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Dairy Manure Anaerobic Digesters

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Department of Ecology Report to the Legislature

Dairy Manure Anaerobic Digesters

by
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Acknowledgements

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Executive Summary

Introduction

This report to the legislature is required by Section 2 of Chapter 238, laws of 2012. Its purpose is to evaluate the degree to which current state air quality regulations consider different feed sources for dairy manure anaerobic digesters and the effects of those different feed sources on the digesters and their emissions.

This review concludes that current regulations are adequate to address the various issues that can result from the use of various feed sources in these dairy manure digesters.

Background information

Some farms use anaerobic dairy manure digesters to supplement the farm income and more easily deal with dairy cow wastewater. Anaerobic digesters are biological systems that convert energy available in dairy manure into combustible digester gas. The digester gas is usually burnt in an engine-generator system to produce heat and electricity. It may also be burned in a hot water/boiler system. When burned, the digester gases produce regulated air pollutants such as sulfur dioxide (SO_2), volatile organic compounds, and nitrogen oxides.

What this report does

The Washington State Legislature directs Ecology to submit a report to the legislature describing how Washington's air quality regulations affect different "feed" (the material used in the digester to produce energy) sources for anaerobic digesters. Ecology evaluated the degree to which air quality control rules consider different feed sources for anaerobic digesters in the permitting process. To assist us in developing this report and to clarify current understanding on the effects of various feed sources on anaerobic digester systems, Ecology contracted with Washington State University (WSU) for a literature review report on the effects on hydrogen sulfide production when using various feed sources and operational choices in an anaerobic dairy manure digester.

This report presents the results of the WSU review report and Ecology's analysis.

Study results

WSU's report indicates that non-manure feed sources and other aspects of the digester system affect the amount of hydrogen sulfide produced by the digester. Other aspects

may include the sulfur content of the dairy's water supply, the sulfur content of the feed sources, usage of the liquid portion of the digester discharge, and the consistency of digester operation. WSU's literature review report is in Appendix A.

Ecology's evaluation

Ecology's evaluation found that state and local air pollution control authority (permitting agencies) rules allow a great deal of flexibility to address the affects of different feed sources on digester emissions. Much of the information used to make decisions is supplied by the owner/operator of the proposed digester system in the Notice of Construction (NOC) application for the project. As part of the application, the applicant must describe the feed sources proposed for use, along with other aspects of the digester system (for example, how digester gas is cleaned and the engine-generator or boiler proposed to be fueled by the digester gas). The application must identify Best Available Control Technology (BACT) requirements for all air pollutants, and must assure that ambient air quality standards are met. During the permit application review and permit writing process, the rules allow a permitting agency to establish operational and other limits to address the emissions from feed sources.

Ecology finds the existing air quality permitting rules of state and local air pollution control agencies are adequate to address the effects of differing feed sources on emissions. When permitting a new digester system, the rules allow us to establish operational requirements adequate to address emissions resulting from the use of various feed sources. The operational requirements are the means to implement BACT and prevent violations of air quality standards.

Purpose of Report

This report to the legislature is to fulfill the requirement in Section 2 of Chapter 238, laws of 2012 (aka SSB5343), which directs Ecology to:

> "submit a report to the appropriate standing committees of the legislature containing information regarding the degree to which current state air quality regulations consider different feed sources for anaerobic digesters and strategies to address the different feed sources used in anaerobic digesters. The department of ecology must consult with interested parties in drafting the report."

This report provides a brief evaluation of how air quality regulations account for the feed sources used in anaerobic digester systems at some Washington dairies. It also includes a review of the published literature on how feed sources used in digesters affect the amount of hydrogen sulfide generated.

Consultation

During the development of the grant contract with WSU to produce a literature review report (Appendix B), Ecology consulted with the local air pollution agency staff and operators of anaerobic digesters. We solicited comment on the proposed scope of work for the contract.

Ecology shared two drafts of this report and the WSU literature report with anaerobic digester operators, the primary anaerobic digester system designer in Washington, and others. We considered all comments we received, and incorporated them in this report as appropriate.

How Does An Anaerobic Manure Digester System Work?

The system

Anaerobic digesters are complex biological systems. They use enclosed tanks and heat to convert the energy available in the digester feed into more microorganisms and byproducts. Among the byproducts are methane, carbon dioxide, ammonia and hydrogen sulfide.

Figure 1 is a graphical depiction of an anaerobic digester system. A manure digester system consists of an open tank where the manure and other feed sources are mixed together before being put in the digester tank. Inside the digester, the manure is heated to a specific temperature. It may be mixed to keep the manure solids and solid components of other digester feed in suspension for easier removal from the digester. After two or three weeks, the digested liquid and solids leave the digester for additional processing. During digestion, gas is produced and removed from the digester tank.

The digester gas is usually burnt in an engine-generator system to produce heat and electricity, or burned in a hot water/boiler system. Heat generated by the hot water/boiler system or engine is used to heat the digester and may provide hot water for other on or off-farm uses.[1] In Washington, the farm sells electricity produced by the engine generator system to the local utility. In other states, some of the electricity produced may be used directly on the farm, with any excess sold. Sometimes the digester gas is cleaned, dehydrated, and compressed for sale to the local natural gas retailer, or used as motor vehicle fuel.

The feed sources

In a typical anaerobic manure digester system, dairy cow manure is transported to the digester, where it is temporarily stored in a digester feed tank. Alternative feed sources such as pre-consumer wastes[2] are delivered to the site and mixed with the manure. Some wastes such as liquid eggs, fish processing waste, and blood can be mixed with the manure directly. Other wastes, such as beer and wine, must be metered into the system over time to avoid upsetting the digester system. The waste is then fed into the digestion tank.

[1] One digester system in Whatcom County provides heat to a commercial greenhouse.

[2] Pre-consumer wastes may be out of date wine, beer, sugared soda, fruit and vegetable processing wastes, discarded fruits and vegetables from grocery stores, off specification or spoiled eggs, fish processing wastes, chicken processing wstes, blood from slaughterhouses, vegetable oil waste from biodiesel production, etc

The process

The digester may have one of two configurations:

- A plug flow[3] or
- A complete mix[4].

Either design can operate at ambient temperature or be heated. In the northern parts of the United States, all anaerobic digesters are heated.

Each configuration has its advantages and disadvantages for the operator. In Washington, the digesters that have been installed have all been heated. All but one has used the plug flow design.

The manure mixture is kept in the tank for a period of time, mixed to keep solids in suspension, and removed about two to three weeks after being put into the digester. The solids are separated from the liquid portion. The solids can be reused as cattle bedding, as a replacement for peat moss in commercial nurseries, or as an organic soil amendment. The liquid portion contains most of the nutrients (nitrogen, potassium and phosphorous compounds) that were contained in the original manure and other wastes. This liquid can be used on the land as allowed by the farm's nutrient management plan. Alternately, there are emerging processes to convert some of the nutrients in the liquid to a solid form that can be sold as dry fertilizer.

Digester gas is produced during the entire digestion process. Typical digester gas is 50 to 60 percent methane; the remainder is mostly carbon dioxide and water. Hydrogen sulfide (H_2S) and ammonia are commonly present in the digester gas as 'trace' contaminants in part per million to part per thousand concentrations. For air quality, the concentration of H_2S in the digester gas is of most concern. When burnt, the H_2S is oxidized to sulfur dioxide, an air contaminant with federally set limits on its concentration in the ambient air.

To produce motor vehicle fuel or pipeline grade natural gas, the digester gas is cleaned and dehumidified. This increases the methane concentration to allow it to be used to replace methane in a natural gas pipeline or compressed gas for motor vehicle fuel. H_2S from the cleaning of digester gas may be emitted to the atmosphere in this step. H_2S is a very odorous toxic air pollutant that has killed workers at concentrations reported at anaerobic digesters.[5]

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[3] A plug flow digester is like a pipe where sthe manure enters at one end and eventually gets shoved to the other end where it leaves. There is no mixing of the material along the length of the pipe. The length and diameter of the pipe determines the detention time of the digester.

[4] A complete mix system has all the digesting material in the tank completely mixed, not unlike a blender. When fresh material is put in the tank, an equal amount is discharged from the tank. The total tank volume defines the detention time of the digester.

[5] See http://www.epa.gov/agstar/documents/safety_practices.pdf , http://www.progressivedairy.com/index.php?option=com_content&view=article&id=7385:stay-safe-in-and-around-anaerobic-digesters&catid=77:manure&Itemid=121, and http://www.epa.gov/agstar/documents/conf07/brown_nellie.pdf As examples of source with information on safety.

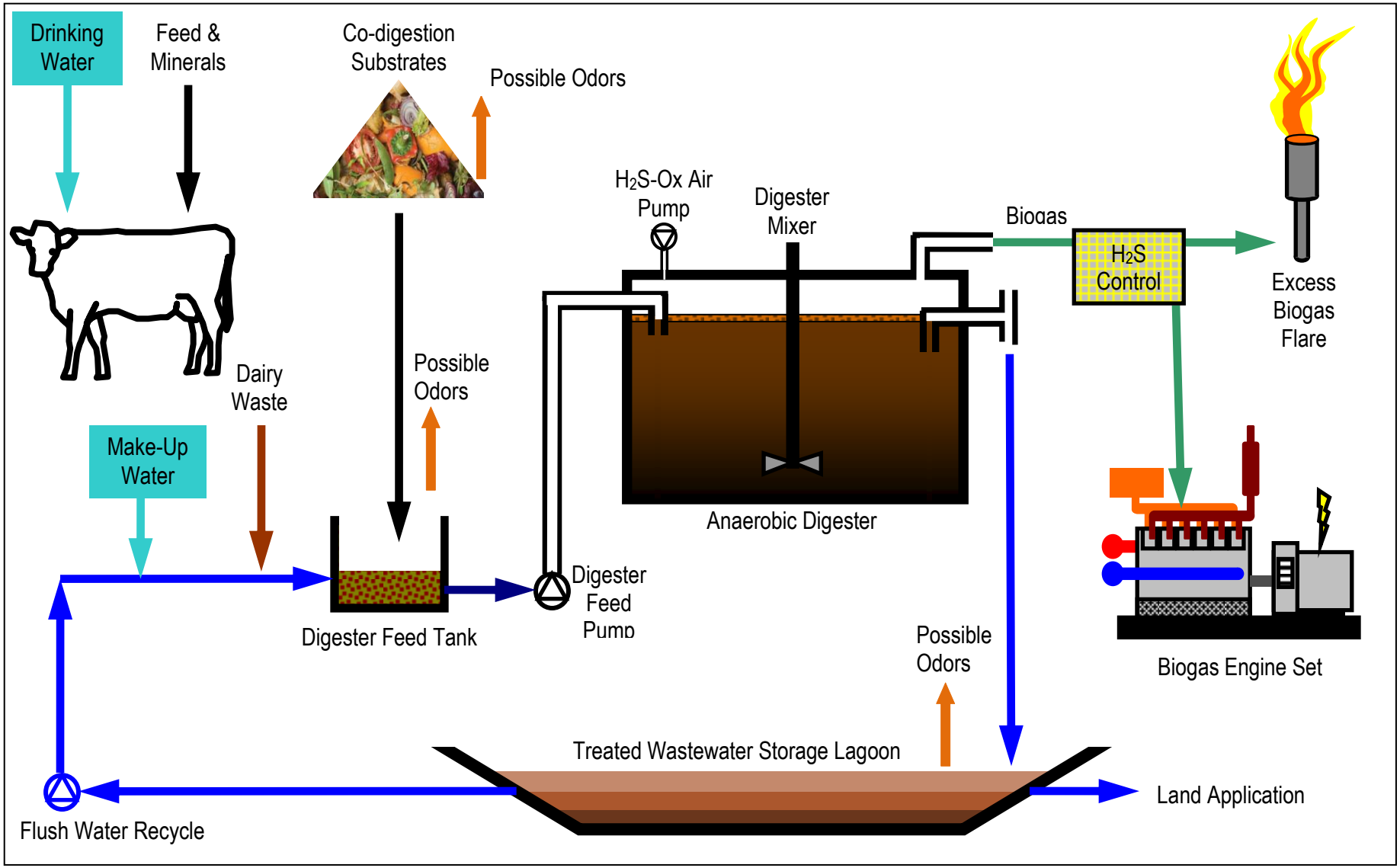

Figure 1: Example flow chart for manure digester system. Biogas is also emitted from the cow. Depending on retention time and storage characteristics, a form of biogas may also be emitted from the digester feed tank. Water flow is shown in blue, fugitive biogas and odors are in brown, and biogas is in green. Solids separation processes have been omitted for clarity, and are different for *Flush*-, *Feedlot*- and *Scrape*-Dairy systems (EPA 2001).

Effects of Digester Feed Sources' on Hydrogen Sulfide Production

WSU's literature review report focuses on:

- The sources of sulfur entering an anaerobic dairy manure digester system and
- The effects of other operational variables on the potential H_2S concentrations in the digester gas.

The review report summarizes over 300 papers discussing anaerobic digestion and H_2S. It makes significant use of the information in 68 of those reports.

Report findings

The review report indicates the cycling and emissions of H_2S is a complex process involving physical and biochemical processes. While the interactions are complex, there some simple findings that show up in the review:

- The evolution of H_2S in an anaerobic digester system is complex.
- Once the sulfur in the re-circulating flush water is at a steady state, most sulfur entering the digester system is in the re-circulating water.
- Digester feeding practices affect production of methane and H_2S in a digester.
    - New feed sources need to be introduced gradually to allow the microorganisms to adapt to the new feed source.
    - Abrupt changes of feed sources can lead to upsets and inconsistent operation of the digester.
    - Batch feeding of a digester leads to upsets of the system causing changes in the production of methane and H_2S. Continuous feeding of a digester works better than batch feeding.
    - Batch feeding of carbohydrates (fruit juices, beer, etc) can increase digester acidity, releasing H_2S into the digester gas.
- Control of digester operations is important to maintain stabile operation, methane and H_2S production. Control of digester operations includes:
    - Maintaining a constant temperature in the digester.
    - Introducing cold feed to a heated digester shocks the system and inhibits digestion until temperature, digester pH, and alkalinity return to normal.
    - Temperature and digester pH and alkalinity are important parameters to control.
    - Digester feed sources may need to be supplemented with trace nutrients to maintain a stable microorganism population.
    - Keep solids in suspension to facilitate removal.
    - Minimize the formation of scum on the digester surface.

- Some feed sources such as fats, oils and grease which do not contain sulfur compounds can result in increased generation[6] of H_2S and methane by the digester.
- The actual digester design (plug flow versus complete mix, heated versus unheated):
    - Is not an important factor in overall H_2S generation.
    - Affects the speed in which an 'upset' digester can be made healthy (plug flow designs take longer).
    - Plug flow designs are more prone to pH and temperature changes at the influent end of the system compared to complete mix systems.
- H_2S control can be easily accomplished by injecting limited amounts of air into the digester head space. However, the operator:
    - Must remove the sulfur from the digester to prevent it converting back to H2S; and
    - Has little direct control of the process inside the digester head space.7

For more details on the findings about the influence of digester feed sources on the production of H_2S by an anaerobic digester, see the report in Appendix B.

[6] Referred to as 'purging' of sulfur from the digester in the review report.
[7] Digester system design could be modified to allow for more visual inspection of the digester contents.

Air Quality Regulations and Feed Sources

Ecology[8] evaluated the degree to which its rules consider the different feed sources for dairy manure anaerobic digesters in the permitting process. The state Clean Air Act and air quality regulations[9] provide only general guidance on how to address differing feed sources and their effects on emissions of air pollutants from digester systems.

NOC permit

Air quality regulations address air pollutant emissions from sources. A Notice of Construction (NOC) air quality permit[10] is required for new sources and modifications to existing stationary sources. Anaerobic digesters and their associated fuel burning equipment are stationary sources of air pollution. If they emit more than a de minimis annual amount of air pollution,[11] they must be permitted as a new source. The NOC application and issued NOC Approval must show that ambient air quality standards are being met, BACT is being used for all air pollutants emitted, and any applicable emission standards in state or federal regulations are being met.

As part of the air quality permit application for a new digester system, the applicant must describe:

- The feed sources they propose to use in their facility and
- Any limitations on the use of those feed sources in the digester system.

Ecology uses this information to develop emission and operating limits that address the emissions from those feed sources.

Flexibility allowed to permitting agencies

Sometimes an applicant does not specify in the permit application the feed sources to be used in the digester. Where the potential feed sources are less well-defined or more variable, the regulations in place allow Ecology to evaluate the emission potentials of possible feed sources. In determining BACT for an anaerobic digester system, the permitting agency will use information about the effects of specific feed sources on digester operation, including methane and H_2S production. Required emission controls are specific to the air pollutants emitted as a result of the use of particular equipment or resulting from the use of specific feed sources. The definition of BACT in the rules allows Ecology to establish operating methods and procedures along with appropriate monitoring, recordkeeping and reporting to assure that those operating and emission requirements are met.

[8] In this discussion, Ecology generically refers to Ecology and the seven air pollution control authorities.
[9] In this discussion, 'air quality regulations' refers to the state and local air pollution control authority regulations.
[10] These may also be called an Order of Approval to Construct. There is also a General Order of Approval that can be issued to simplify the permitting of common and relatively simple facilities and emission units. A General Order of Approval has been issued for anaerobic dairy manure digester systems.
[11] De minimis emission rates are found in WAC 173-400-110(5), also in rules of the local air pollution control authorities.

Ecology used this regulatory flexibility in 2011 to establish a General Order of Approval for dairy anaerobic digesters that streamlines and adds predictability to the permitting process as long as certain conditions are met.

Permitting fugitive and point sources

Several locations in an anaerobic digester system can be sources of air pollution:

- Waste receiving areas
- Digester pressure relief valves
- Digester gas cleaning processes
- Combustion units (engines, flares, boilers)
- Digested material storage and solid/liquid separation steps

Waste receiving areas, digested material storage, and the solid/liquid separation equipment are normally considered fugitive sources[12] of emissions, mostly odors. The digester gas cleaning process may be a fugitive source of emissions (H_2S, odors), or could be a point source[13] of emissions depending on what digester gas cleaning equipment is installed. The combustion units are point sources of emissions (non-fugitive).

The handling of manure and the receipt and temporary storage of alternative feed sources for a digester may generate odors which may be offensive to nearby residents. State air quality regulations address odors as a nuisance that owners and operators of the source should avoid and/or minimize. Best Management Practices have been developed for some types of facilities that generate odors (for example, yard waste composting operations). These Best Management Practices focus on minimizing the odors generated and their impact on property owners in the area around the facility.

Emissions from the combustion sources associated with anaerobic digesters must be permitted before the start of construction. A source must meet the requirements of the permit once it is in operation. As noted above, this permit must assure that ambient air quality standards are met and that BACT is used.

For permits on combustion sources, the permit writer reviews the methods used to limit the emissions of regulated and toxic air pollutants that can be applied to the emission units involved. The emission control methods can range from pre-combustion controls to post-combustion controls to controls that occur during the combustion process itself. It is often less expensive to control a specific pollutant in one part of the system than it is in other parts of the system.

For anaerobic digester systems, it is easier to control sulfur dioxide emissions before the combustion step. This is done by controlling H_2S in the digester gas sent to the combustion unit(s). The Technical Support Document for the Dairy Manure Anaerobic Digester General Order of Approval[14] identifies the common methods used to control H_2S. The General Order of Approval and plant specific Orders of Approval require routine monitoring of the hydrogen sulfide content of the digester gas sent to the combustion

[12] Fugitive emissions are emissions that are not emitted via a specific pipe, duct or opening and as such are not normally required to utilize add-on BACT emission controls.
[13] Point sources of emissions are those that come out of a discrete pipe or opening and are subject to emission contro.
[14] Available at http://www.ecy.wa.gov/programs/air/AOP_Permits/Boiler/GeneralOrders.htm

units. The common method is by use of simple gas samplers (referred to as Draeger™ tubes) or the use of electronic monitoring instruments. The permits require the monitoring results be recorded and reported to or made available to the permitting agency, as described in the Order of Approval.

