Mineral CO_2 Sequestration by Environmental Biotechnological Processes

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Front cover image: Wollastonite particles trapped in a microbial anaerobic granule.

Back cover image: Microbial granules and wollastonite particles under an optical microscope.

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To my mother, Mahin

Summary

The collective pursuit of economic wellbeing has increased the concentration of atmospheric CO_2 causing one of the greatest environmental challenges that humanity is facing today; the climate change. Mineral carbonation of CO_2 is one of the relatively new techniques for sequestration of carbon dioxide emissions. The technology is based on natural weathering of the silicate minerals responsible for capture and sequestration of 100 million tons of carbon every year (1.8-2.0% of the anthropogenic carbon). The main advantages of this method as compared with other available CO_2 sequestration processes such as geological and oceanic sequestration are the permanent and inherently safe process, and the high sequestration capacity due to the vast global reserves of silicate minerals considered as the process feed-stock.

However, the slow kinetics of the CO_2 mineral sequestration process should be overcome before it can be commercially applied. Although it has already been verified that kinetics of the sequestration process can be sufficiently improved by using physical and chemical methods, the energy consumption and the associated costs of the technology are considered substantial compared with other available carbon storage technologies. Therefore, the application of biological processes as a more cost-effective method for enhancing the mineral CO_2 sequestration kinetics has recently been proposed. The research described in this thesis was performed within the framework of the European project funded by the European community's seventh framework programme (FP7). The scientific teams involved in the project investigated sustainable solutions for carbon sequestration using biological processes as a complementary or alternative way to the existing carbon storage and sequestration techniques.

The **aim** of this thesis was to evaluate how alkaline silicate mineral-based CO_2 sequestration can be achieved using two-stage environmental biotechnological processes. The proposed biotechnological processes that enable mineral CO_2 sequestration were studied in terms of rate-determining process steps, sequestration efficiency and technical feasibility. Mineral sequestration of CO_2 in the anaerobic digestion system was further investigated.

In **Chapter 1** the literature published on mineral CO_2 sequestration by biological processes is reviewed. The main advantages of using biological processes over chemical and physical methods are: (i) the biological processes can be operated without intensive process control not requiring elevated pressure and temperature as in physical/chemical methods, (ii) biological processes provide the possibility to use larger size silicate mineral particles compared with the chemical methods because they are typically conducted at lower volumetric rates. An overview is provided on different biological mechanisms that can improve the process kinetics of CO_2 mineral carbonation.

In **Chapter 2**, silicate-mineral based CO_2 sequestration using two-stage environmental biotechnological processes are evaluated. Four proposed biotechnological processes that enable CO_2 sequestration are: (i) anaerobic digestion (AD) (anaerobic fermentation and methanogenesis), (ii) biological nitrogen removal (nitrification and denitrification), (iii) flue gas desulfurization (hydrogen sulfide absorption and oxidation to elemental sulfur), (iv)

bioelectrochemical systems (BES) (anodic organic carbon oxidation and cathodic oxygen reduction). Whereas the acid-producing reaction in these two-stage biological systems, can be used to enhance the dissolution of alkaline silicates minerals, the subsequent alkalinity-producing step can precipitate the carbonate mineral. Wollastonite was chosen as the mineral to use in the experiments because it represents a commonly occurring natural calcium silicate with a simple structure.

Using these biotechnological processes for mineral CO_2 sequestration, the estimated sequestration costs can be reduced to less than half as compared with the physical/chemical methods (from 102 euro/ton CO_2-avoided to 40 euro/ton CO_2-avoided). This cost reduction is attributed to the possibility of applying larger size silicate mineral particles compared with the chemical methods and operation at environmental conditions rather than the elevated temperature and pressure.

The mineral carbonation yield (mole-CO_2/mole-substrate), integration complexity, and commercial added-values offered to each of the proposed systems as result of the integration are compared with one another. The anaerobic digestion process was selected for further research due to its worldwide application, higher CO_2 sequestration yield and additional added-values offered such as improved biogas quality and a self-regulating pH system.

To evaluate the process feasibility, the following key points for further research are identified: (i) improving the rate-controlling reactions of the mineral carbonation of CO_2 (i.e. silicate mineral dissolution and carbonate mineral precipitation) in a two-stage anaerobic digestion system, and (ii) investigating the possibility of achieving the suggested added-values to the AD system as a result of the integration process.

Chapters 3 and **4** research into the kinetics of wollastonite dissolution in the first-stage of the AD process is described, i.e. the anaerobic fermentation process. In **Chapter 3**, the individual effect of process variables such as the concentration and type of organic ligands, alcohols, EPS, pH (2-9), and wollastonite concentration (1-150 g/l) on the dissolution rate of wollastonite are studied by operating a series of chemical batch experiments.

In **Chapter 4**, the dissolution kinetics of wollastonite are investigated in a fed-batch anaerobic fermentation process. An experimental methodology was developed to identify the main factors that fermentative bacteria can have on the dissolution process including the change in ionic strength, production of alcohols, organic ligands and acidity. The method involved operating three vessels containing the same amount of wollastonite as follows: (i) a biotic experiment containing fermentative bacteria, organic ligands and protons, (ii) an abiotic experiment (control 1) with the same concentration profiles of organic acids and alcohols as in the biotic reactor, and (iii) a second abiotic control experiment with the same amount of acidity (by addition of hydrochloric acid) as in the biotic experiment. The results from these two chapters showed that the dissolution of wollastonite in an anaerobic fermentation process is mainly governed by the organic acids produced indicating that the dissolution behavior can be readily explained and may be predicted by the biochemical reactions involved.

Next in **Chapter 5,** the kinetics of calcium carbonate precipitation in the methanogenic phase and in a single-stage AD experiment were studied at 35°C and ambient pressure. It was shown that initial addition of calcite seed crystal can improve the kinetics of the precipitation process during the methanogenic phase. The precipitation of $CaCO_3$ in these methanogenic experiments with calcium acetate improved the biogas quality by increasing the methane

content to 71 % v/v as compared to that of the control experiment (calcium free environment), 53 %.

In the single-stage anaerobic digestion experiments operated at different concentrations of substrate, the poor separation of fermentation and methanogenic phases limited the amount of sequestered CO_2. However, integration of silicate minerals into these experiments resulted in a self-regulating pH system and potential production of biobased materials (chemical grouting material and biofertilizer).

In **Chapter 6** a simplified case was studied, in order to better understand the complicated interconnections among the carbonate components, organic acids, CO_2 mass transfer, and calcium concentration in an integrated AD process with silicate mineral. In this simplified case the integration of a highly soluble mineral ($CaCO_3$) in the anaerobic fermentation process was investigated rather than the slow-dissolving silicate minerals. For this study, a mathematical model was developed with a minimum set of kinetically controlled and equilibrium reactions that was able to reproduce the experimental data of a batch fermentation experiment using finely powdered $CaCO_3$. The calculated pH-pattern strongly resembled the measured pH, suggesting that the chosen set of kinetically controlled and equilibrium reactions were capable of describing the essential properties of the system. A detailed analysis of the reaction system with the aid of the model revealed that the system was most sensitive to four factors: (i) the mass transfer rate of CO_2 to the gas phase, (ii) the biological acid production rate, (iii) the partial pressure of CO_2, and (iv) the Ca^{2+} concentration in the solution. For process design purposes, the sensitivity towards individual variations of these factors on the pH were investigated using the model to a continuously stirred-tank reactor (CSTR) case.

A summary of the obtained results are presented in **Chapter 7**. A set of challenges that remained after this study and recommendations for future research are also presented in this chapter.

Overall, this thesis has proven that two-stage biotechnological processes can effectively enable silicate based mineral carbonation. Application of these biotechnological processes can potentially reduce the sequestration cost as compared with the chemical methods. However, the costs are still high compared with other CO_2 storage technologies and the CO_2 market prices. There is still potential for further cost reduction considering the offered added values to the biotechnological process as the result of integration. Therefore, a comprehensive cost-benefit analysis for each of the suggested biotechnological processes is still required to determine the sequestration cost. In addition, it is warranted to conduct research on CO_2 mineral carbonation process by biotechnological processes with a longer residence time such as in landfill sites (years) compared with the biotechnical processes in this study (days).

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Samenvatting

Het collectief nastreven van economische welvaart gebaseerd op fossiele energie heeft de concentratie van CO_2 in de atmosfeer zo sterk doen toenemen dat de mensheid momenteel voor een van de grootste uitdagingen op milieugebied staat; de wereldwijde verandering van het klimaat. Het mineraal sequestreren van CO_2 is een relatief nieuwe techniek om uitgestoten koolstofdioxide op te slaan. Deze technologie is gebaseerd op de natuurlijke verwering van silicaatmineralen die verantwoordelijk zijn voor het jaarlijks afvangen en opslaan van 100 miljoen ton koolstof (1,8 tot 2,0 % van de door mensen veroorzaakte uitstoot). De grote voordelen van deze methode ten opzichte van overig beschikbare methoden als ondergrondse opslag en opslag in oceanen is het permanente karakter en de inherent veilige toepassing. Verder biedt de enorme, wereldwijde reserve van silicaatmineralen een zeer groot opslagpotentieel. Voordat de techniek commercieel kan worden toegepast, is er een aantal uitdagingen te overwinnen met name in relatie tot het trage verloop van de reactie om CO_2 als carbonaat te binden. Het reactieproces kan fysisch en chemisch voldoende worden versneld, maar de hiervoor benodigde energie en de bijbehorende kosten zijn momenteel nog aanzienlijk hoger dan voor andere beschikbare opslagtechnieken. Om deze reden is er recent voorgesteld om biologische processen toe te passen die op een meer kosteneffectieve manier het proces van CO_2-opslag als carbonaten verbeteren. Het in dit proefschrift beschreven onderzoek is uitgevoerd binnen het zevende kaderprogramma voor onderzoek en technologische ontwikkeling (FP7) van de Europese Unie. Het team van wetenschappers dat betrokken was bij dit project heeft onderzoek verricht naar duurzame oplossingen voor de opslag van koolstofdioxide, waarbij biologische processen als aanvullende of alternatieve methoden werden bekeken voor bestaande opslagtechnieken.

Het **doel** van deze dissertatie was het onderzoeken van de mogelijkheden voor CO_2-opslag op basis van alkalische silicaatmineralen door toepassing van milieubiotechnologische twee-staps processen. De voorgestelde biotechnologische processen die minerale CO_2-opslag mogelijk maken zijn geanalyseerd op de volgende punten: methoden die de reactiesnelheid beïnvloeden, opslagefficiëntie en technische haalbaarheid. Met name de minerale opslag van CO_2 bij anaerobe vergisting is nader onderzocht.
In **hoofdstuk 1** wordt de literatuur besproken die is gepubliceerd over minerale CO_2-opslag door middel van biologische processen. De belangrijkste voordelen van het gebruik van biologische processen ten opzichte van fysische en chemische processen zijn: (i) biologische processen kunnen plaatsvinden zonder uiterst strenge controle van de reacties waarbij verhoogde drukken en/of temperaturen benodigd zijn, zoals het geval is bij fysische of chemische reacties, (ii) bij biologische processen kunnen grotere silicaatdeeltjes worden gebruikt dan bij chemische processen, omdat deze over het algemeen plaatsvinden bij lagere volumetrische conversiesnelheden. Er wordt een overzicht gegeven van verschillende biologische mechanismen die het reactieproces waarbij CO_2 als carbonaat wordt vastgelegd positief beïnvloeden.

Hoofdstuk 2 behandelt de opslag van CO_2 op basis van silicaatmineralen door toepassing van milieubiotechnologische twee-staps processen. Vier voorgestelde biotechnologische processen die de opslag van CO_2 mogelijk maken zijn: (i) anaerobe vergisting (anaerobe fermentatie en methanogenese), (ii) biologische stikstofverwijdering (nitrificatie en denitrificatie), (iii) ontzwavelen van rookgas (absorptie en oxidatie van waterstofsulfide tot elementaire zwavel), (iv) bio-electrochemische systemen (anodische oxidatie van organische koolstof en kathodische reductie van zuurstof). Terwijl de zuurproducerende reactie in deze biologische twee-staps-systemen gebruikt kan worden om het uiteenvallen van alkalische silicaatmineralen te versnellen, kunnen in de hieropvolgende alkalische reactie de carbonaatmineralen precipiteren. Het mineraal wollastoniet is gekozen als silicaatmineraal , omdat het een veelvoorkomend natuurlijk mineraal is met een eenvoudige structuur. Door gebruik te maken van deze biotechnologische processen voor minerale CO_2-opslag kunnen de kosten voor opslag meer dan gehalveerd worden in vergelijking met de fysische/chemische methoden (van 102 euro/ton vermeden uitstoot tot 40 euro/ton vermeden uitstoot). Deze kostenbesparing wordt enerzijds toegeschreven aan de mogelijkheid tot het toepassen van een groter formaat minerale silicaatdeeltjes in vergelijking met de chemische methoden en anderzijds aan het plaatsvinden van de reacties bij standaard omgevingsomstandigheden in plaats van bij een verhoogde temperatuur en druk. Het rendement van het mineraal carboniseren (mol-CO_2/mol-substraat), de complexiteit van de integratie en de potentiele commerciële meerwaarde als gevolg van de integratie van elk van de voorgestelde systemen zijn met elkaar vergeleken. Het anaerobe vergistingsproces werd geselecteerd voor verder onderzoek vanwege de wereldwijde toepassing ervan, een hoger CO_2-opslagrendement en de extra meerwaarden, zoals verbeterde biogaskwaliteit en een zelfregulerend pH-systeem.
Om de haalbaarheid van het proces te evalueren, werden de volgende belangrijke punten voor verder onderzoek geïdentificeerd: (i) verbetering van de snelheidsbepalende reacties van het mineraal carboniseren van CO_2 (i.e., het oplossen van silicaatmineralen en de precipitatie van carbonaatmineralen) in een twee-staps anaeroob vergistingssysteem, (ii) de mogelijkheid tot het daadwerkelijk behalen van de commerciële meerwaarde van het anaeroob vergistingssysteem als gevolg van het integratieproces.
In **hoofdstuk 3** en **hoofdstuk 4** wordt het onderzoek naar het oplossingsmechanisme van wollastoniet in de eerste fase van het anaerobe vergistingsproces beschreven, te weten: de verzuringsreactie. In **hoofdstuk 3** wordt het individuele effect van procesvariabelen op de ontbindingsnelheid/oplossnelheid van wollastoniet bestudeerd door de resultaten van een serie van chemische batch-experimenten te beschrijven. Voorbeelden van relevante variabelen zijn: de concentratie en het type van organische liganden, alcoholen, EPS, pH (2-9) en wollastonietconcentratie (1-150 g/l).
In **hoofdstuk 4** wordt het ontbindingsmechanisme van wollastoniet onderzocht in een fed-batch anaeroob verzuringsproces. Een experimentele methodologie werd ontwikkeld om de belangrijkste factoren te identificeren die fermentatieve bacteriën hebben voor het oplossingsproces, waaronder de verandering in ionische sterkte, de productie van vetzuren, organische liganden en zuurgraad. Drie proceswijzes zijn met elkaar vergeleken: (i) een biotische experiment met fermentatieve bacteriën, en vorming van organische zuren en protonen, (ii) een abiotisch experiment (controle 1) waarbij dezelfde concentraties van organische zuren en alcoholen zijn gedoseerd in de tijd als gevormd in de biotische reactor, en

(iii) een tweede abiotisch experiment (controle 2) met dezelfde pH ingesteld door zoutzuur additie. De resultaten uit deze twee hoofdstukken laten zien dat de ontbinding van wollastoniet in een anaeroob fermentatieproces voornamelijk wordt bepaald door de organisch geproduceerde zuren. Het ontbindingsgedrag kan eenvoudig worden verklaard en voorspeld door de betreffende biochemische reacties.

In **hoofdstuk 5** wordt het reactiemechanisme beschreven van de precipitatie van calciumcarbonaat in de methanogene fase en in een eenstaps anaeroob vergistingsproces bij een temperatuur van 35 °C en omgevingsdruk. Er werd aangetoond dat de toevoeging van calciet kiemkristallen de precipitatiereactie tijdens de methanogene fase kan verbeteren. De precipitatie van calciumcarbonaat in deze methanogene experimenten met calciumacetaat verbeterde de biogaskwaliteit door verhoging van het methaangehalte tot 71% v/v in vergelijking met de gevonden waarde tijdens het controle-experiment (calciumvrije omgeving); 53%.

Bij de eenstaps anaerobe vergistingsexperimenten, uitgevoerd met verschillende concentraties substraat, werd de hoeveelheid opgeslagen CO_2 gelimiteerd door de slechte scheiding van fermentatie en methanogene fasen. Het toevoegen van silicaatmineralen bij deze experimenten resulteerde echter in een zelfregulerend pH-systeem en de potentiële productie van resources (biologsich cement en meststoffen).

In **hoofdstuk 6** wordt een vereenvoudigde opzet gebruikt om beter inzicht te krijgen in de gecompliceerde onderlinge samenhang tussen de carbonzuurevenwichten, organische zuren, de CO_2-massa-overdracht en de calciumconcentratie in een geïntegreerd anaeroob vergistingsproces met silicaatmineralen. In dit vereenvoudigde geval is een redelijk goed oplosbaar mineraal (CaCO3) in het anaerobe vergistingproces onderzocht in plaats van de traag oplossende silicaatmineralen. Voor dit onderzoek werd een wiskundig model ontwikkeld om de experimentele data van een batch fermentatie-experiment met fijngemalen $CaCO_3$ te beschrijven met een minimale set van kinetisch gecontroleerde reacties en evenwichtsreacties. Het berekende pH-patroon vertoont sterke overeenkomst met de gemeten pH-waarden, wat suggereert dat de gekozen set van kinetisch gecontroleerde reacties en evenwichtsreacties in staat is om de essentiële eigenschappen van het systeem te kunnen beschrijven.

Een gedetailleerde analyse van het reactiesysteem met behulp van het model toont aan dat het systeem zeer gevoelig is voor vier factoren: de massaoverdracht van CO_2 naar de gasfase, de biologische zuurproductiesnelheid, de partiële druk van CO_2 en de concentratie van Ca^{2+} in de oplossing. Vanuit het oogpunt van procesontwerp werd de gevoeligheid voor individuele variaties van deze factoren op de pH onderzocht, waarbij het model is toegepast op een situatie met een continu geroerde tankreactor (CSTR).

Een samenvatting van de verkregen resultaten wordt gepresenteerd in **hoofdstuk 7**. Een reeks uitdagingen die na dit onderzoek overblijven en aanbevelingen voor toekomstig onderzoek worden ook in dit hoofdstuk gepresenteerd.

Over het algemeen heeft dit onderzoeklaten zien dat minerale CO2 sequestratie op basis van silicaatminaralen effectief mogelijk is door middel van twee-staps biotechnologische processen. Toepassing van deze biotechnologische processen kan in potentie de kosten voor opslag reduceren ten opzichte van de chemische methoden. De kosten zijn echter nog steeds hoog in vergelijking met andere technologieën voor CO_2-opslag en de CO_2-marktprijzen. Er

zijn zeker mogelijkheden voor verdere kostenreductie als er gekeken wordt naar de eventuele toegevoegde waarde voor het biotechnologisch proces als gevolg van integratie (o.a. hogere kwaliteit biogas). Een uitgebreide kosten-baten analyse voor elk van de voorgestelde biotechnologische processen is daarom nog steeds vereist om de kosten voor opslag te bepalen. Hiernaast is het aanbevolen om onderzoek te verrichten naar de integratie van minerale carbonisatieprocessen in biotechnologische processen met een langere tijdsspanne zoals in stortplaatsen (jaren), naast de tijdsspanne beschreven in deze studie (dagen).

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Chapter 1

General Introduction

1.1 Global carbon cycle and climate change

Human activity has increased the atmospheric concentration of CO_2 from 280 ppm to 360 ppm from pre-industrial to present day, resulting in many environmental and economic disadvantages (Houghton et al., 2001b). This increase of CO_2 level in the atmosphere exceeds the natural variability of the past 1000 years (Crowley, 2000). The main reason for the observed drastic increase of CO_2 levels is due to the consumption of fossil fuels (Houghton et al., 2001a). Global warming, one of the main consequences of a rising CO_2 concentration in the atmosphere, has negatively affected the natural and social systems during the last decades leading to the rise of sea levels, ocean acidification, and in some cases economic losses through the decrease in crop production (Adger et al., 2001).

Building resilience into both human and ecological systems through the use of adaptive management systems has been recently developed as a way to cope with rapid environmental changes caused by global warming (Tompkins & Adger, 2004). It is logical that, to mitigate the global warming, either the production of CO_2 emissions should be reduced and/or effective CO_2 sequestration methods should be implemented. Due to the current dependency of society on carbon-based fuels and the relatively small contribution of alternative energy sources (renewable energy contributed 19 % to the global energy consumption in 2012), it is unlikely that the efforts to decrease green-house gases will be sufficient in the near future to maintain the atmospheric CO_2 concentrations (Broecker, 2007; REN21, 2014). Consequently, it is essential to develop and implement methods for the effective capture and sequestration of CO_2. Currently, the most widely applied mitigation strategies are: geological sequestration in depleted oil and gas reservoirs, ocean storage and terrestrial sequestration as illustrated in Fig. 1.1. However, lack of economic profit, inadequate regulations, and implementation complications, has resulted in limited implementation of many of the proposed mitigation solutions on industrial scale (Herzog, 2001) .

To overcome these challenges, alternative mitigation methods have emerged which mimic the existing natural CO_2 sequestration mechanisms (natural negative feedback mechanisms). A closer look at the global carbon cycle among the reservoirs (e.g. atmosphere, ocean, sediments, geological reservoirs, and biosphere) indicates that from 213 gigatons of carbon (GtC) entering the atmosphere every year, 5.4 GtC has an anthropogenic origin (2.5-3 % of carbon) while the rest are as a result of natural processes such as ocean's and volcanoe's outgassing. Almost all of the carbon entering the atmosphere, is returned to the lithosphere and hydrosphere through the natural negative feedback mechanisms of oceanic and terrestrial uptake, and mineral carbonation (formation of carbonate minerals by weathering of silicate minerals). These natural mechanisms stabilize the carbon levels in the atmosphere providing Earth as a suitable place for humans to live. This implies that the Earth system is dealing with a much larger amount of carbon as compared to the anthropogenic carbon emissions. Therefore, a minor increase in the uptake rate of one of these natural carbon sinks can potentially compensate for the extra anthropogenic CO_2 emissions.

This mindset has led to solutions that are intended to enhance the natural negative feedback mechanisms. Examples of such approaches are: reforestation and distributing finely powdered alkaline silicate minerals over land areas to enhance the biological fixation process and the mineral carbonation (weathering of silicate minerals), respectively (Schuiling & Krijgsman, 2006; Silver et al., 2000). Another similar solution is to increase the capacity of oceanic CO_2 uptake by addition of alkalinity to oceans in order to shift the carbonate equilibrium towards the carbonate ion (Sloan, 2003; Takahashi et al., 1981). The environmental impacts of these solutions on the ecosystems is the most significant factor determining their applicability, which are commonly evaluated by model-based analysis (Koehler et al., 2010).

Among the natural sequestration mechanism, the ocean and terrestrial sequestration have been studied extensively during the last few decades, while application of mineral carbonation as a CO_2 sequestration method, is relatively a newer topic.

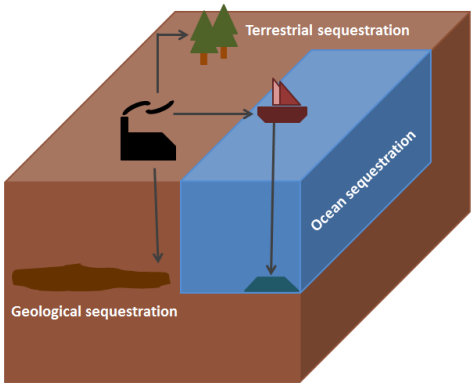

Figure 1.1 The main carbon sequestration options: terrestrial, ocean and geological.

1.2 Mineral CO_2 sequestration

Sequestration of CO_2 by mineral carbonation of CO_2 is based on the natural weathering process of silicate minerals. This process has been responsible for reducing the CO_2 concentration over the geological time scales (Brady, 1991). It is the reaction between saturated rainwater with dissolved CO_2 and alkaline silicate minerals that forms carbonate minerals and thereby reduce the atmospheric CO_2 concentration. Examples of the CO_2 interaction with two common silicate minerals, wollastonite ($CaSiO_3$) and olivine ($(Fe, Mg)_2SiO_4$) are shown in the exothermic reactions of 1.1 and 1.2.

$CaSiO_3$ (s) (wollastonite) + CO_2 (g) → $CaCO_3$ (s) + SiO_2 (s) (ΔHr = -87 kJ/mol) (reaction 1.1)

Mg_2SiO_4 (s) (olivine) + 2 CO_2 (g) → 2 $MgCO_3$ (s) + SiO_2 (s) (ΔHr = -90 kJ/mol) (reaction 1.2)

The fact that 42% wt/wt of the total carbon in the lithosphere of the earth is stored in the form of carbonate minerals shows the high capacity and stability of the mineral carbon storage (Falkowski et al., 2000). Additional advantages of the process as compared to other available carbon dioxide storage options (e.g. geological storage and ocean storage) are the inherently safe nature of the process and high sequestration capacity based on the existing resources of silicate minerals worldwide (Lackner et al., 1995). The main challenge in applying the mineral carbonation process, however, is the slow kinetics of the process. If the kinetics of mineral carbonation process can be enhanced, this natural process can be industrially applied as a CO_2 sequestration option.

As classified in a literature review performed by Huijgen et al, (2005), the mineral carbonation of CO_2 can be performed through different routes: (i) direct routes in which the carbonation process is occurring directly on the silicate mineral in a single step process (O'Connor et al., 2000), and (ii) indirect routes in which the divalent cations (such as Ca and Mg) are initially leached out of the silicate mineral and then carbonated in a separate step (Huijgen et al., 2003; Park, 2005). While the direct route (single step) is operated in form of gas-solid or aqueous environments, the indirect (two-step) carbonation of Mg/Ca route is performed in aqueous environments. In a single process step (direct route) the carbonation of silicate minerals proceeds very slowly. Therefore, many researchers have performed experiments at elevated temperature and CO_2 pressure (e.g. 180 °C and 150 bar) in order to improve the reaction rates (Jonckbloedt, 1998; O'Connor et al., 2000). Operating under these environmental conditions can result in a reaction time in the order of hours (O'Connor et al., 2000). However, the drawback of the single step route remains to be that of the optimal environmental conditions (e.g. temperature and pH) of the precipitation reaction counteracts with the dissolution reaction. As an example, while the dissolution rate of silicate minerals increases at higher temperatures, the solubility of CO_2 in the solution decreases. Therefore, performing the process in two steps results in having comparative conversion rates at relatively milder conditions such as 70 °C and 1 bar (Huijgen et al., 2003). Under standard conditions the rate determining reaction in a two-step aqueous route is leaching of divalent cations from silicate minerals (Huijgen et al., 2003). Therefore, to obtain substantial CO_2 sequestration by the mineral carbonation process, the problem of slow release of divalent cations from alkaline silicate minerals should be overcome. This is why many studies have been devoted to understand the dissolution mechanism of silicate minerals in order to find out the best environmental conditions to obtain higher dissolution rates (Golubev et al., 2005; Murphy & Helgeson, 1987; Schott et al., 2012; Weissbart & Rimstidt, 2000; Wogelius & Walther, 1991). Various options have been proposed with the aim of improving the dissolution kinetics of silicate minerals. Heat activation, size reduction, addition of chemicals,

and surface activation are the (pre)treatments that have been shown to be able to increase the dissolution rate. Most of these treatments increase the dissolution rate through increasing the reactive sites by extending the surface area of the mineral.

A simple process lay-out of chemical mineral CO_2 sequestration is shown in Figure 1.2. As demonstrated in the figure, ex-situ mineral carbonation of CO_2 consists of several key steps as follows: (i) feed-stock preparation, (ii) CO_2 capture and sequestration, and (iii) by-product disposal. A major aspect to be considered, is the associated costs and intensive energy needs of the feed-stock processing: mining, transportation, grinding and pre-treatment.

In the following paragraph the associated cost of each step (expect the mining step) is shortly described. With respect to the transportation, large amount of materials has to be processed within a mineral carbonation process which can result in high costs, for example, for each ton of CO_2 avoided, two tons of wollastonite is required to be processed (this number is 1.8 and 2.6 ton-mineral/ton-CO_2 for olivine and serpentine, respectively). In order to avoid the transport of large quantities of rock, transportation of the carbon dioxide by pipeline or direct capture of CO_2 from the atmosphere is preferable. In these scenarios, the sequestration facilities should be placed close to the mining sites (Huijgen et al., 2007). Grinding of minerals has been reported to consume the largest energy among the mineral processing processes (Gerdemann et al., 2007). Figure 1.3 shows the grinding energy required for size reduction for the case of wollastonite particles (Gerdemann et al., 2007) using a ball mill and stirred media detritor (SMD). It can be observed that reducing the mineral size from 35 μm to 15 μm can greatly influence the process costs by doubling the energy consumption. The energy consumption of the pre-treatment process is dependent on the nature of the process (chemical, physical or biological). The physical pre-treatments are usually rather energy intensive, as an example, 200-300 kWh per ton serpentine ($Mg_3Si_2O_5(OH)_4$) is required to perform thermal activation at 600-650 °C (O'Connor et al., 2000).

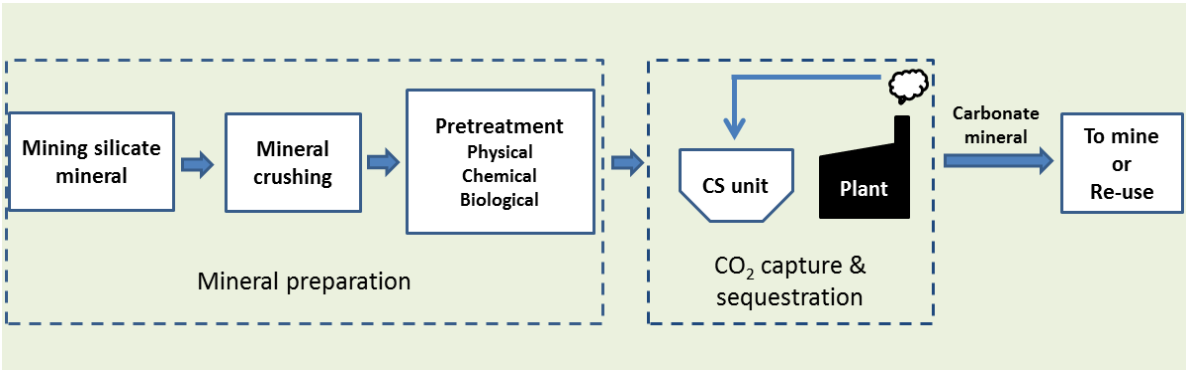

Figure 1.2 Schematic drawing of a mineral CO_2 sequestration process. CS stands for carbon sequestration.

In order for the mineral CO_2 sequesteation to be considered as one of the sequestration options, it is essential to be economically competitive at industrial scales with the other available sequestration methods. Using the current technologies, the sequestration cost is in the range of 57 and 250 euro/ton carbon-sequestered depending on the silicate mineral used (Gerdemann et al., 2007; Huijgen et al., 2007)[1]. This is while the reported cost for geological storage is in the range of 100-190 euro/ton carbon-stored (using a conversion rate of 1.3 dollor = 1 euro) (Anderson & Newell, 2004; Metz et al., 2005). Thus, to achieve a more competitive price further research and development on carbon sequestration by mineral carbonation is required.

The high cost of feedstock processing has led to the search for alternative feed-stock sources such as industrial residues. Examples are slag (steel slag and coal slag), ash (coal fly ash and municipal solid waste incinerator fly ash) and construction wastes (cement and concrete) (Huijgen & Comans, 2005; Renforth et al., 2011). These solid residues contain the divalent cation and alkalinity required for mineral carbonation of CO_2. A study performed by Huijgen et al., 2007 showed that the sequestration cost could be reduced from 102 to 77 euro/ton CO_2-avoided, by using steel slag instead of wollastonite. Recently, the application of biological processes as a cost-efficient method for enhancing the dissolution of silicates and precipitation of carbonates have also been investigated (Bennett et al., 2001; Pokrovsky et al., 2009; Ullman et al., 1996; Uroz et al., 2009; Welch & Ullman, 1993). The following section (1.3) elaborates on the application of biological processes for the mineral carbonation of CO_2.

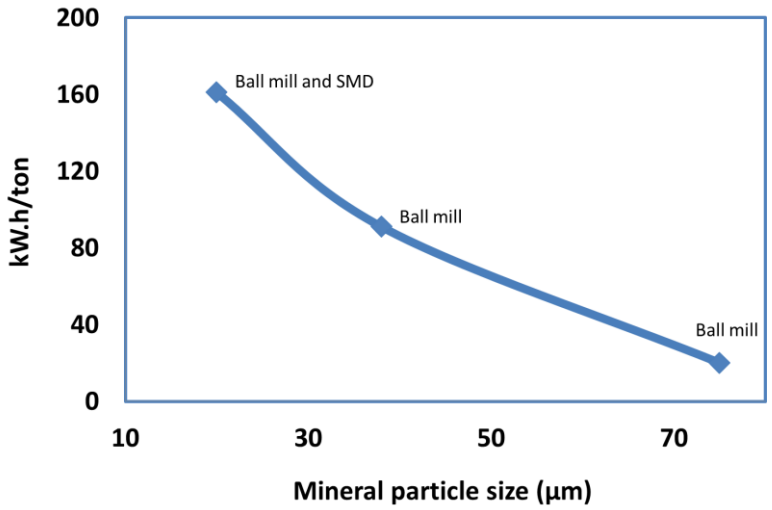

Figure 1.3 The grinding energy required for size reduction of wollastonite particles. Adapted from (Gerdemann et al., 2007). This energy does not include the energy spent for mineral crushing. SMD stands for stirred media detritor.

