Control of calcium carbonate precipitation in anaerobic reactors

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Abstract

Anaerobic treatment of waste waters with a high calcium content may lead to excessive precipitation of calcium carbonate. So far, no proper methods were available to predict or reduce the extent of precipitation in an anaerobic treatment system. Moreover, it also was not clear to what extent precipitation in an anaerobic reactor can be tolerated because adequate knowledge on the structure and quality of methanogenic sludges with high calcium carbonate content was lacking. In this thesis, the feasibility of anaerobic treatment of waste waters with a high calcium content was investigated.

The development of high ash content anaerobic sludges was studied under varying conditions of biomass yield, precipitating quantities of $CaCO_3$ and particle size of the seed sludge. The location of $CaCO_3$ precipitation, within the aggregates or in the bulk solution, appeared to be an important factor determining the quality of high ash content sludges. Precipitation in the bulk solution is considered as favourable for the development of these sludges and can in example be stimulated by seeding with the smallest possible granules or by maintaining the phosphate concentration as low as possible. Operating a reactor containing heavy sludge is often problematical because the crystals will tend to agglomerate, resulting in cementation of the sludge bed. This cementation can be retarded by decreasing the degree of pre-acidification.

Two different options can be distinguished to reduce the extent of calcium carbonate precipitation in an anaerobic reactor. It was found that phosphate, in concentrations of 0.5 - 5 $mgP.l^{-1}$, can drastically reduce the quantity of calcium carbonate precipitation. Additives that only inhibit the formation of calcite and not that of aragonite (e.g. iron) are not suitable, because in that case aragonite, of which the solubility is only slightly lower than of calcite, will accumulate in the reactor. The second option is to remove calcium from the influent in a crystallization reactor reusing the anaerobic effluent alkalinity.

The amount of calcium carbonate precipitation in an anaerobic reactor can be estimated with equilibrium models as developed in this dissertation, which take into account kinetic factors that influence the apparent solubility of calcium carbonate, being the influent composition, the hydraulic retention time and the value of the crystallization rate constant.

In this dissertation, insights have been provided for the design and application of integrated anaerobic-physical-chemical technologies which enable the control of the calcium carbonate precipitation in the anaerobic treatment of waste water with a high calcium content, which appear e.g. in the waste-paper and food processing industries.

Contents

1 General introduction

General aim of the investigation - the aim of the investigation described in this thesis is to develop an integrated anaerobic-physical-chemical technology enabling the control and optimum utilisation of the effects of inorganic precipitation in the anaerobic treatment of waste waters with a high calcium concentration. As a result, anaerobic waste water treatment can be the core technology in closed water circuits, which are characterized by low use of fresh water, due to which the salt concentration (including the calcium salt concentration) tends to increase.

Background; formulation of the problem

The biotechnological technique of anaerobic waste water treatment has become a very successful technology during the last two decades.[62,63] The phenomenon of the formation of highly active and well settleable bacterial aggregates, granular sludge, in upflow anaerobic sludge blanket (UASB) reactors contributed to a major extent to the success of anaerobic treatment techniques. A lot of research was carried out in elucidating the mechanisms of growth of anaerobic granular sludge and in characterization of these aggregates.[2,3,4,22,28] Amongst a variety of other factors, it was found that influent calcium concentrations up to 150 $mgCa^{2+} \cdot l^{-1}$ promote granulation[27,31,34,48,49,66] and also that calcium improves the stability of the granular sludge formed.[33] However, many waste waters contain much higher calcium concentrations, even up to 2.500 $mgCa^{2+} \cdot l^{-1}$.[25,53,58,59] This may lead to an excessive precipitation of calcium carbonate and/or calcium phosphate in an anaerobic reactor, or particularly in the sludge aggregates. Precipitation is often found to result in undesirable situations, such as the formation of a too heavy sludge, loss of specific methanogenic activity of the sludge due to scaling-in of biomass, nutrient deficiency, clogging of discharge pipes and/or scaling in the aerobic post-treatment system.[8,13,25,38,51,57,58,64]

Waste waters with high calcium concentrations for instance prevail in the waste paper processing industry.[37,38,50] The chemical oxygen demand (COD) of the process water of these industries mainly consists of starch. During the waste paper processing, the starch is converted into glucose, which is rapidly further converted into volatile fatty acids by acidifying bacteria. Another important component of waste paper is calcium carbonate, which is used as a filler in the paper. During the waste paper processing, a high calcium process water is generated as a result of the following reaction:[38]

$$2\ CH_3COOH + CaCO_3 \quad \rightarrow \quad 2\ CH_3COO^- + Ca^{2+} + H_2O + CO_2\uparrow \qquad (1)$$

Many efforts were made during the last decade to lower the use of fresh water in industrial processes. In some paper factories, this even resulted in a zero discharge of waste water, consequently in a closed water circuit.[50] In this case, accumulation of calcium will occur in the process water, which might result in scaling problems. Moreover, accumulation of the fatty acids will occur (eq. 1), which causes serious odour problems.[37] These problems can be overcome by applying in-line biological treatment of the process water, e.g. anaerobic treatment.[38] During anaerobic treatment of the process water, the volatile fatty acids are converted into methane and carbon dioxide. The produced carbon dioxide can react with the calcium, resulting in undesirable precipitation of calcium carbonate in the anaerobic reactor and/or in the aerobic post-treatment system.[37,38] For a proper design and operation of such in-line anaerobic treatment systems, adequate knowledge regarding the calcium scaling potential is required.[50]

High concentrations of calcium and/or phosphate also occur in the waste water of citric-acid-, whey-, sugar- and olive-oil factories,[25,39,53] as well as in leachates from landfills. Especially leachates from young landfills are suitable for anaerobic treatment because of their high concentration of volatile fatty acids. However, serious precipitation of calcium and other salts will occur.[51,57,58,59] Waste waters with a high calcium content also arise when waste waters are neutralized with lime, which is the cheapest neutralizing agent.[65,89]

For all above mentioned waste waters, it is of great importance to develop methods which allow the control or prevention of precipitation. In case scaling problems cannot be avoided, the use of anaerobic treatment techniques would be impeded and as a result also less progress would be made to reduce water use. Up to now, no proper methods were available to calculate the amount of precipitation in an anaerobic treatment system. Moreover, fundamental knowledge on the structure and quality of high ash content methanogenic sludge was lacking. This lack of knowledge hinders the implementation and application of sustainable anaerobic technologies for treatment of high calcium waste waters. In this thesis, the feasibility of anaerobic treatment of waste waters with a high calcium content was studied. For this, we focused on the development of a sustainable integrated physical-chemical-biological treatment system. The insights from this research might also lead to applications in other fields where calcium deposition occurs, e.g. in oil production[21,95] or phosphate removal from municipal waste waters.[67]

The phenomenon of calcium precipitation during anaerobic treatment

Quality of high ash content sludge

A high contents of calcium in the influent of an anaerobic reactor can lead to excessive precipitation of calcium carbonate and this may result in severe problems such as *i)* scaling of reactor walls and effluent pipes, *ii)* loss of buffer capacity, *iii)* decreasing efficiency due to sludge washout, *iv)* loss of specific methanogenic activity and *v)* space occupation by inorganic precipitates.[25,57,58] But also positive effects due to the presence of high calcium concentrations were reported. Jördening *et al.*[53] found a distinct improvement of biomass retention in anaerobic fluidized bed reactors due to the presence of calcium concentrations up to 2.5 $gCa^{2+}.l^{-1}$.

The amount of $CaCO_3$ precipitation, the biomass yield and the location where the $CaCO_3$ precipitates are important parameters determining the development and quality of anaerobic sludge. The amount of precipitation strongly depends on the waste water composition and the conversions that take place.[89] The biomass yield depends on the amount of COD degraded and the yield coefficient ($gVSS.gCOD^{-1}$), which varies for different types of COD.[52] The accumulation of biomass compared to the accumulation of calcium carbonate determines the ash content of the sludge at steady state conditions. Ash contents below 60% are considered to be favourable for anaerobic sludge.[65] For sludges with a higher ash content, serious decreases of methanogenic activity were reported.[25,58]

Except the amount of $CaCO_3$ precipitation and biomass yield, particularly the structure of the sludge aggregates needs to be considered with respect to its quality. This structure of the sludge is strongly influenced by the location of calcium carbonate deposition. Precipitation can take place in the bulk solution and/or in the biofilm. Precipitation in the bulk solution is governed by the composition of the bulk liquid medium, which in UASB reactors varies over the height of the reactor, especially when a low upflow velocity is applied.[10] In case the formed precipitates rinse out of the reactor, harmful effects on the sludge structure and quality are unlikely. In case the formed precipitates are retained in the reactor, new biofilms can develop on their surface.[51] On the other hand, also agglomeration of the formed precipitates can take place, which may lead to channelling and clogging problems.[25] Precipitation within the biofilm occurs when the micro-environment in the biofilm is more favourable for precipitation than the environment in the bulk solution.[5] Within anaerobic biofilms, acetic acid is converted in the more weak carbonic acid, leading to elevated pH conditions.[7,26] This increased biofilm pH may initiate calcium carbonate precipitation in the film.[5] As in large granules the core is often depleted from substrate, and therefore does not contribute to the digestion process[3], calcium carbonate precipitation will mainly proceed in the active outer layer of a granule. In case the core of a granule is not depleted from substrate, deposition of precipitates towards the core of a granule is expected, as was observed by Uemura and Harada.[93]

Extent of calcium carbonate precipitation during anaerobic treatment

One of the important parameters determining the feasibility of anaerobic treatment for high calcium waste waters is the amount of $CaCO_3$ that can precipitate. The quantity of calcium carbonate that will precipitate in the anaerobic reactor is preliminary dictated by the concentrations of calcium and carbonate, which depend on a variety of factors, such as the waste water composition (type of substrate, alkalinity) and the conversion reactions occurring in the system.[90] During this thesis, a chemical equilibrium model was developed which enables the calculation of the quantity of calcium carbonate to be expected during anaerobic treatment. Detailed information is provided in chapters 4, 5 and 6. The theory beyond the model can be summarized as follows. In the anaerobic degradation, the biodegradable organic compounds as present in the influent are converted into the completely reduced form of carbon, which is methane (CH_4; oxidation state C -4), and the completely oxidized form of carbon, which is carbon dioxide (CO_2, oxidation state C +4). The TOC/COD ratio (total organic carbon and chemical oxygen demand) is a measure of the state of oxidation of a substrate.[90] Table 1 gives the TOC/COD ratios of several substrates that are often encountered in waste waters.

Table 1. Molecular mass, TOC, COD and state of oxidation of carbon for various substrates

	Molecular mass	Structural formula	Oxidation state C	TOC ($g.l^{-1}$)	COD ($g.l^{-1}$)	TOC/COD
Formic acid	46	CHOOH	+2	0.261	0.35	0.75
Acetic acid	60	CH_3COOH	0	0.4	1.067	0.375
Propionic acid	74	C_2H_5COOH	-0.667	0.486	1.514	0.321
Lactic acid	90	$C_3H_6O_3$	0	0.4	1.067	0.375
Butyric acid	88	C_3H_7COOH	-1	0.545	1.818	0.30
Glucose	180	$C_6H_{12}O_6$	0	0.4	1.067	0.375
Citric acid	192	$C_6H_8O_7$	+1	0.375	0.75	0.5

After the TOC/COD ratio of a substrate has been determined, the quantities of CH_4 and CO_2 formed per gram of COD can be calculated as follows (provided that no growth occurs or other electron acceptors are present)[90]:

$$CH_4\ (mol.l^{-1}) = COD(g.\ l^{-1})/64$$

$$CO_2\ (mol.l^{-1}) = \{(16/3 * TOC/COD) - 1\} * \{COD/64\}$$

In order to calculate the amount of precipitation during anaerobic digestion, the solubility product of calcium carbonate needs to be known, which was often found to differ several orders of magnitude from the theoretical value.[67,90,91] The reason for this discrepancy will be explained below.

Calcium carbonate: thermodynamics, kinetics and the role of additives

Thermodynamics

Calcium carbonate occurs in various polymorphs.[11] These are, in order of increasing solubility, calcite, aragonite, vaterite and amorphous calcium carbonates. The solubility products of calcite, aragonite and vaterite are $10^{-8.4}$, $10^{-8.2}$ and $10^{-7.9}$, respectively, at 30°C and the solubility decreases at higher temperatures.[77] Various amorphous calcium carbonates are described in literature, such as amorphous calcium carbonate and calcium carbonate monohydrate with solubility products of $10^{-6.5}$ and $10^{-7.2}$, respectively, at 30°C.[9,61] Under normal conditions, calcite is the thermodynamically stable form, but at a high degree of supersaturation other, less stable polymorphs will form initially, which will subsequently ripen to form calcite.[55,74,75,84]

During waste water treatment, far less calcium carbonate precipitation usually takes place than is expected based on thermodynamic equilibrium[67,71,90,91] because thermodynamic equilibrium often does not establish. The reason for this is that the kinetics of the precipitation process plays an important role and therefore need to be considered as well.

Kinetics of $CaCO_3$ precipitation

Three main processes can be distinguished in the formation of a solid phase from a supersaturated solution, namely nucleation, crystallization and ripening (Fig. 1).[71,87,88]

Nucleation, or "precipitation" occurs as a result of the interaction between ions or molecules, and it leads to the formation of a critical nucleus. The nucleation process determines the size of the crystals formed. In an unstable, highly supersaturated solution, nuclei can appear spontaneously (this is also called "homogeneous nucleation"). When nuclei are formed on existing particles, the term "heterogeneous nucleation" is used. Because the formation of precipitates on an existing surface requires less energy, heterogeneous nucleation can occur at a lower degree of supersaturation than homogeneous nucleation. For detailed theoretical information on kinetics of nucleation, the concerning literature can be consulted.[29,30,44,88,96,97]

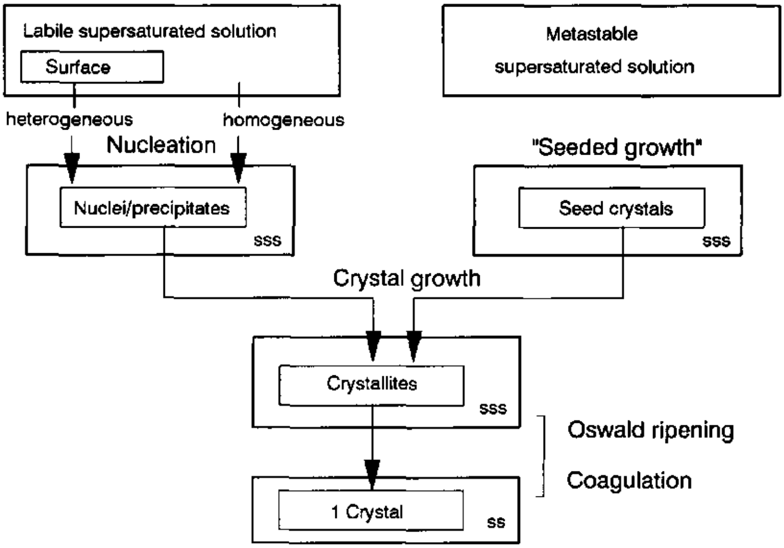

Figure 1. Simplified diagram of the processes involved in solid phase formation of calcium carbonate. sss = supersaturated solution, ss = saturated solution (adapted from: Nancollas and Reddy[71]).

Crystal growth can occur on existing nuclei or added seed crystals. The kinetics of crystal growth are usually studied in a metastable, supersaturated solution.[56] In such a solution, the degree of supersaturation is such low that nucleation is supposed not to occur, but it is sufficiently high for crystal growth after the addition of seed crystals.

One of the primary factors controlling the reaction rate is the degree of disequilibrium. The saturation state, σ, can, in the case of calcium carbonate, be defined as $(Ca^{2+})(CO_3^{2-})$ / $K_{spCaCO3}$. The distance from equilibrium is usually defined as the difference between σ and 1. The general observation is that the rate of precipitation increases with increasing disequilibrium.[69] At different extents of disequilibrium, different mechanisms determine the reaction kinetics. Generally, surface reactions (e.g. diffusion within the hydrated adsorption layer; adsorption at a step; migration along a step; integration at a kink site on the step or dehydration of ions) are most important near equilibrium, while transport control of reaction rates is more important at increasing disequilibrium. In the latter case, possible rate determining steps are transport of lattice ions to the surface by convection; transport of lattice ions to the crystal surface by diffusion through the solution or adsorption of the reactant at the solid/liquid interface.

The crystallization rate can be represented in terms of the general kinetics equation:[73]

$$\text{Rate} = k.s.\sigma^n \tag{2}$$

in which k is the rate constant for crystal growth, s is proportional to the total number of available growth sites on the added seed crystals[6], σ is the degree of supersaturation and n is the effective order of the reaction.

At a high degree of disequilibrium, reaction rates are likely to be controlled by transport limitation and would be described by a simple first-order equation with $n=1$ in equation 2.

At $n=2$, the so called "parabolic rate law" is obtained, which was popularized by the BCF (Burton, Cabrera and Frank[12]) theory of crystal growth at screw dislocations, also known as the spiral growth mechanism. The BCF model is a rather simplistic, but yet convenient, method of picturing surfaces of solids. It treats the solid as being made of "blocks of matter". The lowest energy sites would be in the interior of a crystal, which has no defects. All six faces of a block would be bonded to adjacent blocks. On a perfect infinite surface, one face of a block would be exposed and only five faces can be bonded to adjacent blocks. Blocks along the edge would have two faces exposed, while those on the corners have three faces exposed. The BCF model is important to introduce the idea of steps on surfaces and kinks within the steps (Fig. 2). Because these sites have more exposure than normal surface sites, they are favoured for reaction by attachment, detachment or adsorption of foreign ions.

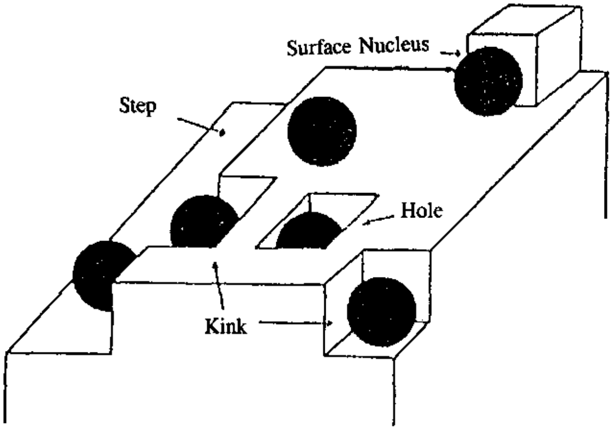

Figure 2. Schematic presentation of crystal surface in BCF model showing adsorbed ions in black.[69]

The parabolic rate law states that if c is the concentration of the dissolved mineral in solution and c_s is the solubility, then (1) growth units enter at surface kinks with a rate proportional to c-c_s, and (2) the kink density varies in proportion to c-c_s. This leads to a growth rate dependence of $(c-c_s)^2$ which is represented by the σ^2 term as seen in equation 2.[76]

The BCF growth theory and the accompanying parabolic rate law were developed for describing near-equilibrium gas-phase crystal growth.[12] However, it also has been used to explain a great deal of crystal growth data outside the range of conditions for which the model originally was intended. The parabolic rate law appears to be a popular explanation of calcite growth kinetics and it often was found to fit well to obtained experimental data.[14,16,35,60] However, it should be applied cautiously because it gives a good fit to almost any initial rate experiment.[24]

While the nonlinear behaviour of growth rate with increasing saturation state is frequently described by the parabolic rate law, recent observations by scanning force microscopy suggest that much of this behaviour can be attributed to the nonlinear rates associated with early-stage surface nucleation.[24] The idea that calcite growth begins with a surface nucleation step is not new. In 1971, Nancollas and Reddy[70] already claimed the formation of surface and bulk nuclei, which they address to as "secondary nucleation". For processes that are surface nucleation controlled, the exponential rate laws are equivalent to equation 2 with $n>2$.

As a result of the *ripening process*, larger crystals are formed. Finely divided solids have a higher solubility than large crystals. As a consequence, small crystals are thermodynamically less stable and tend to recrystallize into large ones.[87,88]

Additives

It is a well-known phenomenon that additives are capable of altering the surface properties of crystals, which leads to changes in nucleation, growth and ripening kinetics. Thereby, changes occur in the shape of crystals as well as in their agglomeration and dispersion behaviour. The effects of additives are generally directly or indirectly related to the adsorption of the additive on the crystal or nucleus surface.

Poisoning of crystal nucleation or growth plays a fundamental role in calcite growth and has a broad impact. In nature, organically-secreted chemicals regulate carbonate biomineralization. Organisms are capable of creating astonishing regulated skeletons of calcium precipitates, because they are able to control the kinetics of nucleation and crystal growth perfectly.[88,98] In industry, phosphate based poisons are often used to control scaling in pipes, heat exchangers and desalination facilities[18,78,81] whereas polyelektrolytes may be used in soda-based detergents to prevent calcium carbonate encrustation on laundry.[93]

Many waste water constituents are known to inhibit the kinetics of calcium carbonate formation.[79] Phosphates and phosphonates are well known inhibitors and have been thoroughly studied.[24,32,42,46,68,78,79,82] Other examples of inhibitors of calcium carbonate crystal growth are the anions sulphate and arsenate; the cations iron, copper, zinc, cadmium and magnesium and the organic additives citrate, nucleic acid and humic acid.[17,20,36,43,68,79,83] Several mechanisms of crystal growth inhibition by additives were proposed in literature, including inhibition of nucleation,[46] adsorption onto growth sites,[68] changes in surface charge and kinetic frustration of cation dewatering.[80]

Phosphate

Phosphate is an important nutrient for anaerobic microorganisms, it is a well known inhibitor of calcium carbonate growth and it is also capable to (co)precipitate with calcium. Moreover, it is a chemical which should not be discharged into surface waters, because it stimulates eutrophication. Therefore, it is an important compound to be considered when studying the feasibility of anaerobic treatment of waste waters with a high calcium content. The fate of phosphate in an anaerobic reactor treating waste waters with a high calcium content is determined by three processes that occur during the anaerobic degradation process: biological assimilation, coprecipitation of phosphate with calcite and/or precipitation of calcium phosphates.

P-removal by biological assimilation or coprecipitation

Anaerobic, non-P-limited biomass contains 6-15 mgP.gVSS^{-1}.[2,85] Assuming a yield for anaerobic microorganisms of 0.02-0.05 g.g^{-1},[52] approximately 0.08-0.54 mgP will be removed from the liquid phase per gram of converted COD. Chemical removal of phosphate by precipitation and/or coprecipitation in the sludge may contribute to a further phosphate removal. Coprecipitation is caused by interaction between dissolved phosphate and the calcite surface during crystal growth of calcite, followed by the incorporation of part of the adsorbed phosphate into the crystal lattice.[20,45] The quantity of phosphate that can coprecipitate was supposed to be dependent on the rate of calcite growth and the rate at which phosphate is adsorbed onto the growing crystals.[41,45] It was recently shown that adsorption of phosphate ions increases with the increase in calcium ion concentration in solution, indicating that phosphate adsorbs on the calcium carbonate surface accompanying Ca^{2+}.[84] In natural waters, coprecipitation of phosphate with calcite highly contributes to phosphate removal, preventing excessive eutrophication.[19,23,40,41,47]

Calcium phosphate precipitation

The Ostwald step rule, or the rule of stages, postulates that the precipitate with the highest solubility (i.e. the least stable solid phase) will form first in a consecutive precipitation reaction. The least stable phase, often an amorphous solid phase, nucleates first because its nucleation rate exceeds that of the more stable phase. In case of calcium phosphates, the polymorphs, amorphous calcium phosphate (ACP), dicalciumphosphate (DCP), octacalciumphosphate (OCP) and hydroxyapatite (HAP) can be distinguished,[73] of which HAP is the thermodynamically most stable phase.[15,54] Generally, amorphous calcium phosphate precipitates first and then recrystallises, eventually via DCP and/or OCP to form hydroxyapatite (HAP; $Ca_5(PO_4)_3OH$).[1] Several additives can inhibit or even hinder the final formation of HAP, such as carbonate, magnesium, P_2O_7 and citric acid.[72] Which calcium phosphate precursor will be formed depends, amongst others, on the pH and the degree of supersaturation.[73,92] For pH values between 7 and 10, ACP is a reasonably well defined compound, with a Ca/P ratio between 1.45 and 1.51.[15,86]

Scope of the thesis

Up to now, no proper methods were available to predict or reduce the extent of precipitation in an anaerobic treatment system. Moreover, it was not clear in how far precipitation in the anaerobic reactor can be tolerated because fundamental knowledge on the structure and quality of high ash content methanogenic sludge was lacking. This lack of knowledge hinders the implementation and proper application of sustainable anaerobic technologies for treatment of high calcium waste waters. Such waters are expected to become much more abundant in the near future as a result of the attempts made in industries to reduce the use of fresh water. This leads to more concentrated process- and waste waters. Therefore, in this dissertation, the feasibility of anaerobic treatment of waste waters with a high calcium content was studied.

Chapters 2 and 3 describe investigations dealing with the development of high ash content sludge under varying conditions of biomass yield, amount of precipitation and particle size distribution of the inoculum. Chapter 2 details experiments carried out with crushed granular sludge and chapter 3 deals with experiments with intact granular sludge as inoculum. It was found that the location of precipitation highly controls the quality of high ash content sludge. In chapter 3, a biofilm model is described, by which factors can be assessed that influence the location of precipitation in an anaerobic reactor.

In chapter 4, it is investigated to what extent calcium carbonate deposition in an anaerobic reactor can be reduced by dosing inhibitors, phosphate and iron, of $CaCO_3$ crystal growth. In this chapter we also present a chemical equilibrium model which allows the calculation of the extent of precipitation, provided the proper apparent solubility of calcium carbonate can be estimated.

In chapter 5, it is investigated to what extent calcium carbonate deposition in an anaerobic reactor can be reduced by calcium removal from the influent. A novel process configuration, comprising an anaerobic reactor combined with a crystallisation reactor, was capable of very selective removal of calcium from the influent, reusing the anaerobic effluent alkalinity.

Chapter 6 describes how to calculate the quantity of calcium carbonate precipitation in an anaerobic reactor by using adapted chemical equilibrium models which take into account important kinetic factors that influence the apparent solubility of calcium carbonate, namely the presence of phosphate, the hydraulic retention time and the composition of the influent.