The effects of different feed sources on H_2S production by an anaerobic digester have been recognized for a number of years. Methods to remove the H_2S from the gas are also common. As noted in the review report in Appendix B, the interaction of feed sources with the digester's microorganisms and the resulting concentration of H_2S in the digester gas is a complex process.

Conclusion

Ecology and the local air pollution control authorities believe we have adequate regulatory authority under the Clean Air Act to regulate dairy manure anaerobic digesters. We also believe we have the ability within the definition of BACT and ambient air quality protection to address the effects of differing feed sources on operations and emissions from dairy manure anaerobic digester systems.

The literature review report provides an outline of actions that can be used to develop best management practices to reduce the potential emissions of H_2S from dairy manure anaerobic digesters while maximizing methane production. Based on the final literature report, the dairy manure digester operators and designers, Ecology, the local air quality agencies, and WSU will be able to develop a set of best management practices for operation of dairy manure anaerobic digesters.

Appendix A. Washington State University Review Report

Hydrogen Sulfide Concentrations in Biogas from Dairy Manure Digesters

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1 Executive Summary

This literature review examines the dynamics of hydrogen sulfide concentrations in dairy manure digester biogas and the contributions of co-digestion substrates. Hydrogen sulfide (H_2S) is a problem because it damages power generation machinery and its combustion product (sulfur dioxide) is a U.S. EPA "criteria pollutant". Furthermore, the large differences in biogas H_2S concentrations between different dairy manure digesters and the high variability of biogas H_2S from individual digesters are still inadequately explained, although the co-digestion substrates are traditionally considered the culprit.

This review combines literature studies with proven chemistry and biology principles to analyze the most important phenomena and specific questions governing dairy manure anaerobic digestion and co-digestion systems. Sulfur sources are identified and the main sulfur conversion processes are described in the context of different parts of a dairy flush-water recycling system.

Sulfur enters the dairy digester in different forms, but organic sulfur (amino acids) and sulfates convert to sulfides within the digester. The amount of H_2S that leaves the digester liquor and enters the biogas depends on the pH, temperature, and biogas flow rate; conditions which depend on the digester design and operation. Mass balances indicate that less than 20% of sulfur leaves a dairy digester as H_2S in the biogas. The remaining sulfur enters the treated water lagoon that serves as a source of recycled water. The bulk of sulfur entering the digester, therefore, comes from lagoon accumulation although in essence it originates from the feed and co-digestion substrates.

Small amounts of air injected into the headspace of some dairy digesters allow the biological conversion of H_2S into elemental sulfur and sulfur oxides. Air helps decrease H_2S concentrations in the biogas, but because the oxidized products remain in the digester liquor, their release as H_2S is sometimes only temporarily delayed.

This review suggests that:

- The sulfur content of the feeds and co-digestion substrates determine the amount of sulfur entering the dairy digester "sulfur-cycle."
- Digester design, digester configuration, and lagoon management practices determine the average biogas H_2S concentrations.
- Short-term digester mixing and temperature changes, and changes in H_2S oxidation conditions within the digester headspace are responsible for the observed variability in biogas H_2S concentrations.

2 Introduction

This report reviews available literature in order to explain the differences and variability of hydrogen sulfide (H_2S) concentration in biogas from the anaerobic digestion of dairy manure with or without other substrates. Presence of H_2S in biogas is a concern to the anaerobic digester owner because it is corrosive to power generation equipment and other metal components. Hydrogen sulfide corrosion occurs either from the acidic oxidation products of H_2S gas in the presence of water (e.g. H_2SO_4), or by direct chemical reaction between H_2S gas and metals. From the environmental standpoint however, the concern is conversion of H_2S to sulfur dioxide (SO_2) during burning or combustion of biogas, which is released into the atmosphere. The Clean Air Act requires the EPA to set national ambient air quality standards, and the EPA currently lists sulfur dioxide and five other major pollutants as "criteria pollutants." For these reasons, removal of H_2S has been identified as one of the technology barriers to biogas production (EPA, 2012). Understanding H_2S release mechanism during anaerobic digestion, might thus provide the most effective approach to source control.

Because of the scarcity of complete dairy digester operational data, the important aspects and specific questions about dairy manure and co-digestion systems were examined using proven chemistry and biology principles.

The process of H_2S release from anaerobic digesters is a confusing and contradictory topic for several reasons:

1. While dairy anaerobic digesters are generally used to stabilize manure wastes, they also facilitate the recycling of treated flush water, which represents most of the sulfur load.
2. Dairy digesters are best understood as part of a system that contains at least four linked elements (Figure 1); cows (& feed), codigestion-substrates, digester, and water recycle system (e.g. lagoon).
3. H_2S release is a complex topic and demands a holistic appreciation and integration of several disciplines; aquatic chemistry, physical chemistry, biology, and engineering.
4. Much of the relevant technical literature is so narrowly focused it constitutes misdirection.
5. Some of the technical literature has significant scientific flaws.
6. Daily sampling of short-duration (e.g. hourly) process fluctuations can produce significant "aliasing" artefacts.
7. As with the last point, discontinuities in digester loading and operation cause similar discontinuities in H_2S concentrations and depending on when a sample is collected different results may be obtained. Loading discontinuities are present because dairies have a daily rhythm, and the waste collection and barn cleaning process is a discrete rather than continuous process.
8. Sulfur concentrations are often described in mass per volume terms without a conventional mass reference, e.g. SO_4^{2-}, H_2S, $S^{2-}$and S^0 (only the last two have equivalent mass and molar masses), making direct comparison difficult; in contrast to wastewater nitrogen where mass is reported as N regardless of the molecular form.
9. A dairy digester's purpose is subordinate to the dairy's main function, so important data are frequently not available.
10. H_2S measurements are sometimes not reliable; H_2S adsorbs onto sample container surfaces or can dissolve in the condensate normally associated with biogas, and there are anecdotal accounts of electronic monitoring instruments generating false signals.
11. Digester operators cannot see the digester fluid surface because the digester is gas-tight and enclosed, so vital observational evidence is missing, e.g. the presence or absence of sulfur stalactites, surface foam, or crust, and the distribution of bubbles within the digester.
12. Clear distinctions must be made between concentration data and mass transfer rates.

This investigation identified several themes: Understanding dairy digesters requires a holistic approach, particularly with regard to their recycled water quality, as well as a synthesis of herd nutrition, microbiology, chemistry, and engineering perspectives. Furthermore, dairy digesters have their own specific trace metal issues but share the "metal/sulfide partition anomaly" and unexplained H_2S variations, of other anaerobic digesters.

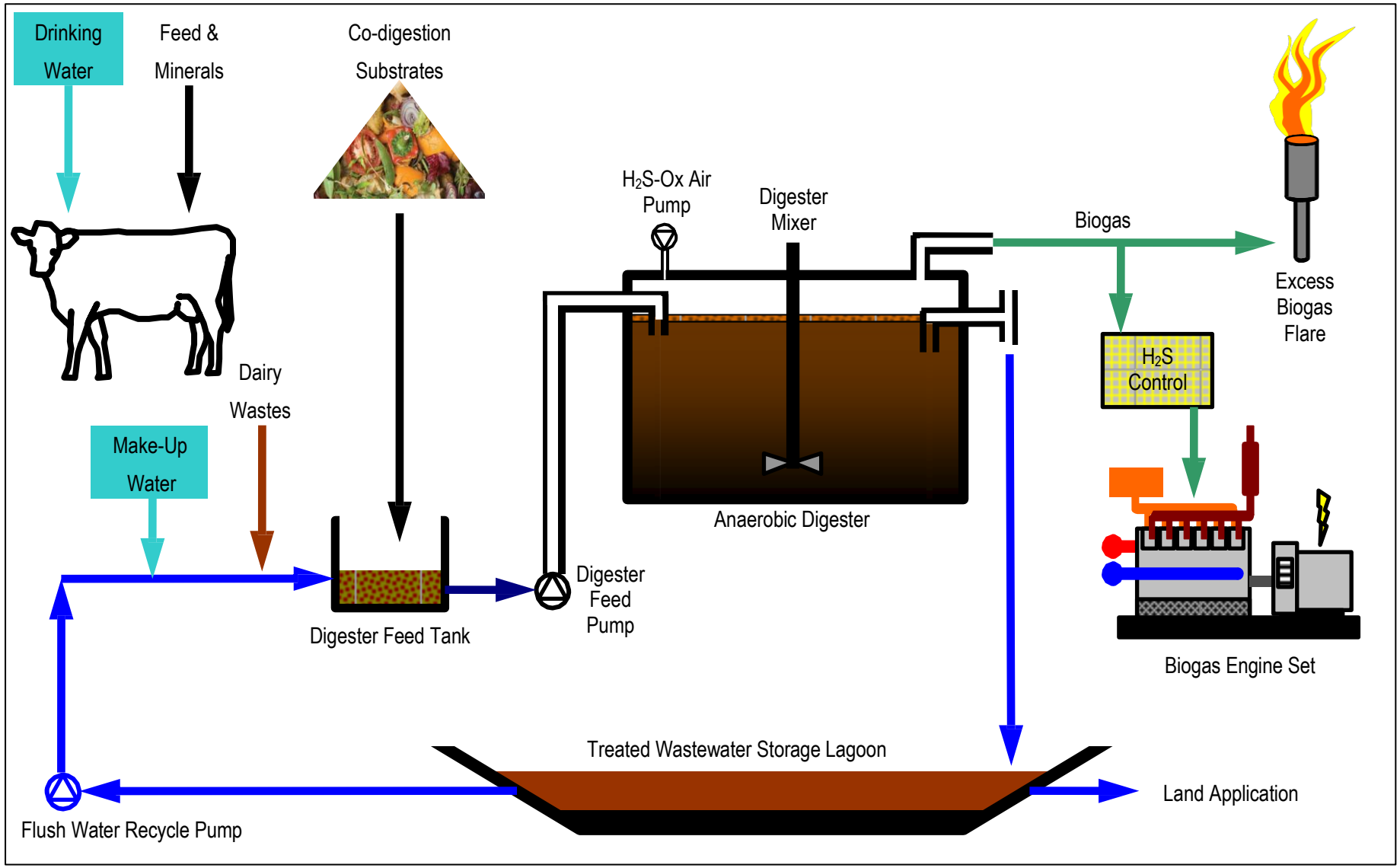

Figure 1. Typical layout of a dairy digester integrated within the main process components. Water flow shown in blue, and biogas in green. Solids separation processes have been omitted for clarity, and are different for *Flush*-, *Feedlot*- and *Scrape*-Dairy systems (EPA, 2001).

2.1 Problem Definition

Dairy biogas data (Table 1) show three distinct patterns, and any successful explanatory scheme must account for all three of these observations:

- Average H_2S concentrations in the biogas differ widely between dairy manure digesters.
- For a specific digester, H_2S varies widely, and higher concentrations levels are not necessarily more stable.
- The variation of H_2S concentration in the biogas is much greater than for methane..

Table 1. Dairy biogas H_2S and methane levels.

Farm	Biogas H_2S			Biogas Methane			pH	Reference
	Avg. ppm	Std Dev. ppm	% Var.	Avg. %	Std Dev. %	% Var.		
Costa Rica	4.8	3.4	71	61.7	24	39*	6.2	(Lansing *et al.*, 2008)
(Several)	141			58.5			-	(Amon *et al.*, 2007)
Ridgeline Farm, NY	800						-	(Bothi, 2007)
AFBI, Hillsborough, UK	1760	548	31	56	1.8	3.2	7.4	(Frost and Gilkinson, 2010)
AA Dairy, NY	1925 1930	893	46	62.5 59.1			- 7.9	(Zicari, 2003) (Martin, 2004)
DDI Farm, NY	1984	571	29	60.3	1.1	1.9	-	(Bothi, 2007)
Sheland Farms, NY	2240			64			-	(Pronto and Gooch, 2009)
Noblehurst, NY	3089	408	13	56.4	0.28	0.5		(Ludington and Weeks, 2008)
Noblehurst, NY	3392	520	15	58.8	3.3	5.6		(Ludington and Weeks, 2008)
Twin Birch, NY	7000						-	(Bothi, 2007)

*This large methane percentage variance is unusual and might be due to the comparatively small size, intermittent loading, and absence of mixing in this digester. The pH 6.2 effluent suggests this digester had failed because pH < 6.7 indicates souring, but soured digesters do not produce 61.7% methane.

Large variations in biogas H_2S concentration, as depicted in Figure 2, are common (Deublein and Steinhauser, 2011). Laboratory studies on the storage of swine wastes have recorded similar variations in H_2S emissions (Ni *et al.*, 2009). Ni *et al.* (2009) explained the bulk of the H_2S release as being due to bubbles rising through the stored manure; however, at least two of these peaks lasted for more than 20 minutes and appeared at times when there were no parallel changes in either carbon dioxide, ammonia, or sulfur dioxide in the reactor headspace. Unfortunately, no methane concentrations were recorded in the study so it is difficult to decide where these H_2S releases originated, but the absence of any simultaneous changes in carbon dioxide and ammonia concentrations in the headspace when the H_2S peaks were observed, suggests that this H_2S did not come from a bubble.

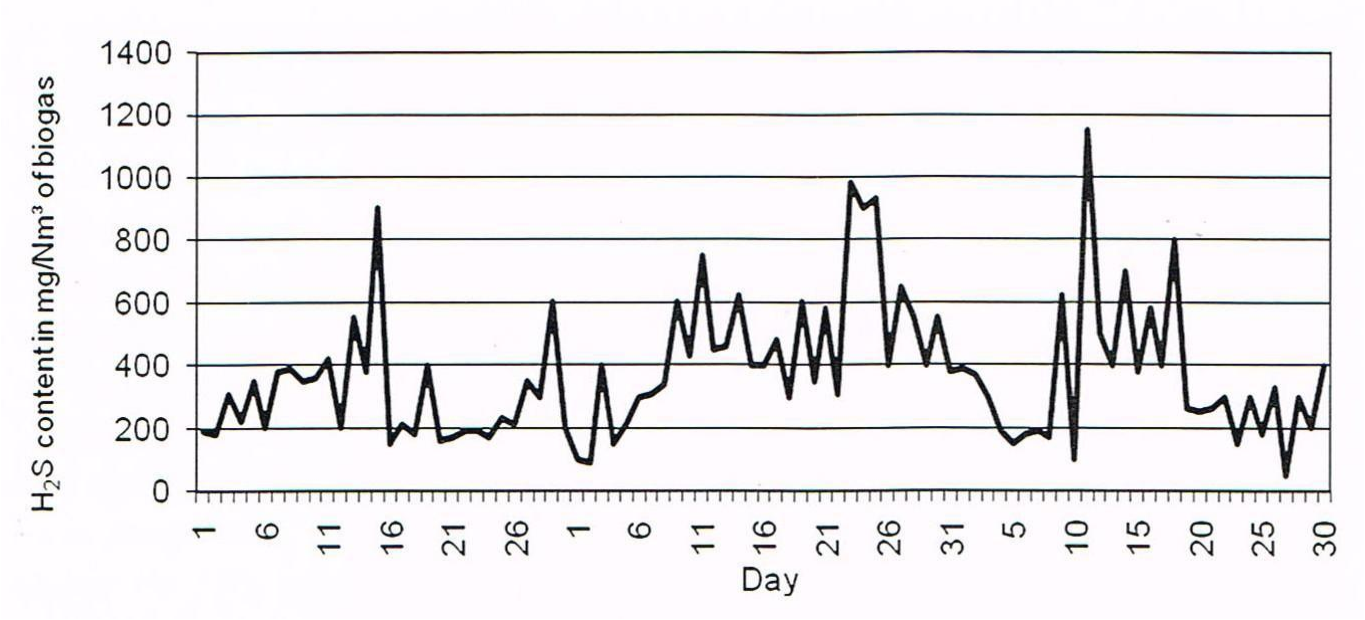

Figure 2. Daily variations in biogas H_2S concentrations from an anaerobic waste digester (Deublein and Steinhauser, 2011).

Despite the obvious biogas H_2S concentration fluctuations (Figure 2), it is misleading to refer to *daily* variations when the actual variation frequency may be much shorter than a day. For instance, when four different anaerobic digesters were tested every twenty-minutes for two-hours, two digesters had ten-fold biogas H_2S concentration spikes that lasted less than forty-minutes (Sklorz, 2002). Furthermore, even though the changes were less dramatic, the other digesters both had H_2S concentration variations greater than 30% within the two-hour monitoring period (Sklorz, 2002). Sklorz's data (2002) raises doubts about all daily digester measurements: If similar biogas H_2S concentration fluctuations are present in other digesters it is likely that any data "patterns" are actually aliasing artifacts caused by an inadequate sampling frequency.

Some practitioners have the opinion that H_2S concentration variations are related to the sulfur content of alternative digester feedstocks (Newman, 2012). This question can be divided into two subquestions: First; do different feedstocks contain different levels of sulfur? Second; what are typical digestion rates for dairy manure and different feedstocks, i.e. how quickly is the feedstock sulfur released?

An initial examination of journal articles, reports and case studies, suggested that H_2S release is a complex and multifaceted subject, and thus needs careful organization to make sense of the different phenomena. Two organizational frameworks are suggested here. The first uses the physical layout of a generic dairy farm (Figure 1), and the second categorizes the different H_2S topics into four broad areas (Table 2): Sulfur(S)-Sources, S-Conversion Processes, Variables affecting S-Transfer, and Variables affecting biogas S-Concentrations. However, many of these different H_2S topics are relevant and play a role within each of the process components (Figure 1). Consequently, a topics-approach is adopted in this review to eliminate duplication.

Three interwoven processes or phenomena need to be considered at each stage of the process: The *Sulfur Cycle* describes the conversion of sulfur into its different forms, as well as the microbes and conditions required for each conversion. The *anaerobic consortia* describes the community of microbes and process steps involved with producing biogas from complex solid and liquid wastes, as well as the growth conditions required by different microorganisms and the consequences of an unbalanced consortium. *Chemical and physical* processes describe the aquatic chemistry and gas transfer principles governing the chemical species interactions. Moreover, while each of these topics is introduced and described separately in this report, elements of all three are present in every stage of the dairy wastewater treatment system.

Table 2. Organizational framework for H_2S-related phenomena.

Sulfur Sources	S-Conversion Processes	Variables affecting H_2S gas transfer to digester headspace	Variables affecting biogas H_2S concentrations in headspace
Recycled flushwater S-content: ○ Drinking water SO_4^{-2}. ○ Rain as SO_4^{-2} source. ○ Evaporation as concentrator. ○ Ice as concentrator. ○ Spring/Fall lagoon turnover. ○ Soil amendment and fertilizer runoff as SO_4^{-2} source. ○ Lagoon processes, e.g. S-losses vs. S-fixing at water surface. ○ Animal health impact of recycle. Cattle feed & variables: ○ True protein (vs. crude protein) and estimation of the S-content of foods. ○ Mineral supplements SO_4^{-2}-source ○ High-Sulfur cattle foods. Co-digestion substrates: ○ Protein content. ○ High S-substrates. ○ Volatile-S substrates and effect of drying. ○ "S-purging" substrates, e.g. fats and oils. Other SO_4^{2-}/H_2S sources: ○ Copper sulfate footbaths. ○ Minerals, e.g. gypsum. ○ Acid Mine Drainage.	The Sulfur Cycle: ○ H_2S from protein by fermenting organisms. ○ H_2S from SO_4^{-2} (and S^0) by Sulfate Reducing Bacteria. ○ Incorporation into biomass by anaerobic organisms. ○ H_2S consumption by Sulfur Oxidizing Bacteria. ○ S-Disproportionation reactions. ○ Digester vs. lagoon processes. Other H_2S-Sinks: ○ Loss with treated liquor. ○ Precipitation as insoluble metal sulfides.	Biogas flow rate, H_2S purging effect, and factors affecting digester biogas flow: ○ Dissociation, pH & pK_a. ○ Henry Coefficient. ○ Role of temperature. ○ Theoretical H_2S prediction. ○ Dairy digester configurations. ○ Digestion consortium & implications of imbalance. ○ Typical digestion rates. ○ Digester feed composition: carbohydrates, fats/oils and proteins. ○ Loading rate and feeding regime. ○ Digester mixing cycle. ○ Methanogen trace-metal nutrient conditions. pH and impact on H_2S availability: ○ Accumulation of volatile fatty acids. ○ Digester buffering and alkalinity. ○ Urea conversion and ammonia production. ○ Relative concentrations of cations and anions.	H_2S Reaction variables: ○ Deliberate oxygen or air injection into digester headspace. ○ Accidental air injection, e.g. air entrainment with feed, or quick draw down. ○ Site of air injection into headspace. ○ Type of digester mixing system (gas recirculation vs. pump or propeller mixer). ○ Biogas flow within headspace. ○ Presence of digester surface foam, scum or crust. H_2S measurement variables ○ Sampling time vs. biogas flow rate. ○ Sample frequency. ○ H_2S sample container adsorption and analytical delay. ○ H_2S dissolving in condensate. ○ Reactions due to light exposure. ○ H_2S sensor problems.