1.3 Background to this thesis

1.3.1 Microbial mediated CO_2 mineral carbonation

A variety of microorganisms can change the environmental conditions in a manner that favors the mineral carbonation processes. These microorganisms include bacteria, archaea, fungi and algae. Application of the microbial processes can enhance the mineral carbonation rates in a more cost-efficient manner as compared to that of chemical and physical methods. Alterations of pH, ionic strength, production of exometabolites such as organic ligands as a result of the microbial activities are among the main factors that can influence the reactions of mineral carbonation process, i.e. silicate mineral dissolution and carbonate mineral precipitation. The focus of this section is on the role of bacteria on mineral carbonation of CO_2.

The role of bacteria in carbonate mineral precipitation is well-known based on the evidence of microbes in rock exhibits in naturally formed carbonate platforms in oceans, lakes and caves (Aloisi, 2008). In these environments, the activity of alkalinity-producing microbes such as phototrophic microbes (e.g. cyanobacteria) induces the precipitation of carbonate. Other alkalinity-producing bacteria such as methanogenesis, denitrifying, and sulfide oxidizing bacteria can have a similar stimulating role on carbonate mineral precipitation (Castanier et al., 1999). In addition to modifications of the medium, it has also been indicated that cell walls and extracellular polysaccharides produced by microbes such as iron-reducing, sulfate reducing and methanogenic bacteria can act as nucleation sites for carbonate crystallization inducing the precipitation (Van Lith et al., 2003).

The effect of bacteria on silicate mineral dissolution is less studied compared with the microbial mediated carbonate mineral formation. Increase of dissolution rate of silicate minerals by bacteria can particularly be important because it is known that under standard conditions, the rate-determining reaction step of mineral carbonation process is the leaching of divalent cations (e.g. Mg and Ca) from the silicate minerals.

Fig. 1.4 gives a schematic representation of different mechanisms for the microbially enhanced dissolution of a silicate mineral (wollastonite). The improvement of the dissolution rate of silicate minerals by microbially produced organic acids is mainly occurring due to the exchange of hydrogen ion for the cation ion the lattice (Fig. 1.4a) (Helgeson et al., 1984; White & Brantley, 1995). Other mechanisms that have been reported to accelerate the leaching rate of cations is adsorption and complexation of organic ligands with reactive sites on the mineral surface (Fig. 1.4b) or the free cations in the aqueous solution (Fig. 4c) (Pokrovsky et al., 2009; Ullman et al., 1996). In addition to the illustrated mechanisms, direct bacterial effects such as biofilm growth can also potentially improve the dissolution process via chelation by metabolites or oxidation-reduction reactions (Bennett et al., 2001; Castanier et al., 1999; Frey et al., 2010; Rogers & Bennett, 2004; Uroz et al., 2009). Assessing the exact role of bacteria on mineral dissolution is a challenging task because environmental factors that are altered by microbial activities and mineral dissolution can influence one another simultaneously (Sand et al., 2001; Vandevivere et al., 1994). One of the motivations for the

current thesis is to develop a method that can differentiate between the chemical and biological effect of microbial activity.

Application of biological processes for mineral carbonation can potentially reduce the operation costs for two main reasons. First, the biological processes can be operated without intensive process control in relatively simple reactors as compared to reactors operated at elevated pressure and temperature. Secondly, biological operation provides the possibility to use larger size silicate minerals compared to chemical methods (i.e., work at lower volumetric rates) for mineral carbonation of CO_2. Another aim of the present study is to investigate the feasibility of using microbial processes for mineral carbonation process.

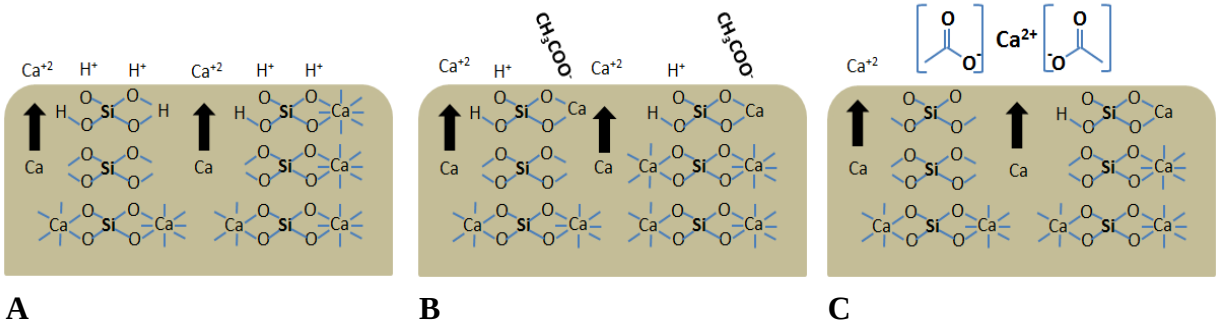

Figure 1.4 Schematic diagram of the mechanims that can enhance the dissolution rate of the wollasotnite. (A) Exchange of proton for the cation in the lattice, (B) adsorption of acetate to calcium site on the wollastonite surface, and (C) complexation of acetate with the Ca^{2+} ion in the solution.

1.4 Aim and overview of this thesis

This study introduces and evaluates mineral carbonation of CO_2 by two-stage environmental biotechnological processes as a potentially cost-efficient option for CO_2 sequestration. The kinetics of the main rate-controlling reactions (silicate mineral dissolution and carbonate mineral precipitation) in the anaerobic digestion system, as one the proposed two-stage biotechnological processes, are experimentally studied and possibilities for improving these reactions are determined. Fig. 1.5 presents an overall schematic of the present thesis.

1.5 The outline of this thesis

Chapter 2 introduces four (two-stage) environmental biotechnological processes that enable mineral carbonation process. The CO_2 sequestration potentials of these four biological technologies are quantitatively analyzed and the relevant factors for process optimization are discussed. The sequestration efficiencies of these options are compared to available (chemical)

sequestration options. Main reactions involved in each of the proposed biotechnological system are given and the potential rate-limiting reactions are pointed-out. Anaerobic digestion process[1] is chosen as the selected process for further research in the present thesis because of its broader technology applicability and CO_2 sequestration yield (mole-CO_2/mole-substrate). As for the silicate mineral, wollastonite ($CaSiO_3$) was selected as the silicate mineral for further research, because it represents a commonly occurring natural calcium silicate with a simple structure. At the end of the chapter, the process cost of the proposed biological route and existing chemical mineral carbonation of CO_2 are compared with one another.

Chapter 3 and 4 are devoted to study the chemical and biological effects of anaerobic fermentation (the first stage of the anaerobic digestion process) on dissolution kinetics of wollastonite. In Chapters 3 the individual effects of the chemical properties of a fermentation process on the kinetics of wollastonite dissolution is determined. Herewith, the influence of pH, wollastonite concentration, organic and inorganic ligands (present in a typical fermentation process) on kinetics of wollastonite dissolution is studied by conducting a series of chemical batch experiments.

The biological effects of the anaerobic fermentation process on wollastonite dissolution rate are characterized in chapter 4. A methodology is described for distinguishing the biological and chemical impacts of fermentative bacteria on wollastonite dissolution kinetics. Using this procedure, the main factors governing the dissolution process of wollastonite in the fermentation process are determined and methods to enhance the dissolution rate in a fermentation process are addressed.

Chapter 5 studies kinetics of $CaCO_3$ precipitation in the methanogenic phase and in a single-stage anaerobic digestion process (including hydrolysis, fermentation and methanogenesis processes). The key reactions controlling the overall efficiency of mineral CO_2 sequestration process by anaerobic digestion are addressed and evaluated. Among them, the effect of calcium carbonate precipitation is experimentally examined. Possibilities for improvement of the precipitation process are identified and their effect on achieving a higher CO_2 sequestration, a higher quality of biogas and production of bio-based products is experimentally shown.

Chapter 6 is dedicated to determine the reactions controlling the pH of fermentation process neutralized by finely powdered calcium carbonate mineral. This chapter shows the possiblities for commercial application of $CaCO_3$ as a cost-effective neutralizing agent. This is done by a better control of the pH via varying the identified parameters such as sparging rate and reactors loading rate. A numerical model is made to analyze the dominant processes that control the pH of a batch fermentation process neutralized by calcium carbonate.

[1] Anaerobic digestion is a collection of processes (hydrolysis, fermentation and methanogenesis) in which bacteria break down degradable material in the absence of oxygen.

Finally, in Chapter 7 the final conclusions on the feasibility of mineral CO_2 sequestration by environmental biotechnological processes are drawn and recommendations for further research are given.

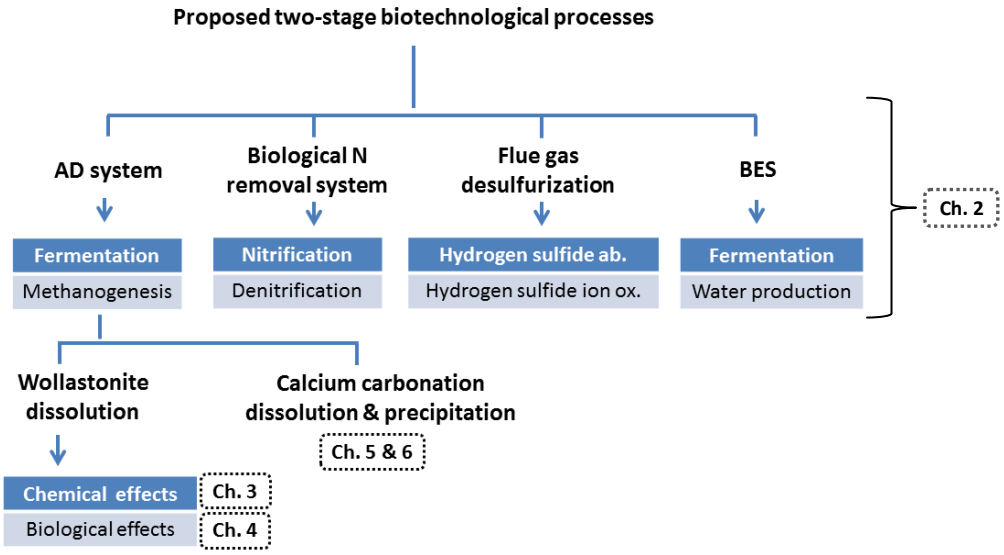

Figure 1.5 An overall schematic of the present thesis. Ch., ab, ox., stand for chapter, absorption, and oxidation, respectively.

Chapter 2

Mineral CO_2 sequestration by environmental biotechnological process

Trends in Biotechnology, (2013) 31(3): 139-146

2.1 Abstract

CO_2 sequestration may be an avenue to mitigate climate change. CO_2 sequestration by mineral carbonation can be achieved by the reaction of CO_2 with alkaline silicates. Here, we evaluate how alkaline silicate mineral-based CO_2 sequestration can be achieved using environmental biotechnological processes. Several biotechnological processes rely on a sequence of (i) an acid-producing reaction such as nitrification and anaerobic fermentation and (ii) an alkalinity-producing reaction such as denitrification and methanogenesis. Whereas the acid-producing reaction can be used to enhance the dissolution of e.g. alkaline calcium silicates, the subsequent alkalinity-producing step can precipitate $CaCO_3$. We quantitatively evaluate the potential of these processes for CO_2 sequestration and propose that optimization of these processes could contribute to climate change mitigation strategies.

2.2 Introduction

2.2.1 Stabilization of atmospheric CO_2 levels by natural mechanisms

CO_2 accumulation in the atmosphere has been suggested to result in global climate change which along with disturbance of the nitrogen cycle and biodiversity loss have been identified as the main environmental challenges facing humanity today (Rockstrom et al., 2009). A variety of strategies have been proposed to mitigate climate change by reducing the atmospheric CO_2 concentration. A closer look at the global carbon cycle shows that from the total amount of carbon entering the atmosphere every year (213 GtC), only 2.5-3 % has an anthropogenic origin (5.4 GtC). The remainder of the carbon is a result of natural activities (Fig. 2.1) (Bowen, 1979; Ehrlich, 2002; Falkowski et al., 2000; Houghton et al., 2001b). Most of the carbon released is returned to the lithosphere and hydrosphere by several natural mechanisms, stabilizing the atmospheric CO_2 concentrations over the geological time-scales. This implies that only a minor increase in the uptake rate of one of these natural mechanisms can compensate for the extra anthropogenic CO_2 emissions. Mineral carbonation of CO_2 (often referred to as mineral CO_2 sequestration) is one of the natural negative feedback mechanisms. It reduces the atmospheric CO_2 concentration by CO_2 reaction with alkaline silicate minerals to form carbonate minerals (Seifritz, 1990). The durability of this process is evident from the distribution of carbon in the lithosphere of the Earth (Fig. 2.1) where approximately half of the total carbon (42% wt/wt) is in the form of limestone ($CaCO_3$) and other kind of carbonates [4]. Although, a portion of the limestone has metamorphic and igneous origins, the large sedimentary portion of these carbonate rocks shows that mineral CO_2 sequestration is a geologically stable process with a high potential for CO_2 sequestration (Holland, 1978; Walker et al., 1981b). The effectiveness of the process for mitigation purposes has however been limited due to the slow kinetics of the CO_2-silicate reactions (Oelkers et al., 2008). The idea of distributing finely powdered alkaline silicate minerals over land areas as a geo-engineering solution is to enhance the efficiency of mineral CO_2 sequestration by increasing the reactive surface area (Schuiling & Krijgsman, 2006). Model based analysis demonstrated that applying this technique can raise the pH of the rivers, which

in response negatively influences the natural environment and decreases the mineral dissolution rates in the long-run (Koehler et al., 2010). Besides using natural ecosystems, specific man-made ecosystems could be particularly suitable for implementing the mineral CO_2 sequestration strategy. Examples of such systems are environmental biotechnological processes such as wastewater treatment facilities that are essentially enhanced natural processes. Here we introduce a new application of various biotechnological processes (i.e. wastewater, waste, and gas treatment facilities) for mineral CO_2 sequestration.

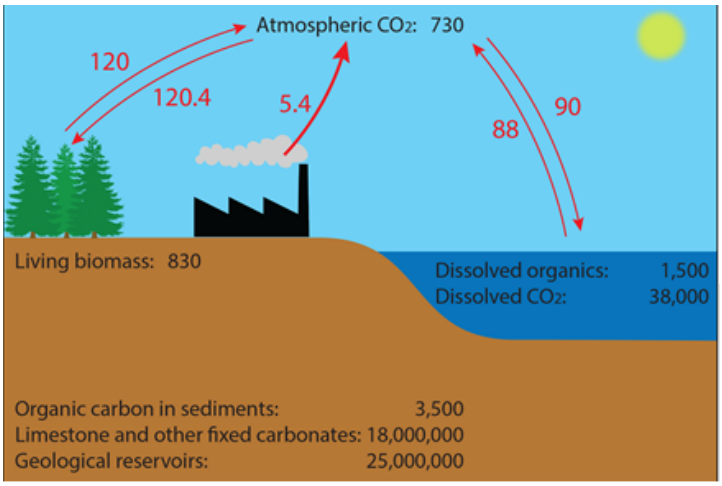

Figure 2.1 Global carbon reservoirs and fluxes (Bowen, 1979; Ehrlich, 2002; Houghton et al., 2001b). Reservoirs are in GtC and fluxes are in GtC/yr.

2.2.2 Challenges for application of mineral CO_2 sequestration

Mineral CO_2 sequestration is a process where atmospheric CO_2 is fixed in the form of carbonates. In order for carbonate ions to precipitate as carbonate minerals, a suitable counter ion should be present. The most common carbonate minerals on Earth contain Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, or Sr^{2+} as counter ion (Deer et al., 1992). These divalent cations, in addition to the alkalinity required for CO_2 conversion to carbonate ions can be considered as the main raw-materials for the mineral CO_2 sequestration process (reaction 2.1). Alkaline silicate minerals such as wollastonite can potentially provide the divalent cation and alkalinity needed for the capture and sequestration of CO_2 at ambient environmental conditions (reaction 2.2) (Daval et al., 2009). There are far more than sufficient alkaline silicate materials available to sequester the equivalent CO_2 of the total known amount of fossil fuels (Graves et al., 2006; Kelemen & Matter, 2008; Lackner et al., 1995). However, the slow release rate of divalent cations from these minerals under neutral and alkaline pH conditions, the same pH at which the carbonate ion (CO_3^{2-}) can form from CO_2 in water, is one the main reasons of limited application of the mineral carbonation process, up to now (Brantley et al., 2003; Lackner, 2003). Therefore, obtaining divalent cations and alkalinity at a high rate is considered among the main challenges for mineral CO_2 sequestration process (Huijgen et al., 2003; Renforth et al., 2011).

$$(Ca/Mg)^{2+}(aq) + CO_2 + 2OH^- \rightarrow (Ca/Mg)CO_3(s) + H_2O(aq) \quad \text{(reaction 2.1)}$$

$$CaSiO_3 + CO_2 \rightarrow CaCO_3 + SiO_2 \qquad \Delta G_r\,(kJ/mol) = -37,\ \Delta H_r\,(kJ/mol) = -87 \quad \text{(reaction 2.2)}$$

2.2.2.1 Enhancing divalent cation and alkalinity release rate of alkaline silicates

To obtain substantial CO_2 sequestration by mineral carbonation, the problem of slow release of alkalinity and divalent cations from alkaline silicate minerals should be overcome. This can be accomplished by introducing chemical compounds such as specific complexing agents or acids to a solution in which alkaline silicates particles are suspended (Kakizawa et al., 2001; Park et al., 2003; Ptáček et al., 2010). Another possibility is to activate the mineral reactants by thermal and mechanical means i.e. increasing the surface area (Maroto-Valer et al., 2005). However, the high costs and intensive energy needs associated with such chemical or physical treatments of silicate minerals have been mentioned as the main drawbacks for application of these methods (Gerdemann et al., 2007; Huijgen et al., 2003; Renforth et al., 2011; Sipilä et al., 2008). Therefore, recently, the application of biological processes as a more cost-efficient method for enhancing the dissolution of alkaline silicates has been investigated (Bennett et al., 2001; Pokrovsky et al., 2009; Rawlings et al., 2003; Ullman et al., 1996; Welch & Ullman, 1993; Wogelius & Walther, 1991). Microbial processes can primarily increase the dissolution rate of silicate minerals by modifying the environmental conditions such as pH reduction by e.g. production of organic acids (Ullman et al., 1996; Uroz et al., 2009). Nitrification and carbohydrate fermentation to volatile fatty acids (VFA) are examples of acid-producing microbial processes that are widely applied in the field of environmental biotechnology. Hence, integration of alkaline silicate minerals into these processes can potentially enhance their dissolution rate (Salek et al., 2013c). The increase of dissolution rate of alkaline silicates can provide the divalent cations needed for the carbonate precipitation. However, the acidity produced by the biological process consumes the alkalinity which is required for carbonate mineral formation (reaction 2.1). Therefore, the process should be combined with a process that generates alkalinity in order to enable carbonate mineralization.

2.2.2.2 Alkalinity source for conversion of CO_2 to carbonate ions

Mineral sequestration of CO_2 requires alkalinity to form carbonate ion (CO_3^{2-}) from the CO_2 gas (reaction 2.1). Various sources of alkalinity have been suggested for CO_2 sequestration purposes. For example, alkaline solid residues from different industries such as steel slag (Huijgen & Comans, 2005; Kelly et al., 2011), cement kiln dust (Huntzinger et al., 2009), and fly ashes (Back et al., 2008; Costa et al., 2007), have been identified as alkalinity sources. The quantity and distribution of these alkaline solid wastes is however limited on a global scale (Huijgen & Comans, 2005). The large deposits of carbonate sediments found in lakes and seas

are a result of various alkalinity producing metabolic processes such as denitrification, methane production, and sulfate reduction (Riding, 2000). Such microbial processes also occur in widely applied environmental biotechnologies such as biological nitrogen removal and anaerobic digestion. In these processes, degradation of organic carbon by denitrifiers and methanogenic bacteria result in a pH increase which stimulate the carbonation of the liquid. Once the divalent cations are provided (e.g. by dissolution of alkaline silicates in an acid-producing process) to the alkaline-producing processes, mineral carbonation of CO_2 can take place.

2.3 Integration of mineral CO_2 sequestration into two-stage biotechnological processes

There are a number of biotechnological processes which involve a sequence of an acid and an alkalinity producing step. Such reaction sequences enable mineral CO_2 sequestration by silicate dissolution and carbonate formation processes. While enhancement of alkaline mineral dissolution can be obtained in the first acid-producing stage, the second alkali-producing stage yields carbonate formation (Fig. 2.2).

Biological waste/wastewater technologies that are characterized by a sequence of an acid- and an alkalinity- producing steps are typically conducted in a single stage reactor in order to prevent large pH variations. Separation of the steps would amend a traditional waste handling facility with CO_2 sequestration capacity. Examples treatments are:

(i) Anaerobic digestion: substrate fermentation to volatile fatty acids and subsequent methanogenesis, and (ii) Biological nitrogen removal: aerobic nitrification and subsequent anoxic denitrification degrading organic carbon. Other examples of sequential acid- and alkalinity producing steps in biotechnological processes are: (iii) Flue gas desulfurization: weak acid hydrogen sulfide absorption and subsequent aerobic oxidation to elemental sulfur; (iv) Bioelectrochemical systems: physical separation of the acid-producing anode reaction and the alkalinity-producing cathode reaction.

In the present study, the CO_2 sequestration potentials of these four biological technologies are quantitatively analyzed and the relevant factors for process optimization are discussed. The CO_2 released during these biological processes can have biogenic or non-biogenic (fossil carbon) origin (Griffith et al., 2009). According to the IPCC guidelines (http://www.grida.no/publications/other/ipcc_tar/) CO_2 emitted as a result of natural organic matter conversion does not contribute to the anthropogenic enhanced greenhouse effect because it has a biogenic basis (Houghton et al., 2001b) . However, reducing the biogenic CO_2 emissions can be an offset for fossil fuels combustion emissions.

To enable better comparison of the four systems mentioned above, the reactions involved in the first and second steps, respectively acid- and alkalinity producing processes, are stoichiometrically balanced for 1 mole of CO_2 sequestration. In addition, for each system, the sequestration efficiency is evaluated based on the amount of substrate (e.g. glucose) consumption required to sequester 1 mole of CO_2. Wollastonite ($CaSiO_3$) was chosen as a

divalent cation and alkalinity source because it represents a commonly occurring natural calcium silicate with a simple structure.

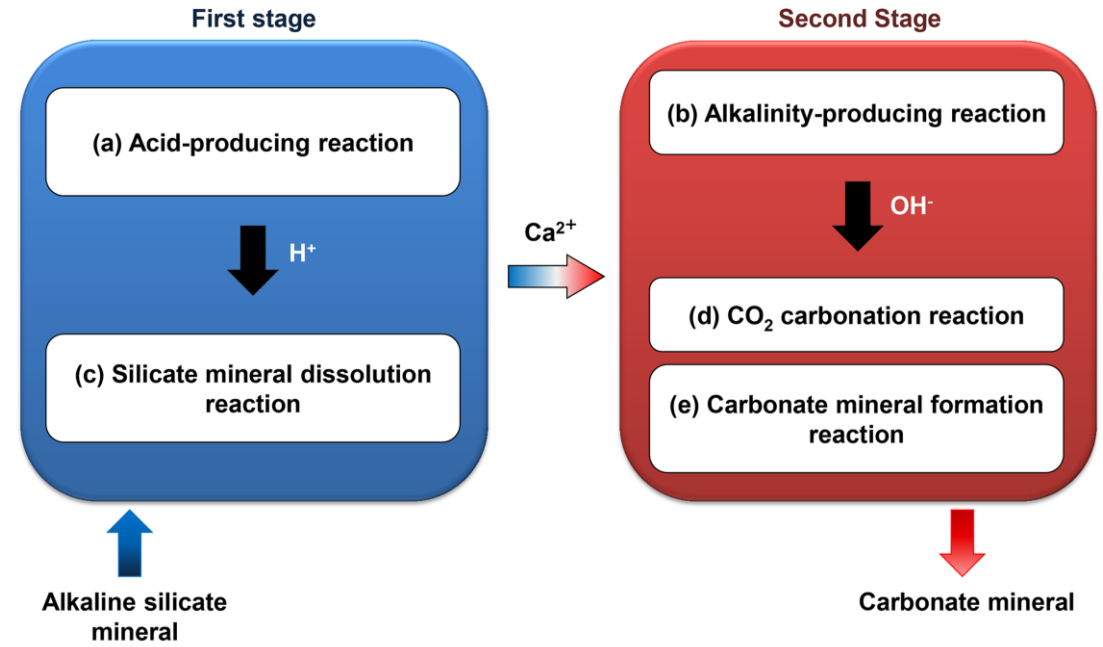

Figure 2.2 Scheme of mineral CO_2 sequestration by two-stage biotechnological processes. Reactions (a) and (b) are biological or chemical (redox) reactions in the treatment technology. These reactions can provide the necessary environmental conditions (i.e acidity and alkalinity) by degrading the waste materials for mineral CO_2 sequestration reactions (c and d). As a result of reactions (c) and (d), carbonate minerals can be formed in the second tank (reaction e).

2.3.1 Two-stage anaerobic digestion process

Anaerobic digestion (AD) is a widely applied biological technology for stabilization of municipal and industrial wastewater sludge and solid wastes. AD is essentially a two-stage system consisting of fermentative production of volatile fatty acids (VFA) from complex organic materials, and subsequent biogas (methane and carbon dioxide mixture) production from the VFA. When operated as a two-stage process, VFA production can provide the necessary acidity for dissolution of silicate minerals and methanogenesis generates adequate alkalinity for carbonate ion (CO_3^{2-}) formation (Fig. 2.4a).

Although, operating the digestion process in two stages has shown better process stability and effluent quality (Song et al., 2004), treatment plants today are mostly operated in a single-stage system for minimizing the construction and operational costs. The increased operational costs of a two-stage digester are associated with the need for alkaline materials such as NaOH and Na_2CO_3 which are used in the first stage to prevent an excessive drop in the pH. Integration of alkaline silicates in the AD can buffer the pH of the acidifying step (Fig. 2.4a), and therefore facilitate the application of two-stage digestion systems.

Fig. 2.4a shows that CO_2 precipitation (as $CaCO_3$) in the second vessel results in a biogas with higher methane content (Datta et al., 2010) of 17 % wt/wt (i.e. half of the produced CO_2 is captured). Depending on the utilization pathway of the biogas, the increase in methane content can offer higher heat and power generation efficiency (Weiland, 2010). In addition, if olivine ($(Fe,Mg)_2SiO_4$) is used as a source of divalent cation and alkalinity, the iron can facilitate mineralization of H_2S as ferrous sulfide (FeS) (Schuiling, 2009). This gives an important added value to the system since H_2S is a corrosive gas. Therefore, if biogas is upgraded to natural gas or vehicle fuel (Börjesson & Mattiasson, 2008) the removal cost of H_2S (as one the main impurities) can be decreased (Abatzoglou & Boivin, 2009). Another added value obtained is the higher content of Mg-, Ca- or Fe-carbonate precipitates in the stabilized residues which may offer a fertilizer with higher quality (Sommers, 1977).

In the first stage of the digestion process, in addition to the generated acidity, the organic ligands produced by fermentation process might also accelerate the dissolution rate of silicate minerals by complexation mechanisms (Drever & Stillings, 1997; Kakizawa et al., 2001; Ullman et al., 1996; Welch & Ullman, 1993). Higher dissolution rate of silicate mineral can increase the overall CO_2 sequestration efficiency of the AD process. This is because the slow process of silicate mineral dissolution is the rate-limiting step of the mineral carbonation of CO_2 (Huijgen et al., 2003).

In landfill sites the organic wastes are also degraded by two-stage anaerobic digestion processes and are therefore potential candidates for integrating mineral CO_2 sequestration.

2.3.1.1 CO_2 sequestration and improved biogas in solid waste treatment systems

The anaerobic fermentation and methanogenic phase at landfill treatment sites occur subsequently in different time zones (Kjeldsen et al., 2002), in which silicate mineral dissolution and CO_2 carbonation processes can take place. The stabilization of organic solid wastes in landfill sites is currently slow due to a number of environmental factors such as excessive drop in pH during the fermentation phase (Kjeldsen et al., 2002). Another property of the landfill sites is the longer residence time (20-25 years) compared to the sludge digestion (~ 30 days) (Wouters et al., 2011). This can provide the possibility to use larger size silicate minerals compared to chemical methods (i.e. with high temperature and pressure) for mineral carbonation of CO_2 which commonly have shorter residence times (e.g. few hours). The mineral carbonation process can be simply integrated by mixing the ground silicate minerals with the solid waste in the beginning of the disposal process.

Integration of the sequestration process into landfill sites can also offer added-values to the system. These added values are improved biogas, reinforcement of soil by carbonate mineral precipitation (Whiffin et al., 2007), and higher stabilization rate of the solid wastes (Šan & Onay, 2001). The latter is because of the alkalinity release from silicate minerals can prevent inhibition of the bacterial activity by excessive drop of pH in the fermentation phase.

2.3.2 Nitrification and denitrification process

Nitrogen removal is a common step used for wastewaters with high nitrogen content in industrial and municipal wastewater treatment plants (WWTPs). Removal of nitrogen from wastewater typically proceeds through ammonium oxidation to nitrate via nitrite (nitrification), followed by the reduction of nitrate to nitrogen gas (denitrification) in which acidity and alkalinity are produced, respectively (Fig. 2.4b). Alkalinity release from activity of denitrifying bacteria has resulted in large carbonate deposits in natural systems (Harold Drew, 1913). Carbonate precipitation as a result of such pH increase could also be applicable for soil reinforcement (van Paassen et al., 2010), and self-healing concrete (De Muynck et al., 2010). In wastewater treatment facilities nitrification and denitrification are commonly operated in two different tanks. Therefore, a CO_2 sequestration process can potentially be integrated in existing treatment plants. The integration can allow for zero CO_2 emission from the nitrogen removal process as shown in Fig. 2.4b.

The activity of the nitrifiers is greatly inhibited at pH values lower than 7 (Anthonisen et al., 1976). This can limit the use of certain silicate minerals such as olivine which only slowly dissolve at pH values above 7 (Pokrovsky & Schott, 2000).

2.3.3 Desulfurization treatment

Desulfurization processes are used to treat gas streams which contain H_2S or SO_2. H_2S is a poisonous gas that occurs in natural gas and biogas. In order to remove it, the gas stream is commonly scrubbed with an alkaline solution (Marcelis et al., 2003). Alternatively, it might be possible to have the H_2S chemically react with alkaline minerals. H_2S is chemically absorbed into the alkaline solution as sulfide ions (HS^- and S^{2-}, Fig. 2.4c). Sulfide removal is subsequently established in a biological oxidation process where *Thiobacillus* bacteria convert sulfide to elemental sulfur and alkalinity under oxygen-limiting conditions.

Since the pH in the first tank should be kept above 7 for the hydrogen sulfide (H_2S) to substantially dissociate (pk_a of 6.96 at 25°C), the overall CO_2 sequestration rate cannot improve because most alkaline silicate minerals dissolve slowly at neutral to alkaline pH values (White & Brantley, 1995). Flue gas desulfurization (FGD) is another desulfurization technology which can potentially reach a higher CO_2 sequestration rate compared to hydrogen sulfide treatment.

2.3.3.1 Mineral CO_2 sequestration by flue gas desulfurization

Another alternative for integration of mineral CO_2 sequestration into desulfurization treatment technologies is the biological flue gas desulfurization (FGD) technology for SO_2 removal (Ruitenberg et al., 1999). SO_2 is a gas mainly released from coal-fired power plants. The

reactions involved in the Bio-FGD process are shown in Fig. 2.3. The main advantage of this system compared to the H_2S removal technology is that the pK_{a1} value of SO_2 conversion to H_2SO_3 in water is much lower (pK_{a1} of 1.8) which indicates that silicate dissolution can be enhanced substantially by operating at lower pH values. This can increase the dissolution rate of silicate minerals to a great extent (White & Brantley, 1995). The disadvantage of such system is the complexity that arises from the compound flows between the process units.

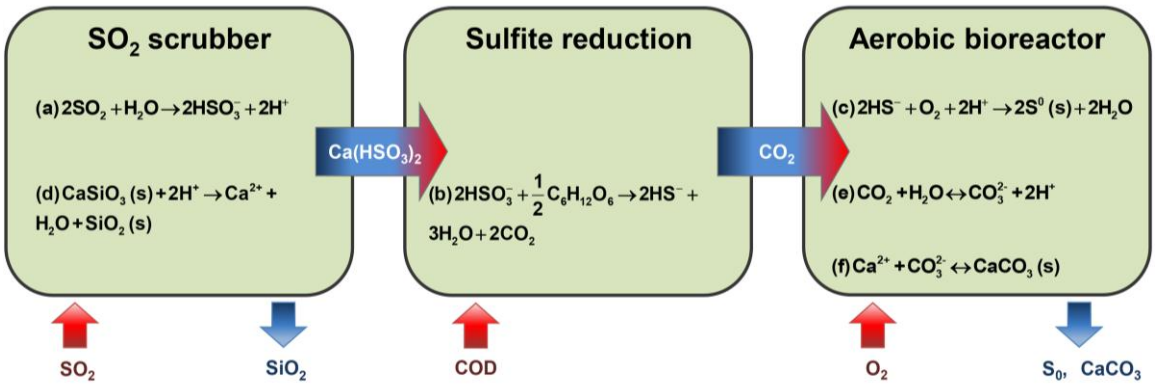

Figure 2.3 A schematic presentation of the involved reactions for stoichiometric sequestration of one mole of CO_2 in biological flue gas desulfurization (bio-FGD) of SO_2 gas. (a) Sulfur dioxide is converted to bisulfite and (d) wollastonite dissolution. Second stage reactions: (b) bisulfite is microbially reduced to hydrogen sulfide ion with concomitant oxidation of glucose. Third stage reactions: (c) hydrogen sulfide ion is oxidized to elemental sulfur and (e) conversion of CO_2 into carbonate ion. (f) $CaCO_3$ precipitation. S^0 and $CaCO_3$ precipitates are removed from the third tank.

