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Anaerobic digestion of alkaline Kaumera Nereda® gum waste residual sludge at pH 9.6 and 0.6 M Na^+ to produce CO_2-free green gas

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Contents

Preface

About 10 months ago I started looking for a project that was part experimental and part modeling to improve my skill on both and see which path I would like the most. I came across the project 'alkaline anaerobic digestion' and was interested in it from the beginning. Eventually I decided that this is what my master thesis will be about. 8 months ago we started the first incubations as the process will be a slow one and had a high chance of failing. During the waiting on the batches my focus was put towards modelling the same process to have a better understanding of what goes on during this unique process.

Once most batches became active at the same time it was an essential skill to multi-task and keep both the experimental work and modelling going. However, with help from various people it became manageable task and really enjoyed the fun, but challenging, time i got to spend on the alkaline anaerobic digestion.

I would like the express my gratitude towards Philipp, Mark, Robbert, Dimitri & others at EBT for active help in forming this thesis and valuable discussions. A special thanks to Xavier for helping me model this one of a kind process and teach me new skills on how to tackle these modelling challenges. Finally I want to thank Walter van Gulik and again Mark van Loosdrecht, Robbert Kleerebezem and Philipp Wilfert for taking a seat at my thesis comité.

Ramon
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Abstract

The first full-scale Kaumera extraction plants are in operation and increases the circularity of WWTP already. However, to reach a goal of zero waste production it is necessary to look into the waste stream of the Kaumera extraction itself. Roughly 30% of organics is extracted in the process and the remaining organics in the waste can be further recovered using anaerobic digestion (AD). In this study the continuation of the alkaline AD was used, instead of neutral digestion. Main reason for the alkaline digestion compared to the neutral digestion is the increase in CH_4 content in the biogas as CO_2 remains in the liquid at pH 9.6. The batch incubation uses the alkaline waste residuals stream of the Kaumera extraction plant in Epe. The digestion was done at haloalkaline conditions (pH 9.6; 0.6 M Na^+). Combinations of inoculum enriched for similar substrates from a previous study and fresh soda lake sediment were used for the AD batch incubations. CH_4 yields varied from 8-28% of total COD going into CH_4. Compared to literature this is on lower side as these conversions are in the range of 35-50%. However, some were incubations with pre-treated substrate and already enriched incubations. Others were neutral digestion of similar substrate and the substrate used in this study. This does show the potential still for a higher conversion of methane in the alkaline digestion of the Kaumera residuals. Based on a titration, the Alkalinity need to keep the pH at 9.6 is 3 g/L of NaOH to prevent a drop from pH 9.64 to 9.34. The overall process observed takes longer than the neutral digestion due to a delay seen in acetate conversion, therefore no bottle-neck in the process could be defined and only a kinetic problem was identified. This could be tackled by transferring the process toward continuous operation, avoiding the slow growth of syntrophic acetate oxidisers once steady state is achieved. The process was modelled using two different methods, where issues surrounding the pH description arise. For the models it is essential to extend the simple buffer capacity description in order to reliably simulate the pH dynamics of the system. As of now information around the microbial community is scarce making the modelling of the alkaline ADM1 a difficult task. To improve the alkaline ADM1 work should be done to determine kinetics of the microbial community and a better description of the substrate with inoculum. The Dry matter (DM) of the process was 0.88%, which is quite low as in full-scale system usually at least 5% DM is used. The low DM used will lower the chance of bottle-necks in the process, thus with an increase of almost 6 times in DM the inhibition threshold of 420 mgNH_4−N will be exceeded. Other implication that need to be solved is the highly saline and high pH waste stream after solids removal. Either this should be recycled back for subsequent digestions or added to the influent of an WWTP assuming it will be diluted enough to not have a major impact anymore. In the future continuous operation should be evaluated as an alternative strategy to prevent the syntrophs from delaying the process and operational parameters like the hydraulic retention time (HRT) and solid retention time (SRT) need to be studied for optimal digestion in such a system.

Nomenclature

Abbreviations

AD	Anaerobic digestion
AGS	Aerobic granular sludge
COD	Chemical oxygen demand
DM	Dry matter
EPS	Extracellular polymeric substance
HPLC	High pressure liquid chromatography
HPLC	Total Kjeldal nitrogen
HRT	Hydraulic retention time
IC	Inorganic carbon
ODM	Organic dry matter
SAOB	Syntrophic acetate oxidising bacteria
sCOD	Soluble chemical oxygen demand
SDS	Soda Lake Sediment
SRT	Solid retention time
tCOD	Total chemical oxygen demand
TS	Total solids
VFA	Volatile fatty acid
VS	Volatile solids
WWTP	Wastewater treatment plant

1. Introduction

In today's society sustainability is a top priority, which includes a circular economy where waste is transformed into valued products (Neczaj & Grosser, 2018). Due to depleting resources and high waste production in 2015 an action plan for circular economy was set-up by the European Commission (European Commission, 2015). The plan is focused on using resources in a more sustainable way. A highlighted issue is the waste management and to treat this as a valuable material to be reintroduced to the economy developing a secondary raw materials market (Kalemba, 2021). This also holds true for wastewater treatment plants (WWTPs) as these produce quite significant amounts of waste sludge, that can be used to create valued bio-based products. The current prime solution to treat wastewater is the TUD invented Nereda® aerobic granular sludge (AGS) technology (Pronk et al., 2018). The Nereda® Wastewater treatment plant uses granules, which allow for a much faster settling time decreasing the size of WWTPs tremendously. However, just like the conventional wastewater plants sludge is produced as a by-product that needs further processing. One option is to use the Kaumera Nereda® gum process, which uses this waste sludge to create a valued bio-based product, called Kaumera (STOWA, 2019). The simplified Kaumera production process is shown in figure 1.1.

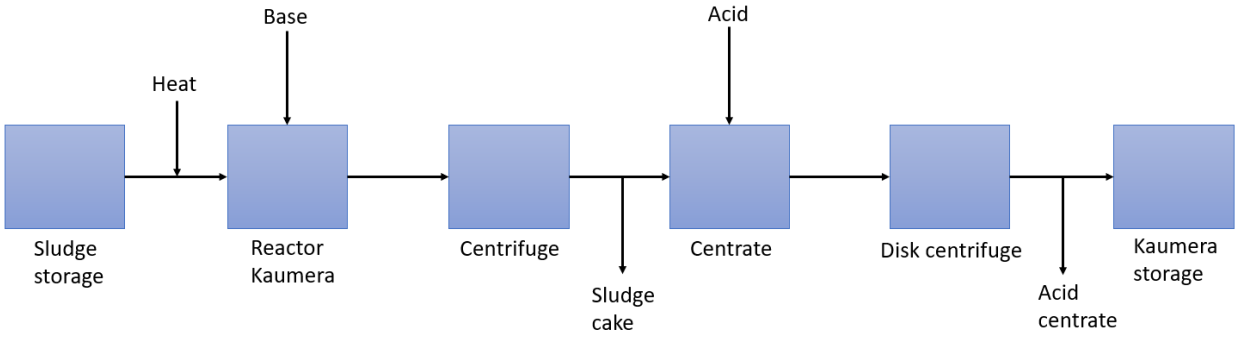


Figure 1.1: Simplified schematic of the Kaumera gum process (STOWA, 2019). The sludge cake is the alkaline residual waste sludge being used in this study as the substrate

First the sludge is heated up to 80 °C and base is added to a final pH between 9-11 in order to extract approximately 30% of the organics as Kaumera (STOWA, 2019), which is essentially the extracellular polymeric substances produced by the bacteria to help with protection from the environmental stresses, mechanical stabilization and the storage of carbon or water (Li et al., 2021). The next step is to centrifuge, creating a liquid phase containing the Kaumera and a waste sludge cake (kaumera residuals). The liquid phase will continue for further processing, but the alkaline cake formed is a waste. This is normally used in anaerobic digestion (AD) to produce biogas and efforts are made to recover nutrients. Using this process valued bio-based products like biogas are formed and the remaining sludge is incinerated to generate heat to be used somewhere else in the process (Kleerebezem, 2016, Owusu-Agyeman et al., 2021). Biogas will exist mainly as mixture containing carbon dioxide (CO_2), methane (CH_4) and water vapour (H_2O), but can also contain nitrogen (N_2), hydrogen (H_2), Ammonia (NH_3) and hydrogen sulfide (H_2S) in neutral conditions(Balat & Balat, 2009). However, the waste sludge from the Kaumera process will have a high pH between 9-11 and high salinity (KOH is used as base in the extraction).

These starting conditions are more suited for alkaline anaerobic digestion instead of neutral digestion. Previous research also showed advantage of alkaline digestion over the neutral digestion process. Alkaline pre-treatment has shown to give increased yields of COD converted into methane due to larger solubilization of COD (Torres & Lloréns, 2008, Vlyssides & Karlis, 2004, S. Wang et al., 2020), which is similar to the alkaline extraction of EPS. One reason to not do the pre-treatment were the cost associated with alkalinity needs, but this will already be done during the Kaumera extraction process. Other studies have also shown that by keeping the pH high it is possible to still generate methane with the main added benefit is creating a high purity methane biogas ($>$ 90%) and keeping the yield between 0.1 to 0.45 g COD CH_4/g tCOD (Sels, 2019) and no trace-amounts of H_2S were found in the biogas (Nolla-Ardèvol et al., 2015). This is due to the high pH keeping the carbon dioxide, hydrogen sulfide & other acidic impurities in the liquid.

The inorganic carbon in the system will be mostly in the bicarbonate (HCO_3^-) and carbonate form (CO_3^{2-}) and the sulfide compounds will follow the same principle as most of it will be in the form of bisulfide (HS^-) instead of hydrogen sulfide (H_2S) (Dickson et al., 2007). However, methane is not affected by pH and therefore will be a dominant component in the biogas similar to natural gas (90-95% CH_4) (Balat & Balat, 2009). Based on these studies it is worth investigating the alkaline anaerobic digestion of Nereda® Kaumera gum alkaline waste sludge from the WWTP from Epe having both the alkaline pre-treatment combined with alkaline AD.

1.1 Alkaline anaerobic digestion

The simplified anaerobic digestion consists of 4 distinctive stages: hydrolysis/solubilization, acidogenenesis, acetogenisis & methanogenesis (Meegoda et al., 2018) shown in figure 1.2. Alkaline anaerobic digestion is mostly similar, but has some differences in the hydrolysis and methanogenesis stages.

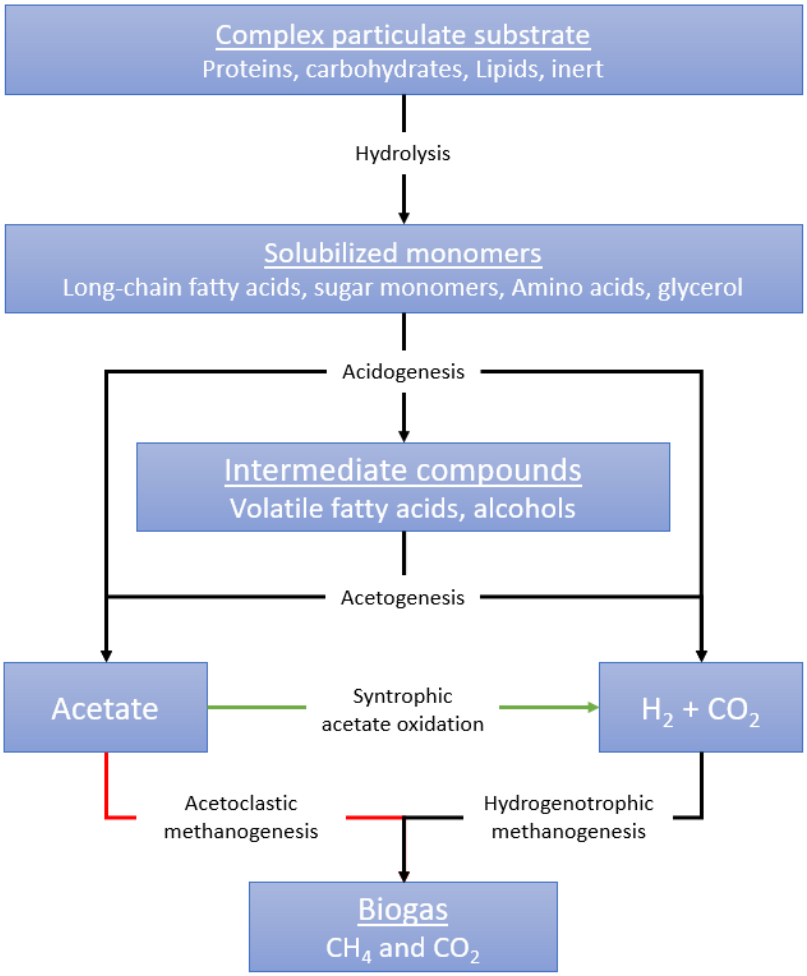


Figure 1.2: General alkaline anaerobic digestion process (Kleerebezem, 2016, Sels, 2019). The acetoclastic methanogenesis pathway (shown in red) is not possible and instead the Syntrophic acetate oxidation (shown in green) will convert the acetate to H_2 and CO_2 to be used by hydrogenotrophic methanogenesis. Not specifically shown in the figure is the possibility of propionate & butyrate oxidation to H_2 & CO_2 and acetate Westerholm et al. (2021) where syntrophic acetate oxidition forms the final H_2 and CO2.

During hydrolysis the complex particulate substrate will be broken down into monomers and solubilized (Kleerebezem, 2016, Meegoda et al., 2018). This is typically done via extracellular enzymes produced by acidogenic bacteria, but is also possible with the use of a pre-treatment step. The substrate to be studied has undergone thermal alkaline pre-treatment, thus hydrolysis and solubilization is probably enhanced due to this. After hydrolysis there are usually amino acids, sugar monomers, long-chain fatty acids and glycerol.

This can be used by the fermentative bacteria in the second stage to form volatile fatty acids (VFAs), carbon dioxide, hydrogen, formate and alcohols. For the VFAs usually acetic acid, propionic acid and butyric acid are produced, but other short-chain fatty acids (like lactate) might also be present. The degradation of amino acids in the acidogenisis will be paired with the release of the ammonium in solution.

These intermediates are then converted in the third stage, Acetogenisis, to either acetate and/or H_2 and CO_2 (Westerholm et al., 2021). These compounds are the main pre-cursors needed for methanogenesis stage. Essentially the current and previous processes are needed to create the limited options of substrates for the methanogens, meaning the H_2 + CO_2 and acetate.

Two metabolic groups of methanogenic archaea are mainly responsible for methanogensis, acetoclasctic methanogens and hydrogenotrophic methanogens. Acetoclastic methanogens are not able to survive the combination of high pH and salinity as seen in other studies (Wormald et al., 2020), meaning acetate will accumulate or needs to be converted to hydrogen that can be used by hydrogenotrophic methanogens. Hydrogenotrophic methanogens seem to be the dominating group in alkaline AD forming the biogas (Wormald et al., 2020). A study by Sorokin et al. (2016) did show that acetate oxidation is possible with syntrophic acetate oxidising bacteria (SAOBs) from soda lakes at extremely haloalkaline conditions. Acetate oxidation itself is an energetically unfavourable process (Sels, 2019), which is why it will need a microbial partner to keep concentration of hydrogen gas low. By coupling the reaction to the hydrogenotrophic methanogens it can keep the hydrogen pressure low enough for both reaction to be viable. Figure 1.3 shows the hydrogen concentration range that will create an overall thermodynamically favourable reaction.

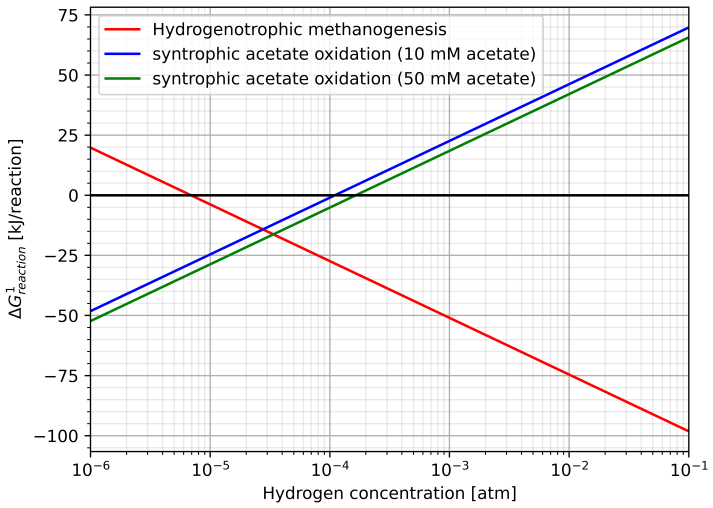


Figure 1.3: General syntrophic acetate oxidation and hydrogenotrophic methanogenesis interspecies hydrogen transfer. To make the thermodynamic graph two different concentration for acetate are chosen (10 & 50 mM) to show the difference in free energy. Concentrations at pH 9.6 for carbonate is 0.30490 M according to the media composition. Partial pressure methane is assumed to be around 0.1 bar.

