

Oxygen Effects in Anaerobic Digestion – A Review

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Abstract: This article reviews the experimental and theoretical studies conducted on the possible effects of oxygen in biogas generating anaerobic digesters. The interactions of oxygen with various biochemical processes associated with anaerobic digestion are discussed together with other relevant aspects. The conventional perception of oxygen being merely toxic in anaerobic digestion (AD) is refuted. Digesters can withstand significant levels of oxygenation without drastic negative impacts. Limited quantities of oxygen can even lead to improved AD reactor performance under certain operating conditions. Co-existence of anaerobic and aerobic cultures in a single bioreactor environment has been demonstrated. It is shown that the partial aeration assisted AD can serve as a beneficial treatment strategy for simultaneous waste treatment and energy generation, for a multitude of organic waste categories.

Keywords: Aeration, anaerobic digestion, hydrolysis, inhibition, methane, oxygen effects.

1. INTRODUCTION

1.1. Anaerobic Digestion

Anaerobic digestion (AD) is a biochemical process that converts a variety of organic matter using naturally occurring microorganisms under oxygen depleted conditions to produce a gaseous mixture mainly composed of methane and carbon dioxide, known as biogas. The biogas results from the microbial degradation of organic matter generally generated by photosynthesis using solar energy. The process can naturally occur in anaerobic environments like river sediments, deep soil or waste landfills. The AD process can also be engineered to take place in a dedicated facility known as an anaerobic digester; primarily a closed vessel kept with a culture of microorganisms, fed regularly with digestible (organic) matter.

It is suggested that the Sumerians used anaerobic cleansing of wastes 5000 years ago [1]. The Chinese may have used “matured” manure as a fertilizer 3000 years ago [2]. The first confirmed historical evidence of using biogas is found related to Assyria, where it was used for heating bath water during the 30th century BP [3]. The first documented engineered anaerobic digester was put into operation in Mumbai, India in 1859 [4], using the process for waste treatment and utilizing biogas for lighting. The fundamental biochemical AD principles were started to be clearly understood only few decades ago. The process has been studied as a genuine scientific matter since the 1930's [3]. Even before that, some well known scientists including Volta, Faraday and Pasteur discovered some primary facts on biogas and its generation process. The definite chemical structure of methane was formulated due to the works of Dalton, Henry, Davy and Avogadro [1].

The anaerobic digestion has now become a well established technology used for handling multitudes of waste categories including those of domestic/municipal, agricultural and industrial origins [5-8]. Compared to alternative treatment methods, the merits of AD include low sludge production, high organic loading rates, energy value of methane and low energy consumption [9, 10]. In the light of current global consciousness of environmental sustainability, AD is regarded as a promising process, both as a renewable energy generation scheme [11-13] and a waste stabilization method [14-17]. Anaerobic digesters further contribute to reduce greenhouse gas emissions [12, 13, 18]. Generation of nutrient rich digestates, which can be used as fertilizer [13, 17-19], is an added advantage. These merits of AD have led to a rapid growth of its application.

The primary problem faced by AD users is its inherent instability [16, 20] as a complex biochemical process [21, 22]. AD is alarmingly sensitive to changes of operating and feed conditions. Accidental or unavoidable oxygen loading is one aspect of this problem. To cope with this inherent instability, it is essential to develop sufficient control techniques. Hence, a comprehensive mechanistic modeling approach towards the process is needed. Various attempts have been made to develop such models on anaerobic digestion and the most important milestone is the standard ADM 1 model [23]. The standard models, however, do not include all necessary aspects to accurately predict the digester behavior under different process disturbances. Lyberatos and Skiadas [24] made a review of early anaerobic digestion models and pointed out that modeling of digester behavior when exposed to an inhibitor (e.g. oxygen) is an area where further attention is necessary. Later modeling approaches published do not seem to make significant improvements in this regard. Botheju *et al.* [25] modified the standard ADM 1 model [23] to include the known oxygen effects as an attempt to investigate oxygen induced disturbances.

AD can also provide benefits from the different means of enhancing the process efficiency [26]. This may improve the

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economy of biogas generation plants enhancing the dissemination of such sustainable solutions. Partial aeration assisted anaerobic digestion has been recognized as a possible way to enhance AD performance [27-30].

1.2. Different Phases of AD

The overall AD process is a combination of a multitude of biochemical and physicochemical reactions. These can be basically categorized as disintegration, hydrolysis, fermentation (acidogenesis), acetogenesis (acetate generation) and methanogenesis (methane generation) [22, 23]. Disintegration involves physical and chemical breakdown of larger particulate matter. Hydrolysis is a step involving the enzyme-mediated chemical/biochemical transformation of long chain polymeric organic matter such as lipids, polysaccharides, protein, nucleic acids and fats into soluble and smaller organic molecules that can be consumed by microorganisms during the fermentation stage. These soluble components include fatty acids, monosaccharides, amino acids, simple aromatics and other short chain compounds [31, 32]. Hydrolysis is catalyzed by the extracellular enzymes excreted by the microorganisms responsible for fermentation/acidogenesis [32-34]. This group of organisms is considered to be composed of a large amount of facultative bacteria [33, 35-38] which can thrive with or without oxygen.

