reactor upsets due to oxygen toxicity have seldom been reported.[3,6] The absence of any detrimental effect of oxygen can be explained due to the fact that anaerobic sludges generally contain facultative bacteria. They can rapidly consume the oxygen present as long as metabolizable substrates are available. Due to the low solubility of oxygen, little substrate is required to eliminate it from the wastewater. Consequently, methanogens located deep inside the biofilm are quite well protected from oxygen exposure.

In this study, the rapid consumption of oxygen by facultative bacteria is demonstrated by the fact that the same effluent redox was found in both reactors. These results are also in agreement with those of previous experiments that demonstrated the tolerance of methanogens in anaerobic sludge exposed to highly aerobic conditions.[9,12] This was the case even when a granular sludge was exposed to oxygen for prolonged periods.[13] A critical question is when oxygen would be a true danger for anaerobic treatment systems. The previous experiments demonstrated that as long as the oxygen is lower than the stoichiometric requirement to oxidize the biochemical oxygen demand (BOD), the oxygen danger would never occur in anaerobic reactors.[12] In practice, this means that there is no inhibition problem because the O_2/BOD ratio will generally be much lower than 1. Additionally, even in the case that the O_2/BOD ratio occasionally exceeds 1, the oxygen effect may not necessarily have to be detrimental since some methanogens have some intrinsic tolerance to oxygen.[9,12,13] Therefore, we can conclude that under practical conditions it is hardly possible that reactor upset will occur due to the toxicity of oxygen present in the wastewater. However, the presence of small amounts of oxygen might represent an important bottleneck for the practical application of the EGSB system, due to the development of fluffy voluminous biomass attached to the surface of the granular sludge. In our study, we observed that such layers did develop and were less active compared with the granules. Excessive accumulation of these fines might result in a poorer settleability and perhaps enhance the tendency for sludge bed buoyancy and flotation. This can particularly be important for the long term operation of the reactor. In this case, the elimination of a relatively small amount of oxygen in the influent might be required.

Our experiments show clear evidence that the mean diameter and the specific methanogenic activity of the sludges changed. The increase of the mean diameter on the one hand can be due to the higher V_{up} applied, as shown in previous research.[7] In our experiment, factors such as the high mixing intensity were clearly important by providing enhanced substrate penetration into the granules. Consequently, the growth over the biofilm detachment by fluid shear forces was favored. On the other hand, bacterial growth can also concentrate on the outer layer of the granule in the case of low substrate level, due to lower penetration. This was observed in UASB reactor experiments where the diameter also increased and the granules showed a hollow core.[1] In that case, growth was also favored because shear forces were significantly lower compared with those prevailing in an EGSB reactor. Changes in the specific methanogenic activity of the sludges can be attributed to substrate levels. Increases were observed in periods of high COD_{in} which is perhaps due to a better penetration of substrate into the granules. On the contrary, less penetration occurs at lower levels of COD_{in}, and consequently, a decrease in the specific activity occurs, since the fraction of viable organisms decreases.

4.2. Problems with sludge retention

While a high treatment efficiency was demonstrated due to the good wastewater-biomass contact, the required sludge hold-up at high levels may represent an obstacle for the feasibility of the EGSB system. The sludge washout and piston flotation were two difficulties we encountered in our experiments. The sludge washout is particularly critical and needs to be resolved. The measures which we took in the laboratory to deal with the sludge washout are not practical for full-scale reactors. Our study revealed that the sludge hold-up problems are related to the mixing intensity, which in turn is associated with the hydraulic and organic loading rates. The sludge hold-up is first controlled by the liquid upflow velocity for any type of granular sludge. If the sludge bed is already expanded up to the top of the reactor, a further increase may result, at least temporarily, in a very-high sludge washout. The first fraction to be forced out of the system contains the particles with the worst settleability. Our experiments confirmed that at hydraulic loads of V_{up} higher than 5.5 m/h, significant sludge washout occurred. There is in fact no benefit in applying V_{up} in excess of 5.5 m/h, because the COD removal efficiency is already optimized in the range of 2.5 to 5.5 m/h. Sludge hold-up is further aggravated by high OLRs because high gas production contributes to increased sludge washout. The V_{gas} itself is not responsible for washout since the sludge bed expansion tests confirmed that exogenous gas bubbles have a minimal effect on the expansion of the sludge bed. The most important mechanism of the enhanced washout is, therefore, likely due to the adherence of small gas bubbles to the granules or the presence of accumulated gas inside them increasing their buoyancy. The floating granules may pass through the settling zone and even escape from the reactor. If no special device is installed to separate the granules from the gas bubbles, limiting the gas production by applying OLRs of less than 7 g COD/$l \cdot$d was found to prevent this kind of washout.

Sludge piston formation was observed when poor sludge bed expansion occurred at low liquid upflow velocity. The dense unexpanded sludge bed causes considerable gas entrapment at the bottom of the reactor. When the buoyancy of the accumulated gas is high enough, there is an incidental flotation of the sludge as a piston. The small diameter of the laboratory-scale reactor may significantly have contributed to this problem. In our study, a minimum V_{up} of 2.5 m/h was required to prevent the sludge piston formation. When the sludge bed was sufficiently expanded, the sludge bed was less dense; consequently, degassing was not hindered.

The observation in our experiments that some sludge erosion occurred can be considered of minor importance. The erosion occurred only temporarily during the start-up and became minimal afterward when surfaces became polished and the granule diameters increased. During the experiments the mechanical strength of the granules were highly preserved despite the increase in granule diameter. This has also been observed in EGSB reactors fed with substrates like VFA-mixture solution and brewery wastewater (in preparation).

Therefore, based on laboratory experiments adequate sludge hold-up is only feasible within a narrow range of operational conditions with the present laboratory-scale EGSB reactor design. Within the optimal hydraulic loading range of 2.5 to 5.5 m/h, there is no problem of piston formation nor sludge washout as long as the OLR is below 7 g COD/$l \cdot$d. However, if OLRs up to 12 g COD/$l \cdot$d or higher are desired, special attention should be given to the sludge washout. Either an improved gas-liquid-solid (GLS) device or an improved type of immobilized biomass suited for high hydraulic and gas loads will be required for this constraint in the applicability of EGSB reactors.

In order to avoid the washout of particles, the present design of the GLS should be adapted. The installation of additional baffles can improve the retention of the granules[17,18] and does not represent excessive additional costs in practice. Installation of a vibrator can be used for the separation of gas bubbles from granules if incidental flotation occurs. Loss of fine eroded biomass can be retained by the use of a sieve-drum or by a sophisticated microscreen.[18] However, since the problem of piston flotation is only expected in laboratory-scale reactors and since granule erosion is expected to only occur temporarily during start-up, the benefits of such measures are perhaps marginal compared with their costs.

Biomass immobilization conforming to the hydrodynamic regime existing in an EGSB reactor can be an option worth considering, instead of utilizing granular sludge. So far all the experiments with EGSB reactors have used granular sludge from UASB reactors. No previous research has been carried out to know which kind of biomass would naturally arise from the conditions inside EGSB in the case of a first start-up. Some previous experiments using the anaerobic attached film expanded bed or fluidized bed process showed that suitable bacterial aggregates could develop on inert carrier materials under a similar operational regime as in the EGSB system.[11,14]

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Chapter 6

Discussion and Conclusions

1. Low Strength Wastewaters and Anaerobic Treatment

Low strength wastewaters account for a significant contribution to the water pollution since large volumes of dilute industrial effluents are produced world-wide. These wastewaters, in general from food processing industries, commonly only contain easily biodegradable organic matter with chemical oxygen demand (COD) concentrations below 2000 mg/*l*. The main components are short-chain volatile fatty acids (VFA), alcohols and carbohydrates. In some cases, proteins, fats and long-chain fatty acids (LCFA) are also present but at lower concentrations. Satisfactory treatment performances obtained with upflow anaerobic sludge blanket (UASB) and expanded granular sludge bed (EGSB) systems reveal that anaerobic processes can be feasible for the treatment of dilute wastewaters. However, some problems have been identified which might explain why those systems have not yet been fully applied so far.

2. The Problems

The problems are wastewater and reactor design related. Important wastewater related problems are: (i) low substrate levels occurring inside the reactor; and (ii) possible presence of dissolved oxygen. The reactor design related problems are concerned with the establishment of a compromise between the requirement of a high retention of biomass and a good wastewater-biomass contact; both of which are dependent on the hydraulic upflow velocity. A high level of hydraulic turbulence and good sludge bed expansion are essential for providing an adequate wastewater-biomass contact, but they endanger the sludge retention.