2.3.4 Bioelectrochemical systems

Bioelectrochemical systems (BES) such as microbial fuel cells (MFCs) have been proposed to treat wastewater treatment at the same time as they produce energy (Angenent et al., 2004). Energy is generated when electrons are transferred via an electric circuit. Bioelectrochemical systems operate in two stages: (i) Organic carbon is completely oxidized to carbon dioxide in the anode chamber by an anaerobic oxidation process resulting in electrons and hydrogen protons. (ii) Water is produced by chemical reduction of oxygen with the transferred electrons and hydrogen protons through a proton exchange membrane (PEM) in the cathode chamber. The produced pH sequence enables mineral CO_2 sequestration integration into the BES (Fig. 2.4d). In the integrated system, in addition to the CO_2 produced in the process, CO_2 from an external source could be supplied and sequestered due to the high carbonation efficiency. Although theoretically the charges of BES are balanced in a silicate free BES system by transport of the protons through PEMs (Fig. 2.4d), without the presence of a buffer solution an obvious difference in pH of the anodic (acidic) and cathodic (alkaline) chambers have been reported (Du et al., 2007; Gil et al., 2003). This is because the proton production rate in the anodic chamber is higher than proton transport and consumption rate in the cathodic chamber

(Gil et al., 2003). Alkaline silicates can alleviate the need for additional buffers that are commonly added to the anode chamber to adjust the pH (Du et al., 2007). In addition, integration of mineral CO_2 sequestration process into the BES systems might allow omitting the membranes between the chambers. This is because the charges can be balanced in both chambers by the silicate dissolution reaction and the CO_2 carbonation reaction (Fig. 2.4d). As complications related to use of membranes such as (bio)fouling are considered to be one of the main problems for efficient application of BES technology (Bogner et al., 2007), this is an important added-value to the BES technology.

Even though the highest current values in BES were achieved at pH values of 7-8 in the first (anodic) chamber, depending on the organisms used the pH can vary between 5 to 9 (Gil et al., 2003). The cathodic chamber was reported to operate within pH range of 7 to 9. Mineral CO_2 sequestration reactions (i.e. silicate mineral dissolution and carbonate precipitation) are not inhibited at these pH values.

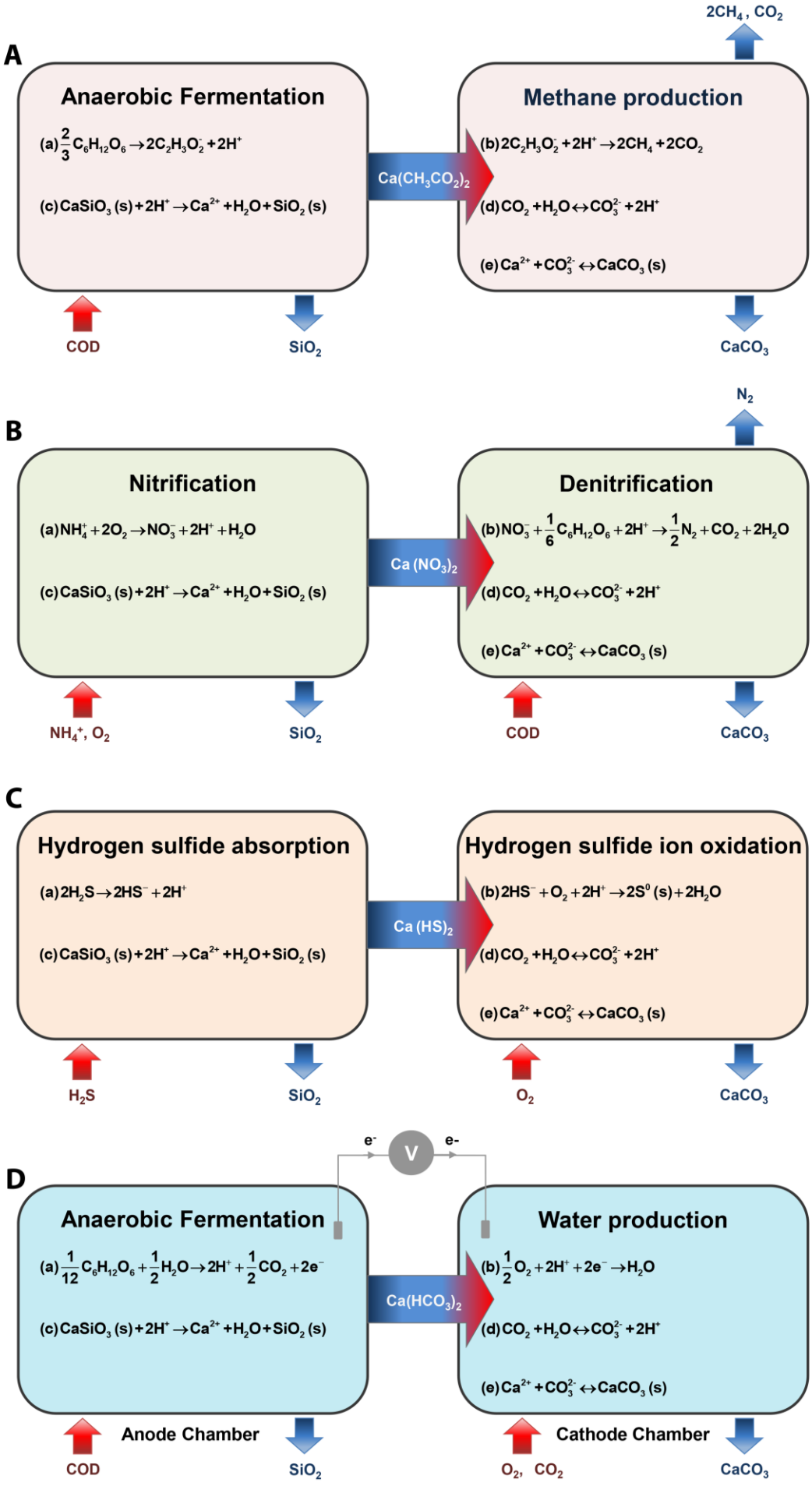
A
Anaerobic Fermentation
(a) $\frac{2}{3}C_6H_{12}O_6 \rightarrow 2C_2H_3O_2^- + 2H^+$
(c) $CaSiO_3(s) + 2H^+ \rightarrow Ca^{2+} + H_2O + SiO_2(s)$
$Ca(CH_3CO_2)_2$
Methane production
(b) $2C_2H_3O_2^- + 2H^+ \rightarrow 2CH_4 + 2CO_2$
(d) $CO_2 + H_2O \leftrightarrow CO_3^{2-} + 2H^+$
(e) $Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_3(s)$
$2CH_4$, CO_2
COD
SiO_2
$CaCO_3$
B
Nitrification
(a) $NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$
(c) $CaSiO_3(s) + 2H^+ \rightarrow Ca^{2+} + H_2O + SiO_2(s)$
$Ca(NO_3)_2$
Denitrification
(b) $NO_3^- + \frac{1}{6}C_6H_{12}O_6 + 2H^+ \rightarrow \frac{1}{2}N_2 + CO_2 + 2H_2O$
(d) $CO_2 + H_2O \leftrightarrow CO_3^{2-} + 2H^+$
(e) $Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_3(s)$
N_2
NH_4^+, O_2
SiO_2
COD
$CaCO_3$
C
Hydrogen sulfide absorption
(a) $2H_2S \rightarrow 2HS^- + 2H^+$
(c) $CaSiO_3(s) + 2H^+ \rightarrow Ca^{2+} + H_2O + SiO_2(s)$
$Ca(HS)_2$
Hydrogen sulfide ion oxidation
(b) $2HS^- + O_2 + 2H^+ \rightarrow 2S^0(s) + 2H_2O$
(d) $CO_2 + H_2O \leftrightarrow CO_3^{2-} + 2H^+$
(e) $Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_3(s)$
H_2S
SiO_2
O_2
$CaCO_3$
D
e-
V
e-
Anaerobic Fermentation
(a) $\frac{1}{12}C_6H_{12}O_6 + \frac{1}{2}H_2O \rightarrow 2H^+ + \frac{1}{2}CO_2 + 2e^-$
(c) $CaSiO_3(s) + 2H^+ \rightarrow Ca^{2+} + H_2O + SiO_2(s)$
$Ca(HCO_3)_2$
Water production
(b) $\frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O$
(d) $CO_2 + H_2O \leftrightarrow CO_3^{2-} + 2H^+$
(e) $Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_3(s)$
COD
Anode Chamber
SiO_2
O_2, CO_2
Cathode Chamber
$CaCO_3$

Figure 2.4 A schematic presentation of stoichiometric sequestration during four biotechnological processes. Anaerobic digestion (A), nitrogen removal (B), desulfurization (C), and bioelectrochemical (D). Among different systems (a) and (b) are biological or chemical (redox) reactions occurring at the treatment facilities, and (c), (d), and (e) are chemical reactions of the mineral carbonation process. (a) and (b) are different in each system, while (c), (d), and (e) remain the same. In system **A,** (a) glucose oxidized to acetate and protons (in this reaction the proton production yield depends on the fermentation product), and (b) fermentation of acetate to methane. In system B, (a) ammonium oxidation to nitrite, and (b) nitrite reduction to nitrogen gas. In system C, (a) hydrogen sulfide is converted to hydrogen sulfide ion, and (b) hydrogen sulfide ion is oxidized to elemental sulfur. In system D, (a) biological oxidation of glucose to CO_2, protons and electrons, and (b) electrons, protons and oxygen react to water.

2.4 Comparison of CO_2 sequestration efficiencies of the two-stage biotechnological processes

The main factor relevant to sequestration efficiencies of the proposed two-stage treatment systems is the yield of production of acidity (i.e. H^+) and alkalinity (i.e. OH^-) from the substrate in the first and second stages. Comparison of the stoichiometries of the four treatment systems, results in the highest CO_2 sequestration efficiency for the bioelectrochemical systems (Table 2.1). This is because the physical separation of the electron donor (e.g. glucose) in the BES process enables the production of one proton per electron donated in the anodic compartment. Thus, BES technology can potentially sequester more CO_2 than what is produced during the organic carbon oxidation (i.e. 200% wt/wt $CaCO_3$-C/organic-C). The other proposed systems (Table 2.1) have lower sequestration efficiencies compared to the BES technology. As an example, in anaerobic digestion, 25% of the organic carbon can be sequestered into carbonate minerals as the remainder is converted to CH_4 and CO_2. Although based on the carbonation yield, the best CO_2 sequestration option is the BES system, existing bioelectrochemical systems suffer from extremely low current densities and therefore further development is required before the BES system becomes applicable. Among the existing options, the more widely applicable technology is the AD process which is readily used for solid waste and wastewater treatment. The global sequestration potential of biodegradable solid waste and wastewater by AD process can be estimated based on its sequestration yield which is 1-kg CO_2/2.9-kg COD. Biodegradation of the organic carbon content of the solid waste and municipal wastewater generated globally each year (Bogner et al., 2007; Sakai et al., 1996) by anaerobic digestion employing silicates is estimated to be able to sequester 1.3-1.5 % wt/wt and 0.2-0.4 % wt/wt of the total anthropogenic CO_2 released to the atmosphere, respectively. This contribution does not include AD treatment of the industrial wastewater such as food, paper and pulp industries with high organic carbon concentrations.

A more comprehensive comparison among the systems should be obtained by considering the added-values (Table 2.1) and added-costs as the result of integrating the CO_2 sequestration process. Full life cycle analysis (LCA) is outside the scope of this study but should be done in the future. For instance, substituting alkaline materials (e.g. Na_2CO_3) with alkaline silicate

minerals will also indirectly reduce the CO_2 emissions by offsetting the CO_2 produced during the production of these alkaline materials.

Table 2.1. Comparison of CO_2 sequestration efficiencies of the proposed two-stage biotechnologies calculated based on the reactions stoichiometry.

Process	$CaCO_3$-C/Organic-C wt/wt %	CO_2 seq./sub. mole/mole	Sub./CO_2-seq. kg/kg	Substrate	Added values
Anaerobic digestion	25	1.5	2.7	$C_6H_{12}O_6$	Biogas improvement Modified sludge property Faster stabilization (at landfills) Added buffering capacity
Nitrogen removal	100[a]	1	0.4	NH_4^+	Added buffering capacity
Desulfurization	33[b]	0.5	1.5	H_2S/SO_2	Added buffering capacity
BES	200	12	0.3	$C_6H_{12}O_6$	Membrane omission Added buffering capacity

[a] Glucose as the organic carbon is degraded during the denitrification process (second stage).
[b] Glucose as the organic carbon is degraded during second stage of the flue gas desulfurization of the SO_2 gas treatment.
Abbreviations: Seq., sequestered; C., carbon; wt., weight; sub., Substrate.

2.5 Advantages of biological mineral carbonation of CO_2 compared to chemical mineral carbonation methods

Integration of the mineral CO_2 sequestration into two-stage environmental biotechnological processes can significantly reduce the process costs in comparison with the evaluated costs of previous aqueous mineral carbonation techniques (Gerdemann et al., 2007; Huijgen et al., 2007). This is because in the proposed biological mineral carbonation (MC) process the costs at several steps; i.e., silicate mineral pre-treatment, CO_2 capture, and the CO_2 mineral carbonation can be avoided or reduced when compared to the chemical MC process (Fig. 2.5). In the mineral pre-treatment step (e.g. mechanical activation) the longer retention times of the biological treatment processes which is of days for wastewater, and of years for solid waste compared to that of hours for the chemical MC processes, allows for using larger mineral

particle sizes. It has been shown that continuous divalent and alkalinity could be released at usefully high rates with a mineral size of 125-250 µm during biological fermentation process (Salek et al., 2013c). By using comparable sizes, the energy-intensive step of stirred media detritor (SMD) which is typically performed for additional size reduction (< 38 µm) of the mined ore may no longer be required. This have a significant impact on the energy requirements since the energy input for SMD step is e.g. about 40 % of the total energy spent for mechanical activation of wollastonite (Gerdemann et al., 2007). For the CO_2 carbonation step, heating and pressurizing of the sequestration unit in chemical methods (e.g. for wollastonite, 200 °C, and 20 bar CO_2 (Huijgen et al., 2007)) are replaced by biological acid production from waste materials. In addition, as the CO_2 is produced inside the biological treatment plant, CO_2 capture, compression, and transportation steps are eliminated from the sequestration process. As Fig. 2.5 demonstrates, by integration into an existing treatment plant constructing a separate sequestration unit is not needed which can reduce the investment costs. Consequently, compared to the evaluated cost of 102 euro/ton CO_2-avoided by wollastonite (Huijgen et al., 2007), applying the biological MC process could reduce the cost to 40 euro/ton CO_2-avoided (~ 60 % reduction). This estimation inevitably involves significant uncertainties, however it suggests that there is great potentials for storing CO_2 as carbonate minerals by integration into environmental biotechnological processes. Even though the sequestration cost has been substantially decreased compared to other cost analysis studies on mineral carbonation, the evaluated price is not yet considered feasible for commercial applications (Heddle et al., 2003). The marketable process products of e.g. $CaCO_3$ and SiO_2 have been suggested as a way to reduce the high costs of mineral carbonation processes. However, the gains achieved by these products cannot offset the costs associated with excessive energy consumption (Gerdemann et al., 2007). Therefore, the obtained added values to the improved biogas quality in AD process, potential omission of the membrane in BES process, and added buffering capacity by alkaline silicates in all of the proposed systems (Table 2.1) can further increase the feasibility of the carbonation process.

There may be also some costs associated with integration of the carbonation process into the treatment plant. For example, extraction of SiO_2 from the first vessel may involve new reactor configurations. In addition, precipitation of carbonate minerals in the second stage results in higher amounts of residue production which can also contribute to the operational costs (Van Langerak et al., 2000).

It should be noted that the loading amount of the treatment plant will not be affected because the properties (i.e. neutralizing capacity/molecular weight) of alkaline silicate minerals (e.g. olivine or wollastonite) are comparable to commonly used alkaline buffers such as calcium hydroxide (CaOH) and sodium bicarbonate (Na_2CO_3).

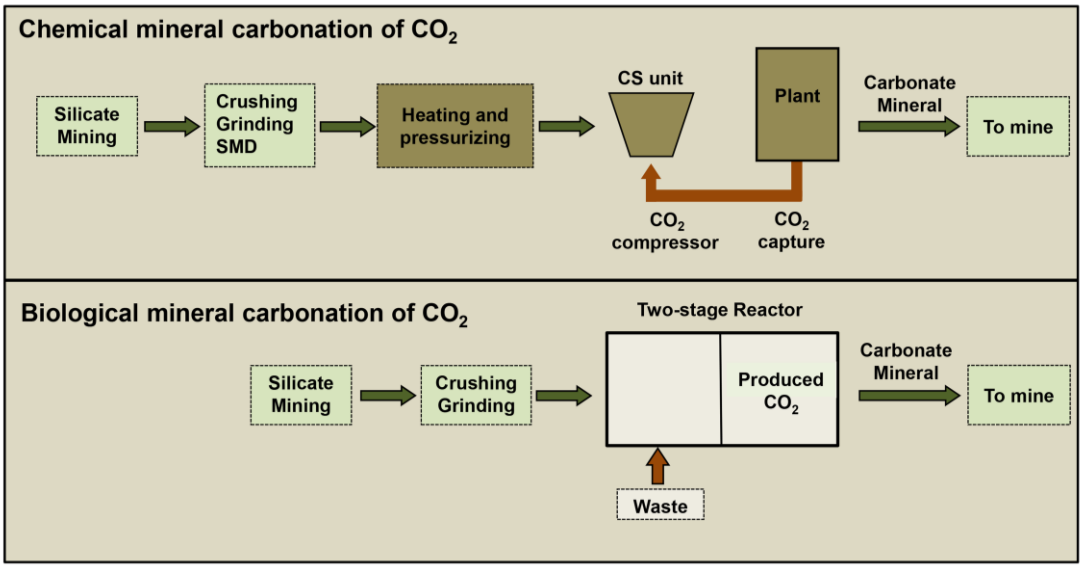

Figure 2.5 Process comparison between biological and chemical mineral carbonation of CO_2. The figure shows flow diagrams of a mineral carbonation of CO_2 by chemical methods and the proposed biological process. The processes of SMD for mineral pre-treatment, heating and pressurizing of the reactor, CO_2 capture, compression and transportation can be avoided as the result of integration into a biological treatment technology.

2.6 Concluding remarks and future perspectives

Application of naturally occurring chemical reactions to sequester CO_2 as mineral carbonate (reaction 2.1) is hampered by the slow reaction kinetics of CO_2 with alkaline silicate minerals. Elevated temperature and pressure using finely powdered silicate minerals accelerate carbonation reactions rates (Maroto-Valer et al., 2005; McKelvy et al., 2004), but the costs are high limiting industrial application. A large fraction of the estimated costs is associated with grinding of the silicate minerals to smaller particles (> 60 % of the total costs) (Gerdemann et al., 2007; Huijgen et al., 2007; Renforth et al., 2011). Application of silicate mineral in a two-stage biotechnological process characterized by acid production in the first stage and acid consumption in the second stage enable CO_2 sequestration with larger mineral particles (e.g. a diameter of 125-250 µm (Salek et al., 2013c) instead of < 38 µm (Gerdemann et al., 2007; Huijgen et al., 2007)) due to the increased reaction rates at lower pH-values as obtained in the first stage. Combined with the lower operational temperature of biotechnological processes and the use of low-cost feedstocks (i.e. waste/wastewater) as raw materials, two stage biotechnological processes can substantially reduce the costs of CO_2 sequestration using alkaline silicate minerals. Comparing to chemical methods, the sequestration costs are estimated to decrease from 102 euro/ton CO_2-avoided (Huijgen et al., 2007) to 40 euro/ton CO_2-avoided (~ 60 % reduction in sequestration costs). Depending on the process considered, the main challenge for integration of mineral carbonation into these biotechnological systems is development of methods for cost-effective transport of Ca^{2+} ions between different vessels, and for withdrawal of silicate and carbonate precipitates from the first and second stages, with minimum operation disturbances.

From the four biotechnological processes proposed, bioelectrochemical system and anaerobic digestion process appear to have higher potentials for application of mineral CO_2 sequestration than biological nitrogen removal and flue gas desulfurization processes. Integration of silicate mineral based CO_2-sequestration into AD and BES systems offered important added values to these systems: improving the biogas quality in anaerobic digestion process, and omission of the need for proton exchange membranes in bioelectrochemical systems. These additional advantages may decrease the costs of the sequestration process below 40 euro/ton CO_2-avoided to a value that will be more feasible for industrial applications (CO_2 tax 10-15 euro/ton (Grimston et al., 2001). More detailed economic analysis is required to estimate how much these added values will in fact decrease the cost of sequestration process. Improving both economic and environmental features of a process (a win-win situation) have shown better prospects for industrial implementation (Xepapadeas & de Zeeuw, 1999).

Chapter 3

Wollastonite dissolution kinetics at 30°C: effects of pH, ligands and wollastonite concentration

3.1 Abstract

Effects of pH (2-9) and wollastonite concentration, organic and inorganic ligands on kinetics of wollastonite dissolution was studied at 30 °C. pH dependency of wollastonite dissolution rate, r ($mol.cm^{-2}.s^{-1}$) to proton activity (aH^+), is given by:

$$r = (5.53 \times 10^{-11})(a_{H^+})^{0.32}$$

Studying the dissolution rate as a function of wollastonite concentration range 1-150 $g.l^{-1}$, showed that the volumetric dissolution rate increased at lower wollastonite concentrations (2-30 $g.l^{-1}$) while at higher mineral concentration range (30-150 $g.l^{-1}$) the observed effects were small. In addition, effect of organic and inorganic ligands (represent exomotonlites and compounds present in a typical anerobic fermentation process) were shown on the dissolution rate of wollastonite. Presence of these compounds increae the dissolution rate by a factor of 2 to 5 times as compared to that of the base experiment with HCl acid. The following relative effectiveness on wollastonite dissolution rate was observed (at pH 5): EDTA > phosphate > succinate > oxalate > gluconate > citrate > acetate > propionate > formate > lactate > butyrate. Other compounds examined such as ethanol, alginate and bacterial medium solution only weakly affected the dissolution kinetics. Having a better understanding of wollastonite kinetics in a fermentation process at pH of 5 can facilitate the integration of mineral CO_2 sequestration in an anaerobic digestion system.

3.2 Introduction

Increase of CO_2 concentration in the atmosphere has caused one of the greatest environmental challenges, climate change. Weathering of alkaline silicate minerals is an important natural feed-back mechanism for balancing the concentration of CO_2 in the atmosphere (reaction 3.1) (Seifritz, 1990).

$$2CO_2 + H_2O + CaSiO_3 \rightarrow Ca^{2+} + 2HCO_3^- + SiO_2 \quad -29.6 \text{ kcal} \qquad \text{(reaction 3.1)}$$

Environmental conditions in the natural environments such as presence of ligands in the soil solutions can enhance the weathering of silicate minerals. The production rate of anthropogenic CO_2 in the past decades is such that the weathering rate of silicate minerals cannot balance the increased production rate (Falkowski et al., 2000). Manipulation of the natural environments with the aim of increase of the weathering rate by addition of for example chemicals or increase the surface area of silicate minerals has shown to negatively influence the natural environment in long-term (Koehler et al., 2010). For example, distributing finely powdered olivine over land areas suggested as mitigation solution for the

climate change by increasing the reactive surface area (Schuiling & Krijgsman, 2006), can potentially raise the pH of the rivers influencing the natural ecosystems. An alternative can be to integrate the dissolution of silicate minerals in readily available biological processes where similar enhancing processes are present. Examples of such systems are environmental biotechnological processes such as the anaerobic digestion process commonly used for industrial and municipal wastewater treatment. This has been proposed in a recent study where mineral CO_2 sequestration is integrated in a two-stage anaerobic digestion process (Salek et al., 2013b). In the integrated process, the acid-producing biological conversion (fermentation process) is used to enhance the dissolution of alkaline silicates in the first stage and the subsequent alkalinity-producing step, methanogenesis, allows precipitation of CO_2 as carbonate minerals (Lindeboom et al., 2013). In this process, the overall efficiency of mineral CO_2 sequestration is controlled by the silicate minerals dissolution reaction as the main rate-limiting process (Huijgen et al., 2003). Biological processes occurring in the fermentation process are able to improve the weathering (dissolution) of silicate minerals by various means: change in solution pH, pCO_2, ionic strength and production of exometabolites like organic ligands. In addition, direct bacterial effects such as biofilm growth can also potentially improve the dissolution process (Castanier et al., 1999; Pokrovsky et al., 2009; Salek et al., 2013c). While the effect of pCO_2 and ionic strength on silicate dissolution rate is well characterized, particularly for Mg and Fe silicates such as olivine (Golubev et al., 2005; Rimstidt & Dove, 1986), the effects of organic ligands on Ca silicates such as wollastonite have been hardly studied and the studies performed were at neutral and weakly alkaline pH (Pokrovsky et al., 2009). The organic ligands are known to enhance the dissolution rate by two key mechanisms: adsorption of ligands to $>CaOH_2^+$ and $>CaOH°$ sites on the mineral surface and complexation with the free Ca ion in the aqueous solution. These interactions are demonstrated in Fig. 3.1. The effectiveness of ligands depends on the nature of their functional groups, molecular structure and thermodynamic stability of the surface complexes they form (Pokrovsky et al., 2009). As an example, ligands with functional groups containing two or more oxygen donors which can form bi- or more multidentate mononuclear surface chelates, have shown to be more effective than other ligands (Ullman et al., 1996). In the present chapter, the kinetics of wollastonite as a common silicate mineral, was studied as a function of pH, wollastonite concentration and fermentation metabolites (organic acids, alcohols and alginate) and chemical compounds (bacterial medium solution and EDTA). This can represent the first stage of the two-stage integrated AD system.

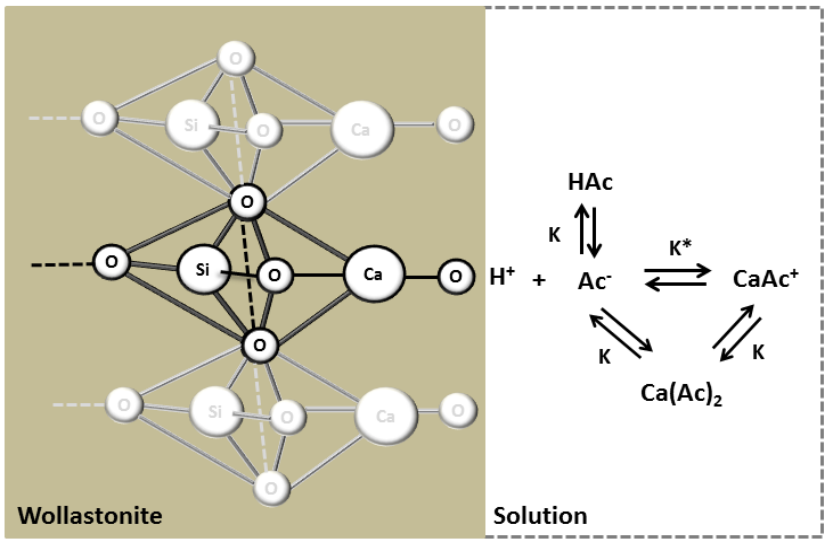

Figure 3.1 The interaction of organic ligand (as an example acetate) with hydrated Ca sites at wollastonite-H_2O interface and the dissolved Ca in the aqueous solution.

3.3. Material and Methods

3.3.1 Reactor operation

The batch experiments were conducted in double jacket glass stirred reactors with standard geometry and working volume of 2 litters (Applikon, The Netherlands). The reactor was temperature controlled at 30±1 °C with a water jacket and a thermostat bath (Lauda, Germany) and mixed at 500 rpm. To insure carbonate free environments, the solution was sparged with N_2 at 1 $l.min^{-1}$ during and 1 hour prior to the experiment. The reactor was equipped with pH (Mettler Toledo), conductivity (Consort C832) and temperature probes.

3.3.2 Mineral characterization

Wollastonite was selected as the model mineral because of its relatively high dissolution rate among silicate minerals (Pellant, 2002) and its simple structural composition ($CaSiO_3$). The obtained mineral from Ankerpoort NV was crushed and sieved in different sizes. Size 65-125 µm was prepared for subsequent experiments as described previously (Salek et al., 2013c). The specific surface area was determined by the Brunauer, Emmet, and Teller (BET) method with N_2 as the adsorptive. The specific surface area was measured to be 0.17 $m^2.g^{-1}$ for the cleaned powdered wollastonite. The composition of the wollastonite is indicated in Table 3.1.

Table 3.1. Bulk composition of the original wollastonite as determined by X-ray fluorescence.

Element	Ca	Si	Fe	Al	Mn	Mg	Be	K	Na	Cl	S	Ba	Cs
wt. %	64.2	34	0.89	0.27	0.17	0.16	0.1	0.07	0.03	0.03	0.02	0.02	0.02

3.3.3 Analytical Techniques

Organic acids and alcohol measurements was conducted by high-performance liquid chromatography (HPLC) as described previously (Salek et al., 2013c). To verify the HPLC measurements for butyrate and ethanol which feature similar HPLC retention times, a gas chromatograph (Chrompack CP 9001, the Netherlands) equipped with a flame ionization detector (FID) was used. The elemental analysis (Ca^{2+}, Si, Mg^{2+}, Al^{3+}, and Fe^{2+}) in the solution was performed by inductively coupled plasma atomic emission spectroscopy (ICP-OES), all samples were acidified with nitric acid (0.5 M) and diluted 10 and 100 times prior to the detection.

3.3.4 Wollastonite dissolution experiments

Dissolution rate of wollastonite was measured as the function of pH (2-9), wollastonite concentration (5-150 $g.l^{-1}$) and various organic and inorganic compounds (acids, base and salt) resulting in three series of batch experiments. All experiments were started with fresh wollastonite and Milli Q plus water. The steady-state dissolution rates were achieved after 17 hours. Therefore, the average dissolution rate was calculated over a period from 17-60 hours (43 h). In this period, the R^2 of all the experiments were equal or higher than 0.998.

The experiments performed at different pH values (2, 3, 4, 5, 6, 7, 8 and 9) and in presence of different (organic and inorganic) compounds were performed at wollastonite concentration of 5 $g.l^{-1}$and 10 $g.l^{-1}$, respectively. The effect of wollastonite concentration (1, 5, 10, 15, 20, 30, 50, 100 and 150 $g.l^{-1}$) and (organic and inorganic) compounds were studied at pH 5. The pH was kept constant by automatic titration of HCl stock solutions (0.1-1 M) or the organic/inorganic acid of interest (e.g. acetic acid) in the solution (Fig. 3.2). Therefore, the effect of organic and inorganic compounds were studied with consequently increase of the ligand concentration.

The examined compounds include the fermentation metabolites such as the organic acids (acetic acid, formic acid, propionic acid, lactic acid, butyric acid and succinic acid), ethanol and alginate (extracellular polymeric substance, EPS). Di/tri carboxylic acids (citric acid, gluconic acid and oxalic acid), chelates (ethylenediaminetetraacetic acid), nutrient media (as describe previously in (Salek et al., 2013c) and phosphoric acid that may be present in an anaerobic fermentation system were also examined. For compounds which do not have acidifying capacity such as ethanol and alginate, the pH was maintained at 5 by dosing HCl acid while the compound was injected continuously (with a certain rate) to the solution. Control and data acquisition of the direct online measurements of acid dosage, temperature,

conductivity, pH and stirring rate was by a bio-controller (BIOSTAT B plus, Sartorius) and MFCS/win (Sartorius Stedim Systems, USA), respectively.

The dissolution rate of wollastonite was calculated based on the dissociated acid injection rate (considering the pK_a value and the pH) and was verified by Ca, Si ions and organic acid measurements. This was possible because the only alkalinity source to neutralize the injected acids was dissolution of wollastonite (reaction 3.2). Considering that every mole of wollastonite neutralizes two moles of protons, the dissolution rate can be calculated based on the dissociated acid injection rate. As the flux of acid dosage in the course of experiments stayed constant within ± 5 % percent, the averaged dissolution rate was calculated by dividing the acid dosed to 2 (based on reaction 2), time (s) and initial surface area (cm^2). All chemical batch experiments were conducted in duplicates and for 100 hours.

$$CaSiO_3 + H_2O + 2H^+ \rightarrow H_4SiO_4 + Ca^{2+} \qquad \text{(reaction 3.2)}$$

Activities and secondary precipitates were calculated by using the computer program, PHREEQC (version 3) (Parkhurst, 1999). As input for PHREEQC the measured concentrations of Si, Ca, acids, and the measured pH and temperature are used.