3 Sulfur Sources

3.1 Recycled flush water S-content:

While the feed substrate is an important source of sulfur for a digester, it may not be the largest source. Sulfur mass-balance studies show that most (between 82 and 85%) of the sulfur remains in the treated digester effluent (Ludington and Weeks, 2008; Pronto and Gooch, 2009). The majority of this treated effluent sulfur will later be recycled from the solids separation process or storage lagoon to flush fresh manure from barns. Thus, this sulfur recycling, constitutes the bulk of the digester sulfur load. The focus thus changes from identifying the main sulfur source to identifying and ranking the "top-up"-source(s). There are two testable implications of this S-maintenance hypothesis. First, if the lagoon storage is the S-source, there will be a seasonal variation in H_2S concentration. Second, the small farms that are more likely to use a "once-through" system (do not recycle their flush water) will have low H_2S; this second observation does appear to be supported by the low H_2S concentrations seen in some of the data (Table 1) (Amon *et al.*, 2007; Lansing *et al.*, 2008).

Although there does not appear to be literature supporting seasonal sulfur variations in dairy lagoons there is evidence of seasonal changes in other lagoon constituents. Seasonal differences in lagoon endotoxin concentrations have been documented (Purdy *et al.*, 2010), with winter levels three times higher than the summer levels. Purdy *et al.* (2010) measured a wide variety of biological, chemical and physical parameters in four different dairy lagoons during the summer and winter, and compared these to two control lakes in the vicinity. Purdy *et al.* (2010) used sulfate as the sulfur parameter and this proved to be unfortunate as the dairy lagoons had BOD, COD, and TOC levels 10 to 100 times higher than the control lakes, and sulfate would be expected to be converted to sulfide by sulfate reducing organisms under these conditions.

One of the few studies showing longer-term H_2S measurements (Zicari, 2003) also supports the notion that winter water may be more concentrated that summer water (Table 3). Zicari (2003) measured the H_2S present in a dairy digester biogas using two different analytical methods and these data do appear to be seasonal (Table 3). Unfortunately, it is difficult to draw conclusions based on these data, as the time difference between the larger H_2S changes is substantial. Of the two methods, Zicari (2003) was more confident in the "tube" readings, and suggested that differences between the electronic sensor and the tube method might be due to leaks in the electronic sensor pipeline sealing.

Table 3. Apparent seasonality in H_2S biogas measurements and differences between measurement methods.

H_2S Gas Detector Tube Readings for AA Dairy Raw Digester Gas (Zicari, 2003)		
Date	Tube Method H_2S ppm	Electronic Sensor H_2S ppm
November 13, 2000	3600	-
March 4, 2001	2200	-
July 1, 2001	3400	-
July 13, 2002	1400	660
July 15, 2002	1400	1380
July 20, 2002	1300	1680
July 27, 2002	1150	1440
August 5, 2002	1200	1280
August 19, 2002	1700	1900
August 22, 2002	1900	-

A sulfur mass balance performed over the Noblehurst Dairy Farm in New York State (Ludington and Weeks, 2008) made three important points. The first was that the digester had an influent sulfur concentration of 0.036% (unclear whether % total solids or total mass) and an effluent sulfur concentration of 0.030%, which showed that 83% of the sulfur was still present in the digester effluent. These last data confirmed that H_2S gas loss from the digester represents a comparatively small proportion of the sulfur load (similar to the Table 4 pattern). Secondly, Ludington and Weeks (2008) note the digester effluent was *sometimes* pumped back to the reception pit to help dilute the solids, so it appears reasonable to expect the digester feed to have a much larger and very different sulfur load on the days when this occurred, compared to days when no digester effluent was recycled. Finally; the mass balance was based on an "as fed Sulfur" of 0.1% of dry weight and this was probably an underestimate as it is only half of the normally recommended 0.2% (Subcommittee on Dairy Cattle Nutrition, 2001).

Apart from the sulfur load implications, Ludington and Weeks' (2008) observation that digester effluent was *sometimes* pumped back to the reception pit to help dilute the solids is important for two reasons. First, it means that the Noblehurst Dairy Farm can control the amount of water returning to the reception pit. The ability to control dilution water is common, and this water can be supplied from the flushwater after solids separation, from treated anaerobic digester water, or from the treated water storage lagoon (EPA, 2001). Second, the observation that dilution water was not always necessary means that either the amount of manure solids or the flushwater volumes change. Differences in the flush and diluent water sources, and amount of solids loading variations, are important because these will cause performance differences in otherwise identical anaerobic digesters.

Table 4. Reported sulfur percentages in three different dairy digesters (Bothi, 2007).

Dairy Farm	Raw Manure Sulfur (%)	Digester Effluent Sulfur (%)	Difference over Digester (%)	H_2S in Digester Gas (ppm)
Twin Birch	0.08	0.04	0.04	7000
AA on 9/19/03	0.05	0.05	0.00	1930
AA on 11/24/03	0.04	0.02	0.02	
DDI	0.03	0.02	0.01	1984

3.1.1 Drinking water SO_4^{-2}

As the median drinking water sulfate levels are similar for both surface- and ground-water supplies (Table 5) (EPA, 2003), it is likely that dairies will be using water of similar quality. However, there is a broad range of sulfate concentrations and higher sulfate concentrations are relatively common. Approximately 4% of drinking water samples contain more than 500 mg SO_4^{2-}/L. SO_4^{2-} is important because it will be converted by Sulfate Reducing Bacteria (SRB) into H_2S once it enters the digester.

3.1.2 Rain as SO_4^{-2} source

Sulfate levels in rainwater appear to be low in the Pacific Northwest (Figure 3), especially when compared to those detected in agricultural drinking water supplies (Table 5), and this comparison suggests that rainwater entering the lagoon will probably not be a significant source of digester sulfate variability other than through reducing the sulfate levels quickly by dilution. Lagoon sulfates will be converted to H_2S by sulfate reducing bacteria in the digester.

3.1.3 Evaporation as concentrator

Evaporation from the storage lagoon will concentrate non-volatile water constituents such as sulfates (dissolved) and elemental sulfur (suspended solids). Potentially volatile constituents such as H_2S will also be concentrated, but whether these volatiles are lost from the lagoon surface or accumulate in lower lagoon layers will be determined by surface mixing conditions and presence of dissolved metals.

Table 5. Sulfate detections and concentrations in streams and ground water (EPA, 2003).

	Detection frequency > MRL*		Detection frequency > HRL*		Concentrations (all samples; mg/L)	
	% Samples	% Sites	% Samples	% Sites	Median	99th Percentile
Surface water						
Urban	100	100	2.6	0.4	20	2000
Mixed	99.9	99.4	0.8	2.2	21	440
Agricultural	99.8	99.7	2.9	3.6	25	670
Forest/rangeland	99.9	99.5	0	0	5	160
All sites	99.8	99.6	1.8	2.7	20	680
Ground water						
Urban	91.1	98.7	5.3	6.4	20	2600
Mixed	89.9	96.6	2.1	2.4	12	940
Agricultural	93.6	99.5	4.3	4.3	24	1200
Forest/rangeland	91.8	97.5	0	0	7	71
All sites	91.6	98	2.7	3.2	17	1300

** The Minimum Reporting Level (MRL) for sulfate in water is 0.1 mg/L and the Health Reference Level (HRL) is 500 mg/L. The HRL is a preliminary health effect level used for this EPA investigation.*

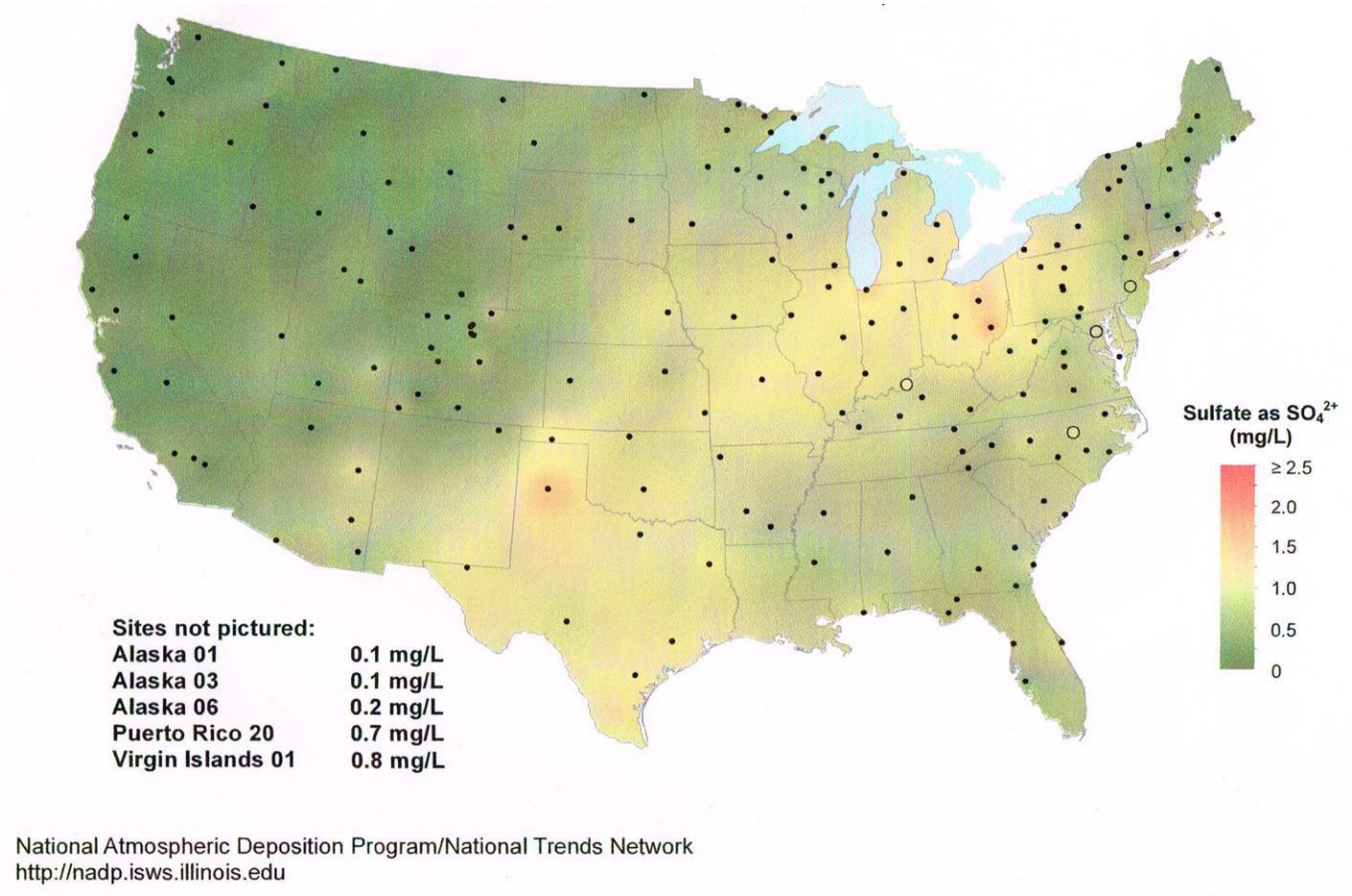

Figure 3. Sulfate levels in rainfall as SO_4^{2-} mg/l for 2011. (Figure from http://nadp.sws.uiuc.edu/ntn/maps.aspx).

3.1.4 Ice as concentrator

Lagoon surface freezing concentrates salts and solids in the lower lagoon levels because pure ice crystals form first and separate from the surrounding water. When a significant ice layer forms on a lagoon surface, the lower layers of unfrozen water become correspondingly more concentrated. This phenomenon would be noticeable by comparatively quick changes in lagoon recycle water concentrations associated with freezing and melting weather events.

3.1.5 Spring/Fall lagoon turnover

Deep lagoons can have significant stratification, which is maintained by density differences between the water layers. However, as the surface water layers warm in spring, or cool in the fall, the surface density approaches that of the lower layers, and this facilitates mixing. Strong surface winds will produce similar water circulation effects, unless ice is present. If turnover does occur, and recycled flush water is being collected from a fixed height within the lagoon, the flush water might show a step-wise quality change, which will be reflected by corresponding digester biogas quality change. This phenomenon is not expected to be a significant source of variability in shallow lagoons.

3.1.6 Soil amendment and fertilizer runoff as SO_4^{-2} source

When sodium ions bind with soil clay particles the soil structure deteriorates, and farmers sometimes use gypsum in combination with lime to restore the soil structure by increasing the calcium concentration. Excess calcium displaces the clay-bound sodium, which is then released as sodium sulfate. Sodium sulfate is more soluble than calcium sulfate and is easily leached from the soil.

Ammonium sulfate fertilizer runoff, if collected by the storage lagoon, may constitute a significant source of sulfates on a dairy farm. Obviously, this contribution will depend on the fertilizer application rate and the area of the fertilized catchment zone. The sulfate contribution from this source will correlate with runoff events caused by precipitation or excessive irrigation.

3.1.7 Lagoon processes, e.g. S-losses vs. S-fixing at water surface

Sulfur-cycling may play a key role in dairy lagoon wastewater stabilization, especially when organic loads are high and little oxygen is present. Sulfides will be oxidized at the water surface into highly soluble (and non-volatile) sulfate by sulfate oxidizing bacteria, or captured as elemental sulfur by photosynthetic organisms (Madigan and Martinko, 2006). The electron-shuttling capability of these lagoon sulfur species might be orders-of-magnitude greater than that of oxygen because sulfate and H_2S are much more soluble than oxygen.

3.1.8 Animal health impact of recycle

Waste removal practices affect both H_2S concentrations and herd health. For instance, dairies in water-scarce areas benefit from having recycled lagoon-stabilized wastewater available as a flush water, but despite this practice being comparatively common (EPA, 2001), there is little quantitative data establishing a pathogen baseline. In one of the more comprehensive lagoon water assessments a variety of quantitative pathogenicity tests was used; bacterial endotoxin (immunogenic residue), selective media to isolate, count, and individually identify colonies of coliform bacteria, Salmonella serotypes, pathogenic fungi, and yeasts (Purdy *et al.*, 2010). Purdy *et al.* (2010) isolated many different pathogens and then recommended against using lagoon water. However, their recommendation is difficult to evaluate because their study was an initial assessment so they could not compare the lagoon water pathogen levels with those found in dairies that are flushed less frequently. Furthermore, they did not incorporate the significant pathogen-reducing impact (typically 99% (Wright *et al.*, 2004)) of anaerobic digestion before lagoon storage.

3.2 Cattle feed & variables:

3.2.1 True protein (vs. crude protein) and estimation of the S-content of foods

The majority of sulfur found in cells is in protein, and more specifically in the methionine and cysteine amino acids. Sulfur is also found in energy harnessing metabolic pathways (e.g. in thioester bonds and iron-sulfur clusters), and specially modified macromolecules (e.g., proteoglycans and sulfolipids) but this quantity is comparatively small (Madigan and Martinko, 2006). As methionine and cysteine are two of the twenty-one most common amino acids, their occurrence frequency is reasonably consistent when large numbers of different proteins are considered, and it appears that this sulfur/protein correlation also works quite well for food sulfur/protein content (Figure 4). Thus, one way to estimate the amount of organic sulfur introduced into an anaerobic digester is to multiply the measured protein content by a known sulfur/protein factor. However, in order for this correlation to be useful it is important to have a good estimate of the protein quantity.

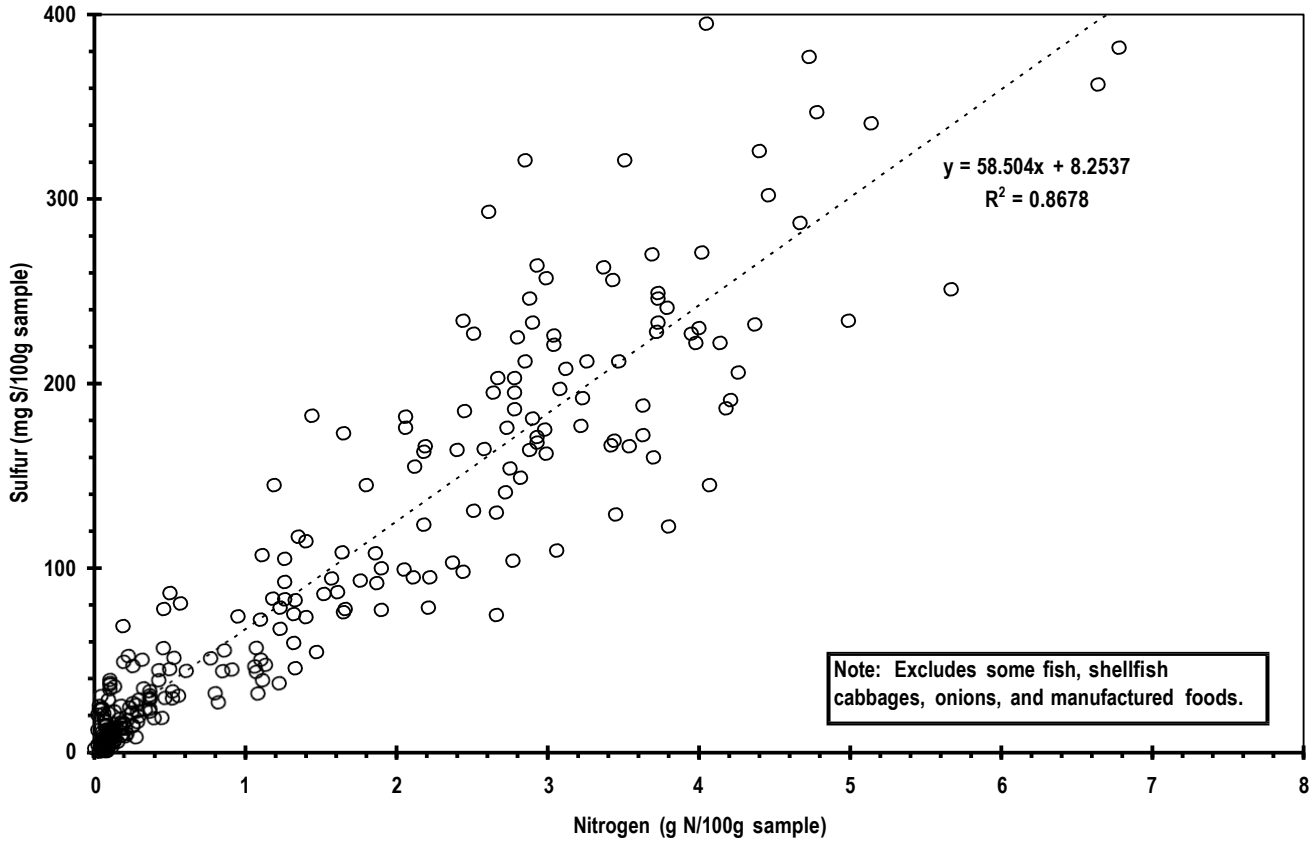

Figure 4. The relationship between sulfur and nitrogen in foods can be seen after combining two different food databases (CoF IDS, 2002; Masters and McCance, 1939).