Such syntrophic association is able to convert acetate into CO_2 and H_2. Furthermore according to Wormald et al. (2020) another factor is the uptake of the acetate. Due to the high pH almost all acetate will be in the dissociated form, preventing trans-membrane diffusion. This would make the uptake of acetate reliant on a active uptake system, whereas normally it could also diffuse freely over the membrane. According to Wormald et al. (2020) it is then also likely to be less energetically favourable than hydrogenotrophic methanogenesis.

During the alkaline digestion there will also be quite some ammonia release due to the high pH. It is known that acetoclastic methanogens have a lower tolerance compared to syntrophic acetate oxidizers (Westerholm et al., 2019). Ammonia released in neutral digestion is less of an issue due to most being in the form of ammonium (NH_4^+), however at a pH of 9.6 most of this will be in the form ammonia (NH_3) that can freely diffuse over the membrane. Once in the cell, where the pH is neutral, it will go back as ammonium and has to be actively transported out of the cell using up energy that could be used for catabolic reactions instead (Sousa, 2017).

Potential issue in AD is VFA failure due to accumulation, but as the high pH creates an issue for the ammonia toxicity and uptake of VFAs it helps with digester failure due to VFAs. Now all of the VFAs are dominantly in the dissociated form preventing free diffusion into the cells. This will prevent the VFAs from disturbing the proton balance (Sels, 2019, Sousa, 2017).

Another important aspect of the substrate is the high salinity, which can have large impact on the microbial community composition (McGenity & Sorokin, 2019). Furthermore the concentration can have negative effects on the effectiveness of the alkaline AD (Nolla-Ardèvol, 2014). This indicates there is a optimum pH and salt concentration for different substrate and according to Nolla-Ardèvol et al. (2012) best production of methane using hydrogen was achieved at pH 9.5 and 0.6 M Na^+, which is also used in the study of Sels (2019). As the dominant predicted species of methanogens will be hydrogenotrophs bacteria it seems to be the best conditions for the alkaline AD. It is also shown by Sels (2019) that the type of ion, K^+ also has a negative effect on the digetion.

1.2 Modelling the alkaline anaerobic digestion

Anaerobic digestion, alkaline or neutral, is a complex process carried out by multiple micro-organisms all having their own growth and substrate uptake characteristics. Adding to this the speciation of all the different compounds where mainly the carbonate system plays a big role. This makes it impossible to intuitively see the dynamics of the system, which means experiments are needed to have some insight in the process. However, AD is a rather slow process taking months to produce data for a single batch. One solution would be to create a model that is able to describe the process to evaluate it (Rozzi, 1984). Over the years different models were created, but differed greatly from one another. The Anaerobic digestion model 1 (ADM1) attempted to combine most of these into one general model. Since then the ADM1 is the standard for AD modelling and subsequent extension are based on the original making comparisons easier between different studies (Batestone et al., 2002, Weinrich & Nelles, 2021).

There is a wide range of applications for the ADM1. One is the ability to predict the process using basic input parameters that can be easily measured & determined according to Kleerebezem (2016). The complex particulate substrate is characterized by percentage of proteins, Lipids & carbohydrates and the inert part of the substrate combined with the initial inorganic carbon and salinity of the stream. The ADM1 describes all 4 fundamental stages of the anaerobic digestion and the physicochemical dependencies are included to iteratively determine the pH and phase transitions processes (Batestone et al., 2002, Weinrich & Nelles, 2021).

1.3 Main goals study

The aim of this study is to answer the following research questions for the Alkaline AD of the alkaline Kaumera waste sludge:

i What is the yield of the alkaline anaerobic digestion (mL CH_4/g VS & COD CH_4/tCOD_{in}) for a batch digestion on Kaumera residual waste sludge from Epe using a bicarbonate/carbonate buffer starting at pH 9.6 and 0.6 M Na^+?

ii What is the dosage of alkalinity needed to keep the pH stable at 9.6 to keep the purity of CH_4 in the biogas above 90% removing the need for post-treatment according to experimental data and model prediction?

iii How do the pH, sCOD, NH_4^+ & VFA change during the alkaline anaerobic digestion on the Kaumera residual waste sludge from Epe and can a bottle-neck be found for the process?

iv What is the characterization of the Kaumera residual waste sludge in terms of carbohydrates, proteins, lipids and inert using their respective COD values to be used in ADM1?

v How well does the simplified excel model and adjusted ADM1 describe the alkaline anaerobic digestion process in order to make predictions on the various compounds released and change in pH for scaling up/optimizing of the process after adding the syntrophic acetate oxidation?

2. Materials and Methods

2.1 Materials

2.1.1 Inoculum

Initially two different inoculums from a previous study ((Sels, 2019)) were taken, which were used to do an alkaline anaerobic digestion using similar conditions. The first inoculum was enriched on aerobic granular sludge (AGS) from the full-scale Nereda® municipal WWTP in Vroomshoop (FS-AGS). The second inoculum is enriched on AGS from a lab-scale sequencing batch fed on acetate, phosphate and ammonium (LAB-AGS). For both the substrate was lyophilised and grounded with pestle and mortar. The third inoculum was soda lake sediment of the Kulunda Steppe from Russia (SLS) Sorokin et al. (2015). All inoculums were made anaerobic by flushing with either argon or nitrogen and stored in the fridge.

From these 3 inoculums a total of 5 mixtures are made to digest the Kaumera alkaline waste sludge. Due to the nature of the substrate and high experiment time multiple batches were run parallel to ensure an active alkaline anaerobic digestion, shown in figure 2.1.

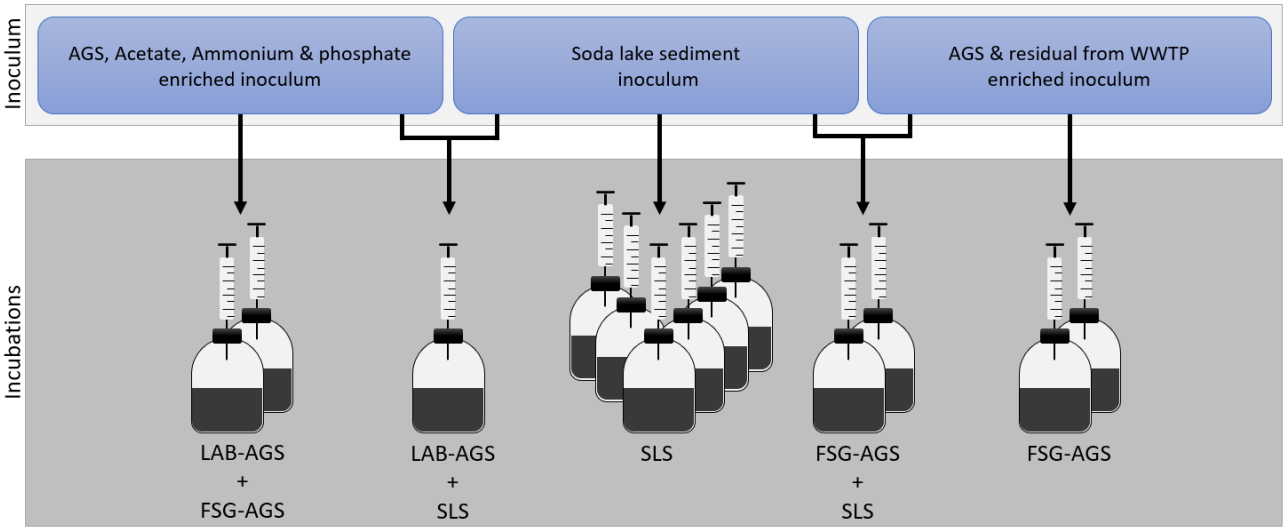


Figure 2.1: schematic of different inoculates used. LAB-AGS + FSG-AGS is not shown with the arrow to keep the figure uncluttered. other combination are shown correctly.

2.1.2 Substrate

The substrate used in this study is collected from the Kaumera Nereda® gum extraction process in Epe. The waste is the residual wet alkaline waste pellet after the centrifugation of the extraction process. It has a sludge-like consistency with a high amount of solids that are insoluble. The characteristics of the waste sludge are shown in table 2.1. The tCOD measurement was done by diluting it approximately 45 times in milli-Q water. After mixing the remaining particles were minimized using a Autotorax mixer and continuous mixing was used during sampling to come close to a homogeneous solution. This is then analysed on a Hach lange (HACH LANGE DR 3900) and incubation was done the HACH LANGE HT 200 S.

Kaumera alkaline waste pellet		
tCOD [g O2/ kg waste sludge]	119.2	± 4.57
TN* [g TN/kg waste sludge]	5.4	± 0.14
TDM* [g TDM/Kg waste sludge]	116.28	± 0.29
ODM* [g VS/ kg waste sludge]	76.22	± 0.18
tCOD/VS [-]	1.56	± 4.57
Ash content [%]	34.5	± 0.06

Table 2.1: Kaumera alkaline residual waste sludge characteristics

Data on the TDM, TN & ODM was done by Arentze, C. (unpublished) on the substrate as well, where tCOD/VS (1.82 tCOD/VS) greatly differed from the method in this study (1.56 tCOD/VS). Both measurements show large COD/VS ratios, which are unrealistic. This is mainly due to inability to completely dissolve the substrate and possible gradients in the sample bottle. Therefore the tCOD used further in the study is determined using the ODM measurement and a COD/VS of 1.34 which is what Sels (2019) has seen for the substrate, which is similar to the substrate used in this study, and is also in the range that is usually seen (1.4 COD/VS).

2.1.3 Media & buffer

The media in this research was made the same as Sels (2019) and Nolla-Ardèvol (2014). The buffer used was a sodium bicarbonate/carbonate with 15 g/L Na_2CO_3 and 20 g/L $NaHCO_3$ giving a final pH of 9.6. Adding NaCl, K_2HPO_4,$MgCl_2$, 1 mMSe/W solution and acidic trace metals according to Pfennig & Lippert (1966). Concentration added to the media are seen in table 2.2. The media/buffer was made anaerobic by sparging with Argon (Ar) for 30 minutes and adding sulfide to reach a concentration of 0.1 mM and storing it at room temperature in an anaerobic environment.

Compound		Concentration
NaCl	[g/L]	3
K2HPO4	[g/L]	1
MgCl2	[g/L]	0.1
Se/W (1mM)	[mL/L]	0.1
Acidic Trace Metals	[mL/L]	1
EDTA (trilon B)	[g/L]	5
$FeSO_4 \cdot 7\ H_2O$	[g/L]	2
$Zn \cdot 7\ H_2O$	[g/L]	0.1
$MnSO_4$	[g/L]	0.04
H_3BO_3	[g/L]	0.3
$CoCl_2 \cdot 6\ H_2O$	[g/L]	0.2
$Cucl_2 \cdot 2\ H_2O$	[g/L]	0.0013
$NiCl_2 \cdot 6\ H_2O$	[g/L]	0.0029
$Na_2MoO_4 \cdot 2\ H_2O$	[g/L]	0.02

Table 2.2: Media composition. The Acidic trace-metals in some incubations are 1000 times higher for $CuCl_2 \cdot 2\ H_2O$, $NiCL_2 \cdot 6\ H_2O$ & $Na_2MoO_4 \cdot 2\ H_2O$. These batches were reïnoculated after the discovery of the high ATM as most of it was assumed to precipitate in the incubation

For some incubations a higher amount for the final three acidic trace metals were added by a factor 1000. These were all reinoculated after the discovery as most of it assumed to be precipitated. All affected batches are mentioned in §2.2.2.

2.2 Methods

2.2.1 Batch set-up

Different incubation were setup using serum bottles with a liquid volume of 50 mL and a headspace of roughly 62 mL. Conditions in the bottle were highly saline and alkaline. The serum bottles were incubated in a oven at 35-37 °C. Bottle were made anaerobic by capping with rubber butyl stoppers and exchanging the headspace with inert argon gas. After relieving the pressure 2-3 drops of neutralized 10% dithionite solution were added to have another oxygen barrier next to the sulfide (appendix A). The serum bottles were not stirred during their incubation and to measure gas production gas-tight syringes were used. Gas was either disposed of after the syringe was full or stored in NaCl brine filled glass vials for later gas analysis. Due to the method of gas quantification a small resistance of the syringe was assumed to create an overpressure resulting in an underestimation of the gas volume. A schematic representation of a unique serum bottle incubation is shown in figure 2.2.

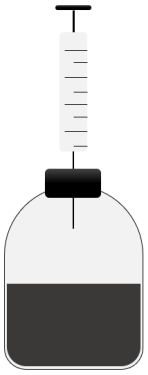


Figure 2.2: Single serum bottle batch incubation

2.2.2 Experiment

Roughly 3.7 g of substrate was added to each serum bottle to match the VS content from the study of Sels (2019) to compare the Kaumera alkaline waste sludge better to the different substrates. After addition of substrate the bottles were aerated with argon using a gas-exchanging system and transferred into the anaerobic chamber.

Different amounts & combinations of inococulum were added due to inactivity of first incubations. Incubation #2 and #4 (LAB-AGS + SLS & FS-AGS + SLS) have a total of 3.5 mL inoculum (7%) and was used to see whether adjusted inoculum with soda lake sediment will be a ideal starting culture. The addition of SLS was to ensure the methanogens the haloalkaline community was present as the enriched inoculum was quite old and stored of a long time. These organism are quite sensitive so could be that the inoculum was not viable anymore. Incubation #1 & #5 (LAB-AGS + FSG-AGS & FSG-AGS) were to see whether the enriched substrate was adjusted enough to also digest the new substrate. The larger volume was due to previous batches with less volume inoculum did not yield any activity (Arentze, C. (unpublished)). Finally incubation #5 was only SLS to see if a non-adjusted community would be better suited for the Kaumera residuals. Main goal was to increase the chance of successful digestion.

The media is the same for all incubations, besides the concentration of ATM. Incubation #1, #2, #4 & #5 all contained high concentration of ATM and incubation #3 had the normal concentration. The controls are only missing the inoculum and one had high ATM (control #1) and the other normal ATM (control #2). An overview of what each incubation and control contains is given in table 2.3. The reason for the odd number of serum bottles was due to later addition of new Inoculum. The SLS consist of two different preparation methods where the sulfide was added directly into the incubations (SLS #1) or in the media.

Incubations	Substrate	Inoculum	Media/Buffer	Number Serum bottles
Incubation #1	3.69 [g] Alkaline res. waste sludge	1 [mL] LAB-AGS + 4 [mL] FSG-AGS	50 [mL]*	2
Incubation #2	3.69 [g] Alkaline res. waste sludge	1 [mL] LAB-AGS + 2.5 [mL] SLS	50 [mL]*	1
Incubation #3	3.7 [g] alkaline res. waste sludge	2.5 [mL] SLS	50 [mL]	6
Incubation #4	3.8 [g] alkaline res. waste sludge	1 [mL] FSG-AGS + 2.5 [mL] SLS	50 [mL]*	2
Incubation #5	3.8 [g] alkaline res. waste sludge	5 [mL] FSG-AGS	50 [mL]*	2
Control #1	3.69 [g] alkaline res. waste sludge	-	50 [mL]*	3
Control #2	3.7 [g] alkaline res. waste sludge	-	50 [mL]	3

Table 2.3: Overview of different incubations done during this study.Substrate was equal for all incubations to match . Different combinations and amounts of inoculum are used to increase the chance of an active incubation. *Media has increased ATM concentration.

2.2.3 Analyses

Biogas was monitored using the syringes to see the accumulation. These measurements were done 4-5 times per week to have a have the best possible visible trend. The initial pressure needed to move the syringe was relieved by moving the plunger up and down to the value it was allowing it to move on its own. After the syringe was filled it was either stored in NaCl saturated water vials or used for Gas analysis. The gas analysis was done on the Gas chromatograph (Agilent Technologies 7890A GC system) using Agilent 19095P-MS6 + 19095P (60 m x 530 μm x μm) for column and He as the carrier gas. This was done to see what ratio the CH_4 and CO_2 was for some of the samples to CH_4 production and to check if CH_4 was produced at all. Detailed protocol on gas storage is found in appendix B.

Weekly and bi-weekly supernatant measurements were done to see the change over time for ammonium, sCOD, VFA & pH. Only for the initial and final sample the orthophosphate and total dissolved inorganic carbon (DIC) were measured. Samples were taken by syringe pre-washed with argon without mixing the culture as mixing showed inhibition of the process in previous studies. Each time roughly 1.5 mL supernatant was removed from the batch incubation. Directly after the sample was taken it was centrifuged if necessary & filtered through an 0.2 μm filter to ensure no biomass was left and stored in the freezer until measurements were taken.

sCOD, PO4-P & total IC were all measured using their respective Hach Lange kits after the before-mentioned preparation. Dilutions were applied to get into the concentration range of their respective kit. Afterward they were measured on the Hach Lange colorimetric methods (HACH LANGE DR 3900). NH_4−N was measured using the colorimetric method of the Galery^{TM} discrete analyser, which diluted the samples automatically to be within range. The VFA were analysed on HPCL (Thermo Scientific Vanquish Detector, Autosampler & Pump and ERC RefractoMax520) using a Aminex® HPX-87H column (300 x 7.8 mm) as the column with an UV-VIS and RI detector. The mobile phase used was 1.5 mM Phosphoric acid (H_3PO_4 and a flow of 0.75 mL/min with a total run time of 45 minutes. Dilution of 5 times was only done to reduce the viscosity of the sample to not cause issues with the sampling in the machine. Finally the pH was monitored using a pH probe (electrochemical analyser consort C6010). An overview of the analyses and dilutions done on the batch incubation is shown in table 2.4 excluding the pH.