Chapter 7 discusses and summarizes the insights gained from the investigation and indicates the relevance of this thesis work to the field.

In hoofdstuk 8 worden de bevindingen van dit proefschrift in het Nederlands samengevat.

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2 Development of high ash content crushed methanogenic sludge in UASB reactors

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Abstract - The feasibility of anaerobic treatment of waste waters with a high calcium content (600 - 1200 $mgCa^{2+}.l^{-1}$) was studied. The influence of the amount of precipitation and the influence of differences in biomass yield were investigated on the development of anaerobic sludge. The experiments were performed in lab scale Upflow Anaerobic Sludge Blanket (UASB) reactors, inoculated with crushed granular sludge. Four reactors were operated during 180 days at a constant organic loading rate of 14 $gCOD.l^{-1}.d^{-1}$, treating completely and partly acidified waste water with different calcium concentrations in the influent. Treatment of completely acidified waste water with a calcium concentration of 1200 $mgCa^{2+}.l^{-1}$ resulted in an unstable COD removal efficiency (60-90%). Furthermore, cementation of the sludge bed occurred after 180 days of operation, leading to operational problems. With completely acidified waste water and a lower calcium concentration in the influent (600 $mgCa^{2+}.l^{-1}$), less calcium precipitated and COD removal efficiency always exceeded 98%. The cultivated sludge had a high ash content, 80-90%, and methanogenic activity remained more or less constant at 0.7 $gCOD.gVSS^{-1}.d^{-1}$. However, scaling of reactor compartments occurred under latter condition. The presence of a fraction of non-acidified substrate, i.e. glucose, decreased the risk of cementation, resulted in better granulation of the methanogenic sludge and seemed to prevent scaling of reactor compartments. Considerable amounts of phosphate were shown to be removed from the influent, most probably by coprecipitation, and incorporated in the anaerobic sludge. The results indicate that anaerobic treatment of high calcium waste waters in UASB reactors is more feasible for partly than for completely acidified substrates.

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Introduction

Anaerobic treatment of waste waters is nowadays widely accepted as a proven technology and extensively used. One of the main factors leading to the success of anaerobic treatment was the introduction of high-rate reactors in which biomass retention and liquid retention are uncoupled.[9,24] Amongst these high-rate reactors, the upflow anaerobic sludge blanket (UASB) reactor is mostly applied. Anaerobic sludge in UASB reactors spontaneously immobilizes into well settling granular sludge. Several studies revealed that calcium, at concentrations ranging from 80 to 150 mg.l^{-1}, stimulates the formation of granules during the start up of UASB reactors.[4,16,26] Many waste waters, however, contain considerably more calcium than 150 mg.l^{-1}, for example due to the utilization of lime, a cheap neutralizing agent.[30] A high calcium content can also result from the industrial production process, which is the case for sugar industries[21] and waste paper industries.[14,17] Also leachates from young landfills contain considerable amounts of calcium.[23] Too high contents of calcium in the influent of an anaerobic reactor can lead to excessive precipitation of calcium carbonate. This might result in severe problems such as *i)* scaling of reactor walls and effluent pipes, *ii)* loss of buffer capacity, *iii)* decreasing efficiency due to sludge washout, *iv)* loss of specific methanogenic activity and *v)* space occupation by inorganic precipitates.[11,22,23] Also positive effects due to the presence of calcium were reported. Jördening *et al.*[21] found improvement of biomass retention in anaerobic fluidized bed reactors due to the presence of calcium in concentrations up to 2.5 $gCa^{2+}.l^{-1}$.

The amount of precipitation, the biomass yield and the location of precipitation seem to be important parameters determining the development and quality of anaerobic sludge during treatment of high calcium waste waters. The amount of precipitation depends on the waste water composition (i.e. calcium concentration and alkalinity) and the conversions that take place.[31] The biomass yield depends on the amount of COD degraded and the yield coefficient ($gVSS.gCOD^{-1}$), which varies for different types of COD.[19] The accumulation of biomass compared to the accumulation of calcium carbonate determines the ash content of the sludge at steady state conditions. Ash contents below 60% are considered to be favourable for anaerobic sludge.[25] For sludges with a higher ash content, serious decreases of methanogenic activity were reported.[11,23] Besides amount of precipitation and biomass yield, the structure of the sludge needs to be considered to evaluate its quality. This structure is influenced by the location of calcium carbonate deposition. Precipitation can take place in the bulk solution and/or in the biofilm. Bulk precipitation is governed by the composition of the bulk liquid, which varies over the height of the reactor, especially in UASB reactors with a low upflow velocity.[8] If washout of the formed precipitates occurs, no harmful effects on the sludge structure and quality are to be expected. In case the formed precipitates are retained in the reactor, new biofilms can develop on their surface.[18] On the other hand, agglomeration of the formed precipitates can take place, which leads to channelling and clogging problems.[11] Biofilm precipitation occurs when the micro-environment in the biofilm is more favourable for precipitation than the environment in the bulk solution. Within anaerobic biofilms, acetic acid is converted in the more weak carbonic acid, leading to elevated pH conditions.[7,13] The increased biofilm pH may initiate calcium carbonate precipitation.[6] In large granules, the core is often depleted from substrate and, therefore, does not contribute to the digestion process.[3] Calcium carbonate precipitation can then be expected in the active outer layer of a granule. When the core of a granule is not depleted from substrate, deposition of precipitates towards the core of a granule is expected.

Calcium can also precipitate with phosphate, which is generally present in waste water. Removal of too much phosphate from the reactor solution might cause reactor failure because phosphate is an essential nutrient for microbial growth.[2]

In the present research, the feasibility of anaerobic treatment of waste waters with a high calcium content was studied. The influence of the amount of precipitation and the influence of differences in biomass yield were investigated on the development of anaerobic sludge. Four lab scale UASB reactors were operated. The reactors were inoculated with crushed granular sludge because it was expected that a small particle size of the sludge will favour precipitation in the bulk solution, resulting in less detrimental effects of the precipitation on the structure and quality of the anaerobic sludge. Reactors R1 and R2 were fed with completely acidified substrate (acetate and butyrate) at influent calcium concentrations of 1200 and 600 $mgCa^{2+}.l^{-1}$ respectively while reactors R3 and R4 were fed with partly acidified substrate (acetate, butyrate and glucose), also at calcium concentrations of 1200 and 600 $mgCa^{2+}.l^{-1}$ respectively. The ash and phosphate content of the sludge as well as its biological activity were followed in time. Phosphate measurements of influent and effluent were performed to study to what extent phosphate will be removed from solution.

Materials and methods

Experimental set-up

The experiments were performed in a temperature controlled room at 30 ± 2°C using four glass UASB reactors with a volume of 4.5 l (approx. 55 cm height, 10 cm i.d.). The UASB reactors were equipped with a reverse funnel phase separator. After passing the gas through a concentrated sodium hydroxide solution for removing carbon dioxide and through a column filled with soda lime pellets with indicator, methane production was monitored by a wet gas meter (Meterfabriek, Dordrecht, The Netherlands). The reactors R1 and R2 were fed with completely acidified substrate and the reactors R3 and R4 with partly acidified substrate. All substrates were neutralized by a mixture of $Ca(OH)_2$ and/or NaOH and/or $NaHCO_3$, in such a way that all reactors were supplied with equal alkalinity in the influent. Operational and influent parameters for the reactors are given in Table 1.

Biomass

The UASB reactors were seeded with anaerobic granular sludge originating from a full scale UASB reactor treating waste water of paper mill industries (Industriewater Eerbeek, Eerbeek, The Netherlands). Prior to seeding, the granular sludge was crushed with a hand blender (Multiquick 350 combi, Braun) under anaerobic conditions. To each reactor, 2 litres of wet sludge was supplied, containing 145 gram VSS in total.

Medium

The mineral medium for the UASB reactors consisted of ($g.l^{-1}$): NH_4Cl (0.75), KH_2PO_4 (0.026 for R1/R2 and 0.087 for R3/R4), $MgSO_4.7H_20$ (0.29), NaCl (0.17), yeast extract (0.008) and 1 ml per litre of a trace element solution according to Zehnder *et. al.*[34] For the methanogenic activity tests, the medium consisted of ($g.l^{-1}$): NH_4Cl (0.28), $MgSO_4.7H_20$ (0.1), KH_2PO_4 (0.25), $CaCl_2$ (0.01), $NaHCO_3$ (4) and 0.1 $ml.l^{-1}$ of a trace element solution according to Zehnder *et al.*[34] All chemicals were of analytical grade (Merck AG, Darmstadt, Germany) except the yeast extract (Oxoid, Unipath Ltd, Basingstoke, England) and resazurin (Fluka Chemie AG, Buchs, Switzerland). The media were prepared in tap water, containing about 35 $mgCa^{2+}.l^{-1}$.

Methods

Sludge bed samples were periodically taken from the bottom and top of the sludge bed. Sampling was done by inserting a tube (2 cm id.) into the sludge bed at the desired level (either bottom or top) after which the samples were siphoned from the reactor.

Methanogenic activity tests were performed in duplicate in 120 ml serum bottles filled with 25 ml of medium and 1 gVSS.l^{-1}. The substrate consisted of 2.5 gCOD-acetate.l^{-1}. The headspace of the bottles was flushed with a mixture of N_2 and CO_2 gas in a ratio 70:30 to create anaerobic conditions and a proper CO_2/HCO_3-buffer to stabilize the pH around 7. The bottles were supplied with a second feed of 1 gCOD-acetate.l^{-1} when about 80% of the COD was degraded. Next, the bottles were flushed again and incubated. The methane production rate was then determined by measuring the percentage of methane periodically in the headspace of the bottles during 4-8 hours. The specific methanogenic activity was calculated from the slope of the methane production curve, and expressed as gCOD.gVSS^{-1}.d^{-1}. The percentage of methane in the headspace was determined as previously described.[28]

Table 1. Operational and influent parameters for the four reactors R1, R2, R3 and R4.

	R1	R2	R3		R4	
Period 1)	---	---	I	II	I	II
OLR 2) (gCOD.l^{-1}.d^{-1})	14	14	14	14	14	14
HRT 3) (hr)	9	9	9	9	9	9
COD (g.l^{-1})	5.25	5.25	5.25	5.25	5.25	5.25
Ac:Bu:Gl 4) (COD ratio)	1:1:0	1:1:0	1:1:2	1:1:1	1:1:2	1:1:1
Calcium influent (mg.l^{-1})	1150	575	1150	1150	575	575
Sodium influent (mg.l^{-1})	0	660	660	430	660	660
HCO_3^- influent (mg.l^{-1})	0	0	1740	1150	1740	1150
Chloride influent (mg.l^{-1})	0	0	1010	670	0	0
PO_4-P influent (mgP.l^{-1}) 5)	6 ± 2	6 ± 2	20 ± 5	20 ± 5	20 ± 5	20 ± 5

1) All reactors were run for 180 days. In R3 and R4, a different substrate composition was used during period I (day 0-56) and II (day 57-180); 2) OLR = organic loading rate; 3) HRT = hydraulic retention time; 4) Ac:Bu:Gl = acetate:butyrate:glucose; 5) Phosphate concentration in the influent fluctuated as a result of dilution problems due to clogging because of precipitation.

Particle size distribution was measured using image analysis. Samples of well-mixed aggregates were brought into a petri dish (d=6 cm). Sludge samples were analyzed with a minimum of 200 granules per sample. The petri dish pictures were digitalized and analyzed on a MAGISCAN 2 image analyzer (Applied Imaging, Tyne and Wear, UK) using the image-analyzing software package GENIAS (v4.6, 1993, Applied Imaging, Tyne and Wear, UK). The radius was calculated from the measured object perimeter assuming ideal spherical particles. Particles smaller than 10 μm in diameter were not considered in the calculations.

For scanning electron microscopy (SEM) analysis, granules were fixed for 2 hours in 2.5% glutaraldehyde. After rinsing two times with sodium cacodylate buffer, the granules were fixed for 1.5 hours in 1% osmium tetroxide. After rinsing with demineralized water, the aggregates were dehydrated in an ethanol series (10, 30, 50, 70, 90 and 100%, 20 min. per step) and subsequently critical-point dried with CO_2. After gold/palladium sputter coating, the aggregates were examined on a SEM (JSM 6300F, Jeol).

Analysis

Chemical oxygen demand (COD) was determined using the 'micro-method'.[20] *VFA* were analyzed by gas chromatography as previously described.[27] *Biogas composition* (CH_4, CO_2 and N_2) was measured taking gas samples of 100 μl from the gas sampling port of the reactor and analyzed by gas chromatography.[27] *Calcium* samples were prepared for measuring both the soluble and the total content. For measuring soluble calcium concentrations, effluent samples were filtrated using a membrane filter with a pore size of 0.45 μm, after which the sample was acidified up to pH 2 with HNO_3 (65%) and stored. For analysis of total calcium, samples of 20 ml were directly transferred into a digestion tube, adding 2.5 ml 65% HNO_3, 7.5 ml 37% HCl and 10 ml distilled water. Hereafter, the mixture was heated for two hours at 80°C, subsequently cooled, and diluted with demineralized water in a volumetric flask of 100 ml. Prior to analysis, all samples were diluted with 0.2% lanthan nitrate solution. Calcium was measured by an atomic absorption spectrometer (Model AA975, Varian, Springvale, Australia), using a lamp at 422.7 nm wavelength. The burning gas for the flame was a mixture of air/acetylene (2:1).[1] *Dry weight and ash content* of the sludge were analyzed using standard methods.[1] *Specific gravity of the sludge (wet density)* was measured by use of a pycnometer according to standard methods (1). *Phosphorus* was determined colorimetrically for filtered samples (0.45 μm) by the ascorbic acid method using a spectrophotometer (800nm).[1] *The phosphorus content of the sludge* was determined from supernatant after destruction. Dried sludge (0.5-1 gram) was destructed adding 2.5 ml 65% HNO_3, 7.5 ml 37% HCl and heated during 1 hour at 80°C, 1 hour at 150°C and 3 hours at 250°C. The supernatant was filtrated and diluted in a volumetric flask of 100 ml. Prior to phosphate analysis, the samples were neutralized to pH 2 with NaOH.

Results

Reactor performance

After inoculation with crushed granular sludge, all four reactors were continuously operated at a fixed organic loading rate of 14 gCOD.l^{-1}.d^{-1}. The achieved COD removal efficiencies, shown in Fig. 1, were generally around 98% for reactors R2, R3 and R4. The performance of reactor R1 was less stable and the COD removal efficiency was generally about 90%. After sampling of sludge, the efficiency of this reactor always dropped slightly, and even down to 60% in the period day 110-150 (Fig. 1).

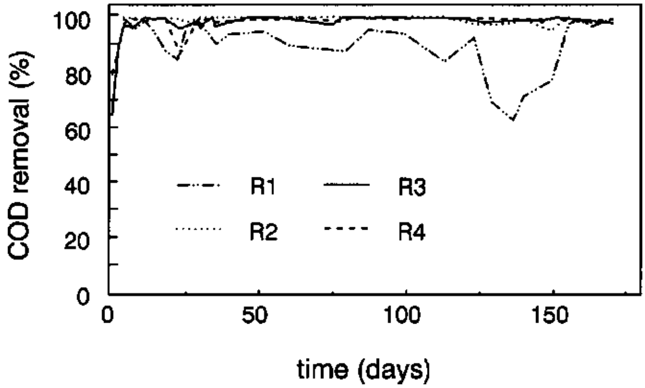

Figure 1. COD removal efficiency of the reactors R1, R2, R3 and R4. Effluent COD consisted mainly of acetate.

Deposition of calcium precipitates

Since the reactors were fed with synthetic waste water with a high calcium content and since bicarbonate was produced from the anaerobic degradation of the substrate and was added in the influent in some reactors (Table 1), calcium carbonate precipitation could prevail. The amount of calcium that precipitated in each reactor varied considerably. Two factors clearly influenced the amount of calcium that precipitated; *i)* The influent calcium concentration; the amount of precipitation was higher in reactor R1, fed with 1200 mgCa.l^{-1}, than in R2, fed with 600 mgCa.l^{-1} (Table 2) and *ii)* The COD removal efficiency; a higher COD removal efficiency resulted in higher bicarbonate concentrations and thus in a higher $CaCO_3$ deposition. E.g. in R1, 265 mgCa^{2+}.l^{-1} precipitated at a COD removal efficiency between 60 and 90%, compared to 706 mgCa^{2+}.l^{-1} during periods of higher removal efficiency (Table 2).

Table 2. Obtained results regarding reactor pH, concentrations of soluble and precipitated calcium and soluble phosphate concentration in the effluent

	R1		R2	R3		R4	
Period[1)]	Eff> 90%	Eff< 90%		I	II	I	II
pH reactor	6.9 ± 0.2	7.2 ± 0.1	7.3 ± 0.2	6.9 ± 0.1	6.9 ± 0.1	6.9 ± 0.1	7.1 ± 0.1
Soluble calcium effluent (mgCa.l^{-1})	434 ± 140	875 ± 65	288 ± 110	713 ± 240	596 ± 60	489 ± 99	402 ± 68
Precipitates in effluent (mgCa.l^{-1})	104 ± 70	53 ± 60	39 ± 50	370 ± 280	38 ± 62	42 ± 60	21 ± 34
Precipitates retained in reactor (mgCa.l^{-1})	602 ± 210	212 ± 120	238 ± 154[2)]	57 ± 289	506 ± 122	39 ± 99	147 ± 102
PO$_4$-P effluent (mgP.l^{-1})	0.2 ± 0.1	2.5 ± 0.6	1.2 ± 0.9	0.4 ± 0.2	5.1 ± 2	3.5 ± 2	6.5 ± 0.7

1) For R1, the data are divided in two periods, efficiency > 90%; data from day 1-120 and 150-180 (Fig. 1)
efficiency < 90%; data from day 120-150

For R2, the data are divided in two periods, according to differences in substrate composition (Table 1)

2) Considerable scale formation of CaCO$_3$ took place on the reactor wall and settler of R2. The thickness at the end of the experiment was about 3mm

In addition to carbonate, also phosphate was removed from the solution. The phosphate removal efficiency fluctuated for the different reactors and also varied with the COD removal efficiency. In reactor R1, the effluent phosphate concentration was only 0.2 mgP.l^{-1} at a high COD removal efficiency (i.e. 90%), while at moderate efficiency (i.e. 60-90%) it remained at a value of 2.5 mgP.l^{-1} (Table 2). In reactors R3 and R4, applied influent concentrations of phosphate were higher (20 mgP.l^{-1}) than in reactors R1 and R2 (6 mgP.l^{-1}). Despite the higher influent phosphate levels, also low concentrations (0.4 - 6.5 mgP.l^{-1}) were found in the effluent of reactors R3 and R4 (Table 2).

Place of deposition and scaling of reactor compartments

Except for reactor R3, 80-100% of the precipitated calcium accumulated in the reactors. The wash out of small calcium precipitates amounted 20-100 mg Ca^{2+}.l^{-1} (Table 2). In reactor R3, 87% of the calcium precipitates washed out of the system, particularly during period I. However, after lowering the glucose fraction in the substrate (period II), the precipitate wash out dropped to 7% (Table 2). Contrary to the other reactors, considerable scaling (2-3 mm) of calcium carbonate occurred in reactor R2 on the glass reactor wall above the sludge bed and on the settler. In reactor R1, the glass wall did not show any scaling and remained clear. In reactors R3 and R4, fed with partially acidified substrate, the reactor parts also remained free of $CaCO_3$. The glass reactor walls were covered with a thin, slimy layer of biomass.

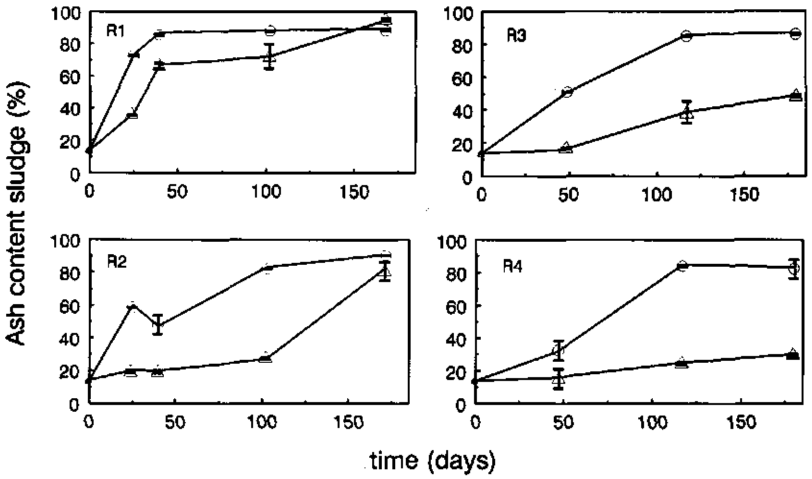

Figure 2. Ash content (% of dry weight) as measured in time for reactors R1, R2, R3 and R4 for sludge sampled from the bottom (○) and the top (△) of the sludge bed.

Sludge development

The increase in ash content of the anaerobic sludge grown in the reactors R1-R4 is shown in Fig. 2. The inorganic fraction increased the fastest in R1; sludge from the bottom and the top of the sludge bed reached an ash content over 70% within two months (Fig. 2). In reactor R2, the ash content increased much slower than in R1, particularly for the top sludge. Nonetheless, the ash content of sludges from both reactors R1 and R2 reached values over 80% after 180 days of operation. In contrast, the rate of increase in ash content in R3 and R4 (fed with VFA and glucose) remained distinctly lower (Fig. 2). The sludges from the top finally reached values of only 50 and 30% for R3 and R4 respectively. The ash content of sludge from the bottom of both reactors reached values of 80% at the end of the experiment (Fig. 2). Together with the ash content, also the dry weight and the density of the sludge increased (Table 3). This allowed equal or even higher concentrations of VSS to be present at the end of the experiment compared to the start up conditions despite the high space occupation by inorganic material. Measurement of the P-content of the sludge revealed a sharp increase in all reactors. The P-content of the seed sludge amounted 12 mgP.gVSS^{-1} and increased during the experiment up to 30 - 192 mgP.gVSS^{-1} after 180 days (Table 3). The maximum specific methanogenic activity remained more or less constant during the experiment and amounted 0.7 gCOD.gVSS^{-1}.d^{-1} for the reactors R1 and R2 and 0.5 gCOD.gVSS^{-1}.d^{-1} for the reactors R3 and R4 (Fig. 3).

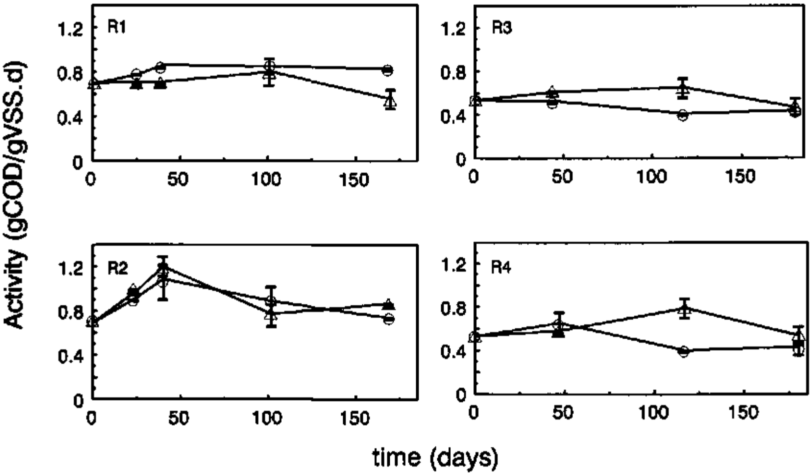

Figure 3. Maximum methanogenic activity (gCOD.gVSS^{-1}.d^{-1}) on acetate as measured in time for the reactors R1, R2, R3 and R4 for sludge sampled from the bottom (○) and the top (△) of the sludge bed.

Table 3. Sludge bed characteristics at the start and the end (t=180 days) of the experiment. Sludge bed volume, dry weight, density, amount of VSS and the phosphorus content are listed for bottom and top sludge and the total sludge bed.

		Volume wet sludge (l)	Dry weight[1] (%)	Density TSS (g.l^{-1})	VSS (% of TSS)	VSS in reactor (gram)	P-content of sludge (mgP.gVSS^{-1})
Start; inoculum		2	8	1055	86	*145*	12
R1	bottom	0.75	90	1878	11	139	192
	top	0.75	70	2294	4	48	61
	total	*1.5*	--	----	--	*187*	---
R2	bottom	0.75	68	2021	9	93	72
	top	0.75	28	1350	18	51	30
	total	*1.5*	--	----	--	*144*	---
R3	bottom	1.3	44	1738	13	129	135
	top	1.3	13	1100	51	95	53
	total	*2.6*	--	----	--	*224*	---
R4	bottom	1.3	33	1442	17	105	149
	top	1.3	8	1100	70	80	46
	total	*2.6*	--	----	--	*185*	---

[1] Dry weight was measured after removal of surface attached water by spreading the sludge during 5 minutes on a grid, which was placed on a hydroscopic tissue (paper napkin).

The results of size distribution measurements of the granules are shown in Fig. 4. In reactors R1 and R2, the size of the cultivated sludge particles hardly increased during the experiment. The median diameter of the inoculum was 0.06 mm while the diameter was 0.09 mm for the top sludge of R1 and 0.11 mm for the bottom and top sludge of R2 after 180 days. The size distribution of the sludge from the bottom of R1 could not be measured, because serious cementation of the sludge bed took place. In fact, it was not longer possible to operate reactor R1 after 180 days. Scanning electron microscopy (SEM) revealed that the cemented sludge consisted of linked small spherical particles with a diameter of approximately 100 μm (Fig. 5a). The surface of the particles consisted of a dense crystal structure with a minor amount of biomass (Fig. 5b). In contrast to reactors R1 and R2, granulation proceeded distinctly better in reactors R3 and R4. By the end of the experiment, the sludge bed in the latter reactors was divided in two equal parts. The bottom part consisted of whitish particles with a median diameter of 1.1 mm and the upper part contained black particles with a median diameter of 0.6 mm (Fig. 4).