Protein is a macromolecule made up of many, typically hundreds, of amino acid subunits. Each amino acid contains a nitrogen atom in their amino part, and amino acids such as asparagine, glutamine, lysine, and arginine also having nitrogen atoms in their side chains (Nelson and Cox, 2005). Just as there is a relationship between sulfur- and protein-mass, there is a consistent relationship for nitrogen and protein, such that the mass of protein is approximately 6.38 times the mass of the organic nitrogen in the protein molecule. A protein estimate based on the measured nitrogen mass, is called the *crude* protein, and is used in the dairy industry to describe the dairy feed, and characterize the waste products. The same nitrogen-containing characteristic of protein can be used to distinguish crude protein from the nitrogen-free carbohydrates and fat content of foods.

C*rude* protein estimates are used because it is difficult to measure the true protein in foods. *Crude* protein is only an approximation of the *true* protein. The crude protein test measures the amount of ammonia released when the organic nitrogen in a sample is converted to ammonia. The test assumes that any ammonia increase is due to the breakdown of amino acids that make up protein. The problem with using the *crude* protein test on manure is that urea nitrogen is also recorded as protein. Furthermore, the RNA and DNA within microbial cells also contain organic nitrogen that can be released as ammonia and recorded as protein. This last point is significant because approximately 25% of the dry mass of the typical microbial cell is RNA, DNA or nucleotides (Madigan and Martinko, 2006). Nucleotides have a nitrogen content about half that of protein; the average nucleotide molecular mass is 660 Daltons and contains 3.75 nitrogen atoms, i.e., has a molar- to N-mass of 660/(3.75×14) = 12.6, compared to protein's 6.38. Thus, as neither urea nor nucleotides contain sulfur, any estimate of the sulfur content of manure based on a crude protein measurement (barring other complications) is expected to be an overestimate, and even though the true protein content of a typical bacterial cell (dry weight) is approximately 55% (Madigan and Martinko, 2006), the overestimate may be substantial.

There are several good sources of dairy manure biogas data, but most do not include H_2S (Kramer, 2004; Mata-Alvarez *et al.*, 2000; Monou *et al.*, 2009). If an estimate of the maximum H_2S were needed it is tempting to use the phosphate composition difference of DNA and proteins, to provide a better estimate of the amount of true protein, and then use this more accurate protein measurement to calculate the sulfur content. Unfortunately, this approach was not feasible in this study because the urea amounts were not known, and phosphate concentrations were quite variable. The latter variability was probably because phosphate anions easily complex with dissolved metals such as magnesium and ferrous ions in the carbonate- and ammonia-rich anaerobic digestion conditions, and precipitate within the digester causing phosphate differences between the digester feed and effluent.

The sulfur content of manure can be difficult to estimate. Manure quality changes significantly before it enters the digester, and the most important change is its water content (Table 6). Hydraulic flushing of fresh manure from barns uses large volumes of water, and while the precise amount is sometimes uncertain (Ludington and Weeks, 2008), it is clearly substantial (Table 6). Technical definitions are important when describing different waste streams, for instance, "slurry" (Table 6) is more concentrated than either the fresh manure or lagoon surface water that was probably used as diluent (Columns 3 vs. 5 in Table 8). Table 7 also suggests that manure can be more concentrated in scraped facilities, although this might be merely a draining/drying process. Another anomaly in Table 7 is that while the scraping "concentration process" approximately doubles most of the manure constituents, the total solids and calcium content increased by factors of three and six respectively. Unfortunately, the original study did not report the sample sizes, so it is not possible to evaluate the significance of these anomalies.

Table 6. Variation of dairy manure production in liters/(Animal Unit per day) depending on consistency of removed manure (Chastain and Camberato, 2004).

	Total Solids Content (TS)							
Manure removed as:	1%	2%	4%	6%	8%	10%	12%	14%
Thick slurry - minimal water						65.4	**54.4**	46.7
Slurry + milking center waste				109.0	**81.6**			
Slurry + parlor Flush			**163.4**	109.0				
Alley flush		**326.8**	163.4					
All facilities flushed	**653.3**	326.8				**Bold designates typical**		

Milking center only	TS	L/AU-day
Twice-a-day (2x) milking	1.70%	28.9
Milking 3-times per day (3X)		43.3
2X milking with flush	0.60%	81.6
3X milking with flush		122.3
Manure and sand bedding	20-38%	45.3

Table 7. Comparison of the nutrient content of solid and semi-solid dairy manure from Barker's (1990) unpublished data from South Carolina farms (Chastain and Camberato, 2004).

Manure Type	Fresh Manure	Scraped Paved Outside Lot	Solids From Settling Basin	Solids From a Stationary Screen
Moisture	86%	67%	89%	80%
Total Solids	14%	33%	11%	20%
	Units below are g per liter			
NH_3-N	0.85	1.00	0.20	0.10
NO_3–N	0.01	0.05	---	---
Organic-N	4.14	6.5	2.9	2.35
Total-N	5	7.55	3.1	2.45
P as PO_4^{3-}	3.3	5.3	2.7	2.1
K	3.4	5.9	0.4	0.8
Ca	1.85	12.3	2.75	2.5
Mg	0.85	1.5	0.35	0.6
Zn	0.0215	0.05	0.095	0.03
Cu	0.005	0.015	0.07	0.05
Mn	0.022	0.04	0.035	0.02
S	0.6	1	0.55	0.4
Na	0.6	0.75	0.1	0.25

What is clear from these tables is that is that if lagoon surface water (Table 8) was used to dilute manure from 14%, down to 3.8% total solids, then a substantial amount of the sulfur in the fresh liquid manure must come from the lagoon or solids seapration proecess. Furthermore, because it is sometimes necessary to agitate and resuspend the lagoon settled solids, any process using lagoon-recycled water during this time would experience substantial sulfur concentration changes.

Table 8. Comparison of the nutrient content of slurry and liquid dairy manure from Barker's (1990) unpublished data from South Carolina farms (Chastain and Camberato, 2004).

Manure Type	Fresh Liquid Manure	Slurry	Milking Center Manure & Wastewater	Lagoon Surface Water	Lagoon Sludge	Agitated Lagoon Liquid & Sludge
Moisture	96.2%	93.0%	98.3%	99.4%	93.9%	97.0%
Total solids	3.8%	7.0%	1.7%	0.6%	6.1%	3.4%
	Units below are g per liter					
NH_3-N	0.66	1.13	0.75	0.38	0.74	0.56
Organic-N	0.78	1.63	0.46	0.22	1.05	0.64
Total-N	1.44	2.76	1.21	0.60	1.80	1.20
P as PO_4^{3-}	1.25	2.24	0.55	0.45	3.53	1.99
K	0.77	2.09	0.77	0.72	0.79	0.76
Ca	0.96	1.20	0.32	0.04	1.44	0.74
Mg	0.34	0.58	0.13	0.16	0.54	0.35
Zn	0.014	0.025	0.005	0.008	0.047	0.028
Cu	0.011	0.006	0.001	0.001	0.043	0.023
Mn	0.012	0.022	0.004	0.006	0.036	0.022
S	0.18	0.37	0.066	0.11	0.43	0.28
Na	0.29	0.38	0.16	0.22	0.17	0.19

3.2.2 Mineral supplements SO_4^{-2}-source

Apart from the sulfur present in most foods, mineral supplements are added to a dairy cows' diet to help maintain high milk production. Recommended intakes differ depending on the cow and production stage (Table 9), and different combinations of minerals are used to achieve nutritional targets. For example, a mix of magnesium sulfate and magnesium oxide can be used to meet the magnesium and sulfur requirements, although more "palatable alternatives" to magnesium sulfate (Epsom salts) are sometimes necessary to help maintain the cows' appetite (Subcommittee on Dairy Cattle Nutrition, 2001).

Mineral supplements are clearly a significant source of several other nutrients and microbiologically important trace metals, and these are important for three reasons. First, dairy cow nutrition aims to balance and optimize the rumen/intestinal biota, which has many similarities to the anaerobic digester biota. Second, most of the minerals are poorly absorbed by the cow, and finally, metals such as iron, copper and zinc, react strongly with H_2S to produce insoluble sulfides, so these metals may represent a significant sulfide sink.

3.2.3 High-S cattle foods

Most of the plant-based foods for dairy cows have phosphorus to sulfur ratios of about 1:0.6, however there are some notable feed-supplement exceptions where the sulfur content is substantially higher (Subcommittee on Dairy Cattle Nutrition, 2001), e.g. blood meal, feather meal, and whey (1:2.6, 1:2.78, and 1.11 respectively). It might be reasonable to expect that dairies using these supplements will have higher manure sulfur concentrations, but this might not be the case in practice because dairy farmers aim to provide a properly balanced diet, sufficient for the cows' milk production stage. Any high protein/sulfur supplement would need to be counterbalanced with the appropriate amount of lower protein/sulfur feed to maintain consistency. Dairy farmers also try to avoid sudden changes as these can upset the cows' digestion process or reduce their appetite.

Dairy cow nutritional stability requirements do not mean that the feedstock can be ruled-out as a source of digester sulfur variation. There are two situations where manure sulfur levels might still change quickly; the first being when a large number of cows joins or leaves the dairy, which changes the average production age and nutritional "weighting" of the herd. The second is when a feedstock is sent directly to drain, e.g. excess whey is discharged into the dairy treatment system.

Table 9. Mineral supplement composition (Subcommittee on Dairy Cattle Nutrition, 2001)

Dairy Cow	Holstein = 680 kg Body weight				Jersey = 454 kg Body weight					
Days in Milk	90	90	90	90	90	90	90	50	120	90
Mineral Element & Units										
Absorbable calcium (g/day)	52.1	65.0	76.5	88.0	50.7	65.2	72.4	65.2	65.2	65.2
Dietary Ca %	0.62	0.61	0.67	0.60	0.57	0.57	0.63	0.66	0.54	0.53
Absorbable phosphorus (g/day)	44.2	56.5	68.8	80.3	41.4	54.1	60.4	52.2	54.6	55.1
Dietary P %	0.32	0.35	0.36	0.38	0.33	0.37	0.36	0.44	0.35	0.34
Mg %	0.18	0.19	0.2	0.21	0.18	0.19	0.2	0.21	0.19	0.19
Cl %	0.24	0.26	0.28	0.29	0.24	0.26	0.27	0.28	0.25	0.25
K %	1.00	1.04	1.06	1.07	1.02	1.03	1.04	1.07	1.03	1.02
Na %	0.22	0.23	0.22	0.22	0.20	0.20	0.20	0.22	0.20	0.19
S %	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Co mg/kg	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11
Cu mg/kg	11	11	11	11	10	10	10	11	10	9
I mg/kg	0.6	0.5	0.44	0.4	0.44	0.4	0.34	0.4	0.36	0.35
Fe mg/kg	12.3	15	17	18	14	16	17	18	16	15
Mn mg/kg	14	14	13	13	12	12	12	13	12	12
Se mg/kg	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Zn mg/kg	43	48	52	55	45	49	51	54	48	47

3.3 Co-digestion substrates:

3.3.1 Protein content

While the correlation between nitrogen and protein may be problematic for dairy manure for the urea and microbial-nucleotide reasons discussed previously, there is a high correlation between sulfur (as a protein proxy) and nitrogen in foods (Figure 4). Published food-nitrogen data (CoF IDS, 2002) were combined with the reported values of sulfur in different food types (Masters and McCance, 1939). Shellfish, cabbages and onions were excluded from this correlation test (Figure 4) because they are known to contain significant quantities of non-protein sulfur and this would obscure the nitrogen/protein-sulfur relationship being investigated here. Dogfish was also excluded, because this fish type (elasmobranchs) is known to retain large amounts of urea.

Based on the combination of food data (CoF IDS, 2002; Masters and McCance, 1939), there are two ways to estimate the amount of sulfur added to a digester. The first converts the measured codigestion nitrogen directly into a sulfur amount using the equation shown in Figure 4, e.g. if a co-digestion substrate (food) contains 3.0g N/100g of sample, then it will probably also contain 180mg of sulfur. The second method would be used if the food nitrogen concentration is not known, i.e. look up the food protein content in a food database (CoF IDS, 2002) and multiply this by 0.0107 gSulfur/gProtein. For example, if a food contains 10g protein, this protein will contain approximately 107mg of sulfur. The 0.0107 Sulfur/Protein factor was found by comparing two data bases (CoF IDS, 2002; Masters and McCance, 1939). The correlation coefficient for protein:sulfur was the same as the correlation between nitrogen and sulfur (Figure 4) because food databases report *crude* protein estimates rather than true protein.

3.3.2 High S-substrates

It is difficult to compare the sulfur content of different foods to manure, because some indices use dry weight while others use food as prepared/served (CoF IDS, 2002; Masters and McCance, 1939). However, if an arbitrary manure sulfur content is chosen (e.g. 100mgS/100gWet Manure), it is possible to rank different foods (Table 10).

Table 10. Food types ranked by decreasing sulfur content (Masters and McCance, 1939) using an arbitrary manure reference of 100mgS/100gManure (Wet).

Food	Manure Ratio	Food	Manure Ratio	Food	Manure Ratio	Food	Manure Ratio
Duck, roast	4.0	Turkey, roast	2.3	Mutton chop	2.0	Smelts	1.7
Peanuts	3.8	Sole, Dover	2.3	Pork, leg	2.0	Beans, haricot, raw	1.7
Hare, roast	3.5	Ham, boiled	2.3	Sole, lemon	2.0	Lamb cutlet	1.7
Beef, topside, stewed	3.4	Chicken, roast	2.3	Salmon	1.9	Peas, split, dried, raw	1.7
Goose, roast	3.3	Cheddar	2.3	Veal	1.9	Egg yolk	1.6
Pheasant, baked	3.0	Stilton	2.3	Turbot	1.9	Mutton, leg	1.6
Brazil nuts	2.9	Dabs	2.3	Dutch cheese	1.9	Hake	1.6
Beef steak, stewed	2.9	Halibut, steamed	2.3	St Ivel cheese	1.9	Beef sausage, fried	1.6
Beef steak, fried	2.7	Haddock, fresh	2.3	Fish paste	1.9	Mackerel	1.6
Herring, fried	2.7	Kippers	2.3	Egg white	1.8	Cocoa	1.6
Liver, calves'	2.6	Sprats, smoked	2.2	All bran, Kellogg's	1.8	Oatmeal	1.6
Liver, ox	2.6	Beef, corned	2.2	Witch	1.8	Kidney, ox	1.5
Whiting	2.6	Haddock, smoked	2.2	Gorgonzola	1.8	Catfish	1.5
Cod, baked	2.6	Herring	2.1	Barcelonas	1.8	Ground ginger	1.5
Parmesan	2.5	Beef, topside	2.1	Heart, sheep's	1.8	Grapenuts	1.5
Fillet, smoked, boiled	2.5	Cod's roe	2.1	Herring's roe*	1.8	Almonds	1.5
Plaice, fried	2.5	Whitebait	2.1	Black sausage	1.7	Kidney, sheep's	1.4
Sardines, tinned in oil	2.5	Gruyère	2.1	Brill	1.7	Meat paste	1.3
Salmon, tinned	2.4	Plaice	2.0	Cod	1.7	Eel	1.3
Bloaters	2.3	Beef steak	2.0	Trout, rainbow	1.7	Peas, dried, raw	1.3

Note. Relative indication values only since moisture contents variable (Masters and McCance, 1939).

Seaweeds contain large amounts of soluble sulfate as well as other water-soluble salts. For instance the average sulfate content for a range of green, brown and red algae seaweeds (Kaliaperumal *et al.*, 1987) was 3.4 g SO_4^{2-} /100g (equivalent to 1.13gS/100g) dried seaweed, which is more than ten-times larger than the average food sulfur content (Figure 5).

3.3.3 Volatile-S substrates and effect of drying

Two food vegetable groups are known to contain volatile organic sulfur; the *Brassicaceae* family (cabbage, mustard and horseradish) contains allyl isothiocyanate, and the *Allium* family (onions and garlic) contains allicin and as these organics are volatile, their sulfur content depends on whether the plants have been crushed, heated, or aired. Volatile sulfur is different from amino acid- and protein-sulfur in that protein sulfur is not volatile at environmental temperatures. The volatile sulfur contribution is substantial for cabbage (16%), onions (30%), horseradish (29%) and mustard and cress (48%) (Figure 5). Thus, very different sulfur levels might be produced from two loads of codigested onions if one load is fresh, and the other has been dried, even if they are the same type of onion.

There are extensive lists of substrate characteristics, unfortunately most do not include sulfur (Labatut *et al.*, 2011; Mata-Alvarez *et al.*, 2000; Monou *et al.*, 2009). However, it may be possible to estimate the sulfur contribution of these different co-digestion substrates by converting the protein faction (via the approximate cysteine and methionine value), by using the known volatile nitrogen:sulfur proportions, or by compensating for the foods with a higher volatile sulfur content (Masters and McCance, 1939).

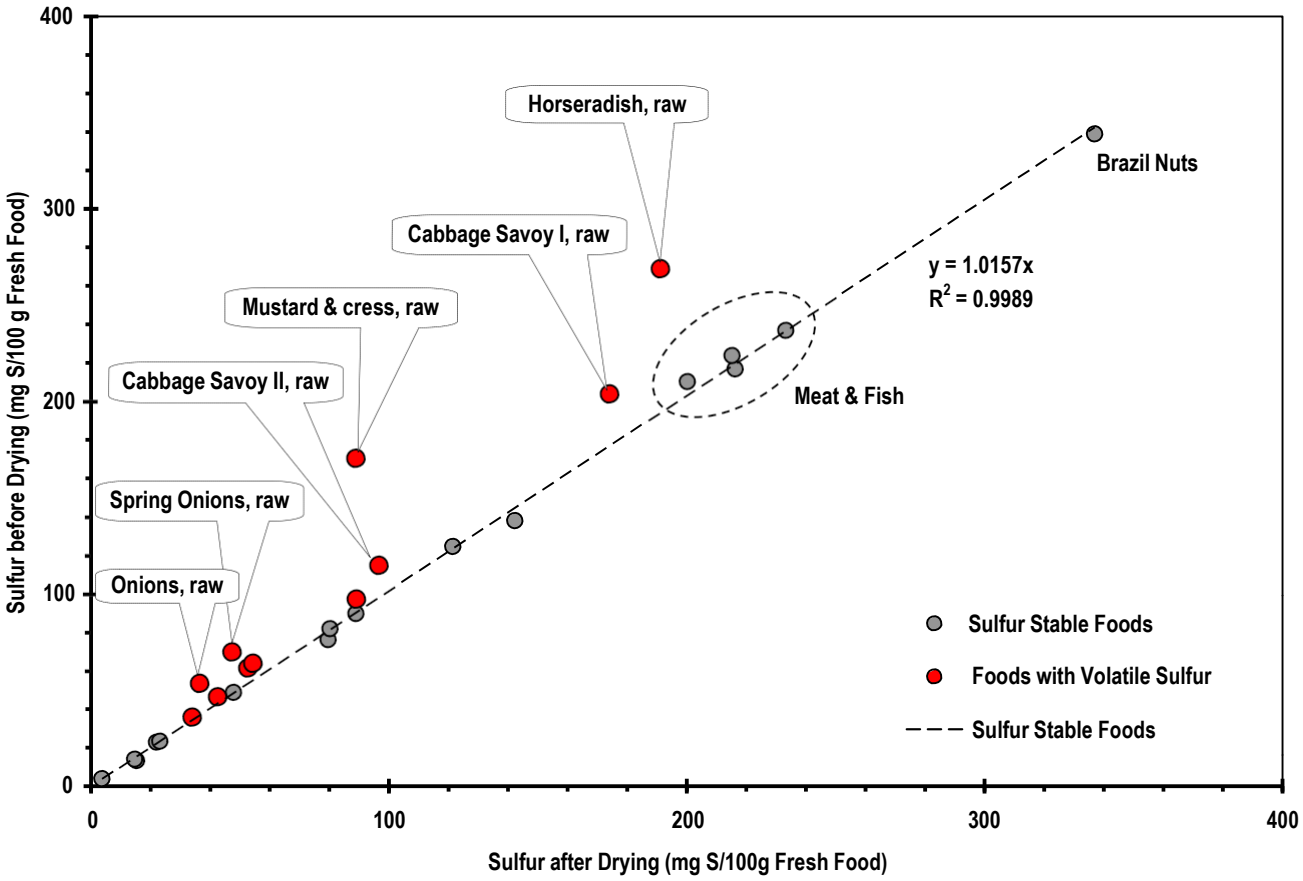

Figure 5. Drying effect highlighting the presence of volatile sulfur in different foods(Masters and McCance, 1939).