Method	Hach lange			HPLC	Discrete analyser
Compound	sCOD	PO4-P	Total IC	VFA	NH4-N
Dilution	20x	50x	20x	5x	20x
Kit/Column	LCK 614	LCK 348	LCK 388	Aminex (R) HPX-87H column (300x 7.8 mm)	R1 & R2
Sample preparation	Centrifugation/ Filtration (0.2 μm)	Centrifugation/ Filtration (0.2 μm)	Centrifugation/ Filtration (μm)	Centrifugation/ Filtration (μm)	Centrifugation/ Filtration (μm)

Table 2.4: Analytics overview methods and dilutions

2.2.4 Modelling the alkaline anaerobic digestion

Main goal of the modeling is to get a description of the alkaline anaerobic digestion process in order to see the change in pH to determine alkalinity needs to keep the pH constant. Furthermore the changes in NH_4−N & VFAs for the digestion of Kaumera alkaline waste sludge are important parameters in anaerobic digestion to see due to their potential toxicity. Finally the carbonate system is an important parameter to follow in this system as most will remain in solution to create the high purity biogas. This model can in turn be used to determine the economic viability of the alkaline digestion process versus the neutral digestion. Another possibility will be the process optimization without the need for lengthy experiments that take up months of time for each incubation. Two unique models are evaluated in this study and compared to one another to determine the ideal strategy.

Simplified black-box excel model - part I

The first part of the model gives the estimated inert fraction (f_i) & overall process rates (k_h) by fitting the gas to the measured data using a summed square of errors. Two different fitting equations are used to achieve this due to the atypical gas accumulation curve seen in alkaline AD.

First the gas it fitted to Equation 2.1 which is a simple first order exponential fit. P(t) [gO2] represent the substrate at time-point t [day], k_h [1/day] is the overall process rate and t the time-point in the anaerobic digestion. P_{c0} [gO2] is the corrected initial substrate using a inert fraction called f_i multiplied by the total initial substrate added (P_0 [gO2]). To determine the CH4 at time-point t, the initial degradable substrate and degradable substrate at time-point t subtracted shown in equation 2.2 where the difference is CH_4 [g O2].

$$\begin{aligned} \frac{dP}{dt} &= -k_h t \\ P(t) &= P_{c0} \cdot \exp\left[-k_h t\right]; P_{c0} = P_0(1 - f_i) \end{aligned} \tag{2.1}$$

$$G_{COD,c}(t) = P(t) - P_{c0} \tag{2.2}$$

Methane production was measured in mL and converted to COD methane according to equation 2.3. V_m [mL/mol] is the molar volume of gas assuming ideal gas conditions at 35 °C and a small overpressure of 100 mbar (25450 mL/L). COD_{CH4} is mol O_2 needed for full combustion of 1 mol methane (2 $\frac{molO2}{molCH4}$) and MW_{O2} the molecular weight of oxygen.

$$G_{COD,m} = \frac{G}{V_m} \cdot COD_{CH4} \cdot MW_{O_2} \tag{2.3}$$

To fit the methane $G_{COD,c}$ was subtracted from & $G_{COD,m}$ and minimized using the summed square of errors (SSE) method (equation 2.4). More weight is added to the initial and final point to ensure the fit will include these points due to atypical gas accumulation. The parameters fitted were the inert fraction (f_i) and rate constant (k_i).

$$0 = G_{COD,m} - G_{COD,c}(t) \tag{2.4}$$

Equation 2.5 is a double Mitscherlich fit that has been shown to fit well to bi-phasic gas accumulation data (Dhanoa et al., 2021, Powell et al., 2020). G(t) [mL] represent the cumulative gas production at time-point t [day]. A_1 [mL] & A_2 represent the two different asymptotic cumulative gas productions. T_1 [day] & T_2 represent the start of the gas production for the first and second phase of methane production. h_1 [1/day] & h_2 [1/day] represents fractional rate constants to their respective accumulation curve. All parameters mentioned except the time-point are fitted using the gas accumulation data. Initial guesses are determined visually for A_1, A_2, T_1 & T_2.

$$G_c(t) = A_1(1 - \exp\left[-h_1(t - T_1)\right] + A_2(1 - \exp\left[-h_2(t - T_2)\right]; t \geq T_1, t \geq T_2 \tag{2.5}$$

Using the same error calculation as in equation 2.4 it is fitted with the measured data.

$$0 = G_m - Gc \tag{2.6}$$

Simplified black-box excel model - part II

The second part of the model is to predict the changing compound during the alkaline AD. In order to do this three balances are set-up that together need be zero, which is again accomplished by a SSE method described in the previous section.

The first balance is the carbonate balance compromising of gaseous CO_2, aqueous CO_2*, HCO_3^-, CO_3^{2-}, produced CO_2 and initial total IC (C_T). The total speciation should equal the sum of produced and initial total inorganic carbon shown in equation 2.7

$$CO_{2,gas} + CO_2^* + HCO_3^- + CO_3^{2-} - (C_{T0} + CO2_{prod}) = 0 \tag{2.7}$$

The speciation of the carbonate system is given by:

$$\mathrm{CO_2(g) \rightleftharpoons CO_2{}^*(aq);} \qquad K_{h,\mathrm{CO_2}} = \frac{[CO_2^*]}{[CO_2(g)]} \tag{2.8}$$

$$\mathrm{Co_2{}^*(aq) + H_2O(l) \rightleftharpoons H^+(aq) + HCO_3{}^-(aq);} \qquad K_{a,\mathrm{CO_2{}^*}} = \frac{[\mathrm{H^+}][\mathrm{HCO_3}-]}{[\mathrm{CO_2{}^*}]} \tag{2.9}$$

$$\mathrm{HCO_3{}^-(aq) \rightleftharpoons H^+(aq) + CO_3{}^{2-}(aq)} \qquad \mathrm{K_{a,HCO_3}} = \frac{[\mathrm{H^+}][\mathrm{CO_3{}^{2-}}]}{[\mathrm{HCO_3{}^-}]} \tag{2.10}$$

The second balance that needs to be reduced to zero is the ammonia/ammonium balance. It consists of gaseous NH_3, aqueous NH_3, $NH_4{}^+$, produced NH_3 and initial $NH_4{}^+$ + NH_3. Just like the carbonate balance the speciation is equal to the total initial and produced ammonia and ammonia, shown in equation 2.14.

$$NH_{3,gas} + NH_{3,liq} + NH_4^+ - ((NH_{4,T0}^+ + NH_{3,T0}) + NH_{3,prod}) = 0 \tag{2.11}$$

Speciation is given by:

$$\mathrm{NH_3(g) \rightleftharpoons NH_3(aq);} \qquad K_{h,\mathrm{NH_3}} = \frac{[NH_3(aq)]}{[NH_3(g)]} \tag{2.12}$$

$$\mathrm{NH_3(aq) + H^+(aq) \rightleftharpoons NH_4{}^+(aq);} \qquad K_{a,NH_4^+} = \frac{[H^+][NH_3(aq)]}{[NH_4^+]} \tag{2.13}$$

The final balance is the charge balance which includes the $HCO_3{}^-$, $CO_3{}^{2-}$, OH^-, H^+, $NH_4{}^+$ and a factor B for residual charge in the system that does not change over time (e.g. Na^+, K^+,...). The netto charge needs to be zero. This gives the following balance:

$$\mathrm{NH_4{}^+ + HCO_3{}^- + 2\ CO_3{}^{2-} + OH^- + H^+ + B = 0} \tag{2.14}$$

The initial concentration of total $NH_4{}^+$ & NH_3, Inorganic carbon and pH is measured at the start of the incubation. And the initial volume of gas is argon using a headspace of 65 mL. Based on these measurements B is determined.

The production of CO_2 and NH_3 is based on the stoichiometric conversion of the substrate. This is determined using the tCOD, ODM and N_{org} creating a theoretical compound to represent the substrate (Kleerebezem, 2016) shown in equation 2.15. A small alteration is made where ammonium bicarbonate is less likely in a highly alkaline system, thus is substituted by ammonia.

$$\mathrm{C_1H_{2.6}O_{0.74}N_{0.13} + 0.06H_2O \longrightarrow 0.59CH_4 + 0.40CO_2 + 0.13NH_3} \tag{2.15}$$

Final step was to relate the pH to the H^+ concentration and using the speciation to determine the OH^- concentration that can be used to determine the alkalinity needs.

The model works, as mentioned before, on the SSE principle to make the error minimal in all balances. The NH_4^+, HCO_3^- and pH are available to be changed by the model and predicted in a way to minimalize the cumulative error of the three balances based on the increase described by the overall stoichiometric balance.

The theoretical methane production and composition can also be determined via this overall substrate composition and is 409 mL CH_4/gVS based on the stoichiometric composition giving 59.6 % of CH_4 and 40.4 % CO_2.

Anaerobic digestion model #1 (ADM1)

The second model to describe the alkaline anaerobic digestion process is the ADM1. This is the most widely used AD model and will therefore be ideal to compare the Kaumera residuals digestion with other processes. However, for the ADM1 more much more info is needed to describe the process properly. Further it tries to describe the intermediate process mechanistically. Using this model the change in VFA can also be predicted. The full description of the model is described in Rosen et al. (2006), Rosen & Jeppsson (2006) and Rosen & Jeppsson (2008).

In the original AMD1 acetoclastic methanogens are active and no acetate oxidation is being utilized. Therefore the first change made to the model is to completely turn off the acetoclastic methanogens by reducing the kinetics to zero. Instead the acetate will be consumed by the syntrophs after the H_2 concentration between 3e-5 - 9e-3 as shown in figure 1.3 having a acetate concentration of 50 mM.

To model the syntrophs a new set of equation was introduced into the AMD1 framework shown in equations to implement the effect of the syntrophs. Equations 2.16 and 2.18 are added to implement the need of the hydrogen concentration that needs to be in a specific range and the inhibition of the pH and need for a minimal concentration of a nitrogen source for growth. pH limitation for the acetate syntrophs is done using a similar approach of the ADM1 shown in equation 2.17. Using the existing limitation based on ammonia toxicity a final equation is left over to describe the full inhibition on the syntrophs shown in equation 2.18

$$I_{H_2,saob} = \frac{1}{1 + K_{S,H_2}/K_{I,H_2,soab}} \tag{2.16}$$

$$I_{pH,saob} = \frac{K_{pH}^{n_{saob}}}{S_{H^+}^{n_{saob}} + K_{pH}^{n_{saob}}} \qquad K_{pH} = 10^{-\frac{pH_{LL,saob}+pHUL,saob}{2}} \qquad n_{saob} = \frac{3.0}{pH_{UL,saob} - pH_{UL,saob}} \tag{2.17}$$

$$I_{saob} = I_{H_2,saob} \cdot I_{IN,lim} \cdot I_{pH,saob} \tag{2.18}$$

Equations 2.19 and 2.20 were added to describe the process rates of growth and decay of the syntrophs, respectively.

$$\rho_{28} = k_{m,aco} \cdot \frac{S_{ac}}{K_{S,aco} + S_{ac}} \cdot X_{saob} \cdot I_{SAO} \tag{2.19}$$

$$\rho_{29} = k_{dec,X_{saob}} \cdot X_{saob} \tag{2.20}$$

Furthermore stoichiometry of the syntrophs was defined by equations 2.21 and 2.22

$$s_{22,c} = -C_{ac} + Y_{saob}\ C_{bac} \tag{2.21}$$

$$s_{22,n} = Y_{saob}\ N_{bac} \tag{2.22}$$

Following reaction equations were added or modified for their respective variable to each compound where the previous shown process rates have an impact on. Some full equations are not shown to remain a clear overview due to their length, but only partly with the changed part in green. Full equations can be found in Rosen & Jeppsson (2008) for S_{IC} and S_{IN}.

$$\begin{aligned}
S_{acetate} =&(1.0 - Y_{su})\ f_{ac,su}\ \rho_5 + (1.0 - Y_{aa})\ f_{ac,aa}\ \rho_6 + (1.0 - Y_{fa})\ 0.7\ \rho_7 + (1.0 - Y_{c4})\ 0.31\ \rho_8 \\
&+ (1.0 - Y_{c4})\ 0.8\ \rho_9 + (1.0 - Y_{pro})\ 0.57\rho_{10} - \rho_{11} - \rho_{28} && (2.23)\\
S_{H_2} =& (1.0 - Y_{su})\ f_{h2,su}\ \rho_5 + (1.0 - Y_{aa})\ f_{h2,aa}\ \rho_6 + (1.0 - Y_{fa})\ 0.3\ \rho_7 + (1.0 - Y_{c4})\ 0.15\ \rho_8 \\
&+ (1.0 - Y_{c4})\ 0.2\ \rho_9 + (1.0 - Y_{pro})\ 0.43\ \rho_{10} - \rho 12 - \rho T8 + (1.0 - Y_{aco})\ \rho_{28} && (2.24)\\
S_{IC} =& \ s_{1,c}\ \rho_1 - ... - s_{13,c}\ rho_{19} - \rho_{T10} - s_{22,c}\ \rho_{28} - s_{23,c}\ \rho_{29} && (2.25)\\
S_{IN} =& \ s_{1,n}\ \rho_1 - ... - s_{13,n}\ rho_{19} - \rho_{T10} - s_{22,n}\ \rho_{28} - s_{23,n}\ \rho_{29} && (2.26)\\
S_I =& f_{sI,xc}\ (\rho_{13} + \rho_{14} + \rho_{15} + \rho_{16} + \rho_{17} + \rho_{18} + \rho_{19} + \rho_{29}) && (2.27)\\
X_{ch} =& f_{ch,xc}\ (\rho_{13} + \rho_{14} + \rho_{15} + \rho_{16} + \rho_{17} + \rho_{18} + \rho_{19} + \rho_{29}) - \rho_2 && (2.28)\\
X_{pro} =& f_{pr,xc}\ (\rho_{13} + \rho_{14} + \rho_{15} + \rho_{16} + \rho_{17} + \rho_{18} + \rho_{19} + \rho_{29}) - \rho_3 && (2.29)\\
X_{li} =& f_{li,xc}\ (\rho_{13} + \rho_{14} + \rho_{15} + \rho_{16} + \rho_{17} + \rho_{18} + \rho_{19} + \rho_{29}) - \rho_4 && (2.30)\\
X_I =& f_{xI,xc}\ (\rho_{13} + \rho_{14} + \rho_{15} + \rho_{16} + \rho_{17} + \rho_{18} + \rho_{19} + \rho_{29}) && (2.31)\\
X_{ace} =& \ Y_{aco} \cdot (X_{ace,dec} - X_{ace,dec}) && (2.32)
\end{aligned}$$

Finally one extra differential equation was added to describe the change over time for syntrophic acetate oxidising bacteria, dX_{soab}/dt. This comes down to reaction 31 as there is no flow in or out.

To calibrate the model toward alkaline AD experimental data and studies on the subject were used. Studies included were Capson-Tojo et al. (2021), Rivera-Salvador et al. (2014), Sels (2019), Wormald et al. (2020) and Nolla-Ardèvol (2014) for initial parameters estimation. Using the experimental data the model was further calibrated and changes are shown in table 2.5.