The granules from the bottom sludge of reactors R3 and R4 had a dense core of calcium carbonate with a thick layer of loosely attached biomass. The surface of most granules was covered with a dense biofilm in which precipitates could not be detected (Fig. 5c). At locations at the surface where the biofilm layer was thin or absent, the underlying layer of crystal plates showed up (Fig. 5d).

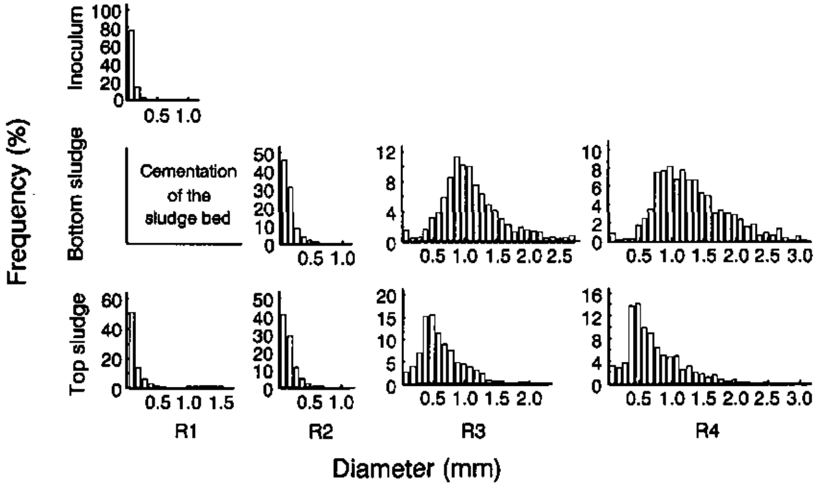

Figure 4. Granular size distribution (frequency (%) of the amount of granules) from the inoculum and the cultivated sludges at the end of the experiments (t = 180) from the bottom and top of the sludge beds of reactors R1, R2, R3 and R4.

Discussion

The results of this study showed that high COD removal efficiencies could be maintained during treatment of waste water with calcium concentrations as high as 1200 $mgCa^{2+}.l^{-1}$ at an organic loading rate of 14 $gCOD.l^{-1}d^{-1}$. Although the ash content of the sludge increased rapidly (up to 90%), the specific methanogenic activity remained at the same level (0.5 - 0.7 $gCOD.gVSS^{-1}.d^{-1}$) during the 180 days of continuous operation. This is in contrast with results of Keenan *et al.*[23] who measured a decrease of the methanogenic activity from 0.56 to 0.25 $gCOD.gVSS^{-1}.d^{-1}$ at a final ash content of the sludge of 78%. El-Mamouni *et al.*[11] even reported a decrease from 0.77 to 0.13 $gCOD.gVSS^{-1}.d^{-1}$ at a final ash content of 50-78%. However, the specific methanogenic activity of sludge from reactors R1 and R2 was 2-3 times lower than of sludge cultivated on similar VFA mixtures in absence of excess calcium.[10,12] This indicates that calcium carbonate precipitation did negatively influence the activity of the sludge. The activity of the sludge from reactor R2 initially increased up to 1.2

$gCOD.gVSS^{-1}.d^{-1}$ but it decreased beyond day 50. This decrease can very likely be attributed to the continuously increasing ash content and density of the sludge, leading to severe scaling-in of the biomass. Compared to reactor R2, the ash content in reactor R1 increased more rapidly, causing faster mass transfer limitations. Latter might explain why higher levels of acetate were found in the effluent of reactor R1 (500 $mgCOD.l^{-1}$) compared to reactor R2 (100 $mgCOD.l^{-1}$) (Fig. 1).

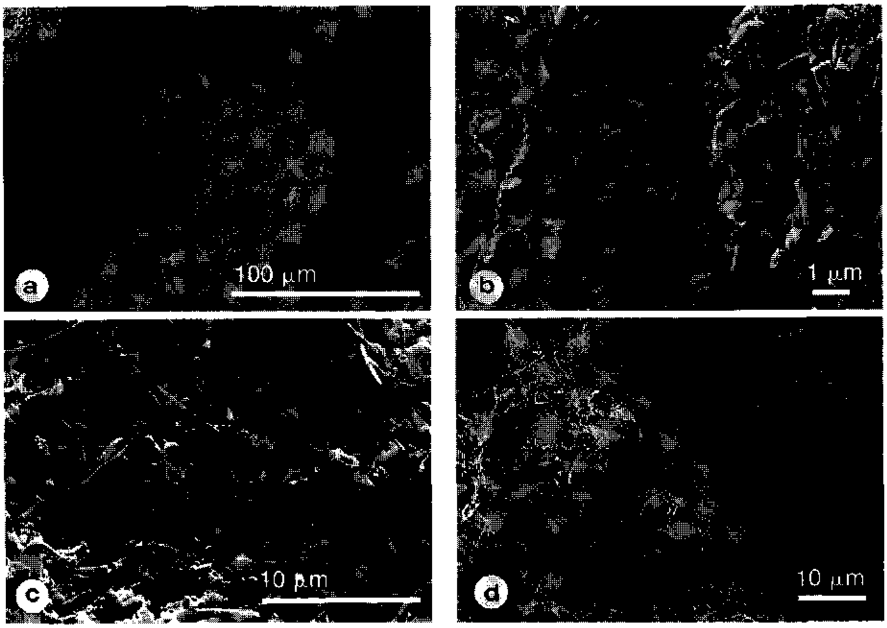

Figure 5. Scanning electron microscope photographs of: (a) cemented sludge from the bottom part of R1 at the end of the experiment which consisted of agglomerated spherical crystal particles, bar = 100μm; (b) detail of the surface of a spherical particle as shown in 5a, showing a dense crystal surface with some attached bacteria, bar = 1μm; (c) granule from the bottom of reactor R3 at the end of the experiment, showing a dense biofilm on the surface, bar = 10μm; (d) granule from the bottom of R3 at the end of the experiment, showing the calcium precipitates as present in the core of the aggregates, bar = 10μm.

Although scaling-in of biomass took place, a serious decrease in methanogenic activity did not occur. Likely, not only biofilm precipitation but also bulk precipitation took place. Bulk precipitation might have been favoured by the small particle size of the sludges. The formed precipitates tended to remain in the sludge bed due to the low upward velocity applied. Subsequently, attachment of biomass could take place on the formed crystal surfaces, which

was indeed confirmed by microscopy. Attachment of biomass to precipitated shells was also observed by Iza *et al.*[18] Very likely, the moderate activity we measured was the average of poorly active, scaled-in biomass and more active biomass as present in thin biofilms on the surface of the formed precipitates. The presence of precipitates did not reduce the concentration of VSS in the reactor, because space occupation by the precipitates was accompanied by a higher dry weight and density of the sludge (Table 3). Consequently, sufficient and active biomass was present, allowing high treatment efficiencies at the applied loading rate of 14 $gCOD.l^{-1}.d^{-1}$. However, in reactor R1, agglomeration of crystals could easily take place due to the high density, low sheer forces and availability of free crystal surface due to poor overgrowth (Fig. 5a). This agglomeration process of crystals is known as ripening[29] and caused severe operational problems.
The sludge from reactor R3 and R4, fed with partly acidified substrate, had a different composition and morphology in comparison to sludges cultivated in reactors R1 and R2, fed with a VFA mixture. The activity of the granules cultivated in reactors R3 and R4 also remained more or less constant in time but did not exceed 0.5 $gCOD.gVSS^{-1}.d^{-1}$. This observed lower methanogenic activity might be attributed to the presence of a relatively large fraction of acidifying biomass[33] and the presence of larger granules, in which mass transfer was probably reduced. The larger size of the glucose-VFA fed granules compared to the VFA fed sludge (Fig. 4) can be attributed to a better granulation process due to the presence of a large fraction of extracellular polymers during growth on glucose.[19] The layered structure we observed, consisting of a core of calcium precipitates with an exterior layer of biomass, was also observed by Uemura and Harada[32] in experiments treating unacidified waste water under thermophilic conditions. This layered structure can be explained by the occurrence of substrate and pH profiles in the sludge particles.[5] The relatively high biomass yield in reactors R3 and R4 very likely prevented cementation of the sludge bed (Fig. 5c).

Regarding phosphate, it can be concluded that much more phosphate accumulation took place in the sludge than could be expected from biological assimilation. Anaerobic, non phosphate limited sludge contains about 10 $mgP.gVSS^{-1}$.[2] In our experiments, P-contents up to almost 200 $mgP.gVSS^{-1}$ were measured, indicating precipitation and/or coprecipitation of phosphate. Based on data of reactor R1 (Table 2), it is likely that coprecipitation took place. At a high COD removal efficiency (>90%), the average calcium and phosphate concentrations were 434 and 0.2 $mg.l^{-1}$ respectively. During a period of lower COD removal efficiency (60-90%), the average concentrations of both calcium and phosphate were higher, 875 and 2.5 $mg.l^{-1}$ respectively. When it is assumed that concentrations of calcium and phosphate in the liquid phase were controlled by the same calcium phosphate polymorph during both periods, the phosphate concentrations should become lower at increasing calcium concentrations. However, the contrary was found. In case of coprecipitation, more phosphate will be eliminated at higher calcium carbonate precipitation rates.[15] The latter is in accordance with our findings.

Conclusions

The feasibility of anaerobic treatment of waste waters with high calcium concentrations depends on the degree and location of calcium precipitation and the biomass yield.

Treatment of completely acidified waste water with a calcium concentration of 1200 $mg.l^{-1}$, at an organic loading rate of 14 $gCOD.l^{-1}.d^{-1}$, results in an unstable COD removal efficiency. The biomass is scaled as a result of the excessive precipitation and the density increases quickly which finally results in cementation of the sludge bed.

Under similar biomass yield conditions but with less calcium in the influent (600 $mgCa^{2+}.l^{1}$), high COD removal efficiencies (98%) can be attained. The inoculation with crushed sludge with a small particle size very likely attributes to this good performance. However, under latter condition, scaling of reactor compartments is favoured.

In the presence of glucose, the feasibility of anaerobic treatment of high calcium waste waters highly increases because precipitates are quickly covered with faster growing acidogens, preventing cementation of the sludge bed. Moreover, treatment of partly acidified waste water results in a better granulation and seems to diminish the risk for scaling of reactor compartments.

Soluble phosphate can be very well removed from the influent. Coprecipitation was found to be the most likely mechanism for the phosphate removal.

Acknowledgements

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3 Impact of location of $CaCO_3$ precipitation on the development of intact anaerobic sludge

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H.V.M. Hamelers & G. Lettinga

Abstract - The development of anaerobic intact granular sludge was studied in UASB reactors under varying conditions of $CaCO_3$ precipitation and biomass yield. Varying precipitating quantities were obtained using different calcium concentrations in the influent and different biomass yields were obtained by feeding with completely or partly acidified substrate. Four reactors were operated at a constant organic loading rate of 13 $gCOD.l^{-1}.d^{-1}$. Additionally, with a biofilm model, key factors were elucidated that determine the location of precipitation within an anaerobic bioreactor, being either the sludge aggregate or the bulk solution. Treatment of completely acidified waste water with high calcium concentrations (780-1560 $mgCa^{2+}.l^{-1}$) resulted in the rapid formation of dense, high ash content granules of which the specific methanogenic activity rapidly dropped and which easily agglomerated, leading to serious cementation of the sludge bed. At a lower influent calcium concentration (390 $mgCa^{2+}.l^{-1}$), treatment of completely acidified waste water resulted in precipitation on reactor compartments, while precipitation within the aggregates did hardly take place. The presence of a fraction of non-acidified substrate retarded cementation of the sludge bed and resulted in development of high ash content sludge with a satisfactory quality. The developed biofilm model was shown to provide a clear, qualitative insight in the factors that affect the location of precipitation, and thereby the quality of high ash content sludge. Moreover, it explains the observed differences in the location of precipitation during the experiments. A low Ca/Na ratio, a small granule diameter and a high crystallization rate constant (=low $CaCO_3$ growth inhibition) were found to stimulate precipitation in the bulk solution, and are considered to be favourable parameters for the development of good quality high ash content sludge.

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Introduction

Anaerobic treatment of waste waters is nowadays accepted as a proven technology, for which the upflow anaerobic sludge blanket (UASB) reactor is mostly applied.[21] In these reactors, the anaerobic biomass spontaneously immobilizes into well settling granular sludge with a good specific methanogenic activity. However, the methanogenic activity might become negatively affected when calcium carbonate precipitates within the granules.[6,15,16] Excessive precipitation of $CaCO_3$ in an anaerobic digester also may lead to cementation of the sludge bed, leading to operational problems.[19]

The risk of calcium carbonate precipitation in anaerobic treatment exists for all waste waters with a high calcium content. Examples of industries where calcium is released to the waste water are the sugar- and waste paper industry.[9,10,14] In anaerobic digestion, organic pollutants are converted to methane and carbon dioxide. Bicarbonate can form calcium carbonate precipitates with the calcium present in the waste water.[28] In addition to carbonate, calcium can also precipitate with phosphate, which is generally present in waste water because it is an essential nutrient for microbial growth.[2] However, phosphate is also an important inhibitor of calcium carbonate crystal growth[8,22] and as shown in previous research, its presence in an anaerobic digester will result in a decrease of the extent of calcium carbonate precipitation.[20]

The extent of precipitation, the biomass yield and the location of precipitation can be considered as important parameters for the development and quality of anaerobic sludge in the treatment of high calcium waste waters. The amount of precipitation depends on the waste water composition, the COD conversion and the kinetics of the $CaCO_3$ precipitation. The latter parameter is particularly influenced by the presence of inhibitors of crystal growth.[20] The ratio between biomass and $CaCO_3$ accumulation determines the ash content of the sludge at steady state conditions. Serious losses of methanogenic activity were reported for sludge with a high ash content[6,16] although also high ash content sludges have been cultivated with a relatively high methanogenic activity.[17,19] This contradiction can be explained considering the structure of the high ash content sludge. This structure is highly influenced by the location where calcium carbonate deposits. Precipitation can take place in the bulk solution but also within the sludge aggregates. Bulk precipitation is primarily governed by the composition of the bulk liquid. It is obvious that if any washout of the formed precipitates occurs, no harmful effects on the sludge structure and quality will occur. In case the formed precipitates are retained in the reactor, new biofilms can develop on the crystal surface.[11] On the other hand, also agglomeration of the formed precipitates can take place, leading to serious channelling and clogging problems.[6,19] Precipitation within the sludge aggregate occurs when the micro-environment in the biofilm is more favourable for precipitation than the environment in the bulk solution.[4] Within the anaerobic biofilms, acetic acid is converted in the weaker carbonic acid, which leads to elevated pH conditions.[5,7] In large granules, the core is often depleted from substrate and, therefore, then does not contribute to the digestion process.[3] Calcium carbonate precipitation in that case can only be expected in the active outer layer of a granule. When the core of a granule is not depleted from substrate, also deposition of precipitates can occur towards the core of a granule, as was observed by Uemura and Harada.[29]

With the present research we aim to gain insight in the factors that determine the location of precipitation and thereby the quality of high ash content anaerobic sludge developing in the treatment of high calcium waste waters. For this we investigated the growth of intact granular sludge under conditions of varying quantities of precipitation and various degrees of biomass yield, which could be achieved by using different calcium concentrations in the influent and by feeding with either completely or partly acidified substrate. The experiments were carried out in reactors with effluent recirculation in order to ensure proper mixing. Additionally, we developed a steady state biofilm model to elucidate the key factors that determine the location of $CaCO_3$ precipitation within an anaerobic reactor.

Materials and methods

Experimental design

Four identical glass, recycled upflow anaerobic sludge blanket (UASB) reactors were used with a reactor volume of 160 ml and a total system volume of 450 ml (Fig. 1). Effluent recirculation was applied to obtain a homogeneous liquid composition in the system, which resulted in an upflow rate of 1.8 $m.hour^{-1}$. The influent flow rate was 1 $l.d^{-1}$ and the recycling flow rate 130 $l.d^{-1}$. The methane production was monitored by liquid displacement after passing the gas through a concentrated sodium hydroxide solution (Fig. 1). The chosen influent composition for the reactors was such that various degrees of precipitation can be attained, resulting in development of sludges with varying ash contents. The reactors were operated at an organic loading rate of 13 $gCOD.l^{-1}.d^{-1}$ and a hydraulic retention time (HRT) of 9 hours. The influent composition of the four reactors is given in Table 1.

Biomass

The UASB reactors were seeded with intact anaerobic granular sludge originating from a full scale UASB reactor treating paper mill waste water (Industriewater Eerbeek, Eerbeek, The Netherlands). Each reactor was supplied with 50 ml of wet sludge, corresponding to 6 g VSS per reactor.

Medium

The mineral medium for the UASB reactors consisted of (g.l^{-1}): NH_4Cl (0.75), KH_2PO_4 (0.065), $MgSO_4.7H_20$ (0.29), NaCl (0.17), yeast extract (0.008) and 1 ml per litre of a trace element solution according to Zehnder *et. al.*.[30] For the batch experiments the medium consisted of (g.l^{-1}): NH_4Cl (0.28), $MgSO_4.7H_20$ (0.1), KH_2PO_4 (0.5), $CaCl_2$ (0.01), $NaHCO_3$ (4) and 0.1 ml.l^{-1} of a trace element solution according to Zehnder *et al.* (1980). The substrates used in the experiment consisted of mixtures of acetate and glucose in a ratio as listed in Table 1. All chemicals were analytical grade (Merck AG, Darmstadt, Germany) except the yeast extract (Oxoid, Unipath Ltd, Basingstoke, England) and resazurin (Fluka Chemie AG, Buchs, Switzerland). The media were prepared in demineralized water.

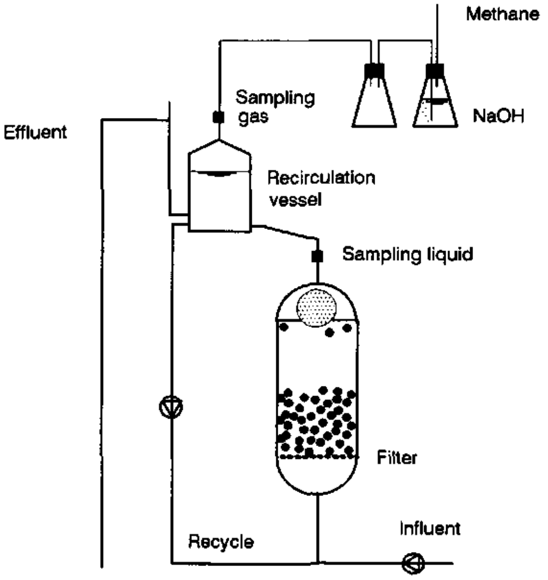

Figure 1. Diagram of the UASB reactors.

Table 1. Process conditions and influent composition for the reactors R1, R2, R3 and R4.

		R1	R2	R3	R4
COD	(g.l^{-1})	5	5	5	5
C_2:glucose	(COD ratio)	1:0	1:0	1:0	3:1
Ca influent	(mg.l^{-1})	1.560	780	390	780
Na influent	(mg.l^{-1})	0	900	1.350	450
P influent	(mgP.l^{-1})	15	15	15	15

Experimental methods

Samples from the sludge bed were periodically taken from the reactors to determine ash content, dry weight and methanogenic activity. *Methanogenic acetoclastic activity* tests were performed in 120 ml serum bottles filled with 25 ml of media and 1 gVSS.l^{-1}. The substrate consisted of 2.5 gCOD-acetate.l^{-1}. The headspace of the bottles was flushed with a mixture of N_2 and CO_2 gas (70:30 v/v) to create anaerobic conditions and a proper CO_2/HCO_3-buffer to stabilize the pH around 7. The bottles were supplied with a second feed of 1 gCOD-acetate.l^{-1} after 80% of the initial substrate COD was degraded. Next, the bottles were flushed again and incubated. The methane production rate was then determined by measuring the percentage of methane periodically in the headspace of the bottles during 4-8 hours. The specific methanogenic activity was calculated from the slope of the curve, and expressed as $gCH_4COD.gVSS^{-1}.d^{-1}$. The percentage of methane in the headspace was determined as described previously.[25] *Particle size distribution* was measured using image analysis.[19]

Analysis

VFA samples were analyzed by gas chromatography as described previously.[24] *Chemical oxygen demand (COD)* was determined according to the 'micro-method' as described by Jirka and Carter.[13] *Biogas composition* (CH_4, CO_2 and N_2) was measured taking gas samples of 100 μl from the gas sampling port of the reactor (Fig. 1) and analyzed by gas chromatography.[24] *Calcium* samples of the effluent were prepared for measuring the soluble calcium content. For this, effluent samples were filtrated using a 0.45 μm membrane filter, after which the sample filtrate was acidified up to pH 2 with HNO_3 (65%) and stored at 4°C. Prior to analysis, samples were diluted with 0.2% lanthan nitrate solution. Calcium was measured by an atomic absorption spectrometer (Model AA975, Varian, Springvale, Australia), using a lamp at 422.7 nm wavelength. The burning gas for the flame was a mixture of air/acetylene (2:1).[1] *Dry weight and ash content* of the sludge were analyzed using standard methods (APHA, 1992). *Phosphorus* in the influent and effluent of the reactors was determined colorimetrically for filtered samples (0.45 μm) by the ascorbic acid method using a spectrophotometer (800nm).[1]

Modelling

A steady-state biofilm model was developed to elucidate factors that determine the location of $CaCO_3$ precipitation within an anaerobic reactor. Two possible locations for precipitation are distinguished in the model: *(1)* within the sludge aggregate and *(2)* in the bulk solution. The bulk solution is considered to be a completely mixed compartment, which is achieved by effluent recirculation. Transport in the biofilm takes place by diffusion (Fig. 2).

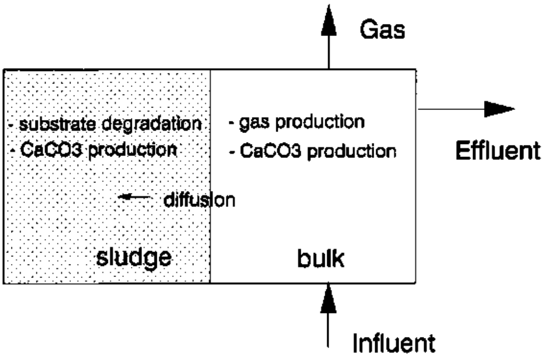

Figure 2. Diagram of the steady-state biofilm model used to gain insight in the factors that determine the location of $CaCO_3$ precipitation in an anaerobic reactor.

The flux from the bulk solution towards the biofilm (J_f) is expressed by:

$$J_f \quad = \quad k_f\,([Ca]_f - [Ca]_b)$$

where:

J_f = flux from the bulk solution towards the biofilm ($mol.hour^{-1}.dm^{-2}$)
k_f = transport coefficient ($dm.hour^{-1}$)
$[C]_f$ = concentration in the biofilm ($mol.l^{-1}$)
$[C]_b$ = concentration in the bulk solution ($mol.l^{-1}$)

The following assumptions were made in the development of the mathematical model:

- Only the degradation of acetate is considered.
- The diffusion coefficient is equal for all substrates and products.
- The concentration at the surface of the granule is equal to that in the bulk solution, allowing the calculation of the transport coefficient, k_f.
- The concentration decreases linearly within the granule and the average concentration is $[C]_f$. The transport coefficient, k_f, then equals D_f / r where D_f = diffusion coefficient in the biofilm ($dm^2.hour^{-1}$) and r = biofilm radius (dm)
- CO_2 and CH_4 are the only components in the gas phase, and all gas is formed in the bulk solution
- Due to the low solubility of methane, it is assumed that all methane formed immediately escapes to the gas phase.

For describing the degradation rate of acetic acid (R_{Ac}) we used Monod kinetics:[27]

$$R_{Ac} = X_f * V_{max} * [HAcT] / (K_s + [HAcT])$$

where:

V_{max} = maximum substrate consumption rate ($mol.gVSS^{-1}.hour^{-1}$)

X_f = microorganism density in the biofilm ($gVSS.l^{-1}$)

K_s = Monod's half-velocity constant ($mol.l^{-1}$)

[HAcT] = total acetic acid concentration in the system ($mol.l^{-1}$)

The rate of calcium carbonate precipitation can be calculated using is represented the following equation:[8,20,23]

$$r_{CaCO3} = k.s\,(\sqrt{IAP} - \sqrt{K_{sp}})^2$$

where:

r	= calcium carbonate accumulation rate	($mol.l^{-1}.s^{-1}$)
k	= growth rate constant	($dm.s^{-1}$)
s	= reactive crystal surface area	($dm^2.mol^{-1}$)
IAP	= ionic activity product $(Ca^{2+})(CO_3^{2-})$	($mol^2.l^{-2}$)
K_{sp}	= solubility product of $CaCO_3$	($mol^2.l^{-2}$)

The applied equilibrium constants of carbonic acid and acetic acid and an overview of input parameters are listed in Table 3.

Results

COD removal efficiency

The COD removal efficiencies of all four reactors are presented in Figure 3. In reactor R1, the COD removal efficiency gradually increased to 99% after 50 days, and deteriorated afterwards, with efficiency drops down to 10% (Fig. 3a). The experiment with R1 was terminated after 165 days because the sludge bed was cemented to such a high extent that it was no longer possible to operate the reactor. However, note that the efficiency was still 70% at day 165. In reactor R2, fed with a lower influent calcium concentration, the COD removal reached 99% immediately after the start-up. Like in reactor R1, the efficiency in R2 was far from stable (Fig. 3b). The experiment carried out with this reactor was terminated after 165 days as well, because of cementation of the sludge bed. Reactor R3, fed with the lowest influent calcium concentration, achieved a COD removal efficiency of 99% after two weeks (Fig. 3c). The experiment with this reactor was terminated after 44 days, because clogging of the inlet filter (Fig.1) resulted in high sheer forces in the reactor and deterioration of the granular sludge, which led to serious wash-out of biomass. The COD removal efficiency in reactor R4, fed with partly acidified substrate, remained very high (97-99%) until day 230. At that day, sludge was removed because the reactor was fully filled with sludge and the efficiency dropped to 90%. Cementation of the sludge bed ultimately also occurred in this reactor (after 260 days), and the experiment was then terminated. The COD removal efficiency was still 95% at that time (Fig. 3d).

Table 2. Minimum and maximum values measured for reactor pH, precipitated quantities of calcium, dissolved phosphate concentrations and $\%CO_2$ in the biogas.