Although extensive lists of co-digestion substrate characteristics are found in review articles and in individual studies, these data need to be interpreted with caution because of the differences in reference units. Review

articles appear to be the most useful in this respect because a standard reference unit is used. For instance, in some cases biogas yield improvements have been used as the common reference unit, for a wide range of cow manure and co-digestion waste combinations, along with the impact of many different pretreatment methods (Esposito *et al.*, 2012; Khalid *et al.*, 2011).

3.3.4 "S-Purging" substrates, e.g. fats and oils

Some foods such as fats, oils, and carbohydrates, have low sulfur content, but high biogas potential. When these types of substrates are digested, the resulting increased biogas volumes help dilute any sulfide present in the biogas and digester. Increased biogas volumes also effectively purge out dissolved sulfides.

3.4 Other SO_4^{2-}/H_2S sources:

3.4.1 Copper sulfate footbaths

Dairy cows can develop a lameness called foot rot, caused by a bacterial infection (*Fusobacetrium necrophorum*) of the hoof. The condition is infectious and can become severe if not treated early. Preventative measures include; maintaining well-drained standing surfaces, trimming the claws, and walking the cows through footbaths. Footbath solutions contain copper- and zinc sulfate, and in some cases formalin. When the footbath solution becomes heavily contaminated (or too dilute), the bath is emptied and if necessary, a new solution is made. However, as the nearest drain point will be the same as that for manure collection, it is reasonable to expect the spent solutions will end up in the anaerobic digester.

3.4.2 Minerals, e.g. gypsum

Apart from cattle-feed supplements, fertilizer and soil amendment components discussed in Section 3.1.6, sulfate may also be present on a dairy farm as solid calcium sulfate (gypsum). Gypsum is the main constituent of wallboard, and is a minor constituent (<5%) of Portland cement. However, it is doubtful that these sources are present in sufficient quantities to maintain a consistent supply of sulfate to the digester feed, because if this were happening it would represent an obvious corrosion problem for the donor structures.

3.4.3 Acid Mine Drainage

Active and inactive mines can be a significant source of sulfate in the form of acid mine drainage. Acid mine drainage is produced by *Thiobacillus* spp. (an autotrophic bacterium), which convert metal sulfides into sulfates in the presence of oxygen. Acid mine drainage water has a low pH and usually contains large quantities of dissolved metals which precipitate when the water pH is raised, giving rise to obvious surface water contaminants such as "yellow boy". Water contaminated with acid mine drainage normally also contains high concentrations of metals such as iron, copper, nickel, and zinc, making it unsuitable for dairy use.

4 S-Conversion Processes

4.1 The Sulfur Cycle:

Sulfur is recycled through the environment by a variety of processes and organisms. Almost all aspects of the sulfur cycle (Table 11) are relevant for the dairy digester and its associated locations such as the lagoon, and cow rumen.

4.1.1 H_2S from protein by fermenting organisms

The desulfurylation reaction (Table 11) occurs when amino acids such as cysteine and methionine decompose, or when dimethyl sulfoxide is converted to dimethylsulfide. The last compound is produced primarily in marine environments as a degradation product of the marine algae osmoregulatory solute dimethylsulfoniopropionate (Madigan and Martinko, 2006), but if seaweed or marine algae is part of the codigestion substrates, similar sulfur releases would be expected.

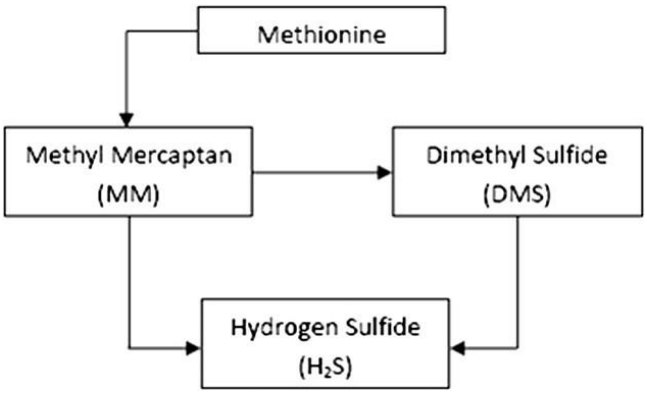

Figure 6. Conceptual model for the generation of volatile sulfur compounds from methionine (Du and Parker, 2012).

Table 11. Stoichiometry, representative organisms, and Gibbs free energies of some sulfur-cycle reactions.

Function	Reaction	Organism & location	ΔG* J/rxn
Desulfurylation	Organic-S → H_2S	Catabolic reaction; many organisms can do this. Lagoon or digester.	-
Sulfate reduction	SO_4^{2-} → H_2S	*Desulfovibrio*, *Desulfobacter*. Anoxic conditions. Lagoon or digester.	-
Sulfur reduction	$S^0 + H_2 \rightarrow HS^- + H^+$	*Desulfuromonas.*	-28.0
Sulfur oxidation	$S^0 + 4H_2O \rightarrow SO_4^{2-} + 3H_2 + 2H^+$	Lagoon or digester.	+124.3
Sulfide oxidation	$HS^- + H^+ \rightarrow S^0 + H_2$	Some chemolithotrophs in digester. Spontaneous in oxygen at pH 7. Purple and green phototrophs in lagoon	+28.8
Sulfide oxidation	$HS^- + 4H_2O \rightarrow SO_4^2 + 4H_2 + H^+$	Sulfur chemolithotrophs (Thiobacillus & Beggiatoa).	+152.2
Sulfate assimilation	SO_4^{2-} → Organic-S	Anabolic reaction; Many organisms can do this. Lagoon or digester.	-
Sulfate reduction	$SO_4^{2-} + 3H_2 + 2H^+ \rightarrow S^0 + 4H_2O$	Lagoon or digester.	-124.3
Sulfate reduction	$SO_4^2 + 4H_2 + H^+ \rightarrow HS^- + 4H_2O$	*Desulfovibrio*, *Desulfobacter.*	-151.9

*Standard conditions at pH 7. (Madigan and Martinko, 2006; Thauer *et al.*, 1977).

Anaerobic degradation pathways also include sulfates and nitrates (Cirne *et al.*, 2008), while others include amino acid conversions and the formation of methanethiol, and dimethyl sulfide (Drennan and DiStefano, 2010). The amino acid deamination and decarboxylation breakdown products have been identified, as well as the excretion pathways associated with the different products (Mackie *et al.*, 1998). However, detailed information on these mercaptan intermediates will probably not be necessary in this review because while it is possible to use the degradation kinetic parameters of methionine, and inhibitory interactions between its process intermediates (Du and Parker, 2012) to determine the different species concentrations, H_2S represents the bulk of the volatile sulfur.

Analysis of dairy digester biogas showed that when the H_2S was present at 0.36 percent, the dimethyl sulfide concentration was less than 0.01% (dry basis) (Zicari, 2003). Furthermore, an increased odor potential and release of volatile sulfur compounds (H_2S, methanethiol, and dimethyl sulfide) is associated with *incomplete* substrate stabilization, e.g. food and landscape wastes (Drennan and DiStefano, 2010). Thus, volatile sulfur intermediates such as mercaptan should be relatively insignificant in a stably operating digester.

4.1.2 H_2S from SO_4^{-2} (and S^0) by Sulfate Reducing Bacteria

Sulfate reducing bacteria (SRB) play a vital role in the anaerobic breakdown of organic substrates whenever sulfate is present because they can consume hydrogen. Hydrogen concentration (partial pressure) is important because it determines the type of volatile fatty acids produced during the breakdown of sugars and higher fatty acids. When sulfate is present, SRB are able to capture more energy from hydrogen consumption compared to the methanogens and as a result SRB can potentially outcompete methanogens (Thauer *et al.*, 1977). SRB outcompete methanogens by lowering the hydrogen concentration and producing more SRB biomass, which further exacerbates the methanogen/SRB imbalance.

4.1.3 Incorporation into biomass by anaerobic organisms

New cells are produced when substrates are consumed. Approximately 55% of the dry mass of bacterial cells is protein, which contains the sulfur amino acids (cysteine and methionine) (Madigan and Martinko, 2006). It is thus possible to estimate sulfur and other materials consumed (Table 16) when particular substrates are degraded (Table 12).

Table 12. Theoretical and observed biological parameters.

Substrate	"Metabolism" (based on electron acceptor)	Theoretical Maximum Growth Rate per day (McCarty, 1971)	Observed Maximum Growth Rate per day (Pavlostathis and Giraldo-Gomez, 1991b)	Theoretical Cell Yields per Mol equivalents (McCarty, 1975)
Hydrogen	Methanogen	0.5	0.05 – 4.07	0.042
Acetate	Methanogen	0.27	0.08 – 0.357	0.045
Carbohydrate	Methanogen*			0.277
Protein	Methanogen*			0.116
Lipid	Methanogen*			0.045
Hydrogen	SRB			0.049
Acetate	SRB	1.0		0.079
Carbohydrate	SRB			0.308
Protein	SRB			0.150
Lipid	SRB			0.078

*Methanogens can only use simple substrates. This table shows "net" metabolisms for a consortium of microoganisms.

Biomass yields depend on digester feed composition and the efficiency of the digester system to convert the feed to new biomass. At a more fundamental level, the substrate (electron donor), electron acceptor, and efficiency of energy conversion will determine the quantity of new biomass produced (McCarty, 1975). However, the measurement of anaerobic organism kinetic/growth parameters, e.g. maximum growth rate, is problematic

because the energy yields are low (Thauer *et al.*, 1977). This slow growth and low biomass yield imply that reported parameters are highly variable (Pavlostathis and Giraldo-Gomez, 1991b). Under these circumstances, it seems reasonable to use theoretical biological parameters based on thermodynamic principles, rather than experimental values (Table 12). A theoretical approach also seems justified when the theoretical yield is based on mass-balance principles, and the controlling efficiencies are approximately consistent for many different organisms (McCarty, 1975). Furthermore, even though methanogens can only degrade simple molecules and not sugars, the use of thermodynamics-based theoretical yields appears to be a better choice because it automatically includes biomass yields for the other cells in the sugar to methane degradation series.

4.1.4 H_2S consumption by Sulfur Oxidizing Bacteria

The same approach (as described in the previous paragraph) can be used to estimate the amount of cell yield from cells using H_2S as an electron donor and oxygen as the acceptor; which yields 0.212 cell equivalents per mole of substrate. H_2S is not listed as a substrate in Table 12, but it is clear from the high (0.212) cell yield that this reaction will be quite favorable if oxygen is present. Apart from cell mass, under certain conditions the sulfur oxidizing bacteria also produce large quantities of elemental sulfur, which can accumulate on the digester roof in the form of yellow sulfur stalactites.

4.1.5 S-Disproportionation reactions

McCarty (1975) did not list sulfur-disproportionation reactions, but if a detailed mass balance analysis is required, disproportionation reactions might be treated as fermentation reactions because the poly-sulfur elemental form (S^0) splits into more oxidized (SO_4^{2-}) and more reduced (HS^-) subunits. There are fermentation yield calculation methods (Rittman and McCarty, 2001), but these will only be needed if further investigations need to examine the sulfur conversions of the reactor headspace.

4.1.6 Digester vs. lagoon processes

Most of the sulfur-cycle reactions that take place within the digester fluid will be anaerobic. However, as it is now common practice to inject small quantities of air into the digester headspace to allow sulfur oxidizing bacteria to oxidize the H_2S (Deublein and Steinhauser, 2011), there may be some sulfur oxidation within the digester headspace. There are anecdotal reports of large elemental sulfur accumulations as stalactites on the inner surfaces of the digester roof, which in turn raises the possibility of disproportionation reactions (Section 4.1.5). Any sulfates in the digester will be quickly converted to H_2S due to the more favorable energetics of this reaction compared to methanogenesis (Thauer *et al.*, 1977). Similar sulfur-cycle organisms might be expected to flourish in both the digester and lagoon. The only exception being the photosynthetic sulfur organisms, which will have sunlight in the lagoon.

4.2 Other H_2S-Sinks:

4.2.1 Loss with treated liquor

Treated/stabilized dairy wastewater will contain sulfur in at least three forms; dissolved H_2S, insoluble metal sulfides, and organic sulfur in the form of microbial protein. As the treated effluent is generally stored in the lagoon (EPA, 2001) there is an opportunity for the metal sulfides and biomass to settle out and it appears that large amounts of sulfide do accumulate in the lagoon sediment (Table 8). When the storage lagoon is emptied (usually annually), the sulfide rich sediment needs to be thoroughly agitated in order to recover the maximum lagoon storage volume. Alternatively, the lagoon can be drained completely and the sediment can be scraped out and trucked away. The impact of this agitation can be seen in differences between the surface water, sludge, and agitated lagoon sulfur concentrations in Table 8.

4.2.2 Precipitation as insoluble metal sulfides

Table 8 shows that a substantial proportion of the circulating sulfur is prevented from recycling to the digester by settling in the storage lagoon, before being agitated back into suspension and pumped away. This situation is somewhat similar to that of domestic wastewater sludge treatment where the bulk of the sulfur is insoluble sulfides that can be trapped in thickener filtrate before digestion occurs (Dewil *et al.*, 2009). However, some caution is appropriate when interpreting mass balances of process studies because it appears that even small disturbances such as temperature fluctuations, may cause a change in the range of sulfur products produced (Iranpour *et al.*, 2005).

Several of the dairy relevant metals are listed in Table 13. Copper is particularly important because of its strong sulfide binding, and its use as copper sulfate to control footrot in dairy cattle. As was noted earlier, any sulfate entering the anaerobic digester would quickly be converted to sulfide by sulfate reducing bacteria. Based on thermodynamics, the low solubility products of CuS and Cu_2S suggest that soluble copper, will be amongst the first metals to form insoluble sulfides, independent of any kinetic or physical process data.

Table 13. Solubility constants for selected metal sulfides (at 25°C and zero ionic strength, I = 0).

Metal Sulfide	Name/Form	Log *Ks	Reference
MnS	Green	0.17	1
	Pink	3.34	1
FeS		-4.16	1
	Troilite	-5.25	2
	Mackinawite	-3.6	2
	Amorphous	-2.95	2
	Pyrrhorite	-5.1	2
FeS_2	Pyrite	-16.4	1
CoS	α	-7.44	1
	β	-11.07	1
NiS	α	-5.6	1
	β	-11.1	1
	γ	-12.8	1
CuS		-22.30	1
ZnS	(α, Sphaelerite, cubic)	-10.93	1
	(β, Wurtzite, hexagonal)	-8.95	1
Cu_2S		-34.65	1

1 = (Dyrssen and Kremling, 1990) and 2 = (Davison, 1991)

Where: $MetalS_{(s)} + H^+ = Metal^{2+} + HS^-$ $\quad *K_s = K_{s0}K_2^{-1}$

$Metal_2S_{(s)} + H^+ = 2Metal^+ + HS^-$ $\quad *K_s = K_{s0}K_2^{-1}$

$K_2 = 10^{-13.9}$ (Stumm and Morgan, 1996)

$pK_a = -\log(K_a)$ by definition

5 Variables affecting H_2S gas transfer to digester headspace

5.1 Biogas flow rate, H_2S purging effect, and factors affecting digester biogas flow:

5.1.1 Dissociation, pH and pK_a

H_2S can have three different ionic forms in aqueous solution depending on the pH; neutral H_2S, mono-anionic HS^{-1}, and di-ionic S^{-2} (Figure 7). Neutral H_2S is the dominant form below the first acidity constant and can volatilize from solution into the headspace of the digester. There is consensus on the first acidity constant value, typically around 1.07×10^{-7} (a pK_{a1} of 6.97) (Dean, 1992) to 9.632×10^{-8} (a pK_{a1} of 7.02) (MINTEQA2, 1998; Sun *et al.*, 2008), but less consensus about the second. The second acidity constant average value is typically reported as 1.26×10^{-13} (Dean, 1992), which is one of the pK_{a2} (12.9) shown in Figure 7. However, aquatic chemists generally recommend reformulating equilibrium expressions to avoid using the second acidity constant (Stumm and Morgan, 1996) because there is a 7-order-of-magnitude variation in the reported pK_{a2} values, ranging from 1.000×10^{-12} to 1.000×10^{-19} (Sun *et al.*, 2008). The influence of this pK_{a2} difference is substantial at higher pH and thus important for some chemical speciation or precipitation modeling investigations. MINTEQA2 database uses 5.01×10^{-18} (MINTEQA2, 1998), but as shown in Figure 7, a pK_{a2} of 17.3 is irrelevant for the dairy manure digestion 6.5 - 8.5 pH range.

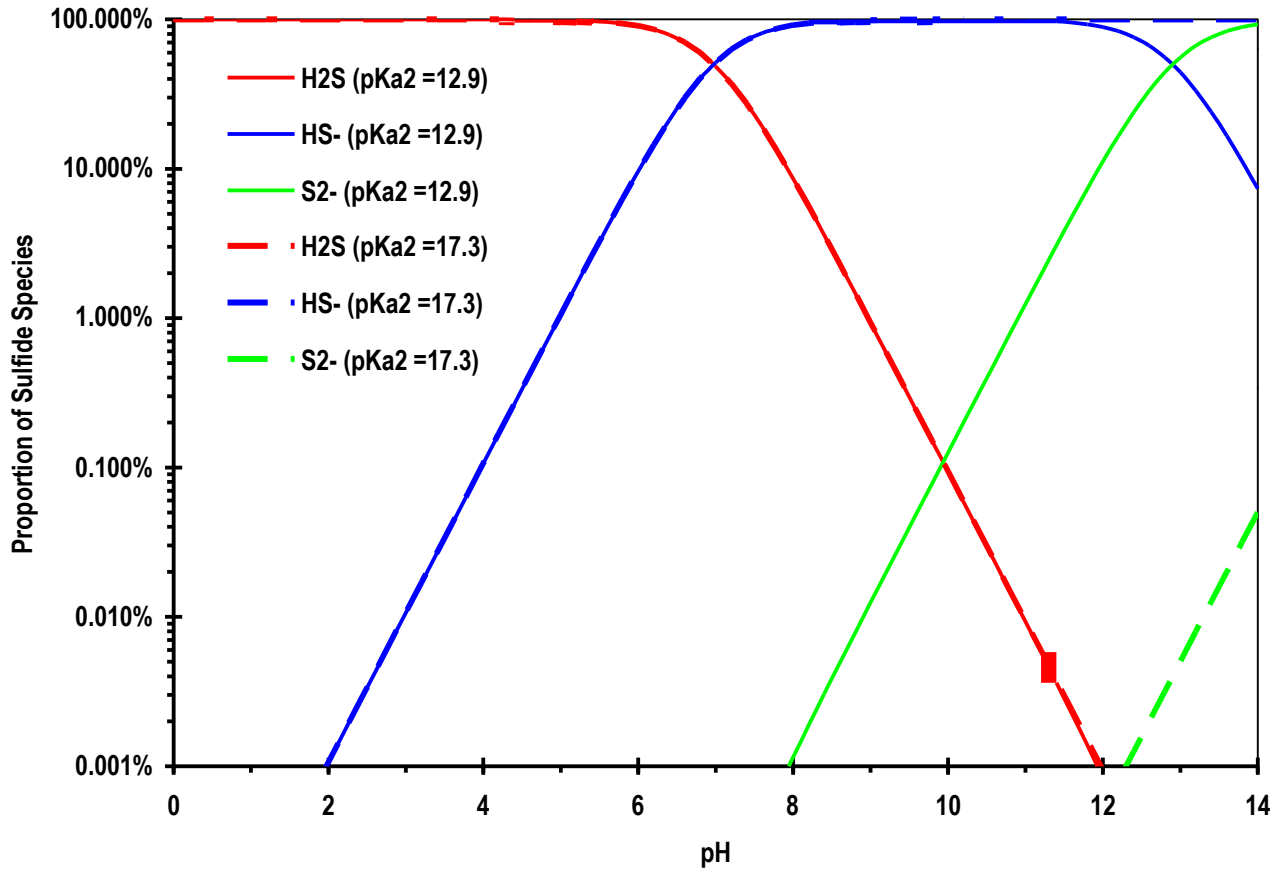

Figure 7. Proportion of different H_2S species at different pH. Note: log proportion axis.