In the third stage of digestion, called acidogenesis/fermentation, the hydrolysis products are degraded to simpler compounds of volatile fatty acids (such as the acids of acetic, propionic, butyric, valeric, caproic and heptanoic), CO₂, H₂ and ethanol [23, 31]. These compounds are the products of fermentative (anaerobic) respiration of acidogenic biomass. The fourth stage of digestion, acetogenesis, involves converting all volatile fatty acids (VFA) and some of the other short chain organic products (e.g. ethanol) into acetate, catalyzed by a separate syntrophic group of strictly anaerobic organisms, called acetogens. In the last stage, methanogenesis, acetate is converted into methane by a group of organisms called acetoclastic methanogens. Meanwhile, a part of the generated CO₂ and H₂ are utilized by another group of methanogens, called hydrogenotrophic methanogens, and again generate methane as the end product [21, 23, 31]. About 70% of methane is generated through the acetic acid pathway [39]. Methanogens belong to the strictly anaerobic microorganism group called archaea: About 19 genera and more than 50 species of methanogens have been identified so far [40]. The final gas mixture, biogas, can contain about 45 – 75% CH₄ and 20 – 50% CO₂ [41, 42, authors' own observations] plus minor amounts of N₂, H₂S, H₂, NH₃, H₂O vapor and O₂ [41-43].

1.3. Effects of Oxygen in AD

It is quite natural that some amount of oxygen can reach anaerobic digesters unintentionally [44, 45] as the reactors are operated within an aerobic open environment, especially through interactions with the surroundings such as by feeding and mixing. Most anaerobic digesters are therefore subjected to minute and varying aerobic loading conditions. The possible effects of such aeration are neither extensively quantified nor handled in standard AD models. A few studies

have, nevertheless, predicted and demonstrated benefits of adding some oxygen in AD [27-29, 44, 46-50].

It is commonly perceived that oxygen acts as an inhibitory and toxic agent [51-55] in AD due to the involvement of strictly anaerobic microorganism group of acetogens and methanogens [40]. Also, aerobic conversion of soluble organic matter into CO₂ by aerobic respiration is likely. Thus, it was believed that reactor instabilities, slow start-ups, low methane yields and even total reactor failures might occur due to oxygen entering anaerobic digesters [44]. Due to this negative perception, inoculums used in anaerobic digesters are even de-aerated before commencing reactor operation; sometimes oxygen scavenging chemicals (e.g. sodium sulfide) are also being added [55-58]. Conversely, improved hydrolysis of particulate matter in AD is observed in the presence of oxygen [49, 59, 60]. All non-soluble and long chain organic matter should go through this initial hydrolysis stage before fermentation or methanogenesis, in which the particulate matter would undergo decomposition and solubilization by the activity of enzymes (such as *protease*, *amylase*, etc.) that are being extracellularly excreted by fermentative (acidogenic) bacteria [34]. Since hydrolysis is often the rate limiting reaction stage when the substrate is composed of particulate organic matter [32, 61, 62], enhanced hydrolysis can greatly benefit the overall process efficiency. It is commonly known that hydrolysis rates are significantly higher under aerobic and anoxic conditions compared to anaerobic conditions [63]. Botheju *et al.* [46] demonstrated the possibility of the existence of an optimum oxygenation level which would yield a maximum methane generation in AD.

The forthcoming sections discuss different aspects and possibilities of using limited oxygenation in AD based on the focus points of 1. Effects on physico-chemical and biochemical processes, 2. Microbial ecology, 3. Operation of partially aerated bioreactors, and 4. Modeling aspects.

1.4. Terminology of Use

The use of oxygen in an anaerobic process needs a specific phrase for the purpose of reference. The term “micro-aeration” has been used for this before, but over the years its usage has become confusing due to the fact that different authors use it with different meanings in their particular perspectives. In literature, the term “micro-aeration” has been found referring to several different conditions such as “*the use of limited amount of air in an anaerobic process* [59]”, “*use of small amount of oxygen dissolved in the medium* [64]”, “*use of oxygen with < 10% O₂ saturation* [65]”, “*use of oxygen up to a certain fraction (10 - 15%) of the total organic load* [30]”, “*dissolved oxygen range where the optimum growth of microaerophilic organisms occur (<1μM - [66])*”, “*a low oxygen transfer rate scenario* [67]” and several more, some with even mathematical definitions [68]. Several other authors, meanwhile, use this term without mentioning a definite range of oxygen. We may, however, encompass the majority of these definitions and usage by calling micro-aeration as a process where “some amount of oxygen is introduced to a basically anaerobic biochemical process”.

In this article we, however, decided to avoid altogether the use of the term “micro-aeration”. This is to be fair among

the different authors, when we describe their findings. Instead, we will use the terms “limited aeration”, “limited oxygenation” or “partial aeration” which we would similarly define as “a process where some amount of oxygen is introduced to a basically anaerobic biochemical process so that there would be both the anaerobic and aerobic biological activities happening in a single bio-reactor”. This definition expects that the process should not become a complete aerobic process (having only aerobic activity) during the reactor operation (as it is to be a “basically anaerobic biochemical process”). However, the definition may allow the process to become, if it does, devoid of any aerobic activity at certain durations of operation. This definition is based on the aerobic and anaerobic activities of a bio-reactor rather than the load or level of oxygen introduction. Hence, it can be a general definition free from reactor configuration and operational effects. We may also use the phrase “oxygen limited conditions” to the situations where the oxygen supply rate is always maintained less than the oxygen utilization rate, so that there would not be any excess oxygen in the liquid phase and the DO reading should always be zero even under continuous oxygen/air supply.