3. Scope of this Dissertation

This dissertation evaluates the applicability of UASB or EGSB reactors for the treatment of low strength soluble wastewaters, focusing on the wastewater related problems. In Chapter 1, an introductory review of the literature is given about the low strength wastewater related problems. In Chapters 2 and 3, the effect of dissolved oxygen on methanogenesis in anaerobic granular sludge was quantified. In Chapters 4 and 5, the effect of low substrate levels on reactor performance was investigated, in order to determine the lowest influent COD concentration (COD_{in}) which is possible for treatment in UASB and EGSB reactors. Furthermore, reactor technological aspects are also presented concerning the requirements for sludge retention and wastewater-biomass contact

4. The Effect of Oxygen

Oxygen is a potential toxic compound to strict anaerobes, especially for the acetogens and methanogens which are the microorganisms at the end of the food chain during anaerobic wastewater treatment. However, it is known from the literature (Chapter 1) that at least some methanogens have some tolerance to oxygen exposure, either in pure or in mixed cultures. Start-up of UASB reactors was successfully conducted with sludges obtained from aerobic activated sludge plants, indicating the presence of anaerobic bacteria in aerobic environments.

The toxicity of oxygen to methanogens in granular sludges was quantified in Chapter 2. Five sludges with distinct properties collected from UASB and EGSB reactors were used for this purpose. The results of batch experiments reveal that methanogens in granular sludge have a high tolerance to oxygen. The concentration of oxygen causing 50% inhibition to methanogenic activity was between 7% and 41% of oxygen added to the head space of flasks which corresponded to 0.05 and 6 mg/*l* of dissolved oxygen prevailing in the media. In order to determine the mechanisms of the O_2 tolerance, the role of the granule size, the respiration rate of the facultative bacteria, the shaking regime and substrate were investigated. The respiration rate of the facultative bacteria present in the granular sludges was the most important mechanism of oxygen tolerance since it was well correlated with highly tolerant sludges. The absence of facultative substrate for respiring O_2 drastically decreased the oxygen tolerance. There was no correlation of O_2 tolerance with the granule size and shaking only affected the least tolerant sludges.

A hypothesis was formulated to describe the oxygen tolerance of methanogens in granular sludges. Facultative bacteria rapidly consume oxygen creating anaerobic microniches inside the granules where the methanogens are well protected against contact with oxygen. This hypothesis can be justified by the poor penetration of oxygen into biofilms. In the literature, O_2 penetration into actively respiring aerobic biofilms has been reported to only reach a depth of 100 to 300 μm.[1,2] The diameter of the granules, being in large excess of that required for creating anaerobic zones, would explain the non-correlation found between oxygen tolerance and the sludge granule size. When substrate was not supplied, facultative respiration was less active. Thus, oxygen could penetrate deeper into the granules and come into contact with the methanogens which would explain the enhanced toxicity. Some tolerance was still evident even in the absence of substrate, indicating that methanogens do have a limited intrinsic tolerance to oxygen. In the literature, several methanogens are reported to contain the enzyme superoxide dismutase (SOD) which neutralizes toxic oxygen radicals.[3,4] Moreover, the existence of redox carriers and growth in aggregates are also reported to be protective factors of strict anaerobes in pure cultures.[5,6]

These results have important implications for the anaerobic treatment of low strength wastewaters. Oxygen toxicity is not expected to occur, because wastewaters usually contain a large excess of biochemical oxygen demand (BOD) required by facultative bacteria to consume the dissolved oxygen present in the wastewaters. Under normal conditions only dissolved oxygen will enter into the system with concentrations below 10 mg/*l*, while dilute wastewaters usually contain at least several hundred milligrams per litre of BOD. The non-detrimental effect of dissolved oxygen in practice was confirmed in the UASB and EGSB studies (Chapter 4 and 5), since COD removal efficiencies and values of effluent redox were very similar in parallel ethanol reactors, one fed with and another without dissolved oxygen in the influent.

Since even higher levels of oxygen can be tolerated, a second implication is that oxygen can be added to anaerobic reactors to enhance the degradation of recalcitrant pollutants. Nonetheless, despite the non-toxicity of oxygen to natural mixed cultures in granular sludges used in wastewater treatment, there is still a concern about the substrate competition between methanogens and facultative bacteria. If too much oxygen were fed into reactor, then all the substrate would be consumed by the facultative bacteria since they have much higher specific activities and growth rates compared with those of the methanogens.

Methanogens without access to substrate will be outcompeted. Thus, the competition can perhaps be regulated by oxygen supply.

In Chapter 3, a study was conducted to investigate the competition for substrate in anaerobic-aerobic co-cultures, where methane production and oxygen consumption occurred simultaneously. First of all, a distinction was made between O_2 intolerant and O_2 tolerant sludges. In the intolerant sludges, each unit of O_2 was reflected in a unit of BOD stolen from methanogens. This would signify that no methane can be expected to be produced if the oxygen supply were in excess of the BOD. Thus, it can be predicted that substrate competition would easily be won by the facultative bacteria in O_2 unlimited conditions. In the tolerant sludges, methane production could be demonstrated even when oxygen was not limiting, suggesting that substrate competition occurs between methanogens and facultative bacteria even in well aerated environments. At dissolved oxygen concentrations of 23, 2 and 1 mg O_2/l, 2%, 15% and 25% of the substrate was converted to methane, respectively. The occurrence of methanogenesis in O_2 unlimited conditions is hypothesized to occur if there is a better transport of substrate to deep positions inside the granule compared with the transport of oxygen. Mass transfer by diffusion depends on the concentration gradient. Oxygen having in practice a maximum solubility of less than 10 mg/*l*, indicates that at most only weak gradients can be developed. Substrate on the other hand has unlimited solubility and was usually applied at 1000 mg COD/*l* or higher. Thus, much higher diffusion gradients could be established for the substrate. The competition for substrate during prolonged periods of O_2 exposure can eventually result in the development of a complex mixed cultures where at least three trophic groups could be identified: the methanogens, the facultative heterotrophic and methanotrophic bacteria. Nevertheless, it was confirmed that since competition for substrate can be regulated, oxygen can be added into anaerobic reactors. In the literature, examples are reported giving evidence for the ability of anaerobic-aerobic co-cultures to degrade recalcitrant pollutants in one bioreactor, instead of using separate anaerobic and aerobic phases.[7,8,9]

5. The Effect of Low Substrate Levels

Low COD_{in} signifies low substrate levels inside the reactor granules, resulting in low activity of biomass. It is inevitable that the reactor contents have a low substrate concentration, since during wastewater treatment the highest possible COD elimination is desired. According to Monod kinetics, the specific sludge activity depends on the substrate concentration. The conversion rates are dependent on the saturation constant K_S, which indicates the affinity of the bacteria for the substrate. Intrinsic values of K_S refers to the transport of substrate into dispersed bacterial cells in perfect suspensions. Apparent K_S values refers to the transport of substrate into biofilms. The values of apparent K_S are higher than those of intrinsic K_S because there is a limited transport of substrate into the biofilm. Consequently, substrate gradients are formed with less substrate available for the biomass inside the granules than in the bulk liquid phase of the reactor. Available substrate at suitable concentrations means that the substrate has to be replenished by forced transport, faster than the consumption by the active biomass. Convective mass transport created by turbulence in the bulk liquid phase can speed up substrate penetration into granules beyond that of diffusion. Thus, the treatment of wastewaters with low COD concentrations requires that an adequate mixing intensity is provided by a high hydraulic turbulence lowering the apparent K_S. The turbulence during the treatment of high strength wastewaters is provided by the high gas production per unit of influent. Regardless of the wastewater strength, the use of UASB and EGSB reactors also requires that the mixing intensity is not too excessive in order to maintain a high retention of biomass.

In Chapter 4, the application of UASB reactors was investigated for the treatment of low strength wastewater containing whey or ethanol as substrate. The high treatment performance of low strength wastewaters in the whey and ethanol reactors can be inferred by the COD removal efficiencies obtained. The efficiencies were above 86% at organic loading rates (OLR) up to 3.9 g COD/*l*.d as long as the influent COD concentration was above 630 mg/*l* for whey-fed reactors. The efficiencies were above 95% at OLRs up to 6.8 g COD/*l*.d as long as the influent COD concentration was above 422 mg/*l* for ethanol-fed reactors. The better performance of ethanol- compared with the whey-fed reactors was due to the presence of proteins and fats in the whey. Acidification instead of methanogenesis was the rate limiting step in the COD removal from whey. From this study, it can be concluded that the lowest acceptable COD_{in} for the UASB treatment of soluble wastewaters is 630 mg/*l* and 422 mg/*l* for whey and ethanol substrates, respectively, in order to obtain efficiencies higher than 85%.