Biological parameters values Alkaline Anaerobic digestion of Kaumera alkaline waste sludge						
Symbol	Parameter	Units	Default value	Source	Calibration value	Source
$k_{hyd,pr}$	hydrolysis of proteins	d^{-1}	0.3	Rosen & Jeppsson (2006), Batestone et al. (2002)	0.673	Simplified Excel model
$k_{hyd,ch}$	hydrolysis of carbohydrates	d^{-1}	0.3	Rosen & Jeppsson (2006), Batestone et al. (2002)	0.673	Simplified Excel model
$k_{hyd,li}$	hydrolysis of lipids	d^{-1}	0.3	Rosen & Jeppsson (2006), Batestone et al. (2002)	0.673	Simplified Excel model
$pH_{UL,aa}$	pH inhibition upper limit amino acid degraders	-	5.5	Rosen & Jeppsson (2006), Batestone et al. (2002)	12	Nolla-Ardèvol (2014)
$pH_{LL,aa}$	pH inhibition lower limit amino acid degraders	-	4.0	Rosen & Jeppsson (2006), Batestone et al. (2002)	8	Nolla-Ardèvol (2014)
$K_{m,fa}$	fatty acid uptake rate by fatty acid degraders	d^{-1}	6.0	Rosen & Jeppsson (2006), Batestone et al. (2002)	8.0	Assumption
$K_{I,H_2,fa}$	H_2 50% inhibitory concentration for fatty acid uptake	kg m^{-3}	5.0e-6	Rosen & Jeppsson (2006), Batestone et al. (2002)	1e-5	Assumption
$K_{I,H_2,c4}$	H_2 50% inhibitory concentration for butyrate/valerate uptake	kg COD m^{-3}	1.0e-5	Rosen & Jeppsson (2006), Batestone et al. (2002)	1e-4	Assumption
$K_{m,pro}$	Propionate uptake rate by propionate degraders	d^{-1}	12.0	Rosen & Jeppsson (2006), Batestone et al. (2002)	2.0	Assumption
$K_{m,ac}$	fatty acid uptake rate by fatty acid degraders	d^{-1}	8.0	Rosen & Jeppsson (2006), Batestone et al. (2002)	0	Assumption
$K_{S,ac}$	Half saturation constant for fatty acid degraders	kg COD m^{-3}	0.15	Rosen & Jeppsson (2006), Batestone et al. (2002)	0	Assumption
K_{S,H_2}	Half saturation constant for hydrogenotrophic methanogens	kg COD m^{-3}	7e-6	Rosen & Jeppsson (2006), Batestone et al. (2002)	1e-6	Capson-Tojo et al. (2021)
pH_{UL,H_2}	pH inhibition upper limit hydrogenotrophic methanogens	-	6.0	Rosen & Jeppsson (2006), Batestone et al. (2002)	10.2	Wormald et al. (2020)
pH_{LL,H_2}	pH inhibition lower limit hydrogenotrophic methanogens	-	5.0	Rosen & Jeppsson (2006), Batestone et al. (2002)	8	Nolla-Ardèvol (2014)
Y_{saob}	Yield syntrophic acetate oxidising bacteria	-	-	-	0.104	Rivera-Salvador et al. (2014)
$k_{dec,X_{soab}}$	First order biomass decay rate syntrophic acetate oxidisers	d^{-1}	-	-	0.02	Assumption
$K_{m,saob}$	Acetate uptake rate by syntrophic acetate oxidisers	d^{-1}	-	-	23.4	Rivera-Salvador et al. (2014)
$K_{s,saob}$	Half saturation constant for syntrophic acetate oxidisers	kg COD m^{-3}	-	-	0.29	Rivera-Salvador et al. (2014)
$K_{I,\ H_2,soab}$	Hydrogen limitation	kg COD m^{-3}	-	-	9e-3	Thermodynamics
$pH_{UL,saob}$	pH inhibition upper limit syntrophic acetate oxidisers	-	-	-	12	Assumption
$pH_{LL,saob}$	pH inhibition lower limit syntrophic acetate oxidisers	-	-	-	8	Assumption

Table 2.5: Biological parameter value changed to the original ADM1 to transform it to an alkaline ADM1 calibrated for the digestion of Kaumera alkaline waste sludge.

Physical parameter changes are shown in table 2.6.

Physical parameter changes for alkaline ADM1			
Symbol	Parameter	unit	value
V_{gas}	Gas volume	m^3	0.00005
V_{liq}	Liquid volume	m^3	0.000065

Table 2.6: Physical parameter used for the digestion of the alkaline AD of the Alkaline Kaumera residual waste.

3. Results

This study tries to give insight into the alkaline anaerobic digestion of the Kaumera alkaline residual waste sludge starting from a pH of 9.6 with high alkalinity and salinity (0.6 M Na^+) during the digestion. Main objectives are to see the conversion yields of tCOD being converted into methane of the Kaumera alkaline residual waste sludge. This has has been determined by monitoring the gas accumulation during the batch incubations. To get an better understanding of the process the SLS incubation was chosen to do an in depth analysis of. Main goals were to find out how much alkalinity would be needed to stay at the pH of 9.6 to create a biogas high in methane. This was done by monitoring the pH during the batch incubation and doing a titration on the buffer to reach a similar pH level. This was also one of the goals of the two different models. Besides the pH other compounds were analysed . This was done to see if and what compounds have the potential to be inhibiting the process for further optimization. Therefore the change in the main VFAs (acetate, propionate, formate, valerate and butyrate), sCOD and NH_4-N were measured at different time points during the incubation. The final question to be answered is the creation of the alkaline AD model that can describe the process compounds including pH, NH_4-N and VFAs. This was done using a simplified black box excel model (excluding VFAs) using a fitting procedure on the gas accumulation and the conventional mechanistic ADM1 using kinetics and a substrate characterization to describe the process.

3.1 Batch incubation green gas yields

For each incubation activity was checked and the final yield was determined based on the tCOD of the substrate. The initial tCOD in the bottles was on average 0.36 gO_2 and used to determine the yield of tCOD being converted into methane. Table 3.1 shows the active batches per incubation with their respective yield. Inactive batches are left out due to creating an non-realistic STD otherwise.

Incubation	**Active incubations**	**Yield**			
		[mL CH4/gVS]		[g COD CH4/ g CODin]	
FSG-AGS*	1/2	43	-	0.0873	-
FSG-AGS + LAB-AGS*	1/2	126	-	0.2461	-
FSG-AGS + SLS*	2/2	141	± 3	0.2754	± 0.0049
LAB-AGS + SLS*	1/1	87	-	0.1701	-
SLS #1	3/3	120	± 22	0.2394	± 0.0448
SLS #2	3/3	102	± 34	0.2045	± 0.0969

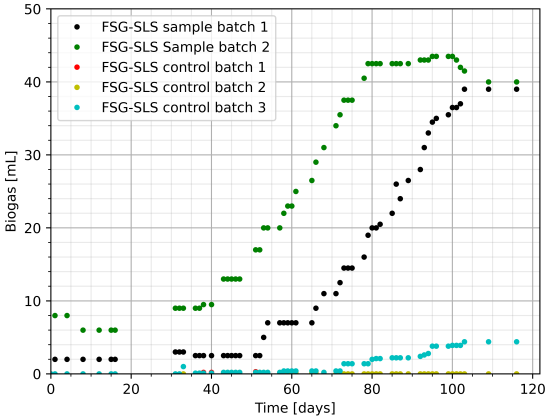
Table 3.1: Methane yield incubations different inoculums. Number of active serum bottle per incubation are shown for each inoculum. Their yield is given in both mL CH_4/gVS and g COD CH_4/ g tCOD initially present. For batches with multiple active batches an average final production was taken and standard deviation was added. *Media contains high concentration of ATM

The gas accumulation of FSG-AGS + SLS and SLS #1 are shown in figure 3.1 as these showed the most promising based on table 3.1. The other gas accumulation graphs are shown in appendix D.

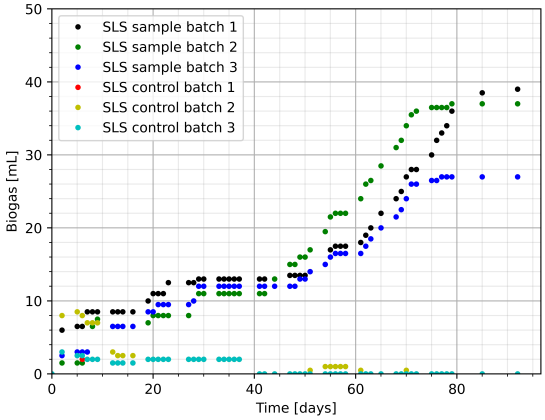
In sub-figure 3.1a the result of the gas accumulation using FSG-AGS + SLS as inocolum is shown after the extra inocolum was added (addition of new inoculum is the new zero-point). During the first 20 days not much is changing in the accumulation of the gas and data for days 20-30 was missing as it was assumed to not be working. A small increase in gas is seen during days 30-40 with a small plateau phase until day 50. After a large accumulation of gas was seen until a plateau was reached of approximately 40 mL gas. The accumulation can be defined bi-phasic, but data is missing in the beginning making it impossible to know for sure. The initial gas production at day one was quite large already. FSG-SLS sample batch 1 is consistently higher compared to sample batch 2, but suddenly decreases at the end of the incubation.

Sub-figure 3.1b gives the results of the incubation containing SLS #1. a clear first accumulation of gas is seen. Production of the first phase is seen during 10-30 days after a small lag-phase of 10 days. Then for days 30-40 a plateau is seen that is followed by another larger accumulation of gas in the days 40 to 70. After this a final plateau is reached of about 35-40 mL for batch sample 1 & 2, but quite lower for sample 3 reaching only 25 mL. Again quite large accumulation in the first days is seen for some samples.

Controls having only the substrate did not show any significant gas accumulation in any of the gas graphs.



(a) Gas accumulation incubation FSG-AGS & SLS.



(b) Gas acculation incubation SLS.

Figure 3.1: Gas accumulation of a) FSG-AGS + SLS of two sample batches and 3 control batches without inoculum. b) 3 samples of the batch incubation SLS#1 with three control samples missing only inocolum.

Based on the yield, number of replicates, length of the incubation and the errors made in the media in some incubations, including FSG-AGS + SLS, it was chosen to use the SLS#1 as the representative batch for all other incubations of the the alkaline digestion of the Kaumera alkaline waste sludge for further analyses.

To ensure that methane was produced two random batches were analysed on the gas chromatograph and results are shown in table 3.2. Sample #1 was 6 mL of total sample and #2 10 mL of total sample. The sample both showed methane and carbon dioxide and the ratio between these two, 92.3% and 97.2% CH_4, were indeed as expected and confirmed that methane is being produced. However, oxygen and nitrogen showed up in large quantities. Nitrogen is not an issue as this is inert gas also used to make systems anaerobic. However, O_2 is a issue as this comprises the anaerobic environment. For the larger sample this seems to go down. This will be elaborated further upon in the discussion as to how this phenomena could have happened.

Sample	#1	#2
CO2	0.98	1.11
CH4	11.77	38.19
O2	61.55	39.06
N2	25.70	21.64
CH4 [%]	92.3	97.2

Table 3.2: Composition of 2 serum bottles off-gas measurements to confirm CH4 production and purity

3.2 In depth analysis incubation SLS #1

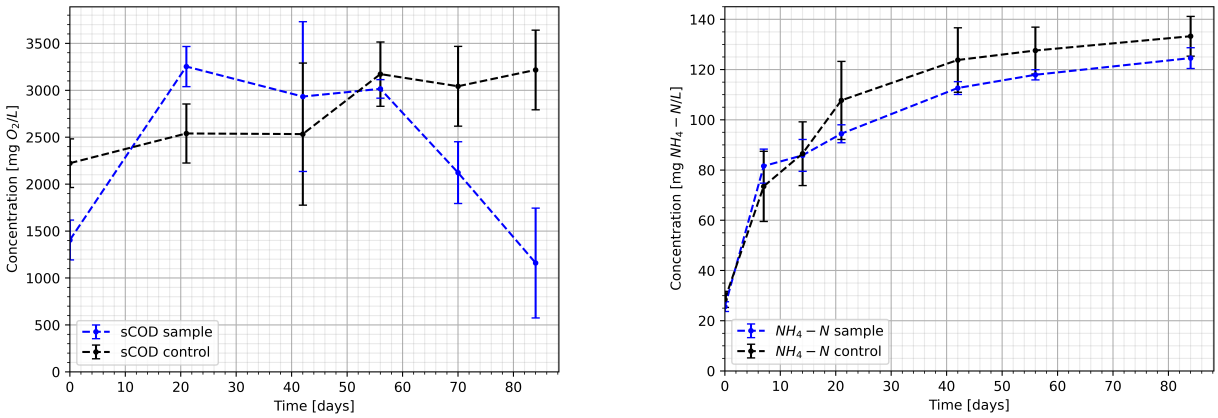
As mentioned in §3.1 SLS #1 was chosen as the batch to represent all other incubations for the digestion of the Kaumera alkaline waste sludge. The sCOD, NH_4-N, VFA and pH were measured over multiple time-point during the digestion of the Kaumera alkaline waste sludge. Finally initial (day 0) and final (day 84) concentration of the total dissolved inorganic carbon (DIC) and orthophosphate released into the liquid are shown.

3.2.1 sCOD & NH_4-N measurement during the alkaline AD

Figure 3.2a shows the dynamics of the sCOD during the alkaline AD. The sCOD available in the beginning is about 1500 mgO_2/L for the sample and increases to around approximately 3000 mgO_2/L in a period of 20 days. From day 20 until 60 no significant changes are seen in the sCOD remaining around 3200 mgO_2/L. At day 60 the sCOD is gradually reduced to around the same value as initially present, coming down to 1200 mgO_2/L. The control start at a higher sCOD value of 2200 mgO_2 and takes 20-30 days longer to reach the 3200 mgO_2 seen in the sample. Another key difference is that once this plateau has been reached it remains this value for the remainder of the batch incubation. The first increase in sCOD is around day 0-20, which correspond to the initial bump seen in the gas accumulation of the batch (figure 3.1b) and the second bump occurs from day 40 until the end of the incubation, which is when the sCOD in the sample goes down. In the control not accumulation is seen and the sCOD also remains at the plateau value.

In figure 3.2b the total dissolved ammonia and ammonium shown as NH_4-N. Initially roughly 20 mg NH4-N NH_4-N is already present in the sample. A quick rise until 80 mg NH_4-N/L happens during the first 10 days for the sample. This is followed by a continuously reducing increase the following 30 days until roughly 110 $mgNH_4-N$ is reached. Finally it reaches a maximum value of just above 120 mg/L over the remaining days for the sample. The control follows the exact same pattern and no significant difference is seen compared to the sample.

What can be seen is that the increase in sCOD and $NH_4{}^+-N$ are quite similar in the beginning of the incubation. This will be further discussed as to why this is the case.



(a) sCOD change during alkaline AD

(b) $NH_4{}^+-N$ change during alkaline AD

Figure 3.2: Change of a) sCOD over the duration of the alkaline AD and b) the change of $NH_4{}^+$. Samples containing the inocolum are shown in blue and control samples are shown in black. The dashed lines are only guidelines and do not predict the compound concentration in between the data points.

3.2.2 VFA measurements during the alkaline AD

The change in the main VFAs of the anaerobic digestion process are also monitored during the alkaline AD and shown in figure 3.3. This include the acetate, butyrate, formate, propionate and valerate.

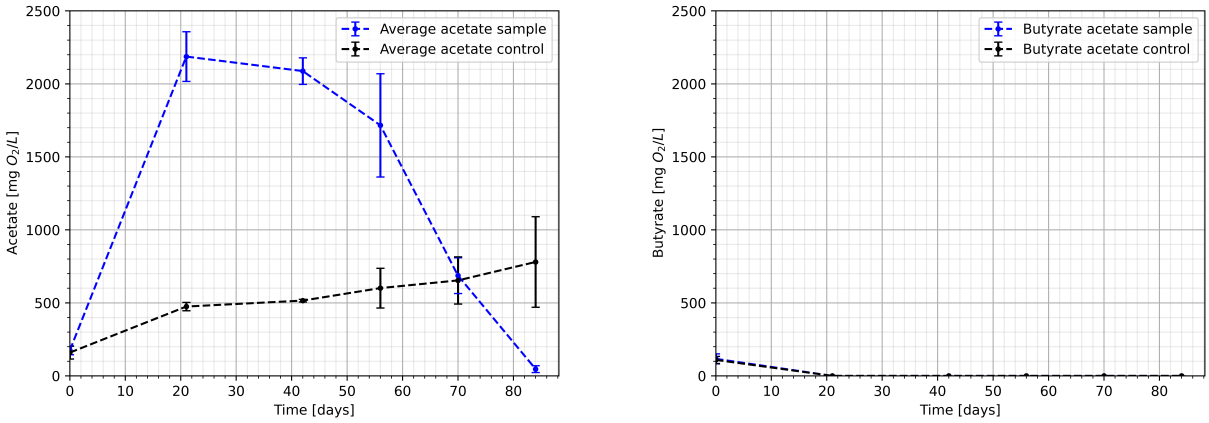
Figure 3.3a shows the change of acetate. Initial concentration of acetate is roughly 200 mgO_2/L, which is about 15 percent of the sCOD present initially (figure 3.1b). This rapidly increases during day 0 to 20 until a value of around 2200 mgO_2/L. sCOD measurements shows a similar increase of roughly 2000 mgO_2 thus mainly consisting of acetate. This acetate concentration then remains constant until day 40 similar to the sCOD concentration. From day 40 acetate is increasingly consumed until it is almost fully consumed. The consumption of the acetate follows the same trend as the sCOD, which also goes down roughly in the same pattern and amount, during days 40-85. Thus acetate accumulates and remains around 2200 mgO_2/L in the system until day 40 before being consumed. The control has a similar starting point and a slight increase of acetate over the full incubation, which is never consumed reaching a final value of roughly 800 mgO_2/L. Main differences are the increased amount of acetate in the sample and the final consumption in the end of the incubation that is not seen in the control.

Figure 3.3b shows the concentration of butyrate in the sample. A small initial concentration of butyrate of less than 150 mgO_2/L is present at the beginning, which is quickly consumed before day 20. The control is identical to the sample.

Next the formate concentration over time is shown in figure 3.3c, which is consistently close to zero and again the control is identical to this similar to the butyrate.