2. OXYGEN EFFECTS ON PHYSICO-CHEMICAL AND BIOCHEMICAL PROCESSES

2.1. Inhibitory and Toxic Effects of O_2 on Methanogens

Methanogenic microorganisms (*archaea*) are categorized as the strictest anaerobes known, with little or no tolerance to oxygen [57, 58]. This can be attributed to the fact that microbial group archaea has the oldest evolutionary history of all prokaryotes, dating back to the geological ages of the Earth when the atmospheric oxygen could not have been yet present [69]. It is claimed that strict anaerobes cannot synthesize the enzyme called *superoxide dismutase* which is used by aerobic organisms to neutralize toxic oxygen ions and radicals such as O_2^{2-} , O_2^- and OH^- [52, 55, 68, 70, 71]. In addition, it is believed that the activities of some of the other important methanogenesis enzymes are also inhibited by oxygen [40]. For example, the pure cultures of the methanogenic species *Methanococcus voltae* and *Methanococcus vannielii* show almost a zero tolerance towards oxygen [72]. Whiteman *et al.* [40] reported that one species of *Methanosarcina* had a half time of survival of only 4 minutes in air equilibrated medium. On the other hand, some methanogenic species can have a certain extent of intrinsic tolerance to oxygen exposure [53]. Kiener and Leisinger [72] reported that the methanogenic species of *Methanobacterium thermoautotrophicum*, *Methanobrevibacter arboriphilus* and *Methanosarcina barkeri* did not show any reduction of colony forming units at the exposure to air for 30 hours; however, the longer exposure rapidly reduced the viability of the cells. It has now been confirmed that some methanogens do have the ability to produce superoxide dismutase enzymes [44, 52]. Accordingly, different methanogenic organisms may differ in their intrinsic capability to tolerate different levels of oxygen presence [73]. Kato *et al.* [44] provides a list of different strict anaerobes with their nominal levels of superoxide dismutase which is the primary enzyme inducing the oxygen tolerance capability of those organisms [71]. It is plausible that some methanogens are evolved to handle different levels of oxygen upon the fact that their habitats are exposed to various oxygen levels throughout a long time

period [55]. According to Gerritse *et al.* [74], when the cultures were adopted for oxygenation over a period of time, the same oxygen levels which were previously inhibitory, were not inducing any inhibition effect anymore.

Inhibitory characteristics of free oxygen towards strict anaerobic organisms present in anaerobic digesters have been investigated by a number of authors [40, 75, 76]. Scott *et al.* [75] experimenting on the oxygen sensitivity of methanogens, tested two different methanogenic cultures, one from rumen and one from an anaerobic digester. Methane production stopped at below 30 nM of oxygen concentration in both of the cultures. The exact low concentration where the oxygen inhibition starts could not be determined due to the limited sensitivity of oxygen probes [75]. This specific concentration, if determined, can be used to model the inhibition effect using an inhibition function (e.g. non-competitive type) together with an inhibition coefficient (K_{i,o_2}).

Due to the generation of highly reactive oxidizing agents such as peroxides and superoxides in the liquid medium [70], it is suggested that not only functional inhibition but also rapid cell lysis [58] of obligatory anaerobic species can occur. As demonstrated by Martin and Savage [77] oxygen can damage the chromosomal DNA of strict anaerobic microorganisms. According to Botheju and Bakke [47], the activity inhibition of methanogens and the complete destruction or cell lysis due to oxygen can be two different aspects of oxygen toxicity. They observed that rigorous initial aeration of an anaerobic inoculum resulted in a three times longer lag period before gas generation started, compared to an unaerated inoculum. This lag duration (3 days) was, however, similar for the different aeration levels denoted by the initial DO levels of 3, 5 and 7 mg/L. This is caused by the inhibition of methanogens due to rigorous initial aeration. The observed inhibition was found to be reversible since the gas generation started eventually. Reversibility of the oxygen inhibition of methanogens was observed also by Gerritse *et al.* [74]. Meanwhile, all the aerated inoculums eventually generated the same amount of methane, but the generation was increasingly slower at higher aeration levels most probably due to the permanent damage of a part of the strict anaerobes [47]. This is opposed to the comments made by Zitomer [53], who conducted an experimental series under limited oxygenation conditions, suggesting that methanogens might not have been killed by the introduction of oxygen but the activity was inhibited for a short period (30 min.). These opposing observations can be explained by the level or intensity of the aeration, as Botheju and Bakke [47] probably exposed their inoculums to much more oxygen than did Zitomer [53].

2.2. Oxygen Shielding Effect and the Oxygen Tolerance of Strict Anaerobes

It is suggested that anaerobic digestion systems can have considerable oxygen tolerance due to several deterrence mechanisms. Accordingly, strict anaerobes in an AD environment may survive and function under limited aeration with no or minor inhibitory effects. Primarily, rapid oxygen consumption ability of facultative fermentative organisms [56] can protect other organisms by scavenging on dissolved oxygen [55]. Microbial aggregates like flocs,

granules and biofilms may shield organisms living deep inside diffusion barriers [54] which stop the full penetration by oxygen. Steep oxygen gradients are created through such microbial aggregates due to the diffusion limitation and also due to oxygen consumption by the facultative or aerobic organisms thriving closer to the surface of the aggregate/biofilm [30, 54, 55, 78, 79].

While experimenting on a reactor arrangement called Membrane Micro-aerated Anaerobic Digester (MMAD), Rathnasiri *et al.* [78] and Botheju *et al.* [79] pointed out that oxygen supplied by aerated water circulation through a polydimethyl siloxane (PDMS) membrane tube did not reach the anaerobic bulk phase of the digester, when a biofilm layer was formed on the outer surface of the membrane. A modeling and simulation study carried out on this case [79], revealed that facultative organisms growing closer to the membrane surface consumed the supplied oxygen, leaving near zero dissolved oxygen values at the biofilm-digester bulk liquid interface. Methanogenic and acetogenic organisms were mainly accumulating at this biofilm-bulk liquid interface. Another interesting finding was that the membrane attached biofilm increased the oxygen mass transfer rate through the membrane compared to a clean membrane case [78]. This is due to the increased concentration driving force created by rapid oxygen consumption of facultative organisms in the biofilm.