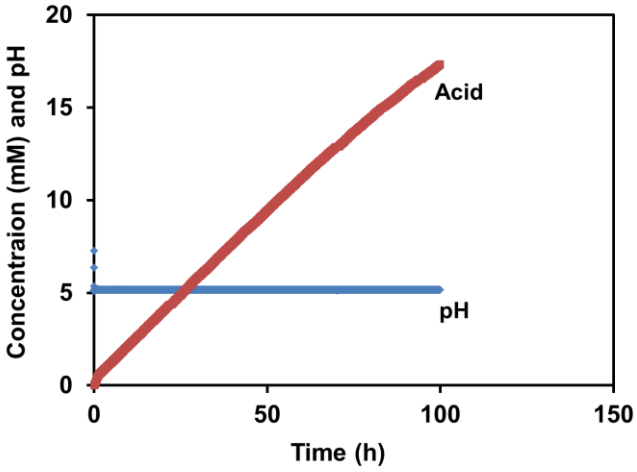

Figure 3.2 Typical chemical batch experiment with (organic or inorganic) acid titration. The acids was titrated continuously into the reactor to keep the pH constant at 5 while the wollastonite concentration is constant.

3.4 Results and Discussion

Dissolution rate of wollastonite (63-125 µm) as a function of pH (2-9) and wollastonite concentration (1-150 $g.l^{-1}$) is shown in Fig. 3.3a and Fig. 3.3b, respectively. The dissolution rate of wollastonite decreases at higher pH values with almost no dissolution above pH 8. This correlation is in agreement with previously reported results (Pokrovsky et al., 2009; Rimstidt & Dove, 1986; Salek et al., 2013c). An equation (3.1) was derived based on the dependency of the dissolution rate (*r*) to the pH value:

$$r = (5.53\times10^{-11})(a_{H^+})^{0.32} \quad \text{(equation 3.1)}$$

where a_{H+} is the activity of H^+ and 5.53×10^{-11} is the dissolution constant in $mol.cm^{-2}.s^{-1}$. The R^2 value was 0.99 when fitted to the experimental data. Based on these results, operating anaerobic fermentation process (the first stage) at lower pH values such as pH 5 can increase the efficiency of the overall mineral CO_2 carbonation in an integrated two-stage anaerobic system with wollastonite.

Fig. 3.3b displays that specific dissolution rate ($mol.cm^{-2}.s^{-1}$) is higher at lower wollastonite concentrations (at pH 5). This is more evident at lower concentration range (i.e. 1-30 $g.l^{-1}$ wollastonite) in which the dissolution rate of wollastonite differs by a factor of 2 between 1 $g.l^{-1}$ and 30 $g.l^{-1}$. As can be seen in the figure, the influence of wollastonite concentration on dissolution rate at higher concentration values (30-150 $g.l^{-1}$) is relatively weaker. Higher aqueous activities of constituent metals (Ca and Si) and/or precipitation of secondary precipitates such as SiO_2 on the mineral surface can be the reason for the lower observed dissolution rate at higher wollastonite concentration values. Decrease of dissolution rate (at pH 2 and 70°C) at higher concentration of constituent metals (Mg) was observed in other studies on enstatite ($MgSiO_3$) (a silicate mineral having a similar structure to wollastonite i.e. containing infinite chains of [SiO_4] tetrahedral) although, the silica activity had no influence on the dissolution rate (Oelkers & Schott, 2001). Formation of a surface precursor complex (as the main rate-controlling reaction of the dissolution process) from proton/magnesium exchange reactions has been reported as the reason for decrease of dissolution rate at higher Mg concentrations (Oelkers & Schott, 2001). Dependency of forsterite (Mg_2SiO_4) dissolution rate on Si concentration at alkaline conditions was observed in the study of (Pokrovsky & Schott, 2000). However, at the acidic condition (pH 2) and 25°C, forsterite dissolution rate was not affected by Si and Mg aqueous activities because it appeared that the indicated responsible processes for dissolution rate (surface protonation and braking octahedra chain linking Mg-O bonds) are independent of Mg and Si activities (Oelkers, 2001).

The output results of the PHREEQC software indicates precipitation of secondary precipitates (chalcedony and quartz) at all the concentration experiments including the experiment having the lowest wollastonite concentration (1 $g.l^{-1}$). Therefore, it is unlikely that SiO_2 precipitation on the mineral surface has been the cause for decrease of wollastonite dissolution rate at higher concentrations.

The dissolution rate of wollastonite in presence of different organic and inorganic compounds at pH 5 were compared to that of the base experiment using HCl (Fig. 3.4). The related properties of the ligands examined and their effect on dissolution rate are presented in table 3.2. Last two columns of the table stating aqueous stability constant (log K aq) and ligand adsorption constant (log K*Ca-L) of the compounds have been adopted from the literature (Pokrovsky et al., 2009).

Among the examined compounds, EDTA showed the highest effect on the dissolution rate (i.e. increasing the dissolution rate by 5 times as compared to the base experiment). This can be due to the formation of very stable chelate rings by EDTA with surface Ca ions (Pokrovsky et al., 2009) which is also expected from the high values of aqueous stability constant (log K aq) and the surface Ca ion adsorption constant of EDTA (table 3.2). Citrate and gluconate have also shown similar Ca surface complexation abilities and therefore have demonstrated high impact on the dissolution process (Fig. 3.4).

At pH 5, phosphoric acid is mainly in the form of dihydrogen phosphate ($H_2PO_4^-$) which has been reported to have high catalyzing effect at pH of 6.8 on wollastonite dissolution (Pokrovsky et al., 2009) (following the same trend as in present study after EDTA). Although these ligands can also form Ca-ligand complexation in the aqueous solution (Fig. 3.1), the main enhancing effect is due to the ligand adsorption on the mineral surface. This is because breaking the Ca-O bond on the wollastonite surface is known to be the main limiting step of wollastonite dissolution (Pokrovsky et al., 2009). Comparison among the other examined ligands indicates that the effectiveness of organic ligands such as oxalate and succinate having two functional groups is higher on the dissolution process as compared to that of the monofunctional organic ligands (formate, acetate, propionate, lactate and butyrate). This result is in agreement with the previously reported data that propose compounds with higher functional groups promote dissolution to a much higher extent than monofunctional ligands (Golubev et al., 2006; Stumm, 1997).

Table 3.2. Properties of the ligands used in the present study and their aqueous stability constants (log K_{aq}) and surface Ca ion adsorption constants (log $K^*_{Ca\text{-}L}$) taken from the database. (#), NA, and DR stands for number, not available, and dissolution rate, respectively.

Compound	# Carbon (s)	# Proton (s)	Dissociated acid at pH 5 %)	Concentration at 60 h (mM)	Times more DR than base experiment	log $K_{aq\ (Ca\text{-}L)}$	log $K^*_{Ca\text{-}L}$
HCl	0	1	100	11.5	1.0	NA	NA
Butyric acid	4	1	60.2	24.1	2.4	NA	Na
Lactic acid	4	1	93.2	18.0	3.0	1.5	1.6
Formic acid	1	1	94.7	17.6	3.0	1.4	1.5
Propionic acid	3	1	57.0	36.3	3.9	NA	NA
Acetic acid	2	1	64.0	35.8	4.5	1.2	1.7
Citric acid	4	3	98.8	15.3	4.5	3.5	2.4
Gluconic Acid	6	1	93.2	26.9	4.6	NA	NA
Oxalic acid	2	2	100	15.8	5.7	2.8	2.3
Succinic Acid	6	2	86.3	21.9	5.0	2.0	2.1
Phosphoric Acid	0	3	99.9	26.7	5.4	NA	NA
EDTA	10	6*	100	57.0	5.0	10.7**	3.5
						5.8	4.7
						3.7	4.3

* The fully protonated H_6EDTA^{2+} form only at very low pH.
**Different dissociation levels of $EDTA^{\cdot}$

Over the experimental period, the the ligands are titrated in the solution reaching the concentration indicated in 5th column of table 3.2 at 60h. The neutralization rate by wollastonite (i.e. wollastonite dissolution rate) in the course of experiments stayed constant (17-60 h) within ± 5 percent. This suggests that the dissolution rate showed low dependency to the concentration of the ligands. Although, continuation of the experiment to 100 h showed slight decrease in the dissolution rate particularly for the more effective ligands such as EDTA (the average dissolution rate at 17-60 h was 18 % higher than 60-100h).

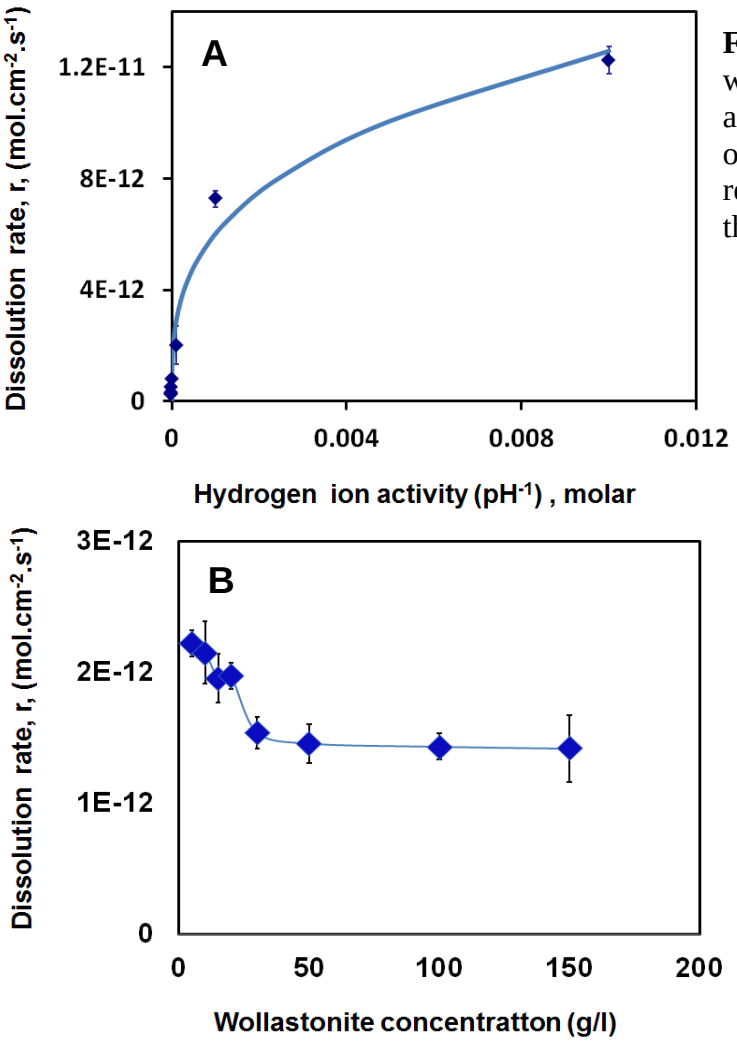

Figure 3.3 Dissolution rate of wollastonite at: pH range of 2–9 (A), and wollastonite concentration range of 1-150 g.l^{-1} (B). The solid line represents the best fit of the data to the equation.

Presence of some of the examined ligands in the solution lead to a decrease of wollastonite dissolution rate. For example, increase of ethanol concentration (from 0-60 mM) had a negative effect on dissolution rate by 7-11 % as compared to the base experiment. In addition, alginate (11 mM at 60 h) decreased the dissolution rate by 16 % during the experiment. Pokrovsky et al (2009) also observed negative influence of alginate on wollastonite dissolution above 0.1 mM. Potentially this is due to the formation of a polymeric alginate coating on the mineral surface. Nutrient media weakly affected the dissolution rate of the

wollastonite (< 1%) at pH of 5. Although the exact impact of mixture of organic ligands on wollastonite dissolution rate is yet to be determined, the observed increase of dissolution rate in the presence of the anaerobic fermentation products such as acetic acid, suggests that the overall efficiency of an integrated AD system can be improved by the fermentation process. This is mainly because the dissolution rate of the silicate minerals is indicated as the main rate controlling process in the mineral CO_2 sequestration process (Huijgen et al., 2003). A higher dissolution rate can also decrease the amount of silicate mineral required which can positively influence the feasibility of the process.

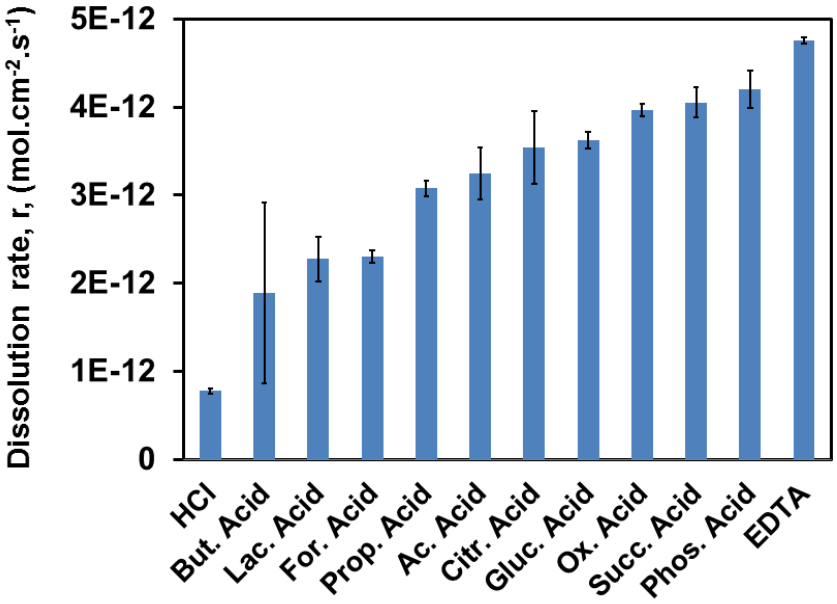

Figure 3.4 Increase of dissolution rate of wollastonite in presence of organic and inorganic ligands as compared to the base experiment with HCl.

3.5 Conclusions

Results of this study indicate that the dissolution rate of wollastonite can be enhanced as a result of the microbial activities in an anaerobic fermentation process through production of acids and organic ligands (e.g. acetate). Since organic ligands with two functional groups showed higher enhancement on the dissolution process compared to that of the monofunctional ligands (e.g. butyrate and propionate), directing the fermentation process towards production of these compounds can potentially increase the overall efficiency of the CO_2 sequestration in an integrated anaerobic digestion system with silicate minerals.

Chapter 4

Determining the impacts of fermentative bacteria on wollastonite dissolution kinetics

Applied microbiology and biotechnology

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4.1 Abstract

Silicate minerals can be a source of calcium and alkalinity, enabling CO_2 sequestration in the form of carbonates. For this to occur, the mineral needs to be first dissolved in an acidifying process such as the biological process of anaerobic fermentation. In the present study, the main factors which govern the dissolution process of an alkaline silicate mineral (wollastonite, $CaSiO_3$) in an anaerobic fermentation process were determined. Wollastonite dissolution kinetics was measured in a series of chemical batch experiments in order to be able to estimate the required amount of alkaline silicate that can neutralize the acidifying fermentation process. An anaerobic fermentation of glucose with wollastonite as the neutralizing agent was consequently performed in a fed-batch reactor. Results of this experiment were compared with an abiotic (control) fed-batch reactor in which the fermentation products (i.e. organic acids and alcohols) were externally supplied to the system at comparable rates and proportions, in order to provide chemical conditions similar to those during the biotic (fermentation) experiment. This procedure enabled us to determine whether dissolution of wollastonite was solely enhanced by production of organic acids or whether there were other impacts that fermentative bacteria could have on the mineral dissolution rate. The established pH profiles, which were the direct indicator of the dissolution rate, were comparable in both experiments suggesting that the mineral dissolution rate was mostly influenced by the quantity of the organic acids produced.

4.2 Introduction

The study of silicate mineral dissolution has attracted much attention during the past decade because these minerals can provide the necessary raw-materials (i.e. alkalinity and divalent cations) for CO_2 sequestration by mineral carbonation (Seifritz 1990). Application of microbial processes have been proposed as a cost-efficient method to enhance silicate mineral dissolution rate when compared to chemical and physical methods (Huijgen and Comans 2005). Since alkaline silicates dissolve more rapidly in lower pH environments (White and Brantley 1995), the biological process of anaerobic fermentation could potentially increase the dissolution rate of the mineral as a result of acid production (Ullman et al. 1996). This can offer an important benefit since anaerobic fermentation is a microbial process that is widely applied in biotechnological systems. Therefore, silicate minerals can potentially be integrated into such biotechnological systems providing the necessary raw materials for mineral CO_2 sequestration. In order to be able to describe the dissolution process in the anaerobic fermentation, the biochemical reactions that govern the dissolution of silicate minerals in such systems should be determined. Apart from acid production which can enhance the dissolution rate of silicate minerals by proton-promoted mechanism (White and Brantley 1995), anaerobic fermentative microbes might also affect the dissolution process through other mechanisms such as production of extracellular polymeric substance (EPS), or change of ionic strength (Bennett et al. 2001; Ehrlich 1996; Icenhower and Dove 2000; Pokrovsky et al. 2009). Ligand-interaction with organic ligands (e.g. acetate), is another mechanism whereby

the dissolution rate of silicate minerals can be accelerated (Pokrovsky et al. 2009; Ullman et al. 1996; Uroz et al. 2009). In addition, it has been reported that when microbes are attached to the mineral surface they are able to influence the dissolution of silicate minerals by acidolysis, chelation or oxido-reduction reactions which are defined as direct impacts (Bennett et al. 2001; Berthelin 1988; Frey et al. 2010; Rogers and Bennett 2004; Sand et al. 2001; Uroz et al. 2009). Assessing the exact role of microbes on mineral dissolution is a challenging task since environmental factors that are altered by microbial activities and mineral dissolution can influence one another simultaneously (Sand et al. 2001; Vandevivere et al. 1994). The main objective of the present study was to develop an experimental system to determine the main processes which can influence alkaline silicate mineral dissolution in an anaerobic fermentation process. The dissolution rate of wollastonite, which was used in the experiments as a model silicate mineral, was studied in an anaerobic fermentation experiment (biotic experiment). Subsequently, the fermentation products (i.e. organic acids and alcohols) produced during the biotic fermentation experiment were externally supplied to an abiotic (control 1) experiment at comparable rates (Fig. 4.1). We hypothesized that if the fermentative bacteria have additional impacts other than organic acid production on dissolution of alkaline silicate minerals, the dissolution rate in the biological fed-batch reactor would differ from that in the abiotic fed-batch reactor (control 1). Since organic acids can enhance dissolution rate of mineral by a proton-release mechanism or by acting as ligands, we determined the role of organic acids on the dissolution rate of wollastonite by operating an additional abiotic fed-batch reactor in the presence of inorganic acid that acts as the proton source, i.e. HCl (control 2). Comparison of the two abiotic control experiments with and without organic acids, could indicate whether organic acids only affect the dissolution process because of the acidity produced, or also due to the ligand-interaction (Fig. 4.1).

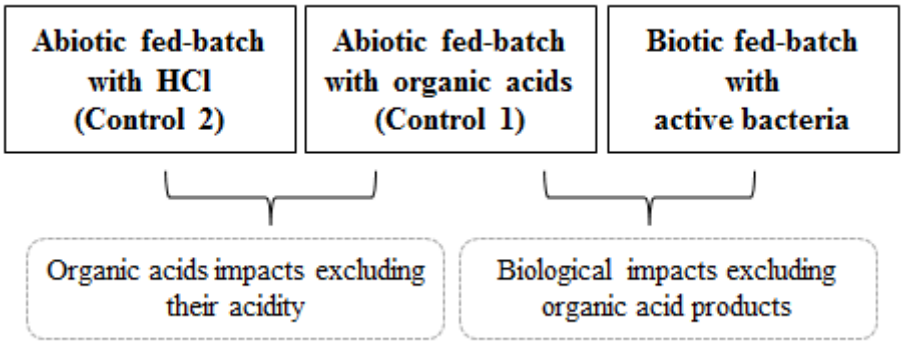

Figure 4.1 Experimental design of the fed-batch experiments.

4.3 Material and methods

Two types of experiments were performed: batch (abiotic) and fed-batch (abiotic and abiotic) experiments.

4.3.1 Reactor operation

All experiments (batch and fed-batch) were conducted in double jacket glass reactors with standard geometry and a working volume of 2 L (Applikon, The Netherlands). Each reactor was temperature controlled at 30 ± 1 °C with a water jacket and a thermostat bath (Lauda, Germany). The medium of the reactor was stirred at a rate of 500 rpm. Fig. 4.2 shows the schematic set up of a fed-batch reactor.

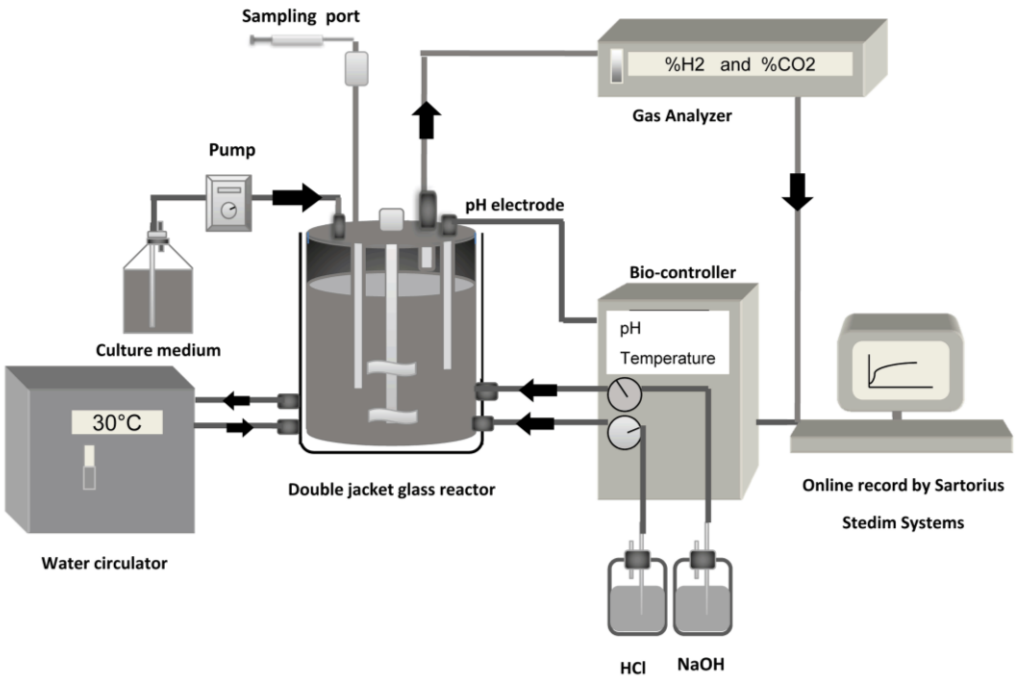

Figure 4.2 Schematic setup of the biotic fed-batch reactor.

4.3.2 Mineral characterization

To be able to neutralize the pH of the anaerobic fermentation process wollastonite was selected as the model mineral, since it was reported to be one of the more soluble silicate minerals (Murphy and Helgeson 1987). Wollastonite was provided by Tata Steel Company in the Netherlands. The crushed wollastonite was sieved in a clean environment in a fume hood. The size fraction of 125- to 250-µm was selected for all experiments. The particles were washed with deionized (DI) water at high pressure while being shaken in a sieve (125-µm)

until the effluent water was clear. The samples were dried for 24 hours in an oven at 100 ºC and then stored in a sealed plastic bag until used in an experiment. The specific surface area of the particles was determined by the Brunauer, Emmet, and Teller (BET) method with N_2 as the adsorbent. The specific surface area of cleaned powdered wollastonite was 0.1 $m^2.g^{-1}$. The composition of wollastonite was analysed by X-ray fluorescence (XRF) technique (Table 4.1), and X-ray diffraction (XRD) technique was used to investigate the structure of the precipitates at the end of the fed-batch experiments.

Table 4.1 Bulk composition of the original wollastonite as determined by X-ray fluorescence**.**

Component	Ca	Si	Fe	Al	Mg	Ti	Ba	P	S	Mn	Na	Ar	Cr
wt. %	65.2	32.2	0.82	0.81	0.34	0.09	0.07	0.07	0.06	0.05	0.04	0.04	0.02

4.3.3 Analytical techniques

A gas detection system was coupled to the bioreactor outlet to measure concentrations of O_2, H_2 and CO_2 during the experiment, with a Rosemount Analytical NGA 2000 MLT 1 Multi-component analyser (infrared detector). The pH, the N_2 flow rate, the stirring rate, and the temperature were controlled by a bio-controller (BIOSTAT B plus, Sartorius). Data acquisition of the direct online measurements of O_2, CO_2 and H_2 concentration, acid dosage, temperature, pH, and stirring rate was by MFCS/win (Sartorius Stedim Systems, USA) as illustrated in Fig. 4.2. In order to measure the elemental concentrations (Ca^{2+}, Si, Mg^{2+}, Al^{3+}, and Fe^{2+}) in the solution, samples taken from the reactor were first acidified with 1 ml hydrochloric acid (1 M), and then analysed with the inductively coupled plasma atomic emission spectroscopy (ICP-OES) technique. Organic acids and alcohols produced in the fermentation and glucose concentrations were determined by HPLC, using an Animez HPX-87H column from Bio Rad (T = 60ºC) coupled to an UV and a RI detector, while 0.01 M phosphoric acid was used as the eluent. A chrompack CP 9001 gas chromatograph (Chrompack, The Netherlands) equipped with a FID was used to verify the HPLC measurements and to distinguish butyrate and ethanol which feature similar HPLC retention times.

4.3.4 Batch experiments

A series of chemical batch experiments were conducted with 2.5 g-wollastonite.l^{-1}, to quantify chemical properties of the wollastonit. 2.5 g.l^{-1} was chosen to avoid potential re-precipitation of Si and Ca precipitates such as SiO_2. Before starting the batch experiments, the water solution was sparged with N_2 for 24 h with 1 L.min^{-1} at 700 rpm stirring speed. During the initial experimental hours the dissolution rate was not stable; therefore the dissolution rate was quantified over a 20 h-interval between the 4th to 24th hour of the experiment. The pH

was controlled by a pH electrode (pH ± 0.1) attached to an automatic titration device, using 0.5, 0.25 or 0.125 M HCl stock solutions depending to the desired pH value. All batch experiments were conducted in triplicate and were started with fresh wollastonite mineral (2.5 $g.L^{-1}$) and ultrapure water (Milli Q Plus water).

4.3.5 Fed-batch experiments

For this set of experiments, one biotic and two abiotic (control) fed-batch reactors were run. All fed-batch experiments were performed at a wollastonite concentration of 70 $g.L^{-1}$. The biotic fed-batch experiment was inoculated with 0.05 L of anaerobically digested sludge obtained from a local municipal wastewater treatment plant. Therefore, a mixed culture of anaerobic fermentative bacteria was expected to be present in the reactor. As a result of this inoculation in the biotic experiment the dissolution of wollastonite could be affected by presence of active fermentative bacteria and the microbially produced organic acids and alcohols. To one of the abiotic (control 1) experiments, the same fermentation products (i.e. organic acids and alcohols) that were produced during the biotic experiment were added at similar rates and in similar proportions. This experimental design enabled study of the effect of organic acids and alcohols to the exclusion of other possible effects of the fermentative bacteria on the dissolution rate (Fig. 4.1). In order to determine the effects of dissociated organic acids (i.e. organic ligands such as acetate), another abiotic fed-batch experiment (control 2) was run using HCl as neutralizing agent in place of the organic acids (Fig. 4.1). In this experiment, the proton dosage rate using 0.5 M HCl stock, was similar to the rate of proton generation in the abiotic experiment (control 1). For both abiotic (controls) fed-batch experiments, the reactor apparatus, organic acids and medium stock solutions were autoclaved prior to use in experiments. In all fed-batch experiments, the alkalinity source, which was the wollastonite, was added at the beginning of the experiment. This was because continuous addition of crushed silicates by pumps was not possible due to the solid nature of the mineral.

4.3.6 Dissolution rate measurement

Wollastonite dissolution rate was measured based on two methods which were proton consumption rate and the rate of release of Ca^{2+} (reaction 4.1). As shown in reaction 1, one mole of wollastonite consumes two moles of protons and releases one mole of calcium. Since wollastonite was used as the alkalinity source in the experiments, the acid that was supplied to or generated in the reactor was neutralized by the dissolution of the mineral. Therefore, the dissolution rate of wollastonite at stable pH was calculated from the amount of acid dosed which was verified by Ca ion measurements.

$$CaSiO_3 + H_2O + 2H^+ \rightarrow H_4SiO_4 + Ca^{2+} \qquad \text{(reaction 4.1)}$$

4.3.7 Stock solution preparation

Glucose and mineral salts solution were added continuously to the biological fed-batch reactor. The glucose solution and the solution containing the mineral salts were prepared and fed separately to the reactor in order to prevent microbial growth in the stock solutions before feeding. Glucose was added at a rate of 1 $g.L^{-1}.d^{-1}$ (i.e. 0.7 $mg.min^{-1}$). The constituents of the mineral salts solution were added at the rates of ($mg.L^{-1}.d^{-1}$): NH_4Cl 670, KH_2PO_4 390, NaCl 146, $Na_2SO_4.10H_2O$ 65, $MgCl_2.6H_2O$ 60, $ZnSO_4.7H_2O$ 2.6, $NiCl_2.6H_2O$ 2.4, $CuCl_2.2H_2O$ 1.1, $MnCl_2.2H_20$ 1, $Na_2MoO_4.2H_2O$ 0.05, $CoCl_2.6H_2O$ 0.48, H_3BO_3 0.04, and EDTA 25.

A stock solution of organic acids and alcohols was prepared for the abiotic fed-batch experiment (control 1). Continuous dosing of the stock solution into the abiotic fed-batch experiment should have resulted in the same concentration profiles of organic acids and alcohols of the biotic reactor. This was verified by measuring the concentration of fermentation products during the control 1 experiment. Taking into consideration that the liquid volume in the bioreactor was constantly increasing because of the continuous feeding of the glucose and mineral salts solutions, we calculated with approximations the concentration of organic acids and alcohols when preparing the stock solutions for the abiotic fed-batch experiments, using the following equation (4.1):

$$C_t = \frac{C_0 V_0 + Q C_i t}{V_0 + Qt} \qquad (4.1)$$

Where C_t, C_i and C_0 are respectively, the concentration of fermentative products in the abiotic fed-batch experiment (mM), in the stock solution (mM), and the initial concentration in the reactor (mM). The Q is the dilution factor in $L.d^{-1}$, V_0 is the volume in L at time 0, and t is the time in days. The concentration in the stock solution was obtained by fitting the equation to the measured concentrations of organic acids and alcohols in the biotic experiment.

4.4 Results

4.4.1Quantification of wollastonite dissolution kinetics by chemical batch experiments

A series of chemical batch experiments was conducted to quantify the dissolution kinetics of wollastonite. With the results obtained from these batch experiments, the amount of wollastonite required to neutralize the acids produced by fermentative bacteria during the biotic fed-batch experiment could be estimated. The dissolution kinetics of wollastonite (2.5 $g.L^{-1}$) in distilled water in the pH range of 3 to 8 is shown in Fig. 4.3. The results obtained showed that at lower pH values the dissolution rate of wollastonite increased which was in agreement with previously reported results (e.g. Pokrovsky et al. 2009; Weissbart and

Rimstidt 2000). The average specific dissolution rates of wollastonite in a pH range of 3 to 8 derived from the chemical batch experiments were expressed by the following equation (4.2):

$$r_w = k_w(a_{H^+})^{p_w} \qquad \text{(equation 4.2)}$$

Where r_w , a_{H^+} and k_w are respectively, wollastonite surface specific dissolution rate (mol.s^{-1}.m^{-2}), proton activity, and the dissolution rate constant of wollastonite (mol.s^{-1}.m^{-2}) at 30°C. The power number was determined to be $p_w = 0.43$. The rate constant (k_w) was 5.52×10^{-11} mol.s^{-1}.cm^{-2} and the R^2 value was 0.96 when fitted to experimental data. Using the results obtained from the batch experiments, the specific proton consumption rate of wollastonite (mol.h^{-1}.g^{-1}) for each pH value was calculated (Table 4.2).

During the chemical batch experiments, it was also observed that the dissolution rate of wollastonite decreased on average by almost 0.25 % per hour (data not shown). Decrease of dissolution rate of silicate minerals with time was also previously reported in laboratory experiments (Stillings and Brantley 1995; Welch and Ullman 1993; White and Brantley 2003). The time dependency of dissolution of the silicate minerals was attributed to different factors such as production of secondary precipitates, accumulation of leached layers, and increase of surface area (White and Brantley 2003). This decrease in dissolution rate during the experiment should be considered when estimating the amount of wollastonite that needs to be added to the biotic fed-batch experiment for pH neutralization.

The results from the batch experiments confirmed that the protons consumed and Ca ions released to the solution followed the overall stoichiometry of reaction 4.1 (the release of each mole of Ca from the wollastonite related to the consumption of two moles of protons). The same stoichiometric behaviour (i.e. $\frac{Ca^{2+}}{H^+} = 0.5$) was also observed in previous studies (Rimstidt and Dove 1986; Xie and Walther 1994). Therefore, the release rate of Ca ions and proton consumption rate were linked to one another.

Table 4.2 Wollastonite needed to neutralize completely the protons produced during fermentation. The amount of wollastonite required was estimated from its specific proton consumption rate and the biological proton production rate.

pH	Wollastonite specific H^+ consumption rate ($mol\text{-}H^+.d^{-1}.g^{-1}$)[a]	Required wollastonite ($g.L^{-1}$)[b]	Quantity of wollastonite required for duration of experiment ($g.L^{-1}$)[c]
8	2.8×10^{-4}	57	81
7.5	3.2×10^{-4}	50	70
7	4.6×10^{-4}	34	49
6	8.4×10^{-4}	19	27
5	1.1×10^{-3}	15	21
4	3.2×10^{-3}	5	7
3	9.9×10^{-3}	1.6	2

a Wollastonite specific H^+ consumption rate by dissolution of wollastonite based on chemical batch experiments results.
b Wollastonite required to neutralize the acid-production in the anaerobic fermentation process which was estimated based on the assumption of 1.5 $mol\text{-}H^+.mol\text{-}glucose^{-1}$ conversion.
c The total amount of wollastonite required to neutralize the fermentation acids over the duration of the experiment, is listed in the fourth column of the table. Because of the decrease in dissolution rate of wollastonite, due to probably production of secondary precipitates on the mineral surface with time (which was observed in the chemical batch experiments), extra mineral was required to be supplied to the reactor in order to keep the pH constant.