The log scale in Figure 7 shows that the H_2S proportion changes very quickly at pH 7.8 of a typical dairy digester. For more quantitative determinations, the standard acid/base equilibrium formula can be used to determine the proportion of different H_2S species present at each pH. For instance the proportion of undissociated (and therefore volatile) H_2S increases by 158% as the pH drops from 7.8 to 7.6, but H_2S decreases by 37% if the pH rises

from 7.8 to 8.0. These calculations show that even small pH decreases have a dramatic impact on H_2S gas transfer. The formula for undissociated H_2S is:

Proportion of (volatile)	H_2S	=	$1/(1 + K_{a1}/[H^+] + K_{a1} \times K_{a2}/[H^+]^2)$
Where:	K_{a1}	=	First ionization coefficient = 1.45×10^{-7} @ 35°C
	K_{a2}	=	Second ionization coefficient = 5×10^{-18} @ 35°C
	$[H^+]$	=	Proton concentration, i.e. $[H^+] = 10^{(-pH)}$

As suggested above, temperature compensations are important, but for accurate results activity effects must also be included (Smith and Chen, 2006).

5.1.2 Henry Coefficient

The ratio of H_2S concentration in the digester liquid, compared to the concentration (partial pressure) found in the digester headspace is described by the Henry Coefficient. H_2S has a Henry Coefficient similar to that of carbon dioxide (Smith and Stöckle, 2010). It is the ability to dissociate in solution that makes gases such as carbon dioxide, H_2S, and ammonia especially soluble. The Henry coefficient describes the balance between the gas over the solution, and the dissolved gas, so if the dissolved gas dissociates to form other species, more gas must enter the solution to restore the balance.

5.1.3 Role of temperature

Temperature affects dissolved H_2S concentrations because it changes the digester biological, physical and chemical conditions. The most important impact is biological through changing the growth rate of micororganisms. The approximate rule is that growth rates double for each 10°C rise, and mesophilic organism growth rates do not change much between 35 and 40°C (Rittman and McCarty, 2001). Temperature affects several crucial parameters in different ways (Rittman and McCarty, 2001), but a simplistic interpretation of the acidogenic and methanogenic relative growth rates will suffice to illustrate the role of temperature.

If the acidogen and methanogen relative growth rates are 5:1 respectively (Pavlostathis and Giraldo-Gomez, 1991a), and the other biokinetic parameters are identical, there would need to be five times as many methanogens as acidogens to provide balanced processing. However, as the cow rumen selects for acidogens and against methanogens, manure is expected to contain an excess of acidogens. Thus, if there is an imbalance at 25°C that produces an excess of one unit acid per unit time, when the temperature of the manure is raised to 35°C there will be an excess acid production of two units acid per unit time. If this increased acid production overwhelms the buffering capacity the pH will drop and the methanogen growth rate will fall, further exacerbating the instability. While it is oversimplified, this example illustrates why it is important to provide supplemental methanogens (recirculated biomass) and ample buffering to the point where new substrate enters the digester.

Temperature increases cause water viscosity and gas solubilties to decrease, in contrast to diffusion rates and mineral solubilities, which increase. The overall effect of these physical changes on digester microorganisms may be complex, but will depend on which of these phenomena is currently rate limiting.

Temperature also influences chemical conditions not just through the ionization coefficients but also the Henry coefficient of H_2S. Temperature's impact on dairy digesters, is through influencing the solubility of gases and the ionization coefficient of ammonia which changes from a pK_a of 9.25 @ 25°C to 8.95 @ 35°C (Smith and Chen, 2006). A 35°C temperature was used here as representative of the mesophilic range of 20 to 40°C (68 to 104°F) (Batstone *et al.*, 2002a). This apparently small change must be considered alongside the dramatic changes of H_2S solubility with a small change in pH, as well as the fact that ammonia is present in high concentrations in dairy digesters.

Anaerobic digesters are designed to operate at a fixed temperature, but there are several reasons why the temperature control may be less than ideal in practice: Substrate is pumped into the digester at temperatures

well below the operational target, heat transfer to high-solids digester liquor is problematic because solids can foul the heat-exchanger surfaces, and the heat exchanger may contain relatively low-temperature circulating fluid. Thus, the digester temperature cannot be assumed constant.

5.1.4 Theoretical H_2S prediction

When the principles of aquatic chemistry and standard gas transfer laws are combined, it is possible to investigate how the biogas H_2S concentration changes with changes in the biogas volume to feed volume ratio (McCarty, 1964). The relationship between biogas/feed volume and H_2S concentration in the biogas for typical dairy digester conditions is shown in Figure 8. Two main conclusions can be drawn from this family of curves. First, H_2S gas concentrations change more quickly in low pH digesters. Second, H_2S increases with decrease in biogas/feed volume; particularly at low biogas/feed volume ratios. Actual biogas/feed volumes for dairy digester will be more than ten, but the gas production process will be spread out over the complete retention time.

The biogas/feed curves are particularly important for plug-flow digesters because these digesters have regions of high gas output and other regions of lower gas output, meaning that the H_2S concentration will be different in the biogas across the length of the digester. The impact of these differences along the length of the plug-flow may be exacerbated or eliminated depending on the digestion conditions, because the H_2S concentrations will be largest near the start of the plug-flow digester. In a well-balanced digester there is little pH depression at the influent entry point where the new (and thoroughly preheated) feed enters. In the opposite of the ideal situation, a slightly overloaded plug-flow digester will have a pH drop near the feed entry point, and this situation will be worse if the feed is not warm enough for the methanogens to be fully active. If the digester feeding were intermittent, the position of the biogas takeoff point might also play a role in determining whether a H_2S pulse is diluted before leaving the digester and entering a combustion process.

5.1.5 Dairy digester configurations

The two main types of suspended growth digester configurations used in the dairy industry are plug-flow, and completely mixed digesters. Plug-flow digesters are effectively long pipes, with influent entering at one end, and treated effluent leaving at the other. In contrast, completely mixed digesters are large tanks in which the influent is mixed throughout the digester contents. There are advantages and disadvantages to both designs. For instance, the advantage of plug-flow digesters is that they have higher efficiencies and reaction rates near the substrate entry point. This same characteristic might be a disadvantage for anaerobic digesters plug-flow digesters because plug flow reactors are generally more susceptible to overload and toxins than completely mixed digesters (Rittman and McCarty, 2001). This is particularly relevant for H_2S transfer because high reaction rates might overwhelm the digester alkalinity and cause a pH drop, which would increase H_2S release.

Completely mixed digesters achieve effective mixing throughout the digester liquor. Any changes in feed concentration, composition and temperature are moderated more effectively in a completely mixed digester than the equivalent plug flow digester. This means there will be less chance of a process upset and that gas output and composition should be more consistent. In the case of a completely mixed reactor with a hydraulic retention time of 20 days, each day's feed only represents 5% of the tank volume.

There is another significant configuration difference between dairy digesters and those of domestic wastewater treatment. Domestic wastewater treatment plants are required to have duplication of equipment and parallel processing ability (redundancy), whereas dairy treatment facilities sometimes have a single large digester (Coats, 2012). This issue is important for older digesters that have reduced digester volume due to accumulated solids, because these are more prone to overloading, and once this happens there will be no alternative processing capability to process the soured liquor. Redundancy also allows digesters to be taken off-line so they can be properly drained and maintained.

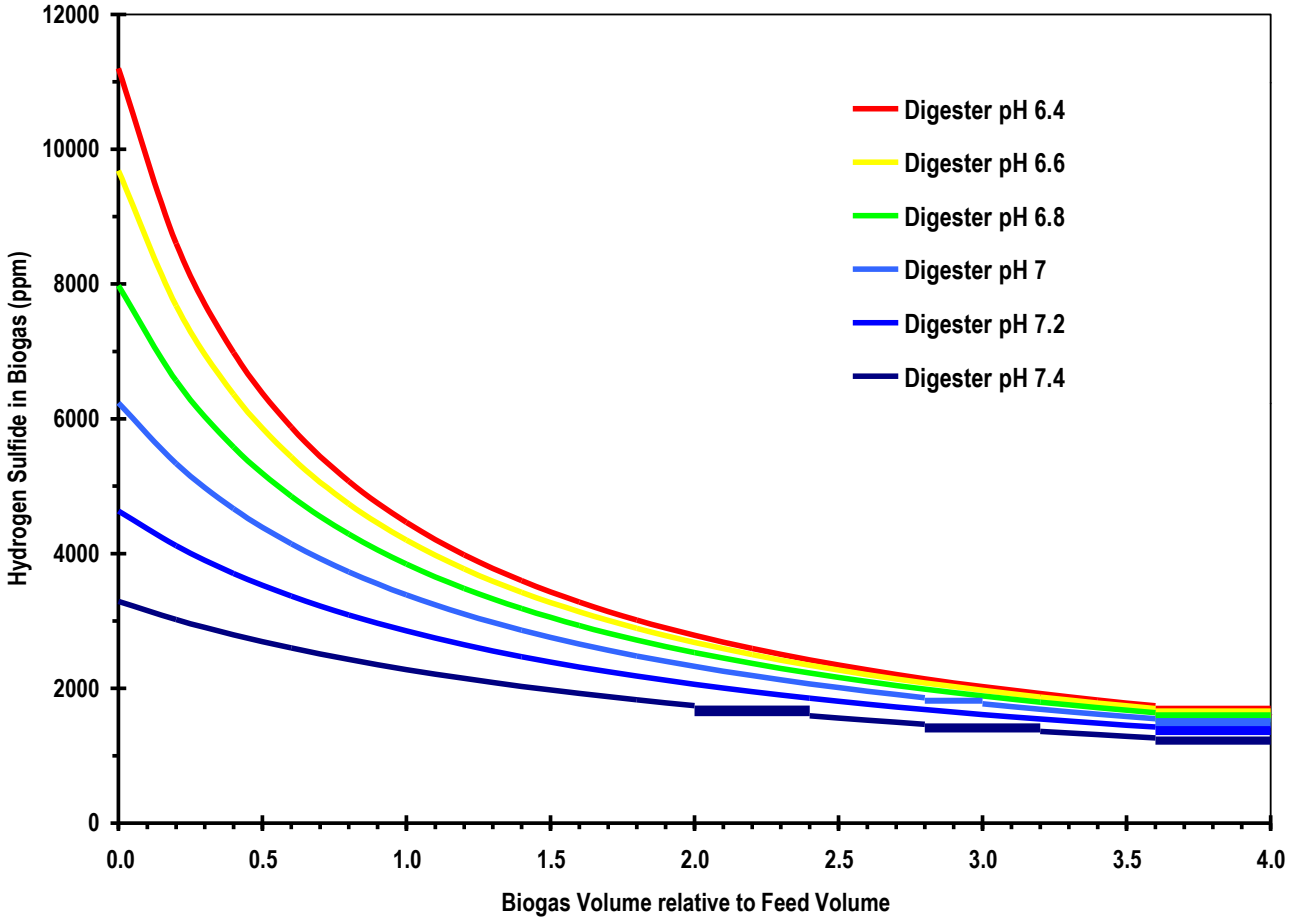

Figure 8. Predicted change in biogas H_2S concentration based on digester pH and biogas flow rate.

It is possible to combine the principles of aquatic chemistry, gas transfer, microbiological responses to changing temperatures, and the physical arrangement of different digester configurations, to investigate how these different phenomena might interact in practice. Such a model might be used to develop and test appropriate best management practices. This is however, beyond the scope of the current study.

5.1.6 Digestion consortium & implications of imbalance

A wide range of organisms participates in the complete stabilization of complex wastes under anaerobic conditions. As little energy is released through anaerobic substrate conversions (Thauer *et al.*, 1977), the organisms involved tend to be specialized, and appear to have little metabolic flexibility. The initial steps require physical changes, and the complex wastes must be disintegrated, and then hydrolyzed (dissolved) through the action of enzymes. Long polymers, such as proteins, starches, and long-chain fatty acids, are broken into individual subunits, which must be further processed before acetate and hydrogen become available for consumption by the methanogens (Figure 9). While the network of reactions outlined in Figure 9 (Angelidaki *et al.*, 1999) may initially appear complex, it is by necessity a simplification that best describes the balanced operation of a network of organisms, and omits some of the important reactions which occur during unbalanced operation (Smith, 2009).

At the molecular level, the anaerobic digestion process can be described and visualized as a production line, with organisms at each step processing a steady stream of substrates. The production line concept is useful because it accurately captures what happens when parts of the process are interrupted. During normal operation, there is a steady flow of substrates and products through this network, but if the process is interrupted, the substrates arriving at the bottleneck, accumulate quickly, and can in turn cause further upsets. For instance, when volatile

fatty acids accumulate, the pH can drop quickly and further compromise the performance of critical microorganisms. If these conditions persist, the acid tolerant fermenters will continue to produce organic acids and the rest of the process remains "stuck". Furthermore, the low pH will favor the release of H_2S into the biogas (Figure 7).

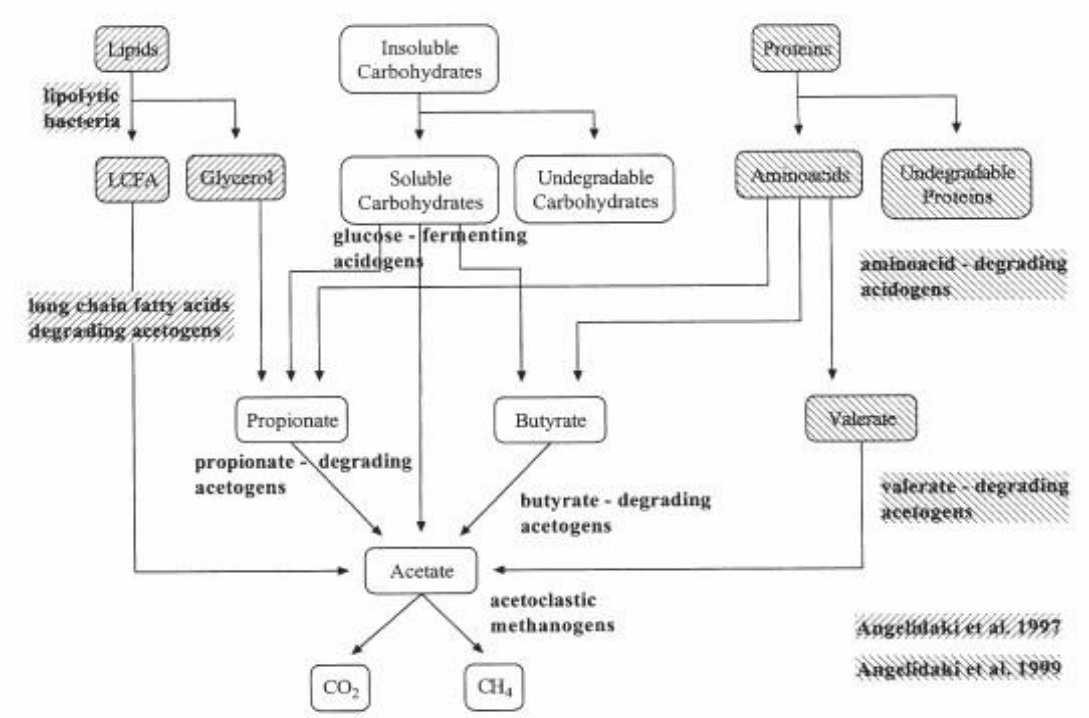

Figure 9. Representative reactions during anaerobic digestion of substrates (Angelidaki *et al.*, 1999).

A second type of metabolic disruption can be understood in terms of the reactions described in Table 14, which shows the reaction stoichiometry and Gibbs free energy available under "normal" wastewater conditions. Using monosaccharide acidogenesis (third line**,** Table 14) as an example, both reactions have similar Gibbs free energies, but the butyrate reaction produces two-, rather than the acetate reaction four-hydrogen molecules. If both reactions convert the same amount of glucose in a fixed time, the acetate producer will produce more hydrogen. If this hydrogen waste gas accumulates, it will eliminate the thermodynamic impetus, slowing and ultimately stopping the forward reaction. During these initial stages, the butyrate producing reaction will continue, and the ratio of butyrate to acetate volatile fatty acids will change. If there are not enough butyrate-consuming organisms nearby, butyrate will accumulate, the pH will drop and more H_2S will be released (Figure 7).

Process imbalances involving hydrogen gas or acetate accumulation have at least three more consequences significant to the release of H_2S from dairy wastes. The first is that sulfate reducing organisms can compete more effectively for hydrogen than the methanogens (Thauer *et al.*, 1977), so if sulfate is present the sulfate reducing bacteria will proliferate. The second consequence is that sulfate reducing organisms produce sulfide which can inhibit the activity of both methanogens *and* sulfate reducing organisms (O'Flaherty *et al.*, 1999); resulting in unstable, oscillating systems (Vavilin *et al.*, 1994). The third consequence is when sulfide reacts with trace metals to produce stable precipitates, this reduces microorganism growth rates especially in digesters where trace metal was already growth limiting.

Table 14. Representative reactions and organisms present in an anaerobic dairy digester.