The UASB reactors was shown to have no problem with the retention of sludge. However, the mixing intensity was presumably not so high since liquid upflow velocities (V_{up}) were always below 0.1 m/h, which are not enough for turbulence and sludge bed expansion. Likewise, there was little natural mixing since the biogas production was low. Thus, the mixing intensity was not sufficient in order to provide adequate wastewater-biomass contact. Biomass at concentrations higher than the 5 g/*l* volatile suspended solids (VSS) used in the UASB experiments, can be utilized in practice, allowing the application of higher OLRs. Thus, higher natural mixing can be expected due to further biogas production. Consequently, it can be anticipated that even COD_{in} values lower than 630 and 422 mg/*l* can be tolerated for whey and ethanol reactors, respectively.

In Chapter 5, the effect of low substrate levels was also studied in EGSB reactors fed with ethanol as substrate in two distinct ranges of COD_{in}, 100-200 and 500-700 mg/*l*. An increased V_{up} (up to 21 m/h) provided by effluent recirculation was applied in order to obtain a high expansion of the sludge bed and high levels of hydraulic turbulence. Moreover, the problem of sludge retention was also investigated.

The feasibility of the EGSB for the treatment of dilute wastewaters was demonstrated by the high treatment performance obtained, with COD removal efficiencies ranging from 80% to 97% at OLRs up to 12 g COD/*l*.d and at COD_{in} values as low as 100 mg/*l*. The high treatment performance obtained in the EGSB reactors depended on three main control parameters: the V_{up}, the OLR and the COD_{in}. The treatment performance is optimized when applying V_{up} in the range between 2.5 and 5.5 m/h (recirculation ratios up to 8). In this range, an adequate wastewater-biomass contact occurred. The requirement for a high retention of biomass was also fulfilled. At values of V_{up} lower than 2.5 m/h the COD removal efficiency decreased because there was a mass transport limitation of substrate migrating into the granules resulting in apparent K_S values higher than 28 mg COD/*l*. At values of V_{up} higher than 2.5 m/h, the biofilm diffusion limitation was overcome by convective mass transport with faster replenishment of substrate than the consumption by the active biomass. The higher V_{up} applied resulted in a remarkable apparent K_S value which was found to be as low as 9.8 mg COD/*l*. This value is comparable to the intrinsic K_S (18-30 mg COD/*l*) of the common occurring acetoclastic methanogen, *Methanothrix soehngenii*, confirming that mass transport limitations were overcome. The high efficiency obtained with concentrations as low as 100 mg/*l* is explained by the low value of apparent K_S. At values of V_{up} higher than 5.5 m/h, high levels of hydraulically assisted sludge washout occurred. The hydraulically assisted sludge washout can be controlled by limiting the V_{up} to less than 5.5 m/h. The amount of gas production per unit of influent was not significant for providing natural turbulence. However, the buoyancy forces of gas bubbles attached to biofilms caused sludge flotation. The problem of gas assisted sludge flotation was prevented by limiting the OLR to 7 g COD/*l* which lowers the biogas production. If a higher OLR is desired, a better system of sludge retention is required. There is a great need to improve the existing gas-solid-liquid (GSL) separator or to develop a new type of biomass immobilization conforming to the hydrodynamics of the EGSB. Possible strategies to improve the GSL would be the installation of additional baffles in the weirs and the use of sieve-drums or sophisticated microscreen, to deal with over-expanded beds and sludge flotation. The detachment of gas bubbles from biofilms could be enhanced by installing a vibrator in the GSL. The attachment of biomass to a dense mobile or fixed carrier which would anchor the biomass against flotation are alternatives worth considering.

One of the objectives of this dissertation was to determine the lowest COD_{in} which can effectively be removed in UASB and EGSB reactors treating wastewaters containing simple substrates. Table 1 shows the theoretical values of lowest acceptable COD_{in} levels that were calculated using Monod kinetics, assuming a COD elimination efficiency of 85% at various OLRs. The lowest feasible COD_{in} which can be treated in UASB and EGSB reactors would be 187 mg/*l* and 13 mg/*l*, respectively, at an OLR of 5 g COD/*l*.d. At higher OLRs, the lowest COD_{in} which can be tolerated is higher. This signifies that low strength wastewaters of less than 2000 mg COD/*l* containing simple substrates, can effectively be treated at OLRs up to 20 g COD/*l*.d in UASB reactors. Compared with the UASB reactor, the EGSB reactor can handle the same OLR at much lower values of COD_{in}. Since extremely low values of COD_{in} can efficiently be treated, the EGSB reactor could be considered as a polishing off step for residual BOD of any treatment system treating higher strength wastewaters. The EGSB reactor can also be considered as an alternative to the aerobic activated sludge process for treating dilute wastewaters since the EGSB process is able to handle OLRs of 5 g COD/*l*.d which are higher than that of the aerobic process. Furthermore, the EGSB opens the possibility of utilizing the anaerobic wastewater technology to treat very dilute contaminated groundwater with less than 100 mg/*l* of organic pollution.

Care should be taken considering the extremely low values of COD_{in}. The lowest value for the EGSB of 13 mg/*l* would result in a dissolved O_2/BOD ratio approaching 1, which can then perhaps result in an environment where the facultative bacteria can outcompete the methanogens.

Energy recovery from biogas can be of importance in UASB reactors since the economic benefits due to the methane production are quite considerable. In the case of the EGSB, such benefits would significantly be lower due to the relatively high amount of dissolved methane lost in the effluent, at values of COD_{in} below 100 mg/*l*. However, most wastewaters of the food processing industry are expected to have COD_{in} values greater than 100 mg/*l*.

Table 1. The theoretical lowest COD_{in} which can be applied to UASB and EGSB reactors for the treatment of wastewaters containing simple substrates, assuming the parameters outlined in [a].

Reactor	Apparent K_S (mg COD/*l*)	OLR (g COD/*l*.d)	COD_{in} (mg/*l*)	COD_{ef} [b] (mg/*l*)	Yield of methane [gas/(liquid+gas)] [c]
UASB	145 [d]	5	187	28	0.51
		10	483	73	0.81
		15	967	145	0.91
		20	1933	290	0.95
		25	4833	725	0.98
EGSB	10 [e]	5	13	2	0
		10	33	5	0
		15	67	10	0
		20	133	20	0.31
		25	333	50	0.73

a assuming VSS = 20 g/*l*; V_{max} = 1.5 g COD/g VSS.d; T = 30°C; and COD removal efficiency = 85%
b calculated using Monod kinetics, assuming effluent COD as the reactor substrate concentration
c assuming 75 mg COD/*l* as methane dissolved in the effluent and (0.96 · 0.85 · COD_{in}) as total methane produced (gas phase + liquid phase); 0.96 refers to total methane yield from COD eliminated; 0.85 refers to COD removal efficiency
d average value found in literature for UASB granular sludge[10,11]
e value observed in EGSB reactors of this study (Chapter 5)

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Hoofdstuk 6

Discussie en Conclusies

1. Verdunde Afvalwaterstromen en Anaërobe Zuivering

Verdunde afvalwaterstromen dragen significant bij aan de watervervuiling aangezien wereldwijd grote hoeveelheden industriële effluenten geproduceerd worden. Dit afvalwater, meest afkomstig van de levensmiddelen industrie, bevat in het algemeen makkelijk afbreekbare organische bestanddelen met een chemisch zuurstof verbruik (CZV) lager dan 2000 mg/*l*. De voornaamste componenten zijn lagere vluchtige vetzuren (VFA), alcoholen en koolhydraten. In sommige gevallen zijn tevens eiwitten, vetten en hogere vetzuren aanwezig maar in lagere concentraties. Bevredigende zuiveringsrendementen verkregen met anaerobic sludge blanket (UASB) en expanded granular sludge bed (EGSB) systemen laten zien dat anaërobe processen rendabel kunnen zijn voor de zuivering van verdunde afvalwaterstromen. Er zijn echter problemen, wat verklaart waarom deze systemen tot nu toe nog niet volledig worden toegepast.

2. Problemen

De problemen zijn gerelateerd aan het afvalwater en het reactorontwerp. Belangrijke problemen betreffende het afvalwater zijn: (i) lage substraat concentraties in de reactor en (ii) de mogelijke aanwezigheid van opgelost zuurstof in de reactor. De problemen in het reactorontwerp betreffen het maken van een compromis tussen de vereiste hoge retentie van biomassa en een goed water-slib contact; die beide afhankelijk zijn van de hydraulische opstroomsnelheid. Een zekere mate van turbulentie en expansie van het slibbed is essentieel voor voldoende water-slib contact, maar is nadelig voor de slibretentie.