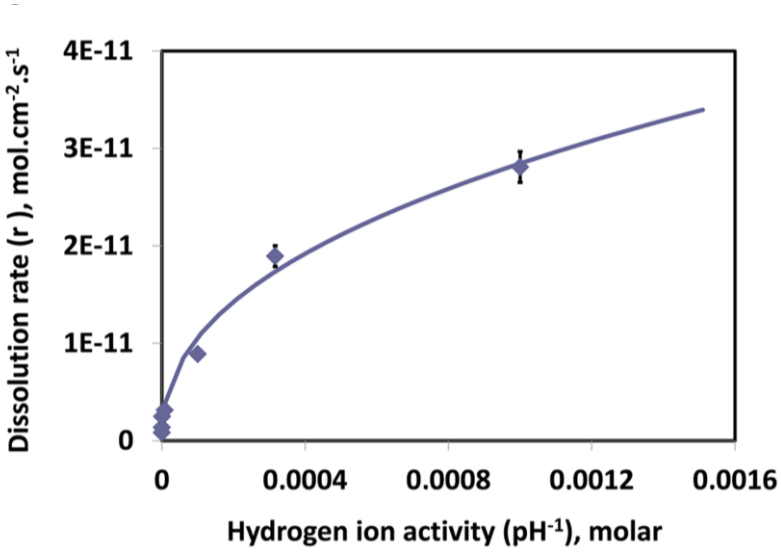

Figure 4.3 Dissolution of wollastonite (mol.cm^{-2}.s^{-1}) in chemical batch experiments at pH range of 3-8. The solid line represents the best fit of the data to the equation.

4.4.2 Wollastonite as neutralizing agent in a fermentation process

Impacts of anaerobic fermentative bacteria on the alkaline silicate dissolution were investigated by adding a certain amount of wollastonite to an anaerobic fermentation process. In order to ensure that the microbial culture was not inhibited, the fermentation experiment should be run at a stable pH value. In an acid-producing system with wollastonite as neutralizing agent, stable pH is achieved when all protons produced are balanced by the alkalinity released from dissolution of wollastonite. In order to know how many protons should be neutralized, the proton production rate in the fermentation fed-batch experiment was estimated. The proton production rate in the biotic fed-batch reactor could be estimated by knowing the ratio of protons produced to the glucose consumed in the fermentation process. A study performed by Temudo et al. (2007) showed that a fermentation process in the pH range of 4 to 8.5 would result in 1.5-2 moles of protons per mole of glucose fermented. Therefore, with 1 g.L^{-1}.d^{-1} glucose dosage in the biotic fed-batch experiment and assuming a production of 1.5 mole of H^+ per mole of glucose^{-1}, the rate of proton production by fermentative microbes was estimated to be 8.3 mmol-H^+.L^{-1}.d^{-1}. According to the specific proton consumption rate (mol-H^+.d^{-1}.g^{-1}) of wollastonite at different pH values (Table 4.2) which were known from the batch experiments, the amount of mineral required to neutralize the protons produced, at constant microbial activity, could be calculated. A pH value close to the neutral pH (pH 7.5) was chosen for the fermentation fed-batch experiment because at this pH value most of the organic acids are in their dissociated form, and the fermentative activity is not inhibited. At the chosen pH of 7.5, 50 g wollastonite per liter was sufficient to neutralize the protons estimated to be produced by the fermentation (Table 4.2).

However, as was found in the abiotic batch experiments, the dissolution rate of wollastonite slowed for different reasons such as production of secondary precipitates on the mineral

surface. Such slowing of dissolution rate meant that more wollastonite was needed to be added in order to keep the pH stable for the experimental period (i.e. 7 days). Based on results of the batch experiments, there was on average 0.25 % decrease in dissolution rate per hour. Therefore, an additional amount of mineral, calculated for each pH (see Table 4.2), was required to maintain the desired pH. Consequently, 70 $g.L^{-1}$ wollastonite was added at the beginning of the experiment to the fed-batch reactor with 1 $g\text{-}glucose.l^{-1}.d^{-1}$ to obtain a stable pH value of 7.5 during the experimental period (170 h).

4.4.3 Wollastonite dissolution by anaerobic fermentative bacteria

To analyse the wollastonite dissolution during the fermentation process the pH profile of the biotic fed-batch experiment was studied. This was possible in our fed-batch experiments, in which the organic-acid production rate was constant, because the pH profile reflected the proton consumption rate and, therefore, the wollastonite dissolution rate. As shown in Fig. 4.4a, the pH initially increased to 7.9 due to dissolution of wollastonite. The pH stayed at 7.9 for the next 9 h because of the bacterial lag phase. Thereafter, the pH rapidly decreased to 6.2 as a result of organic acids production by the fermentative activity (Fig. 4.4b). A subsequent relatively fast increase in pH to a value of 6.7 at 20 h was due to the accelerated dissolution rate of wollastonite at lower pH values (Fig. 4.3). The observed rapid decrease and increase of pH value during the initial hours (from 0 h to 20 h) was due to the low buffering capacity of the solution. During the next 70 hours, the pH remained relatively stable at 6.7 ± 0.1. This was attributed to the establishment of a balance between mineral dissolution and biological acid production. Then, the pH slightly decreased to 6.5 and remained stable once more for nearly 55 hours (from 115 h to 170 h). As the proton production stayed constant during the overall experiment, the slight decrease of pH during the period of 90 h to 110 h was probably due to the smaller availability of mineral. During the overall experiment, the ratio of calcium ion released to proton consumed was confirmed to be $\frac{Ca^{+2}}{H^{+}} = 0.5$ (Fig. 4.6a). As shown in Fig. 4.4c, dissolution of wollastonite ($CaSiO_3$) resulted in continuous release of Ca ion. However, Si ion showed no increase over time suggesting Si re-precipitation or incongruent form of mineral dissolution.

In order to assure that all organic carbon dosed in the biotic experiment was taken into account the carbon balance was calculated. Biomass, organic products and CO_2, accounted for 96 ± 3% of the carbon supplied to the system.

4.4.3 Determining the impacts of fermentative bacteria on wollastonite dissolution rate

Anaerobic fermentative bacteria can potentially affect the dissolution of wollastonite by different factors e.g. production of protons, ligands, EPS, and change of ionic strength (Bennett et al. 2001; Ehrlich 1996; Icenhower and Dove 2000; Pokrovsky et al. 2009). To exclude impacts of the organic acids produced during the fermentation process from other

impacts of fermentative bacteria, the same fermentation products of the biotic experiment were externally supplied to an abiotic fed-batch experiment (control 1) at comparable concentrations. As shown in Fig. 4.5a, the pH profile of the abiotic (control 1) reactor followed the same trend as that of biotic fed-batch reactor (Fig. 4.4a), only at a somewhat higher pH (average pH difference of 0.76 ± 0.06). The accountable proton quantity for such pH difference was negligible (less than 1 %) compared to the total neutralized protons from the dissociated organic acids (Fig. 4.5b). Therefore, the rate of proton consumption (i.e. rate of wollastonite dissolution) in the biotic experiment was similar to that in the abiotic fed-batch experiment (in the presence of organic acids). This indicates that the fermentative bacteria enhanced the dissolution of wollastonite mainly by the action of the organic acids they produced during fermentation.

To examine whether the dissociated organic acids influence wollastonite dissolution by ligand-interaction mechanism or by releasing protons, an additional abiotic fed-batch experiment (control 2) was conducted in the absence of organic acids (Fig. 4.1). In this experiment HCl was used as the source of acidity source. The proton dosage rate was the same as in the abiotic fed-batch experiment (control 1) as shown in the figure (Fig. 4.7). The established pH profile (i.e. proton consumption rate) exhibited the same range and profile as that of the abiotic fed-batch experiment (Fig. 4.5a). It was also confirmed that the Ca^{2+} released to H^+ consumed was similar to the ratio of $\frac{Ca^{2+}}{H^+} = 0.5$, in control 2 experiment (Fig. 4.6b). Because the dissolution rates of wollastonite in the presence of organic acids (that can act as organic ligands) and in the presence of inorganic acid, i.e. HCl, were similar, it indicated that the protons released by organic acids was the main factor for the wollastonite mineral dissolution in the anaerobic fermentation process.

The Ca ion showed comparable release rate in the biotic and the abiotic fed-batch (control 2) experiments (Fig. 4.4c and Fig. 4.5d). In the abiotic fed-batch experiment with organic acid addition (control 1) however, Ca ions appeared to be released at a slower rate (Fig. 4.5c). Since similar proton consumptions were observed in all fed-batch experiments, and Ca ion release and proton consumption were shown to be linked to one another ($\frac{Ca^{2+}}{H^+} = 0.5$), the lower Ca ion concentration in the control 1 was probably due to experimental measurement error.

Finally, the dissolution behaviour of wollastonite was studied by comparing the concentration of dissolved Ca and Si ions. The concentrations (mM) of Ca and Si ions in abiotic fed-batch experiments (Fig. 4.5c and Fig. 4.5d) showed a large difference, similar to what was observed in the biotic fed-batch experiment.

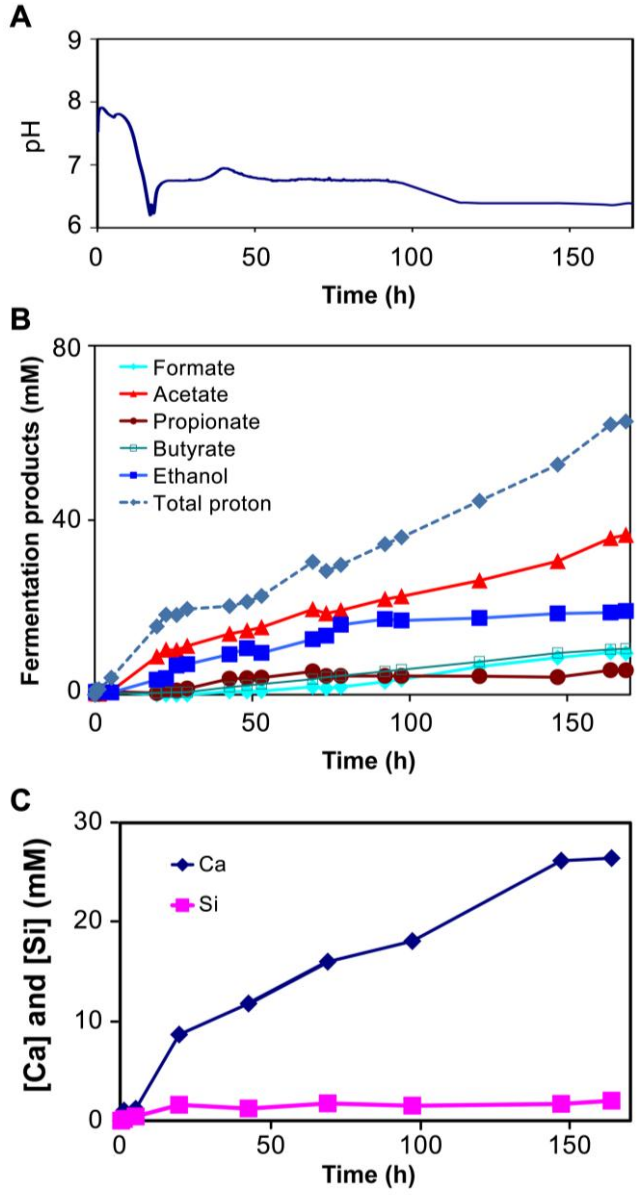

Figure 4.4 Quantification of wollastonite dissolution kinetics in the biotic fed-batch reactor. The pH profile (A), the concentration of fermentation products (mM) and the total proton production (mM) (B), and cumulative concentrations of Ca ion and Si ion released by dissolution of wollastonite (C).

4.5 Discussion

4.5.1 Kinetics and applications of wollastonite dissolution in an anaerobic fermentation process

The results of this study showed that the activity of the fermentative bacteria resulted in continuous dissolution of wollastonite. Conducting biotic and abiotic (control 1) fed-batch experiments examined whether fermentative bacteria dissolve the silicate minerals solely by production of organic acids or by additional processes. Having identical organic acids and concentrations in both fed-batch reactors (Fig. 4.4b and Fig. 4.5b), with and without the presence of bacteria, could indicate other possible effects that planktonic bacteria may have on dissolution of wollastonite besides the effect of the organic acids they produced. If fermentative bacteria had shown additional positive impacts on the dissolution rate than production of organic acids, a higher pH would have been expected in the biotic fed-batch

experiment. This is because, the fed-batch experiments were designed in a way that the pH profile directly reflected the wollastonite dissolution rate. Since the proton production rate were nearly the same in the fed-batch reactors (see total proton production in Fig. 4.4b and Fig. 4.5b) it was the proton consumption by dissolution of the wollastonite that determined the pH. This experimental design has the advantage of directly assessing the dissolution rate by looking at pH-values. Comparison between the pH values established in the biotic and abiotic experiments revealed that comparable dissolution rates were observed in fed-batch experiments. Therefore, the dissolution of wollastonite was mainly enhanced by the organic acids produced. Operation of an additional abiotic fed-batch experiment in the absence of organic acids but in the presence of protons (control 2), showed that the organic acids were increasing the dissolution rate through the protons associated with them and not through complexation by their anions. Noticeable chelation impacts of the organic ligands on silicate mineral dissolution rate have been reported at relatively high concentrations of these compounds (Pokrovsky et al. 2009; Vandevivere et al. 1994; Wogelius and Walther 1991). As an example, the study of Pokrovsky et al. (2000) showed that the necessary concentration for acetate in order to increase the dissolution rate of wollastonite was above 0.1 M. In the fed-batch experiments, acetate (the most plentifully produced organic ligand) reached the maximum concentration of 0.03 M, at the end of the experiment. The low concentration of organic acids in our experiments was possibly the reason for the observed low impact of organic ligands on the dissolution rate.

The results of the present study showed that the fermentative organisms enhance wollastonite dissolution mainly by biological proton production. Consequently, alkaline silicate mineral dissolution and behaviour can be readily explained and may be predicted by the chemical reactions involved.

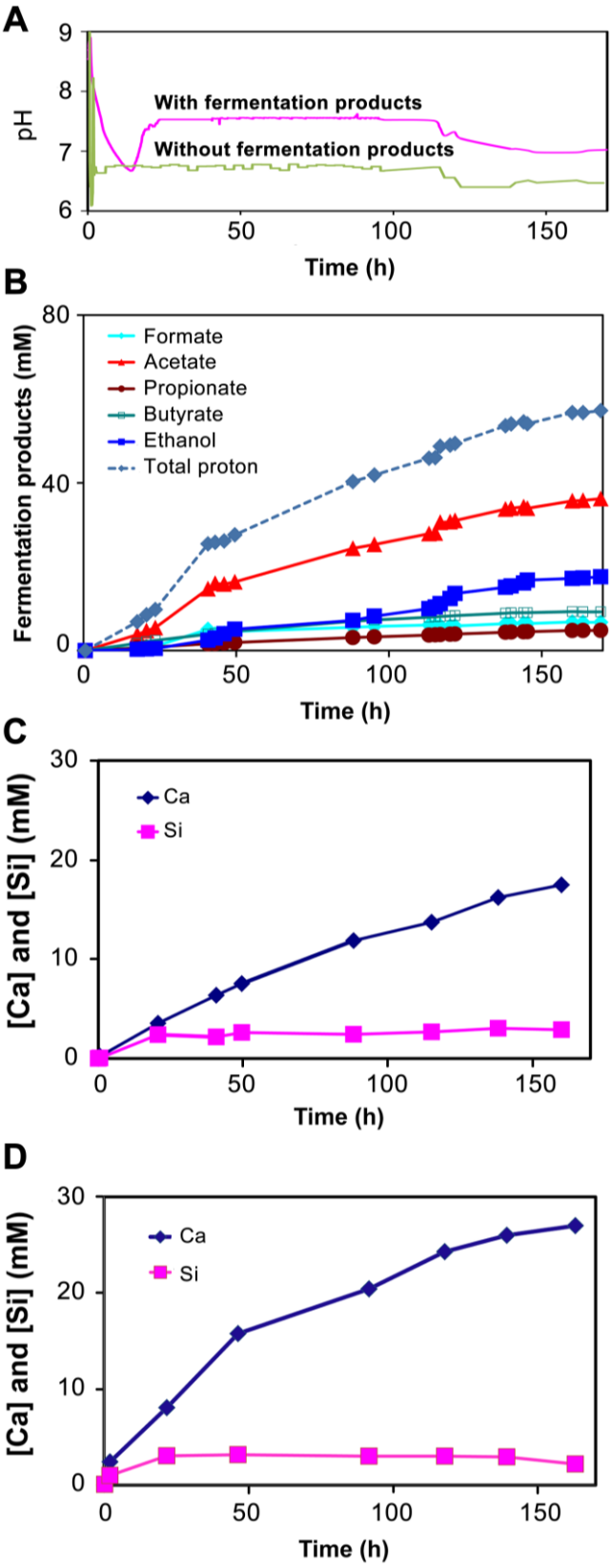

Figure 4.5 Quantification of wollastonite dissolution kinetics in the abiotic fed-batch reactors. The pH profile of the abiotic fed-batch experiments in presence of the added organic acids (control 1), and in the absence of the added organic acids (control 2) (A). The concentration of fermentation products added (mM) and total added protons (mM) to the abiotic fed-batch reactor (B). Cumulative concentrations of Ca ion and Si ion released by dissolution of wollastonite in abiotic fed-batch experiments: in the presence of the added organic acids (C) and in absence of the added organic acids (D).

4.5.2 Si precipitation or/and incongruent dissolution of wollastonite

In the biotic and abiotic fed-batch experiments it was observed that while calcium ion concentration increased, the concentration of silicon ion stayed low during the wollastonite dissolution which could have been due to Si re-precipitation in form of secondary minerals or incongruent wollastonite dissolution by two mechanisms described in previous studies (Green and Luttge 2006; Murphy and Helgeson 1987; Weissbart and Rimstidt 2000; Xie and Walther 1994). In order to examine the Si re-precipitation mechanism, the structure of the precipitate at the end of the experiments was examined by XRD analysis. Calcium silicates (Ca_2SiO_4) and silicon dioxide (SiO_2) were formed during the experiments in low quantities (data not shown). However, because of the large difference between Si and Ca concentrations, the low quantities of Si precipitates did not appear to account for the lost Si concentration in the solution. Therefore, incongruent dissolution of wollastonite was more probable. Further research is required to confirm this interpretations.

As a result of Si or Ca precipitation, the commonly used method to calculate dissolution rate of silicate minerals based on elemental (e.g. Ca or Si ion) release rate (Ullman et al. 1996; Vandevivere et al. 1994), may result in underestimation of the dissolution rate. Verification of the dissolution rate by the alkalinity release rate corresponding to the rate of dissolution of alkaline silicates can increase the measurement accuracy.

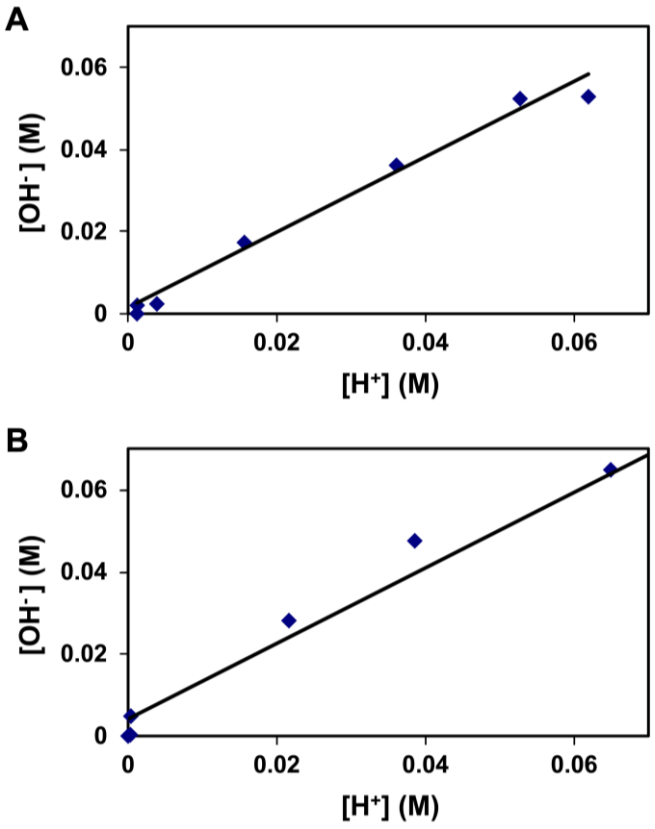

Figure 4.6 Cumulative equivalent OH^- concentration (M) obtained from Ca ion released because of wollastonite dissolution vs. equivalent H^+ released (M) by dissociated organic acids, in the biotic fed batch experiment (A). Cumulative equivalent OH^- concentration (M) obtained from Ca ion released because of wollastonite dissolution vs. equivalent H^+ released (M) by HCl in the control 2 experiment (B).

4.5.3 Application of the alkaline silicate dissolution in fermentation process

Dissolution of calcium silicate minerals such as wollastonite in a fermentation process can provide the raw materials for a CO_2 sequestration process. As observed in the present study, continuous release of Ca ion (i.e. divalent cation) (Seifritz 1990) can be achieved by introducing alkaline silicates into an anaerobic fermentation.

If the Ca ions come into contact with carbonate ions (CO_3^{2-}) they can precipitate in the form of carbonate calcium ($CaCO_3$) based on the solubility product of e.g. amorphous calcium carbonate which is 4×10^{-7} M^2 at 25 °C (Brečević and Nielsen 1989). The storage of CO_2 in the form of carbonate minerals has been proposed as a sustainable CO2 sequestration method for climate change mitigation (Seifritz 1990). The same concept can be used for practical application of the present study. In natural environments, fermentation process occurs prior to the methanogenic conversion in which carbon dioxide, and alkalinity are produced. Alkalinity generated during methanogenic process can chemically convert the atmospheric CO_2 to carbonate ion (CO_3^{2-}). Therefore, calcium carbonate ($CaCO_3$) can precipitate with Ca ion which is obtained from dissolution of calcium-silicates (e.g. wollastonite) in the fermentation process. This would indicate a potential use of fermentative and methanogenic bacteria for precipitation of calcium carbonate minerals (Datta et al. 2010). Since fermentation and methanogenic processes are commonly used to treat biodegradable waste and sewage sludge, mineral CO_2 sequestration process can potentially be integrated into waste and wastewater treatment facilities. Whereas the silicate minerals are dissolved during the fermentation process in the first stage, carbonate minerals can be formed in the second stage of methane production.

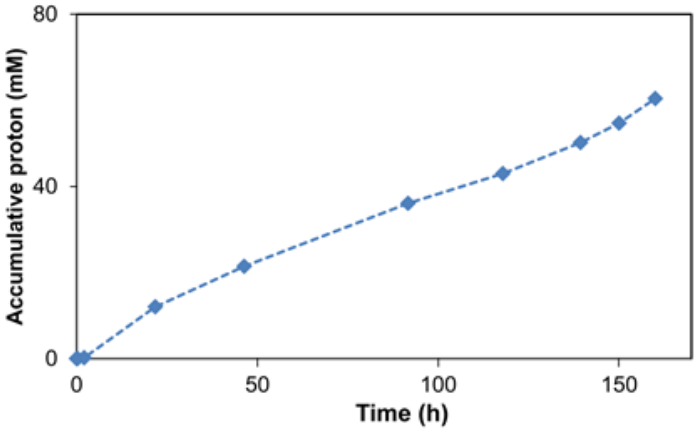

Figure 4.7 The proton dosage rate in the abiotic fed-batch experiment (control 2) which was the same as in the control 1 experiment.

4.5.4 Optimization of the wollastonite dissolution by a fermentation process

The fermentation process can be further optimized in order to achieve higher dissolution rate of alkaline silicate minerals. The fermentation process could be operated at higher substrate supply rate or lower alkaline mineral concentrations which will result in lower pH values accelerating the dissolution rate. Directing the fermentation process to obtain more effective organic acids with more carboxyl group such as succinic acid might also increase the solubility of the minerals through a ligand-promoted mechanism (Drever and Stillings 1997; Welch and Ullman 1993). In addition, biofilm production on mineral surface might offer microenvironments with high concentration of organic acids which might result in higher release rate of divalent cations (Hiebert and Bennett 1992; Liermann et al. 2000; Ullman et al. 1996).

Chapter 5

Kinetics of $CaCO_3$ precipitation in an anaerobic digestion process integrated with silicate minerals

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5.1 Abstract

Integration of silicate minerals in the anaerobic digestion (AD) process has recently been proposed as a way to sequester CO_2, neutralize the pH and produce biobased products such as biogas and biofertilizers. The objective of this study was to investigate the kinetics of $CaCO_3$ precipitation in an anaerobic digestion process integrated with wollastonite. The first series of experiments studied the precipitation kinetics during the methanogenic phase by addition of calciumacetate to the anaerobic batch reactors. $CaCO_3$ precipitation initiated when the ion activity product of Ca^{2+} and CO_3^{2-} exceeded calcium carbonate monohydrate solubility product (K_{sp} of 5.7×10^{-8} at 35°C). Addition of calcite seed crystal at the start of the experiment showed that the precipitation could be initiated earlier by reducing the induction period. In these experiments, precipitation of CO_2 in the form of $CaCO_3$ showed an 52 % increase in CH_4 content of the biogas (71 ± 0.5 % v/v CH_4) compared to the control experiment fed with sodium acetate (53 ± 0.5 % v/v CH_4).

In a second set of experiments a single-stage anaerobic batch digestion with 25 g/l wollastonite (63-125 µm) at two different substrate concentrations (5.5 g/l and 17.5 g/l insoluble starch) was studied. In these experiments wollastonite dissolution and calcite precipitation was observed, but the limited separation of fermentation and methanogenesis phases resulted in a limited increase in methane content of the biogas. Although the precipitation was observed at lower supersaturation, the crystal growth followed the same pattern as in previous experiments with acetate. In both sets of experiments, a self-regulating pH could be achieved in the system as the result of the presence of wollastonite which caused the pH to remain in the range of 5.7-7.9. Obtaining an improved biogas and a self-regulated pH in a digester contribute to the advantage of CO_2 sequestration by means of silicate minerals in anaerobic digestion systems.

5.2 Introduction

5.2.1 Mineral carbonation of CO_2 by biotechnological processes

Mineral CO_2 sequestration (also known as mineral carbonation of CO_2) is one of the natural negative feedback mechanisms responsible for capture and sequesteration of 100 million tons of carbon every year (1.8-2 % of the anthropogenic carbon) (Dunsmore, 1992; Walker et al., 1981a). The process is based on natural weathering of the silicate minerals (Seifritz, 1990). The effectiveness of the process for climate change mitigation purposes is however limited due to the slow kinetics of the CO_2–silicate reactions (Oelkers et al., 2008). Lackner et al. 1995 introduced a number of chemical and physical methods to increase the rate of the process by accelerating the weathering rate of silicate minerals and precipitation rate of carbonate minerals (Lackner et al., 1995). Application of biological processes has also shown to be a cost-effective approach for accelerating CO_2 sequestration by mineral carbonation (Bennett et al., 2001; Cailleau et al., 2004; Castanier et al., 1999; Huijgen et al., 2003; Mirjafari et al., 2007; Pokrovsky et al., 2009). Four biotechnological processes of: anaerobic

digestion (AD), nitrogen removal, desulfurization and bioelectrochemical systems characterized by a sequence of an acid- and an alkalinity- producing steps were specified to be able to adopt the mineral CO_2 sequestration process (Salek et al., 2013b). Considering the technology applicability and CO_2 sequestration yield (mole-CO_2/mole-substrate), the anaerobic digestion process appears to have the highest potential for application of mineral CO_2 sequestration among the proposed biotechnological processes (Salek et al., 2013b). In addition to the CO_2 sequestration, integration of silicate mineral-based CO_2 sequestration into AD systems was suggested to offer important added values to the system: self-regulating pH system and production of biobased products such as methane enriched biogas and biofertilizers (Salek et al., 2013b; Schuiling, 2009; Vanherk et al., 1989). In a study performed by Lindeboom et al. (2013) on autogenerative high pressure digestion systems (AHPD), the addition of wollastonite (particle size of 20 µm) resulted in improvement of methane content of the biogas up to 88 % v/v at pressure of 9 bar and a self-regulating pH system (Lindeboom et al., 2013). The neutralization capacity of wollastonite was also confirmed in other studies on fermentation process (Fernández-Caliani et al., 2008; Salek et al., 2013c).

Main challenges for implementation of the wollastonite in an anaerobic digestion system are: (i) the high costs associated to silicate minerals grinding (Gerdemann et al., 2007), (ii) the precipitates residues in the reactor vessels, and (iii) the slow kinetics of silicate mineral dissolution and carbonate mineral precipitation. To address these challenges, in the current study, we performed experiments with relatively larger silicate particles (i.e. 63-125 µm) compared to the previous studies (Huijgen et al., 2006; Lindeboom et al., 2013) while studying the residual composition and the kinetics of wollastonite dissolution and $CaCO_3$ precipitation.

5.2.2 Theoretical Aspects

To be able to fully achieve the benefits proposed (improved biogas quality, self-buffering system, and production of fertilizing worth residual) in an single-stage anaerobic digestion system with silicate minerals as shown in the overall reaction 5.1, the chemical and biological reactions should occur in a right order (Table 5.1). This means that the fermentation process should initially provide sufficient acidity for dissolution of silicate minerals (e.g. wollastonite) and release of divalent cations (Table 5.1, reactions 5.2 and 5.3) (Salek et al., 2013c; Ullman et al., 1996). The produced CO_2 in this stage is unlikely to be captured, because of the low conversion of CO_2 to the carbonate ion due to the low pH. Therefore, it is favorable to choose for fermentation pathways where no CO_2 is produced similar to the case of homolactic fermentation (i.e. one molecule of glucose is converted to two lactic acid and no CO_2, $C_6H_{12}O_6 \rightarrow 2C_3H_6O_3$). In the next phase, the released divalent cations such as calcium ion and the produced alkalinity by the methanogenesis activity result in capturing the CO_2 in form of carbonate minerals (Table 5.1, reactions 5.4 and 5.5). A delay in CO_2 precipitation due to precipitation kinetics or low concentration of ion activity product (IAP) of (Ca^{2+}) (CO_3^{2-}) causes loss of CO_2 to the atmosphere and therefore limited improvement of the biogas quality. This can be prevented by having a closed system where CO_2 cannot escape to the air

resulting in a higher partial pressure of CO_2 in the reactor (Lindeboom et al., 2013). Pressurized reactors can be used but they are economically not preferred. The kinetics of $CaCO_3$ precipitation can be improved through several methods such as addition of pre-nucleation clusters (Cacciuto et al., 2004; Gebauer & Cölfen, 2011).

In addition to enhancement of $CaCO_3$ precipitation kinetics, another way to increase the efficiency of CO_2 capture and sequestration, is formation of more stable forms of $CaCO_3$ polymorphs which decrease the chance of carbonate mineral re-dissolution and the potential escape of CO_2 to the atmosphere. $CaCO_3$ is known to occur in unstable and more stable polymorphs. The $CaCO_3$ polymorphs in decreasing stability are: calcite, aragonite, vaterite, monohydrocalcite, ikaite and amorphous calcium carbonate (Meiron et al., 2011; Radha et al., 2010). Although $CaCO_3$ deposition in anaerobic reactors is commonly occurring and therefore it has been extensively studied (Langerak et al., 1999; Lier & Boncz, 2002), its interconnections with silicate mineral dissolution and biogas improvement have not been studied. In this study we have investigated the kinetics of calcium carbonate precipitation and its effects on biogas enrichment from starch fermentation and pH neutralization in an integrated AD system with wollastonite.

Table 5.1. The main chemical and biological reactions occurring in an integrated AD system with wollastonite.

Number	Reaction
5.1	$\frac{2}{3n}(C_6H_{10}O_5)_n + \frac{2}{3}H_2O + CaSiO_3(s) \rightarrow SiO_2(s) + CO_2(g) + 2CH_4(g) + CaCO_3(s)$
5.2	$\frac{2}{3n}(C_6H_{10}O_5)_n + \frac{2}{3}H_2O \rightarrow \frac{2}{3}C_6H_{12}O_6$
5.3	$\frac{2}{3}C_6H_{12}O_6 \rightarrow 2C_2H_3O_2^- + 2H^+$
5.4	$CaSiO_3 + 2H^+ \rightarrow Ca^{+2} + H_2O + SiO_2$
5.5	$2C_2H_3O_2^- + 2H^+ \rightarrow 2CH_4 + 2CO_2$
5.6	$Ca^{2+} + CO_2 + H_2O \rightarrow CaCO_3 + 2H^+$

5.3 Materials and Methods

5.3.1 Experimental set-up

All experiments were conducted in a double jacket glass reactor with standard geometry and a working volume of 2 L at atmospheric pressure. The reactor was temperature controlled at 35 ± 1 °C with a system of temperature probe, water jacket and a thermostat bath (Lauda, Germany). The reactor was equipped with pH (Mettler Toledo), conductivity (Consort C832) and temperature probes.