Function	Reaction	Organism/Reaction Type	Ref. *	ΔG** kJ/rxn
Hydrolysis	$Substrate_{(s)} \rightarrow Substrate_{(aq)}$ e.g. Starch → Glucose	Celluloytic and other hydrolytic bacteria, e.g. *Clostridium* spp., *Fibrobacter succinogenes* and *Ruminococcus albus*.	1	-
Monosaccharide acidogenesis	$Glucose + 2H_2O \rightarrow Bu^- + 2HCO_3^- + 2H_2 + 3H^+$	Fermentative bacteria , e.g. *Clostridium* spp.	1 & 3	-319
	$Glucose + 4H_2O \rightarrow 2Ac^- + 2HCO_3^- + 4H_2 + 4H^+$	Fermentative bacteria		-284
Amino acid acidogenesis	$AA + H_2O \rightarrow VFA + HCO_3^- + H_2 + NH_4^+$	Fermentative bacteria, e.g. *Clostridium* spp.	1	-
Long chain fatty acid acetogenesis	$Cap^- + 4H_2O \rightarrow 3Ac^- + 4H_2 + 2H^+$	Secondary fermenters, e.g. *Syntrophonomas wolfei*	1	-
Valerate acetogenesis	$Val^- + 2H_2O \rightarrow Pr^- + Ac^- + 2H_2 + H^+$	Secondary fermenters, e.g. *Syntrophonomas wolfei*	1 & 4	-
Butyrate acetogenesis	$Bu^- + 2H_2O \rightarrow 2Ac^- + 2H_2 + H^+$	Syntrophic organism + β-Oxidation	1 & 3	-17.6
Propionate acetogenesis	$Pr^- + 3H_2O \rightarrow Ac^- + HCO_3^- + H_2 + H^+$	Secondary fermenters, e.g. *Syntrophobacter wolinii*	1 & 3	-5.5
Aceticlastic methanogenesis	$Ac^- + H_2O \rightarrow CH_4 + HCO_3^-$	Methanogens, e.g. *Methanosarcina* and *Methanosaeta*	1 & 3	-24.7
Hydrogen-utilizing methanogenesis	$4H_2 + H^+ + HCO_3^- \rightarrow CH_4 + 3H_2O$	Methanogens, e.g. *Methanosarcina, Methanococcus* and *Methanobacterium.*	1 & 2	-3.2
Acetogenesis	$4H_2 + 2HCO_3^- + H^+ \rightarrow Ac^- + 4H_2O$	Homoacetogens, e.g. *Clostridium aceticum* and *Acetobacterium wieringae*	1 & 2	-7.1

Where: AA is amino acid, Ac^- is acetate, Bu^- is butyrate, Cap^- is caproate, Pr^- is propionate, Val^- is valerate, and VFA is volatile fatty acid.

*1 (Madigan and Martinko, 2006), 2 (Schlegel, 1988), 3 (Thauer *et al.*, 1977), and 4 (Batstone *et al.*, 2002b).

**1 mM fatty acids, 20mM HCO_3^-, 10μM Glucose, 0.0001Atm H_2, and 0.6Atm CH_4, (Madigan and Martinko, 2006).

5.1.7 Typical digestion rates

Substrates must be broken into smaller particles (disintegrated) and disssolved (hydrolyzed) before microorganisms (Figure 9) can use them. All subsequent substrate conversions depend on the initial disintegration and hydrolysis steps, so these early processes can constrain the digester processing rates. When disintegration or hydrolysis is rate limiting, all substrate conversion rates are affected, regardless of whether these are methane generation or sulfide production processes. This also means that the H_2S production process might be similarly constrained and if an accurate measure of the rate-limiting process is found, this same rate-limit will apply to the H_2S concentration changes.

Table 15. Typical kinetic parameters for anaerobic digestion as reviewed by Anaerobic Digestion Model 1 Task Group (Batstone *et al.,* 2002a).

Substrate	Disintegration (per day)	Carbohydrate (per day)	Protein (per day)	Lipid (per day)	Temperature (°C)
Dairy waste		0.13	0.24		35
Cattle manure	0.13*				6
Food waste	0.41				37
Fish waste			0.1-0.15		33

* This process is not biological, so its rate is not expected to double with each 10°C rise.

If the Table 15 rates occur in practice the H_2S concentration will change comparatively slowly in response to a change in digester substrate, i.e. only 41% of food waste will disintegrate each day in an anaerobic digester at 37°C. There is a substantial rate mismatch between the 41% example (Table 15) and the *worst case* H_2S concentration change quoted by Deublein and Steinhauser (2011), which showed a twelve-fold increase within one day. (Days 10 and 11 on the right-hand-side of Figure 2 have H_2S concentrations of 95 and 1155 mg/Nm^3 respectively.) If this significant H_2S increase was caused by an increase in the amount of pre-hydrolyzed (liquid) digester feed, there would be a similarly dramatic increase in biogas volume, a carbon dioxide concentration increase, as well as a significant change in the chemistry of the digester liquor.

On the other hand, if the H_2S increase was caused by an increase in the amount of organic sulfur in the digester feed, there would be a similarly dramatic increase in biogas volume, a carbon dioxide concentration increase, as well as a significant change in the chemistry of the digester liquor. Finally, if the H_2S increase was caused by a sudden sulfate addition, there would be a simultaneous but small drop in biogas volume and methane concentration, and a significant change in the chemistry of the digester liquor. None of these three reasons appears plausible because a H_2S concentration change caused by substrate changes or sulfate addition would also produce a sustained change in digester biogas and liquor chemistry for at least one hydraulic digester retention time (typically 20 days), and this is not seen in the data (Figure 2).

Slow disintegration and hydrolysis kinetics suggest that the 12-fold H_2S concentration change was either not an accurate measure of the process, or was caused by a sudden change in the way sulfur is stored within the digester. Neither of these causes would change the biogas flow rates, methane or carbon dioxide concentrations, or the digester liquor chemistry.

5.1.8 Digester feed composition: carbohydrates, fats/oils and proteins

Dairy manure has a high alkalinity, and this can buffer the digestion by-products that otherwise cause digester instability. Of the three food types, carbohydrates have the most potential to cause a pH drop because hydrogen gas, a digestion intermediate, has an inhibitory effect on the conversion of acetate (Thauer *et al.*, 1977). Dairy manure digesters generally have large numbers of organisms that can convert carbohydrates because carbohydrates are a significant part of the dairy herd diet. Carbohydrate converting organisms represent a substantial portion of the cows' intestinal biota. Carbohydrates are expected to produce 790 liters of biogas per kg volatile solids, of which 50% will be methane (Baserga, 1998).

Large amounts of biogas are produced when fats and oils are digested and this has the beneficial effect of stripping H_2S from the system. Waste milk contains some animal fats so there will already be some microorganisms habituated to digesting fats and oils. Fats are expected to produce 1250 liters of biogas per kg fat, of which 68% will be methane. If the fat is partially oxidized, the biogas yield may drop to 1000 L/kg-fat, but the methane proportion will remain high at 70% (Baserga, 1998). While digesters need time to adjust to fat and oil, it has been shown that even toxic fats such as sodium oleate can be treated successfully if they are first precipitated as the calcium complex (McCarty, 1964).

Dairy manure contains small amounts of microbial, plant and animal proteins, so some protein digestion capability is already present. Protein digests to methane, carbon dioxide, ammonia, and H_2S. High ammonia and H_2S levels, and foaming can occur with sudden protein introduction (Speece, 1996). Proteins are expected to produce 700 L/kg volatile solids, of which 71% will be methane (Baserga, 1998).

In all cases, it is vital that the digester be given time to habituate to changes in substrate, and new substrates should be introduced gradually. Operational tests such as measuring the Ripley Ratio (Ross *et al.*, 1992) can indicate how the digester buffer capacity is changing, and whether excessive alkalinity is being consumed. If large quantities of a pure carbohydrate, fat or oil are fed to the dairy digester routinely, the digester operator will need to assess whether other complex nutrients need to be supplemented.

5.1.9 Loading rate and feeding regime

Anaerobic digesters operate best when loaded consistently, and the operational emphasis is on making any changes gradually to give the anaerobic consortium time to adapt. In some cases however, the digester hydraulic load can change over time, even when the operator supplies an identical amount of feed everyday. Digesters can accumulate large amounts of grit, sand and solids on the bottom of the reactor (due to mineral precipitation, failure of upstream solids separation, or inadequate mixing energies). Dairy digesters can also accumulate a thick surface crust of floatable debris (made up of straw fragments, bedding materials, etc.) and these floating solids can occupy so much reactor space that the digester can become overloaded.

Hydraulic loading is especially important for dairy digesters because dairy flush water can be extremely cold. When too much cold flush water enters the digester within a short time, the heat exchangers may not be able to re-heat the digester fast enough to avoid local process upsets. Microorganisms such as the methanogens are very susceptible to temperature changes and their activity is severely affected by sudden changes in excess of 2 to 3°C (Ross *et al.*, 1992).

5.1.10 Digester mixing cycle

Mixing helps ensure that new substrate is distributed evenly within the digester, and that heat is spread effectively. Mixing can be achieved in different ways; some dairy digesters recirculate biogas, others use pumps or propellers, and still others use external draft tubes. If the mixing cycle is not continuous, then there may be detectable changes in the biogas production during the day and this will be reflected by changes in the biogas H_2S concentration. If the mixing is not effective and parts of the digester remain cold, the biogas from these sections should be expected to have a higher H_2S concentration. Similarly, ineffective mixing may allow the accumulation of excessive substrate in small areas of the digester. The net effects are a reduced biogas volume and higher H_2S concentration (Figure 8).

5.1.11 Methanogen trace-metal nutrient conditions

It has long been known that metals such as calcium and magnesium are vital for the stable operation of anaerobic digesters (McCarty, 1964). There are also some essential trace metals which are often insufficient in industrial wastewaters. The absence of essential trace metals is operationally indistinguishable the presence of toxins, so it is recommended that trace metals sufficiency be confirmed first (Speece, 1996). In this case, dairy manure and codigestion wastes are expected to contain ample trace metals (iron, zinc, and copper), however, these might become unavailable if they react with H_2S and form insoluble sulfides (Stumm and Morgan, 1996).

Methanogenic organisms have particular trace metal requirements, but there is some debate as to exactly which of these is important. In this study, the chemical composition of typical methanogens (Scherer *et al.*, 1983; Whitman *et al.*, 1982) was used to determine appropriate test ratios for the different trace metals (Table 16). This is important because the standard microbial biomass empirical formula ($C_5H_7O_2N$) used for mass balances in waste treatment facilities (Ekama *et al.*, 1984) does not include sulfur, phosphorus, or trace metals. In contrast, the Table 16 (column 5) ratios allow an estimation of all of these components; $C_{71}H_{124}O_{21}N_{16}Na_{1.7}K_{1.2}P_{1.0}S_{0.5}$,$Mg_{0.24}$, etc.

Column values less than one, (sixth column, Table 16) indicate a potential to limit microbial growth due to a trace metal deficiency. Low levels of sodium and potassium are not a concern (sixth column, Table 16) because these metals probably leached out of dairy manure during the composting process (Zicari, 2003). The low concentrations of cobalt, nickel, and molybdenum indicate that there may be some benefit to supplementing these metals for the methanogens in this dairy digester.

The availabilities of the trace metals (Table 16) were tested under different conditions of pH, H_2S concentration and redox potential. The MinteqA2 speciation model (MINTEQA2, 1998) was used to test the dairy cattle mineral supplement metals (Subcommittee on Dairy Cattle Nutrition, 2001) after dilution in the flush water volume. MinteqA2 only provides an estimate of equilibrium conditions and its authors stress that it has several limitations; but despite these limitations, the model is useful for indicating whether a particular metal's dominant complex is a carbonate, phosphate, sulfide or hydroxide, or how the concentration of species might change under changing

conditions. MinteqA2 was used here to determine which species might not be present in sufficient quantity to meet the methanogens' nutritional requirements. Dissolved di-cations of Ca, Mg, Zn, Fe, Mn, Co, and Cu were used as an "availability index", and the test conditions included a low redox potential (Eh of -300mV), and an overpressure of 35% CO_2 (Table 17).

Table 16. Data on elemental composition of methanogenic organisms used to estimate trace metal requirements.

Element	Concentration mg/L (Scherer *et al.*, 1983)	Concentration mg/L (Whitman *et al.*, 1982)	Mean % of Dry Weight	Standardize Molarity on Phosphorus	Dairy Compost metals (Zicari, 2003) as ratio to Std. P Molarity
H	55000	65000	7.39%	124.4	
C	370000	440000	49.9%	70.5	
O	148000	176000	19.9%	21.2	
N	95000	128000	13.6%	16.5	
Na	3000	40000	2.3%	1.702	0.219
K	1300	50000	2.7%	1.174	0.770
P	5000	28000	1.8%	1.000	1.000
S	5600	12000	1.0%	0.547	1.089
Mg	900	5300	0.34%	0.239	6.120
Cl	3400	4800	0.50%	0.238	
Ca	8	4500	0.24%	0.100	32.589
Fe	700	2800	0.20%	0.0599	4.282
Zn	50	630	0.037%	0.0095	1.461
Ni	65	180	0.014%	0.0041	0.425
Se	14	320	0.018%	0.0038	
Cu	10	160	0.009%	0.0024	8.112
Co	10	120	0.007%	0.0020	0.044
Mo	10	70	0.004%	0.0008	0.386
Mn	5	25	0.002%	0.0005	46.081

Table 17. Speciation software (MinteqA2) estimates of the availability of different trace metals.

Test concentrations used in MinteqA2 tests									
Species	HS^-	PO_4^{3-}	Ca^{2+}	Mg^{2+}	Zn^{2+}	Mn^{2+}	Fe^{2+}	Co^{2+}	Cu^{2+}
Mol/L	1.26E-03	2.44E-03	3.14E-03	1.68E-03	1.59E-04	4.85E-05	5.99E-05	3.91E-07	3.44E-05
A. Dissolved ion concentrations (Mol/L) at pH 7.9 and using different HS^- concentrations									
HS^-	HS^-	PO_4^{3-}	Ca^{2+}	Mg^{2+}	Zn^{2+}	Mn^{2+}	Fe^{2+}	Co^{2+}	Cu^{2+}
0.000631	3.52E-04	1.65E-03	2.33E-05	1.13E-05	4.08E-08	2.46E-09	1.14E-12	3.52E-14	1.50E-14
0.00126	9.81E-04	1.65E-03	2.33E-05	1.13E-05	1.14E-07	2.46E-09	9.87E-13	1.26E-14	3.25E-13
0.00251	2.23E-03	1.65E-03	2.33E-05	1.13E-05	2.61E-07	2.46E-09	1.08E-12	5.55E-15	3.82E-12
0.00501	4.73E-03	1.65E-03	2.33E-05	1.13E-05	5.61E-07	2.46E-09	1.32E-12	2.62E-15	3.64E-11
B. Dissolved ion concentrations using a HS^- concentration of 0.00126 Mol/L at different pH									
pH	HS^-	PO_4^{3-}	Ca^{2+}	Mg^{2+}	Zn^{2+}	Mn^{2+}	Fe^{2+}	Co^{2+}	Cu^{2+}
7.0	9.81E-04	7.31E-04	5.98E-05	1.37E-03	3.91E-07	2.03E-09	6.27E-11	1.50E-14	1.36E-13
7.3	9.81E-04	1.34E-03	2.89E-05	3.28E-04	2.62E-07	1.22E-09	1.59E-11	1.18E-14	2.24E-13
7.6	9.81E-04	1.50E-03	2.35E-05	5.84E-05	1.73E-07	1.60E-09	3.99E-12	1.13E-14	2.89E-13
7.9	9.81E-04	1.65E-03	2.33E-05	1.13E-05	1.14E-07	2.46E-09	9.87E-13	1.26E-14	3.25E-13
8.2	9.81E-04	2.39E-03	1.23E-05	5.47E-06	7.54E-08	2.68E-09	2.39E-13	1.53E-14	3.60E-13

MinteqA2 screening shows that the change in metal ion concentration under changing H_2S concentration and pH conditions is not simple. Metals like copper are very sensitive to changes in the HS^- concentration (Table 17, A) while calcium, magnesium, and manganese show no change. When there are comparatively large amounts of metals available, there is little change in HS^-, Co^{2+} or Cu^{2+} with pH (Table 17, B), while Mg^{2+} and Ca^{2+} decrease because pH increases the amount of complexing carbonate.

Two significant anomalies were noticed during the MinteqA2 screenings:

1. When free metals are present, un-dissociated H_2S concentrations do not reach a level where it transfers into the biogas.
2. When H_2S concentrations are sufficient to maintain a biogas "presence", the metal availabilities are extremely low, and well below the levels required to maintain methanogen growth.

These anomalies in turn suggest two hypotheses:

1. The conditions coded into MinteqA2 had significant omissions or mistakes.
2. H_2S and free metals are present in different parts of the digester but do not complex because they do not meet.

The first hypothesis was rejected because other researchers (Callander and Barford, 1983; McFarland and Jewell, 1989) have noted a similar anomaly. The second hypothesis might be true at the macro- and/or micro-scale. Macro-scale separation would be when the H_2S escaped into the biogas, but in a different part of the digester from the metals; this was initially rejected because H_2S is released early in the anaerobic digestion process and the bulk of the H_2S remains in solution. The micro-scale separation hypothesis appears to have some merit and other researchers (McFarland and Jewell, 1989) have suggested that biofilms may indeed play a protective role.

Some biofilm will be present in suspended growth digesters because small amounts of extracellular polymers help hold the bacterial flocs together. However, it is not sufficient to have a *protective* biofilm; the biofilm must also be extensively negative charged in order to repel H_2S anions, and attract and concentrate cationic metals. An alternative form of the micro-scale separation hypothesis would be that microbial siderophores (metal-trapping biological chelates) are produced to compete for soluble metals. However, this last suggestion does not appear to solve the issue of metal diffusion or transport, which will be a significant barrier if highly insoluble metal precipitates are present. Moreover, the presence of strong chelates that promote metal solubility do not necessarily guarantee increased metal availability to the microbes (Callander and Barford, 1983).

The debate as to which metals are stimulatory and which are toxic makes it difficult to decide whether process instabilities are likely. For instance, copper, zinc and nickel salts were all once thought to be quite toxic to the anaerobic digestion process, as were both sulfide and heavy metals if these were unbalanced. Slightly excess H_2S was recommended to ensure complete metal removal (McCarty, 1964). However, substances such as iron, nickel, copper, zinc, cobalt, and molybdenum all play recognized roles in particular enzyme functions and are therefore essential nutrients, but become inhibitory at higher concentrations, depending on whether the substance was present as an ion or as the carbonate (Deublein and Steinhauser, 2011). Solubility does not guarantee availability, because some chelates and soluble complexes bind metals so effectively they are not available for uptake by the organisms (Aquino and Stuckey, 2007).

A third observation noted during the MinteqA2 screenings was that nickel was not present in the cattle mineral supplement (Subcommittee on Dairy Cattle Nutrition, 2001). As the dairy farmer is trying to favor acetogenic- over methanogenic-organisms in the cows' intestines, it makes sense to exclude nickel as an potentially important methanogenic nutrient. Furthermore, nickel is already present in leafy vegetables and has been reported in the dairy compost tested by Zicari (2003), but at a deficient ratio of 0.425, which is comparatively minor compared to cobalt's 0.044 (Table 16).

5.2 pH and impact on H_2S availability

5.2.1 Accumulation of volatile fatty acids

Although volatile fatty acids may be weak acids with a pK_a of about 4.8 (Stumm and Morgan, 1996), they are still one of the primary determinants of anaerobic digester pH because their concentrations can be significant in overloaded digesters. Furthermore, while the VFA pK_a is high, it is far enough away from operational digesters pH (7 to 8) for the acid to be almost completely ionized under operational conditions. VFA accumulation must be avoided as this can neutralize alkalinity and cause a pH drop which will significantly increase the amount of volatile H_2S in solution (Figure 7).

5.2.2 Digester buffering and alkalinity

The most important buffers for the anaerobic dairy manure digester are bicarbonate (neutralized carbon dioxide), and dissolved ammonia. The concentration of these buffers determines how much the pH drops when volatile fatty acids are produced. When present in large quantities, these buffers ensure that the pH is stable. However, there might still be situations, e.g. when the methanogens have been poisoned and VFA accumulate quickly, when even the large amounts of dairy alkalinity might be neutralized.

5.2.3 Urea conversion and ammonia production

Apart from nickel's role as a methanogenic trace metal nutrient, it is also an essential constituent of the urease enzyme. Urease activity determines how much and how quickly ammonia is released from dairy wastes (Muck, 1981). Ammonia release kinetics are important for the dairy anaerobic digester because they determine the buffering available for the volatile fatty acids in the digester feed. Ammonia has been suggested to play a major role in buffering the digester liquor during gas transfer (Ni *et al.*, 2009).

5.2.4 Relative concentrations of cations and anions

An anaerobic digester's pH represents a balance between the positive and negative charges in solution and thus any changes in the relative concentrations of cations and anions have a pH effect (Stumm and Morgan, 1996). Furthermore, in cases where volatile fatty acids accumulate due to metal toxicity, the presence of particular ions, e.g. potassium, can reduce this toxic effect (McCarty, 1964).