	R1		R2		R3		R4	
	min	max	min	max	min	max	min	max
pH reactor	6.7	7.2	7.2	7.6	7.5	8	7.1	7.4
Ca precipitated $mgCal^{-1}$	260	860	230	630	190	290	280	430
PO_4-P effluent ($mgP.l^{-1}$)	0.6	3.9	0.6	6.3	4.4	6.5	2.0	5.0
% CO_2 in biogas	10	20	8	14	5	7	15	20

Quantity of calcium carbonate precipitation

Table 2 presents the results of the reactor pH, the precipitated quantities of calcium, the effluent phosphate concentrations and the pCO_2 of the biogas. The precipitated quantities of calcium carbonate in each of the reactors fluctuated widely, mainly because of the fluctuations in COD removal efficiency (Fig. 3).

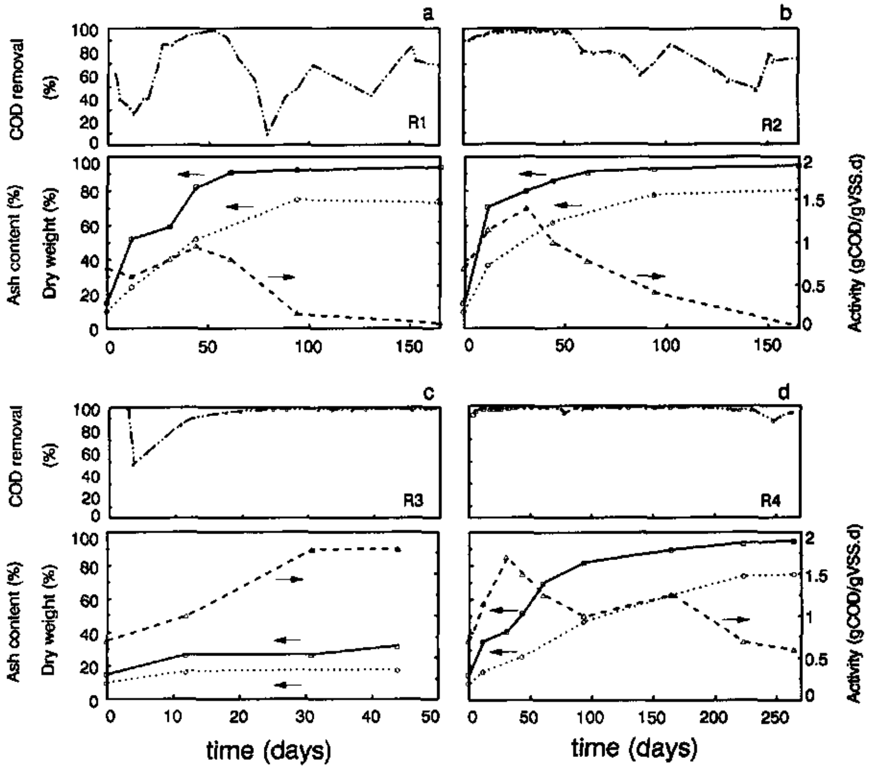

Figure 3. Cause of the assessed COD removal efficiency (¨ ¨--), ash content (——), dry weight (···) and methanogenic activity (---) of the sludge in UASB reactors 1-4.

Location of calcium carbonate precipitation

In the reactors R1 and R2, calcium carbonate precipitated mainly within the sludge granules. The crystals became visual at the surface of the granules after 120 days in both reactors. These surface crystals showed a tendency to agglomerate, and due to that, cementation of the sludge bed commenced. Together with the onset of agglomeration, also scaling of the marble (phase separator, Fig. 1) became apparent. In contrast, hardly any calcium carbonate precipitated within the sludge in reactor R3 but instead it precipitated mainly on the glass reactor wall, on the wall of the recirculation vessel, on the settler (marble) and within the teflon filter at the bottom of the reactor, which consequently was clogged frequently (Fig. 1). Like in reactor R1 and R2, in reactor R4 the calcium carbonate precipitated within the sludge aggregates. However, the first signs of granule cementation became visible only after 230 days.

Sludge characteristics

Since in reactors R1 and R2, calcium carbonate precipitated within the sludge aggregates, the sludge ash content in these reactors increased rapidly, reaching 90% within 50 days (Fig. 3a/3b). Beyond day 50, the dry matter content of the sludge continued to increase in both reactors, from 50% to 70%. This shows that the granules became completely filled with precipitates, as was confirmed by microscopic observations of cross sections of the granules (pictures not shown). The maximum specific methanogenic activity of the sludge in reactors R1 and R2 increased during the first 50 days, but from then onwards it continuously decreased. At the end of the experiment, hardly any activity was left (Fig. 3a/b). The average diameter of the sludge granules remained at 2.1 mm during the 165 days of operation.

In reactor R3, very little calcium carbonate precipitated in the sludge aggregates and the ash content only increased to 32% (Fig. 3c). In case calcium carbonate would have precipitated exclusively in the sludge, the ash content should have increased to 70% at day 44. The methanogenic activity of the sludge in this reactor increased from 0.7 to 1.8 $gCOD.gVSS^{-1}.d^{-1}$ in this short experimental period (Fig. 3c). The average diameter of the granules in R3 was 1.8 mm at the start of the experiment, and could not be measured at the end of the experiment because deterioration and washout of the sludge had taken place.

In reactor R4, operated at an influent calcium concentration of 780 $mgCa.l^{-1}$ and partially fed with glucose, less calcium carbonate precipitated than in R1 and R2 (Table 2). Moreover, the biomass yield was higher due to the presence of glucose. As a result of these factors, a slower increase of the sludge ash content prevailed compared to the sludge in R1 and R2. However, despite that, the ash content of the sludge also reached a value of 95% after 260 days of operation. During the first 40 days of the experiment, the sludge activity increased to 1.7 $gCOD.gVSS^{-1}.d^{-1}$ and thereafter, it gradually dropped to 0.6 $gCOD.gVSS^{-1}.d^{-1}$ (Fig. 3d). After 94 days of operation, when the ash content of the sludge amounted to 80%, we observed a porous structure of the granules. However, after 165 days of operation, the granules were completely filled with calcium precipitates except at one spot, where a pit allowed transport of substrates and products towards the core of the granule. The outer part of the granules consisted of a thick layer of biomass, and the inner part of a dense core of calcium carbonate and scaled-in biomass. About forty-five days later (at day 210), the entire sludge bed tended to float. Most of the granules were stuck together by a slimy, stringy biomass structure. This layer was barely permeable for gas. Although most of the granules could easily be separated from each other, some of them were agglomerated tightly by crystal bindings. Clearly visible crystals were spread over the surface of many of the granules, and most of them were covered with a thin biomass layer. This biomass layer seems to prevent crystal agglomeration. Due to the high density of the granules, and because the reactors were filled up completely with sludge, any distinct abrasion did not occur, allowing cementation to take place. When the operation of the reactor was terminated at day 260, approximately half of the sludge bed was agglomerated by crystal bindings. During the experiment, the diameter of the granules in R4 increased from 2 to 3.1 mm at the termination of the experiment. Even at that time, the activity was still 0.6 $gCOD.gVSS^{-1}.d^{-1}$ and the COD removal efficiency 95% despite the very high ash-content (95%) of the sludge.

Discussion

Qualitative interpretation of experimental data

In reactors R1 and R2, fed with acetate as substrate and with calcium concentrations of respectively 1560 and 780 $mgCa.l^{-1}$, precipitation exclusively proceeded within the granules and not in the bulk solution. As a result, the granules became completely filled up with precipitates. Due to that, the mass transfer became very low and the specific methanogenic activity dropped accordingly. However, when operated at a lower calcium influent concentration (R3; 390 $mgCa.l^{-1}$) than R1 and R2, any significant precipitation within the sludge aggregates did not occur, but instead, the $CaCO_3$ mainly precipitated onto glass and plastic reactor parts. The presence of a fraction of non-acidified substrate (glucose) in the influent, clearly had a strong effect. Although ultimately the ash content of the sludge reached a value of 95%, it increased much slower in reactor R4 than in R1 and R2 and the methanogenic activity remained at a reasonable value (Fig. 3d). Appearing crystals at the surface of the granules did not show a strong tendency to agglomerate. The main reason for that can be found in the fact that they were rapidly covered with a layer of fast growing acidifying biomass. This layer delays the cementation of the sludge bed. The main question arising from these experiments is why in reactors R1, R2 and R4 the precipitation occurred within the sludge aggregates, while in R3, it proceeded on glass and plastic reactor parts. The reason for this should be found in differences between the feed solutions of R1, R2, R4 and R3. To explain the observed discrepancies in location of precipitation, the different conditions applied for the four reactors were simulated with the developed biofilm model. The used input parameters are summarized in Table 3.

Table 3. The constants used in simulations with the biofilm model.

Constant		Value	Unit
k_1	equilibrium constant for H_2CO_3/HCO_3^-	4.10^{-7}	$mol.l^{-1}$
k_2	equilibrium constant for HCO_3^-/CO_3^{2-}	4.10^{-11}	$mol.l^{-1}$
k_H	Henry's constant	29.3	$mol.l^{-1}.atm^{-1}$
K_a	equilibrium constant for HAc/Ac$^-$	1.10^{-5}	$mol.l^{-1}$
K_{sp}	solubility product of calcite	$10^{-8.5}$	$mol^2.l^2$
V_{max}	maximal conversion rate for HAc	$3.3.10^{-3}$	$mol.gVSS^{-1}.hour^{-1}$
X_f	microorganism density in biofilm	120	$gVSS.l^{-1}$
K_s	Monod's constant for HAc conversion	0.0001	$mol.l^{-1}$
Q	flow rate	0.04	$dm^3.hour^{-1}$
V_f	sludge volume	0.05	l
V_b	bulk volume	0.1	l
k'	crystallisation rate constant (=k.s)	$360 - 7.2.10^5$	$l.mol^{-1}.hour^{-1}$
k_f	transfer coefficient	function of r	$dm.hour^{-1}$
A_f	total sludge area in the reactor	function of r	dm^2
r	biofilm radius	various	dm
D_f	diffusion coefficient in the biofilm	$6.67.10^{-4}$	$dm^2.hour^{-1}$

To simplify the calculations, it was assumed that all granules were 3 mm in diameter, and that the crystallization rate constant, k, was 50 $l.mol^{-1}.s^{-1}$.[20] The calculated rates of calcium precipitation for the biofilm and for the bulk phase are shown in Fig. 4a. They appear to be in the same order of magnitude as the calculated rates (Table 2). By dividing the rate of precipitation in the biofilm over that in the bulk solution, a ratio factor is obtained which allows the estimation of the most likely location of precipitation (Fig. 4b). At a ratio of 1, rates are equal and there is no preference for biofilm or bulk precipitation. At a ratio < 1, there is a tendency for precipitation in the bulk solution, while at a ratio >1, precipitation in the biofilm is favoured. The calculated values for the ratio factor shown in figure 4b reveal the ratio is lowest (0.25) for R3, indicating that precipitation is least likely to occur within the biofilm in this reactor, which was indeed found. Consequently, the model looks a suitable tool to explain where $CaCO_3$ precipitation will proceed despite the fact that assumptions and simplifications were made in the present model. So we assumed equal and constant crystallization rate constants for all four reactors. However, in reality, this constant depends on the phosphate concentration in the reactor, because it is an inhibiting compound for calcium carbonate crystal growth.[20] As the phosphate concentration varied in all four reactors (Table 2), k was not constant during the experiments. Moreover, a uniform granule diameter of 3 mm was assumed, while in reality, the size of the granules was more diverse (data not shown). The effect of those two factors on the location of precipitation, and consequently on the quality of high ash content sludge, will be discussed below.

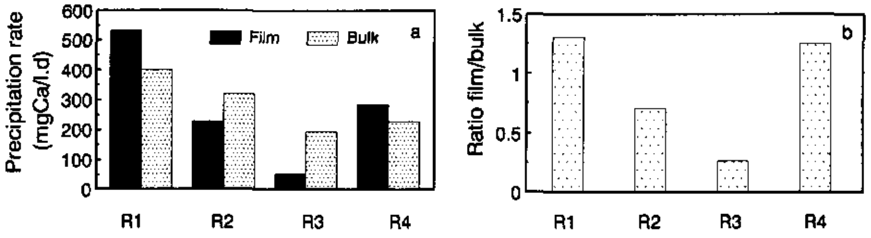

Figure 4. **(a)** Calculated steady-state precipitation rates in the bulk solution and biofilm phase in reactors R1-R4. The COD concentration was $5 g.l^{-1}$, and the Ca/Na composition as follows:

R1: Ca=0.04M, Na=0M R2: Ca=0.02M, Na=0.04M

R3: Ca=0.01M, Na=0.06M R4: Ca=0.02M, Na=0.02M

(b) Calculated ratio between the precipitation rates in the film and bulk solution for the four reactors.

Effects of the crystallization rate constant and granular size on the location of precipitation

From the discussion above, it is clear that the Ca/Na ratio of the influent is an important parameter determining the location of precipitation in an anaerobic reactor. But, as explained above, a substantial effect can also be expected from the granular size and the crystallization rate constant with respect to the location of the precipitation. This can be demonstrated, though also qualitatively, with the developed biofilm model. For this purpose, simulations were carried out with k values varying between 1 and 200 $mol.l^{-1}.s^{-1}$, which are, according to earlier findings, reasonable values in anaerobic digestion under conditions of high (5 $mgP.l^{-1}$) and low phosphate concentrations (0-1 $mgP.l^{-1}$).[20] Moreover, we made simulations for granule diameters varying between 0.75 and 6 mm. The values for the remaining applied parameters are summarized in Table 3. The results of the simulations are presented in Figure 5. It appears that for small granules (0.75 mm), and at low values of k' (1-100 $mol.l^{-1}.s^{-1}$), there does not exist any clear preference for bulk or biofilm precipitation. Only at very low inhibition by phosphate (k'=200), there exists some tendency towards bulk precipitation. For larger granules, and under conditions of smaller crystallization rate constants, k, precipitation in the biofilm tends to dominate (Fig. 5). These examples illustrate that the location of precipitation particularly will be controlled by the rates of conversion and mass transport processes, which are, amongst others, influenced by the granular size, the crystallization rate constant and the composition of the feed solution.

Possibilities to cultivate a high ash content sludge with appropriate methanogenic activity

In our present experiments, we measured a significant loss of activity for the sludge in R1 and R2. These findings are in fact quite in contradiction with those of previous experiments, where no drop of methanogenic activity was found.[19] However, in these previous experiments, conducted with similar feeds compared to the present experiments, we used a seed sludge with a distinct smaller diameter, e.g. only 0.06 mm compared to 2 mm in this study. Apparently, the smaller size is the size of the granules in the seed sludge the more bulk precipitation will dominate. Consequently, less scaling-in of biomass will prevail, and the less will be the drop in specific methanogenic activity. This prevalence of bulk precipitation in case of smaller aggregates could be confirmed on the basis of simulations with the biofilm model. However, with both types of sludge, crushed and intact granules, a serious agglomeration of sludge occurs when treating completely acidified waste water, leading to severe operational problems. On the other hand, when the biomass yield is high, e.g. in presence of partially acidified feed solutions, cementation of the sludge bed will be strongly retarded, as was shown previously[19] and also in the present experiments. The addition of non acidified substrate thus improves the quality of high ash content sludge because it retards agglomeration.

Also other researchers described big controversies in specific activity of high ash content sludges. Keenan *et al.*[16] and El-Mamouni *et al.*[6] found a serious loss of activity during the development of high ash content sludge, contrary to Ketunnen *et al.*[17]. From the discussion as mentioned above, it will be clear that the contradictions can be explained considering the factors that determine the location of precipitation. However, due to a lack of detailed information in these studies, regarding e.g. granular size and presence of compounds inhibiting calcium carbonate crystal growth, a detailed evaluation of those results cannot be provided.

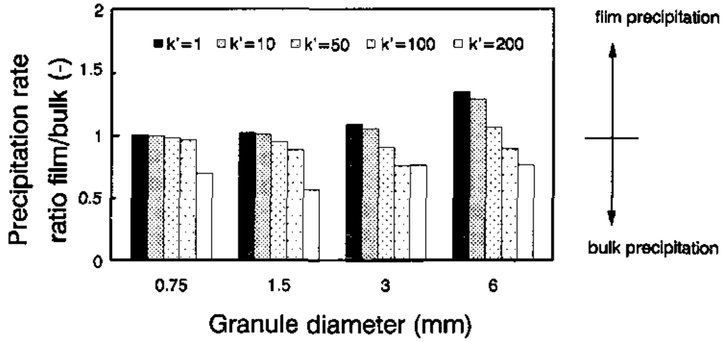

Figure 5. The effect of granular size on the precipitation rates in the film divided over the precipitation rate in the bulk (ratio film/bulk) at different k' values. The calculated COD removal efficiency fluctuated due to differences in granular size. The calculated values were as follows: 0.75mm (COD%=99); 1.5mm (COD%=98); 3mm (COD%=92); 6mm (COD%=74).

With respect to the cultivation of a high quality "high ash content sludge", it can be concluded that biofilm precipitation should be minimized. The results of simulations with the biofilm model reveal that this can be accomplished by e.g. 1) using seed sludge with small aggregates; 2) applying a low Ca/Na ratio and 3) not inhibiting crystal growth, which can be accomplished by providing a low phosphate concentration in the reactor. The biofilm model we developed is certainly an appropriate tool, to provide a good qualitative insight in the factors that determine the location of precipitation. In would obviously be attractive to make a quantitative model, however, many additional kinetic factors need to be considered for this purpose. For instance, one factor that should be considered in a quantitative model is the available crystal surface in the reactor. The present model assumes that the crystallization rate constant, k'(=k.s), is constant for the film and bulk phase during the entire experiment. However, values of k' will increase once appropriate surface area for crystal growth (s) is formed,[18] which will e.g. lead to enhanced biofilm precipitation once some crystals are formed there. Thus, in order to make quantitative predictions, a dynamic model should be developed, which is far beyond the scope of this research.

Conclusions

Starting up a reactor using intact granular sludge in case of feeds consisting of completely acidified waste water and with a calcium concentration of 780-1.560 mgCa.l^{-1}., will result in the rapid formation of dense, high ash content granules of which the specific methanogenic activity will rapidly deteriorate, resulting in a poor COD removal efficiency.

When operating the same system at a lower influent calcium concentration, 390 mg.l^{-1}, less precipitation occurs within the sludge aggregates. Instead, precipitation takes place on glass and plastic reactor parts.

The presence of non acidified substrate leads to the development of high ash content sludge of still quite satisfactory quality. The sludge activity remains at a reasonably high level (0.6 gCOD.gVSS^{-1}. d^{-1}) even at a prolonged period of operation. As a result of the higher biomass yield, cementation of the sludge bed is significantly retarded, which can be attributed to the occurrence of biofilms on the crystal surfaces.

The developed steady-state biofilm model provides a clear, qualitative insight in the factors that affect the location of precipitation and it explains the observed differences in the location of precipitation.

Factors which promote precipitation in the bulk solution phase are (1) a small granular size; (2) a low concentration of phosphate; and (3) a low calcium/sodium ratio at equal alkalinity. By stimulating precipitation in the bulk solution, the quality of the sludge can be maintained satisfactory, despite the fact that the ash content comes up to high levels.

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4 Influence of phosphate and iron on the extent of $CaCO_3$ precipitation during anaerobic digestion

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Abstract - It was investigated to what extent calcium carbonate deposition in an anaerobic reactor can be reduced by dosing inhibitors, phosphate and iron, of $CaCO_3$ crystal growth. At several concentrations of the additive, the extent of precipitation was assessed in continuous experiments with lab-scale reactors. Phosphate concentrations in the reactor as low as 0.5 - 5 mg total-P.l^{-1} were found to severely inhibit $CaCO_3$ precipitation. On the contrary, iron did not inhibit the deposition of $CaCO_3$, which was found to be due to the fact that iron, in contrast to phosphate, only inhibits the growth of calcite and not the formation of aragonite. The results led to the conclusion that only additives, inhibiting the formation of as well aragonite and calcite, can be used as effective inhibitors during anaerobic digestion. A developed chemical equilibrium model was shown to be a useful tool to calculate the extent of calcium carbonate deposition during anaerobic digestion provided the proper apparent solubility product of calcium carbonate can be estimated.

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Introduction

For concentrated industrial waste waters, anaerobic treatment is often the key to cost-effective treatment. During anaerobic treatment of waste waters with a high calcium and/or phosphorus content, precipitation is likely to occur. High calcium concentrations in waste water i.e. occur in the waste paper industry, especially when waste water loops are closed for reuse.[1] Precipitation in an anaerobic system generally leads to serious problems, like $CaCO_3$ scaling on reactor walls, loss of specific methanogenic activity, cementation of the sludge bed and clogging of effluent pipes.[2-4] With chemical equilibrium models, the amount of calcium carbonate deposition in an anaerobic reactor can be estimated. Those models assume establishment of equilibrium of precipitates within treatment time spans. However, the actually prevailing solubility products often differ one or two orders of magnitude from the thermodynamic value for different types of waste waters,[5,6] which can be attributed to kinetic inhibition of calcium carbonate formation by waste water constituents.[7] Traces of several additives can reduce the reaction rate constant of calcium carbonate deposition by several orders of magnitude.[8] Although numerous publications on the role of inhibitors on calcium carbonate formation kinetics exist,[8-12] the applicability of such inhibitors to diminish the extent of calcium carbonate deposition during anaerobic digestion was never studied so far. Amongst others, phosphate and iron are known to be very effective inhibitors of calcium carbonate crystallization.[8,9] Both components are usually present in the influent of anaerobic digesters as they are needed for the growth of micro-organisms. Therefore, phosphate and iron were selected in this work to study their influence on the extent of calcium carbonate deposition during anaerobic digestion.

The aim of this research was to study the possibility of dosing phosphate and/or iron in the influent of an anaerobic reactor to diminish the extent of calcium carbonate deposition.

Theoretical aspects

Calcium carbonate

Calcium carbonate can be found in different polymorphs, which in order of increasing solubility are calcite, aragonite, vaterite, hydrated and amorphous calcium carbonate. Calcite, aragonite and vaterite have solubility products of $10^{-8.4}$, $10^{-8.2}$ and $10^{-7.9}$ $mol^2.l^{-2}$ respectively at 30°C.[15] Calcium carbonate monohydrate has a solubility product of $10^{-7.2}$ $mol^2.l^{-2}$ at 30°C[16] and for amorphous calcium carbonate it is $10^{-6.5}$ $mol^2.l^{-2}$.[15] Calcite is the thermodynamically stable $CaCO_3$ polymorph, however, formation of the other, metastable, phases during spontaneous precipitation from a highly supersaturated solution is a common phenomenon.[16] The presence of impurities can have important consequences on the formation and transformation behavior of the metastable phases.[10]

Kinetics of $CaCO_3$ crystal growth

The driving force for a crystallization reaction is the extent of the supersaturation of the solution. If surface diffusion or integration of ions at kink sites are the rate determining step, the rate expression of calcium carbonate growth can be expressed as:[9,17-19]

$$r_{CaCO_3} = k \, . \, s \left(\sqrt{IAP} - \sqrt{K_{sp}}\right)^2 \tag{1}$$

where:
r_{CaCO3} = calcite accumulation rate $(mol.l^{-1}.s^{-1})$
k = crystal growth rate constant $(dm.s^{-1})$
s = reactive crystal surface area $(dm^2.mol^{-1})$
IAP = ionic activity product $(Ca^{2+})(CO_3^{2-})$ $(mol^2.l^{-2})$
K_{sp} = solubility product of $CaCO_3$ $(mol^2.l^{-2})$

The value of k can be drastically reduced by the presence of additives, inhibiting crystal growth. Regarding the estimation of s, it is important to notify that the crystal growth depends on the density of growth sites on the surface and not on the specific surface area of the crystals,[20] which makes it a parameter difficult to assess.

Inhibition mechanism of crystal growth

Inhibition of crystal growth by trace amounts of additives generally is assumed to be caused by the blocking of surface sites (active growth sites, i.e. terraces, steps or kinks) by adsorbed molecules or ions of the additive. A reversible adsorption can be described by a Langmuir isotherm:[8,12]

$$\Theta_i = \frac{k_{ads} . c_i}{k_{ads} . c_i + 1} \tag{2}$$

where:
Θ_i = fraction of surface sites covered by the additive (-)
c_i = concentration of additive $(mol.l^{-1})$
k_{ads} = adsorption constant $(l.mol^{-1})$

With the further assumption that the growth rate constant k decreases with increasing coverage corresponding to:

$$k = k_0 (1 - \Theta_i) \tag{3}$$

where:
k_0 = growth rate constant without additive (dm.s^{-1})
k = growth rate constant with additive (dm.s^{-1})

substitution of equation 2 and 3 in equation 1 gives:

$$r_{CaCO_3} = k_0 \cdot s \cdot \left(1 - \frac{k_{ads} \cdot c_i}{k_{ads} \cdot c_i + 1}\right) \cdot \left(\sqrt{IAP} - \sqrt{K_{sp}}\right)^2 \tag{4}$$

Both supersaturation and inhibition influence the rate of crystallization (Eq. 1). To study the influence of inhibitors on the crystallization rate, independent from supersaturation, we define r_x to evaluate the results of the experiments:

$$r_x = \frac{r_{CaCO_3}}{\left(\sqrt{IAP} - \sqrt{K_{sp}}\right)^2} = k_0 \cdot s \cdot \left(1 - \frac{k_{ads} \cdot c_i}{k_{ads} \cdot c_i + 1}\right) \tag{5}$$

Materials and methods

Experimental set-up

The experiments were performed in a temperature controlled room at 30 ± 2°C. The glass Expanded Granular Sludge Bed (EGSB) reactors had a working volume of 5.7l (150 cm height, 5 cm i.d.) and were equipped with a reverse funnel phase separator. After passing the gas through a concentrated sodium hydroxide solution for removing carbon dioxide and through a column filled with soda lime pellets with indicator, methane was monitored by a wet gas meter. Three reactors were operated. All reactors were fed with a mixture of acetate and butyrate (1:1 COD). The influent COD concentration was 5.3 gCOD.l^{-1}. The applied hydraulic retention time (HRT) was 20 hours.