Limited aeration caused no oxygen inhibition of the anaerobic microorganisms in a UASB (up-flow anaerobic sludge blanket) reactor, but only led to sulfide oxidation, according to a study by Zhou *et al.* [80]. Granular sludge in the UASB reactors could shield the methanogenic organisms from oxygen exposure, since they mainly grow inside the granules [54].

Studies have shown that at least some of the methanogenic organisms have an intrinsic capability of tolerating limited oxygen exposure for extended time periods [51, 55, 72, 81, 82]. Hence, together with this intrinsic tolerance, in a mixed consortium like the environment in an anaerobic digester, the methanogenic species should have a considerable ability to tolerate oxygen due to the presence of other (facultative) bacteria which help to reduce the oxygen stress on strict anaerobes. Survival of methanogens under low aeration conditions in a fully suspended medium in the absence of any microscopically observable flocs or aggregates has been described by Zitomer and Shrouf [52] and also by Zitomer [53]. They confirmed that the majority of biomass was planktonic organisms. This observation cannot be explained by the existence of anaerobic micro-niches in biomass aggregates protected by diffusion barriers [53, 54], but by the oxygen shielding effect of facultative biomass. According to Kato *et al.* [55], a high correlation has been found between the oxygen tolerance and the oxygen uptake activity of the facultative bacteria in anaerobic granular sludge. But, a certain oxygen tolerance was still present even when the aerobic activity of the facultative organisms was absent [55]. This may be due to the intrinsic oxygen tolerance of strict anaerobes and/or the oxygen diffusion barriers through sludge granules. The above studies propose that the oxygen tolerance of many AD systems stems from a combination of several factors.

Conklin *et al.* [81] experimented on the effects of oxygen exposure on anaerobic digester sludge caused by recuperative thickening. They concluded that short term oxygen exposure did not significantly reduce the acetoclastic methanogenic activity. Prolonged oxygen exposure (over 7 days) did, however, decrease the methanogenic biomass activity with an oxygen lethality constant of 0.23 days^{-1} for acetoclastic methanogens. Even with the lower methanogenic activity, long term oxygen exposure did not reduce the long term performance of the digester, but the ability of the digester to handle shock loads (digester reserve capacity) was significantly reduced by this long term oxygen exposure [81]. Peter and Conrad [83] concluded that, though methanogens cannot function in the presence of oxygen, they can survive longer time durations at oxygen exposure. Gerritse and Gottschal [84] studied the growth of the two methanogenic species *Methanobacterium formicum* and *Methanosarcina barkeri*, in mixed cultures by feeding a limited concentration of oxygen ($450 \mu\text{M O}_2$ per hour). Their results showed a decrease in the methane formation rate compared to a complete anaerobic case, even though the growth of methanogenic organisms continued.

Shen and Guiot [54] observed that, in the long term, influent dissolved oxygen was inhibitory to acetogens and methanogens in a UASB type anaerobic/aerobic coupled reactor only when its concentration reached a certain high level, such as 8.1 mg/L in their experiment. This oxygen level certainly was a high value considering the high hydraulic loading rates of UASB reactors, and should have resulted in an oxygen non-limiting condition in the reactor liquid phase. Based on a model simulation, Botheju *et al.* [79, 85] concluded that typical dissolved oxygen concentrations found in influent streams of CSTR (continuous stirred tank reactor) type reactors could not cause a significant impact on digestion performance.

According to Hungate and Macy [73], oxygen was removed as rapidly as it entered an anaerobic habitat when enough substrate was present. Kato *et al.* [55] suggested that the presence of enough substrate was important for the oxygen tolerance of anaerobic cultures. Scott *et al.* [75] reported that an increased O_2 uptake was noticed when an anaerobic inoculum was fed glucose. A certain lower level of oxygen tolerance was still noticed at no substrate feed conditions [55]. This can be due either to some intrinsic oxygen tolerance of organisms as mentioned before or to the presence of some substrates (including the decaying biomass) within the inoculum. More particulate matter in the inoculum may have been converted into soluble substrate due to oxygen enhanced hydrolysis [27, 49]. The possibility of some abiotic chemical oxygen uptake (e.g. sulfides) is also suggested as an explanation for such oxygen tolerance at no feed conditions [55, 86]. Further, diffusion barriers due to aggregated biomass can always exclude the inhibitory oxygen effects as mentioned before, even under substrate limiting excess oxygen conditions. The effect of mixing on the oxygen tolerance of granular sludge was also studied by Kato *et al.* [55]. The conclusion was that intense shaking reduced the oxygen tolerance in most cases. Mixing can increase the oxygen mass transport into the locations protected by diffusion barriers but dense aggregates can prevent oxygen penetration even with strong mixing.

It has been reported that the population densities of methanogens in some paddy field soils do not change upon the seasonal changes of flooded anaerobic periods and dried aerobic periods [53, 57]. This demonstrates the capability of methanogens to survive rather long durations of aerobic conditions. The availability of facultative and aerobic organisms together with organic substrates and also the diffusion barriers through soil particles may provide a significant protection to methanogens, as described before. Simon [20] stated that methanogens mixed with solid organic wastes demonstrated a high tolerance to aeration and did not show a significant reduction in methanogenic activity after the aeration period. Most probably, methanogens could have been well protected within the particulate solid matter avoiding exposure to much of the aeration.