5.3.2 Operation

In total, five anaerobic batch experiments were performed: three experiments with acetate and two experiments with starch to study the precipitation in the methanogenic phase and in the anaerobic digestion, respectively. Among the three experiments with acetate, two were conducted with 5.2 ± 0.1 g/l calcium acetate ($Ca(CH_3COO)_2$) in which one was supplemented with 5.5± 0.1 g/l calcite seed crystals. The third experiment was performed as a control in a calcium free environment by addition of 1.1 g/l sodium acetate ($NaCH_3COO$) to the reactor.

The subsequent series of experiments were carried out with 25 ± 0.2 g/l wollastonite (63-125 µm particle size) at two different substrate concentrations: 5.5 ±0.1 g/l and 17.5 ±0.1 g/l potato starch $(C_{12}H_{22}O_{11})_n$ (Fluka). The wollastonite was pre-treated (ground and washed) prior to the experiments as described previously (Salek et al., 2013c). The surface area of the cleaned powdered wollastonite was measured to be 0.17 m^2/g using the Brunauer, Emmet, and Teller (BET) method with N_2 as the adsorptive. The composition of the wollastonite was determined to be (% wt/wt): 64.2 Ca^{2+}, 34.0 Si^{4+}, 0.89 Fe^{3+}, 0.27 Al^{3+} and 0.16 Mg^{2+} by X-ray fluorescence (XRF) (concentrations of elements lower than 0.1 % are not indicated).

To ensure anaerobic conditions, N_2 gas was sparged in the headspace of the reactor with the rate of 0.5 L/min meaning that the gases produced (CO_2 and CH_4) were stripped out from the gas phase. The reactor was mixed at a rate of 200 rpm with an impeller. The pH was not controlled in the experiments.

5.3.2.1 Stock solution preparation

Mineral medium containing growth nutrients and trace elements was prepared in demi water and supplied to the reactor prior to the batch experiments. Nutrient concentrations in the experiments with acetate were (g/l): 0.04 KH_2PO_4, 0.04 $Na_2HPO_4.2H_2O$, 0.15 NH_4Cl, 0.30 NaCl, 0.05 $MgCl_2.6H_2O$ and 0.18 $Na_2S.9H_2O$, and for the experiments with starch were (g/l): 0.15 KH_2PO_4, 0.04 $Na_2HPO_4.2H_2O$, 0.52 NH_4Cl, 0.30 NaCl, 0.05 $MgCl_2.6H_2O$ and 0.18 $Na_2S.9H_2O$. To minimize potential precipitation of calcium phosphate in the reactor, phosphate addition to the medium was minimized by estimating the phosphate requirements for growth of biomass according to the organic carbon supplied (0.016 mol-P/mol-C). Final concentrations of the trace elements in experiments with acetate were (in mg/l): 50.4 H_3BO_3, 50.4 $ZnCl_2$, 38.3 $CuCl_2.2H_2O$ 50.4 $CoCl_2.6H_2O$, 92.4 $NiCl_2.6H_2O$, 5037.8 $MnCl_2.4H_2O$, 39.5

$Na_2MoO_4.2H_2O$, 91.2 $AlCl_3.6H_2O$, 1007.6 Na_2SeO_3 and 2740 $FeCl_3.6H_2O$. In the experiments with starch, the trace element concentrations were 5 fold higher.

5.3.2.2 Inoculation

Anaerobic granular sludge (Purac, Gorinchem) was used in all experiments as inoculum to ensure a high concentration of methanogenic archaea. In order to begin with the same type and concentration of biomass, a simple protocol was followed to prepare the inoculum in all experiments. A metallic filter of 2 mm pore size was used to remove excess water of the granular sludge. Subsequently, 50 g of the filtered sludge were ground with a grinder (Janke Kunkel Ultra Turrax T25 grinder) for 3 minutes. The ground sludge was then sieved through a 0.125 mm pored sieve, 200 ml demi water was added on the sieve to facilitate the sieving. The filter cake was discarded while the filtrate was used as the inoculum.

5.3.3 Analytical Methods

A gas detection equipment was coupled to the bioreactor outlet to measure CH_4 and CO_2 concentration on-line (Rosemount Analytical NGA 2000 MLT 1 Multi-component analyzer). The N_2 flow rate, stirring rate, and temperature were controlled by a bio-controller (Biostat B plus, Sartorius). Data acquisition of the on-line measurements for pH, conductivity, temperature, gas composition, stirring rate, and gas flow rate was made by MFCS/win (Sartorius Stedim Systems, U.S.A.). Sampling for the detection of the dissolved species including volatile fatty acids (VFAs), calcium, silicon, phosphate and ammonium ions were done by taking a 2 ml sample from the sample port of the reactor and passing through a 0.45 μm syringe filter (Milipore). The samples were stored for the maximum period of 2 days at 4 °C before measurements.

The concentrations of glucose, ethanol, glycerol and volatile fatty acids (VFAs) including acetate, butyrate, propionate, lactate, valerate and succinate were determined by high-performance liquid chromatography (HPLC), using an Animex HPX-87H column from Bio-Rad at 60ºC coupled to an UV and a RI detector using phosphoric acid 0.01 M as the eluent. Hach Lange LCK 327, LCK 350, LCK 302-303, and LCW 028 were used for detection of calcium (Ca^{2+}), orthophosphate (PO_4^{3-}), ammonium (NH^+_4), and silicon (Si) concentrations, respectively. Samples for calcium detection were pre-acidified with HCL (1 M) to pH 2 for breaking the calcium complexes and enabling the measurement of total calcium. In addition to the periodic Ca^{2+} measurements, precipitation of $CaCO_3$ was also monitored by on-line measurement of electrical conductivity of the solution using a conductivity meter (Consort C832). This was possible due to the relatively high concentration and limiting molar conductivity (molar conductivity at infinite dilution) of Ca^{2+} (11.9 $mS.m^2/mol$) compared to other available ions in the experiments such as acetate (4.09 $mS.m^2/mol$) (Wright, 2007). Therefore, a decrease in conductivity was found to be a reliable indicator for the precipitation process throughout the experiment.

Sampling and measurement of total solids (TS) and volatile suspended solids (VSS) was done by taking 5 ml liquid sample from the reactor following the standard methods for the

examination of water and wastewater (Clesceri et al., 1998). Excluding the biomass concentration from the VSS (i.e. total organic carbon), the concentration of the insoluble starch was estimated. Biomass production was determined indirectly by NH^{+}_{4} uptake by the anaerobic bacteria using the stoichiometric conversation of ammonium to biomass.

X-ray diffraction (XRD) was used to determine the type crystalline precipitates at the end batch experiments. XRD patterns were recorded in a Bragg-Brentano geometry in a Bruker D5005 diffractometer equipped with Huber incident-beam monochromator and Braun PSD detector. Data collection was performed at room temperature using monochromatic Cu radiation (Kα1 λ = 0.154 nm) in the 2θ region between 10° and 90°, step size 0.038 degrees 2θ.

Activities were used (instead of the measured concentrations) to calculate the ion activity product (IAP) for precipitation calculations and plotting the graphs. Activities were calculated from the measured concentrations using ORCHESTRA (a geochemical equilibrium calculator, version 2012) (Meeussen, 2003) following the Davies equation and the standard equilibrium calculations (Bethke, 2008). The following ions and parameters were used as inputs for the ORCHESTRA: total inorganic carbon (H_2CO_3 + HCO_3^- + CO_3^{2-}), Ca^{2+}, acetate-tot, propionate-tot, butyrate-tot, PO_4^{3-}-tot, NH_4^+-tot and the measured pH. Besides the acid base equilibria, the following complexes are taken into account in the calculations: $CaH_2PO_4^+$, $CaHCO_3^+$, $CaNH_3^{2+}$, $CaOH^+$, $CaPO_4^-$, $Ca[Acetate]^+$, $Ca[Butyrate]^+$, $Ca[NH_3]_2^{2+}$, $Ca[Propionate]^+$ and OH^-. Precipitation of $CaCO_3$ is not included in the model.

The mass transfer constant (k_la) of CO_2 was used to calculate the carbonate ion (CO_3^{2-}) concentration and the total amount of carbonate system components (carbonate (CO_3^{2-}), bicarbonate (HCO_3^-) and carbonic acid (H_2CO_3)) in the solution. The k_la of CO_2 was measured by using the gassing-in method in a separate biomass-free experiment (in duplicates). To do this, the k_la value for oxygen was determined by using the same reactor and environmental conditions as in the experiments (i.e. N_2 headspace sparging of 0.5 L/min and stirring rate of 200 rpm at 35 °C). Using this method, the K_la value was determined to be 1.97 h^{-1} for O_2. This value was multiplied with 0.9 taken from the study of (Heijnen J. , 2011) in order to obtain the liquid-gas mass transfer coefficient for carbon dioxide, 2.2 h^{-1}.

5.4. Results

5.4.1 Precipitation of $CaCO_3$ during the methanogenic phase

To study the kinetics of calcium carbonate precipitation during the methanogenic phase, batch experiments with calcium acetate ($Ca(CH_3COO)_2$) were conducted. As shown in Fig. 5.1a and 1b, the consumption of acetate by methanogenesis resulted in an increase in the pH value and the CO_2 partial pressure following reaction 5.5 stated earlier in the introduction. This had consequently increased the carbonate ion activity in the solution by shifting the carbonate equilibrium towards the carbonate ion (CO_3^{2-}) which was calculated using the Orchestra model (Fig. 5.1b). The carbonate concentration increased until the point that the ion activity

product $(Ca^{2+})(CO_3^{2-})$ exceeded the solubility product of calcium carbonate monohydrate (also known as monohydrocalcite) with the pK_{sp} of 7.2 at 35^0C (Fig. 5.1a). The $CaCO_3$ precipitation was evident from the simultaneous decrease in conductivity, pH, Ca^{2+} activity (mol/l) and he CO_3^{2-} activity (mol/l). From this point (24h) onwards while the acetate was still consumed by the methanogens, the activity of CO_3^{2-} started to decrease due to the precipitation process. The ion activity product $(Ca^{2+})(CO_3^{2-})$ followed a decreasing trend until the solubility product of calcite crystal was reached (pK_{sp}=8.5 at 35^0C). The decrease in conductivity and pH also confirmed the $CaCO_3$ precipitation until 95 h when substrate was depleted and the $CaCO_3$ precipitation stopped. After substrate depletion, the pH starts to raise due to the CO_2 stripping. To examine whether the precipitation kinetics could be enhanced, in the subsequent batch experiment calcite seed crystals (5.5 ± 0.1 g/l) were added initially to the medium (Fig 5.1c and Fig 5.1d). This was according to the reported results which have shown that in presence of crystal nuclei or seed particles, the crystallization process is induced (Cacciuto et al., 2004; Gebauer & Cölfen, 2011). Fig. 5.1c shows that the induction period (i.e. the time between the occurrence of supersaturation and the formation of nuclei) is shortened by 10 hours compared to the previous experiment even though the same extend of super saturation was found before the precipitation was initiated.

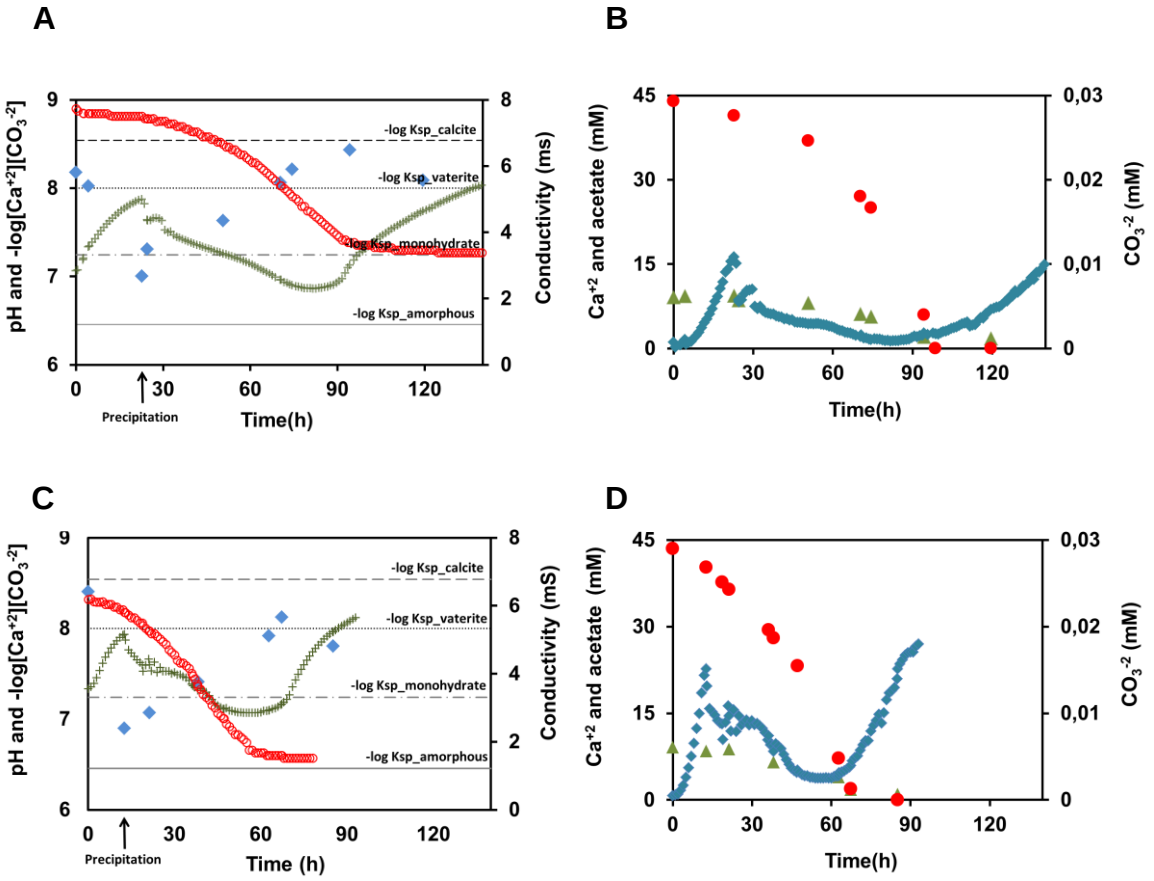

Figure 5.1 Analysis of the precipitation profiles of the experiments with acetate by: Ca^{2+}-CO_3^{2-} ion activity products (♦), pH (+) and conductivity (○) in the experiments: without calcite seed crystals (A), with calcite seed crystals (C). The horizontal straight lines in these figures are the negative logarithm of the solubility products of calcium carbonate polymorphs. Activities of Ca^{2+} (▲), acetate (●) and CO_3^{2-} ions (♦) in the experiments; without calcite seed crystals (B) and with calcite seed crystals (D).

Mineral carbonation of CO_2 (carbonate ion formation and precipitation) in these experiments increased the CH_4 content of the biogas to 71 ± 0.5 % v/v compared to a control batch experiment fed with sodium acetate (i.e. Ca^{2+} free environment) yielding 53 ± 1 % v/v CH_4 (Fig. 5.2a and 5.2b). The observed improvement of biogas quality is according to the maximum theoretical value indicated for CO_2 capture and sequestration in an open-system AD system with wollastonite (Salek et al., 2013b). In these experiments, biomass, organic acids, carbonate system components (HCO_3^-, H_2CO_3 and CO_3^{2-}) and CO_2 (g) accounted for 95 ± 0.2 % and 94 ± 0.5 % of the carbon and electron supplied to the system, respectively.

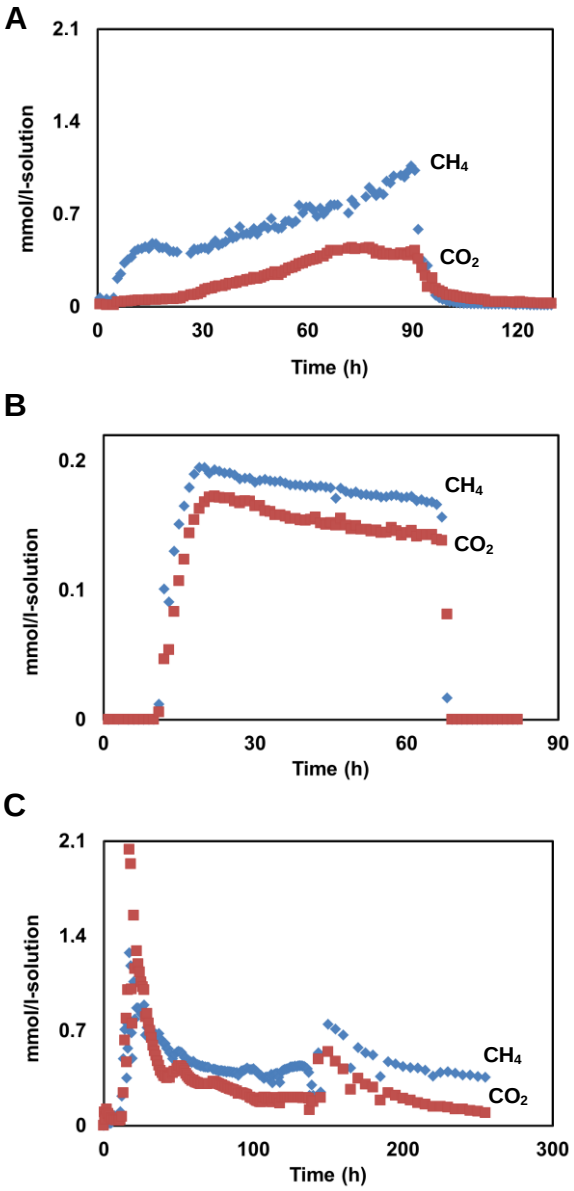

Figure 5.2 The concentrations of CO_2 and CH_4 gases in experiment with calcium acetate (A), the control experiment with sodium acetate (B), and the experiment with 5.5 g/l starch (C). The composition The experiment with 17.5 g/l starch has similar gas composition to the experiment with 5.5 g/l starch.

5.4.2 $CaCO_3$ precipitation in an anaerobic digestion with wollastonite

In the next series of anaerobic batch experiments, the carbon and calcium source were selected to be insoluble starch and wollastonite (63-125 µm), respectively. This represents a single-stage anaerobic digestion treatment process integrated with a silicate mineral. Thus, in addition to the processes of methanogenesis and $CaCO_3$ precipitation which were present in the previous set of experiments (reactions 5.5 and 5.6), three other reactions including hydrolysis, fermentation and wollastonite dissolution (reactions 5.2 to 5.4) were expected to occur in the reactor. Two concentrations of substrate (5.5 g/l and 17.5 g/l starch) were examined at the same wollastonite concentration (25 g/l) to study the $CaCO_3$ precipitation and biogas quality under the increasing acidifying conditions (i.e. higher dissolution rate of wollastonite).

Figs. 5.3a and 5.3c show that the pH of the experiments with starch were maintained between 5.6 and 7.8 throughout the experiments because of the biological and chemical reactions 5.2 to 6 (introduction section). The pH in these experiments followed a similar pattern with an initial decrease as a result of organic acid production by the fermentation process (Fig 5.3a and Fig 5.3c, phase 1) and then a short stable phase (phase 2). A similar trend of the pH was also observed in a fermentation study with 1g/l/d glucose with wollastonite (125-250 µm) (Salek et al., 2013c). In the subsequent phase (phase 3) the pH value increased in response to the wollastonite dissolution (Weissbart & Rimstidt, 2000) and the methanogenesis process.

The decrease of conductivity in this phase observed in Fig. 5.3a is probably in response to conversion of VFA to gases. However, it is unclear why in the experiment with 17.5 g/l starch, at the time of decrease in conductivity, the VFA concentration is increasing (Fig 5.3c and Fig 5.3d).

In phase 4, the simultaneous decrease of conductivity, pH and -log $[Ca^{2+}][CO_3^{2-}]$ presented in Fig. 5.3a and Fig. 5.3c, suggests that the $CaCO_3$ precipitation was initiated. In the last phase (phase 5) where the substrate was no longer consumed, the pH raised gradually because of the slow CO_2 stripping out of the system. While all VFA produced was consumed in the experiment with 5.5 g/l starch (200 mM starch carbon), a considerable amount of propionate was accumulated (i.e. 100 mM propionate-carbon) at the end of the experiment with 17.5 g/l starch (Fig 5.4a and Fig 5.4b).

As shown in Fig. 5.3a and Fig. 5.3c, similar precipitation patterns to experiments with acetate were observed with initial formation of less stable polymorphs and subsequent stable crystalline forms. XRD analysis of the residue at the end of the digestion confirmed the production of calcite in the reactors (data not shown). Table 5.2 (2^{nd} row) presented the XRF analysis of the residue. In both cases, biogas quality did not reach higher than 56 ± 0.3 % v/v CH_4 content (Fig. 5.2c). The carbon and electron balances of the products (CO_2, CH_4, biomass, organic and inorganic acids) from these two experiments showed more than 90 % of the supplied substrate could be calculated back.

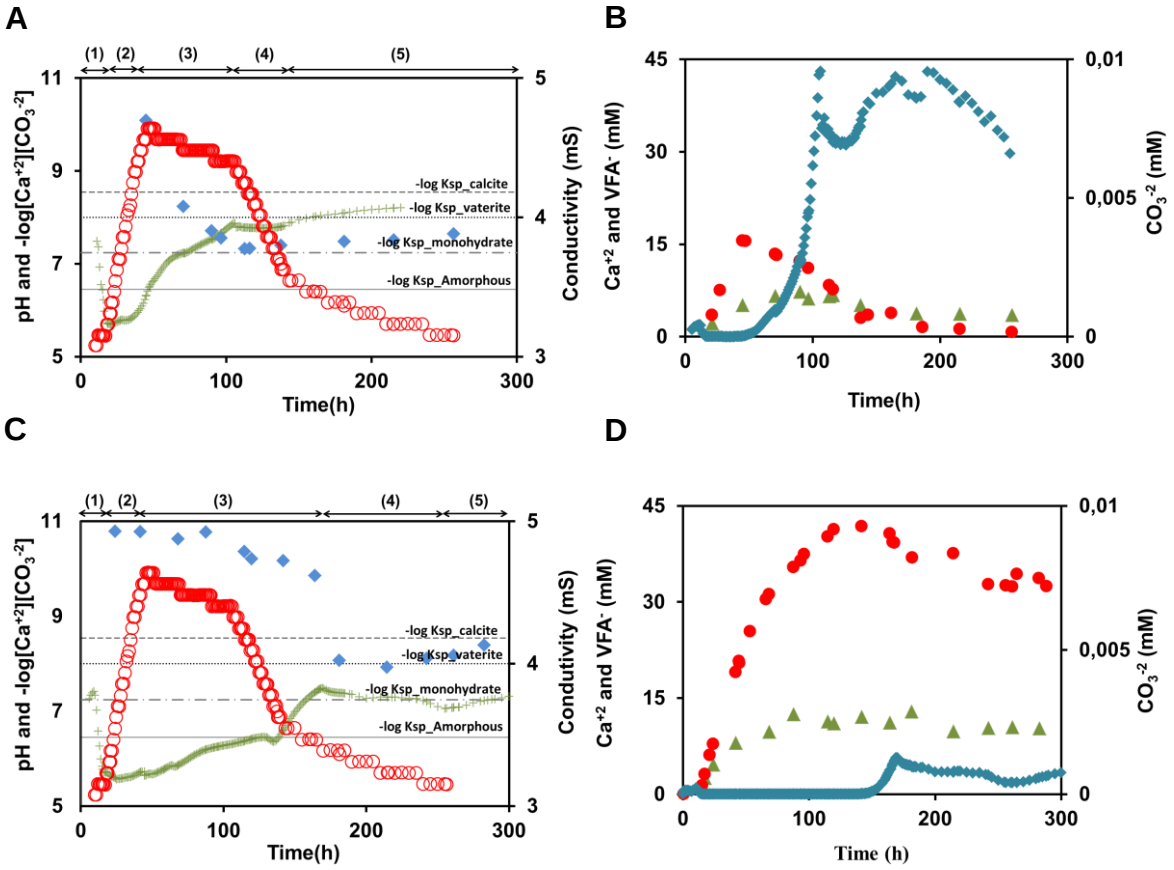

Figure 5.3 Analysis of the precipitation profiles of the anaerobic experiments with starch by: Ca^{2+}-CO_3^{2-} ion activity products (♦), pH (+) and conductivity (○) in the experiments : with 5.5 g/l starch concentration (A), with 17.5 g/l starch concentration (C). Different phases (1 to 5) in the experiments with starch are mentioned in the brackets on top of the figures. Activities of Ca^{2+} (▲), CO_3^{2-} (♦) and sum of the dissociated VFA^- (acetate, propionate, lactate, butyrate) (●) in the experiments: with 5.5 g/l starch concentration (B), with 17.5 g/l starch concentration (D).

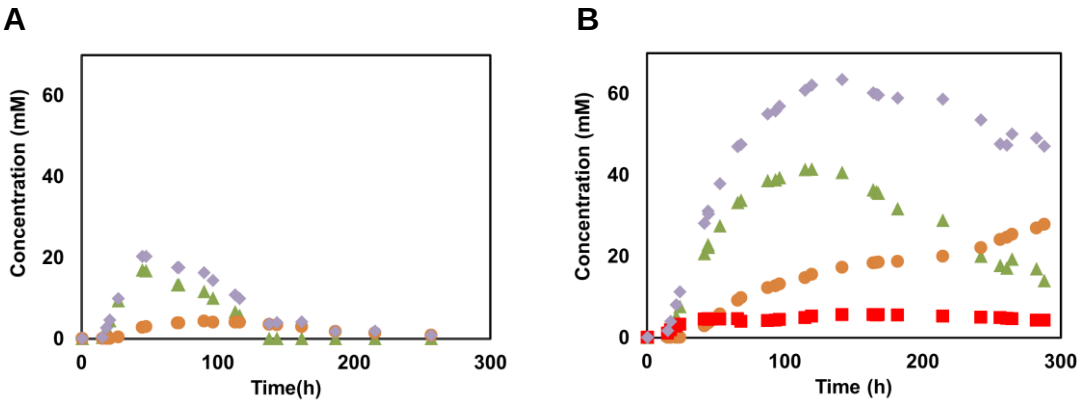

Figure 5.4 Concentration of organic acids including (●) total propionate, (■) total butyrate, (▲) total acetate, and (♦) the sum of the organic acids during the experiments with (A) 5.5 g/l starch and (B) 17.5 g/l starch. Concentration of the organic acids less than 0.5 mM are not shown in the figure.

5.5. Discussion

5.5.1 Mineral carbonation of CO_2 by an anaerobic digestion process

The results of the present study confirmed that the kinetics of mineral carbonation of CO_2 can be improved by increasing the rates of silicate mineral dissolution and carbonate mineral precipitation.

5.5.1.2 Kinetics of $CaCO_3$ precipitation in a single-stage anaerobic digestion with wollastonite

The kinetics of calcium carbonate precipitation could be improved mainly by the activities of methanogenic archaea in the anaerobic digestion. The precipitation profile obtained by the ion activity product $(Ca^{2+})(CO_3^{2-})$, in the performed experiments with calciumacetate and starch (shown in Figs. 5.1a, 5.1c, 3a and 5.3c), suggests the common pattern of $CaCO_3$ precipitation with initial formation of less stable polymorphs and the subsequent ripening to the calcite precipitate (Gebauer & Cölfen, 2011; Xiao et al., 2009). Studying the type of $CaCO_3$ precipitate by comparing the ion activity product of Ca^{2+} and CO_3^{2-} ions to the solubility products of the polymorphs were done in the previous studies as well (Gebauer & Cölfen, 2011; Lindeboom et al., 2013; Nehrke, 2007; Sawada, 1997).

Transformation of less stable polymorphs to stable crystals decreases the chance of re-dissolution and possible release of CO_2 to the atmosphere. An alternative way to prevent loss of CO_2 to the atmosphere and therefore increase of the CO_2 capture efficiency, is to induce the kinetics of $CaCO_3$ precipitation. An initial introduction of calcite crystals (crystal seed) to the medium of the experiment showed that the precipitation kinetics could be improved by

reducing the induction period i.e. the time between the occurrence of supersaturation and the formation of nuclei (Figs. 5.1a and 5.1c). The added calcite seeds may have acted as nucleation spots for the amorphous calcium carbonate clusters. Occurrence of nucleation on the calcite seed surface as a secondary nucleation was also reported previously (Nancollas & Reddy, 1971).

In the experiments with starch, the $CaCO_3$ could initiate the precipitation at a lower ion activity product (Ca^{2+})(CO_3^{2-}) compared to the experiments with acetate. Therefore, the ion activity product did not exceed the monohydrate calcium carbonate solubility product achieving the nucleation at lower supersaturations (Fig 5.3a and 5.3c, compared to Fig 5.1a and 5.1c). In these experiments, the wollastonite particles might have acted as particles providing heterogeneous nucleation sites for calcium carbonate clusters. In addition, different biological conditions such as presence of fermentative bacteria, higher bacterial concentration could also be the reason for the observed different precipitation behavior. The bacteria are known to absorb the ambient metal ions to their surfaces due to the negative surface charge properties and enhance the nucleation of carbonates with these metal ions (Schultze-Lam et al., 1996).

5.5.1.2 Enhancement of wollastonite dissolution rate by an anaerobic digestion process

The dissolution rate of wollastonite was mainly enhanced due to the activity of fermentative bacteria. Wollastonite dissolution rate (mol/cm^2/s) in the experiments with 5.5 g/l and 17.5 g/l starch concentrations were, respectively, 11 and 18 folds higher than the calculated dissolution rate in abiotic conditions (Rimstidt & Dove, 1986). The dissolution rate was calculated based on the average Ca^{2+} ion release rate during the fermentation phase of the experiments (0-90 h, Fig 5.3a). The equation (5.1) used to calculate the dissolution rate in abiotic conditions is (adopted from Rimstidt & Dove, 1986 study): $R_{H^+} = -Ak_+(a_{H^+})^{0.4\pm0.07}$ (5.1) where R_{H^+} is the hydrogen ion consumption rate (mol/s), A is the total interfacial surface area (m^2), k_+ is the reaction constant with the value of $10^{-5.42\pm0.4}$(mol/m^2/s) and a_{H^+} is the activity of hydrogen ion (Rimstidt & Dove, 1986). The equation 5.1 calculates the dissolution rate as a function of hydrogen ion activity (pH) which is based on the widely accepted mechanism of hydrogen exchange for the calcium ion in the lattice (Murphy & Helgeson, 1987). Enhancement of wollastonite dissolution rate in the experiments with starch compared to the abiotic conditions is potentially due to the chelation and complexation effect of the dissociated organic acids (organic ligands) in the solution (Murphy & Helgeson, 1987; Pokrovsky et al., 2009; Vandevivere et al., 1994). This can be the reason for the higher observed dissolution rate in the experiment with 17.5 g/l starch with concentration of dissociated organic acids reaching 60 mM as compared to the 5.5 g/l starch case with maximum of 20 mM dissociated organic acids (Fig 5.4).

5.5.2 Production of biobased products: energy and material

5.5.2.1 Biogas improvement

The results from the experiments with acetate showed that in the case of a highly soluble Ca^{2+} containing compound such as $Ca(CH_3COO)_2$ and production of sufficient alkalinity, the maximum theoretical improvement of biogas (as the bioproduct) could be achieved (i.e. half of the produced CO_2 is sequestered) (Fig 5.2a).

However, in the experiments with starch the methane content of the biogas could only improve a little (56 ± 0.3 % v/v CH_4). In these experiments, the methanogenic archaea were not inhibited by the low pH and therefore they were active from the beginning of the experiment together with the fermentative bacteria (Fig 5.2c and reaction 5.5). The CO_2 produced during these initial phases could not be retained in the system due to the low pH and insufficient Ca^{2+}. Consequently, at the time that there was enough calcium available in the solution, most of the CO_2 had already escaped to the atmosphere resulting in limited improvement of the biogas quality. This resulted in the high remaining Ca^{2+} ion concentration observed at the end of these experiment (Fig 5.3b and Fig 5.3d). In a study performed by Lindeboom et al. 2013, the loss of CO_2 was prevented by operating a closed system in autogenerative high pressure digestion (AHPD) with wollastonite where CO_2 partial pressure was increased in time resulting in high content of CH_4 in the biogas (88 % v/v CH_4) (Lindeboom et al., 2013).

An optimization of a single-stage open anaerobic digestion system is possible by lower stripping of CO_2 and better separation of fermentation and methanogenesis phases. Separation of fermentation and methanogenesis phases in a single-stage allow reactions 5.2 to 5.6 to occur in the right sequence. This means that first the fermentation process provides sufficient divalent cations (Ca^{2+}) and then the methanogenesis provides the alkalinity required for the carbonation of the produced CO_2. Using a methanogenic culture that is more susceptible to the low pH can provide a better separation among the phases.

5.5.2.2 Potential production of chemical grouting material and biofertilizer

XRD and XRF composition analysis of the residues at end of the integrated experiments with starch and wollastonite show their potential usage as chemical grouting material for stabilizing and strengthening the subsurface soil deposits. The presence of calcium silicate, calcite, quartz and trace compounds (< 2 %) of iron oxide (Fe_2O_3), dolomite ($MgCa(CO_3)_2$) and aluminum oxide (Al_2O_3) shows comparable composition to that of the chemical grouting materials particularly the silicate-based grouting material (Table 5.2) (Oyler, 1984; Warner, 2004). The silicate based grout can have great varieties in compositions and chemistries depending on the purpose of their usage. The grout can be made from a mixture of cement, fly ash, sand, acrylic gels, polyurethane and surfactants (Clarke, 1988; Oyler, 1984; Warner, 2004). Table 5.2 shows concentration range of these compounds used in the silicate-based chemical grouting in comparison with the residual composition.