Propionate is shown in figure 3.3d where the sample shows a small accumulation. Propionate starts at roughly 300 mgO_2/L and increases slightly until day 40. After day 40 a small decrease in propionate concentration is seen and at day 70 it start reducing at a faster rate until less than 50 mgO_2 is left over.

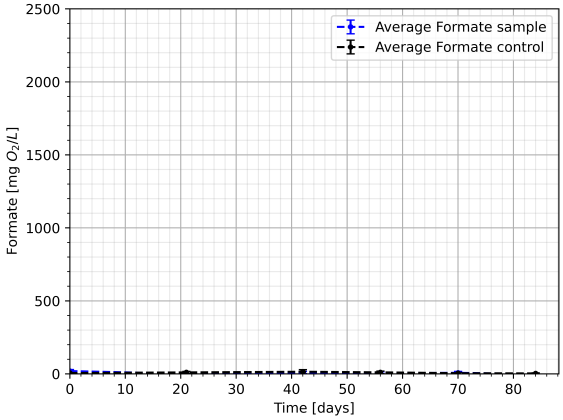
Lastly the valerate is shown in figure 3.3e, which is either not produced or immediately consumed as no measurable amounts are seen over the full incubation period.



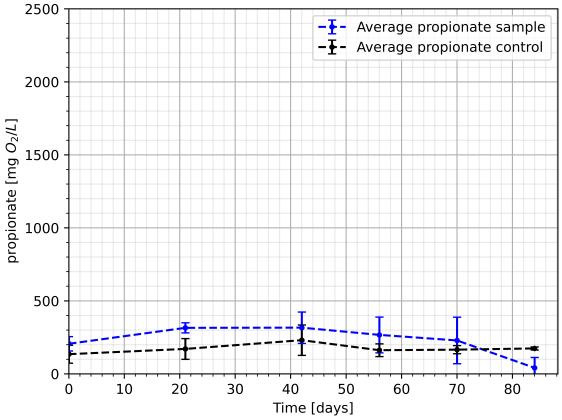
(a) Acetate concentration over time.

(b) Butyrate concentration over time

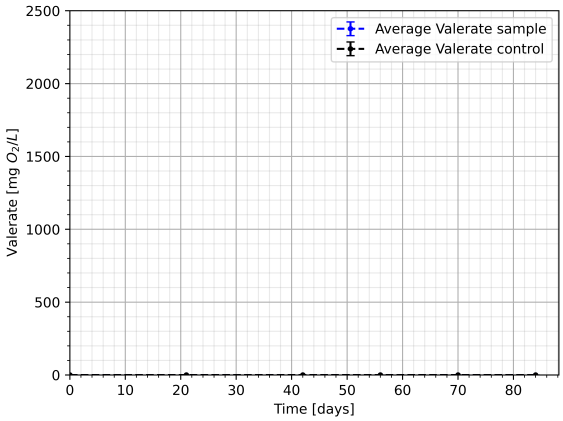
Figure 3.3: Change of VFAs over time in terms of COD. A) acetate changes over time, B) butyrate changes over time, C) formate changes over time and D) Propionate changes over time. Dashed lines are guidelines and do not represent intermediate values.



(c) Formate concentration over time



(d) Propionate concentration over time



(e) Valerate change during alkaline AD

Figure 3.3: Change of VFAs over time in terms of COD. a) acetate, b) butyrate, c) formate, d) Propionate and e) valerate concentration during the alkaline digestion process. Dashed lines are guidelines and do not represent intermediate values.

3.2.3 pH measurements during the alkaline AD

The pH measurements are shown in figure 3.4. The pH in the sample decreases along the batch incubation until a final value of 9.34 is reached. the control sample has a small decrease until pH 9.6 giving a rough pH difference of 0.3 during the alkaline AD digestion to methane in the well buffered system.

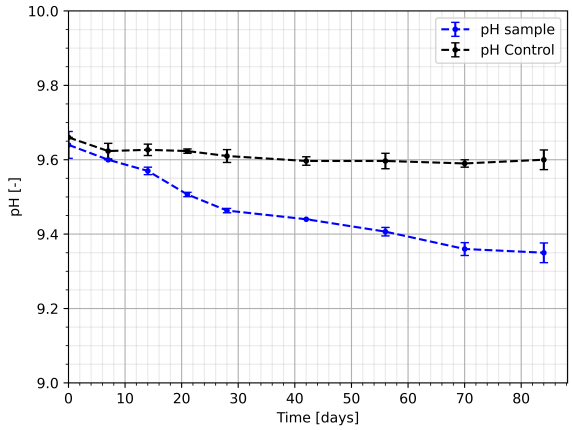


Figure 3.4: pH change over time. dashed lines are guidelines and do not represent intermediate values of the pH.

To decrease the buffer solution towards 9.34 an addition of 75 mM HCl was needed. As the batch incubation reaches a final pH of 9.34 starting from 9.64 a equivalent amount of NaOH would be necessary to keep the pH at 9.64.

3.2.4 Phosphate and total IC measurements

Dissolved inorganic carbon (DIC) and total phosphate in the liquid phase were measured at the start and end of the experiment (day 84), where the end of the experiment is once the gas accumulation does reaches a plateau. An increase of 4.9 g/L is seen for the DIC in the sample and only 0.8 g/L in the control sample.

The orthophosphate measurement was adjusted for the phosphate added in the media at the beginning of the batch incubation in the form of K_2HPO_4. A difference of 31.67 mg/L and 23.33 mg/L orthophosphate is seen for the sample and control, respectively.

	Start incubation	**End incubation**	**Change**
PO4-P (sample)	55.06 ± 27.5 (mg/L)	86.73 ± 10.1 (mg/L)	31.67 (mg/L)
PO4-P (control)	59.06 ± 11 (mg/L)	82.31 ± 6.35 (mg/L)	23.33 (mg/L)
Total IC (sample)	12.3 ± 1.55 (g/L)	17.1 ± 1.14 (g/L)	4.9 (g/L)
Total IC (control)	13.2 ± 0.55 (g/L)	13.8 ± 0.60 (g/L)	0.8 (g/L)

Table 3.3: Starting and final concentration total phosphate and total IC of the Alkaline anaerobic digestion of Kaumera alkaline waste sludge. With the media 164.94 $HPO_4{-}P$ was added and is removed from the initial and final measurements.

3.3 Modelling Alkaline anaerobic digestion of the Kaumera alkaline residual waste sludge

3.3.1 Simplified alkaline AD model

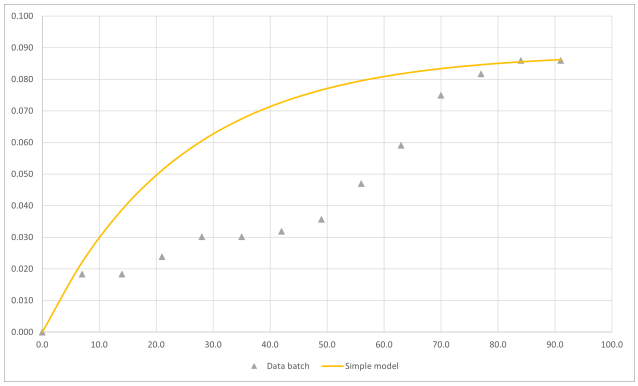
In this section of the results a simplified black-box model and the mechanistic alkaline ADM1 implementation is shown. The main goal was to find out which model would be suitable to simulate the anaerobic digestion of the Kaumera residuals. Further to find out what the alkalinity needs are for the process to work.

Simplified alkaline AD model gas fit

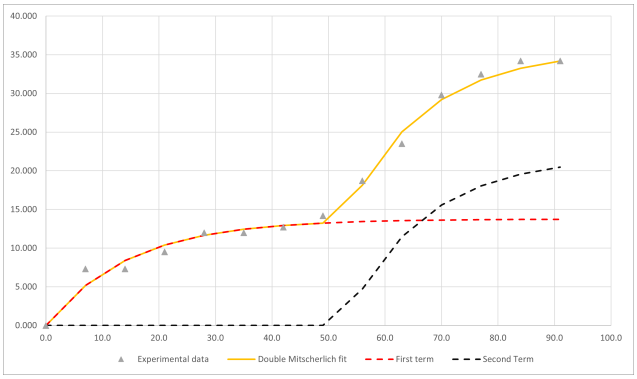
The simplified black-box model assumes a overall stoichiometric conversion of an theoretical compound representing the Kaumera residuals towards CH_4, CO_2 and NH_3 shown in §2.2.4.

Figure 3.5a shows the fit using equation 2.1 using the GRG Nonlinear solver from excel. This gives a inert fraction (f_i) of 0.76 assuming all biodegradable material is converted into methane. the overall process rate from this fit is about 0.041 d^{-1} (K_h).

The second fitting model uses equation 2.5 and gives individual process rates for each gas accumulation phase which is 0.674 d^{-1} for the first bump and 0.720 d^{-1} for the second phase.



(a) First order gas fit.



(b) double Mitscherlich gas fit (Dhanoa et al., 2021, Powell et al., 2020).

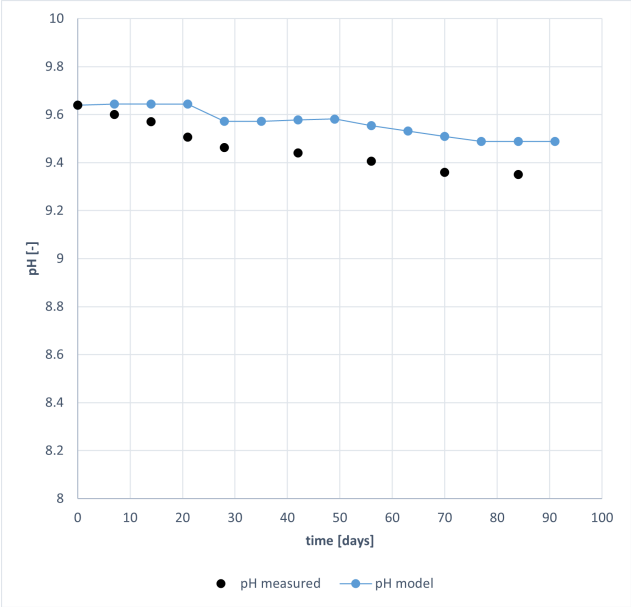
Figure 3.5: Methods to fit the atypical gas accumulation curve. a) A first order fit where the grey dots are the average gas production of the batch incubation and the yellow line the first order fit equation. b) A double Mitscherlich fit (Dhanoa et al., 2021, Powell et al., 2020) where the grey dots represent the gas accumulation data and the yellow line is the double Mitscherlich equation prediction. The orange and black dashed lines represent the two Mitscherlich terms that fit their respective gas accumulation phase.

Simplified alkaline AD model predictions

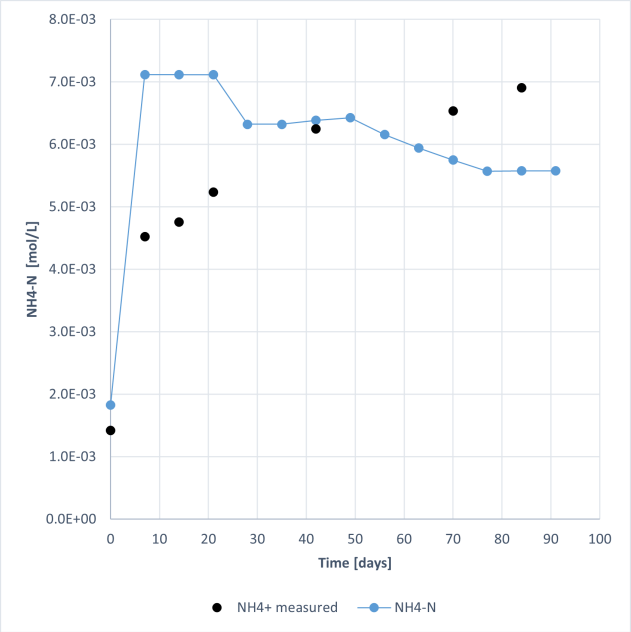
Based on the gas fit and the stoichiometry production of NH_3 and CO_2 was determined. Using the carbonate balance, ammonia balance and charge balance prediction of the $NH_4{-}N$ and pH were made. This again uses the solver of excel to force the balances towards zero allowing only the pH, NH_4^+ and HCO_3 to change in the system. Initial guesses used for this prediction were a pH 9.6 and HCO_3^- of 0.3 mol/L and 2e-3 mol/L for NH_4^+. The initial guesses are based on the initial measurements made for the pH and HCO_3^-. The initial guess of IN was taken by adding the produced NH_3 according to the stoichiometry to the $NH_4{-}N$ at the beginning and taking the middle value of this.

Prediction of the simplified black-box model are shown in figure 3.6. The experimental data is represented by the black dots and the model prediction is shown by blue lines, where dots represent the prediction at that time-point. In figure 3.6a the pH is shown. The pH does not change for the first couple of days until day 30. Afterward it is somewhat constant before decreasing again at day 50. The pattern of the pH is loosely followed, but consistently too high compared to the experimental data.

For the NH_4-N production a large overestimation in the beginning is seen. This slowly goes down towards the experimental value at day 40. However from this point the NH_4-N undershoot the experimental data. Both changes happen when the pH starts to change as well. The prediction is not following the data at all as the data rather suggest a high initial increase followed by a slower increase until the incubation has finished.



(a) pH prediction simplified alkaline AD model



(b) NH_4-N prediction simplified alkaline AD model

Figure 3.6: Prediction of a) the pH and b) NH_4-N

3.3.2 ADM1

The alkaline ADM1 is a mechanistic model trying to describe the syntrophic acetate oxidation using inhibition factors, like hydrogen & pH and ammonia limitation to describe the growth of the syntrophic micro-organisms.

To define the substrate a fractionation was performed with according to Kleerebezem (2016) for a biodegradability of 0.24, which is an output from the excel model and for a potential biodegradability of 0.36 which is seen for neutral digestion. A neutral digestion was also performed by (Arentze, C., unpublished) that showed a biodegradability of 0.36. Based on the tCOD, ODM and N_{org} it is also possible to determine a rough fractionation of the substrate consisting of carbohydrates, proteins & lipids. Combined with measurements on the supernatant at the first day and media concentration for the DIC. an overview of the characterization is shown in 3.4 for both biodegradabilities.

By using the assumed tCOD based on 1.34 tCOD/VS and measured VS an fractionation is done for the substrate. This resulted in 50% proteins, 45% lipids and 5% of carbohydrates for the particulate and the inert fraction of particulate is used is the inert fraction from the simplified black-box model gas fitting (0.24).

The amount of amino acids is assumed to be zero at the beginning as most of it will likely be in the particulate. The VFA were measured on the HPLC and the fatty acids and sugars were distributed according to the distribution of lipids and carbohydrates. An 10 % inert sCOD was assumed for the system.

Ammonia and DIC are based on the initial measurement and carbonate & bicarbonate added to the system, respectively.

Substrate characterization of the Kaumera alkaline waste sludge				
			Value biodegradability	
Symbol	Parameter	Unit	0.24	0.35
S_{su}	Sugar concentration	kg COD m^{-3}	0.068	0.068
S_{aa}	Amino acid concentration	kg COD m^{-3}	0.000	0.000
S_{fa}	Fatty acid concentrarion	kg COD m^{-3}	0.699	0.699
S_{va}	Valerate concentration	kg COD m^{-3}	0.000	0.000
S_{bu}	Butyrate concentration	kg COD m^{-3}	0.117	0.117
S_{pro}	Propionate concentration	kg COD m^{-3}	0.206	0.206
S_{ac}	Acetate concentration	kg COD m^{-3}	0.175	0.175
S_{H_2}	Soluble hydrogen concentration	kg COD m^{-3}	0.000	0.000
S_{CH_4}	Soluble methane concentration	kg COD m^{-3}	0.000	0.000
S_{IC}	Soluble inorganic carbon concentration	kmol m^{-3}	0.380	0.380
S_{IN}	Soluble ammonia & ammonium concentration	kmol m^{-3}	0.0018	0.0018
S_I	Soluble inert COD	kg COD m^{-3}	0.1405	0.1405
X_{ch}	Particulate carbohydrates	kg COD m^{-3}	0.0604	0.088
X_{pr}	Particulate proteins	kg COD m^{-3}	0.712	1.041
X_{li}	Particulate lipids	kg COD m^{-3}	0.622	0.906
X_I	Inert particulate	kg COD m^{-3}	4.414	3.776

Table 3.4: substrate fraction for the ADM1 based on the theoretical compounds & measurements

The output of the alkaline ADM1 implementation is shown in figure 3.7 and 3.8.

Sub-figure a) in the top left shows the sCOD available in the system during the digestion. What can be seen is that the pattern follows the experimental data quite well, but the amount of sCOD is lower than expected. The initial increase of sCOD is not seen as the plateau is reached much faster.

In the top right (sub-figure b) the NH_4-N release can be seen into the system. The initial release is well described by the system followed by a slow increase along the rest of the incubation. It does show a slight overestimation and even around day 70 a small drop can be seen, meaning something is consuming a bit of either NH_3 or NH_4^+. Afterwards it is again increasing and reaching a final concentration of about 0.009 mol NH_4/L.