2.3. Use of Limited Aeration in Anaerobic Biochemical Conversions

In addition to its potential use in AD, limited aeration has been suggested to be advantageous in many other anaerobic biochemical conversion processes. Testing 2,3-butanediol production by the facultative anaerobe *Enterobacter aerogenes* as a model anaerobic process, Zeng and Deckwer [68] suggested that the use of limited aeration can help reduce the formation of toxic metabolites such as lactic acid and ethanol. They further showed the possibility of increased biomass and productivity under limited aeration, but excessive aeration could reduce the productivity by higher aerobic respiration and also inducing inhibition [68]. Another evidence for this product formation shift due to oxygen is found with the aero-tolerant lactic-acid bacteria *Enterococcus sp.* found in termite guts. This bacterium mainly produces lactate when fed glucose under anaerobic conditions, but when oxygen is added to the system mainly acetate is produced [87]. Testing the growth of the fermentative bacteria *Lactococcus lactis* under complete anaerobic, aerobic and limited aeration conditions, Nordkvist *et al.* [64] reported that the predominant lactate production culture showed a shift in by-product formation from formate, ethanol and acetate under complete anaerobic conditions to acetate, acetoin, pyruvate and CO₂ under aerated and limited aeration conditions. They further reported that the maximum lactate yield and the minimum biomass yield were obtained under limited aeration conditions, compared to anaerobic and aerobic conditions. These studies indicate the possibility of using oxygen for controlling (by-) product formation in anaerobic biochemical processes.

As reported by Ghaly and El-Taweel [88], oxygen at very low concentrations is required by anaerobic yeast cultures to synthesize certain lipids needed to build cell walls. A similar finding was reported by Tango and Ghaly [89]. According to them, oxygen at low concentrations is essential for synthesizing oleic acid and ergosterol which are essential membrane components for certain anaerobic bacteria and, hence, stimulate the growth under anaerobic conditions. Franzen *et al.* [67] reported the need of trace amounts of oxygen for the yeast *Saccharomyces cerevisiae* in synthesizing sterols and unsaturated fatty acids. They further observed that limited aeration can be used to enhance the production of ethanol and minimize the formation of by-products like glycerol. According to Franzen *et al.* [67] high aeration levels are, on the other hand, found to be

disadvantageous due to the high aerobic respiration rates leading to increased biomass and CO₂ generation. They highlighted the need of determining the optimum oxygen limited condition by using a unit-less parameter like respiratory quotient which is defined as the ratio between the CO₂ evolution rate and the oxygen utilization rate. Experimenting on the effects of limited aeration on the growth of yeast *Candida pseudotropicalis* and production of ethanol during batch fermentation of cheese whey, Ghaly and El-Taweel [88] showed that increasing the rate of limited aeration decreased the lag period of growth and increased the specific growth rate, maximum cell number and also the substrate utilization rate. Meanwhile, the increased limited aeration increased the ethanol yield up to a certain aeration rate (up to 0.1 vvm. -volume of air per volume of culture per minute). Above that, the yield started to decrease as a result of the consumption of more substrate for biomass production, reducing the available substrate for ethanol production. It is known that some beer fermenting breweries use limited aeration effects to enhance their products (P. G. Rathnasiri - personal communication). Tango and Ghaly [89] studied the effects of limited aeration on the lactic acid production from cheese whey using anaerobic bacteria specie *Lactobacillus helveticus*. The findings are very similar to those by Ghaly and El-Taweel [88]. Limited aeration significantly improved the specific growth rate and enhanced the lactic acid yield while reducing the lag period of growth. The maximum cell number and also the substrate utilization rate increased with increasing aeration rate. Increasing aeration rate also increased the specific growth rate for any given initial lactose concentration, indicating that the limited aeration resulted in a culture that is more tolerant to substrate inhibition. After an optimum limited aeration rate of 0.1 vvm., the lactic acid yield started to decrease with increasing aeration. This is considered to be due to two factors: (i) utilization of more substrate (lactose) for cell synthesis (aerobic respiration) instead of product (lactic acid) formation (fermentation), and, (ii) oxygen toxicity to bacterial cells (apparent at the limited aeration rate of 0.15 vvm) which lead to an increased death rate of microbes. Using the facultative bacterium *Klebsiella pneumoniae*, Chen *et al.* [90] studied the different possible bioconversion pathways of glycerol into 1,3-propanediol (trimethylene glycol). Interestingly, a detailed stoichiometric and energetic analysis revealed that it was plausible to achieve a maximum theoretical yield of 0.85 mole 1,3-propanediol per mole glycerol under a limited aeration condition in comparison with the yield of 0.72 mol/mol in fully anaerobic conditions. They further concluded that a limited aeration condition could be more ideal for the intended bioconversion than the strict anaerobic or fully aerobic environment. In the study of Chen *et al.* [90], a partially aerated condition was realized by aerating the culture at the beginning to its oxygen saturation with an aeration rate of 0.4 vvm. No aeration was allowed during the growth phase thereafter. Chen *et al.* [90] suggested that many enzymes found in facultative bacterial cells are similarly active in both the anaerobic and aerobic environments.

In order to conduct some biochemical conversions advantageously under limited aeration conditions, Liden *et al.* [45] suggested using varying (ramped) aeration rates to tally with the dynamically varying conditions in the reactor.