The influence of phosphate on the extent of $CaCO_3$ deposition was studied in reactor R1 and

R2. Both reactors were supplied with different levels of phosphate in the influent, ranging from 0-30 mgP.l^{-1}. Phosphate was supplied as KH_2PO_4. The influent of reactor R1 was neutralized with $Ca(OH)_2$, resulting in an influent calcium concentration of 1200 mgCa.l^{-1}. Reactor R2 was neutralized with 600 mgCa.l^{-1} and 630 mgNa.l^{-1}. Both reactors were seeded with anaerobic sludge originating from lab scale UASB reactors. This sludge had a high ash content (80-90%) and a moderate activity, 0.5 gCOD.gVSS^{-1}.d^{-1}.[4] The sludge was crushed with a handblender (Multiquick 350 combi, Braun) under anaerobic conditions. The amount of sludge added in each reactor was 600 ml, containing 75 gVSS. To fluidize the heavy sludge, an upflow velocity of 24 m.hr^{-1} was applied by recirculation (ratio 1:140).

Reactor R3 was used to study the influence of iron on the extent of $CaCO_3$ deposition. For this purpose, several concentrations of iron were supplied in the influent, ranging from 0 - 500 mgFe.l^{-1}. Iron was dosed as $FeCl_2$. Calcium and sodium were added in concentrations of 600 and 630 mg.l^{-1} respectively. Reactor R3 was seeded with anaerobic granular sludge from Industriewater Eerbeek B.V., the Netherlands. The ash content of this sludge was 15% and the activity 0.7 gCOD.gVSS^{-1}.d^{-1}. The amount of sludge added to reactor R3 was 1.5l, containing 125 gVSS. In order to expand the granular sludge, an upflow velocity of 7 m.hr^{-1} was applied by recirculation (ratio 1:40).
The calcium, phosphate and iron concentrations and the pH of the influent and the effluent were regularly measured during operation, as well as the pCO_2 of the biogas.

Medium
The mineral medium used was described elsewhere.[4] The doses of phosphate and iron fluctuated in time for the three reactors and are indicated in the text and figures.

Analytical Methods
Analyses of Volatile Fatty Acids (VFA), biogas composition (CH_4, CO_2 and N_2), calcium and phosphorus were described elsewhere.[4] Iron was analyzed by AAS according to standard methods.[21] X-ray diffraction analysis was performed using a Guinier camera and CokÁ radiation.

Modelling
A chemical equilibrium model was developed to calculate the speciation in the anaerobic reactor. Activity coefficients were calculated according to Davies and calculations were made by iteration, using the multivariable method of Newton-Rhapson.[22]

Input parameters for the model are COD of the influent and the effluent (gCOD.l^{-1}), sodium (M), calcium (M), chloride (M) and the TOC/COD ratio of the substrate. It was assumed that all converted COD was used for the production of methane, consequently the yield of biomass was neglected. The ratio TOC/COD allows to predict the average oxidation state of organic carbon in the waste water, and from that the specific amount of methane and carbondioxide produced can be calculated.[5] Mass balances for electroneutrality, sodium, chloride, calcium, acetic acid, inorganic carbon, methane and gas were used. The last balance was introduced to calculate the speciation of inorganic carbon in the gas and liquid phase. It was assumed that methane and carbondioxide are the only components of the gaseous phase. Considered species in the model are H^+, OH^-, Na^+, Ca^{2+}, Cl^-, HAc, Ac^-, H_2CO_3, HCO_3^-, CO_3^{2-}, CO_{2gas}, CH_{4gas} and Vg' (total gas volume), the complexes $CaCO_3^0$ and $CaHCO_3^+$ and the solid phase $CaCO_3$. For used equilibrium constants is referred to Plummer and Busenberg.[13]

Results

Influence of phosphate on $CaCO_3$ deposition

The reactors R1 and R2 were operated at an organic loading rate of 6-8 gCOD.l^{-1}.d^{-1}. During the time of operation (150 days), a COD removal efficiency of 90-95% was obtained. Based on model calculations with measured input parameters and assuming thermodynamic equilibrium ($pK_{spCaCO3}=8.4$), calcium concentrations of 265 ± 35 and 42 ± 13 mgCa^{2+}.l^{-1} are to be expected in the effluent of R1 and R2 respectively. However, considerable higher concentrations of calcium were found in the effluent of both reactors, up to 748 and 276 mg.l^{-1} for R1 and R2 respectively. The extent of calcium carbonate precipitation turned out to depend on the phosphate dosage. The higher the concentration of phosphate in the influent, the less calcium carbonate precipitated. This clearly demonstrates that the presence of phosphate drastically reduces the calcium carbonate deposition, especially in reactor R1 (Fig. 1). In reactor R2, the amount of precipitation fluctuated considerably at phosphate dosages of 15 mgP.l^{-1} (Fig. 1). To explain this phenomenon, it needs to be considered that inhibition of calcium carbonate growth is likely caused by the soluble phosphate concentration in the reactor. Due to phosphate removal, the reactor phosphate concentration is much lower than the influent concentration (Fig. 2). So, at an initial phosphate dosage of 15 mgP.l^{-1}, the amount of phosphate in the effluent of R2 fluctuated from 3 to 5 mgP.l^{-1} (Fig. 2). This explains the variance in extent of calcium precipitation at a phosphate dosage of 15 mgP.l^{-1} (Fig. 1). Calculation of the apparent solubility of calcium carbonate in the reactors

($pIAP_{CaCO3}$) allows a comparison of the extent of phosphate inhibition in both reactors. The results shown in figure 3 clearly reveal that at phosphate levels in the reactors between 0.5 - 5 mgP.l^{-1}, the apparent solubility of calcium carbonate is equally increased in both R1 and R2. Phosphate can thus be effectively used in practice to diminish the extent of calcium carbonate precipitation in anaerobic digestors.

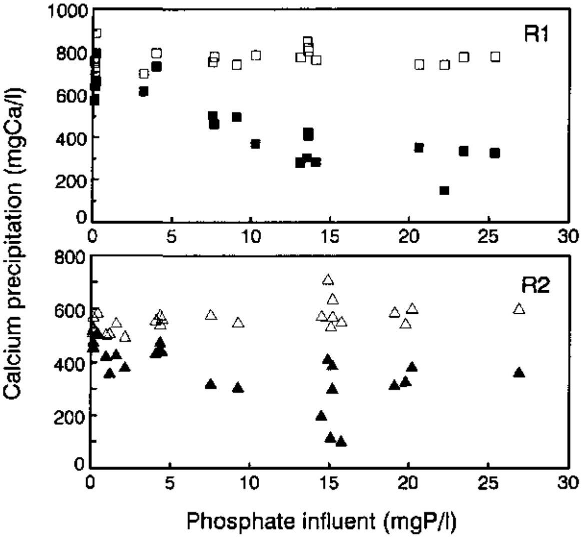

Figure 1. Measured amount of calcium precipitation for R1 (■) and R2 (▲) and calculated maximal amount of precipitation (at $pK_{sp}=8.35$) for R1 (□) and R2 (Δ) as function of the phosphate concentration in the influent.

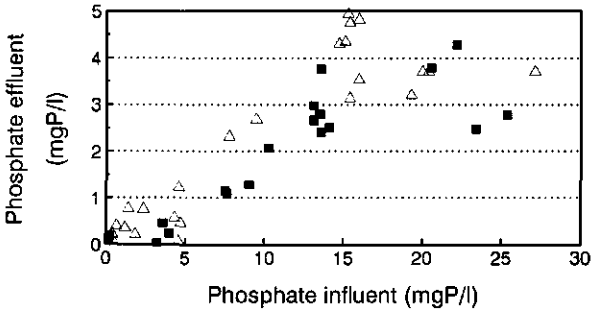

Figure 2. Phosphate concentration in the effluent as a function of the phosphate concentration in the influent as measured for R1 (■) and R2 (Δ).

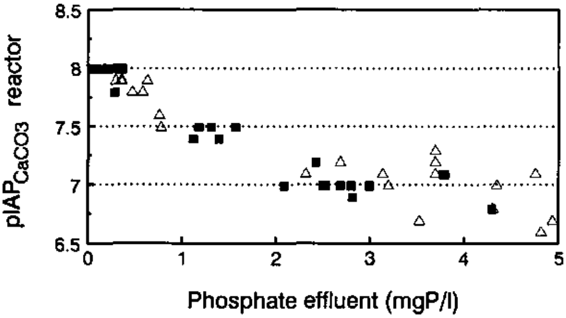

Figure 3. Measured ionic activity product (IAP) of $CaCO_3$ in reactor R1 (■) and R2 (Δ) as a function of the total concentration of phosphate in the effluent (= reactor concentration).

The pH in the reactors R1 and R2 was dependent on the extent of precipitation that took place. The lower the amount of precipitation, the higher was the pH. In R1, the pH ranged from 6.5 to 7.1 and in R2 from 6.9 to 7.5.

The sludge was analyzed by X-ray diffraction analysis at the termination of the experiment. The mineral fraction of the sludge from R1 an R2 consisted mainly of calcite, a minor fraction (< 1%) consisted of monohydrocalcite. Crystalline calcium phosphate polymorphs were not detected.

Calculation of the adsorption constant, k_{ads}, and the crystal growth rate constant, k_0'

In case adsorption of phosphate at the growth sites of the crystal would affect the growth rate of calcite, the inhibiting effect of phosphate can be described by an adsorption isotherm according to Langmuir.[12] Several ionic phosphate species might adsorb to the crystal surface, but in case of calcite, the complex $CaHPO_4^0$ was recently found to be the most likely adsorbing and inhibiting specie.[23] Assuming $CaHPO_4^0$ to be the adsorbing specie, both the adsorption constant (k_{ads}) and the growth rate constant at zero inhibition ($k_0' = k_0 . s$) can be calculated according to equation 5 with a non-linear least square fit method (Fig. 4). The calculated values for the adsorption constants, k_{ads}, for reactors R1 and R2 amounted to $1.2*10^6$ $l.mol^{-1}$ and $7.5*10^5$ $l.mol^{-1}$ respectively, and the values of the crystal growth rate constant at zero inhibition, k_0', amounted to 223 for R1 and 95 $l.mol^{-1}.s^{-1}$ for R2.

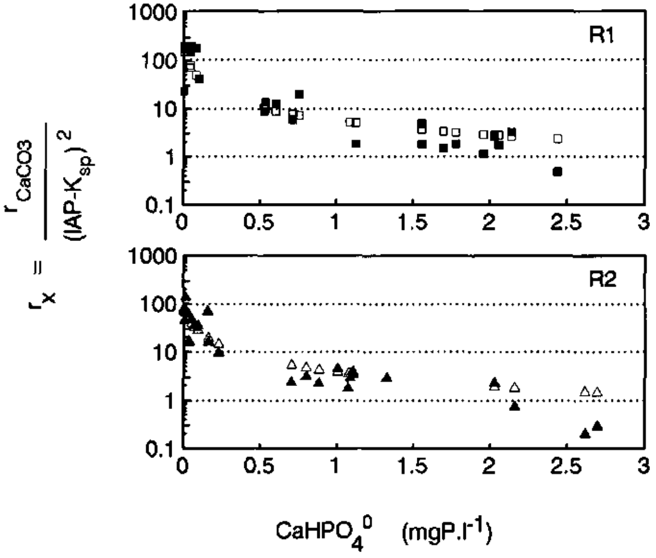

Figure 4. Measured values of r_x (R1 (■); R2 (▲)) as function of the $CaHPO_4^0$ concentration and estimated values of r_x (R1 (□); R2 (Δ)) as determined with a least square linear fit method assuming adsorption according to Langmuir (Eq. 5). Estimated values of k_{ads} (l.mol^{-1}) are 1.2*10^6 (R1) and 7.5*10^5 (R2). Estimated values of k_0' (l.mol^{-1}.s^{-1}) are 223 (R1) and 95 (R2).

Influence of iron on $CaCO_3$ deposition

Like reactors R1 and R2, reactor R3 was also operated at an organic loading rate of 6-8 gCOD.l^{-1}.d^{-1} and a COD removal efficiency > 95%. The reactor was supplied with iron concentrations in the influent ranging from 0-500 mgFe.l^{-1} (Table 1). The results in Table 1 reveal that dosage of iron in the range 20-50 mgFe.l^{-1} did not significantly decrease the amount of calcium carbonate precipitation. Only dosage of 500 mgFe.l^{-1} resulted in a distinct decrease of the calcium carbonate precipitation. However, on the basis of calculations with the equilibrium model, it was found that this effect could not be attributed to an inhibitory effect of iron, but that it was due to removal of carbonate by precipitation of $FeCO_3$, with an apparent solubility of $10^{-9.0}$ mol^2.l^{-2}. The results in Table 1 show that iron, in contrast to phosphate, hardly influences the apparent solubility of calcium carbonate in the reactor, and thus is not a proper additive to diminish the extent of calcium carbonate deposition in an anaerobic reactor.

Table 1. Measured iron and calcium concentration of the influent and the effluent of reactor R3 during periods in which the influent iron concentration was varied and the apparent solubility product of calcium carbonate in the reactor (=IAP_{CaCO3}).

		Iron influent mgFe.l^{-1}	Iron effluent mgFe.l^{-1}	Calcium influent mgCa.l^{-1}	Calcium effluent mgCa.l^{-1}	$pIAP_{CaCO3}$
Period:						
I	(n=3)	0	0.2 ± 0.1	542 ± 8	123 ± 9	7.6 ± 0.0
II	(n=4)	22 ± 2	4 ± 2	571 ± 18	78 ± 12	8.0 ± 0.1
III	(n=3)	47 ± 9	3 ± 1	573 ± 14	88 ± 3	7.9 ± 0.1
IV	(n=8)	503 ± 80	50 ± 20	528 ± 21	273 ± 28	7.8 ± 0.1
V	(n=3)	0	2 ± 1	544 ± 4	72 ± 5	8.1 ± 0.0

The sludge characteristics of reactor R3 turned out to be different from R1 and R2. The mineral fraction mainly consisted of aragonite (85-95%) and only a minor fraction (5-15%) of calcite. No crystalline siderite or iron-oxides were detected. Reactor R3 was started up with granular sludge without calcium carbonate. At the end of the experiment, many small crystals (<0.1 mm i.d.) were formed and retained in the sludge bed (Fig. 5). However, and quite important, no crystals were observed within the granules.

Discussion

Influence of phosphate on the extent of calcium carbonate deposition

The results of this study reveal that the crystallization of calcium carbonate in an anaerobic reactor is severely inhibited at phosphate concentrations in the range 0.5 - 5 mgP.l^{-1} (Fig. 3). At phosphate concentrations exceeding 4 mgP.l^{-1}, the apparent solubility product of calcium carbonate in the reactors ($10^{-6.6}$ - $10^{-7.1}$) even approached the value as measured for amorphous calcium carbonates ($10^{-6.5}$)[15] (Fig. 3). This indicates that amorphous calcium carbonate was formed under all conditions as applied during our experiments, but that the rate limiting step, being the transformation to calcite, was severely inhibited by the presence of phosphate. The calculated adsorption constants, k_{ads}, for reactor R1 and R2 were $1.2*10^6$ l.mol^{-1} and $7.5*10^5$ l.mol^{-1} respectively, which is slightly higher than the value $2.1*10^5$l.mol^{-1} as found in earlier phosphate adsorption experiments with calcite.[11] However, Hartley *et al.*[11] calculated k_{ads} assuming that the sum of all phosphate species is inhibitory. As the adsorption of phosphates significantly increases at increasing calcium concentrations in solution, k_{ads} values can only be compared by selecting the proper inhibiting species, i.e. neutral species ($CaHPO_4^0$) for calcite.[23] The estimated values of k_0' were 223 for R1 and 95 $l.mol^{-1}.s^{-1}$ for R2. A difference in k_0' values can easily occur because the measured rate constant, k_0', is a combination of k_0 and s. Theoretically, k_0 is a constant, but the reaction site density (s) might vary in R1 and R2 because of the different supersaturation, due to i.e. different influent calcium concentrations.[24]

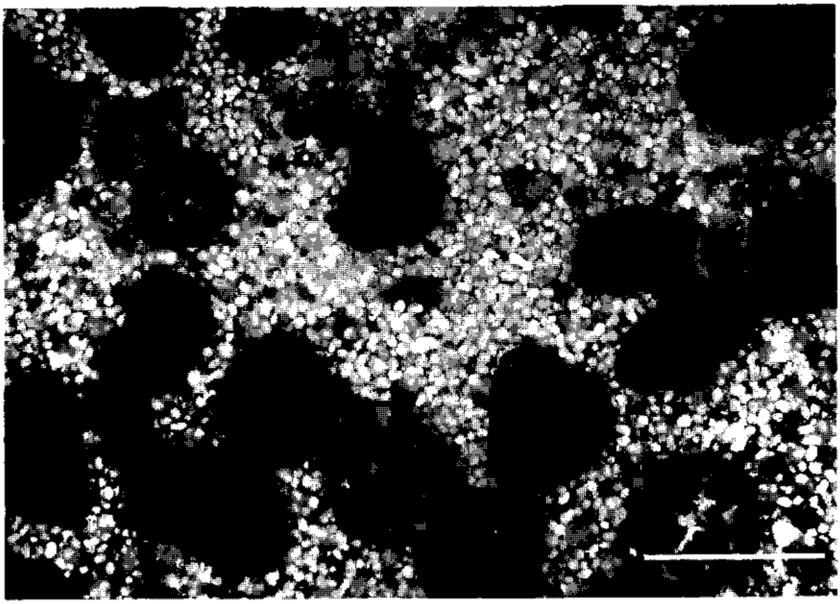

Figure 5. Overview of the morphology of the sludge bed in R3 after 100 days of operation. Intact granular sludge is present, together with retained freshly formed crystal particles, which mainly consist of aragonite. No crystals were observed within the granules. Bar indicates 1 mm.

The importance of the reactive surface area (s) on the kinetics of the $CaCO_3$ deposition is also evident from the results as obtained for reactor R3 (Table 1). No iron was dosed to the reactor at the start and the end of the experiment, and thus the amount of precipitation was expected to be equal during both periods. However, the calculated $pIAP_{CaCO3}$ was different, being 7.6 at the start of the experiment, when any crystal surface area was absent, and 8.1 at the end of the experiment, when many small crystals were present (Fig. 5). This implies that the apparent solubility of $CaCO_3$ in anaerobic reactors drops significantly in the presence of appropriate crystal surface area for growth, which is in accordance with earlier findings.[25]

Thus, the effect of phosphate on calcium carbonate formation can be expressed as a function of the concentration in the reactor, using Langmuir adsorption isotherm models. However, the influence of the available reactive crystal surface area (s) in the reactor on the kinetics of the calcium carbonate deposition cannot be quantified with the obtained results.

Influence of iron on extent of calcium carbonate deposition

The presence of iron as additive showed no effect on the extent of calcium carbonate deposition although the contrary was expected because it is known that trace amounts of 10^{-9} M Fe^{2+} already inhibit calcite formation.[8] Indeed, iron appeared to inhibit calcite formation in our reactors, as only 5-15% calcite was found in the sludge (compared to 99% in presence of phosphate as inhibitor). However, during iron dosing, most of the calcium carbonate (85-95%) was present as aragonite, the growth of which is not strongly inhibited by the presence of iron.[12,26] Thus in the reactors, iron inhibited the formation of calcite, but not of aragonite. As the solubility of aragonite is only slightly higher than for calcite, iron addition did not effectively decrease the extent of calcium carbonate formation in the reactors (Fig. 6). In general, it can be concluded from this result that only additives inhibiting the growth of as well aragonite and calcite can be used as effective inhibitors during anaerobic digestion. Phosphate seems to fulfill this requirement according to our results which is in correspondence with literature data.[27]

Implications for practice

The results of this study show that phosphate is an effective inhibitor of calcium carbonate crystallization, and can be dosed in the influent of an anaerobic digester to diminish the extent of calcium carbonate deposition. However, of the dosed phosphate, only a small fraction will remain in the reactor solution, because part will be removed by biological phosphate assimilation, phosphate precipitation and/or coprecipitation (Fig. 2). Further insight in the phosphate removal processes is needed to optimize the phosphate dosing, allowing minimal deposition of calcium carbonate to be achieved. Once the remaining phosphate concentration in the reactor, k_{ads} and k_0' can be reliably estimated, the apparent solubility of calcium carbonate in the reactor can be calculated (Equation 4). Once this apparent solubility of calcium carbonate can be estimated, also the amount of calcium carbonate deposition to be expected in an anaerobic digester can be calculated, as will be shown in a next paper.

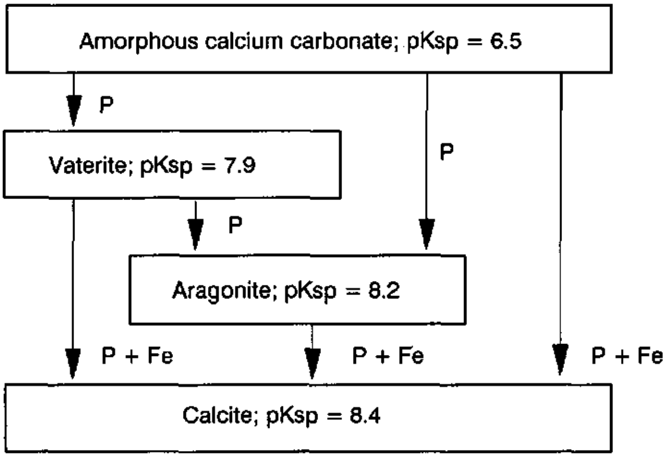

Figure 6. Schedule of calcite formation and inhibition by phosphate and iron in an anaerobic reactor. Amorphous calcium carbonate will be formed once the product $(Ca^{2+})(CO_3^{2-})$ exceeds the value $10^{-6.5}$ mol^2.l^{-2}. The recrystallization to the stable calcite can be either directly or via the intermediates vaterite and/or aragonite. The research in this paper showed that phosphate inhibits all crystallization steps, while iron only inhibits the final conversion to calcite. Thus, dosing of iron resulted in an accumulation of aragonite, but did not reduce the extent of precipitation because the solubility of aragonite is only slightly higher than for calcite.

Conclusions

The influence of the additives phosphate and iron on the extent of calcium carbonate precipitation was studied during continuous experiments in lab-scale anaerobic expanded granular sludge bed (EGSB) reactors. Phosphate, at concentrations in the reactor as low as 0.5 - 5 mg total-P.l^{-1}, was shown to severely inhibit the extent of $CaCO_3$ precipitation and can be effectively used in practice to minimize $CaCO_3$ deposition in anaerobic digestors. On the contrary, iron did not effectively inhibit the deposition of $CaCO_3$. This was shown to be due to the fact that iron, in contrast to phosphate, only inhibits the growth of calcite and not the formation of aragonite. From this results it can be concluded that only additives, inhibiting the formation of both aragonite and calcite can be used as effective inhibitors during anaerobic digestion.

A developed chemical equilibrium model was shown to be a useful tool to calculate the extent of calcium carbonate deposition during anaerobic digestion once the proper apparent solubility product of calcium carbonate can be estimated. The apparent solubility of $CaCO_3$ was shown to be dependent on at least two kinetic factors: *(1)* the phosphate concentration in the reactor and *(2)* the available reactive crystal surface area for growth. The kinetics of calcium carbonate formation due to phosphate inhibition in an anaerobic reactor can be approached with a Langmuir adsorption isotherm model.

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5 Influent calcium removal by crystallization reusing anaerobic effluent alkalinity

E.P.A. van Langerak, H.V.M. Hamelers & G. Lettinga

Abstract - Waste waters from the waste paper industry and landfill leachates contain high calcium concentrations. Treatment of those waste waters in an anaerobic system will lead to extensive precipitation of calcium carbonates. Problems due to accumulation of calcium carbonate in anaerobic reactors can be avoided by applying a crystallization reactor to remove calcium from the influent. This solution, however, is expensive due to the need to dose alkalinity. In this paper, the possibility of reusing the produced alkalinity by recirculating anaerobic effluent was investigated. Calculations and experiments were carried out to investigate to what extent calcium could be removed from the influent. Experiments were carried out in a 4.5 l UASB reactor and a 0.8 l crystallization reactor, seeded with sand. The influent contained up to 1800 mg.l^{-1} calcium and the substrate was acetic acid. Removal of calcium from the influent could be successfully accomplished. Recirculation of the anaerobic effluent supplied sufficient bicarbonate alkalinity for crystallization. Consequently, no extra chemicals (i.e. Na_2CO_3 and/or NaOH) were needed. A chemical equilibrium model, with adapted pseudo solubility products, is a very useful tool to determine the proper recycle ratio of effluent for optimal performance.

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Introduction

Anaerobic treatment of waste waters is nowadays widely applied and considered a proven technology. However, anaerobic treatment of waste waters with high contents of calcium in the influent may lead to extensive precipitation causing serious operational problems. In the sludge bed, it may result in channelling and a dramatic loss of methanogenic activity. Furthermore, scaling of reactor walls and effluent or recirculation pipes can occur.[4,8,9] High concentrations of calcium are found in several waste waters due to the utilization of lime, a cheap neutralizing agent.[16] High calcium concentrations can also result from the industrial production process which is, for example, the case for waste paper industries.[7] Also leachates from young landfills contain considerable amounts of calcium.[9] Subject of our research is the development of a method for removal of calcium from the influent by crystallization reusing anaerobic effluent alkalinity. Influent calcium can be removed in crystallization reactors (CR), which are widely used for the central softening of drinking water[6] and for the removal of phosphate.[13] Crystallization reactors are fluidized bed reactors with grains (i.e. sand) on which the crystallization of calcium carbonate takes place. The grains have to move freely in the upward flow of the water to prevent cementation. Proper initial mixing is required to obtain a metastable state of supersaturation in the solution. A too high local supersaturation will lead to formation of calcium carbonate precipitates in the bulk solution and should be prevented. To maintain a sufficient reactive surface for crystallization, some of the grown grains should be removed regularly and replaced by smaller-diameter seeding grains.[6] In the present paper, the possibility of using a crystallization reactor for removal of calcium from the influent is studied. To reduce the need of chemicals, the produced bicarbonate alkalinity from the anaerobic reactor was used by recirculation of the effluent.