Sub-figure c shows the pH dynamic estimation. This is not close to what it should be as a pH of almost 8 is reached. The final pH determined experimentally is 9.34, underestimating the pH by at least 1 point. The pattern it shows is the same of the experiment, but the change is much smaller in the experiment, pointing towards an buffer capacity issue in the simulated model.

Sub-figure d and e shows the Valerate and butyrate in the system, respectively. This is either not produced during the incubation or consumed immediately by the microorganisms. The model does predict a small increase of valerate at the beginning of the incubation but is quickly consumed before the next measured data point. Butyrate does show an initial concentration, but is also consumed before the next measured data point. According to the data these compounds are predicted well for the ADM1, but in between day 0-21 no data is available to see whether valerate and butyrate accumulate before being consumed fully.

Sub-figure f shows propoinate in the system. Accumulation is well represented, but at day 70 consumption of the small amount of propionate should occur. This is not predicted by the model right now and shows rather that the propionate is not degraded.

Finally the acetate concentration is shown. Here it is shown quite well how first acetate is accumulated during the initial phase of the digestion and start to get consumed during the final 40 days of digestion of the Kaumera residuals. However, a small underprediction of the accumulation of acetate is observed. This is also the problem seen in the sCOD.

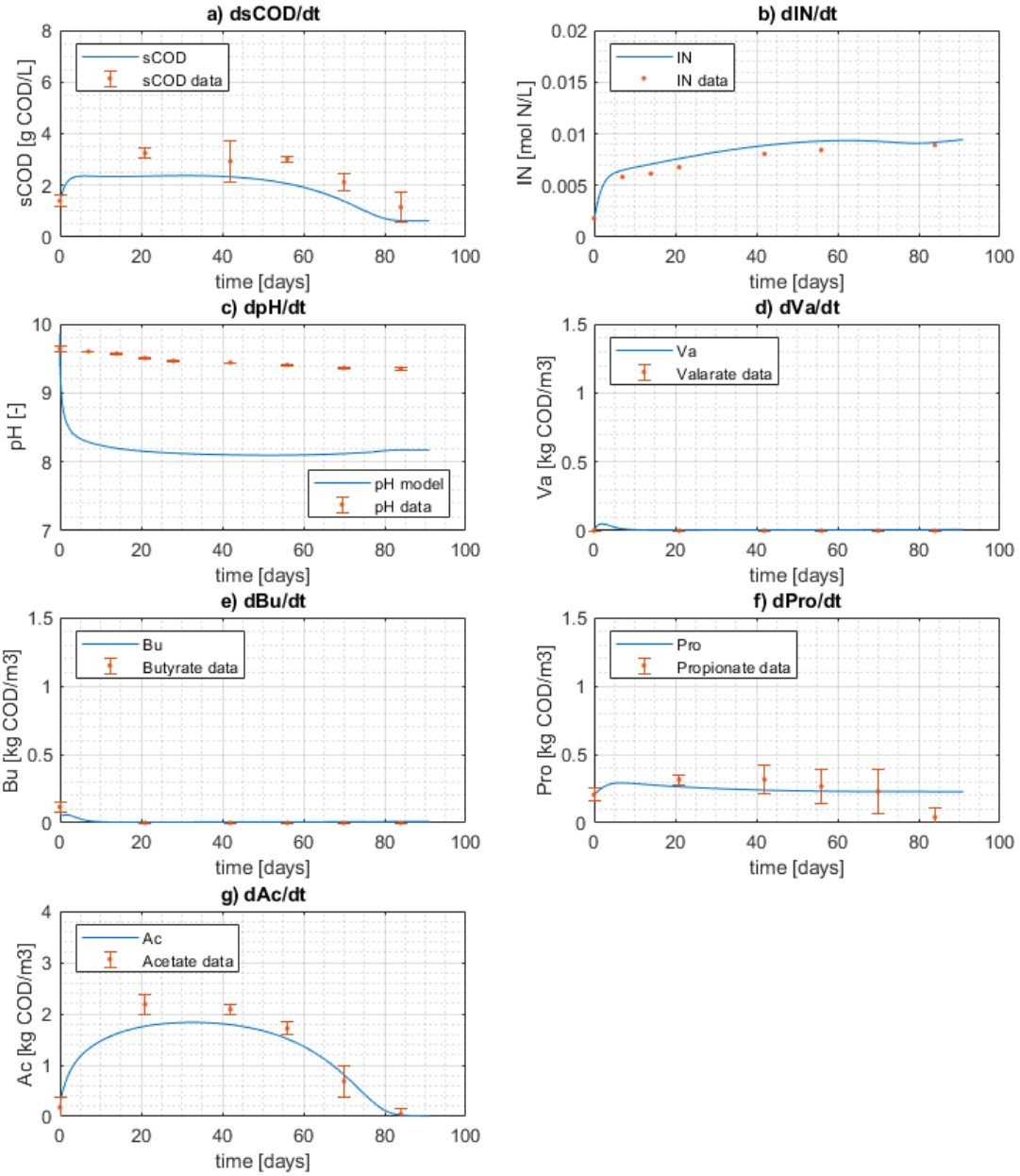


Figure 3.7: Prediction ADM1. the change over time for a) sCOD, b) NH_4-N, c) pH, d) valerate, e) butyrate, f) propionate and g) acetate. dashed lines are guidelines and do not represent intermediate values of the pH.

A major difference compared to the simplified black-box model is that gas prediction is possible as this is not used to determine the production of CO_2 and CO_2. Figure 3.8 shows the prediction of methane in sub-figure a. This is however massively over predicted, but the pattern the experimental data shows can be somewhat seen. The hydrogen concentration is also shown in sub-figure b. This shows the two small curves of hydrogen release. one small peak and a second much larger peak. Finally on the bottom left the growth of the syntrophs is shown and a increase after day 20 is seen, when acetate is at the highest concentration measured.

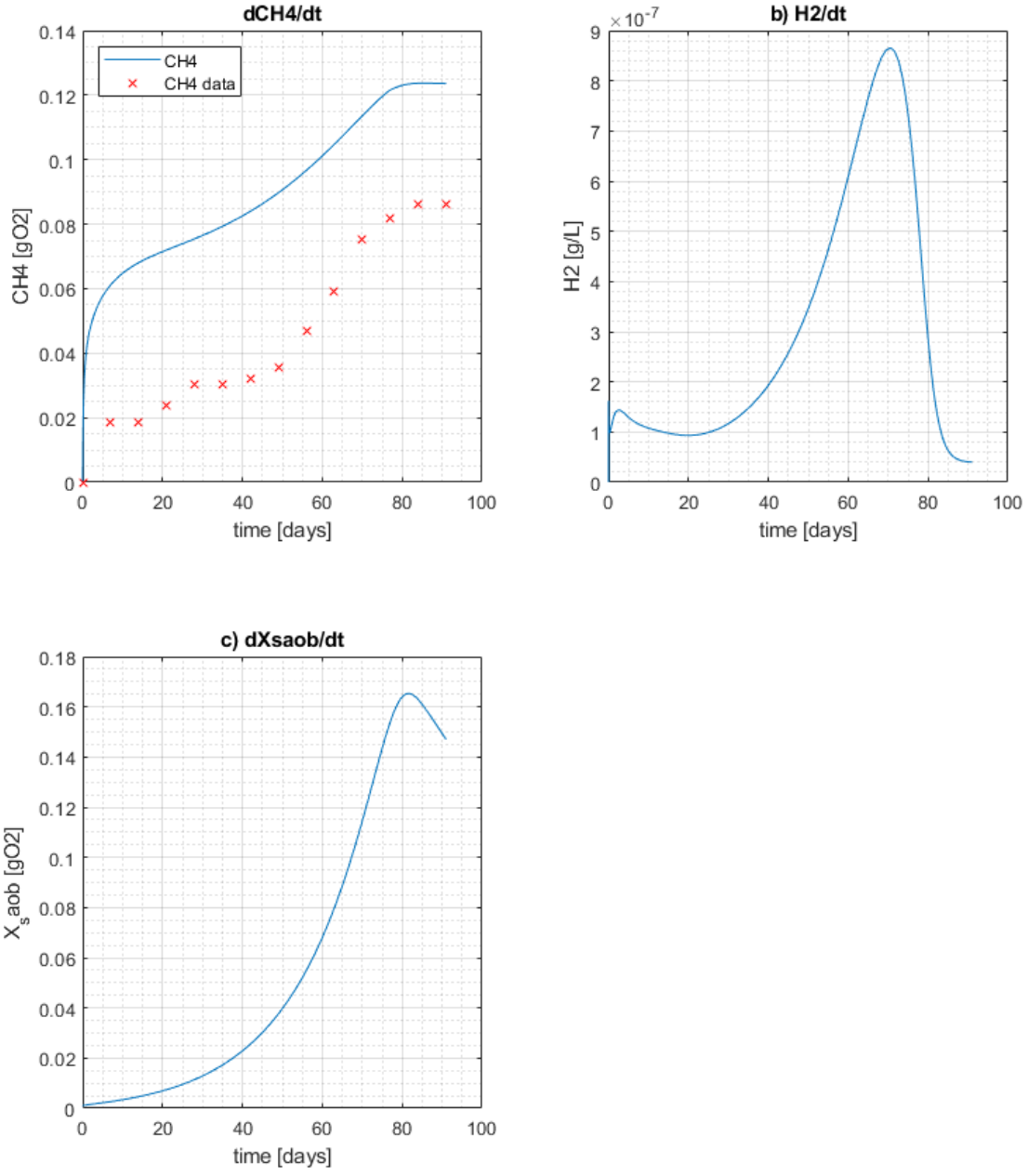


Figure 3.8: Prediction ADM1. where a) shows the CH_4 production, b) the change of hydrogen over time H_2 and c) the change of syntrophic acetate oxiders. dashed lines are guidelines and do not represent intermediate values of the pH.

4. Discussion

The main goal of the study was to get insight into the alkaline AD of the Kaumera alkaline waste sludge. This was achieved through batch incubation using in total 5 different inoculums and choosing the best performing batch to perform additional analysis on. Simultaneously an attempt was made to create a batch model that can describe the anaerobic digestion of the substrate with two different methods that can path a way for scaling up the system. First the biogas production of the alkaline Nereda® Kaumera residuals is discussed based on the different yields achieved between the different batches, the pattern of the gas accumulation and finally the composition of the gas. However, the main focus of the discussion will be on the biogas production and in depth analysis of the best performing digestion, which has the soda lake sediment (SLS) as the inoculum. Then the alkalinity necessity is evaluated and quantified for this specific system. Afterwards both batch models are evaluated based on their results and ability to describe the process measured in the experiments. To conclude the thesis a potential theoretical full scale digester of the Kaumera alkaline waste sludge is discussed to be followed up and what additional research and/or challenges need to be overcome in order to succeed.

4.1 Biogas production

The different and amount of inoculums were initially used to increase the chance of successful incubations (see table 2.3 for sample ID). This was done as the experiment takes a long time of 3 months to complete at minimum. However, after some time all different incubations showed at least one batch with activity. The differences between these batches are shortly discussed. Afterwards a potential explanation on the observed pattern is performed and finally the results of the first active batches on the gas composition is further elaborated on. For the controls a missing batch incubation containing only inoculum was missed. As the soda lake sediment was centrifuged multiple times and the pellet re-suspended most sCOD should be washed away (Appendix A). However this should be validated by an additional experiment and should be taken into account when looking at the yields obtained in this study. For the enriched inoculum it is hard to say whether there was still some sCOD in them as they were stored for more than 2 years. Again, this should be validated by an additional experiment, but due to time limitation it cannot be included in this thesis unfortunately.

Biogas yield

Average yield, length of incubation, number of active replicates and consistency of each incubation are used to determine the performance of the batch incubation. Active batches produced a total volume of biogas in the range of 10-45 mL over the different incubations. Based on the substrate added for each incubation a yield was determined for each incubation and an overview is given in table 3.1.

The lowest conversion of tCOD into methane was seen in the batch containing the FSG-AGS inoculum with only 8.73% tCOD being converted into CH_4. Only 1 out of a total of 2 serum bottles showed activity making this the least performing batch incubation. Reasoning for this could be due to the sample preparation used for the enrichment inoculums. For each enrichment inoculum substrate added was lyophilised, grounded and dried where the substrate used in this study is untreated wet sludge directly out of the Kaumera process. This could lead to a microbial community that is lacking amounts or certain groups of micro-organisms giving a low conversion towards methane. It is also reported by Sels (2019) that wet substrate gave for some substrates a worse conversion of tCOD to methane compared to pre-treated dried substrate.

The FSG-AGS + LAB-AGS inoculum did show already some better results, converting 24.61 % of tCOD into methane and is second highest of all incubations. However, with this incubation still only one of the two incubations showed activity in the end. The increase in yield might be due to the combination of the different inoculums increasing the bio-diversity of the community giving it higher versatility in digestion different substrates.

Finally some incubations were done using completely fresh soda lake sediment from Russia. This was to have a higher quality of inoculum as the other inoculums were already stored for 2 years. An added benefit here is the non-adjusted microbial community that can still be 'molded' instead of an already adjusted community as with the previous discussed batches.

Two new combinations with this fresh soda lake sediment were made called FSG-AGS + SLS and LAB-AGS + SLS to both increase the amount and quality of the inoculum. Combination with the LAB-AGS showed the second lowest conversion of 17.01%, but only 1 batch was done using this combination making it hard to say anything meaningful about it. The combination of FSG-AGS + SLS showed a conversion of 27.54 % of tCOD into methane making this the highest yield of all batches. Additionally all serum bottles (2) showed activity. Finally a batch incubation was done using only soda lake sediment as inoculum with two different methods. The two slightly differed in the time-point where sulfide was added. for SLS# it was added directly to the serum bottles and for SLS#2 it was added to the media. All incubations in the end were active and 23.94% and 20.45% of tCOD were converted into methane, respectively.

This could suggest that the ideal starting community would be the combination of the FSG-AGS + SLS, but only a duplo was done while for the SLS#1 a triplo has been performed. Looking at figure 3.1 it is also shown that 1 batch is considerably lower than the other batches, giving a much lower average yield. Furthermore the lenght of the SLS#1 shows a shorter incubation time of roughly 20 days compared to the FSG-AGS with SLS. Adding the fact that the SLS #1 contains the correct ATM concentration it was chosen to represent the alkaline AD of the alkaline Kaumera residual waste

Samples for NGS sequencing were sent for all different starting inoculums and batches after plateau in gas production was reached, but unfortunately the results will only arrive after the end of the thesis due to issues starting up the batch process. Therefore these results are not included in the thesis and only speculation can be done on the reasoning for the observed yields. Using the microbial community analysis it might give more insight into what organisms are present and what these are able to do with the substrate supplied.

Using the paper of Kleerebezem (2016) it is possible to determine the theoretical yield of methane (Th_{CH_4}) if all organic carbon would be degraded. For this only the organic dry matter of the substrate and theoretical compound (see §2.2.4) is needed. For the alkaline Kaumera residuals this is 52 L/kg sludge, which can be redefined as 682 mL biogas/gVS. Using the theoretical biogas composition determined by the stoichiometry 409 mL CH_4/gVS can be achieved if all organics are converted to methane.

The yield of the Kaumera residuals seems to higher than the determined production of methane based of the theoretical percentage of CH_4. This can be due to the inability to get a tCOD measurement with low standard deviation of the insoluble substrate and the need to use an assumption for the tCOD.

Currently only a handful of studies are done on alkaline AD using different substrate and also limited studies on the neutral digestion of the substrate.

One of the alkaline studies done is by Sels (2019). Compared to this study the incubation shows a lower yield for all the other substrates, but the AGS-FS as substrate comes close with 130 mLCH_4/gVS. The residue-FSG and residue-LAB show higher productions and yields. This could be that the residue contain more easily degradable organics, than the aerobic granular sludge from the full-scale.

One reason the incubations outperform the substrate used in this study could be the use of the pre-treated substrate, which is grounded, dried and lyophilised as stated by Sels (2019). This would make the substrate more readily available as mentioned in before. Another reason the comparison is not completely fair is that all incubations are enriched already, being the second or third incubation. It is shown by the study that for some the the biodegradability increases over the subsequent incubations. This could also be the case for the digestion the Kaumera alkaline residual waste sludge.

The substrate used in this study does seem to already outperform the algae *Sprirulina* used in the study of Nolla-Ardèvol (2014) as only 85 CH_4 mL/gVS is achieved for the incubation, while for the Kaumera residuals this is already 119.5 mL CH_4/gVS. However the Na^+ used in this study is much higher (2.0 M Na^+) than used in this study (0.6 M Na^+). This could explain as higher salt concentration can inhibit the process.

A neutral digestion was done by Guo et al. (2020). The production the production of 296 mL CH_4/gVS and is more than double that of the Kaumera residuals, which is 120 mL CH_4/gVS.