This can be a suitable technique to exploit the maximum benefits of aeration, but it demands advanced control systems together with online measurements of reactor dynamics. Botheju *et al.* [46] pointed out that there exists an optimum oxygenation level giving rise to the maximum methane yield of an anaerobic digestion system but the optimum point may vary depending on several factors including the reactor biomass concentration and feed composition.

These findings show that limited aeration can be used as a way of improving anaerobic bio-conversions but optimization of the degree of aeration is necessary to achieve maximum benefits. The above findings also indicate that oxygen is essential for the stability of the cell membrane at least for certain anaerobic microbes. Further research would be helpful to confirm to what extent limited aeration can be used to optimize product formation of the bacterial and archaeal cultures typically found in anaerobic digesters.

2.4. Aerobic Substrate Consumption

With the availability of oxygen, facultative biomass can switch their metabolic function to aerobic respiration instead of fermentation, using readily available organic substrates such as monosaccharides, amino acids, long chain fatty acids (LCFA) and also volatile fatty acids (VFA) [91, 92]. This will generate CO₂ as the metabolic end product instead of the VFAs under anaerobic fermentation. The facultative organisms can rapidly switch between aerobic respiration and anaerobic fermentation [68].

Methane generation reduced with increasing oxygen loads in a CSTR exposed to stepwise oxygen loading increases from zero to 2.5%, 5% and 10% (as % of COD input) [28]. When the oxygenation levels were reversed from 10% to 0% in the same stepwise manner, the methane generation recovered rapidly at each step, showing no sign of significant biomass inhibition or damage due to oxygenation. Also the digester dynamics related to gas generation showed almost no hysteresis in increasing and decreasing oxygenation under the conditions tested. Increased CO₂ generation and reduced CH₄/CO₂ ratio are observed at increasing oxygenation. It is concluded that the oxidation of soluble organic matter by aerobic respiration lead to these results. Johansen and Bakke [49] also observed increased CO₂ production due to aeration while experimenting on the effects of limited aeration on hydrolysis in AD. Additional CO₂ generation under limited aeration conditions is noted also by Hao *et al.* [59] while experimenting on the effects of partial aeration in hydrolysis stage of AD. Shen and Guoit [54] reported that the methanogens in a granular sludge survived oxygen exposure for 18 days when the substrate was supplied. It is conceivable that the supply of substrate leads to the consumption of oxygen by facultative organisms and reduces the oxygen stress on methanogens. They further reported reduced methane generation and increased CO₂ generation due to aerobic substrate consumption. Joss *et al.* [93] mentioned that apart from aerobic respiration and CO₂ generation, partial aeration in AD does not influence other biochemical interactions significantly.

It is also likely that, if the oxygen exposure level is sufficiently high, facultative organisms would, in the long run, out-compete strict anaerobes by rapid substrate uptake

due to the high specific activities and growth rates. This can act as another form of inhibition of methanogens by substrate competition and would lead to reduced methane generation. Conklin *et al.* [81] reported that long term oxygen exposure led to reduced digester reserve capacity, probably caused by less methanogenic activity.

2.5. Oxygen Effects on Hydrolysis

Johansen and Bakke [49] showed that limited aeration can successfully be used to enhance hydrolysis during anaerobic digestion. These experiments were conducted in 500 ml batch reactors by supplying air with a rate of 500 ml/d. The follow-up analysis revealed that about 50% increase in hydrolysis (based on COD) was obtained in the aerated reactors compared to the positive controls of strict anaerobic reactors maintained under the same operating conditions (mesophilic, 37 °C). The ratio of extra hydrolysis to oxygen utilization is calculated to be about 0.4 mg C/mg O₂. The increase in hydrolysis with limited aeration was only observed related to the carbohydrates and proteins, but no significant influence was found on lipids. Despite the enhanced hydrolysis, the aerated reactors produced 50% lower methane compared to the strict anaerobic control reactors. Increased hydrolytic effect may have been undermined by the faster aerobic respiration in the presence of an increased level of oxygen. Johansen and Bakke [49] pointed out the necessity of optimizing the oxygen supply in order to avoid excessive oxidation of hydrolyzed products to CO₂. The biomass production was also found to be five times higher for the partially aerated reactors than that of the strictly anaerobic ones. While studying the effects of pre-aeration on the thermophilic anaerobic digestion of organic fraction of municipal solid wastes, Charles *et al.* [94] observed increased enzymatic activity related to the *cellulase* and *protease* (two hydrolytic enzymes) during the pre-aeration period. Drastic reductions of VFAs and soluble COD were also noted due to the aerobic substrate consumption. This is consistent with the findings of Botheju *et al.* [28] and Johansen and Bakke [49].

Wood feeding termites carry out an anaerobic mineralization process in their guts. Termites' extraordinary capability of degrading lignocellulosic substrate is believed to be derived from the rich microorganisms biota found in their guts and the diffusion of O₂ through their gut wall [87]. This is a natural evidence for the relation between extreme hydrolysis capabilities and free oxygen. Zitomer and Shrout [52] described a batch experimental series where an aerated reactor showed a significantly higher residual soluble COD compared to an anaerobic and mildly aerated reactors. Release of soluble matter from microbial cells was suggested. Effects of two different aeration levels (0.4 and 0.08 vvm.) on the solubilisation of biological sludge by the aerobic thermophilic bacterium *Bacillus stearothermophilus* under the thermophilic condition of 65 °C were investigated by Hasegawa *et al.* [34] in a continuous flow reactor. Both aeration rates resulted in approximately similar VSS (volatile suspended solids) solubilisation of about 40% after an HRT (hydraulic retention time) of 1 day. The VFA accumulation, on the other hand, was significantly higher for the low aeration rate than high aeration. The high aeration condition could have further oxidized the generated VFA while the low aeration rate was sufficient enough for the solubilisation

but was not enough for the subsequent VFA oxidation. Choosing the appropriate aeration level for solubilization without VFA oxidation is therefore obtainable. The enhanced solubilisation effects noticed by Hasegawa *et al.* [34] can also be contributed partly to thermal hydrolysis, in addition to the aeration effect.