3. Doel van dit Onderzoek

Dit proefschrift evalueert de toepasbaarheid van UASB of EGSB reactoren voor het behandelen van verdunde afvalwaterstromen, toegespitst op de problemen gerelateerd aan het afvalwater. In Hoofdstuk 1 wordt een introductie overzicht van de literatuur gegeven van aan verdunde afvalwater gerelateerde problemen. In Hoofdstuk 2 en 3 wordt het effect van opgelost zuurstof op de methanogenesis in het anaërobe korrelslib gekwantificeerd. In Hoofdstuk 4 en 5 wordt het effect van het verdunde afvalwater op het rendement van de reactor onderzocht, om de laagst mogelijk influent CZV concentratie (CZV_{in}) te bepalen voor behandeling in UASB of EGSB reactoren. Verder komen aan bod reactor-technische aspecten die de slibretentie en slib-water contact betreffen.

4. Effect van Zuurstof

Zuurstof is een potentieel toxische stof voor strikt anaëroben, vooral voor acetogenen en methanogenen die aan het einde van de voedselketen van het anaërobe afbraakproces staan. Uit literatuur blijkt (Hoofdstuk 1) dat enige methanogenen minstens een weinig tolerant zijn voor blootstelling aan zuurstof, hetzij in een pure cultuur of een gemengde cultuur. Het opstarten van UASB reactoren was succesvol met slib uit aërobe actiefslib reactoren, wat aangeeft dat er anaërobe bacteriën aanwezig zijn in aërobe omstandigheden.

De toxiciteit van zuurstof voor methanogenen is gekwantificeerd in Hoofdstuk 2. Vijf slibsoorten met karakteristieke eigenschappen verkregen uit UASB en EGSB reactoren zijn voor dit doel gebruikt. De resultaten van batch-experimenten laten zien dat methanogenen in anaëroob korrelslib een hoge tolerantie hebben voor zuurstof. De concentratie O_2 die een 50% reductie van de methanogene activiteit tot gevolg heeft lag tussen de 7 en 41% toegevoegd zuurstof in de bovenstaande gasfase, corresponderend met 0,05 en 6 mg/*l* opgelost O_2 in het medium. Om het mechanisme van zuurstof-tolerantie te bepalen is de rol van korrelgrootte, respiratie snelheid van de facultatieve bacteriën, het effect van schudden en het substraat bepaald. De respiratie snelheid van de facultatieve bacteriën in het korrelslib bleek de belangrijkste factor voor de zuurstoftolerantie, aangezien dit goed correleerde met hoog tolerante slibsoorten. De afwezigheid van substraat voor facultatieve respiratie van zuurstof verminderde de zuurstoftolerantie drastisch. Er is geen correlatie gevonden tussen zuurstoftolerantie en korrelgrootte terwijl schudden alleen effect had op de minst tolerante slibsoorten.

Er is een hypothese opgesteld die de zuurstoftolerantie van methanogenen in korrelslib beschrijft. Facultatieve bacteriën consumeren met hoge snelheid O_2 waarbij er plaatselijk een anaërobe microklimaat wordt gecreëerd binnenin de korrel, waar de methanogene bacteriën beschermd worden tegen zuurstof contact. De slechte penetratie van O_2 in biofilms rechtvaardigt deze hypothese. Uit de literatuur blijkt dat het indringdiepte van zuurstof in actief respirerende aërobe films slechts 100 tot 300 μm bedraagt.[1,2] Dit kan verklaren waarom er geen relatie is gevonden tussen de korrelgrootte en de zuurstoftolerantie, de korreldiameter verschilt immers ordengroottes met de in de literatuur gevonden indringdieptes. Wanneer er geen substraat werd aangeboden daalde de facultatieve activiteit. Als gevolg daarvan kon zuurstof dieper binnendringen in de korrels en de methanogenen remmen, wat de verhoogde toxiciteit verklaart. Enige tolerantie was zelfs bij afwezigheid van substraat aanwezig, wat aangeeft dat methanogenen zelf een beperkte tolerantie hebben voor zuurstof. In de literatuur wordt vermeld dat methonogenen het enzym superoxide dismutase bevatten (SOD) dat zuurstof radicalen neutraliseert.[3,4] Verder wordt vermeld dat het bestaan redox-carriers en de groei in aggregaten beschermende factoren zijn in pure culturen van anaëroben.[5,6]

Deze resultaten zijn van groot belang voor het anaërobe zuiveren van verdunde afvalwaterstromen. Toxiciteit van zuurstof wordt niet verwacht daar er meestal een groot overschot aan biologisch zuurstof verbruik (BZV) aanwezig is, wat nodig is voor de facultatieve bacteriën om het opgeloste zuurstof uit het afvalwater te respireren. Onder normale omstandigheden zal alleen O_2 in het systeem voorkomen als de zuurstof concentratie lager is dan 10 mg/*l*, terwijl verdund afvalwater in het algemeen meer dan honderd milligram BZV per liter bevat. De onschadelijkheid van opgelost zuurstof in de praktijk werd bevestigd door onderzoek met UASB en EGSB systemen (Hoofdstuk 4 en 5). Uit dit onderzoek bleek dat vergelijkbare CZV verwijderingsrendementen en effluent redoxpotentialen werden behaald met vergelijkbare reactoren gevoed met ethanol, de een met en de ander zonder opgeloste zuurstof in het influent.

Omdat het mogelijk is om met hogere concentraties aan opgelost zuurstof te werken, geeft dit de mogelijkheid om O_2 toe te voegen aan anaërobe reactoren om de afbraak van recalcitrante stoffen te bevorderen. Niettegenstaande het feit van de onschadelijkheid van zuurstof bestaat er de mogelijkheid van substraat competitie tussen methanogenen en facultatieve bacteriën. Wanneer er te veel O_2 beschikbaar is in de reactor kan al het substraat verbruikt worden door facultatieve bacteriën, omdat deze bacteriën een veel hogere specifieke activiteit en groeisnelheid hebben in vergelijking met methanogenen. Methanogenen zonder beschikbaar substraat worden weg geconcurreerd. De competitie kan dus eventueel geregeld worden via de zuurstoftoevoer.

In Hoofdstuk 3 is onderzoek verricht naar de competitie voor substraat met anaërobe-aërobe co-culturen, waarin zowel methaanproductie als zuurstofconsumptie optrad. In eerste instantie is een onderscheid gemaakt tussen zuurstoftolerant en zuurstof-intolerant slib. Bij intolerant slib werd al het zuurstof uitgedrukt als BZV die niet beschikbaar is voor methanogenen. Dit zou betekenen dat er geen methaanproductie kan worden verwacht als de zuurstoftoevoer groter is dan de BZV. Op deze manier kan makkelijk worden voorspeld dat de substraat competitie gewonnen wordt door facultatieven wanneer er geen zuurstoflimitatie is. Bij tolerante slibsoorten kon worden aangetoond dat er methaanproductie optrad wanneer geen zuurstoflimitatie was, wat doet vermoeden dat er competitie is tussen methanogenen en facultatieven zelfs onder zuurstofrijke omstandigheden. Bij een concentratie aan opgelost O_2 van 23, 2 en 1 mg O_2/l werd resp. 2%, 15% en 25% van het substraat omgezet in methaan. Verondersteld wordt dat methaanproductie bij zuurstof-ongelimiteerde omstandigheden zal optreden als er een beter transport is van substraat naar het binnenste van de korrel in vergelijking met zuurstoftransport. Diffusie is afhankelijk van het concentratiegradiënt. Zuurstof heeft in de praktijk een maximale oplosbaarheid van minder dan 10 mg/l, met als gevolg dat er slechts een beperkt gradiënt kan bestaan. Substraat daarentegen is onbeperkt oplosbaar en werd meestal toegepast bij een concentratie van 1000 mg/l of hoger met als gevolg dat een veel groter concentratiegradiënt werd ingesteld. De competitie voor substraat over langere perioden van O_2 blootstelling kan uiteindelijk resulteren in het ontwikkelen van een complexe mengcultuur waarin minstens drie verschillende groepen kunnen worden geïdentificeerd: methanogenen, facultatief hetrotrofen en methanotrofe bacteriën. Toch werd bevestigd dat zuurstof kan worden toegevoegd aan anaërobe reactoren omdat de competitie voor substraat kan worden gereguleerd. In de literatuur worden voorbeelden gegeven die de afbraak van recalcitrante stoffen in anaërobe-aërobe co-culturen bevestigen.[7,8,9]

5. Effect van Lage Substraatconcentraties

Een lage CZV_{in} concentratie betekent een lage concentratie in de slibkorrels, resulterend in een lage activiteit. Het is onvermijdelijk dat in de reactor een lage CZV concentratie heerst omdat de hoogst mogelijke CZV verwijdering gewenst is. Volgens Monod kinetiek hangt de specifieke activiteit af van de substraatconcentratie. De omzettingssnelheid is afhankelijk van de verzadigingsconstante K_S, die de affiniteit van de bacteriën voor het substraat weergeeft. Intrinsieke waarden voor de K_S betreffen het substraattransport in gedispergeerde bacteriecellen in ideale suspensies. De schijnbare K_S betreft het substraattransport in biofilms. De waarde van de schijnbare K_S ligt hoger dan die van de intrinsieke K_S omdat er substraattransportlimitatie in de biofilm optreedt. Dit leidt tot de vorming van een grotere concentratiegradiënt, met minder beschikbaar substraat voor de biomassa binnenin de korrels dan in de bulk vloeistoffase van de reactor. Beschikbaar substraat in geschikte concentraties betekent dat het substraat aangevuld moet worden door geforceerd transport, sneller dan de consumptie door de bacteriën. Geforceerd massa transport door turbulentie van de vloeistoffase kan de substraatpenetratie in de korrels versnellen ten opzichte van diffusie. Daardoor is het essentieel dat voldoende menging optreedt door hoge hydraulische turbulentie waardoor de schijnbare K_S verlaagd wordt. De turbulentie bij de behandeling van geconcentreerde afvalwaterstromen wordt bewerkstelligd door de hogere gasproduktie per eenheid influent. Afgezien van de concentratie van het afvalwater, is het voor UASB en EGSB reactoren van belang dat de menging niet te intensief is om een hoge slibretentie te waarborgen.