This can also be seen in the neutral digestion of the substrate done by Arentze, C (unpublished) as there the conversion is 36% already and 263 mL CH_4/gVS, showing that the substrate has more potential for degradation. This also means that right now the alkaline digestion of the substrate is worse compared to the neutral digestion.

The digestion done by STOWA (2019) also used Kaumera residuals from Epe, but did a neutral digestion. This digestion reaches a methane production of almost 300 mLCH_4/gVS. This is also close to the measured production seen by Arentze, C. and Guo et al. (2020). Showing again the potential for this substrate.

Even though the yield is quite low compared to the other studies using similar substrates it is not a lost cause. The neutral digestion show that more conversion is possible and the study of Sels (2019) shows that enrichments can have an increase of methane production for alkaline AD showing that there is still potential in this process.

Substrate	**Kaumera alkaline residual waste sludge**	**AGS-FS**	**residue-FSG**	**residue-LAB**	***Spirulina***	**Granular sludge**	**Kaumera alkaline residual waste sludge**	**Kaumera alkaline residual waste sludge**
Source	-	Sels (2019)	Sels (2019)	Sels (2019)	Nolla-Ardèvol (2014), Sels (2019)	Guo et al. (2020)	(Arentze, C., unpublished)	STOWA (2019)
Type of digestion	Alkaline digestion					Neutral digestion		
Yield [g CH_4/g COD_{in}]	0.24	0.3	0.4	0.5	-	-	0.35	-
Yield [mL CH_4/gVS]	119.5	130 ± 25	200	240 ± 30	85	296 ± 15	263	230
Th_{CH4} [mL CH_4/gVS]	409	550	570	570	627	-	409	-
Percentage of Th_{CH4} [%]	29	24	35	42	14	-	39	-

Table 4.1: Yields incubation compared to literature

Biogas composition

The biogas composition is thoroughly described by Sels (2019) & Nolla-Ardèvol (2014), but can also be derived from speciation of inorganic carbon over the pH. However, it was measured directly for some samples to confirm the production of methane. Interestingly a significant amount of O_2 and N_2 was found in these measurements as well. Only argon was additionally used to make the serum bottles anaerobic, thus these gasses were unexpected. This can happen due to contamination when transferring the gas to the gas chromatograph (GC), but should be lower than 5% and not 39-62%. One explanation for this is that argon and O_2 will come of the column closely together and are hard to separate in general without a specific method (Ansón et al., 2009). This might show up as oxygen while it is actually argon and only a small amount of oxygen contamination would be present that could account for the nitrogen with oxygen. Based on the nitrogen and oxygen ratio in air (roughly 4:1) and nitrogen measured this would get an oxygen contamination of 6.4% for sample #1 and 5.4% for sample 2, which are more reasonable as contamination due to gas transfer to the GC. As it is also impossible to have both methane production and oxygen present it is most likely that this contamination is after the digestion as CH_4 and CO_2 is present as well.

Therefore only the ratio of methane and carbon dioxide is used from these results. This gives indeed a ratio of 92-97%, thus having a purity similar to natural gas Balat & Balat (2009), which is also seen in the other studies mentioned in before Nolla-Ardèvol et al. (2012), Sels (2019).

Gas accumulation

The further discussion on the incubation will be focused only on the incubation containing the SLS#1 inoculum and will now be referred to as SLS.

The pattern of biogas accumulation are consistent over all batches and similar to the pattern seen in Sels (2019). First a lag phase occurs until day 10 after which the initial gas accumulation occurs. This is most likely the degradation of butyrate and valerate into acetate, H_2 and CO_2 (Q. Wang et al., 1999, Westerholm et al., 2021). The hydrogen that comes free with this can be used by the hydrogenotrophic methanogens to create the initial bump of gas production. After the initial gas production phase another lag phase occurs for about 10-20 days. Simultaneously, acetate and propionate accumulate in the system showing that acetoclastic methanogens are either inactive or unable to keep up with the large amount of acetate that is most likely due to the high pH as this makes the concentration NH_3 much larger than in neutral systems (Nolla-Ardèvol, 2014). Another reason for acetoclastic methanogens being inactive is that it is likely less favourable due to the need for active uptake according to Wormald et al. (2020). As the hydrogen is not affected by pH this might form an advantage for the hydrogenotrophs to be able to still have diffusion, while acetoclastic methanogens require active uptake. It should be investigated whether diffusion will out-perform active uptake of acetate by acetoclastic methanogens to validate this. Butyrate & valerate are not observed and formate is lower than 50 mgO_2 and does not accumulate. This make the degradation toward acetate, H_2 and CO_2 the most likely reason for the initial bump (3.3), which should be considered to measure in a subsequent experiment to verify this hypothesis.

The increase of sCOD, VFA and NH_4-N indicates that the process is also partly happening in the control group. This suggest that a microbial community is still active in the substrate. A possibility is that *Clostridia* spores are able to survive this harsh treatment of the Kaumera extraction (80 °C; pH of 11) as these are quite resistant to harsh environments (Fontana et al., 2020). It is likely also the reason for the partly digestion seen in the sample as well. However, in the control no gas accumulation is seen, thus the need for the inoculum seems to still be required for the final step of the anaerobic digestion.

After the first initial biogas production a lag phase of approximately 20 days is observed. At end of the first second lag-phase acetate is starting to get consumed around day 40 until day 50. After day 50 the consumption start to pick up speed until no more acetate is left over over a period of 30 days, which is roughly the same length of the second gas accumulation curve. This is likely the acetate oxidation toward the hydrogen. The lag phase seems to be due to the syntrophs not being able to grow during the initial gas production phase as syntrophs need low H_2 in order to make the interspecies hydrogen relation with the hydrogenotrophic methanogens possible, shown in §1.1. This is further confirmed by the acetate consumption during the same period, shown in figure 3.3a. Once all acetate has been consumed, biogas production halts as well. Thus the hydrogenotrophic methanogens first need to convert the H_2 to get the concentration of this low enough in order to let the syntrophs grow.

It seems to be the caste that the bi-phasic gas accumulation curve comes from delay in acetate conversion toward H_2. In the study of Sels (2019) it is seen that even in further enrichment a bi-phasic gas accumulation remains. This can be due to the syntrophs dying in such extent during the initial gas production phase it is necessary to grow all over again from a low concentration of biomass.

The propionate consumption does start at a later stage in the digestion process. It looks like another syntrophic relationship is responsible for this (Westerholm et al., 2021). Elevated acetate and hydrogen concentrations are inhibiting such a syntrophic relationship, which corresponds well with the data as this only occurs once most of the acetate is consumed already (between day 70-90).

The initial hydrogen production from fermentation and conversion to acetate and propionate seem to be responsible for the initial bump seen in the gas accumulation graphs. The final syntrophic conversions to hydrogen form most likely the hydrogen for the second bump seen in the gas accumulation graphs. This is again backed up by the sCOD measurement as the increase in COD is almost equal to the acetate and propionate measured at each time-point, which is converted to H_2.

Thus, the yield of the Kaumera residuals for the first batch incubations is roughly 24% of the tCOD is being converted into methane. This is rather low compared to other studies doing the same process showing yields between 30-50 %, but these are different substrates of which some are pre-treated and from enriched incubations with well-adjusted microbial communities to their specific substrate. The yields shown in this study are from the first series of active batches and the neutral digestion shows an higher conversion of 36 % for the same substrate. This gives a strong indication that further enrichments might show increased conversion rates for the alkaline process. The process itself is most likely done in two separate phases. First all degradable materials is converted towards acetate and/or propionate, producing already some CH_4, which is subsequently fully converted toward CH_4 as well.

4.2 Process potential bottle-necks

In this part the in depth analysis of SLS is further discussed and potential bottle-necks of the process are shown. All samples taken here are taken from the supernatant of the SLS. The analysis done is for a batch system, but in paragraph §4.5 implication for a potential full-scale continuous digester are discussed.

sCOD

The sCOD is available throughout the full alkaline digestion of the alkaline Kaumera waste sludge as shown in 3.2a. After some time this goes down toward the initial value after the gas accumulation is stopped as well. Looking at figures 3.3a & 3.3d it shows that all of the sCOD that goes into solution is compromised of these specific compounds as the total COD values are approximately equal (2000 mgO_2/L). Therefore it shows that the sCOD is not a limiting factor in the process as this is readily available during the whole process. However, some of the sCOD remains after digestion is completed. Only 10% of this sCOD is allocated toward the remaining VFA, leaving the other 90% undefined.

What can be seen in §1.1 is that with an increase in acetate the range of the inter-species hydrogen transfer mechanism becomes more favourable. This could mean that for the low acetate concentration it becomes thermodynamically unfavourable to support growth of the syntrophs. As the sCOD at the start is around the same value as at the end of the incubation it might be that this sCOD is not biodegradable at all. Another option might be the release of inhibitory compounds like the NH_4-N, which will be further discussed in §4.2. Most likely it is a combination of the aforementioned options, but to determine this a characterization of the sCOD at the end of the batch should be done.

If the initial or third option are right it would most likely decrease due to adjustment of the microbial community toward the substrate. This is seen for some enrichments in Sels (2019), making it a likely option. It could also be a combination of all aforementioned options.

NH_4-N

The NH_4-N is the most common inhibitory compound for alkaline digestion due to the increase in the NH_3 as a resultant of the high pH of 9.6. Around 50 % will be in the form of ammonia that can freely diffuse over the membrane of the micro-organisms. For this study a threshold for ammonium toxicity at pH 9.6 of 420 $mgNH_4-N/L$ (Sels, 2019) was taken.

This is not reached as the final concentration is 120 $mgNH_4-N/L$, which is reached quite fast. In the first 20 days most of the NH_4-N is released, showing that degradation of the substrate is quite fast. Due to both not reaching the maximum threshold value and quick release of this it is not seen as a potential bottle-neck.

However, the system has a quite low DM of only 0.88% due to having the same VS content as used in Sels (2019). As already mentioned in the beginning of the paragraph it will be discussed what this would mean for a larger full-scale digester where the substrate DM is much higher.

VFA

Another potential bottle-neck can occur in the VFA production and/or consumption. For formate no measurable amounts were found, which either means nothing is produced at all or it is quickly consumed. The latter seems reasonable as formate can be directly used in the methanogenesis (Pan et al., 2016). For butyrate a small amount is initially present but is already in undetectable ranges for subsequent measurements on the HPLC, thus seems not to be limiting in the process as it is quickly consumed.

Acetate and formate however are both accumulating in significant amounts and reach a total concentration of 2200 mgO_2/L and 300 mgO_2/L, respectively. This shows that the bottle-neck right now is in the step of converting these intermediates into pre-cursors for the final step, methanogenisis. This makes sense as in neutral digestion this is normally done using acetoclastic methanogens reducing the need for the syntrophic relationships that are known to be quite slow Sorokin et al. (2016), Westerholm et al. (2021). This is likely the reason that the second lag phase occurs making the overall digestion take a longer time to be completed.

To conclude it is most likely that the process is heavily delayed by the accumulation of the acetate and propionate and no 'real' bottle-neck is present right now. The NH_4-N is not reaching toxic levels and sCOD seems to be present until the end of the incubation and are therefore most likely not limiting right now. However, once the delay due to acetate and propionate are no longer there these might become an issue again. This will be further explored in §4.5.

4.3 Alkalinity need and pH

The change in pH plays an important role in the alkaline digestion as this has impact on important parameters like the ammonia concentration & VFA toxicity, methane purity in the produced biogas and effectiveness of the different microbes during the digestion.

The pH in the digester drops about 0.3, which is not much. This in mainly due to the fact that the digestion is performed in a highly buffered system. This will prevent most of the fluctuation in the pH. The amount of DM present is also relatively low (0.88 % DM) compared to a realistic full-scale digester (5%).

Based on the pH measurements the pH continuously drops, thus the total amount of alkalinity needed can be determined by titration. 75 mM of HCL was needed to drop the buffered system without substrate to a pH of 9.34, which is the final pH of the sample. When the pH of the sample was measured initially a pH of 9.64 was measured, thus not changing the pH much when added to the media. Therefore assuming the 75 mM of NaOH necessary to drop the buffer down to pH of 9.34 is the same with substrate as it would be in the substrate. This would translate towards 3 g/L NaOH for the full incubation.

Thus based on the titration and final pH measured an alkalinity need of 3 g/L NaOH would be needed to keep an constant pH during the alkaline digestion.

4.4 Modeling the alkaline AD of the Kaumera alkaline waste sludge

The modeling of the process was to create a simple way to see the effect of small changes to the system without the need of a lengthy experiment that would take months to complete and analyse costing valuable time and resources. Here the performance of the models is accessed and compared to each other.

The gas fit of the simplified model works poorly for the first order gas fit. This is due to the second lag phase giving a bi-phasic gas accumulation curve. As the inert fraction is not dependant on the intermediate values it still hold true. The overall hydrolysis factor is not useful in this situation as the accumulation does not follow an first order rate. The double Mitscherlich gas fit will be much more suited to determine these rates due to the ability to fit bi-phasic gas accumulation much better. The first rate can be used to determine the hydrolysis rate and is used for the ADM1. The second part of the curve could be related to the process rate of the syntrophic acetate oxidation. The process rate of the first accumulation curve can be used in the ADM1 to get the rate of hydrolysis. As the NH_4-N is released by protein degradation and it is released at almost fully in the beginning of the batch (day 0 to 20) one could say this represents the hydrolysis rate of the substrate. This can be used in the alkaline ADM1 to describe better the hydrolysis of the particulate in the system.

The predictions of the simplified black-box model work poorly for both the pH and NH_4-N. The pattern shown in the experimental data does come back in the model prediction. However, the model predicts the pH too high. This is due to the missing VFA in this simplified version. The effect of the VFA should be included as this would significantly drop the pH as acetate accumulates to 2200 mgO_2/L. The reason they were not included was due to the inability to measure the VFA during the thesis up until the last month. So in order to make the model make a better prediction it would need the weak acid-base chemistry of the VFA in the system. Right now the only compounds giving a drop in pH is the produced CO_2 and this is not sufficient as seen in the graphs.

The NH_4-N is first overpredicted and subsequently underpredicted. In a neutral system usually ammonium carbonate is formed, but as the pH is rather high it was assumed all is converted towards NH_3. This might cause the system to over predict the amount of ammonia in the system in the beginning giving also an higher pH. However, this is hard to say as the effect of pH and ammonia concentration together are quite unpredictable and pH is not well described yet. In order to have a better prediction is it required to improve the overall stoichiometric reaction and buffer capacity description in the system.

The ADM1 shows already better the change of compounds in the system as VFA are taken into account. The sCOD is under predicted together with the acetate, which can point towards multiple things. The most likely being acetate conversion happening already during the first gas accumulation curve. This is also seen in the gas production that is much higher in the beginning than in the experimental data. Acetate consumption at an early stage would show up as a decrease in the plateau value of acetate itself and for sCOD. The acetate would be converted to H_2 and CO_2 and the sCOD would go down. Simultaneously methane will go up overpredicted in this case. The CH_4 production is also overpredicted in general in the system, which could be due to a too generous biodegradability of the already present sCOD.

Another issue that could show an overprediction in the CH_4 is an ill defined substrate. Most of the substrate was measured, but some of the sCOD is unknown and needed to be assumed. It might be that this is assigned to fatty acids, which is degradable in the system. However, this might be inert sCOD or not biodegradable sCOD under the conditions of the digestion. This shows that a better substrate characterization on the sCOD is necessary to improve the prediction of the model.

The NH_4-N is well described with only a small overprediction. This can be due to missing NH_3 stripping in the model. To better predict this a description for the removal of ammonia should be added. The small decrease seen at day 70 could be the syntrophs growing still and using it as a nitrogen source.

Propionate is not consumed in the end. This could be due to a poor definition of the inhibitions of the multiple compounds. The ADM1 has a complex system of inhibition for hydrogen, pH and NH_3. This makes the system hard to calibrate and not much data is known on this very specific system. Therefore a better understanding and kinetics of the microbial community is needed to improve the alkaline ADM1. Studies to pH limitation, kinetics and inhibitions are needed to give an accurate prediction of the model.

Finally the ADM1 also has a simple description of the buffer capacity, based only on carbonate. This is not sufficient for the alkaline digestion as the pH overshoots towards a pH of 8, while the date shows a pH of 9.34 is reached instead. Similar to the simplified model a better description would be needed to ensure pH is predicted correctly.

Overall there needs to be a better understanding of composition of the Kaumera residuals and the kinetics of the microorganisms involved in the process to achieve a better description of the alkaline AD of the Kaumera residuals. Unless this can be done no viable answer can be given on the alkalinity need from either of these models.

4.5 Implication of study for full scale alkaline fermentation

This study, along other studies, should give an insight into a potential full scale digester able to generate methane from the alkaline Kaumera waste sludge. The topic discussed here is a theoretical digester that will be used to treat a stream containing 5% DM, which is conventionally used in these type of full-scale operations.

The first implication seen is the bi-phasic gas production. This is due to the batch system requiring the syntrophs to grow each time an incubation is done. This can be negated in a continuous system where eventually a steady state is reached. This way the cell death during the initial phase and growth during the second phase can be completely removed. This is for now also the major reason for the delay seen in the alkaline AD of the alkaline Kaumera residuals.