Somewhat contrasting results of the aeration effects on hydrolysis are also presented: Hao *et al.* [59] found that high (“sufficient”) aeration promoted the hydrolysis of biodegradable carbohydrates and proteins, while lower “insufficient” aeration impaired the hydrolysis potential of the system. Nguyen *et al.* [95] also concluded that no evidence was available in their study to suggest a positive effect of limited aeration on hydrolysis. They, however, observed increased AD biogas production when limited aeration was used as a pre-treatment. Goel *et al.* [96] reported that there was no significant difference in enzymatic activity under anaerobic and aerobic phases of a digestion system consisting of sequential batch reactors.

It can be hypothesized that more acidogenic biomass leads to more hydrolysis, since hydrolysis is carried out by the extracellular enzymes excreted by acidogens. In the presence of oxygen, facultative acidogens can have a higher yield, leading to a higher population and more enzymes. Giannakis *et al.* [35] reported that supplying of additional facultative biomass into the hydrolysis/acidogenesis stage of a two phase biogas reactor led to enhanced performance of the acidogenic reactor which then made a clear positive impact on the methanogenic reactor. It is also conceivable that the facultative organisms excrete a higher amount of enzymes in the presence of oxygen. Another plausible scenario is that oxygen itself or some of its radicals may act as agents carrying out a chemical hydrolysis, but this is unlikely to be important at limited aeration conditions, since no or very little oxygen is available in the liquid phase. Nevertheless, chemical hydrolysis effects of some strong oxidizing agents like H_2O_2 in bio-gasification processes have been observed [97].

2.6. Sulfide Oxidation

Several studies [29, 80, 86, 98] have demonstrated that limited aeration can be used to oxidize the sulfides generated in anaerobic digesters operating on S containing wastes. In anaerobic digestion, S compounds are reduced to HS^- and H_2S by the biological activity of anaerobic sulfate reducing bacteria (SRB) [99]. Minimizing the sulfides content benefits the process by minimizing the toxic effect of aqueous sulfides on methanogenic and acetogenic organisms [29, 48, 80, 98] and also eliminates the formation of toxic, odorous and corrosive H_2S gas [11, 86]. SRB organisms compete with methanogens for organic substrates including acetate and hydrogen. Hence, S reducing activity leads to reduced methane yields [48, 80].

Zee *et al.* [86] using a fluidized bed anaerobic reactor (1.7 L) fed with low-sulfate vinasse, observed that the provision of an airflow of $0.7 - 0.9 \text{ m}^3/\text{m}^3 \cdot \text{d}$ corresponding to O_2/S molar ratios in the range of 8 - 10, resulted in a complete removal of H_2S presence in the biogas. They suggested that sulfide oxidation can happen both by biological and non-biological mechanisms, confirmed by batch test studies involving active cultures, sterilized cultures

and no biological cultures at all. The sulfide was observed to be primarily oxidized to elemental sulfur, thiosulfate and possibly polysulfate. Further oxidized forms such as sulfate did not occur under the low oxygenation conditions tested. Thiosulfate production was higher in the batch tests involving limited-aerobic culture. Sulfide removal rate was also faster in batch tests involving the partially aerated culture than the strict anaerobic culture. Zee *et al.* [86] further noticed that sulfide reappearance can also happen sometime after the sulfide oxidation, due to the activity of S reducing anaerobic organisms. However, this sulfide reappearance rate was always lower than the oxidation rate. The authors further concluded that oxygen uptake for sulfide oxidation could effectively over-compete the aerobic COD oxidation at these low aeration rates. Introduction of a low oxygen flow ($0.013 - 0.024 \text{ L O}_2/\text{L} \cdot \text{d}$) into pilot scale CSTR reactors (200 L) fed with waste-water treatment plant (WWTP) sludge was capable of removing H_2S in biogas almost completely without affecting the biogas generation, methane content or COD removal efficiency, according to a study carried out by Polanco *et al.* [29]. They pointed out that the contact between O_2 and the aqueous sulfides or the mass transfer also play an important role in the overall success of the approach. During a similar study on the partially aerated sulfide removal in AD [98] the level of air injection was precisely controlled by ORP (oxidation reduction potential) measurements so that oxygen inhibition of methanogens would not occur. H_2S content of the biogas was almost completely removed and more than 98% of the oxidized sulfide was recovered as elemental S with negligible sulfate production. These observations are similar to those made by Zee *et al.* [86]. Limited aeration (3 - 6 ml/L. min.) was used in a laboratory scale UASB reactor (10.5 L), fed evaporator condensate from a sulfite pulp mill, to overcome the serious sulfide inhibition, as reported by Zhou *et al.* [80]. Sulfide was oxidized mainly to elemental S which was non-toxic for microorganism. H_2S stripping caused by aeration was also thought to be playing a role in observed reduction of sulfides. Although the stoichiometric oxygen content needed to oxidize 1 mg of S was 0.38 ml, a much higher aeration rate was needed due to the mass transfer limitation in the reaction system [80]. The possibility of mass transfer limitations in sulfide oxidation was pointed out by Polanco *et al.* [29] too. Tang *et al.* [100] also reported that limited aeration of an anaerobic digester led to considerable reduction of the concentration of H_2S in the biogas. The aerated digester had a H_2S content of 5 ppm. as compared to the strictly anaerobic reactor having 700 ppm. Conducting a batch experimental series under strictly anaerobic and oxygen limited conditions, Zitomer and Shroot [52] observed that strictly anaerobic culture broths were black in color all the time while the oxygen limited broths became brown over time. This was explained as less reduction of sulfate and/or iron to ferrous sulfide under oxygenated conditions.