In Hoofdstuk 4 is de toepassing onderzocht van UASB reactoren voor de behandeling van verdund afvalwater, met wei of ethanol als substraat. Uit de gevonden hoge CZV verwijderingsrendementen kan worden geconcludeerd dat er een hoog zuiveringsrendement wordt behaald, met verdund afvalwater in de reactoren gevoed met wei en ethanol. De rendementen waren hoger dan 86% bij organische belastingen tot 3,9 g CZV/*l*.d wanneer de influent CZV concentratie boven de 630 mg/*l* lag, voor de reactoren met wei als substraat. Het verwijderingsrendement lag boven 95% bij organische belastingen tot 6,8 g CZV/*l*.d en de influent CZV concentratie boven 422 mg/*l*, voor de reactoren met ethanol als substraat. De hogere rendementen van de ethanol-gevoede reactoren in vergelijking met de wei-gevoede reactoren was het gevolg van de aanwezigheid van eiwitten en vetten in de wei. De snelheidsbeperkende stap was de acidificatie in plaats van de methanogenesis bij de afbraak van wei. Uit dit onderzoek blijkt dat de laagst mogelijk CZV_{in} concentratie voor behandeling van opgelost afvalwater in UASB reactoren 630 mg/*l* en 422 mg/*l* is voor resp. wei en ethanol substraten om een zuiveringsrendement van minstens 85 % te behalen.

De UASB reactoren vertoonden geen problemen met slibretentie. De menging was waarschijnlijk niet zo groot omdat de opstroomsnelheid altijd lager dan 0,1 m/h was, wat niet voldoende is om turbulentie en slibexpansie te veroorzaken. Bovendien was de menging door gasproductie laag. Hierdoor was de menging niet voldoende om in een goed water-slib contact te voorzien. Slib met een concentratie hoger dan 5 g/*l* gesuspendeerde organische stof (VSS) kan worden toegepast in UASB experimenten, waarbij hogere slibbelastingen kunnen worden opgelegd. Aldus mag een grotere natuurlijke menging worden verwacht door een hogere gasproductie. Het ligt dan voor de hand dat zelfs een CZV_{in} lager dan 630 en 422 mg/*l* kan worden geaccepteerd voor resp. wei en ethanol reactoren.

In Hoofdstuk 5 is het effect van lage substraatconcentraties onderzocht in EGSB reactoren gevoed met ethanol als substraat in twee CZV_{in} concentratie ranges, 100-200 en 500-700 mg/*l*. Een verhoogde opstroomsnelheid (tot 21 m/h) door middel van effluent recirculatie werd toegepast voor de benodigde expansie van het slibbed en turbulentie. Tevens werd het probleem van slibretentie onderzocht.

De geschiktheid van het EGSB systeem voor de behandeling van verdund afvalwater bleek uit de hoge CZV verwijderingsrendementen die varieerden tussen 80 en 97% bij een organische belasting tot 12 g CZV/*l*.d en een CZV_{in} concentratie van ten minste 100 mg/*l*. De hoge zuiveringsrendementen van de EGSB reactoren hing af van de drie hoofdbedrijfsvoeringsparameters: de opstroomsnelheid, de organische belasting en de influent CZV concentratie. De zuivering is optimaal bij een opstroomsnelheid tussen 2,5 en 5,5 m/h (recirculatieverhouding tot 8). In dit gebied was er een goed water-slib contact. Tevens was de slibretentie voldoende hoog. Bij een lagere opstroomsnelheid dan 2,5 m/h daalde de CZV verwijdering wegens massa transportlimitatie van het substraat in de korrels resulterend in een schijnbare K_S hoger dan 28 mg CZV/*l*. Bij hogere opstroomsnelheden dan 2,5 m/h werd diffusielimitatie voorkomen door geforceerd massatransport met een snellere aanvoer van substraat dan de consumptie door de actieve biomassa. Deze hogere opstroomsnelheid gaf een opmerkelijk lage schijnbare K_S van 9,8 mg CZV/*l*. Deze waarde is vergelijkbaar met een intrinsieke K_S (18-30 mg CZV/*l*) van de veel voorkomende acetoclastische methanogeen, *Methanothrix soehngenii*, wat bevestigd dat massa transportlimitatie werd voorkomen. Het hoge rendement bij een influentconcentratie tot 100 mg CZV/*l* wordt verklaard door de lage schijnbare K_S. Bij opstroomsnelheden hoger dan 5,5 m/h trad sterke slibuitspoeling op. De slibuitspoeling kan beperkt worden door geen opstroomsnelheden boven 5,5 m/h toe te passen. De gasproductie per liter influent was niet voldoende voor natuurlijke menging. Toch trad er slibflotatie op door hechting van gasbelletjes aan slib. Om flotatie te voorkomen werd de organische belasting beneden de 7 g CZV/*l*.d gehouden wat de gasproductie verlaagt. Wanneer een hogere organische belasting gewenst is, moet worden voorzien in een betere slibretentie. Er is sterk behoefte aan een verbeterd drie-fasenseparatiesysteem of een verbeterde immobilisatie van de biomassa conform de hydrodynamische aspecten van de EGSB. Een mogelijkheid om de fasenscheiding te verbeteren is het plaatsen van schotten in de overstortrand en het gebruik van een trommelzeef of microzeef. Om gasbellen van biomassa los te krijgen is het plaatsen van een vibrator in de driefasenscheider een optie. De immobilisatie van biomassa aan zwaar of vast drager materiaal kan ook overwogen worden als maatregel tegen flotatie.

Een doelstelling van dit proefschrift was het bepalen van de laagst mogelijke influent CZV van afvalwater met simpele substraten die effectief kan worden verwijderd in UASB en EGSB reactoren. In Tabel 1 staan de theoretisch laagst mogelijke CZV_{in} waarden, berekend gebruik makend van Monod kinetiek, bij een gesteld zuiveringsrendement van 85% en verscheidene organische belastingen. De laagst haalbare CZV_{in} die kan worden behandeld in een UASB en EGSB systeem is resp. 187 mg/*l* en 13 mg/*l*, bij een organische belasting van 5 g CZV/*l*.d. Bij hogere organische belastingen dient de laagst mogelijke CZV_{in} hoger te zijn. Dit betekent dat verdund afvalwater met een CZV lager dan 2000 mg/*l* bestaande uit simpele substraten effectief gezuiverd kan worden bij organische belastingen tot 20 g CZV/*l*.d in UASB reactoren. Vergeleken met de UASB kan een EGSB systeem bij een gelijke organische belasting een veel lagere influent CZV aan. Omdat extreem lage influent CZV concentraties effectief kunnen worden gezuiverd, kan de EGSB reactor gebruikt worden als nazuivering voor residu BZV van elk zuiveringssysteem voor geconcentreerd afvalwater. De EGSB reactor is tevens een alternatief voor aërobe actiefslib-systemen gebruikt voor verdund afvalwater, omdat het EGSB proces meestal is ontworpen voor organische belastingen van 5 g CZV/*l*.d, wat hoger is dan de organische belasting voor aërobe processen. Tevens opent de EGSB de mogelijkheid voor het anaërobe zuiveren van zeer verdund vervuild grondwater met minder dan 100 mg CZV/*l* organische verontreiniging.

Bij zeer lage influent CZV concentratie zoals bij een EGSB systeem van 13 mg/*l*, kan dit leiden tot een opgelost O_2/BZV verhouding van bijna 1, wat misschien leidt tot omstandigheden waarbij facultatieve bacteriën de methanogenen weg concurreren.