6 Variables affecting biogas H_2S concentrations in headspace

6.1 H_2S Reaction variables:

The variation frequency and trends in biogas H_2S concentration can suggest potential sources. For instance, although consistent dairy herd diet composition may not explain the daily variance, it has a major influence in the average dairy H_2S concentration. Similarly, a well-mixed digester at consistent temperatures would have a consistent H_2S release, and only the downstream phenomena would be expected to impose variation.

There appears to be little scientific or technical literature describing the phenomena occurring in the digester headspace, although it is possible to apply known biology and engineering principles. There are anecdotal accounts of sulfur accumulations on the digester headspace surface, but these accumulations are difficult to monitor because the digester remains closed and gas-tight most of the time.

6.1.1 Deliberate oxygen or air injection into digester headspace

The preferred method of biogas H_2S concentration reduction is degradation of H_2S by microorganisms within the reactor headspace, particularly for smaller agricultural digesters (Deublein and Steinhauser, 2011). Deublein and Steinhauser (2011) point out that it is only necessary to add a small amount of air because the catalyzing species *Thiobacillus* and *Sulfolobus* are ubiquitous and exhibit indirect-, and direct reactions:

Indirect reactions: $2H_2S + O_2 \rightarrow 2S + 2H_2O$

$2S + H_2O + 3O_2 \rightarrow 2H_2SO_4$

Direct reaction: $H_2S + 2O_2 \rightarrow 2H_2SO_4$

H_2S oxidizing organisms are aerobic but also need inorganic salts as well as trace elements and a moist surface for immobilization; about one square unit area is required to desulfurize 20 units volume of biogas (Deublein and Steinhauser, 2011).

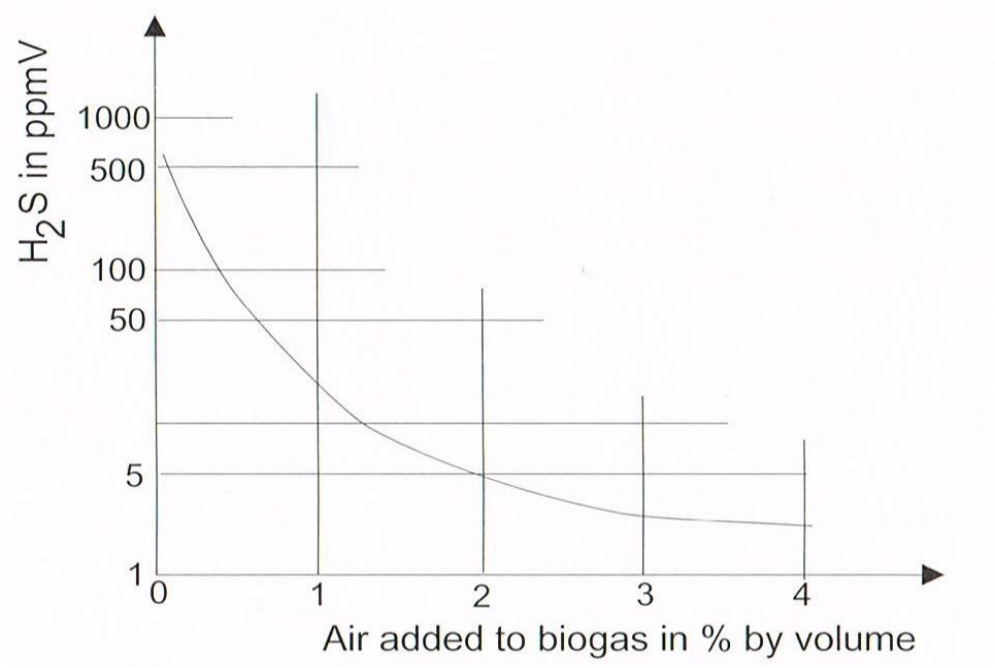

Figure 10. Correlation between biogas H_2S content and air injected into the bioreactor (Deublein and Steinhauser, 2011).

Several points can be made about the Deublein and Steinhauser (2011) guidelines:

1. If at least 75% of the H_2S in biogas is oxidized to pure sulfur and only 25% to sulfate (Deublein and Steinhauser, 2011), this indicates that the indirect oxidation reactions are preferred. The energetics of these digester headspace reactions, therefore, need to be tested (Thauer *et al.*, 1977) to find out, which is more likely under different conditions.
2. While Figure 10 is idealized, the H_2S/air injection relationship shows a monotonic response, which suggests either that only direct sulfur oxidation reactions are occurring or that digester headspaces are sufficiently heterogeneous to obscure a two-peak response.
3. No kinetics are shown in Figure 10.
4. The productivity of a $1m^2$ surface to $20m^3$ biogas treatment system would depend on the gas velocity over the surface because the biogas velocity would change the gas transfer rates.
5. Sulfuric acid would liberate carbon dioxide from solution.
6. Unless removed from the reactor headspace, both the elemental sulfur and sulfur oxides generated by oxidation will be reduced again at a later stage. This means that headspace oxidation processes are more of a delaying, than a removal measure.

6.1.2 Accidental air injection, e.g. air entrainment with feed, or quick draw down

Substantial H_2S reductions are possible with relatively small amounts of added air (Figure 10). Although, it is possible that air bubbles might be entrained by the feed pump, or be drawn into the digester if there is a sudden drawdown, these events should be rare in practice. Air intake might also be less likely for a floating- as opposed to a fixed lid digester.

6.1.3 Site of air injection into headspace

The air concentration will be highest near the point of its injection, and because biogas movement is the only circulation method, it should make a difference where the air is injected. As was mentioned earlier, the presence of air is not the only determinant for H_2S oxidation; a growth surface and source of dissolved nutrients is also needed (Deublein and Steinhauser, 2011). Dissolved nutrients will not be available on the gas-exposed surfaces of the headspace, so organisms are expected to favor surfaces that are occasionally submerged or splashed. However, if large quantities of biogas are released in a particular part of the digester, this biogas will flush air away from digester surfaces, cause anoxia, and reduce the H_2S conversion rate.

6.1.4 Type of digester mixing system (gas recirculation vs. pump or impellor mixer)

Some digesters are stirred by withdrawing biogas from the headspace and pumping this to the digester bottom so that the rising bubbles mix the digester contents. If the air injected into the headspace for H_2S oxidation is drawn into this recirculating air, this oxygen will be consumed within the digester liquor by aerobic biological reactions other than H_2S oxidation. Moist growth surfaces inside the headspace have only three sources of reduced substrate: H_2S, methane, and trace quantities of hydrogen, compared to the digester liquor where a wide range of substrates and intermediates are available. Furthermore, any sulfide oxidation that did occur in the digester liquor would produce sulfur or sulfate which would be quickly reduced again. For these reasons, digesters that are mixed by gas recirculation should be expected to have different H_2S removal characteristics from digesters that use pumps or impellers for mixing.

6.1.5 Biogas flow within headspace

Biogas might be quite stagnant in parts of the digester headspace, and this will affect the rate at which oxygen is transferred to the H_2S oxidizing organisms present on the moist reactor surfaces. When the biogas is moving fast the stagnant layer of gases surrounding the surface-attached microorganisms will be thinner and therefore less of an oxygen barrier. Surface condensation may also play a role in oxygen transfer. Furthermore, if this headspace is restricted (e.g. by sulfur-stalactites or a surface crust) the biogas velocities will be substantially different in the restriction area.

6.1.6 Presence of digester surface foam, scum or crust

Foam, scum and surface crust share one common characteristic; they isolate the headspace from the digester liquor. Otherwise, they are very different phenomena. Foam comprises small bubbles and is a problem because it sometimes attaches to the active gas-producing organisms lifting them out of solution and into a less active foam region. Foaming is a common problem associated with digester startup, or sudden increase in protein substrate, and as a result, foam control measures should be part of all anaerobic digester designs (Speece, 1996). Apart from the problems caused by foam occupying digester space (shortening the hydraulic retention time) and clogging biogas pipes, foaming is especially problematic for H_2S oxidation because there will be minimal gas transfer between the gases in individual bubbles. Foam will also block air access to the normally exposed surfaces colonized by the sulfur oxidizing bacteria.

Scum is different from foam or crust because it has a greasy/thicker consistency. If co-digestion wastes contain large quantities of fats and oils, these may separate and rise to the surface of the digester to form a surface layer. Once this happens there will be minimal contact between the digester liquor and the scum layer, so the scum may persist for some time. Crusts, made up of straw fragments and bubble-filled plant residue, can accumulate on the surface of the digestive liquor and in the case of dairy digesters, occupy a significant amount of space. Surface accumulations persist because they are lighter than the surrounding fluids, and although biogas erupts through these layers, the surface layer will be more quiescent than the better-mixed digester liquor. Specific digester design measures must be taken to break up these layers.

The contribution of floating material to the digestion process is not clear. For instance, while excessive foam, scum or surface crust might be expected to cause problems by occupying digester volume and thereby shortening the hydraulic retention time of the digester, their upper surfaces also effectively double the surface areas available for colonization by sulfur oxidizing bacteria. Thus, changes in the amount and extent of these surface layers might have a significant impact on the amount of H_2S in the biogas (where headspace air injection is used). Furthermore, if H_2S is oxidized to sulfuric acid on the surface of a crust, this acid might hydrolyze large quantities of the crust and cause it to break up.

Oxidation of H_2S significantly changes the biogas H_2S concentrations but the operator has little real control of this important process. Furthermore, as the oxidized sulfur products remain in the digester headspace (or fall back into the liquor), this process serves to delay rather than remove H_2S.

6.2 H_2S measurement variables

6.2.1 Sampling time vs. biogas flow rate

Section 5.1.4 described how biogas H_2S concentrations change as the biogas flow rate changes when the dissolved H_2S concentrations are constant (Figure 8). However, there is another more important process that will cause significant variations in the biogas H_2S concentrations. The H_2S producing bacteria are a different group of organisms from the methane-producing bacteria, which explains the disconnection between H_2S and methane production. When dissolved H_2S concentrations increase before significant quantities of methane are produced, the initial biogas flow will contain substantially larger quantities of H_2S than later flows. This phenomenon is expected to be significant because sulfate reducing organisms have a thermodynamic advantage over methanogens (Thauer *et al.*, 1977), and amino acid fermentation and subsequent H_2S release must occur before simpler compounds are available for the methanogens. Furthermore, when new substrate is pumped into the digester, there will be an initial temperature drop and the methanogens will be particularly sensitive to this. These different H_2S and methane production rates will combine to produce a H_2S concentration that starts high, and then tapers off. The duration of this H_2S pulse will depend on the frequency of digester feeding and efficiency of the digester mixing and heat exchangers.

6.2.2 Sample frequency

Dairy manure and codigestion substrates often contain large amounts of solids. These solids can cause clogging in solids separation equipment, so the receiving tanks and feeding equipment are not generally operated unsupervised for extended times. This means that digester feeding and receiving tank recharge will most probably occur during the day when supervision is available, and a daily feeding routine is imposed on the digester. These high solids feeds and intermittent flows, stress the digester and heat exchange equipment, and combine to produce loading and temperature changes.

Loading and temperature changes, cause variations in the gas flow rates and quality. Furthermore, in cases where digester mixing is not continuous there will also be substantial variations in digester biogas.. Biogas H_2S concentrations from operational digesters show ten-fold increases within the twenty minute intervals (Sklorz, 2002). Under these conditions the choice of sampling time becomes the key determinant of biogas "quality". The usual recommendation is to collect samples at twice the frequency of the variation (Holman, 2001), but most dairies with daily digester feeding regimes collect only one sample each day. Most biogas data when sampled daily is thus prone to aliasing, and the generation of artificial patterns that may not properly reflect the average biogas quality.

Digester feeding, mixing- and temperature-patterns will need to be documented to determine the variation range. Daily biogas H_2S measurements will be representative only where there are minimal diurnal changes in digester conditions.

6.2.3 H_2S sample container adsorption and analytical delay

H_2S adsorbs onto Tedlar biogas sampling bags (Bothi, 2007). Variations in H_2S concentrations in biogas samples collected in gas sampling bags, therefore may be due to the sample collection and storage. The H_2S adsorption onto Tedlar bags was found to be time-dependent as well as concentration dependent; with 1000ppm H_2S biogas samples showing an 80% loss of H_2S within 24 hours and with 2500 and 5000ppm H_2S samples showing a loss of approximately 60% within 24 hours, respectively (Bothi, 2007).

6.2.4 H_2S dissolving in condensate

Wide (+ and -6 °C on 30 °C) diurnal temperature fluctuations have been recorded in the biogas arriving in a sulfur oxidation test facility (Zicari, 2003). If biogas was saturated at 36 °C and cooled to 24 °C, approximately 72 mL of water would condense from this biogas onto the surfaces of the biogas piping. Some of the soluble gases will leave the biogas and dissolve in this condensate depending on the respective gases partial pressures. Carbon dioxide, H_2S, and ammonia will be particularly susceptible to dissolving in this condensate resulting in their concentration changes as biogas moves through the pipes.

Condensation estimates will be underestimates because the calculations are usually based on the average biogas temperatures, when the condensation temperature on the pipe surfaces will be lower than this. Apart from gas concentration changes, another practical question concerns what happens to the condensate water that contains carbonate, sulfide and ammonia; whether it runs back to the digester or is drained from the system?

6.2.5 Reactions due to light exposure

H_2S has long been known to decompose if exposed to ultraviolet light (Avery and Forbes, 1936). Biogas samples should thus be protected from the light to ensure accurate measurements.

A second form of light reaction is mediated by purple sulfur bacteria and while this is not expected to affect H_2S concentrations in the enclosed sections of the dairy digester, or in sample bags, it can play a major role in converting H_2S into sulfate within anaerobic lagoons (Belila *et al.*, 2012).

6.2.6 H_2S sensor problems

On-line electronic H_2S sensors need periodic testing because they can become inaccurate. Zicari (2003) noted that when he compared an electronic sensor H_2S reading with that of a gas-tube test the electronic sensor was "consistently higher" (Table 5) but concluded that this might have been due to poor sealing in the sensor gas tubes (Zicari, 2003).

7 Conclusions

It has proven difficult to secure substantial operational data for this study; however, there are sufficient dairy sulfur mass-balances and lagoon observations to conclude that dairy anaerobic digesters get most of their sulfur load through the recycling of lagoon or solids separation effluent. Based on the foregoing conclusion, seasonal H_2S biogas trends consistent with the dairy lagoon evaporation, precipitation, and irrigation patterns should be expected. Daily digester loading that causes uneven temperature distributions (excessive flush water delivery), gas flows, pipeline condensation, and changes in operation of the sulfide oxidation system, are likely to be the contributors to the daily H_2S fluctuations. Careful targeting and selective removal of the most sulfide-rich lagoon sediments might be sufficient to reduce this cycling. Digester operators will be able to identity several more sulfur elimination opportunities once the digester is seen as just one part of the multi-component dairy farm.

Certain co-substrates, such as seaweed, can introduce substantial sulfur loads into the digester and operators should avoid feeding these to the digester. High carbohydrate loadings may trigger souring if dosed fast enough to overwhelm the alkalinity reserves of dairy digesters; these are, however, relatively easy to manage.

While it may appear that trace metal nutrition to target particular organisms holds promise, the notion of metal supplementation must be approached with caution, because it could adversely affect herd health, crop application alternatives, and nutrient management plans. If metal concentrations do need to be increased, it would be more sustainable to identify the metal chemistry processes, and eliminate some of the current metal sinks.

There are significant process uncertainties and questions surrounding the operation and control of H_2S oxidation within the headspace of dairy digesters. However, there does not appear to be any real long-term advantage to this process if the oxidation products remain inside the digester.

Where detailed H_2S data are available, these have shown large (ten-fold) concentration changes within the space of twenty minutes. It is unlikely that H_2S concentration variations of this size, frequency and speed, are caused by changes in digester substrates or changes in the average sulfide content of the digester feedwater. Furthermore, if the biogas sampler is not aware of these underlying patterns the collected H_2S data will be meaningless. Thus, the first step to understanding the sudden biogas H_2S concentration changes, and building an accurate picture of the digester biogas quality will be to document the feeding, heating and mixing control patterns and machinery.

8 Answers to questions:

What is the effect on H_2S production of including or excluding copper sulfate foot wash or other inorganic sulfur compounds in the digester feed or the water supply?

Copper sulfate is used in footbaths to help prevent foot rot in cows. The traditional formulation contains copper sulfate and formalin, but there are also chelated copper and zinc commercial formulations. When copper sulfate enters an anaerobic digester, the sulfate component is reduced by sulfate reducing bacteria to sulfide, which reacts to form insoluble copper sulfide precipitate as either CuS or Cu_2S (Table 13). The 1:1 and 2:1 stoichiometry would depend on whether divalent copper is reduced before it reacts with sulfide, but as both sulfides have extremely low solubility products, it is likely that the copper is completely removed by precipitation.

Which feedstock tends to produce more or less reduced sulfur compounds in anaerobic digesters compared to digesters fed only manure? What is the cause of the difference? Which feedstock could be used to reduce H_2S generation in a digester?

Answers to these questions can be found in sections 3.2 and 3.3 of this review.

Is there a difference between starting an empty digester compared to normal operation?

Yes. Anaerobic digesters need to be started gradually to ensure they do not become overloaded. Dairy wastes have more alkalinity than industrial or domestic wastes. However, during startup it is vital to ensure that operational temperatures are maintained and that the digester is properly stabilizing its current organic load before increasing this load further. Foaming issues are also a normal part of startup (Speece, 1996).

One digester facility is receiving a material that contains ferric chloride and producing low H_2S concentrations in the digester gas. Previously the operator tried to add ferric chloride directly to the digester feed, but did not achieve the same effect. Why?

More information is needed to answer this question. For instance, exactly how was the $FeCl_3$ added to the digester feed? If solid $FeCl_3$ was sprinkled into the digester feed, an insoluble sulfide may have encapsulated the granules, or the heavy granules may have sunk into the bottom sediment. Either outcome would prevent the $FeCl_3$ from dissolving properly.

An observation developed during review of materials to draft a permit indicates that plug flow and complete mix digesters produce different quantities of H_2S, even using what looks like the same types of feedstock. Is the observation true? If so, why?

If all other components (e.g. heat exchangers and lagoon conditions) are identical, a plug flow digester might be expected to experience larger variations in digester conditions especially near the influent point, where the temperatures and pH might drop further (and release more H_2S) than a complete mix digester. On the other hand, as plug flow digesters can produce more stable wastes, they will release more diluting biogas per unit substrate. If the plug flow digester is operated without a significant pH dip in the digester influent area, then it might be able to out-perform a complete mix reactor. However, the measures adopted to avoid the pH drop effectively turn the plug flow into a complete mix reactor. The key lies in avoiding temperature and pH fluctuations in the influent area, and this is difficult to achieve because of the high solids content of dairy wastes.

Feedstock management: Given knowledge of the sulfur content of various materials, are there preferred methods of feedstock blending/management (BMPs) that can reduce overall sulfur concentrations in the gas? Are there poor management practices that can increase sulfur concentrations in the gas?

In the presence of sufficient nutrients, fats and oils (if properly emulsified to reduce separation) will provide a flow of low-sulfur biogas and purge excess sulfide from solution. *Excessive* protein needs to be avoided as it adds both sulfur and nitrogen, and there are already sufficient quantities of both in dairy waste. Even though carbohydrates do not contain sulfur, carbohydrates still need to be added gradually because they can depress the digester pH and cause a sudden increase in the biogas H_2S. Large amounts of sulfides can be prevented from recycling to the digester by selectively removing accumulated lagoon sediments. Apart from the substrate composition, the digester loading rate should be kept constant and any changes should be made gradually. Feed water volume must be kept to the minimum that allows effective pumping, heat transfer, and mixing.

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