Residual of many AD systems is already used as biofertilizer, however the presence of extra calcium and silicon ions can increase the quality of the produced biofertilizer (Salek et al., 2013b; Sommers, 1977). In addition, wollastonite and $CaCO_3$ can act as neutralizing agents regulating the pH of the agricultural soil. Further research is required to investigate the possible undesirable compounds presence in the residual as a result of impurities in the wollastonite ore such as nickel. The additional costs associated with potential pretreatment required such as addition of surfactant in the case of chemical grouting material before using the residual as a bioproduct should also be considered.

Table 5.2. Bulk composition (wt.%) of the AD residue of the 5.5 g/l starch experiment and silicate-based chemical grouting material as determined by X-ray fluorescence.

Component	SiO_2	CaO	Fe_2O_3	P_2O_5	MgO	Al_2O_3	Na_2O	Cl	MnO	Organic reactant	TX-100 surfactant
					(wt.%)						
AD Residue	51.8	46.1	0.73	0.46	0.26	0.21	0.12	0.1	0.1	0.5-1	0
Chemical grout	50-70	0-30	1-3	0	1-3	3-5	20-30	0	0-2	4-6	0.1

5.5.3 Self-regulating pH system

The processes of wollastonite dissolution and calcium carbonate precipitation neutralize the fermentation and methanogenesis biological processes, respectively (reactions 5.3 to 5.6). The increase of dissolution rate of wollastonite at lower pH values (e.g. pH 5) and the precipitation rate of $CaCO_3$ at higher pH values (e.g. pH 8), result in a self-regulating pH system that can maintain the pH in a favorable range for the bacteria. This can be observed in the experiments with acetate where the pH was increased until the point that $CaCO_3$ precipitation initiated (maximum pH 7.9). Hereafter, the pH showed a decreasing trend by shifting the carbonate equilibrium towards CO_3^{2-} (Fig 5.1a and 5.1c). This is particularly beneficial for wastes containing high ammonium (NH_4^+) concentrations such as animal manures where high pH values can cause ammonia (NH_3) toxicity for bacteria. In the experiments with starch, the increase of wollastonite dissolution rate was observed at lower pH values (minimum pH 5.7) resulting in increase of pH (Fig 5.3a and 5.3c). Such self-regulating pH was also observed in previous studies performed with silicate minerals (Lindeboom et al., 2013). Achieving a self-regulating pH system reduces the costs associated to operational monitoring.

5.6 Conclusions

This study showed that the anaerobic digestion process can improve the kinetics of CO_2 mineral carbonation by increasing the rates of silicate mineral dissolution and carbonate mineral precipitation. Enhancing the kinetics of precipitation and formation of more stable

calcium carbonate precipitates increase the efficiency of CO_2 sequestration. Precipitation of $CaCO_3$ in the experiments using a highly soluble Ca containing compound improved the biogas quality by increasing the methane content by 52 %. However, the poor separation of fermentation and methanogenic phase in single-stage anaerobic digestion experiments with wollastonite, limited the biogas improvement. In general, increase of dissolution rate of wollastonite due to the biotic environment, obtaining higher caloric value biogas, a self-regulating pH system and potential production of biobased materials (biochemical grouting material and biofertilizer) were the added values observed in the AD system as a result of silicate mineral integration

Chapter 6

pH control in biological systems using calcium carbonate

Biotechnology and bioengineering (2015) 112(5): 905-913

6.1 Abstract

Due to its abundance, calcium carbonate ($CaCO_3$) has high potentials as a source of alkalinity for biotechnological applications. The application of $CaCO_3$ in biological systems as neutralizing agent is however limited due to potential difficulties in controlling the pH. The objective of the present study was to determine the dominant processes that control the pH in an acid-forming microbial process in the presence of $CaCO_3$. To achieve that, a mathematical model was made with a minimum set of kinetically controlled and equilibrium reactions that was able to reproduce the experimental data of a batch fermentation experiment using finely powdered $CaCO_3$. In the model, thermodynamic equilibrium was assumed for all speciation, complexation and precipitation reactions whereas rate limited reactions were included for the biological fatty acid production, the mass transfer of CO_2 from the liquid phase to the gas phase and the convective transport of CO_2 out of the gas phase. The estimated pH-pattern strongly resembled the measured pH, suggesting that the chosen set of kinetically controlled and equilibrium reactions were establishing the experimental pH. A detailed analysis of the reaction system with the aid of the model revealed that the pH establishment was most sensitive to four factors: the mass transfer rate of CO_2 to the gas phase, the biological acid production rate, the partial pressure of CO_2 and the Ca^{2+} concentration in the solution. Individual influences of these factors on the pH were investigated by extrapolating the model to a continuously stirred-tank reactor (CSTR) case. This case study indicates how the pH of a commonly used continuous biotechnological process could be manipulated and adjusted by altering these four factors. Achieving a better insight of the processes controlling the pH of a biological system using $CaCO_3$ as its neutralizing agent can result in broader applications of $CaCO_3$ in biotechnological industries.

6.2 Introduction

Neutralization of biological acid production by $CaCO_3$ is a common process occurring in natural and man-made ecosystems such as seawater (Feely et al., 2002; Morse & Arvidson, 2002) and waste streams with high calcium concentration such as wastewaters of pulp and paper industry, whey permeate and municipal landfill leachates (Kennedy et al., 1988; Rintala & Puhakka, 1994; van Langerak et al., 1998). The commercial application of $CaCO_3$ as a neutralizing compound is however limited because of difficulties in controlling the pH due to presumed slow kinetics of $CaCO_3$ dissolution and instability of the pH (Maree & Duplessis, 1994; Watten et al., 2007). Commercial application of $CaCO_3$ as neutralizing agent can be of great interest in the context of production of bio-based products (material, chemical and energy) where the cost efficiency of the process justifies their ultimate feasibility (Hatti-Kaul et al., 2007). Reducing the cost of each step can contribute to the development of the bioproduct (van Wyk, 2001). Examples of (organic) acid producing microbial processes are acetic acid and lactic acid for production of deicing salt and bioplastic, respectively (Angenent et al., 2004), where $CaCO_3$ can potentially be substituted for the current neutralizing agent used such as sodium carbonate (Na_2CO_3) (Wang et al., 2002). Some of the difficulties of buffering the pH by $CaCO_3$ might be due to the lack of understanding the

system instead of the $CaCO_3$ buffering capacity itself. Although there have been extensive studies on $CaCO_3$ dissolution and precipitation in H_2O-CO_2-$CaCO_3$ systems (Arakaki & Mucci, 1995; Buhmann & Dreybrodt, 1987; Dreybrodt et al., 1996; Morse et al., 2007; Plummer & Wigley, 1976; Svensson & Dreybrodt, 1992), fewer investigations have been reported on the kinetics of $CaCO_3$ dissolution (and its buffer capacity) in biological systems where CO_2 and organic products are produced. A good understanding of the interactions among processes that control the pH can provide a firm basis for practical application of $CaCO_3$ as a neutralizing agent in biological processes. The overall reaction of an acid-producing fermentation neutralized by $CaCO_3$ dissolution is shown in reaction 6.1 using acetate as an example and displayed in more detail in Fig. 6.1. As shown in the figure, the reactions that can potentially influence the pH in this system are: $CaCO_3$ dissolution, biological production of organic acids and their speciation, CO_2 stripping from the reactor, and the complexation, precipitation/dissolution of the main organic and inorganic ions present in the system. Among these processes, the kinetically controlled reactions are more likely to control the pH. These processes are: biological production of organic acids, mass transfer of CO_2 from the liquid to the gas phase and dissolution of $CaCO_3$. The reason for it is that while the mass transfer constant (k_la) for CO_2 and the specific product production rate (q_s) (for a common mixed culture anaerobic process) are on the order of 1×10^{-2} s^{-1} and 2×10^{-4} s^{-1}, respectively (Merkel & Krauth, 1999; Zeebe & Wolf-Gladrow, 2001); the rate constants of other reactions like speciation, complexation and precipitation/dissolution are by nature occurring at much higher rates and therefore can be considered practically instantaneous. As an example, the rate constant of the relatively slower reaction of $HCO_3^- + H^+ \rightarrow CO_2$ (aq) is still in the order of 12 s^{-1} (Dreybrodt et al., 1996; Zeebe & Wolf-Gladrow, 2001). The process of $CaCO_3$ dissolution can also be seen as an equilibrium reaction when finely powdered calcite is used because it is no longer surface controlled (solution volume to surface area of powdered $CaCO_3$, V/A ratio, is lower than 10^{-6} m^3/m^2) (Dreybrodt et al., 1996). Due to the interactions of all these processes (kinetically controlled reactions and reactions in equilibrium), it is not obvious what are the individual effects of different factors on the pH (Merkel & Krauth, 1999). The objective of this study was therefore to investigate if the pH pattern in a batch fermentation process can be described using a mathematical model based on the processes described. The results obtained from the model were extrapolated to a continuously stirred-tank reactor (CSTR) in order to investigate the effect of process parameters on the pH of a more practical case.

$$2\,CH_3COOH + CaCO_3 \rightleftarrows Ca^{2+} + 2CH_3COO^- + H_2O + CO_2 \uparrow \qquad \text{(reaction 6.1)}$$

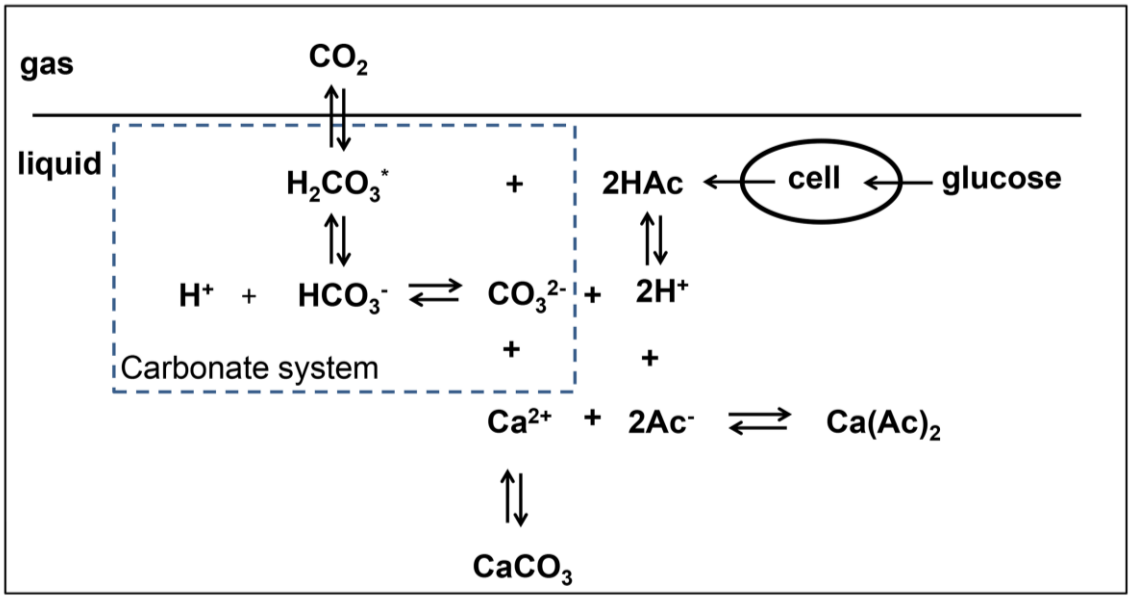

Figure 6.1 Schematic overview of the main reactions involved in the organic acid (acetate as an example) producing system using $CaCO_3$ as neutralizing agent. The inorganic carbon is mostly excreted in form of CO_2 (aq) from the cells membrane (Ho et al., 1987). HAc and Ac^- are representative for monocarboxylic acids and carboxylate, respectively, such as acetic acid and acetate. H_2CO_3* is denoted for sum of the H_2CO_3 and CO_2 (aq).

6.3 Material and Methods

6.3.1 Experimental set-up

The fermentation experiment was conducted in a double jacket glass reactor with working volume of 2 liters. The experiment was carried out in a batch mode in order to examine the $CaCO_3$ buffering capacity in an gradient of substrate and products concentration. The temperature of the reactor was controlled at 30 ± 1 °C with a water jacket and a thermostat bath (Lauda, Germany). The mixing rate was 500 rpm and the broth was sparged with 0.5 $l.min^{-1}$ N_2 gas.

6.3.2 Analytical Techniques

The analytical techniques were described previously (Salek et al., 2013a). Briefly, the concentrations of CO_2, H_2 and O_2 were measured using Rosemount Analytical NGA 2000 MLT 1 Multi-component analyzer (infrared detector) and the data acquisition of the direct online measurements of the gases, temperature, pH and stirring rate was done by MFCS/win (Sartorius Stedim Systems, USA). Fermentation products and glucose concentrations of the samples taken from the reactor were determined by High-performance liquid chromatography (HPLC). The ammonia concentrations of samples were measured by flow injection analysis colorimetry.

6.3.3 Medium and Culture

Glucose, calcium carbonate and mineral salts solution were added to the reactor before starting the experiment. Glucose concentration in the reactor was 60 $g.l^{-1}$. To ensure sufficient amount of $CaCO_3$ was available for the complete conversion of glucose to organic acids, 33 $g.l^{-1}$ $CaCO_3$ was added to the reactor. The used $CaCO_3$ was in powder form with 99% purity, provided from Merck Chemicals. The medium was composed of (in $g.l^{-1}$): NH_4Cl 10.7, KH_2PO_4 1.3, NaCl 4.4, $Na_2SO_4.10H_2O$ 3.2, $MgCl_2.6H_2O$ 1.8, EDTA 0.7 and trace elements (in $mg.l^{-1}$): $FeSO_4.7H_2O$ 46.5, $CaCl_2.2H_2O$ 9, H_3BO_3 1.2, $Na_2MoO_4.2H_2O$ 1.6, $ZnSO_4.7H_2O$ 76.9, $CoCl_2.6H_2O$ 14.5, $CuCl_2.2H_2O$ 33, $MnCl_2.2H_2O$ 30.6, $NiCl_2.6H_2O$ 7.5. The inoculum was obtained from a Sluisjesdijk anaerobic digestion plant located in Rotterdam, the Netherlands.

6.3.4 Model description

6.3.4.1 General framework

The model considers two types of reactions, kinetically controlled reactions (R_i) and reactions that are in thermodynamic equilibrium at all times (Re_i). All reactions considered in the model are listed in Table 6.1. The rate dependent reactions are the production of organic acids during the fermentation (reaction 6.2), the mass transfer of CO_2 and H_2 from the liquid phase to the gas phase (transfer) (ractions 6.3 and 6.4) and the convective transport of gases (CO_2, H_2 and N_2) out of the gas phase (transport) (reactions 6.5 and 6.6). Other reactions such as speciation, complexation and precipitation/dissolution reactions are modeled as equilibrium reactions (reactions 6.6 to 6.40). The liquid phase and the gas phase in the model are assumed to be perfectly mixed. Modeling of the biological system was lumped into one reaction (reaction 6.2) because the main goal of the model is to investigate the effect of the organic acid production on the chemical system and not to model the biological processes in detail. In the model, the biological processes of organic acids and CO_2 production are considered among the rate controlling processes whereas the chemical dissociation of organic acids are assumed to be in equilibrium. The complexation and precipitation processes resulting in concentration of compounds below 5 mM are not included in the model. In addition, the reaction between the dissolved CO_2 and hydroxide was neglected because the pH during the experiment stayed below 8 (Astarita et al., 1983).

In order to couple equilibrium and rate dependent reactions two types of concentrations are considered in the model. Equilibrium reactions are expressed in derived concentrations (C_i^D) and rate dependent reactions are expressed in total concentrations (C_i^T). This choice is based on the fact that compounds can be in a different protonation, complexation or mineral form depending on the state of the system. As an example, CO_3^{2-} may also exist in the forms of HCO_3^-, H_2CO_3 or $CaCO_3$. A derived concentration represents the concentration of one of these forms while a total concentration represents the mass balance over all forms of the compound. Additionally, total concentrations are expressed in one of their forms called the primary state. The derived concentrations are derived from the total concentrations and the

considered equilibrium reactions following the method described by (Bethke, 2008). Hereby, the primary states of the total concentrations determine the reference states in the total proton balance ($C_{H+}{}^{T}$) (zero level method). The total concentrations *i* considered in the gas phase are expressed as partial pressures (p_i).

Table 6.1. Rate dependent (reactions 6.2-6.6) and equilibrium reactions (reactions 6.7-6.40) included in the model.

Biological acid production (reaction 6.2)

$$1C_6H_{12}O_6 + Y_i \cdot NH_3 \rightarrow Y_i \cdot C_2H_4O_2 + Y_i \cdot C_3H_6O_3 + Y_i \cdot C_2H_6O + Y_i \cdot C_4H_6O_4 + Y_i \cdot C_3H_6O_2 + Y_i \cdot CO_2 + Y_i \cdot H_2 + Y_i \cdot X$$

Gas-liquid transfer (reactions 6.3-6.4)

$$CO_{2,gas} \rightleftarrows CO_{2,liquid} \qquad H_{2,gas} \rightleftarrows H_{2,liquid}$$

Gas-liquid transport (reactions 6.5-6.6)

$$CO_{2,gas} \rightarrow CO_{2,outside} \qquad H_{2,gas} \rightarrow H_{2,outside}$$

Speciation (reactions 6.7-6.15)

$$C_2H_4O_2 \rightleftarrows C_2H_3O_2^- + H^+ \qquad NH_4^+ \rightleftarrows NH_3 + H^+$$

$$C_3H_6O_3 \rightleftarrows C_3H_5O_3^- + H^+ \qquad H_2O \rightleftarrows OH^- + H^+$$

$$C_4H_6O_4 \rightleftarrows C_4H_5O_4^- + H^+ \rightleftarrows C_4H_4O_4^{2-} + H^+ \qquad H_2CO_3 \rightleftarrows HCO_3^- + H^+ \rightleftarrows CO_3^{2-} + H^+$$

$$C_3H_6O_2 \rightleftarrows C_3H_5O_2^- + H^+ \qquad H_3PO_4 \rightleftarrows HPO_4^- + H^+ \rightleftarrows HPO_4^{2-} + H^+ \rightleftarrows PO_4^{3-} + H^+$$

$$HSO_4^- + H^+ \rightleftarrows SO_4^{2-} + H^+$$

Complexation (reactions 6.16-6.36)

$$Na(C_2H_3O_2) \rightleftarrows Na^+ + C_2H_3O_2^- \qquad NaSO_4^- \rightleftarrows Na^+ + SO_4^{2-}$$

$$Ca(C_3H_5O_2)^+ \rightleftarrows Ca^{2+} + C_3H_5O_2^- \qquad NaHPO_4^- \rightleftarrows Na^+ + HPO_4^{2-}$$

$CaHPO_4 \rightleftarrows Ca^{2+} + HPO_4^{2-}$

$CaHPO_4{:}2H_2O \rightleftarrows Ca^{2+} + HPO_4^{2-} + 2H_2O$

$CaHPO_4\,[s] \rightleftarrows Ca^{2+} + HPO_4^{2-}$

$CaH_2PO_4^+ \rightleftarrows Ca^{2+} + H_2PO_4^-$

$Ca_4(HPO_4)_3{:}3H_2O \rightleftarrows 4Ca^{2+} + 3HPO_4^{2-} + 3H_2O$

$CaPO_4^- \rightleftarrows Ca^{2+} + PO_4^{3-}$

$Ca_3(PO_4)_2\,[beta] \rightleftarrows 3Ca^{2+} + 2PO_4^{3-}$

$Ca(NH_3)_2^{2+} \rightleftarrows Ca^{2+} + 2NH_3$

$NaHCO_3 \rightleftarrows Na^+ + HCO_3^-$

$NH_4SO_4^- \rightleftarrows NH_4^+ + SO_4^{2-}$

$NaCO_3^- \rightleftarrows Na^+ + CO_3^{2-}$

$Ca(C_2H_3O_2)^+ \rightleftarrows Ca^{2+} + C_2H_3O_2^-$

$Na_3H(CO_3)_2 \leftrightarrow 3Na^+ + HCO_3^- + CO_3^{2-}$

$Ca_5(PO_4)_3(OH) \leftrightarrow 5Ca^{2+} + 3PO_4^{3-} + OH^-$

$CaNH_3^{2+} \rightleftarrows Ca^{2+} + NH_3$

$CaHCO_3^+ \rightleftarrows Ca^{2+} + HCO_3^-$

$CaOH^+ \rightleftarrows Ca^{2+} + OH^-$

Precipitation/dissolution (reactions 6.37-6.40)

$CaCO_3 \rightleftarrows Ca^{2+} + CO_3^{2-}$

$CaCO_3 \leftrightarrow Ca^{2+} + CO_3^{2-}$

$CaSO_4 \leftrightarrow Ca^{2+} + SO_4^{2-}$

$CaSO_4 \rightleftarrows Ca^{2+} + SO_4^{2-}$

6.3.4.2 Numerical integration

The changes in concentrations of compounds in the gas phase and the liquid phase caused by the rate dependent reactions are calculated using equations 6.1 and 6.2. The indicated reaction rates ($R_i^{fermentation}$, $R_i^{transfer}$ and $R_i^{transport}$) in these equations are calculated by using equations 6.3 to 5. The rate of fermentation $R_i^{fermentation}$ for compound *i* is calculated as specified in equation 6.3. The parameters $q_{s\text{-}max}$, K_s and Y_i in this equation were obtained by fitting the experimental data to the volatile fatty acids (VFAs) production in time. Glucose is considered as the limiting substrate (C_s) during the experiment. The mass transfer rate ($R_i^{transfer}$) of the gases CO_2 and H_2 are calculated by equation 6.4 while for other compounds the mass transfer rate was considered zero. The mass transfer constant k_la for CO_2 was measured by using the gassing-in method in a separate biomass-free experiment having the same rate of mixing and gas sparging (500 rpm and 0.5 $l.min^{-1}$ N_2 gas, respectively) as the fermentation experiment. The k_la value for hydrogen is deduced from the k_la value for CO_2 by equation 6.6. Diffusion coefficients for CO_2 (D_{CO2}) and H_2 (D_{H2}) were taken from Noorman et al. 1992. Henry coefficients (H_i at 30 C^o) were taken from (Ferrell & Himmelblau, 1967). All parameter values used in the model are listed in Table 6.2. The partial pressure of compound *i* in the gas phase is dependent on the dynamics of the mass transfer ($R_i^{transfer}$) and the conductive transport of it out of the gas phase ($R_i^{transport}$). The rate of convective transport of gas affecting the partial pressure of compound *i* is calculated using equation 6.5 where constant total pressure and volume of the gas phase is assumed. Compound N_2 is only considered in the gas phase and not in the liquid phase and therefore its $R_i^{transfer}$ is set equal to the sparging rate in $mol.h^{-1}$.

During the numerical integration of equations 6.1 and 6.2 the derived concentrations are calculated for each time step. This is performed by coupling between ORCHESTRA (a geochemical equilibrium calculator) (Meeussen, 2003) and MATLAB. ORCHESTRA calculates the derived concentrations based on the chosen equilibrium reactions and the total concentrations at the time step in MATLAB. Fully coupled kinetic and equilibrium reactions are important for accurate calculation of parameters such as pH, inhibitory concentrations and the mass transfer.

The inhabitation of fermentative bacteria at low pH values due to the protonated VFA's is quantitated using equation 6.7 taken from (Kleerebezem et al., 2010) where the inhibition constant (K_i) is 1.5 x 10^{-4} $mol.L^{-1}$ and the VFA concentration (C_{HVFA}) is the sum of all protonated VFA. The inhibition factor (f_{HVFA}) is multiplied with equation 6.3 and has a range between 0 and 1.

$$\frac{dC^T_i}{dt} = R_i^{fermentation} - R_i^{transfer} \qquad (6.1)$$

$$\frac{dp_i}{dt} = \left(R_i^{transfer} - R_i^{transport}\right)\frac{R \cdot T \cdot V_l}{V_g} \qquad (6.2)$$

$$R_i^{fermentation} = Y_i \cdot q_s^{max} \cdot \frac{C_s^T}{K_s + C_s^T} \cdot C_x^T \quad (6.3)$$

$$R_i^{transfer} = k_l a_i \cdot \left(C_i^D - \frac{p_i}{H_i} \right) \quad (6.4)$$

$$R_i^{transport} = \frac{p_i}{\sum p_i} \cdot \sum R_i^{transfer} \quad (6.5)$$

$$kla_{H2} = kla_{CO2} \times \left[\frac{D_{H2}}{D_{CO2}} \right]^{0.5} \quad (6.6)$$

$$f_{HVFA} = \frac{K_i}{K_i + C_{HVFA}} \quad (6.7)$$

Table 6.2. Model parameters and initial conditions.

Symbol	Value	Units	Purpose	Source
Mass transfer kinetic parameter values				
D_{CO2}	1.6×10^{-9}	$m^2.s^{-1}$	CO_2 diffusion coefficient	(Noorman et al., 1992)
D_{H2}	4.5×10^{-9}	$m^2.s^{-1}$	H_2 diffusion coefficient	(Ferrell & Himmelblau, 1967)
H_{CO2}	0.029	$mol.l^{-1}.atm^{-1}$	Henry constant (25 C^o)	(Ferrell & Himmelblau, 1967)
H_{H2}	7.8×10^{-4}	$mol.l^{-1}.atm^{-1}$	Henry constant (25 C^o)	(Ferrell & Himmelblau, 1967)
Reactor design parameters				
V_l	2	L	Volume liquid phase	
V_g	0.95	L	Volume gas phase	
P	1	atm	Pressure gas phase	
$R_{N2}^{transfer}$	0.5	$L\ min^{-1}$	Sparging rate of N_2	

Table 6.3. Model parameters and initial conditions obtained from the experimental data.

Symbol	Value	Units	Purpose	Source
Fermentation kinetic parameter values				
q_{smax}	0.14	C-mol.C-mol^{-1}.h^{-1}	Max*. specific substrate uptake rate	Estimated (current study)
K_s	1×10^{-4}	mol.l^{-1}	Substrate affinity constant	Estimated (current study)
Y_{C2H4O2}	0.19	mol.mol^{-1}	Max. acetate yield on substrate	Estimated (current study)
Y_{C3H6O3}	1.13	mol.mol^{-1}	Max. lactate yield on substrate	Estimated (current study)
Y_{C2H6O}	0.42	mol.mol^{-1}	Max. ethanol yield on substrate	Estimated (current study)
Y_{C4H6O4}	0.02	mol.mol^{-1}	Max. succinate yield on substrate	Estimated (current study)
Y_{C3H6O2}	0.03	mol.mol^{-1}	Max. propionate yield on substrate	Estimated (current study)
Y_{CO2}	0.63	mol.mol^{-1}	Max. CO_2 yield on substrate	Estimated (current study)
Y_{H2}	0.56	mol.mol^{-1}	Max. H_2 yield on substrate	Estimated (current study)
Y_X	0.8147	mol.mol^{-1}	Max. biomass yield on substrate	Estimated (current study)
Mass transfer kinetic parameter values				
k_la_{CO2}	120	h^{-1}	CO_2 mass transfer constant	Current study
k_la_{H2}	201	h^{-1}	H_2 mass transfer constant	Calculated (Eq. 4)
Initial concentrations				
$C^{T(0)}_s$	0.338	mol.l^{-1}	Glucose	
$C^{T(0)}_{,C2H4O2}$	0.025	mol.l^{-1}	Total acetate	

$C^{T(0)}_{,C3H6O3}$	0.005	$mol.l^{-1}$	Total lactate
$C^{T(0)}_{, C2H6O}$	0	$mol.l^{-1}$	Ethanol
$C^{T(0)}_{,C4H6O4}$	0	$mol.l^{-1}$	Total succinate
$C^{T(0)}_{,C3H6O2}$	0	$mol.l^{-1}$	Total propionate
$p^{(0)}_{CO2}$	0.0175	Atm	CO_2 in gas phase
$p^{(0)}_{,H2(g)}$	0	Atm	H_2 in gas phase
$p^{(0)}_{,N2(g)}$	0.0207	Atm	N_2 in gas phase
$C^{T(0)}_{,NH3}$	0.2	$mol.l^{-1}$	Total ammonium
$C^{T(0)}_{,X}$	0.04	$mol.l^{-1}$	Total microbial biomass
pH^0	6.8		pH
$C^{T(0)}_{,Ca2+}$	0.33	$mol.l^{-1}$	Calcium
$C^{T(0)}_{,Na+}$	0.08	$mol.l^{-1}$	Sodium
$C^{T(0)}_{,Cl-}$	0.19	$mol.l^{-1}$	Chloride
$C^{T(0)}_{,gypsum}$	0.04	$mol.l^{-1}$	Gypsum
$C^{T(0)}_{,H2O}$	55.6	$mol.l^{-1}$	Water
$C^{T(0)}_{,H3PO4}$	0.01	$mol.l^{-1}$	Total phosphate

*Maximum

6.4 Results

6.4.1 pH profile of an anaerobic fermentation neutralized with $CaCO_3$

The pH profile of an anaerobic batch fermentation with 60 $g.l^{-1}$ glucose and 33 $g.l^{-1}$ $CaCO_3$ is shown in Fig. 6.2a (dotted line). The pH during the experiment decreased from 6.8 to 6. A decrease in pH during carbohydrate fermentation has been also observed in other studies where powdered (Taylor & Nasr-El-Din, 2003) or coarse (Koenig & Liu, 2002; Maree et al., 2004; Maree & Duplessis, 1994) $CaCO_3$ was used as the neutralizing agent. After the substrate (glucose) was consumed (18 h), the remaining CO_2 was sparged out of the broth resulting in a rapid increase of the pH. During the fermentation process, the degraded glucose was converted to organic acids and compounds (mainly lactate, ethanol and acetic acid), biomass, H_2 and CO_2 (Figs. 6.2b and 6.2c). Sum of these compounds accounted for 92$\pm$3 % of the carbon supplied to the system. Using the results obtained from the experimental data,

the fermentation and mass transfer kinetic parameter values for the model could be estimated as stated in Table 6.3. The same initial concentrations of compounds and products as in the experiment were used in the model (Table 6.3). The reason for the observed initial concentrations for lactate and acetate is that there was a pre-cultivation experiment performed before the fermentation experiment to activate the culture and avoid the bacterial lag phase. The measured CO_2 in the experiment (Fig. 6.2c) originates partially from the bacterial activity and partially from $CaCO_3$ dissolution. Therefore, the biological yield of CO_2 was calculated through the difference between the measured CO_2 and the CO_2 released by $CaCO_3$ dissolution. This estimated biological CO_2 production matches with the sum of the CO_2 produced per organic acid based on their associated metabolic pathways (e.g. $C_6H_{12}O_6$ → 2 C_2H_5OH + 2 CO_2). As shown in Fig. 6.2a, reproducing the pH of the fermentation with the model showed high resemblance to the experimental pH. This suggests that the correct parameters and reactions were considered in the model.

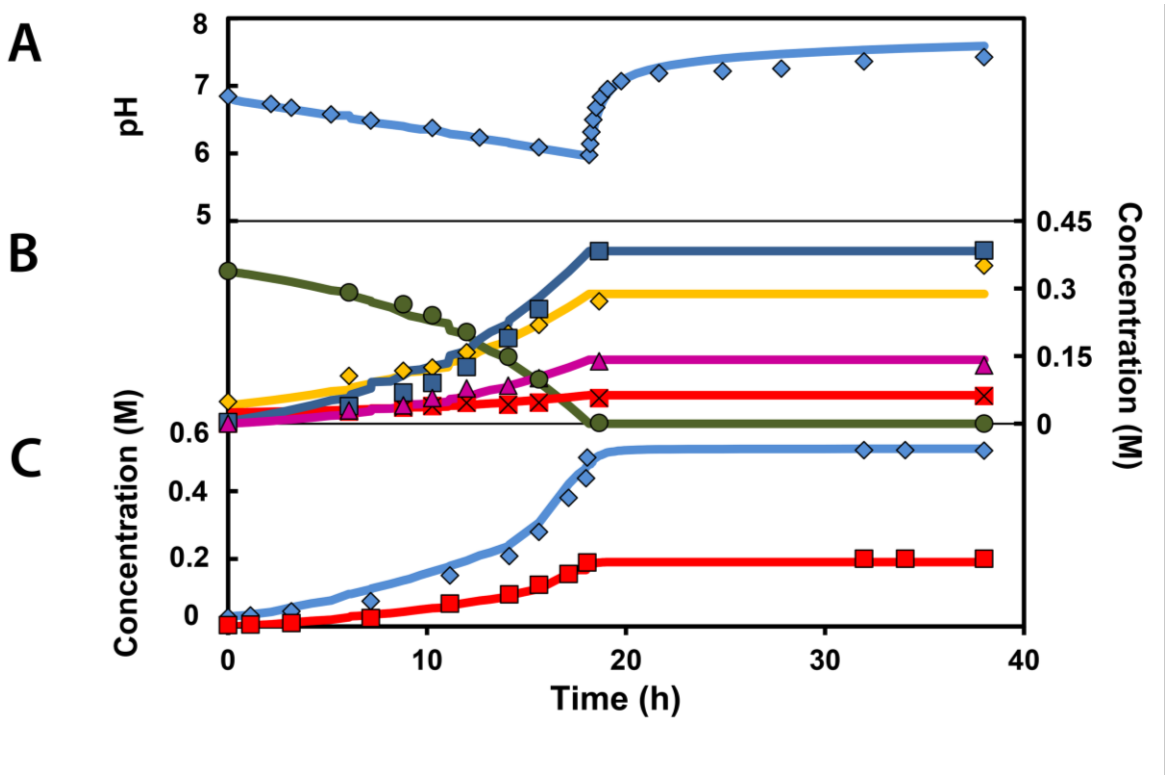

Figure 6.2 The measured and simulated pH profile of the anaerobic batch fermentation (A). Concentrations of substrate (○), biomass (◊), and fermentation products (including dissociated and undissociated): lactate (□), ethanol (Δ), and acetate (×) (B). The products of butyrate, succinate and propionate with lower concentrations than 0.01 M are not shown in the graph. Accumulative CO_2 (including biogenic and abiogenic) (◊) and H_2 (□) concentrations during the batch experiment (C). Solid lines are calculated by the model and the dotted lines are experimentally measured.