Energiewinning van biogas kan van belang zijn in UASB reactoren omdat het economische voordeel aanzienlijk kan zijn. Bij EGSB reactoren zou dit voordeel aanmerkelijk lager uitvallen, wegens het verlies van opgelost methaan via het effluent, bij influent CZV waarden lager dan 100 mg/*l*. De meeste afvalwaterstromen van de levensmiddelen industrie bevatten meer dan 100 mg CZV/*l*

Tabel 1. De theoretisch laagst mogelijke CZV_{in} die kan worden toegepast in UASB en EGSB reactoren voor de zuivering van simpele substraten, veronderstellend de parameters vermeld in [a].

Reactor	Schijnbare K_S (mg COD/*l*)	Organische belasting (g COD/*l*.d)	CZV_{in} (mg/*l*)	CZV_{ef} [b] (mg/*l*)	Methaanopbrengst [gas/(liquid+gas)] [c]
UASB	145 [d]	5	187	28	0,51
		10	483	73	0,81
		15	967	145	0,91
		20	1933	290	0,95
		25	4833	725	0,98
EGSB	10 [e]	5	13	2	0
		10	33	5	0
		15	67	10	0
		20	133	20	0,31
		25	333	50	0,73

a gesteld VSS = 20 g/*l*; V_{max} = 1,5 g CZV/g VSS.d; T = 30°C; en CZV verwijderingsrendement = 85%
b met behulp van Monod kinetiek berekend, gesteld effluent CZV is de reactor substraatconcentratie
c gesteld dat 75 mg CZV/*l* als methaan opgelost in het effluent en (0.96 · 0.85 · COD_{in}) als totaal geproduceerd methaan (gasfase + vloeistoffase); waarin 0,96 staat methaanopbrengst van de verwijderde CZV; 0,85 staat voor het verwijderingsrendement
d gemiddelde waarde uit de literatuur voor UASB korrelslib[10,11]
e waarde gevonden in EGSB reactoren bij dit onderzoek (Hoofdstuk 5)

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Capítulo 6

Discussão e Conclusões

1. As Águas Residuárias de Baixas Concentrações e o Tratamento Anaeróbio

As águas residuárias diluídas contém baixas concentrações orgânicas, porém contribuem significativamente para a poluição das águas em muitos países, devido às grandes quantidades de determinados efluentes líquidos industriais produzidas. Estes despejos são produzidos principalmente em indústrias alimentícias e contém predominantemente matéria orgânica facilmente biodegradável, cujas concentrações são menores que 2000 mg/*l* de demanda química de oxigênio (DQO). Os principais componentes são os ácidos graxos voláteis simples (AGV), alcoóis e carboidratos. Em certos casos também se encontram presentes proteínas, gorduras e ácidos graxos de longa cadeia (AGLC), porém em concentrações bem menores. Os estudos com os sistemas que empregam o reator anaeróbio de leito granular de fluxo ascendente (RAFA ou UASB - upflow anaerobic sludge blanket) e o reator de leito granular expandido (RELGE ou EGSB - expanded granular sludge bed) têm apresentado resultados mostrando desempenhos satisfatórios no tratamento de águas residuárias diluídas. Esta revelação é uma prova de que os processos anaeróbios podem ser perfeitamente viáveis para o tratamento dessas águas residuárias. Entretanto, alguns problemas constatados talvez expliquem por que tais sistemas até hoje não tenham sido tão largamente empregados na prática.

2. Os Problemas

Os problemas do tratamento anaeróbio dos despejos diluídos estão relacionados com as características das águas residuárias em si e com a concepção dos reatores. Os principais problemas relacionados com as águas residuárias são: (i) os baixos níveis de substrato existentes dentro do reator e (ii) a possível presença de oxigênio dissolvido. Os problemas com a concepção dos reatores envolvem a parte de processo e são relativos ao estabelecimento de um equilíbrio entre os requisitos de alta retenção de biomassa e um bom contato entre a água residuária e o lodo. Ambos os requisitos são dependentes da velocidade ascensional do líquido (V_{as}). Para prover um contato adequado entre a água residuária e o lodo são necessários um alto grau de turbulência hidráulica e uma boa expansão do leito de lodo. Entretanto, ambos acarretam uma situação delicada para a retenção do lodo.

3. Objetivos desta Tese

A viabilidade dos reatores RAFA e RELGE para o tratamento de águas residuárias solúveis de baixa concentração foi avaliada nesta tese, focalizando-se os problemas relacionados com as características das águas residuárias. Para esses problemas foi feita uma revisão da literatura, a qual se encontra como introdução no Capítulo 1. Os efeitos do oxigênio dissolvido sobre a metanogênese em lodo granular anaeróbio foram quantificados experimentalmente e se encontram descritos nos Capítulos 2 e 3. Os efeitos de baixos níveis de substrato sobre o desempenho dos reatores foram estudados com o objetivo de se determinar a menor concentração de DQO no afluente (DQO_{af}), a qual seria possível de se tratar em reatores RAFA e RELGE. Os resultados se encontram descritos nos Capítulos 4 e 5. Em adição, certos aspectos tecnológicos relativos a reatores também são discutidos, no tocante aos requisitos de retenção de lodo e contato água residuária/biomassa.

4. Os Efeitos do Oxigênio

O oxigênio é um composto tóxico em potencial para os microrganismos estritamente anaeróbios, em especial para as bactérias acidogênicas e metanogênicas. Estes microrganismos se encontram ao final da cadeia alimentar durante o tratamento anaeróbio de águas residuárias. Entretanto, sabe-se da literatura (Capítulo 1) que ao menos algumas bactérias metanogênicas possuem uma certa tolerância à exposição ao oxigênio tanto em culturas puras como em culturas mistas. Uma indicação da presença de bactérias anaeróbias em ambientes aeróbios é a partida de reatores RAFA, já demonstrada com êxito através do uso de lodo obtido de tanques aerados de estações de lodos ativados.

A quantificação da toxicidade do oxigênio para as bactérias metanogênicas em lodos granulares se encontra descrita no Capítulo 2. Foram utilizados para esse propósito cinco lodos de distintas propriedades, todos coletados de reatores RAFA ou RELGE. Os resultados em ensaios de batelada revelam que os lodos granulares exibem uma alta tolerância para com o oxigênio. As concentrações de oxigênio que causaram 50% de inibição à atividade metanogênica variaram entre 7% e 41% de oxigênio adicionado à fase gasosa dos frascos ao início do ensaio e correspondentes, respectivamente, às concentrações de 0.05 e 6 mg/*l* de oxigênio dissolvido na fase líquida ao final do ensaio. Com o objetivo de se determinar os mecanismos da tolerância ao oxigênio foi avaliada a influência do diâmetro do grânulo, da taxa de respiração das bactérias facultativas, do regime de agitação dos frascos e do substrato. O mecanismo mais importante para a tolerância ao oxigênio foi a presença das bactérias facultativas nos lodos granulares. Isto foi constatado devido à forte correlação entre a taxa de respiração dessas bactérias com os lodos mais tolerantes. A ausência de substrato para as bactérias facultativas diminuiu drasticamente o grau de tolerância. Não houve qualquer correlação entre a tolerância e o diâmetro dos grânulos e a agitação afetou somente os lodos menos tolerantes.

Para se descrever a tolerância das bactérias metanogênicas ao oxigênio em lodos granulares formulou-se uma hipótese. As bactérias facultativas, ao consumirem rapidamente o oxigênio, propiciam a formação de micro-nichos no interior dos grânulos, onde as bactérias metanogênicas ficam, por conseguinte, bem protegidas contra o contato com o oxigênio. Esta hipótese se justifica pela pouca penetração do oxigênio para bem dentro dos grânulos. Na literatura se encontra relatado que a penetração do oxigênio para o interior de biofilmes aeróbios ativos não chega a alcançar mais do que 100 a 300 μm.[1,2] Esta poderia ser a explicação de não se encontrar qualquer correlação entre o tamanho dos grânulos e a tolerância ao oxigênio, uma vez que o diâmetro dos grânulos seria excessivamente grande comparado com as dimensões necessárias para a formação de zonas anaeróbias. Adicionalmente, quando não se forneceu substrato, a taxa de respiração das bactérias facultativas foi bem menor e, por conseguinte, o oxigênio pôde penetrar profundamente nos grânulos e entrar em contato com as bactérias metanogênicas, o que explicaria o aumento da toxicidade. O fato de que alguma tolerância foi observada mesmo na ausência de substrato, é uma indicação de que as bactérias metanogênicas realmente exibem alguma tolerância intrínseca ao oxigênio. Na literatura se encontra relatado que diversas bactérias metanogênicas possuem a enzima dismutase superoxidase (DSO), a qual neutraliza os radicais tóxicos de oxigênio.[3,4] Ademais, também se relata que a existência de transportadores de redox e o crescimento das bactérias em agregados são fatores de proteção às bactérias estritamente anaeróbias em culturas puras.[5,6]

Estes resultados tem implicações importantes para o tratamento anaeróbico de águas residuárias de baixas concentrações. A toxicidade do oxigênio não é prevista na prática, uma vez que essas águas residuárias contém uma demanda bioquímica de oxigênio (DBO) em quantidade bem superior àquela requerida para as bactérias facultativas consumirem o oxigênio presente na forma dissolvida. O oxigênio dissolvido entrará no sistema somente em concentrações abaixo de 10 mg/*l* em condições normais, ao passo que as águas residuárias diluídas contém pelo menos centenas de miligramas de DBO por litro. O efeito não-detrimental do oxigênio dissolvido na prática foi confirmado nos estudos com os reatores RAFA e RELGE (Capítulos 4 e 5). As eficiências em termos de DQO removida e os valores de redox do efluente foram muito similares em dois reatores operando em paralelo e alimentados com etanol, porém um com (e outro sem) oxigênio dissolvido no afluente.