Methods

Experimental set-up

The experiments were performed in a temperature controlled room at 30°C. Two glass reactors were used: a bioreactor and a crystallization reactor. The bioreactor (UASB-type) had a volume of 4.5 l, a height of 55 cm and a diameter of 10 cm. The crystallization reactor consisted of two parts; the bottom part had a volume of 0.2 l, a height of 60 cm and a diameter of 2 cm, and the upper part (for sedimentation) had a volume of 0.6 l, a height of 30 cm and a diameter of 5 cm (Fig.1). The effluent was recycled in order to mix the bicarbonate produced in the bioreactor with the calcium present in the influent and to fluidize the grains in the crystallization reactor. The bioreactor was started up with 1.8 litres of granular sludge with an ash content of 15%, obtained from Industriewater Eerbeek. The crystallization reactor was started up with sea sand, the particles of which had a diameter ranging from 0.1 to 0.3 mm. The mineral medium consisted of ($g.l^{-1}$): NH_4Cl (0.75), $MgSO_4.7H_20$ (0.29), NaCl (0.17) and 1 ml per litre of a trace element solution according to Zehnder *et. al.*.[18] The substrate used during the experiment was acetic acid, neutralized with $Ca(OH)_2$ and NaOH (Table 1). All chemicals were of analytical grade (Merck AG, Darmstadt, Germany) except the resazurin (Fluka Chemie AG, Buchs, Switzerland). The media were prepared in tap water.

The reactors were operated for 31 days and sampled twice a week. The experiment can be subdivided into two periods. During period I, the organic loading rate of the bioreactor was fixed at 11 $gCOD.l^{-1}.d^{-1}$ and the hydraulic retention time at 20 hours. During period II, the influence of lowering the retention time on the performance of the system was studied. To apply equal sludge loading rates during both periods, correction took place for sludge yield during period I and the organic loading rate was set at 13 $gCOD.l^{-1}.d^{-1}$ during period II. For further experimental conditions during period I and II is referred to Table 1.

Analysis

Acetate and biogas composition were analyzed as describe elsewhere.[5] For measuring soluble calcium concentrations, effluent samples were filtrated using a membrane filter with a pore size of 0.45μm, after which the samples were acidified up to pH 2 with HNO_3 (65%) and stored at 4°C. For analysis of total calcium, samples of 20 ml were transferred into a digestion tube, adding 2.5 ml 65% HNO_3 and 7.5 ml 37% HCl and 10 ml distilled water. Next, the mixture was heated for two hours at 80°C, cooled, and diluted with distilled water in a volumetric flask of 100 ml and stored at 4°C. Prior to analysis, all stored samples were diluted with 0.2% lanthan nitrate solution. Calcium was measured by an atomic absorption spectrometer (Model AA975, Varian, Springvale, Australia) according to standard methods.[1] For scanning electron microscopy (SEM) analyses, crystals were air-dried and some were cleaved. After gold/palladium sputter coating, the crystals were examined on a SEM (JSM 6300F, Jeol).

Modelling

A chemical equilibrium model was used to calculate the amount of calcium carbonate precipitation in the bioreactor and the crystallization reactor. For each reactor, separate equilibrium equations and mass balances were formulated (Table 2). Both systems were connected and the equilibrium state of the system could be iteratively calculated with the Newton Rhapson procedure. Corrections for ionic strength took place, activity coefficients were calculated according to Davies equation.[15] For equilibrium equations and constants used is referred to literature.[12] Mass balances for electro neutrality, sodium, chloride, calcium, acetate, methane, gas and inorganic carbon were used in the model (Table 2). Required input parameters are calcium (M), sodium (M), chloride (M), COD and acetate in the influent ($gCOD.l^{-1}$); acetate in the effluent ($gCOD.l^{-1}$), the recycling factor of liquid ($Rf=Qr/Qi$); the recycling factor of gas (Rf_g) and the TOC/COD ratio. The latter indicates the average oxidation state of organic carbon in the waste water, from which the specific amount of methane and carbon dioxide produced can be predicted.[17] It was assumed that no substrate was converted in the crystallization reactor and the gas phase consisted of CH_4 and CO_2.

Table 1. The flow rates, recycling factors, influent composition, loading rates of COD and calcium, hydraulic retention times and upflow velocities as applied during periods I and II.

	Period I	Period II
	Days 0-24 n=6	Days 24-31 n=3
Influent flow rate, Q_i ($l.d^{-1}$)	5.3 ± 0.3	10.7 ± 0.1
Recycling flow rate, Q_r ($l.d^{-1}$)	279 ± 9	292 ± 9
Recycling factor, Rf (Q_r/Q_i) (-)	53 ± 1	27 ± 1
Acetic acid in influent ($mgCOD.l^{-1}$)	9100 ± 360	5386 ± 144
Calcium in influent ($mg.l^{-1}$)	1828 ± 63	1129 ± 60
Sodium in influent ($mg.l^{-1}$)	1058 ± 66	570 ± 37
Organic loading rate UASB ($gCOD.l^{-1}.d^{-1}$)	11 ± 1	13 ± 1
Calcium loading rate system ($mgCa.l^{-1}.d^{-1}$)	1828 ± 100	2279 ± 105
HRT in bioreactor (hours)	20 ± 1	10 ± 1
HRT in crystallization reactor (hours)	0.9 ± 0.1	0.45 ± 0.05
V_{up} in bioreactor ($m.h^{-1}$)	1.5 ± 0.1	1.6 ± 0.1
V_{up} in crystallization reactor ($m.h^{-1}$)	38 ± 1	40 ± 0.1

Results and discussion

System performance

The COD removal efficiency of the bioreactor amounted 98%, and no COD was degraded in the crystallization reactor. The pH in both reactors was 7.1 ± 0.1 during period I and slightly lower (7 ± 0.1) during period II. The percentage of CO_2 in the gas phase of the bioreactor was 24 and 22% during period I and II respectively. The percentage of CO_2 in the gas phase of the crystallization reactor was always slightly higher by 0.6-0.8%. The removal efficiency of calcium in the system amounted 96 and 91% during period I and II respectively. During both periods, the precipitation took dominantly place in the crystallization reactor. The calcium removal in this reactor was 97-100% of the total removal (Table 3). During period II, the concentration of suspended calcium carbonate increased strongly. This was clearly visible in the reactor: all flows were cloudy because of a white precipitate that was recycled. Unfortunately, an exact balance of suspended calcium carbonate could not be made as dispersed matter accumulated on the settler of the UASB reactor. During the sampling procedure, part of the settled particles were swirled and determined to be suspended calcium carbonate in the effluent. This explains why more calcium was measured in the effluent than in the influent of the bioreactor, especially during period II (Table 3).

Table 2. Balances and equations to calculate the equilibrium in a combined system of a bioreactor and a crystallization reactor.

Bioreactor	EN	$= 0$
	Na_t	$= [Na]_i$
	Cl_t	$= [Cl]_i$
	Ca_t	$= [Ca]_{cr}$
	Ac_t	$= [Ac]_e/64.02$
	C_{CH4}	$= 1/(Rf+1) * ([COD]_i-[Ac]_e)/64$
	Vg'	$= 0$
	C_t	$= ([COD]_i-[Ac]_e)/64 * (16/3*TOC/COD-1)/(Rf+1)$ $+ [CO_3]_{cr} + [HCO_3]_{cr} + [H_2CO_3]_{cr}$
Crystallization reactor	EN	$= 0$
	Na_t	$= [Na]_i$
	Cl_t	$= [Cl]_i$
	Ca_t	$= 1/(1+Rf)*[Ca]_i + Rf/(1+Rf)*[Ca]_r$
	Ac_t	$= 1/(1+Rf)*[Ac]_i/64.02+Rf/(1+Rf)*([Ac]_r+[HAc]_r)$
	C_{CH4}	$= Rf_g*[CH_4]_{g,r}$
	Vg'	$= 0$
	C_t	$= Rf/(Rf+1)*([H_2CO_3]_r+[HCO_3]_r+[CO_3]_r) + Rf_g*[CO_2]_{g,r}$

EN = electro neutrality; t = total; g,i,r,cr = concentration in gas phase, influent, recirculation flow, effluent CR; Rf/Rf_g = recirculation factor liquid phase/gas phase; Vg' = gas balance

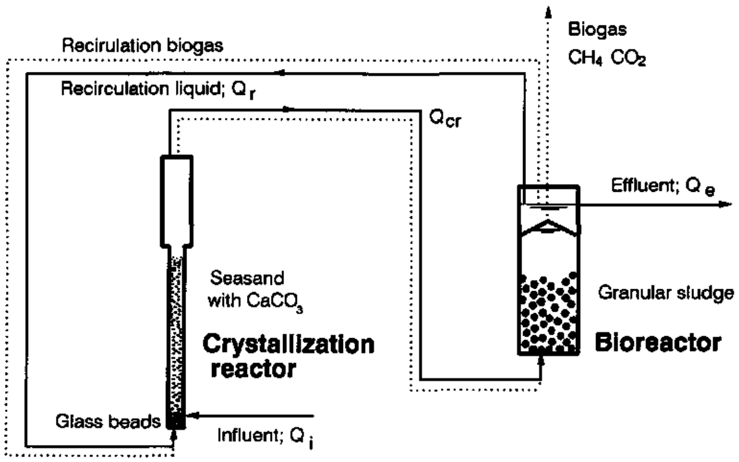

Figure 1. Diagram of the two-reactor system, consisting of a bioreactor and a crystallization reactor. I/e (influent/effluent), r (recycled flow) and cr (effluent of crystallization reactor).

Equilibrium model

A comparison between the results of calcium measurements and calculations showed that they did not correspond well. Measured concentrations were considerably higher than the calculated values (Table 3). A better fit between measurements and calculations could be obtained by adjusting the solubility product of calcium carbonate (pK_{sp}) in the model. Introduction of pK_{sp} values in the range from 7.6 to 7.9 resulted in good correspondence between measured and calculated effluent values. However, the percentage of precipitation occurring in the crystallization reactor (CR%) was underestimated when lower values of pK_{sp} were introduced in the model (Table 3). Both the measured concentrations and the measured calcium removal in the crystallization reactor fitted with model calculations when a higher pK_{sp} value (0.2 units) was introduced for the crystallization reactor only (Table 3). In this case, also measured and calculated values for pH and pCO_2 were found to fit very well.

Kinetics of calcium carbonate deposition

The need to introduce lower pK_{sp} values in the model indicates that thermodynamic equilibrium was not reached in the system. The pseudo solubility products reflect the kinetics of the precipitation and crystallization processes that took place. The Ostwald step rule postulates that the precipitate with the highest solubility (i.e. the least stable phase) will form first in a consecutive precipitation reaction.[15] In case of calcium carbonate, hydrated amorphous phases will form first during precipitation from highly supersaturated solutions.[2] The amorphous phase, which is generally a fine precipitate with a disordered lattice, will transform in the metastable polymorphs vaterite or aragonite by recrystallization. Subsequently, those metastable polymorphs convert (age) slowly into the most stable form; calcite.[15] The last recrystallization step to calcite is considered to be the rate-determining step.[11] Likely, conversion of metastable polymorphs into calcite was not completed during our experiments and the solubility we actually measured in the system was a mixture of the solubility of amorphous calcium carbonate ($pK_{sp}=6.5$);[2], vaterite and calcite ($pK_{sp}=7.9$ and 8.4 resp.).[12] This was confirmed by electron microscopic analysis of the grains. If no precipitation but only immediate crystallization of calcite takes place on seed grains, which occurs at metastable levels of supersaturation, needle shaped crystals can be expected.[3] However, we observed a typical "cauliflower structure" for all grains (Fig. 2). This structure was also observed during the softening of drinking water and assumed to be the result of a combination of precipitation, crystallization and agglomeration processes.[14]

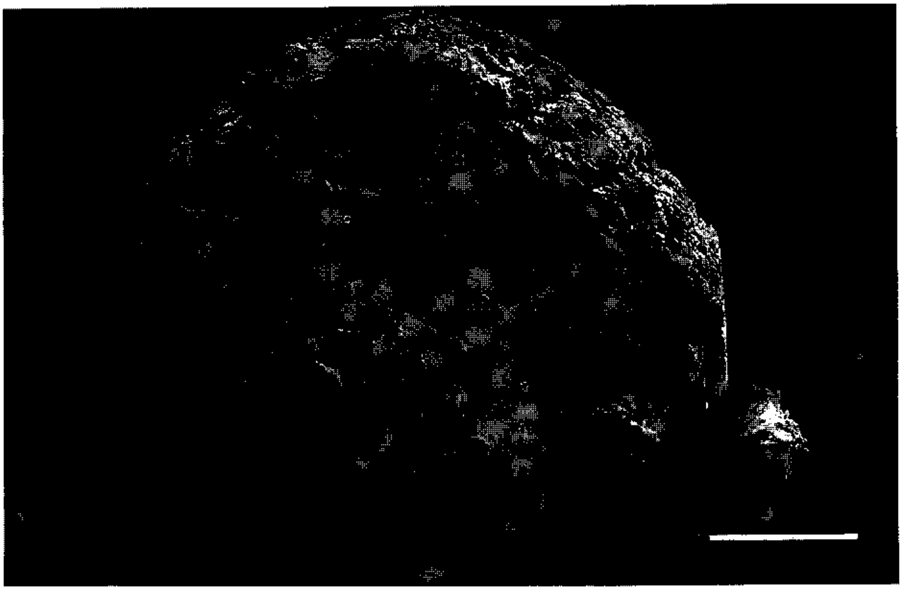

Figure 2. SEM picture of a cleaved grain from the crystallization reactor, showing a typical "cauliflower structure". The seed grain (sand) is visible in the core. Bar indicates 1mm.

The crystallization rate of calcite depends on three factors: *1)* the supersaturation *2)* the growth rate constant, which can be dramatically reduced by traces of certain inhibiting compounds and *3)* the available surface area for crystal growth.[10] As a large surface area for crystal growth was available in the crystallization reactor, it is likely that the rate of calcite formation was higher in this reactor than in the bioreactor. This higher rate could be simulated with the model by introducing a higher pK_{sp} value for the crystallization reactor (Table 3).

Influence of hydraulic retention time

During period II, the overall calcium removal efficiency of the system decreased slightly (Table 3). However, this decrease can not be attributed to the lower retention time applied but can be explained by the relatively low sodium alkalinity that was supplied during this period (Table 1). Due to the presence of less alkalinity, the maximal calcium removal efficiency decreased, as was calculated at thermodynamic equilibrium conditions (Table 3). Also the fact that higher pK_{sp} values had to be used to fit the model with the results indicates that a saturation state closer to equilibrium was achieved during period II, despite the lower retention time. This is likely to be caused by the higher calcium loading rate that was applied (Table 1), resulting in a higher precipitation rate during period II (103 $mgCa.l^{-1}.h^{-1}$) compared to period I (88 $mgCa.l^{-1}.h^{-1}$). Probably, the higher precipitation rate also stimulated the formation of suspended calcium carbonate in the bulksolution.

Feasibility of the process and the model

To evaluate the feasibility of influent calcium removal in a crystallization reactor (CR), reusing anaerobic effluent, it is of essential importance to consider the kinetics of the calcium deposition, as will be shown with some examples. Differences in the kinetics can be simulated in the model by introducing different pseudo solubility products of calcium carbonate (pK_{sp}). In case of thermodynamic equilibrium in both reactors ($pK_{sp}=8.4$), the maximal removal of calcium in the CR is only 80% and decreases severely when lower pK_{sp} values for both reactors are used (Fig.3). However, introduction of higher pK_{sp} values for the CR only (reflecting a higher crystallization rate) leads to considerable higher maximal removal efficiencies of calcium in this reactor, and results in a better calcium removal at lower recirculation rates (Fig.3). In practice, calcium carbonate precipitation can be allowed in the UASB reactor to a certain extent. For example, 90% removal of the influent calcium in the CR would be sufficient to keep the ash content of the sludge below 40% during period I. This 90% removal could not be reached at thermodynamic equilibrium, and would be even more difficult if crystallization would occur slower and at the same rate in both reactors. However, the higher the crystallization rate in the CR compared to the bioreactor, the lower is the recirculation factor needed for 90% removal of calcium in the CR (Fig.3). At a pK_{sp} of 8.0 for the CR and 7.6 for the UASB, a recirculation factor of 8 is already sufficient to keep the ash content of the anaerobic sludge below 40%.

Table 3. Average measured values of calcium concentration and calcium removal at several locations in the reactor system and average calculated data with different solubility products of $CaCO_3$ (pK_{sp}).

	Period I				**Period II**			
	Measured values	Calculated values model with varying pK_{sp}			Measured values	Calculated values model with varying pK_{sp}		
		UASB 8.4 CR 8.4	UASB 7.6 CR 7.6	UASB 7.6 CR 7.8		UASB 8.4 CR 8.4	UASB 7.9 CR 7.9	UASB 7.9 CR 8.1
Influent CR								
Calcium (mg.l^{-1})								
soluble	110	54	110	110	133	65	120	114
suspended	*18*				*153*			
Influent UASB								
Calcium (mg.l^{-1})								
soluble	78	23	89	76	100	52	113	95
suspended	*9*				*109*			
Effluent UASB								
Calcium (mg.l^{-1})								
soluble	77	19	76	76	100	45	102	95
suspended	*19*				*165*			
Ca removal								
System (%)[1]	96	99	96	96	91	96	91	92
CR (%)[2]	97	89	62	100	100	65	39	100
UASB (%)[3]	3	11	38	0	0	35	61	0

[1] Calcium removal efficiency entire system: $(1 - Ca_{effluent}/Ca_{influent}) * 100\%$

[2] Calcium removal in crystallization reactor (CR) (% of total precipitation in system)

[3] Calcium removal in bioreactor (UASB) (% of total precipitation in system)

Thus for optimal removal of calcium in the CR, it is very important to optimize the kinetics of crystallization in the CR, which can i.e. be achieved by supply of sufficient surface area for crystal growth. By optimizing the kinetics of crystallization in the CR, the required recirculation rate for optimal performance can be minimized. The chemical equilibrium model can be an excellent tool to calculate the proper recirculation factor for optimal performance once a good estimation of the required pK_{sp} values for both reactors can be made.

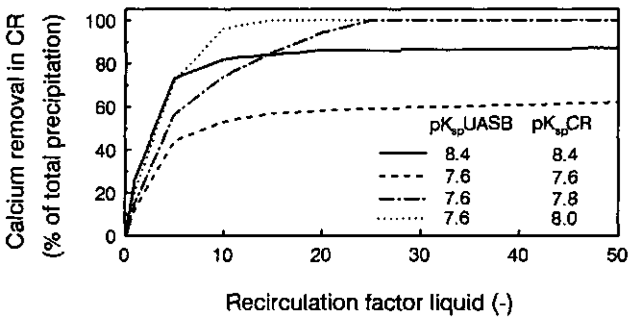

Figure 3. Influence of recirculation factor on the percentage of calcium removal in the crystallization reactor, as calculated with different values of the solubility product of calcium carbonate (pK_{sp}). Calculations were performed with average data from period I (Table 1).

Conclusions

Removal of calcium from the influent of an anaerobic reactor can be accomplished using a combined system of an anaerobic reactor and a crystallization reactor. Almost all the calcium (97-100% of the total precipitation) could be removed in the crystallization reactor, preventing detrimental calcium carbonate accumulation in the anaerobic reactor. By recirculating the anaerobic effluent, sufficient bicarbonate can be supplied for crystallization. Consequently, no extra chemicals (i.e. Na_2CO_3 and/or NaOH) are needed.

Based on a thermodynamic equilibrium model, only 65-90% of the total precipitation was expected to occur in the crystallization reactor. Furthermore, calculated effluent concentrations of calcium were too low when thermodynamic equilibrium was assumed. By introducing lower and different values of pK_{sp} for both reactors, it was possible to fit the model with the measured results. The adapted pK_{sp} values used were shown to reflect the kinetics of the precipitation and crystallization processes that took place. The available surface area for crystal growth in the crystallization reactor turned out to be an important factor influencing the kinetics and determining the feasibility of the process. When factors influencing the kinetics in both reactors can be quantified, proper values of pK_{sp} can be estimated. Once values of pK_{sp} for both reactors can be predicted, the chemical equilibrium model is a very useful tool to determine the proper recycle ratio of effluent for optimal removal of calcium in the crystallization reactor.

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6 Phosphate removal and kinetics of $CaCO_3$ precipitation in anaerobic reactors

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Abstract - Anaerobic treatment of waste waters with a high calcium concentration often leads to undesirable precipitation of calcium carbonate. The amount of precipitation is often far less than expected based on thermodynamic equilibrium. The aims of the present research were to gain insight in the factors that determine the apparent solubility of calcium carbonate and to develop models that predict the extent of precipitation, which would be of great help in the design of systems for the anaerobic treatment of high calcium waste waters. As it is known that the precipitation kinetics and the apparent solubility of calcium carbonate are strongly dependent on the phosphate concentration, this study also focusses on the phosphate removal mechanisms occurring in an anaerobic treatment system. Phosphate removal in an anaerobic reactor was found to take place by biological P-assimilation, co-precipitation and precipitation of the calcium phosphates ACP or OCP. This implies that phosphate should be dosed in excess in order to achieve optimal phosphate inhibition in the reactor. The precipitation rate and the apparent solubility of calcium carbonate were shown to be dependent on the hydraulic retention time, the composition of the influent and the crystallization rate constant ($k' = k.s$). The latter depends on the phosphate concentration in the reactor and the availability of surface area for crystal growth. These kinetic parameters could be impeded in a chemical equilibrium model, which was shown to be a useful tool for prediction of the extent of calcium precipitation during anaerobic treatment.

Introduction

High concentrations of calcium occur in various industrial waste waters, for instance in the effluent from the paper industry.[8,9,13] Anaerobic treatment of waste waters with a high calcium content may lead to undesirable precipitation of calcium carbonate in the reactors.[6,15,27] The amount of calcium carbonate precipitation during anaerobic treatment can be effectively reduced by dosing phosphate,[16] because it is an effective inhibitor of calcium carbonate crystal growth.[5,7,11,18,22] The extent of precipitation is one of the major factors determining the feasibility of anaerobic treatment of high calcium waste waters. Therefore, it would be of great practical importance for the design of anaerobic systems to develop models that predict the extent of precipitation. The available equilibrium models up to now do not consider kinetic aspects of precipitation, and thereby do not allow a proper estimation of the apparent solubility product of calcium carbonate.[27] As it was often found that far less calcium usually precipitates than expected based on thermodynamic equilibrium,[16,17,27,28] a detailed study on the factors that influence the extent of precipitation is needed.

As mentioned above, the precipitation kinetics and the apparent solubility of calcium carbonate both strongly depend on the phosphate concentration in the anaerobic reactor. The concentration of phosphate in the reactor is usually distinctly lower than in the influent because part will be removed by biological or chemical mechanisms.[16] This implies that phosphate should be dosed in excess in order to achieve optimal phosphate inhibition in the reactor. Detailed insight in the phosphate removal mechanisms is thus required for the control and calculation of calcium carbonate precipitation in an anaerobic reactor. Phosphate can be removed from the solution by: *1)* biological assimilation, *2)* precipitation and *3)* co-precipitation. As methanogenic bacterial cells contain 6-15 mgP.gVSS^{-1},[1,24] a removal of 0.08 - 0.54 mgP per gram of COD converted can be obtained (assuming a yield of methanogens of 0.02 - 0.05 g.g^{-1}). With respect to the precipitation of calcium phosphate, hydroxyapatite (HAP) is the thermodynamically most stable polymorph, but it is generally assumed that other phases such as di calcium phosphate dihydrate (DCPD), amorphous calcium phosphate (ACP) and octa calcium phosphate (OCP) serve as precursor phases.[4,29] In the co-precipitation mechanism, phosphate adsorbs to a calcium carbonate crystal lattice. Next, only a fraction of the adsorbed phosphorus will be incorporated into the growing crystals.[10,23]

The apparent solubility of calcium carbonate thus depends, amongst other factors, on the presence of phosphate. Also the availability of reactive surface area for crystal growth is an important factor affecting the kinetics of calcium carbonate crystal growth.[2,14,16] The presence of phosphate affects the value of the crystallization rate constant, k, which can be estimated using a Langmuir adsorption model.[16] The calcium carbonate accumulation rate in an anaerobic reactor then can be calculated according to the equation:[16]

$$r_{CaCO_3} = k_0 \cdot s \cdot \left(1 - \frac{k_{ads} \cdot c_i}{k_{ads} \cdot c_i + 1}\right) \cdot \left(\sqrt{IAP} - \sqrt{K_{sp}}\right)^2 \qquad (1)$$

where:
r_{CaCO3} = $CaCO_3$ accumulation rate ($mol.l^{-1}.s^{-1}$)
k_0 = growth rate constant without additive (P) ($dm.s^{-1}$)
s = reactive crystal surface area ($dm^2.mol^{-1}$)
k_{ads} = adsorption constant ($l.mol^{-1}$)
c_i = concentration of additive (P) ($mol.l^{-1}$)
IAP = ionic activity product (Ca^{2+})(CO_3^{2-}) ($mol^2.l^{-2}$)
K_{sp} = solubility product of $CaCO_3$ ($mol^2.l^{-2}$)

In order to be able to estimate the calcium carbonate accumulation rate, the constants k_0, s and k_{ads} need to be established, together with the prevailing phosphate concentration in the reactor. The latter depends on the influent concentration of phosphate and the extent of phosphate removed.

The aims of this research were *(1)* to gain a better insight in phosphate removal mechanisms in an anaerobic reactor, because it enables the prediction of the phosphate concentration in the reactor, which strongly affects the extent of calcium carbonate precipitation and *(2)* to establish the relation between precipitation kinetics and apparent solubility of calcium carbonate such that chemical equilibrium models can be used to predict the amount of calcium deposition in an anaerobic reactor.