The initial substrate has a DM of 11%, which has been diluted about 14.2 times in the incubation giving 0.88 % DM. This is quite a bit lower compared to the conventional 5% percent that is usually taken for digestion waste sludge and would give some implications based on the results of this study.

A challenge that immediately arises is the ammonia toxicity, which was already at 25-30 percent of the toxic threshold for the 0.88% DM. This means that if an increase of minimally 6 times to match the DM content of an normal digester this would be about 720 $mgNH_4-N$ and exceeds the threshold by 320 $mgNH_4-N/L$ almost doubling it as this is at 420 $mgNH_4-N$. Therefore it would be necessary to increase the liquid volume by a factor of 1.71 to reach maximum concentration based on the toxicity threshold value. Another option is to continuously strip the ammonia out of the system keeping a low concentration in the liquid itself.

For now it seems that the ammonia toxicity will be the determining factor for the hydraulic retention time as this seems to be the compound that will most likely be the limiting compound in the full digester.

The VFA would also increase together with the accumulation of acetate and proportionate by six times. The increased amount of acetate could lead to an more ideal environment for the interspecies relation between hydrogenotrophic methanogens and syntrophs if the H_2 concentration remains low enough. An issue that could arise is that propionate will not be degraded anymore, due to acetate always being in the system. This would lose some of the biodegradability of the substrate and lower the methane production. In general an implication will be that sCOD is not fully consumed in an continuous system and therefore lost. This lost sCOD could have been used for production of methane, but instead is wasted now. A recycling loop could be implemented to counter this to some extent.

Another factor that will be largely affected by the increase of DM is the pH drop. As mentioned in §4.3 about 3 g/L of NaOH was needed for a incubation. This would be increased 6 giving an alkalinity need of 18 g/L. This needs to be evaluated economically to see whether the investment in pH is worth the increase in biogas purity, removing the need for purification that is needed in the neutral digestion. The price of downstream processing should be higher than the investment of alkalinity to make this process viable. Furthermore it is necessary for the microbial community to have a high carbonate and bicarbonate concentration, but reasons for this is yet

unknown. This would also increase the cost of the incubation, but should also lower the amount of alkalinity needed to keep the pH constant at 9.6.

Finally we have the salt implication. During this study Na^+ is the salt used, however in the Kaumera extraction process KOH will be used instead of NaOH. As mentioned in Sels (2019) the change of NaOH with partly KOH already start to give issues in the digestion. Another issue that arises from the salt concentration is the waste stream that is left over at the end. Part of this could be recycled back into the system, but the K^+ would increase over time until Na^+ is fully removed. This cannot happen as halophiles, even though Na^+ can be toxic, need it. Part of the sodium can be exchanged for Na^+, but this has been seen to have a negative impact on the process (Sels, 2019). An solution could be the addition of NaOH as the alkalinity, but a minimal Na^+ needs to be determined to see whether this strategy is viable. Another option could be to mix the waste stream with the incoming wastewater after removing the solids. If the amount of incoming waste water is large enough, then the pH and salinity of the waste stream might be diluted enough and this way also the produced ammonia is removed in the WWTP.

Through the initial and final measurement of orthophosphate it can be seen that in digester of 5% DM efforts towards phosphate recovery can be made. The 30 mg orthophoshpate released in the low DM done in this study becomes 180 mg orthophosphate in a 5% full-scale digester. Showing another potential for recovery in the Kaumera residuals.

Thus, a system that can be used for a full-scale digester would most likely be an continuous system that would need 18 g/L of NaOH dosing . Overall more sCOD will be lost in this system, but the speed could greatly increase due to the removal of the delay due to syntrophic growth as this is now at a steady state value. The removed sCOD could then be recycled into the loop. The waste stream might be recycled or added to the influent of a WWTP diluting it and effort could be made to get the phosphate out.

4.6 Outlook

Right now the main issue with the substrate is the characterization of it as tCOD has a large standard deviation. Measurements differ greatly due to the insoluble nature of the sludge making it hard to get an homogenized solution to do analysis on. Further a more detailed description of the substrate can help identifying the troublesome compounds that could limit the digestion process.

Next a start towards continuous digestion needs to be made in order to have a potential for a full-scale process. This can be achieved by starting the system in a batch like done before and once the exponential phase has been achieved feeding every 20 days can be a starting point. This semi-continuous process should than be converted in the end towards a full continuous system. This experimental data can again be used for the modeling part to convert this into a continuous system.

For the modeling it is important to take a much closer look into the charge balance and the alkalinity definition as this is currently too simple. For now only the carbonate and bicarbonate are used to define this, but compounds like K_2HPO_4 will also impact the buffer capacity of the system. Additionally with the simplified black-box model it cannot be assumed that VFA are not impacting the system as massive accumulation occurs having a drastic impact on the system.

Furthermore a better characterization of biodegradability and what is exactly in the substrate would greatly enhance the capabilities of the model. This includes the inoculum as well as right now this needs to be assumed, while it has a large impact on the system. More info on the kinetics of the different organisms in this specific environment should make the model more robust as for now not much info is known on this. Most of the kinetics need to be chosen based on assumption and logical thinking. This way a model can be defined, but it hard to validate as experimental data is needed to calibrate. For now the model can show an insight into what these kinetic parameters should be approximately, but experiments on this are needed to validate these numbers.

The buffer capacity description in both models rely only on the carbonate and bicarbonate in the system. This is a rather weak system to describe the buffer capacity, but is usually sufficient in neutral digestion. However, due to the unique conditions used in the ADM1 it should be expended upon to have a proper estimation of the pH in the system, which is now lacking.

Finally it is essential to transform the model into an continuous operation as this would be the ultimate end goal for this process due to reasons given before. Once all of this is implemented it can help optimize the process toward the best economical potential to see whether this process can be used instead of the conventional neutral digestion.

5. Conclusion

In this study it is shown that the degradation of the alkaline Kaumera residuals is possible without any pre-treatment steps at a high pH (pH $>$ 9) and salinity of 0.6 Na^+ using a highly buffered system. It is possible to get a degradation of 24 % tCOD into CH_4 of the substrate for this first batch incubations having a methane purity between 92-97%. This is less than the neutral digestion of the exact same substrate that reaches 36% degradation, showing there is potential for increase in the alkaline digestion. In other studies even higher degradation of 30-50 % for their substrate is reached in alkaline digestion, but for some pre-treatment is used for the substrates and shown that this increases the conversion yield.

The process is at the moment delayed through the slow growing syntropic acetate oxidising bacteria and no real bottle-neck in the process could be identified due to only the delay being present. ammonia toxicity of 120 $mgNH_4$ does not reach the inhibitory threshold. Incubation times ranged from 80-110 days producing up to 119.5 mL CH_4/gVS. A total accumulation of acetate of 2200 mgO_2 is seen together with a smaller accumulation of propionate of 200 mgO_2 before consumption. In the end 1000 mgO_2 is left over, which cannot be converted due to either inhibitory compounds or as it is inert.

pH in the system drop only by 0.3 due to small DM of 0.88 % and high buffer capacity. Based on the final pH a titration was done to reach a similar pH giving a need of 75 mM NaOH resulting in a need of 3 g NaOH/L. The models did not show a well enough description of the pH so no answer can be given here. The buffer capacity description in the models will need to be extended beyond the carbonate system to properly predict the pH dynamic in this unique system.

The characterization of the substrate is done roughly in to 50% proteins, 45% lipids and 5% carbohydrates. The most common VFA were identified and implemented into the characterization. However due to the nature of tCOD measure for this specific substrate it is necessary to find an alternative method to better define the substrate. Also a more elaborate analysis of the Kaumera alkaline residual waste sludge is needed to identify all the sCOD to have a better description of the process.

The modeling of the process is able to somewhat describe the process, but has some issues. The buffer capacity description in both models is too simple for such an unique system regarding the buffer capacity. Right now an methane production is overestimated and the pH is shown lower than what is seen in the experiments. Also more research into the kinetics of the alkaline microbial community should be done in order to improve the model as this data right now is unknown.

In conclusion the process seems to have potential in terms of biogas production with subsequent batch incubations and scaling up of the system. The model can show the patterns, but more info around the kinetics and influent characterization are needed to have a better description of the system.

For subsequent steps a continuous operation should be studied to see whether this can solve the kinetic issues of acetate consumption. Further the models both need a better description in order to be able to predict the pH dynamics and also a continuous model needs to be created. Additionally kinetics of the microbial community should be determined to have a better understanding of the processes controlling this complex system. Finally a better characterization of the alkaline Kaumera residuals and inoculum will greatly improve the prediction that can be made using the alkaline ADM1 implementation. This would subsequently allow for an economical evaluation of this process to be compared to the neutral digestion giving more insight into the viability as a full-scale process in the future.

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Appendices

A. Protocol dithionite, sulfide & Inoculum preparation.

50 mL Dithionite preparation (10% w/v)

Materials:

- Sodium dithionite (Na2S2O4)
- Sodium carbonite (Na2CO3)
- Magnetic stirrer
- Inert gas (Ar/N2)
- Serum bottle
- Butyl rubber stopper
- 3 Syringes (1 mL) + 3 needles (small to to not leave holes)

Method: For 50 mL liquid volume:

i Weigh Add 4 g Na_2CO_3 to make a 8% (w/v) solution

ii Flush with inert gas (Ar/N2)

iii Place on magnetic stirrer

iv Weigh (under airflow system) and add 5 g $Na_2S_2O_4$ (will release CO2) to have an 10% (w/v) solution

v After neutralization close the bottle with butyl rubber stopper and cap the bottle

vi Flush headspace with inert gas (Ar/N2) using 2 needles using the headspace exchanger

vii Keep overpressure in the bottle to be able to get aliquots

Sulfide preparation (75 mM)

Materials:

- Sodium sulfide (Na_2S)
- Serum bottle
- Butyl rubber stopper
- Inert gas (Ar/N2)
- Syringe

Method: For 50 mL liquid volume:

i Add 50 mL milli-Q, cap it and flush with inert gas (Ar/N2)

ii Weigh 0.9 g of Na_2S and add to an open syringe (no plunger) and close it with the plunger cap

iii Puncture the anaerobic serum bottle and get water in the syringe to dissolve the Na2S

iv Once dissolved put all liquid back in the bottle to create a small overpressure to be able to get aliquots

Soda lake sediment preparation

Materials needed:

- Falcon centrifuge
- Soda lake sediment
- 2 Falcon tubes (50 mL)
- Syringe + needle
- Anoxic/Anaerobic media

Methods:

i Add 10 mL slurry mix to the falcon tube

ii Add 20 mL anoxic media to the falcon tube

iii Centrifuge at max speed for 10 minutes

iv Decant all liquid

v Add another 20 mL anoxic media and thoroughly resuspend the pellet

vi Centrifuge at 500 rpm for 1 minute

vii Take top 10 cm (top section; colloidal section)

viii Add to desired reactor

B. Gas storage and analysis protocol

Materials:

- needles (D<0.2 nm)
- Butyl rubber stopper
- 10 mL glass vials containing water saturated with NaCl (26%) containing no bubbles
- syringe valve

Method:

i wash needles with argon 3 times

ii close the valve and puncture butyl stopper serum bottle

iii open valve and take gas sample from serum bottle and afterwards close it

iv hold the brine vial upside down and puncture the butyl stopper

v add another needle for the liquid to escape

vi open the valve and exchange the brine for the gas

vii close the valve and remove the extra needle, afterwards remove the gas syringe

viii store indefinitely upside down

C. Model assumptions

Assumptions alkaline digestion model - ADM1

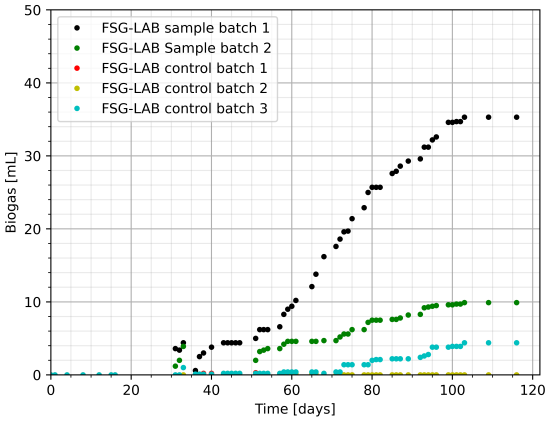
- Bacterial mass is represented by $C_5H_7NO_2$
- K_a values for the organic acids do not change over the 0-60 °C temperature range
- All biochemical processes are irreversable
- All psycho-chemical reactions are reverseable
- Acetoclastic methanogens will not be present at the beginning of the incubation and not able to grow in these conditions ((Wormald et al., 2020))
- Hydrogenotrophic methanogens are the only active microbial group able to convert substrate towards methane during the alkaline digestion process (Wormald et al., 2020)
- Produced acetic acid will be consumed by syntrophic acetate oxidising bacteria ((Capson-Tojo et al., 2021))
- No amino acids are present in the beginning, thus no organic nitrogen in solution
- All DIC present in the beginning comes from the buffer added
- Initial biomass concentration is low and calibrated to fit the data.
- Hydrogen and pH inhibition are similar to the other inhibitions processes ((Capson-Tojo et al., 2021))
- liquid volume does not change over time
- Gas volume does not change over time
- tCOD is measured of the substrate and assumed similar in all batches
- All other assumption for the original ADM1 file (Batestone et al., 2002, Rosen & Jeppsson, 2008)

Assumptions simplified black-box excel model

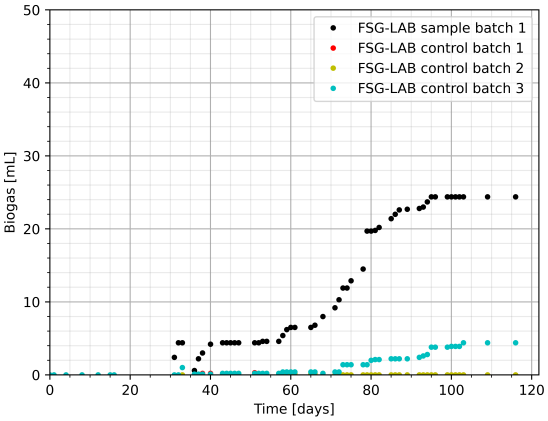
- K_a values do not change over the 0-60 °C temperature range
- Substrate is defined into one theoretical compound
- Substrate is converted to CH_4, CO_2 or NH_3 according to the theoretical compound and remaining part is inert
- Biogas produced is equal to methane production
- No pressure increase happens
- HCO_3^-, CO_3^{2-}, OH^-, H^+, $NH_4^+{}_1$ and correction factor B are used to describe the charge in the system.
- Assume all psycho-chemical reactions are reverseable
- liquid volume does not change over time
- Volume changes according to gas production and initial volume
- tCOD is measured of the substrate and assumed similar in all batches
- N content of proteins is standardize under 0.26 mol N/mol C
- Oxidation state of standard lipids used is 5.7 mol e^-/mol C
- VMG under these condition is 25.45 L_{gas}/mol
- methane COD conversion factor is 2 mol O_2/mol CH_4

D. Gas accumulation graphs

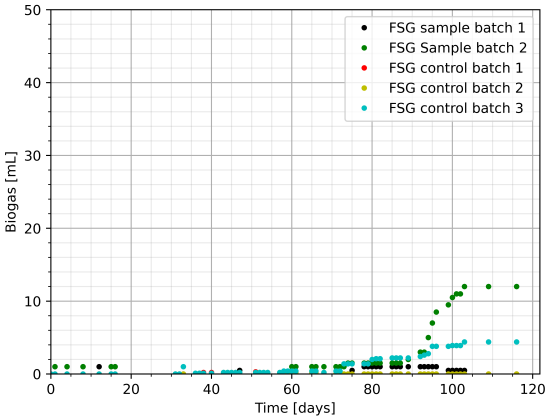
Gas accumulation graphs for the remaining batch incubations.



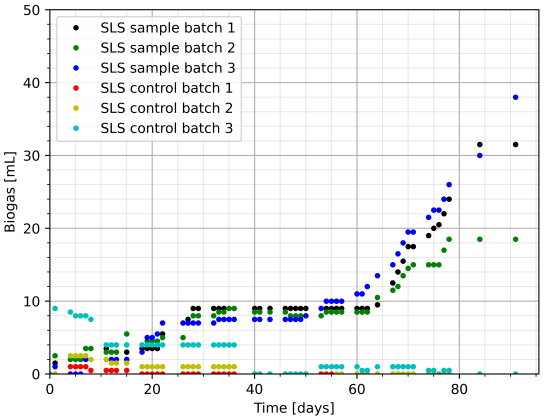
(a) Gas accumulation FSG-AGS & LAB-AGS.



(b) Gas accumulation LAB-AGS & SLS.



(c) Gas accumulation FSG-AGS.



(d) Gas accumulation SLS#2

Figure D.1: Change of VFAs over time. A) acetate changes over time. B) Butyrate changes over time. C) Formate changes over time. D) Propionate changes over time.