Uma vez que podem ser toleradas altas concentrações de oxigênio, uma segunda implicação seria a sua adição em reatores anaeróbios para melhorar a degradação de poluentes recalcitrantes. Não obstante, apesar da não toxicidade do oxigênio às culturas mistas naturais em lodos granulares utilizados no tratamento anaeróbio, existe ainda a questão da competição por substrato entre as bactérias metanogênicas e as facultativas. Caso o oxigênio fosse adicionado em demasia, todo substrato seria consumido pelas bactérias facultativas, uma vez que elas têm atividade e taxa de crescimento específicas muito maiores do que as das bactérias metanogênicas. As bactérias metanogênicas seriam, portanto, facilmente superadas durante a competição por substrato. Logo, esta competição talvez poderia ser regulada pela quantidade de oxigênio a ser adicionado.

No Capítulo 3 se encontra descrita a pesquisa para se estudar a competição por substrato em co-culturas anaeróbicas e aeróbicas, nas quais foi verificada a ocorrência simultânea de produção de metano e consumo de oxigênio. Primeiramente foi feita uma distinção entre lodos intolerantes e lodos tolerantes ao oxigênio. Nos lodos intolerantes cada unidade de O_2 refletiu-se numa unidade de DBO a menos para as bactérias metanogênicas. Isto significaria a previsão de nenhum metano produzido caso o oxigênio adicionado fosse maior do que a DBO. Ou seja, pode-se prever que, em condições ilimitadas de oxigênio, a competição por substrato seria vencida facilmente pelas bactérias facultativas. Nos lodos tolerantes pôde-se demonstrar a produção de metano mesmo quando o oxigênio foi ilimitado, o que sugere a ocorrência de competição por substrato entre as bactérias metanogênicas e as facultativas, mesmo em ambientes bem aerados. As percentagens de substrato convertido para metano foram 2%, 15% e 25%, respectivamente, para as concentrações de oxigênio dissolvido de 23, 2 e 1 mg O_2/l. A hipótese de ocorrência de metanogênese em condições ilimitadas de oxigênio é que existe um transporte de substrato mais eficiente para as posições mais profundas do grânulo em comparação com o transporte de oxigênio. O transporte de massa por difusão depende do gradiente de concentrações. O fato de que a máxima solubilidade do oxigênio na prática ser menor que 10 mg/*l*, indica que no máximo somente gradientes fracos podem ser formados. Por outro lado, o substrato tem uma solubilidade ilimitada e foi usado em geral com concentrações acima de 1000 mg DQO/*l*. Assim, pôde-se estabelecer gradientes muito maiores para a difusão do substrato. A competição por substrato, após um longo tempo de exposição ao oxigênio, resultou ao final do período na formação de culturas mistas complexas, nas quais ao menos três grupos tróficos puderam ser reconhecidos: as bactérias metanogênicas, as facultativas heterotróficas e as bactérias metanotróficas. Não obstante, confirmou-se que, podendo regular a competição por substrato, o oxigênio pode ser realmente adicionado em reatores anaeróbios. Na literatura são dados exemplos evidentes da habilidade de co-culturas anaeróbias e aeróbias para se degradar poluentes recalcitrantes em um único reator, ao invés de se utilizar fases anaeróbias e aeróbias separadas.[7,8,9]

5. Os Efeitos dos Baixos Níveis de Substrato

As baixas concentrações de DQO no afluente significam baixos níveis de substrato no interior dos grânulos nos reatores, o que resulta em baixa atividade dos lodos. Uma vez que o objetivo é remover o máximo possível da DQO durante o tratamento de águas residuárias, é inevitável que o reator contenha baixos níveis de substrato. Segundo a cinética baseada no modelo de Monod, a atividade específica do lodo depende da concentração do substrato. As taxas de conversão são dependentes da constante de saturação K_S, a qual indica a afinidade das bactérias pelo substrato. Os valores intrínsecos de K_S se referem ao transporte de substrato para o interior de células de bactérias dispersas em suspensões perfeitas. Os valores aparentes de K_S se referem ao transporte de substrato para dentro dos biofilmes. Os valores de K_S aparente são maiores do que os do K_S intrínsico devido ao transporte limitado de substrato para o interior dos biofilmes. Como consequência, são formados gradientes de substrato, havendo menos substrato disponível para a biomassa no interior dos grânulos do que aquele existente no liquor misto do reator. A necessidade de substrato em concentrações adequadas exige que o seu reabastecimento seja efetuado por transporte forçado, a velocidades maiores que o seu consumo pela biomassa ativa. A aceleração da penetração do substrato para o interior dos grânulos, acima daquela somente por difusão, pode ser efetuada por meio do transporte convectivo de massa criado por turbulência no liquor misto do reator. Logo, o tratamento de águas residuárias de baixa concentração de DQO requer uma intensidade de mistura adequada, a qual é obtida por meio de alta turbulência hidráulica e com isso, consequentemente, poder diminuir o valor de K_S aparente. A turbulência conseguida no tratamento de águas residuárias de alta concentração é obtida através da alta produção de gás por unidade de afluente. Independente da concentração do afluente, os reatores RAFA e RELGE também requerem que a intensidade de mistura não seja excessiva, uma vez que é necessário manter uma alta retenção de biomassa.

O estudo da aplicação de reatores RAFA para o tratamento de águas residuárias contendo baixas concentrações de soro de leite ou etanol se encontra descrito no Capítulo 4. O alto desempenho obtido pode ser verificado pela elevada eficiência na remoção de DQO. No caso dos reatores alimentados com soro de leite as eficiências foram acima de 86% para cargas orgânicas volumétricas (COV) de até 3,9 g DQO/*l*.d, desde que a DQO_{af} estivesse acima de 630 mg/*l*. No caso dos reatores alimentados com etanol as eficiências foram acima de 95% para valores de COV até 6,8 g DQO/*l*.d, desde que a DQO_{af} estivesse acima de 422 mg/*l*. O melhor desempenho dos reatores alimentados com etanol, em comparação com aquele dos reatores alimentados com soro de leite, foi obtido devido à presença de proteínas e gorduras no soro de leite. Ao invés da metanogênese, a acidificação do soro de leite foi a taxa limitante para a remoção da DQO. Pode-se concluir deste estudo que a mínima concentração de DQO_{af} aceitável para o tratamento de águas residuárias solúveis em reatores RAFA, a fim de se obter eficiências acima de 85%, é 630 mg/*l* e 422 mg/*l*, para soro de leite e etanol como substrato, respectivamente.

A retenção de lodo não se constituiu em qualquer problema nos reatores RAFA. Entretanto, o grau de mistura não deve ter sido intenso, uma vez que os valores de V_{as} sempre se mantiveram abaixo de 0,1 m/h, os quais não são suficientes para causar turbulência e a expansão do leito de lodo. De maneira semelhante, o grau de mistura natural também deve ter sido baixo, uma vez que a produção de biogás foi pequena. Por conseguinte, a intensidade de mistura não foi suficiente para prover um contato adequado entre as águas residuárias e a biomassa. Na prática, podem ser utilizados concentrações de biomassa maiores que os 5 g/*l* de sólidos suspensos voláteis (SSV) usados nos experimentos com os reatores RAFA, permitindo assim a aplicação de maiores valores de COV. Logo, pode se esperar uma maior intensidade de mistura, devida à produção extra de biogás. Em consequência, pode-se prever que mesmo DQO_{af} menores que 630 e 422 mg/*l* podem ser tolerados, para reatores RAFA alimentados com soro de leite e etanol, respectivamente.

No capítulo 5 descrevem-se os estudos sobre os efeitos de baixos níveis de substrato nos reatores RELGE, usando etanol como substrato em duas faixas distintas de DQO_{af}, 100-200 e 500-700 mg/*l*. Os altos valores de V_{as} (até 21 m/h), obtidos através de recirculação do efluente, foram empregados com o objetivo de se obter uma grande expansão do leito de lodo e um alto grau de turbulência hidráulica. Além disso, pesquisou-se também o problema relacionado com a retenção do lodo.