Materials and methods

Experimental set-up

The experiments were performed in a temperature controlled room at 30 ± 2°C. The glass Expanded Granular Sludge Bed (EGSB) reactors used in the experiments had a working volume of 5.7l (150 cm height, 5 cm i.d.) and were equipped with a reverse funnel phase separator. The biogas produced was scrubbed using a concentrated sodium hydroxide solution for removing carbon dioxide and through a column filled with soda lime pellets with indicator, and then the methane was monitored by a wet gas meter. Two reactors were operated and fed with a mixture of acetate and butyrate (1:1 COD). The influent COD concentration was 5.3 $gCOD.l^{-1}$. The applied hydraulic retention time (HRT) was 20 hours. Both reactors were supplied with different levels of phosphate (KH_2PO_4) in the influent, ranging from 0-30 $mgP.l^{-1}$. The influent of reactor R1 was neutralized with $Ca(OH)_2$, resulting in an influent calcium concentration of 1200 $mgCa.l^{-1}$. Reactor R2 was neutralized with 600 $mgCa.l^{-1}$ and 630 $mgNa.l^{-1}$. Both reactors were seeded with crushed anaerobic sludge originating from lab scale UASB reactors. It was a high ash content sludge (80-90%) and its activity amounted to 0.5 $gCOD.gVSS^{-1}.d^{-1}$. The sludge was crushed with a handblender (Multiquick 350 combi, Braun) under anaerobic conditions. In each reactor, 600 ml of sludge was supplied, containing 75 gVSS. In order to fluidize the heavy sludge particles, an upflow velocity of 24 $m.hr^{-1}$ was applied (recirculation ratio 1:140). The calcium and phosphate concentrations and the pH of the influent and the effluent, as well as the pCO_2 of the biogas, were measured during operation of the reactors.

Medium and analytical methods

The mineral medium used was the same as described elsewhere.[15] The applied amounts of phosphate fluctuated in time for the two reactors and are indicated in the text and the figures. Analyses of Volatile Fatty Acids (VFA), biogas composition (CH_4, CO_2 and N_2), calcium and phosphorus were described elsewhere.[15]

Modelling

A chemical equilibrium model was used to calculate the speciation in the anaerobic reactor. Activity coefficients were calculated according to Davies and calculations were made by iteration, using the multi variable method of Newton-Rhapson. A detailed description of the model is provided in the chapters 4 and 5. For the purpose of the present study, the model was extended with phosphate species and kinetics of $CaCO_3$ precipitation.

Results and discussion

Phosphate removal mechanisms

Reactors R1 and R2 were fed with increasing amounts of phosphate in the influent. The results on the phosphate removal for R1 are shown in Figure 1. Those obtained for R2 showed a similar trend (data not shown). Biological phosphate assimilation could only contribute to the removal of 0.5 - 3 mgP.l^{-1} under the applied conditions, for this it was assumed that methanogenic bacterial cells contain 6-15 mgP.gVSS^{-1} [1,24] and have a yield of 0.02-0.05 g.g^{-1}. As the phosphate removal was generally much higher (Fig. 1), apparently precipitation and/or co-precipitation contributed to the removal. To determine which process (precipitation and/or co-precipitation) and which precipitate (HAP, OCP, ACP or DCPD) governed the solubility of phosphate in the reactors, calculations were performed with the equilibrium model for different phosphate species. Results of these simulations were compared to measured data (Fig. 1). At low phosphate dosage (0 - 10 mgP.l^{-1}), phosphate concentrations below 1 mgP.l^{-1} were measured in the effluent. Only part of this removal can be attributed to biological assimilation, the rest should be due to the formation of HAP (Fig. 1), although any HAP could not be detected in the sludge by X-ray analysis. This is in correspondence with other investigations, where no distinct pattern of CaP was found in anaerobic sludge.[30] The reactor solution appeared to be undersaturated with respect to the metastable phases OCP, ACP and DCPD (Fig. 1), which indicates that co-precipitation could have contributed to the P-removal at phosphate dosages up to 10 mgP.l^{-1}.[10] At higher phosphate dosages (10 - 25 mgP.l^{-1}), the measured phosphate concentrations were within the range of solubility between OCP and ACP (Fig. 1). The formation and transformation behavior of calcium phosphates is pH dependent[20,25,29] and as the pH was not constant during the experiments, slight differences in prevailing metastable phases are to be expected.

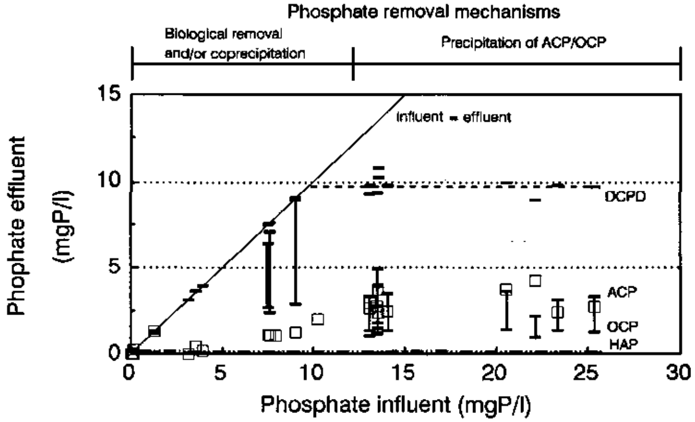

Figure 1. Measured (□) and calculated concentrations of phosphate in the effluent as a function of the phosphate concentration in the influent. In the calculations, determined apparent values of IAP_{CaCO3} were used. Calculations were performed with the solid phases DCPD (—), ACP (– upper marker), OCP (– lower marker) and HAP (–••–) for reactor R1.

Thus, in order to quantify phosphate removal, the biological phosphate assimilation should be included in the model, together with the precipitating species ACP or OCP. Moreover, in order to elucidate the importance of co-precipitation at low phosphate dosages, and high precipitation rates, further research will be needed.

Other factors influencing the extent of calcium carbonate deposition

To establish which other factors influence the apparent solubility of calcium carbonate, and thus the extent of $CaCO_3$ deposition, a calcium balance over the reactor was made:

$$\frac{d[Ca]}{dt} = Q_{in} * [Ca]_{in} - Q_{effl} * [Ca]_{effl} - V * r_{CaCO3} \tag{2}$$

where:
$d[Ca]/dt$ = calcium accumulation rate ($mol.s^{-1}$)
$[Ca]_{in/out}$ = calcium concentration, influent and effluent ($mol.l^{-1}$)
Q = flow ($l.s^{-1}$)
V = volume of system (l)
r_{CaCO3} = calcium carbonate crystallization rate ($mol.l^{-1}.s^{-1}$)

By substituting equation 1 in equation 2 and assuming that the carbonate concentration in the reactor solution is constant, the following expression for the apparent solubility product of calcium carbonate is found:

$$IAP = \frac{1}{4.\left(k' - \frac{1}{HRT'}\right)^2} \cdot \left(2k'\sqrt{K_{sp}} - \sqrt{\left(4k'^2 K_{sp} - \left(4k' - \frac{4}{HRT'}\right)\right) \cdot \left(\frac{IAP_{in}}{HRT'} + k'K_{sp}\right)}\right)^2 \tag{3}$$

where:
IAP = apparent solubility product of $CaCO_3$ ($mol^2.l^{-2}$)
IAP_{in} = $[Ca]_{influent} * [CO_3]_{reactor}$ ($mol^2.l^{-2}$)
K_{sp} = solubility product of $CaCO_3$ ($mol^2.l^{-2}$)
HRT' = HRT * f_2 * $[CO_3]_{reactor}$ ($mol.s.l^{-1}$)
f_2 = activity correction coefficient (-)
k' = k.s = crystal growth rate constant ($l.mol^{-1}.s^{-1}$)

The apparent solubility of calcium carbonate, IAP_{CaCO3}, in the reactor thus depends on k', the hydraulic retention time (HRT) and IAP_{in}. The latter is a measure of the composition (saturation state) of the influent and is defined as the influent calcium concentration multiplied with the carbonate concentration in the reactor. The concentration of carbonate in the reactor depends on the conversions that take place, and is thus not known in advance. However, by introducing equation 3 in the chemical equilibrium model, the concentration of carbonate in the reactor can be calculated iteratively at defined input values of k' (which depends on the phosphate concentrations and the availability of surface area for crystal growth) and the hydraulic retention time.

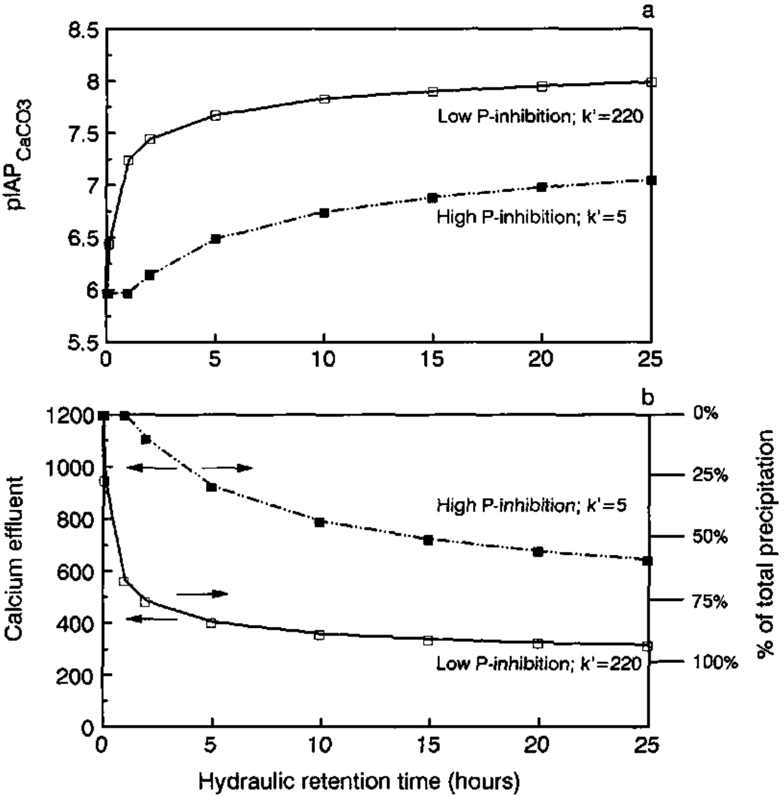

Figure 2. Simulations for the conditions as applied in reactor R1. **(a)** Apparent solubility product of calcium carbonate ($pIAP_{CaCO3}$) as function of the hydraulic retention time (HRT) for high (k'=5) and low (k'=220) inhibition by phosphate. **(b)** Calcium concentrations in the effluent (also expressed as % of maximal possible precipitation) as function of the hydraulic retention time for high (k'=5) and low (k'=220) inhibition by phosphate.

Linking kinetics and apparent solubility

With equation 3, it now becomes possible to predict the apparent solubility of calcium carbonate (IAP_{CaCO3}) in the reactor as a function of the hydraulic retention time and growth rate constant of calcium carbonate. This expression thus gives the desired link between the kinetics of precipitation and the apparent solubility of calcium carbonate. As an example, we calculated the apparent solubility of calcium carbonate for the influent conditions as applied in R1 at different hydraulic retention times (0-25 hours) and at different degrees of inhibition by phosphate. For low phosphate inhibition, we used k' =220 and for high inhibition k' =5.[16] Fig. 2a shows the apparent solubility of calcium carbonate as a function of the hydraulic retention time and Fig. 2b shows the corresponding calculated calcium levels in the effluent and the percentage of maximal possible precipitation. At low inhibition by phosphate (k' =220), the $pIAP_{CaCO3}$ sharply increases during the first hour (Fig. 2a), within which 70% of the maximal precipitation already takes place (Fig. 2b). The calculated apparent solubility of calcium carbonate after 25 hours amounts 10^{-8} $mol^2.l^{-2}$, which is in correspondence with earlier observations.[16] This example shows that, even in absence of inhibitors, a very long retention time (> >25 hours) is required to reach complete thermodynamic equilibrium (pIAP=8.4). In the presence of phosphate (k' =5), the increase of $pIAP_{CaCO3}$ in time is much slower. Now only 60% of the maximal precipitation will take place within a retention time of 25 hours. The $pIAP_{CaCO3}$ is then about 7, which is in correspondence with earlier measured values as well.[16]

The results from this research enable the prediction of the extent of precipitation, once the characteristics of the waste water are known. Besides inhibitors, also the retention time has an important influence on the extent of precipitation, especially in the presence of phosphate. To improve the model, it is recommended to establish a quantitative link between k' (k.s) and the phosphate concentration in the reactor (Eq. 3), which will allow an iterative calculation of k' once the phosphate concentration in the reactor is known.

Conclusions

In order to obtain phosphate concentrations in an anaerobic reactor medium which provide optimal inhibition of calcium carbonate crystal growth, it is generally necessary to dose excess phosphate to the waste water because phosphate will be eliminated from the solution by biological P-assimilation, co-precipitation and precipitation of calcium phosphates. Amorphous calcium phosphate (ACP) and octa calcium phosphate (OCP) were shown to be the most likely calcium phosphate polymorphs to be considered. Co-precipitation of phosphate seems to occur at relatively low dosing of phosphate, 0-10mgP.l^{-1}, (= at high precipitation rates), but to quantify its contribution, more research is needed.

The precipitation rate and the apparent solubility of calcium carbonate were shown to depend on the hydraulic retention time, the composition of the influent and the crystallization rate constant (k'=k.s). The latter depends on the phosphate concentration in the reactor and the availability of surface area for crystal growth. These kinetic parameters could be impeded in a chemical equilibrium model, which was shown to be a useful tool for prediction of the extent of calcium precipitation during anaerobic treatment.

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7 Discussion and conclusions

Introduction

High concentrations of calcium occur in the waste water of paper-, citric acid-, whey- and sugar factories[5,6,7,11,14] as well as in leachates from landfills.[12,16,17] During anaerobic treatment of these waste waters, methane and bicarbonate are produced. The produced bicarbonate can precipitate with the present calcium. Precipitation of calcium carbonate in anaerobic reactors often results in undesirable situations, such as the formation of a too heavy sludge bed, loss of specific methanogenic activity of the sludge, nutrient deficiency, clogging of discharge pipes and/or scaling in the aerobic post-treatment system.[5,7,11,12,16,17,22]

Anaerobic treatment of waste waters is a sustainable technology because energy from the biodegradable organic waste fraction is recovered as methane gas. Therefore, it would be highly profitable to achieve that this technology also can be applied for high calcium waste waters, the more so because they will become more abundant in the near future due to emphasis put on reuse of water and application of closed water circuits, which will result in more concentrated waste- and process waters.

So far, no proper methods were available to predict or reduce the extent of precipitation in an anaerobic treatment system. Moreover, it also was not clear to what extent precipitation in an anaerobic reactor can be tolerated because adequate knowledge on the structure and quality of methanogenic sludges with high calcium carbonate content was lacking. This lack of knowledge hindered the implementation and application of the anaerobic treatment technology for high calcium waste waters.

In this PhD dissertation, we investigated the feasibility of anaerobic treatment of waste waters with a high calcium content. The development of high ash content sludges was investigated under varying conditions of biomass yield, precipitating quantities of $CaCO_3$ and particle size of the seed sludge. The location of precipitation, e.g. within the aggregates or in the bulk solution, appeared to be an important factor determining the structure and quality of high ash content sludges. Next, emphasis was paid to the prediction of the amount of precipitation in an anaerobic reactor, and options to reduce the extent of calcium deposition were investigated. It was shown that the amount of precipitation can be calculated using chemical equilibrium models in which the kinetics of calcium carbonate precipitation is an important parameter. The extent of calcium deposition can be diminished either by dosing proper inhibitors of $CaCO_3$ crystal growth, like phosphate, or by removing calcium from the influent by using a crystallization reactor.

Development of high ash content sludge

Impact of amount and location of precipitation on the quality of high ash content sludge

In chapters 2 and 3, the effects of variable amounts of precipitation and biomass yield were investigated, both for crushed and intact methanogenic sludge. The amount of precipitation depends, amongst others factors, on the waste water composition and the presence of inhibitors of $CaCO_3$ crystal growth, as is explained in chapters 4, 5 and 6. The biomass yield depends on the amount of COD degraded and the yield coefficient ($gVSS.gCOD^{-1}$), which varies for different types of COD.[13] The ratio between $CaCO_3$ and biomass accumulation determines the ash content of the sludge at steady state conditions.

A serious loss of methanogenic activity was found during the development of high ash content intact granular sludge, with feeds consisting of completely acidified substrate (Chapter 3). However, with a similar acidified feed, but with crushed granular sludge as seed, a high ash content sludge with still quite satisfactory and stable quality was cultivated

(Chapter 2). Controversies regarding the activity of high ash content sludges were also observed by other authors.[5,16,17]

The controversies in methanogenic activity of high ash content sludges can be explained considering the structure of the sludge, and in particular, the location of the $CaCO_3$ precipitates. In case precipitation in the bulk solution predominates, the formed precipitates can either wash-out or be retained in the reactor. Wash-out of $CaCO_3$ precipitates was observed in the reactors inoculated with crushed sludge (Chapter 2). The wash-out amounted to 10-20% of the total precipitated amount (Chapter 2, Table 2). The $CaCO_3$ precipitates that were present in the bulk solution and were retained in the reactor served as nuclei, on which biofilms developed. Any serious drop of specific methanogenic activity did not occur under these circumstances, although the ash content of the sludge reached values up to 95%. Apparently, bulk precipitation was favoured by the small particle size of the seed sludge and as a result, relatively little scaling-in of biomass occurred. This hypothesis was confirmed by the results as described in chapter 3. Under almost identical conditions, except for distinctly larger granular seed sludge aggregates, precipitation almost exclusively occurred within the sludge aggregates and wash-out of precipitates was not observed. This exclusive precipitation within the aggregates turned out to be very detrimental because the granules became completely filled-up with calcium carbonate, which seriously impeded the transport of substrate and products within the granules. As a result, the specific methanogenic activity of the sludge rapidly decreased, and this soon resulted in a considerable reduction of the COD removal efficiency of the system. Calculations with a developed steady-state biofilm model confirm the findings that the smaller the size of the seed sludge granules, the more likely will be the precipitation in the bulk solution.

Besides the granular size, also the kinetics of $CaCO_3$ precipitation were found to affect the location of the precipitation. The results presented in chapter 4 clearly show that the kinetics of $CaCO_3$ precipitation are highly influenced by the presence of phosphate. In the presence of phosphate, using intact granular sludge as seed, precipitation exclusively occurred within the aggregates (Chapter 3, reactors R1+R2), whereas under similar conditions, but in the absence of phosphate, precipitation took mainly place in the bulk solution (Chapter 4, reactor R3, Fig. 5). Those results clearly show that also with intact granular sludge as seed, precipitation in the bulk solution can predominate when phosphate is absent. Calculations with the biofilm model confirmed that the more phosphate is present, the more precipitation is expected within the sludge aggregates.

The quality of high ash content sludge thus clearly depends on the location where the $CaCO_3$ precipitates. Precipitation in the bulk solution can be considered as favourable for the development of high ash content sludges, because scaling-in of biomass is then relatively small and appropriate activity is left. Therefore, it is highly advisable to use the smallest possible granules for the start up in case of treatment of calcium-rich waste waters. Calcium carbonate precipitation in the bulk solution is also enhanced at low phosphate concentrations, however, the presence of phosphate on the other hand drastically reduces the total amount of precipitation, as was shown in chapter 4. Whether or not the dosing of phosphate will be advantageous in practical situations can be evaluated with the models as developed in this study, because they provide a reasonable prediction of the amount and the location of precipitation.

Cementation

The results in chapter 2 showed that a high ash content sludge (95%) with appropriate activity can be cultivated, provided a seed sludge with a very small particle size is used for start-up. Nevertheless, cementation of the sludge bed ultimately became a serious bottle neck in the operation of the reactors when they were fed with completely acidified waste waters. Cementation started once the sludge ash content reached high levels (80-95%) and when a large free crystal surface area was present in the reactor. Under the applied conditions, e.g. low upflow velocity, these crystals showed a strong tendency to from one big agglomerate (Chapter 2, Fig. 5a), which is thermodynamically determined. As a result, channelling and stagnation occurred in the reactors and the operation had to be terminated. In chapters 2 and 3, it was shown that cementation can be effectively retarded by reducing the degree of pre-acidification. When the waste water contained non-acidified substrate (e.g. glucose), a significant amount of acidifying bacteria was grown in due to their relatively higher biomass yield than methanogens. As a result, crystal surfaces in the reactor became rapidly covered with a film of acidifying biomass, and this prevented the crystal agglomeration and thereby retarded cementation. Moreover, the results in chapter 2 showed that the presence of a fraction of non-acidified substrate promotes granulation.

It therefore can be concluded that a lower degree of pre-acidification is quite favourable for the treatment of waste water with a high calcium concentration, because it prevents cementation and promotes granulation. Another possibility to prevent cementation might be found in application of higher upflow velocities.

Extent of precipitation

Calculation of the amount of precipitation

The amount of $CaCO_3$ that precipitates is an important parameter in determining the feasibility of anaerobic treatment for high calcium waste waters. This quantity is primarily dictated by the concentrations of calcium and carbonate, which depend on factors like the waste water composition and the type of conversion reactions proceeding in the system.[22] Moreover, the extent of $CaCO_3$ precipitation is obviously determined by the "apparent solubility product" of $CaCO_3$, which was often found to differ several orders of magnitude from the theoretical value.[11,18,22] Consequently, far less calcium usually precipitates than is expected on the basis of thermodynamic equilibrium.

In this PhD dissertation, we investigated which factors influence the apparent solubility of calcium carbonate, and developed a model enabling the calculation of the amount of precipitation during anaerobic treatment. The results in chapter 4 clearly reveal that the apparent solubility of $CaCO_3$ depends on the phosphate concentration in the reactor. Phosphate already was known to be an important inhibitor of calcium carbonate crystal growth.[10,19,20,21] We found that at concentrations of 5 $mgP.l^{-1}$ in the reactor, the apparent solubility product reached values close to $10^{-6.5}$ $mol^2.l^{-2}$, which corresponds to the solubility of amorphous calcium carbonate.[3] This is in agreement with findings of other researchers, who also reported that calcium carbonate only precipitates in the amorphous form in the presence of phosphates.[18] It was demonstrated that the value of the crystallization rate constant, k, depends on the phosphate concentration and can be estimated using a Langmuir adsorption model. Moreover, the results in chapters 4 and 5 also showed that the apparent solubility of $CaCO_3$ is affected by the available reactive surface area for crystal growth.

In chapter 6, we established a quantitative link between the apparent solubility and the precipitation kinetics of calcium carbonate. The kinetics of calcium carbonate precipitation were shown to depend on the hydraulic retention time, the composition of the influent and on the crystallization rate constant ($k'=k.s$). The latter depends on the phosphate concentration and the available reactive crystal surface. A chemical equilibrium model was developed including these kinetic parameters along with equilibrium conditions. This model proved to be a very useful tool for predicting the extent of calcium precipitation during anaerobic digestion.

Possibilities to reduce $CaCO_3$ precipitation by supply of inhibitors of crystal growth

As mentioned above, the results in chapter 4 revealed that the quantity of calcium carbonate precipitation in an anaerobic reactor can be drastically reduced by increasing the phosphate concentration in the reactor. At phosphate concentrations of 0.5 - 5 $mgP.l^{-1}$ in the reactor medium, the crystallisation rate of calcium carbonate is inhibited to such an extent that far less precipitation occurred than was expected based on a state of thermodynamic equilibrium (Chapter 6, Fig. 2). To achieve maximal reduction of precipitation, it is advisable to maintain concentrations of >5 $mgP.l^{-1}$ in the anaerobic reactor medium. Crystallization of calcite is then completely prevented, and the apparent solubility of calcium carbonate in the reactor approaches the value for amorphous calcium carbonate ($pK_{sp}=6.5$). In order to realise such a high concentration in the reactor, it will be necessary to dose phosphate in excess, because phosphate removal will occur by biological P-assimilation[1] and by co-precipitation[9] and/or precipitation[4] of calcium phosphates. Investigations in chapter 6 showed that amorphous calcium phosphate (ACP) or octa calcium phosphate (OCP) were the most likely calcium phosphate polymorphs to consider.

In addition to phosphate, other additives, e.g. iron, are mentioned in the literature that can inhibit the formation of calcium carbonate.[8,19,20] However, the results presented in chapter 4 revealed that addition of iron salts was not effective in reducing the extent of calcium carbonate precipitation during anaerobic treatment. The reason for the absence of a clear effect could be attributed to the fact that iron only inhibits the formation of calcite and not that of aragonite, which was predominantly formed in the sludge bed. As a matter of fact, it can be concluded that only additives inhibiting the formation of both aragonite and calcite are suitable for reducing the extent of calcium carbonate precipitation in an anaerobic reactor.

Reduction of $CaCO_3$ precipitation by removal of calcium from the influent

Besides by adding inhibitors, an alternative solution to reduce the extent of precipitation in an anaerobic reactor can be found in removal of calcium from the influent. As shown in chapter 5, a crystallisation reactor placed in front of the anaerobic reactor represents an attractive option for removal of calcium from the influent. By recirculation of the effluent, sufficient bicarbonate could be supplied for the precipitation of a significant fraction of the present calcium. In this way, no extra chemicals (e.g. Na_2CO_3 and/or NaOH) were needed. The calcium removal efficiency of the crystallisation reactor was much higher than expected on the basis of calculations as carried out with an equilibrium model as developed for the two-reactor system. This can very likely be attributed to more favourable precipitation kinetics in the crystallisation reactor because of the presence of a large specific crystallization surface area. The chemical equilibrium model developed for a two-reactor system (bioreactor plus crystallisation reactor) turned out to be a very useful tool to calculate the calcium removal from the influent. It enables the calculation of the recycling factor at which optimum calcium removal occurs at minimal dosing of chemicals.

Conclusions and recommendations

Conclusions

The insights gained from the investigations as described in this dissertation enable the control of the precipitation of calcium carbonate in anaerobic reactors. Ash contents of methanogenic sludges up to 90% can be tolerated provided the formed precipitates are not exclusively located within the aggregates, so that the sludge retains a satisfactory activity. However, on the other hand, reactors containing heavy sludges suffer easily from operational problems because cementation of the sludge bed might take place. Cementation of the sludge is drastically retarded in the presence of a fraction non-acidified substrate (e.g. glucose) in the influent and this represents a possible tool to improve the feasibility of anaerobic treatment of high calcium waste waters.