6.4.2 Changes in the concentrations of compounds influencing the pH

A better insight in the pH neutralization process can be achieved by looking at the changes of concentrations of compounds which could potentially influence the pH value (Fig. 6.1). The model can help to calculate the changes in concentrations of these compounds that could not easily be measured experimentally including the total dissociated organic acids, carbonate system components (H_2CO_3* denoted for the sum of H_2CO_3 and dissolved CO_2, HCO_3^- and CO_3^{2-}), Ca^{2+} ion and partial pressure of CO_2. Fig. 6.3 shows the changes of concentration of these compounds in the course of the fermentation process. As it is apparent from Fig. 6.3a, the Ca^{2+} ion concentration followed the increase in concentration of the total dissociated organic acids. In response to this increase (in Ca^{2+} ion concentration), the CO_3^{2-} ion concentration decreased with a comparable rate (Fig. 6.3b). The fast conversions of carbonate components (i.e. CO_3^{2-} to HCO_3^- and $H_2CO_3^*$) kept the concentration of bicarbonate low in the experiment (Fig. 6.3c). However, the observed increase in concentrations of $H_2CO_3^*$ and CO_2 in the gas phase (Figs. 6.3c and 6.3d) could indicate a relatively slower rate of H_2CO_3* transfer (from the liquid to the gas phase) or a slower convective transport of CO_2 (out of the gas phase) compared to the other reactions. It is observed from these results that the concentrations of all mentioned compounds are linked to one another and can directly or indirectly influence the pH.

The model was used to analysis the individual effect of different parameters on the pH value (sensitivity analysis). In order to do this, the batch model was extrapolated to a CSTR that served as a reference to test different scenarios. In each scenario only one parameter affecting the pH was changed while all other parameters are kept constant. Process parameters of the CSTR model are the same as in the batch model except that an in/outflow for the liquid phase is added with a flow (F) = 0.0976 L h^{-1}, an inlet substrate concentration ($C_{s\text{-}in}$) = 0.01 mol L^{-1} and an inlet ammonia concentration ($C_{s\text{-}in\text{-}NH3}$) = 0.01 mol L^{-1}. The values of these parameters were selected based on the commonly measured values in a continues wastewater treatment plant (CSTR) (Metcalf et al., 2002). All (CSTR) scenarios started from the same initial conditions as the batch model and were run until steady state is reached. The sensitivity analysis pointed out four main parameters influencing the pH: the mass transfer rate (i.e. k_la), the sparging rate (i.e. pCO_2), the inlet substrate concentration ($C_{s\text{-}in}$) and the Ca^{2+} concentration (C_{ca2+}) (Fig. 6.4). As depicted in Fig. 6.4a, increasing the sparging rate which results in decrease of pCO_2 in the gas phase has increased the pH value. A qualitatively comparable relationship was found for the mass transfer rate of CO_2 from the liquid to the gas phase which can be seen by changing the k_la value. As shown the figure, the increase in the dissolved calcium concentration and substrate concentration has decreased the pH value. Since the Ca^{2+} always requires a counter ion, it is not possible to directly increase its concentration without effecting other parameters in the system. Hence in the scenarios where the effect of Ca^{2+} concentration on pH was tested, the Ca^{2+} concentration was increased indirectly by increasing the steady state concentration of VFA (i.e. $C_{s\text{-}in}$). It was necessary to keep the pCO_2 constant (pCO_2 = 0.039 atm) and the k_la very high to observe the individual

effect of Ca^{2+}. This effect on the pH value becomes very strong at higher $C_{s\text{-}in}$ values where $CaCO_3$ and therefore the buffering capacity of the system is depleted.

Decrease of pH to low values (for the Ca^{2+} concentration and the $C_{s\text{-}in}$ parameters) inhibit the fermentative bacteria due to the high concentrations of protonated VFA. Including the inhibitory effect of the low pH on the fermentative bacteria (equation 6.7), the graph would look like Fig. 6.4b. As shown in this graph, the pH stayed at higher values compared to Fig. 6.4b. This gives a more realistic description of the CSTR for scenarios with low pH.

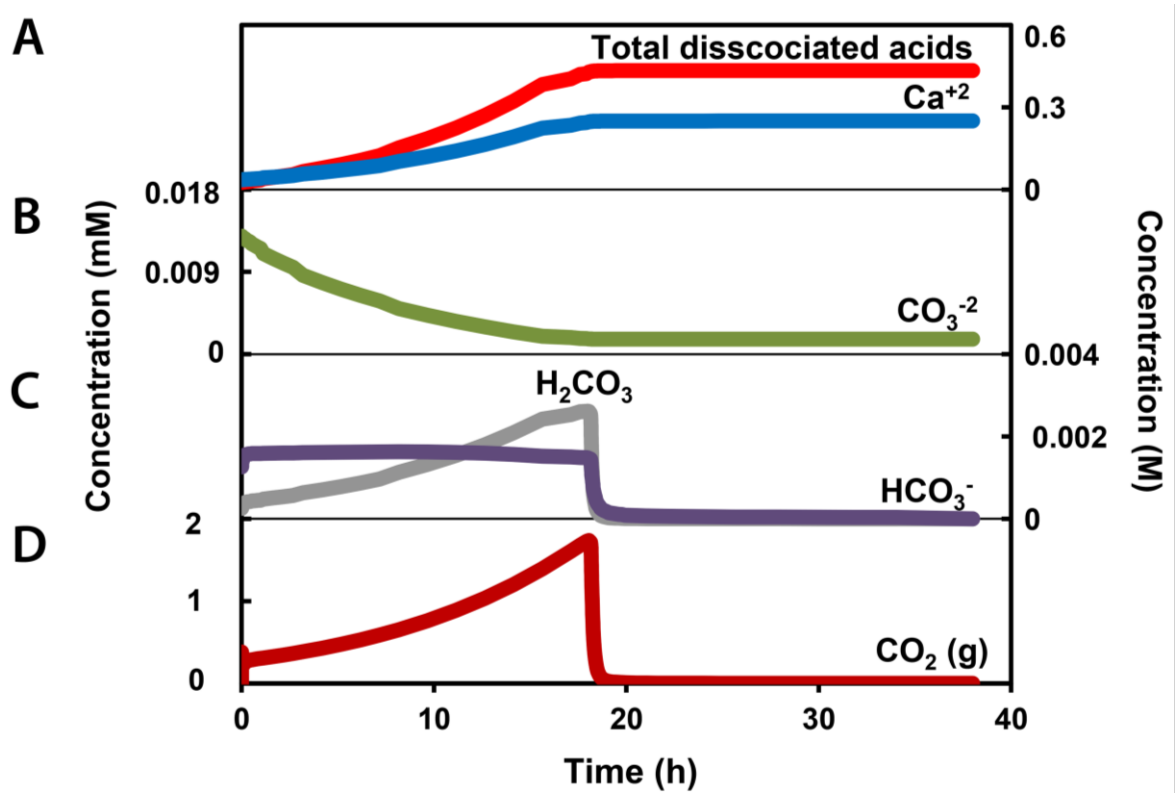

Figure 6.3 Changes in the concentrations of compounds in the fermentation process calculated by the model. The concentrations of Ca^{2+} ion and total produced dissociated organic acids (A), carbonate ion (B), bicarbonic ion and carbonic acid (C), and partial pressure of the CO_2 in the headspace of the reactor (D).

6.5 Discussion

6.5.1 The key reactions controlling the pH of a fermentation process neutralized with $CaCO_3$

Identifying the reasons for the decrease of pH in the fermentation process requires an understanding of the complex interactions between the carbonate system and the biological fermentation process. The strong resemblance between the pH profile of the experimental measurements and the numerical model showed that the model fits the data well.

Analysis of the reaction system with the aid of the model identified two main factors as the reasons for the pH decrease. As first the increase in concentration of CO_2 in the gas phase which consequently resulted in higher pCO_2. As depicted in Figs. 6.2b and 6.2c, the microbial activity in the batch fermentation process resulted in exponential increase in the production rate of organic acids and $H_2CO_3^*$. This in addition to $CaCO_3$ dissolution caused an exponential increase in the concentrations of $H_2CO_3^*$ and CO_2 gas in the headspace (Fig. 6.3d). This occurred because either the rate of convective gas *transport* (out of the gas phase) was not high enough to keep the pCO_2 low (equation 6.2) or the mass transfer of CO_2 from the liquid to the gas phase (k_la) was not high enough to keep the $H_2CO_3^*$ concentration low (equation 6.3). In either case, the exponential increase of pCO_2 and/or $H_2CO_3^*$ shifts the carbonate system equilibrium towards CO_3^2 and therefore an increase in the proton concentration in the liquid phase (i.e. decrease of pH) which can be observed in Fig. 6.1. In mathematical modeling of anaerobic digestion the mass *transfer* of CO_2 from the liquid to the gas phase is commonly introduced as one of the dominant factors influencing the pH mainly because of its slower reaction rate compared to the other carbonate system reactions (Campos and Flotats 2003; Merkel and Krauth 1999). However, in the experiment performed in this study, the extent of oversaturation in the k_la value was small compared to the changes in pCO_2. This was because of the relatively high N_2 sparging rate (0.5 $l.min^{-1}$) and mixing rate (500 rpm) of the experiment which had resulted in a high k_la value (120 h^{-1}) compared to the commonly used fermentation systems where the k_la value is in the range of 0.2 to 5 h^{-1} (Metcalf et al. 2002). Since the oversaturation is small it seems like the convective transport of CO_2 out of the gas phase plays a significant role on the pCO_2 and the decrease of pH.

A closer look at the results obtained by the model indicates that in addition to the effect of the pCO_2, there is another parameter which also influences the pH. The following explanation describes this parameter. The exponential production of carboxylates (i.e. dissociated organic acids such as lactate) in the batch fermentation process were ionically neutralized by the Ca^{2+} ion which was generated by dissolution of the $CaCO_3$. The increase of Ca^{2+} ion concentration can be observed in Fig. 6.3a. In order for the Ca^{2+} ion to continuously neutralize the produced carboxylates, the $CaCO_3$ must dissolve in a comparable rate as the organic acids production. For this to occur, the CO_3^{2-} ion concentration was forced to decrease exponentially to keep the $[Ca^{2+} \times CO_3^{2-}]$ below the solubility product of the calcium carbonate avoiding re-precipitation of the calcium carbonate (Fig. 6.3b). This decrease of CO_3^{2-} concentration caused the system to redistribute its protons accordingly to thermodynamic equilibrium (solubility and acidity constants) resulting in an increase in proton concentration or pH decrease which is apparent from the equation: $K_{a1,H2CO3^*} \times K_{a2,HCO3} = \frac{[CO_3^-] \times [H^+]^2}{[H_2CO_3^*]}$ (6.8). Equation 6.8 shows that at equilibrium conditions when $H_2CO_3^*$ is kept constant and CO_3^- decreases the proton concentration has to increase. This highlights the importance of Ca^{2+} ion concentration (which is directly related to the substrate concentration) on the pH and it indicates that the decrease in the pH is inevitable when using $CaCO_3$ as a neutralizing agent. Consequently, the increase of dissolved calcium concentration will always result in a certain pH slope.

6.5.2 Practical application of $CaCO_3$ in biotechnological processes

The partial pressure of CO_2 in the gas phase, biological acid production and Ca^{2+} ion concentration were pointed out as the main factors establishing the pH. In addition to these main factors, mass transfer rate of CO_2 from the liquid to the gas phase (i.e. k_la) was also considered as a parameter which could potentially affect the pH. Influence of these parameters on the pH were individually analyzed in Fig. 6.4a by fixing the other interdepending parameters such as $C_{s\text{-}in}$, pCO_2, and NH_3. This situation is similar to anaerobic digestion systems, in industry, which are typically established in open systems and in continuous stirred-tank reactor (CSTR) bioreactors. These systems are characterized by constant loading rate ($C_{s\text{-}in}$), very low k_la-values and a low CO_2 partial pressure as corresponds to a very high sparging rate. Therefore, the results presented in Fig. 6.4a could be extrapolated to similar biotechnological systems. As shown in Fig. 6.4a, having a higher sparging rate and mass transfer rate (i.e. k_la) increased the pH value, while a lower Ca^{2+} ion concentration and substrate consumption rate resulted in decrease of the pH. A decrease of pH to low values (e.g. pH of 4) cause protonation of volatile fatty acids that would inhibit the bacterial activity (Fig. 6.4b) and therefore it should be avoided (Kleerebezem et al., 2010). These results confirmed that if the kinetic properties of the parameters establishing the pH were identified properly, the pH of an acidifying microbiological process neutralized with powdered $CaCO_3$ can be accurately predicted. Consequently, the pH can be controlled by altering the operational conditions that can influence the identified parameters. For example. the k_la value can be modified by changing the agitation rate, size of sparging bubbles, and vessel geometry such as reactor height or diameter. Adjustment of pH of acid producing biotechnological systems by changing the carbonate system equilibrium has been practiced in other studies as well, for example aeration of solutions having high carbonate contents caused an increase of pH (El-Mamouni et al., 1995; Eshchar et al., 2006; Kim et al., 2004; Van Langerak et al., 2000) or regulating the solutions alkalinity by varying the pCO_2 (Watten et al., 2007).

It should be noted that control of the pH with the use of coarse $CaCO_3$ particles or alkaline silicate minerals is probably different than what was explained in the current study due to the kinetic limitations in mineral dissolution. Application of calcium containing silicate minerals such as wollastonite ($CaSiO_3$) in acid-producing biological processes has recently been introduced as a method for CO_2 sequestration and pH neutralization (Salek et al., 2013a; Salek et al., 2013b). Study the neutralization capacity of particulate $CaCO_3$ and alkaline silicate minerals was out of the scope if this study, however the interconnections among the carbonate components and CO_2 transfer process and calcium concentration stays similar and therefore can be extrapolated for these cases. It should be also noted that when using $CaCO_3$ as neutralizing agent, although scaling may impose problems upon degradation of Ca-VFA, it may on the other hand enable regeneration of neutralizing agent for the VFA production process. Further research is required to validate this hypothesis.

A better understanding of the $CaCO_3$ dissolution in acidifying microbiological processes can increase the practical applications of $CaCO_3$ as neutralizing agent in biotechnological processes such as anaerobic fermentations where bio-based chemicals such as lactic acid and

acetic acid are produced (Hatti-Kaul et al., 2007) or in wastewater treatment plants (the anaerobic digestion process). This can substantially reduce the operational costs since $CaCO_3$ has 60-80 % lower price (Halmann & Steinfeld, 2006) compared to the commonly applied alkaline materials in anaerobic systems which are soda ash (Na_2CO_3), caustic soda (NaOH), and hydrated lime ($Ca(OH)_2$) (Isenburg & Moore, 1992; Metcalf et al., 2002). In addition, usage of $CaCO_3$ results in a self-regulating pH system as the pH of the system cannot reach higher than 8.5 due to the nature of $CaCO_3$ excluding the requirement for active pH-control through base dosage. It is important to know that using calcium based alkalinity in an acid producing process, may induce massive $CaCO_3$ precipitation in a subsequent processing step where the organic acids are consumed in for example the methanogenic process of the anaerobic digestion process (Salek et al., 2013a; Van Langerak et al., 2000). This may impose operational problems, but when operated adequately can also be seen as an opportunity to regenerate alkalinity and exclude the need for continuous supply of alkalinity from external sources.

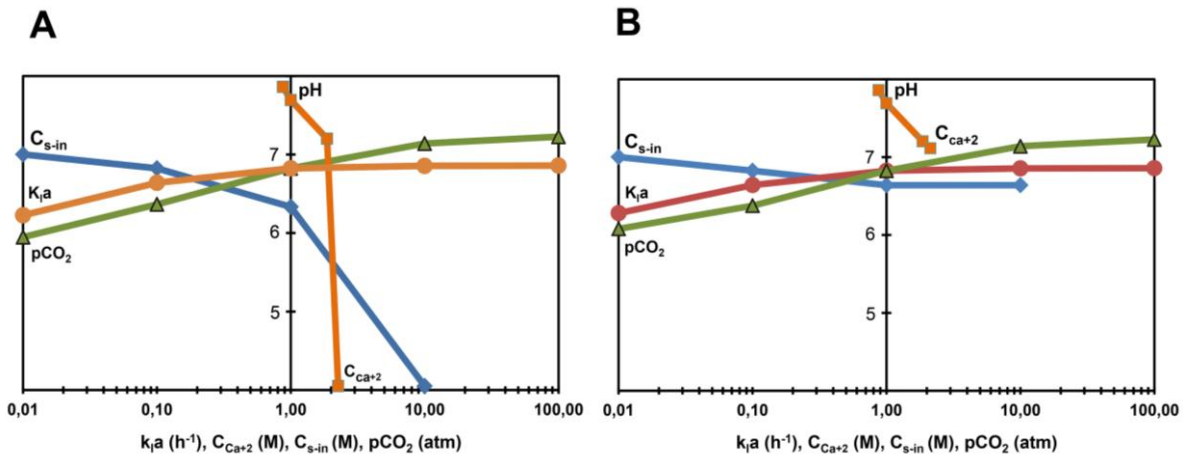

Figure 6.4 Individual influence of pCO_2 (○), C_{Ca2+} (□), $C_{s\text{-}in}$ (◊) and k_la (**Δ**) on the pH value of an anaerobic fermentation process in a continues stirred-tank reactor using the model. Without (A) and with (B) including the inhabitation factor of low pH values on fermentative bacteria (equation 6.7).

6.6 Conclusions

A numerical model was made to analyze the dominant processes which control the pH of a batch fermentation process neutralized by $CaCO_3$. The modeled pH showed strong resemblance with the experimental pH and was able to identify the factors controlling the pH. The mass transfer rate (k_la), the sparging rate (pCO_2), the inlet substrate concentration ($C_{s\text{-}in}$) and the Ca^{2+} concentration (C_{Ca2+}) were identified as the main factors establishing the pH. Although the values of pCO_2, k_la and $C_{s\text{-}in}$ affect the pH range, the calcium concentration independently sets the distribution of protons over the compounds and therefore the pH profile (pH slope). Using this information the pH of a continuous biotechnological process of CSTR system was adjusted by varying the identified parameters. The insights provided from

this study can facilitate the application of $CaCO_3$ as a cost-effective neutralizing agent by a better control of the pH via varying the identified parameters such as sparging rate (k_la) and reactors loading rate ($C_{s\text{-}in}$). This can be particularly useful in fermentation industries such as production of bio-based chemicals such as acetic acid and lactic acid.

NOMENCLATURE

k_La	volumetric mass transfer coefficient (h^{-1})
P	pressure (Pa)
R	gas constant ($J . K^{-1} . mol^{-1}$)
k	rate constant (h^{-1}, $m^3 . mol^{-1} . h^{-1}$)
K	equilibrium constant ($mol . m^{-3}$, $mol^2 . m^{-6}$, $rn^3 . mol^{-1}$)
K_i	protonated acids inhibition constant ($mol.m^{-3}$)
K_s	substrate affinity constant ($mol.l^{-1}$)
C	concentration ($mol . m^{-3}$)
C^*	maximum solubility in the liquid phase
C_s	glucose concentration ($mol . m^{-3}$)
C_x	bacterial biomass ($mol . m^{-3}$)
a	activity ($mol . m^{-3}$)
D	diffusion coefficient ($m^2.s^{-1}$)
H	Henry coefficient ($Pa,m^3.mol^{-1}$)
I	ionic strength ($mol . l^{-1}$)
r	net conversion rate ($mol . m^{-3} . h^{-1}$)
R	gas constant ($J . K^{-1} . mol^{-1}$)
T	absolute temperature (K)
V	volume (m^3)
t	time (h)
x	mole fraction
Y	yield ($mol.mol^{-1}$)
q_{smax}	maximum specific uptake rate ($mol.mol^{-1}.h^{-1}$)

Chapter 7

Conclusions and directions for future work

7.1 Conclusions and directions for future work

Atmospheric CO_2 levels are primarily balanced by the rate of volcanic input and the rate of output by chemical weathering of silicates on the time scales longer than a million years (White & Brantley, 1995). The contribution of anthropogenic CO_2 (5.4 GtC) to total CO_2 entering the atmosphere every year (213 gigatons of carbon, GtC) is about 2.5–3%. This implies that only a minor increase in weathering rate of silicate minerals by technical means can compensate for the anthropogenic CO_2 emissions.

In this thesis, the applicability of two-stage environmental biotechnological processes for silicate mineral-based CO_2 sequestration was evaluated. The main aspects covered in this thesis were: (i) evaluation of mineral sequestration of CO_2 by two-stage environmental biotechnological processes; (ii) kinetic studies of two-stage anaerobic digestion (the selected process among the proposed two-stage biotechnological processes) with silicate mineral; (iii) improvement of the efficiency of mineral sequestration of CO_2 in the anaerobic digestion system by enhancing the rate-determining processes; (iv) development of a mathematical model for an anaerobic digestion case integrated with a highly soluble alkaline mineral (calcium carbonate). This chapter provides a summary of the results obtained in addition to directions for future research. The covered topics are:

- Mineral CO_2 sequestration by two-stage environmental biotechnological processes
- Mineral CO_2 sequestration by two-stage anaerobic digestion:
    - Impacts of fermentative bacteria on wollastonite dissolution kinetics (first-stage)
    - Kinetics of $CaCO_3$ precipitation in a methanogenesis process (second-stage)
- Integration of silicate mineral into a single-stage AD system
- Mathematical modelling of an anaerobic digestion system with calcium carbonate
- Directions for future research

7.2 Conclusions

7.2.1 Mineral CO_2 Sequestration by two-stage environmental biotechnological processes

We have proposed four two-stage environmental biotechnological processes which enable mineral sequestration of CO_2. These biotechnological processes are characterized by a sequence of an acid- and an alkalinity-producing step which can integrate the two-step aqueous process of mineral CO_2 sequestration (i.e. silicate dissolution process and carbonate mineral precipitation process). These two-stage biotechnological processes are: (i) anaerobic digestion (AD) (anaerobic fermentation and methanogenesis), (ii) biological nitrogen removal (nitrification and denitrification), (iii) flue gas desulfurization (hydrogen sulfide absorption

and oxidation to elemental sulfur), (iv) bioelectrochemical systems (BES) (anodic organic carbon oxidation and cathodic oxygen reduction).

The main advantages of using biotechnological processes over the existing chemical methods for mineral CO_2 sequestration (at elevated temperature and pressure), are cost reduction and potentially lower energy consumption. This is because in the proposed biological mineral carbonation process the costs at several steps (i.e. silicate mineral pre-treatment, CO_2 capture and the CO_2 mineral carbonation) can be reduced when compared with the chemical methods. Consequently, the sequestration costs were estimated to decrease from 102 euro/ton CO_2-avoided by the available chemical methods to 40 euro/ton CO_2-avoided (60 % reduction in sequestration costs).

Considering the technology applicability and CO_2 sequestration yield (mole-CO_2/mole-substrate), the BES and the AD processes appeared to have a higher potential for application of mineral CO_2 sequestration among the four proposed biotechnological processes. In addition, integration of mineral carbonation process into AD and BES, improves the biogas quality and omitted the need for proton exchange membranes, respectively. These added values can potentially reduce the costs to below the estimated cost (40 euro/ton CO_2-avoided). We have selected the AD process for further studies because it is a widely applicable technology for solid waste and municipal wastewater treatment, while the BES in not yet a commercially applicable technology (Chapter 2).

7.2.2 Mineral CO_2 sequestration by two-stage anaerobic digestion

Anaerobic digestion process enables mineral sequestration of CO_2 by the anaerobic fermentation and methanogenesis processes. When the AD system is operated as a two-stage process, the fermentation process provides the necessary acidity for dissolution of silicate minerals and the methanogenesis process generates adequate alkalinity for mineral carbonate of CO_2. In response, the alkaline silicate minerals enhance the AD system by neutralizing the acid-producing fermentation process, and improving the biogas quality. We have performed a series of kinetic studies, to determine the effects of biological processes of an AD system on silicate mineral dissolution and carbonate mineral precipitation, and vice versa; i.e. the effect of mineral CO_2 sequestration on the AD process (Chapters 3-5).

7.2.2.1 Impacts of fermentative bacteria on wollastonite dissolution kinetics (first-stage)

Fermentative conversions have been proposed to influence the silicate mineral dissolution process by indirect mechanisms including the proton-promoted mechanism, organic ligand complexation, and change of ionic strength and by direct mechanisms caused by attached microbes on the mineral surface such as acidolysis, chelation and oxidoreduction reactions (Bennett et al., 2001; Sand et al., 2001; Uroz et al., 2009). The individual effect of pH, wollastonite concentration and fermentation products (organic ligands and alcohols) on the

kinetics of wollastonite dissolution was studied at 30 °C by performing a series of chemical batch experiments. Experimental results showed that organic ligands with two functional groups such as succinate could enhance the dissolution process more than monofunctional ligands (e.g. butyrate and propionate). The results showed that the dissolution rate of wollastonite could be significantly enhanced (> 10 times) by acids and organic ligands production in an anaerobic fermentation process.

To determine whether the anaerobic fermentative bacteria could have other effects in addition to the production of acids and organic ligands on the dissolution of wollastonite, an experimental methodology was developed including a biotic and two abiotic control experiments. Production of EPS, presence of fermentative cells in the medium, or the ionic strength variations are examples of kinds of effects that fermentative bacteria can have on the dissolution process. By using the developed methodology, it was shown that at low organic acid concentrations, the fermentative organisms enhanced wollastonite dissolution mainly by biological acid production while at higher organic acid concentrations, in addition to the proton-promoted mechanism, chelation of organic ligands also have a positive effect on the dissolution rate. This indicates that silicate mineral dissolution and its behavior can be explained and described by the involved chemical reactions (chapters 3 and 4).

7.2.2.2 Kinetics of $CaCO_3$ precipitation in a methanogenesis process (second-stage)

The methanogenesis process can mainly affect the carbonate precipitation process by increasing the pH and providing nucleation sites for calcium carbonate crystals. Kinetics of $CaCO_3$ precipitation is especially important since a delay in CO_2 precipitation can cause loss of the CO_2 to the atmosphere and therefore decrease the overall efficiency of the CO_2 sequestration. In this thesis, the kinetics of calcium carbonate precipitation during the methanogenic phase was studied by the addition of calcium acetate to an anaerobic batch reactor. The precipitation profile obtained by the ion activity product (IAP) ($[Ca^{2+}]\times[CO_3^{2-}]$), in the performed methanogenic experiments, followed the common pattern of $CaCO_3$ precipitation with initial formation of less stable polymorphs and the subsequent ripening to the calcite precipitate. Initial addition of calcite seed crystal in the bioreactor improved the $CaCO_3$ precipitation process by shortening the induction period. The precipitation of $CaCO_3$ in the methanogenesis experiments showed that the methane content in the biogas could increase to 71 % v/v as compared with that of the control experiment (calcium free environment) with 53 % v/v methane content (Chapter 5).

7.2.3 Integration of silicate mineral into a single-stage AD system

Mineral CO_2 sequestration in a single-stage AD system was studied by integrating wollastonite in anaerobic batch experiments fed with different concentrations of insoluble starch to study the sequestration process under the increasing acidifying conditions.

The efficiency of CO_2 sequestration was limited in these experiments due to the poor separation of fermentation and methanogenic phases. This means that the acidity and alkalinity required for wollastonite dissolution and carbonate mineral formation, respectively, could not be achieved in the experiments. However, an increase of 18 times in the dissolution rate of wollastonite by anaerobic fermentation process, obtaining higher caloric value biogas, a self-regulating pH system and potential production of biobased materials (biochemical grouting material and biofertilizer) help to justify the integration of silicate minerals in the AD systems for CO_2 sequestration (Chapter 5).

7.2.4 Mathematical modelling of an anaerobic digestion system with calcium carbonate

A mathematical model has been developed with a minimum set of kinetically controlled and equilibrium reactions to study an AD system with a highly soluble alkaline mineral, $CaCO_3$. This system represented a simpler case compared to the slow-dissolving silicate minerals in an AD system. This study helped us to better understand the complicated interconnections among the calcium concentration, organic acids, carbonate components, CO_2 mass transfer. The model was able to reproduce the experimental data of a batch fermentation experiment using finely powdered $CaCO_3$. The strong resemblance between the experimental and the predicted pH by the model indicated that the chosen set of equilibrium reactions and kinetically controlled were capable of describing the essential properties of the system. The identified factors that the system was most sensitive to were: (i) the mass transfer rate of CO_2 from the liquid to the gas phase, (ii) the sparging rate, (iii) the inlet substrate concentration, and (iv) the calcium ion concentration. A better understanding of the interconnections among the carbonate system components with other parameter in the system achieved by the model can be extrapolated to the more complicated case of AD system integrated with silicate minerals.

In addition, the insights provided from this study can facilitate the application of $CaCO_3$ as a cost-effective neutralizing agent by a better control of the pH via varying the identified parameters such as sparging rate and reactors loading rate. This can be particularly useful in fermentation industries for production of bio-based chemicals such as acetic acid and lactic acid (Chapter 6).

7.3 Directions for future work

7.3.1 Further cost-reductions

Application of biotechnological processes for mineral CO_2 sequestration is estimated to reduce the costs (to less than half) as compared with the sequestration costs of chemical methods based on silicate minerals (from 102 euro/ton CO_2-avoided to 40 euro/ton CO_2-avoided). However, the costs are not yet considered feasible for the commercial application relative to other available CO_2 storage technologies (Anderson & Newell, 2004; Huijgen et al., 2007). The offered added values to the AD and BES processes can potentially reduce the

costs to below 40 euro/ton CO_2-avoided. A comprehensive cost-benefit analysis for each of the suggested biotechnological processes is required to calculate the sequestration cost per ton CO_2 avoided.

7.3.2 Technical improvements

Based on the results of this study, the CO_2 sequestration efficiency in an AD system can be improved through several process modifications: (i) in the anaerobic fermentation process (first-stage), since organic ligands with two functional groups showed higher enhancement on the dissolution process compared with that of the monofunctional ligands; directing the fermentation process towards production of these compounds can increase the dissolution rate and release of divalent cations for the methanogenic phase; (ii) in a single-stage AD system, a gradient of pH (subsequent low and high pH) which is caused by separation between the fermentation and methanogenesis phases in the reactor can result in sufficient dissolution of wollastonite and mineral carbonation of the CO_2.

There are a number of challenges that needs to be overcome before biotechnological processes can be commercially applied for mineral CO_2 sequestration. Depending on the process considered, one of the main challenges for integration of mineral carbonation into these biotechnological systems is the development of a method for cost-effective transport of Ca^{2+} ions between different vessels. Moreover, decrease in the dissolution rate (mol/cm^2/s) of silicate minerals in time due to re-precipitation of SiO_2 on the surface of the mineral or incongruent mineral dissolution, can result in accumulation of ineffective silicate mineral particles in the reactor. This can decrease the overall efficiency of the CO_2 sequestration. Another technical issue to be considered is selective removal of silicate and carbonate precipitates from the first and second stages, respectively, with minimum operation disturbances. A possible method is to use an external gravity separator which separates the biomass and minerals based on their density difference.

7.3.3 Long-term application of mineral CO_2 sequestration in solid waste treatment systems

The slow kinetics of mineral carbonation reactions makes it a proper fit for integration into the slow biological processes of landfill treatment sites. The anaerobic fermentation and methanogenic phase at landfill sites occur subsequently in different time zones, in which silicate mineral dissolution and CO_2 carbonation processes can take place. The longer residence time (20–25 years) in these systems, compared with the anaerobic digestion process (20-30 days) can provide the possibility to use larger size silicate minerals reducing the process costs. The mineral carbonation process can simply be integrated by mixing the ground silicate minerals with the solid waste at the beginning of the disposal process. Integration of the CO_2 sequestration process into landfill sites offers additional advantages: improved biogas, reinforcement of soil by carbonate mineral precipitation, and higher stabilization rate of the

solid wastes. The latter is because the alkalinity released from silicate minerals can prevent inhibition of the bacterial activity through an excessive drop of pH in the fermentation phase. These potential treatment applications warrant further research on integration of silicate minerals in solid waste treatment.

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Curriculum Vitae

Shiva S. Salek was born in 18 October 1983 in Tehran, Iran. Following her interests, she did her BSc studies on the subject of enviromental engineering at Tehran University. Right after obtaining her BSc degree in August 2005 with the thesis title of "Nitrite removal from underground wastewater in Tehran", she moved to Singapore to start her master studies. During the two years of her master program in Singapore, she received complete financial support from A*STAR agency. In 2007, she graduated from the department of chemical engineering, National University of Singapore (NUS) with the thesis title of "Hydrogen production by granules enrichment in an acidogenic fermentation process" under the supervision of Prof. Ng Wun Jern.

After graduation in August 2007, she continued to work at NUS as a researcher for 1 year and 9 months on a project entitled: "Removal of emerging contaminants of wastewater using genetically modified bacteria" with the guidance of Prof. He Jianzhong.

In May 2009, she moved to the Netherlands to start her PhD under the supervision of Prof. dr. ir. Mark C.M. van Loosdrecht and Prof. ir. Robbert Kleerebezem in the department of biotechnology, at Delft University of Technology (TU Delft). From May 2013 till present she has been working at Lely Industries (Maassluis, the Netherlands) as a product engineer on the topic of dairy manure treatment. The project involves start-up and development of a dairy manure refinery system which can offer economic and environmental advantages to the farmer.

List of publications and presentations:

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Patent

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