Demonstrou-se a viabilidade do RELGE para o tratamento de águas residuárias diluídas através da obtenção de um elevado desempenho. As eficiências de remoção da DQO variaram de 80 a 97% para valores de COV até 12 g DQO/*l*.d e valores de DQO_{af} tão baixos quanto 100 mg/*l*. Três principais parâmetros de controle foram responsáveis pelo elevado desempenho obtido no RELGE: V_{as}, COV e DQO_{af}. A otimização do desempenho é obtida quando se utiliza V_{as} entre 2,5 e 5,5 m/h (razão de recirculação de até 8 vezes). O contato adequado entre as águas residuárias e a biomassa, além da exigência de uma alta retenção de biomassa, foram obtidos nesta faixa. A remoção de DQO diminuiu para valores abaixo de 2,5 m/h porque houve limitação de transporte de substrato para dentro dos grânulos, o que resultou em valores de K_S aparente acima de 28 mg DQO/*l*. A limitação de difusão no biofilme foi superada para valores de V_{as} superiores a 2,5 m/h devido ao transporte convectivo de substrato, com um reabastecimento muito mais rápido do que o consumo pela biomassa ativa. O resultado do emprego de alto valores de V_{as} foi um valor de K_S aparente excepcionalmente baixo de 9,8 mg DQO/*l*. Este valor é comparável ao do K_S intrínsico (18-30 mg DQO/*l*) da mais comum das bactérias metanogênicas acetoclásticas, *Methanothrix soehngenii*, o que confirma a superação das limitações de transporte de massa. A elevada eficiência obtida com concentrações tão baixas quanto 100 mg DQO/*l* se justifica pelo baixo valor de K_S aparente. Para valores acima de 5,5 m/h, ocorreu a perda de lodo no efluente devido às condições hidráulicas intensas. Esta perda de lodo pode ser controlada limitando-se V_{as} a valores menores que 5,5 m/h. A quantidade de gás produzida por unidade de afluente não foi significativa para melhorar a turbulência natural. Entretanto, ocorreu a ascensão de lodo devido às forças de empuxo provocadas por bolhas de gás aderidas aos biofilmes. O problema de ascensão de lodo devido ao gás foi evitado limitando-se a COV a 7 g DQO/*l*.d, uma vez que isto diminuiu a produção de biogás. Caso o objetivo seja a aplicação de maiores valores de COV, então será necessário um sistema de retenção do lodo mais eficiente. Uma grande melhoria no separador gás-sólido-líquido (GSL) existente se faz necessária, ou então é primordial que se desenvolva um novo tipo de imobilização de biomassa que se adapte às condições hidrodinâmicas do RELGE. Para se combater a possível perda de lodo por excessiva expansão do leito de lodo ou por flotação de lodo, uma possível estratégia para se melhorar o GSL seria a instalação de lâminas amortecedoras defronte aos vertedores do efluente e o uso de cilindros com malhas de retenção ou micro-peneiras sofisticadas. A liberação de bolhas de gás aderidas aos biofilmes poderia ser melhorada com a instalação de um vibrador no GSL. Merece consideração também a possibilidade de imobilização da biomassa em suportes de alta densidade, móveis ou fixos, para servir de âncora à biomassa contra a flotação de lodo.

A determinação da menor DQO_{af} possível de se remover efetivamente em reatores RAFA e RELGE no tratamento de águas residuárias contendo substratos simples foi um dos objetivos desta tese. A Tabela 1 mostra os mais baixos valores teóricos de DQO_{af} aceitáveis, os quais foram calculados na cinética baseada em Monod e assumindo uma eficiência de 85% para vários valores de COV. A mínima DQO_{af} viável para o tratamento em RAFA e RELGE seria 187 mg/*l* e 13 mg/*l*, respectivamente, para COVs de 5 g DQO/*l*.d. Para valores maiores de COV os valores mínimos admissíveis de DQO_{af} também seriam maiores. Isto significa que as águas residuárias de concentrações menores que 2000 mg/*l* contendo substratos simples podem ser efetivamente tratados em RAFAs até valores de COV de 20 g DQO/*l*.d. Em comparação, os reatores RELGE têm capacidade de tratar a mesma COV, porém a menores valores de DQO_{af}. Tendo em vista que valores extremamente baixos de DQO_{af} podem ser tratados eficientemente, os reatores RELGE podem ser também considerados como uma etapa de polimento para a eliminação da DBO residual de qualquer sistema de tratamento de águas residuárias de alta concentração. O reator RELGE também pode ser considerado como uma alternativa para o processo de lodos ativados no tratamento de águas diluídas, uma vez que o RELGE tem capacidade para COV acima de 5 g DQO/*l*.d, a qual já é maior do que a capacidade daquele processo aeróbio. Além disso, o RELGE abre a possibilidade de se utilizar a tecnologia do tratamento anaeróbio para se remover compostos orgânicos diluídos em águas subterrâneas, contendo menos que 100 mg DQO/*l*.

Em se considerando os valores mínimos mais extremos de DQO_{af} deve-se tomar uma certa precaução. O mínimo valor de 13 mg DQO/*l* para o RELGE resultaria num valor próximo de 1 para a relação oxigênio dissolvido/DBO, o que talvez então acarretaria um ambiente onde as bactérias facultativas poderiam superar as bactérias metanogênicas.

Tabela 1. Valores mínimos de DQO_{af} que teoricamente podem ser utilizados em reatores RAFA e RELGE para o tratamento de águas residuárias contendo susbstratos simples; assume-se os parâmetros delineados em [a].

Reator	K_S aparente (mg DQO/*l*)	COV (g DQO/*l*.d)	DQO_{af} (mg/*l*)	DQO_{ef} [b] (mg/*l*)	Produtividade de metano [gás/(líquido+gás)] [c]
RAFA	145 [d]	5	187	28	0,51
		10	483	73	0,81
		15	967	145	0,91
		20	1933	290	0,95
		25	4833	725	0,98
RELGE	10 [e]	5	13	2	0
		10	33	5	0
		15	67	10	0
		20	133	20	0,31
		25	333	50	0,73

a assume-se SSV = 20 g/*l*; V_{max} = 1,5 g DQO/g SSV.d; T = 30°C; e eficiência de remoção de DQO = 85%

b calculado usando a cinética de Monod, assumindo a DQO do efluente (DQO_{ef}) como sendo igual à concentração do substrato no reator

c assumindo 75 mg COD/*l* como perda de metano dissolvido no efluente e (0,96 × 0,85 × DQO_{af}) como metano total produzido (fase gasosa + fase líquida); o valor 0,96 se refere ao metano total produzido da DQO removida; o valor 0,85 se refere à eficiência de remoção da DQO

d valor médio obtido da literatura para o caso de lodos granulares de reatores RAFA[10,11]

e valor obtido neste estudo (Capítulo 5) para o caso de reatores RELGE

A recuperação de energia do biogás pode ser importante para os reatores RAFA, uma vez que os benefícios econômicos devido à produção de metano serem razoavelmente significativos. No caso do RELGE tais benefícios seriam bem menores para o caso de DQO_{af} abaixo de 100 mg/*l*, devido à quantidade relativamente alta de metano dissolvido que seria perdida com o efluente. Todavia, é de se esperar que a maioria das águas residuárias das indústrias de processamento de alimentos tenham concentrações de DQO_{af} maiores que 100 mg/*l*.

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Curriculum vitae

The author of this dissertation was born in Irapuru, São Paulo, Brazil. He received the basic education in Irapuru and in Umuarama, Paranaguá and Curitiba, Paraná. He obtained his degree in Civil Engineering at the Federal University of Paraná and in Administration Sciences at the Faculty Catholic of Administration and Economy, Curitiba. His master of science degree with *magna cum lauda* was granted from the University of São Paulo (Department of Hydraulics and Sanitary Engineering) at São Carlos. The topic of the master thesis was on the treatment of (low strength) poultry processing wastewaters with the activated sludge process. He worked as civil engineer in consulting company developing design and plans in the field of water supply, and domestic and industrial wastewater treatment systems. Further, he worked mainly with low cost sanitation in faculties of civil and sanitary engineering in São Paulo, and since 1985 he is a faculty member of the Department of Civil Engineering at the Federal University of Pernambuco, Recife. In January, 1989 he started his Ph.D. studies at the Department of Environmental Technology at the Landbouwuniversiteit in Wageningen, The Netherlands. After June, 1994 he reassumes his academic position in Recife and he hopes he can conduct research on the topic of low cost sanitation and anaerobic wastewater treatment. The address will be:

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