The extent of precipitation of calcium carbonate in an anaerobic reactor can be effectively reduced by adding phosphate, which is an inhibitor of calcium carbonate crystal growth. As biological assimilation, co-precipitation and precipitation will lead to phosphate removal, it may be necessary to supply phosphate in excess to achieve maximal reduction of the amount of $CaCO_3$ precipitation. Only additives inhibiting the formation of both aragonite ánd calcite are suitable to reduce the extent of calcium carbonate precipitation in an anaerobic reactor. Another means to reduce, or even prevent, the precipitation of calcium carbonate can be found in using a crystallisation reactor to remove calcium from the influent.

The amount of calcium carbonate precipitation in an anaerobic reactor can be estimated with equilibrium models we developed, which take into account kinetic factors that influence the apparent solubility of calcium carbonate, e.g. the influent composition, the hydraulic retention time and the crystallisation rate constant ($k' = k.s$). The latter factor depends on the presence of inhibitors, like phosphate, and on the availability of crystal surface area (s). Also the influent calcium removal and location of precipitation can be estimated with developed models.

With respect to the general aim of this dissertation, it can be concluded that with the work conducted in this thesis, sufficient insights have been provided for the design and application of integrated anaerobic-physical-chemical technologies which enable the control of the calcium carbonate precipitation in the treatment of waste water with a high calcium content. In the meantime, it has been shown in practise that anaerobic waste water treatment can be very successfully applied in closed water circuits with a high calcium concentration.[6,7]

Recommendations

Although the developed equilibrium models already are very suitable for predicting the quantity of calcium carbonate precipitation in an anaerobic system, it seems possible and therefore highly recommended to further improve them, such that phosphate removal and inhibition can be more reliably estimated. For this, a quantitative link between k' (=k.s) and the phosphate concentration in the reactor should be assessed, because this will allow an iterative calculation of k' once the phosphate concentration in the reactor is known.

Chemical phosphate removal in the post-treatment system, mediated by the expulsion of CO_2, deserves special attention, because high levels of phosphate in the final effluent cannot be tolerated in view of eutrophication problems when discharging such effluents.

In practise, compounds like sulphate and protein are often encountered in waste waters and production of sulphide and ammonium will take place during anaerobic treatment. As both compounds will influence the equilibrium in the system, it is highly recommendable to extent the models with processes of sulphate reduction and degradation of proteins.

References

See chapter 8.

8 Discussie en conclusies

Inleiding

Hoge calcium concentraties komen voor in het afvalwater van de papier-, citroenzuur-, wei- en suikerindustrie[5,6,7,11,14] alsmede in het percolatiewater van stortplaatsen.[12,16,17] Tijdens anaërobe zuivering van deze afvalwaters worden methaan en bicarbonaat gevormd. Het gevormde bicarbonaat kan vervolgens precipiteren met het aanwezige calcium in het afvalwater. Precipitatie van calcium carbonaat in anaërobe reactoren resulteert vaak in ongewenste situaties, zoals de vorming van een te zwaar slibbed, verlies van specifieke methanogene activiteit van het slib, gebrek aan nutriënten, verstopping van afvoerleidingen en/of kalkafzetting in een aërobe nabehandelingstrap.[5,7,11,12,16,17,22]

Anaërobe zuivering van afvalwater is een duurzame technologie omdat energie uit organisch afval wordt teruggewonnen als methaangas. Daarom is het van groot belang om er voor te zorgen dat deze technologie ook kan worden toegepast voor de behandeling van afvalwaters met een hoog calcium gehalte, te meer daar deze afvalwaters in de nabije toekomst steeds talrijker zullen worden omdat veel aandacht wordt besteed aan het hergebruik van water. Het sluiten van waterkringlopen komt daarom steeds vaker voor, wat leidt tot het ontstaan van geconcentreerde proces- en afvalwaters.

Tot op heden waren geen geschikte methoden beschikbaar om de mate van precipitatie in een anaëroob systeem te voorspellen of te voorkomen. Ook was het niet duidelijk tot op welke hoogte precipitatie in een anaërobe reactor kan worden getolereerd omdat kennis omtrent de structuur en kwaliteit van methanogeen slib met een hoog calciumcarbonaat gehalte ontbrak. Deze kennisleemte belemmerde de toepassing van de anaërobe zuiveringstechnologie voor de behandeling van afvalwaters met een hoog calcium gehalte.

Dit proefschrift had tot doel om de geschiktheid van anaërobe zuivering, eventueel in combinatie met fysische chemische technologieën, vast te stellen voor de behandeling van afvalwater met een hoog calcium gehalte. De ontwikkeling van slib met hoge asgehaltes werd bestudeerd onder variabele condities qua biomassa aanwas, hoeveelheid $CaCO_3$ precipitatie en korrelgrootte van het entslib. De plaats van de precipitatie, t.w. in de aggregaten of in de bulk oplossing, bleek een belangrijke factor te zijn die de structuur en kwaliteit van slib met een hoog asgehalte bepaalt. Verder werd aandacht besteed aan de berekening van de hoeveelheid precipitatie in een anaërobe reactor, en werden verschillende opties onderzocht om de hoeveelheid precipitatie te verminderen. Het bleek dat de hoeveelheid precipitatie goed kan worden berekend met ontwikkelde chemische evenwichtsmodellen, waarin de kinetiek van de precipitatie werd opgenomen omdat die een cruciale rol bleek te spelen. De mate van precipitatie bleek te kunnen worden verminderd middels het toevoegen van geschikte additieven die de kristalgroei van $CaCO_3$ remmen, zoals bijvoorbeeld fosfaat, of middels verwijdering van calcium uit het influent met behulp van een kristallisatiereactor.

Ontwikkeling van slib met een hoog asgehalte

Invloed van de hoeveelheid en plaats van precipitatie op de kwaliteit van slib met een hoog asgehalte

In de hoofdstukken 2 en 3 werd de ontwikkeling van slib met een hoog asgehalte bestudeerd onder verschillende condities qua hoeveelheid precipitatie, biomassa aanwas en korrelgrootte verdeling van het entslib. De hoeveelheid precipitatie hangt onder andere af van de afvalwater samenstelling en van de aanwezigheid van remmers van de kristalgroei van $CaCO_3$, zoals werd beschreven in de hoofdstukken 4, 5 en 6. De biomassa aanwas wordt bepaald door de hoeveelheid CZV die wordt afgebroken en de aanwascoëfficiënt ($gVSS.gCZV^{-1}$), die varieert voor verschillende typen CZV.[13] De verhouding tussen $CaCO_3$ en biomassa accumulatie bepaalt het asgehalte van het slib tijdens steady state condities.

Na opstarten van de reactoren met intact korrelslib en voeding met volledig verzuurd substraat werd een drastisch verlies van methanogene activiteit geconstateerd (Hoofdstuk 3). Echter, onder vergelijkbare omstandigheden, maar na opstarten van de reactor met vermalen slib, werd slib gekweekt met een redelijke methanogene activiteit (Hoofdstuk 2). Tegenstrijdigheden betreffende de activiteit van slib met een hoog asgehalte worden ook gevonden door andere onderzoekers.[5,16,17]

Om de verschillen in kwaliteit, en dan met name de activiteit, van slib met een hoog asgehalte te kunnen verklaren dient de structuur van het slib te worden beschouwd, en dan met name de locatie van de $CaCO_3$ precipitaten. Wanneer precipitatie uitsluitend plaatsvindt in de bulkoplossing kunnen de gevormde precipitaten uitspoelen of in de reactor achterblijven. Uitspoeling van $CaCO_3$ precipitaten werd waargenomen in de reactoren die geënt werden met vermalen slib (Hoofdstuk 2). De uitspoeling bedroeg 10-20% van de totale geprecipiteerde hoeveelheid (Hoofdstuk 2, Tabel 2). De $CaCO_3$ precipitaten die in de reactor achterbleven bleken te dienen als groeikernen, waarop zich een biofilm ontwikkelde. Een drastische afname van methanogene activiteit trad onder deze omstandigheden niet op, ondanks het hoge asgehalte, 95%, van het slib. Deze resultaten lijken erop te duiden dat precipitatie in de bulkoplossing plaats vindt wanneer een reactor wordt opgestart met kleine korrels (vermalen slib) als entmateriaal, wat leidt tot relatief weinig inschaling van biomassa. Inderdaad bleek dat het opstarten van de reactor met grotere aggregaten (intact korrelslib) uitsluitend leidde tot precipitatie ín de slibkorrels onder verder vrijwel identieke condities (Hoofdstuk 3). Deze precipitatie in de korrels bleek zeer nadelig te zijn, de korrels werden volledig volgebouwd met calcium carbonaat, wat het transport van substraten en produkten naar en in de korrel ernstig belemmerde. Als gevolg hiervan daalde de methanogene activiteit van het slib snel, wat leidde tot een aanzienlijke vermindering van het CZV verwijderingsrendement van het systeem. Berekeningen met het in dit onderzoek ontwikkelde steady-state biofilmmodel bevestigden dat naarmate de korrels kleiner zijn er meer precipitatie in de bulkoplossing kan worden verwacht.

Naast de korrelgrootte blijkt ook de kinetiek van de $CaCO_3$ precipitatie in belangrijke mate te bepalen waar de afzetting plaats zal vinden. De resultaten zoals beschreven in hoofdstuk 4 toonden duidelijk dat de kinetiek van de $CaCO_3$ precipitatie sterk wordt beïnvloed door fosfaat. In de aanwezigheid van fosfaat, met intact korrelslib als entmateriaal, vond de precipitatie uitsluitend plaats binnen in de korrels (Hoofdstuk 3, reactor R1 en R2) terwijl onder vergelijkbare omstandigheden, maar in afwezigheid van fosfaat, de precipitatie voornamelijk plaatsvond in de bulkoplossing (Hoofdstuk 4, reactor R3, Fig. 5). Deze resultaten laten dus duidelijk zien dat, wanneer fosfaat afwezig is, ook na opstarten met intact korrelslib de precipitatie in de bulkoplossing kan plaatsvinden. Berekeningen met het biofilm model bevestigen dat naarmate meer fosfaat aanwezig is, er meer precipitatie ín de korrels kan worden verwacht.

De kwaliteit van slib met een hoog asgehalte blijkt dus in hoge mate te worden bepaald door de plaats waar de $CaCO_3$ precipitatie plaatsvindt. Precipitatie in de bulkoplossing blijkt het gunstigst voor de ontwikkeling van slib met een hoog asgehalte omdat onder deze omstandigheden relatief weinig inschaling van biomassa plaatsvindt en voldoende methanogene activiteit van het slib wordt behouden. Voor de opstart van anaërobe reactoren waarin precipitatie kan worden verwacht is het daarom aan te bevelen om gebruik te maken van zo klein mogelijke korrels als entmateriaal. Om precipitatie in de bulkoplossing te stimuleren is het tevens aan te bevelen om zo min mogelijk fosfaat te doseren, echter, het toevoegen van fosfaat leidt juist weer tot een drastische vermindering van de totale hoeveelheid precipitatie, zoals is beschreven in hoofdstuk 4. Of het toevoegen van fosfaat al dan niet aan te bevelen is in de praktijk hangt af van de samenstelling van het afvalwater en de omzettingen die plaatsvinden. Met de ontwikkelde modellen kan zowel een schatting worden gemaakt van de plaats als van de hoeveelheid precipitatie, en kan vervolgens worden afgewogen of het toevoegen van extra fosfaat al dan niet nodig zal zijn.

Verstening

De resultaten in hoofdstuk 2 toonden dat een slib met een hoog asgehalte, 95%, kan worden gekweekt met een redelijke activiteit wanneer de reactoren werden opgestart met vermalen slib als entmateriaal. Echter, verstening van het slibbed bleek uiteindelijk tot zodanige operationele problemen te leiden dat de experimenten moesten worden gestopt. Verstening van het slibbed trad op wanneer het asgehalte van het slib waarden bereikte van 80-95%, waarbij een zeer groot vrij kristaloppervlak in de reactor aanwezig was. Onder de toegepaste condities, zoals bijvoorbeeld een lage opstroomsnelheid, vertoonden deze kristallen de neiging om aaneen te groeien tot één groot agglomeraat (Hoofdstuk 2, Fig. 5a). Als gevolg hiervan ontstonden gemakkelijk voorkeursstromen en raakten reactoren zodanig verstopt dat ze niet meer bedreven konden worden. In de hoofdstukken 2 en 3 werd aangetoond dat verstening danig kan worden geremd door de mate van voorverzuring van het influent te verlagen. Wanneer het afvalwater een fractie onverzuurd substraat bevat (bijvoorbeeld glucose) zal een significante hoeveelheid verzurende bacteriën ingroeien, die een hogere biomassa aanwas hebben dan methanogenen. Dit heeft tot gevolg dat aanwezig kristaloppervlak in de reactor snel kan worden bedekt met een laagje biomassa, wat het aaneengroeien van de kristallen belemmert en daarmee de verstening vertraagd. Bovendien werd in hoofdstuk 2 aangetoond dat de aanwezigheid van een fractie onverzuurd substraat de korrelvorming bevordert.

Er kan dus worden geconcludeerd dat een lage mate van voorverzuring gunstig is voor de behandeling van afvalwater met een hoog calcium gehalte omdat het verstening van het slibbed voorkomt en leidt tot een betere korrelvorming. Een andere mogelijke oplossing om verstening te voorkomen is het toepassen van hogere opstroomsnelheden.

Mate van precipitatie

Berekening van de hoeveelheid precipitatie

De haalbaarheid van de toepassing van de anaërobe zuiveringstechnologie voor de behandeling van afvalwaters met een hoog calcium gehalte wordt in belangrijke mate bepaald door de hoeveelheid $CaCO_3$ precipitatie die op zal treden. Deze hoeveelheid wordt bepaald door de hoeveelheden calcium en carbonaat in de reactor, die weer afhangen van de afvalwatersamenstelling en de omzettingen die plaatsvinden.[22] Bovendien wordt de mate van $CaCO_3$ precipitatie in hoge mate bepaald door de waarde van het "schijnbare oplosbaarheidsprodukt" van $CaCO_3$, dat vaak enkele ordes van grootte blijkt te verschillen van de theoretische waarde van het oplosbaarheidsprodukt.[11,18,22] Hierdoor vindt vaak veel minder precipitatie plaats dan verwacht wordt, uitgaande van het thermodynamische evenwicht.

In dit proefschrift hebben we onderzocht welke factoren dit schijnbare oplosbaarheidsprodukt van calcium carbonaat beïnvloeden tijdens anaërobe zuivering. Vervolgens werd een model ontwikkelt waarmee de hoeveelheid precipitatie tijdens anaërobe zuivering kan worden berekend. De resultaten in hoofdstuk 4 tonen dat het schijnbare oplosbaarheidprodukt van $CaCO_3$ in hoge mate wordt bepaald door de fosfaatconcentratie in de reactor. Het was reeds bekend dat fosfaat een zeer goede remmer is van de kristalgroei van calcium carbonaat.[10,19,20,21] Bij een fosfaat concentratie in de reactor van 5 $mgP.l^{-1}$ benaderde het schijnbare oplosbaarheidsprodukt de waarde van $10^{-6.5}$ $mol^2.l^{-2}$, wat correspondeert met de oplosbaarheid van amorf calcium carbonaat.[3] Dit is in overeenstemming met bevindingen van andere onderzoekers, die tevens vonden dat calcium carbonaat alleen in de amorfe vorm precipiteert in de aanwezigheid van fosfaat.[18] De aanwezigheid van fosfaat in de reactor beïnvloedt de waarde van de kristallisatie-snelheidsconstante, k, die kan worden geschat met behulp van een Langmuir adsorptie model (Hoofdstuk 4). De resultaten in de hoofdstukken 4 and 5 tonen dat het schijnbare oplosbaarheid van $CaCO_3$ tevens wordt beïnvloed door de aanwezigheid van actieve kristal groeiplaatsen.

In hoofdstuk 6 beschrijven we de kwantitatieve samenhang tussen de schijnbare oplosbaarheid en de precipitatie kinetiek van calcium carbonaat. De kinetiek van de calcium carbonaat precipitatie wordt bepaald door de hydraulische verblijftijd, de samenstelling van het influent en de waarde van de kristalgroeisnelheidsconstante ($k' = k.s$). Deze laatste wordt bepaald door de fosfaat concentratie in de reactor en de aanwezigheid van actieve groeiplaatsen voor kristalgroei (s). Het ontwikkelde chemische evenwichtsmodel, waarin deze kinetische parameters kunnen worden opgenomen, blijkt een zeer goed middel te zijn om de mate van precipitatie in een anaërobe reactor te berekenen.

Vermindering van de mate van $CaCO_3$ precipitatie door het toevoegen van remmers van kristalgroei

De resultaten in hoofdstuk 4 toonden dat de mate van calcium carbonaat afzetting in een anaërobe reactor drastisch verminderd wordt in de aanwezigheid van fosfaat. Bij fosfaatconcentraties van 0.5 - 5 $mgP.l^{-1}$ in de reactorvloeistof wordt de kristallisatie van calcium carbonaat zodanig gehinderd dat veel minder neerslag wordt gevormd dan te verwachten valt in geval van thermodynamische evenwicht (Hoofdstuk 6, Fig.2). Om een optimale reductie van de hoeveelheid precipitatie te verkrijgen is het aan te bevelen om concentraties >5 $mgP.l^{-1}$ in de anaërobe reactor te handhaven. De kristallisatie tot calciet wordt dan volledig verhinderd en de schijnbare oplosbaarheid van $CaCO_3$ in de reactor benadert dan de waarde van amorf calcium carbonaat ($pK_{sp}=6.5$). Om deze hoge concentraties in de reactor te bereiken zou het nodig kunnen zijn om een overmaat fosfaat te doseren, omdat fosfaatverwijdering op zal treden middels biologische P-assimilatie[1] en co-precipitatie[9] en/of precipitatie van calcium fosfaten[4]. Het onderzoek in hoofdstuk 6 toonde aan dat amorf calcium fosfaat (ACP) of octa calcium fosfaat (OCP) de meest waarschijnlijke fosfaat polymorfen zijn die de oplosbaarheid van fosfaat in een anaërobe reactor bepalen.

Naast fosfaat worden in de literatuur andere additieven, zoals bijvoorbeeld ijzer, genoemd die de vorming van calcium carbonaat remmen.[8,19,20] Echter, de resultaten in hoofdstuk 4 laten zien dat het toevoegen van ijzer de schijnbare oplosbaarheid van calcium carbonaat tijdens anaërobe zuivering niet beïnvloedt. De reden hiervoor is hoogstwaarschijnlijk dat ijzer alleen de vorming van calciet remt, en niet de vorming van aragoniet, dat onder deze omstandigheden dan ook in grote hoeveelheden in het slibbed werd aangetroffen. Hieruit kan worden afgeleid dat alleen additieven die zowel de vorming van calciet als aragoniet remmen geschikt zijn om de mate van calcium carbonaat precipitatie tijdens anaërobe zuivering te verminderen.

Vermindering van de $CaCO_3$ precipitatie door calcium verwijdering uit het influent

Naast het toevoegen van additieven om de mate van calcium neerslag te verminderen, kan worden overwogen om het calcium uit het influent te verwijderen. In hoofdstuk 5 wordt een reactorconfiguratie beschreven, bestaande uit een kristallisatiereactor en een anaërobe bioreactor, waarmee het mogelijk bleek om calcium uit het influent te verwijderen. Middels het recirculeren van effluent kon voldoende bicarbonaat worden verkregen om een aanzienlijke hoeveelheid calcium uit het influent te verwijderen. Door het toepassen van effluent recirculatie bleek het niet nodig om extra chemicaliën, zoals bijvoorbeeld Na_2CO_3 en/of NaOH, te doseren. Het verwijderingsrendement van calcium in de kristallisatiereactor bleek aanzienlijk hoger te zijn dan verwacht op basis van berekeningen zoals uitgevoerd met het evenwichtsmodel dat we ontwikkelden voor het twee-reactor systeem. De oorzaak hiervoor kan hoogstwaarschijnlijk worden gevonden in het feit dat de precipitatiekinetiek in de kristallisatiereactor gunstiger was dan in de bioreactor vanwege de aanwezigheid van een groot specifiek kristaloppervlak met veel groeiplaatsen. Het ontwikkelde chemische evenwichtsmodel voor dit twee reactor systeem blijkt een zeer nuttig instrument te zijn waarmee de mogelijkheden tot het verwijderen van calcium uit het influent kunnen worden bepaald voor verschillende typen afvalwaters. Zo kan bijvoorbeeld met het model de recirculatiefactor worden bepaald, waarbij de calcium verwijdering plaats kan vinden met een minimale dosering van extra chemicaliën.

Conclusies en aanbevelingen

Conclusies

De inzichten van het onderzoek zoals beschreven in dit proefschrift leiden ertoe dat het fenomeen "precipitatie in anaërobe reactoren" wordt begrepen en daarmee kan worden beheerst. Asgehaltes van methanogeen slib tot zelfs 90% kunnen worden getolereerd mits de gevormde precipitaten zich niet uitsluitend hebben afgezet in de korrels, zodat het slib een redelijke activiteit heeft behouden. Echter, reactoren met een zwaar slibbed hebben vaak te kampen met operationele problemen, omdat het gevaar bestaat dat voorkeursstromen ontstaan en dat uiteindelijk verstening van het slibbed optreedt. Risico's met betrekking tot verstening van het slibbed worden drastisch verminderd in de aanwezigheid van een fractie onverzuurd substraat (bijvoorbeeld glucose) in het influent.

De mate van $CaCO_3$ precipitatie in een anaërobe reactor kan effectief worden verminderd door het toevoegen van fosfaat, een additief dat de kristalgroei remt. Omdat biologische P-assimilatie, coprecipitatie en precipitatie zullen leiden tot gedeeltelijke verwijdering van het fosfaat kan het nodig zijn om een overmaat fosfaat te doseren om een optimale reductie van de hoeveelheid $CaCO_3$ neerslag te verkrijgen. Alleen additieven die zowel de vorming van calciet als van aragoniet remmen zijn geschikt om de hoeveelheid calcium carbonaat neerslag in een anaërobe reactor te verminderen. Een andere manier om de hoeveelheid neerslag te verminderen, of zelfs volledig te voorkomen, is het verwijderen van calcium uit het influent met behulp van een kristallisatiereactor en recirculatie van het anaërobe effluent.

De hoeveelheid te verwachten calcium precipitatie in een anaërobe reactor kan worden berekend met de chemische evenwichtsmodellen die werden ontwikkeld tijdens dit onderzoek. In deze modellen wordt rekening gehouden met kinetische factoren die de schijnbare oplosbaarheid van calcium carbonaat beïnvloeden. Deze factoren zijn de influent samenstelling, de hydraulische verblijftijd en de kristallisatiesnelheidsconstante, $k' = k.s$. Deze laatste parameter wordt beïnvloed door de aanwezigheid van kristalgroeiremmers, zoals fosfaat, en de aanwezigheid van geschikt kristaloppervlak. Ook de mate van calcium verwijdering uit het influent met behulp van een kristallisatiereactor en de plaats van precipitatie kunnen worden bepaald met behulp van de ontwikkelde modellen.

Kijkende naar de doelstelling van dit proefschrift kan worden geconcludeerd dat het onderzoek voldoende inzichten heeft opgeleverd om te kunnen komen tot het ontwerp en de toepassing van gecombineerde anaërobe-fysische-chemische technologieën, waarmee de calcium carbonaat precipitatie tijdens de zuivering van afvalwater met een hoog calciumgehalte kan worden beheerst. Tijdens de duur van het onderzoek is in de praktijk gebleken dat anaërobe zuivering succesvol kan worden toegepast in gesloten waterkringloopsystemen met een hoog calcium gehalte.[6,7]

Aanbevelingen

Hoewel met de ontwikkelde evenwichtsmodellen de hoeveelheid calcium precipitatie reeds redelijk kan worden voorspeld lijkt het mogelijk, en daarmee aan te bevelen, om ze zodanig verder uit te breiden, dat de verwijdering van remming door fosfaat betrouwbaarder kunnen worden berekend. Hiertoe dient een kwantitatief verband te worden vastgesteld tussen k' (=k.s) en de fosfaatconcentratie in de reactor, zodat k' iteratief berekend kan worden als de fosfaat concentratie in de reactor bepaald is.

Speciale aandacht is verder nodig voor de chemische fosfaat verwijdering in een eventueel aanwezige aërobe nabehandelingstrap. Als gevolg van het uitdrijven van CO_2 uit de vloeistof zal de pH hier stijgen, wat waarschijnlijk zal leiden tot calcium carbonaat en/of fosfaat precipitatie. Fosfaat verwijdering op deze plaats zou gunstig zijn omdat een hoge fosfaatconcentratie in het uiteindelijke effluent niet kan worden getolereerd wegens risico's met betrekking tot eutrofiëring van het oppervlaktewater.

In de praktijk komen verder vaak stoffen als fosfaat en eiwitten voor in afvalwater, die tijdens anaërobe behandeling worden omgezet in o.a. sulfide en ammonium, beide stoffen die de ligging van het chemische evenwicht in de reactor zullen beïnvloeden. Het is daarom aan te bevelen de modellen uit te breiden met processen als sulfaatreductie en de afbraak van eiwitten.

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Dankwoord

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Curriculum Vitae

De auteur van dit proefschrift, Emerentiana Petronella Adriana (Anita) van Langerak, werd op 24 april 1969 geboren in Hooge Zwaluwe. In 1987 behaalde zij het Atheneum-B diploma aan het Dongemond College in Raamsdonksveer. In datzelfde jaar begon zij met haar studie Milieuhygiëne aan de Landbouw Universiteit Wageningen. In augustus 1993 werd deze studie voltooid met als specialisaties anaërobe waterzuivering en microbiologie. Haar stage bracht zij door in Brazilië aan de Universiteit van Paraïba in Campina Grande en bij Cetrel in Salvador, Bahia, waar zij zich bezig hield met de aërobe zuivering van petrochemisch afvalwater.

Van augustus 1993 tot januari 1998 was zij werkzaam als toegevoegd onderzoeker aan de vakgroep Milieutechnologie van de Landbouw Universiteit Wageningen. De resultaten van dit onderzoek zijn beschreven in dit proefschrift.

Van januari tot juni 1998 was zij werkzaam als cursusdocent bij het Groenhorst Trainings Centrum Technologie te Ede. Zij werkte mee aan de verzorging van de cursus "Recherche en Milieu Specialisme" in opdracht van de Regio Politie Midden- en West Brabant.

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