2.7. Effects on Intermediate Products (VFA)

According to Botheju *et al.* [28], introduction of partial aeration may lead to a reduced accumulation of VFA in anaerobic digesters. Introduction of aeration (2.5% O_2 load as % of feed COD) in a semi-continuously fed anaerobic digester (fed a synthetic substrate mixture containing starch,

peptone and yeast extract) having 33 days HRT (hydraulic retention time) and a 0.33 kg COD/m³. d OLR, immediately resulted in a significant reduction (80%) of total VFA content. Further increasing the O₂ load to 5% and 10% led to even less VFA. The high initial VFA level was not recovered when the oxygen load was removed. This suggests that oxygenation can be helpful for stabilizing anaerobic digesters in their start-up period or during periods of reactor instability characterized by high accumulation of VFA. Following a three stage aerobic-anaerobic-aerobic sequential batch treatment of organic solid wastes, Simon [20] observed that the primary aeration stage considerably reduced the VFA accumulation and, thus, avoided digester souring of the anaerobic stage, resulting in a better methanogenic phase.

Rathnasiri *et al.* [78] observed a synchronized behavior of VFA with cyclic introduction of oxygen into a type of anaerobic reactor called Membrane Micro-aerated Anaerobic Digester (MMAD); higher oxygen utilization rates were detected corresponding to high VFA levels and vice versa. Zhou *et al.* [80] observed reduced VFA accumulation due to limited aeration in a UASB reactor. Effluent COD content was also reduced after aeration. Aerobic oxidation of reaction intermediates such as VFA and H₂ was suggested by Zitomer and ShROUT [52] too. Chu *et al.* [51] suggested the possibility of VFA and hydrogen oxidation due to the oxygen presence in their membrane granular sludge bed reactor operated under oxygen limited conditions. Gerritse *et al.* [74] also observed aerobic consumption of acetate and propionate when they increased the oxygen supply rate into a co-existing anaerobic and aerobic culture. According to an experiment conducted by Hasegawa *et al.* [34] to investigate the effects of pre-aerating biological sludge before biogasification, VFA oxidation was found to occur under higher aeration conditions while maintaining low aeration levels led to the accumulation of VFA.

It has been suggested that aeration might increase the proportional content of acetate in total VFA generation. Chu *et al.* [101] reported about 70 - 80% acetate content in total VFA by using an air flow rate of 0.126 volume of air per volume of sludge per hour, in a thermophilic *aerobic* digestion process. In comparison, a complete anaerobic fermentation process only generated acetate in the proportion of ~ 40 - 50% [101]. That study further suggested VFA oxidation due to aerobic respiration. It is possible that the observed higher acetate content can be caused by the inhibition of methanogens under oxygenated conditions which would otherwise have consumed acetate. Aerobic VFA consumption on the other hand can similarly use up all the types of VFAs [28] and, therefore, do not offset the acetate proportion. Chu *et al.* [101], nevertheless, argued the possibility of an alternative oxygen assisted biochemical process which generated acetates, in addition to the common fermentation. However, strong experimental proof of the existence of such a biochemical pathway was not provided. VFA generation in *aerobic* sludge digestion processes is reported by several authors [101-104], mainly caused by low aeration and the creation of anaerobic zones where the fermentation can occur.

According to Tang *et al.* [100], limited aeration of 7.5% (v/v) of the biogas generation rate did not lead to any difference in VFA concentration. Joss *et al.* [93] too

observed that the amount and composition of VFA did not change when supplying oxygen into an anaerobic digester.

The contradicting results published can be explained as follows: It is likely that generation of additional VFA by the increased activity of acidogens and the consumption of VFA due to aerobic respiration and methanogenesis occurs concurrently in anaerobic digesters which are being partially aerated. Hence, the outcome of VFA concentration depends on the relative rates of generation and consumption of VFA. This will vary under different test conditions. Under stable and sound operating conditions, however, the consumption rate of VFA by aerobic respiration and methanogenesis should supersede the rate of VFA generation.

2.8. Effects on pH

Aeration in AD can, as documented above, lead to reduced VFA accumulation and thereby prevent pH drops. A stripping effect on CO₂ due to aeration can also prevent pH drops. According to Zitomer and ShROUT [52], partially aerated complete-mixed digesters recovered from a pH drop more quickly than un-aerated reactors. Guiot *et al.* [105] observed CO₂ stripping due to aeration, resulting in significantly higher pH in an aerated UASB reactor (aeration in recycle stream) compared to a strictly anaerobic reactor. Simon [20] reported that when pre-aeration was not used, pH dropped to a lower value of 5.2 in a reactor treating solid organic wastes. Botheju *et al.* [27], also observed a high initial pH drop in an un-aerated anaerobic reactor, compared to the aerated ones.

According to an experiment described by Zitomer and ShROUT [52], when given a shock load of 4 g COD/L.d to a series of bench scale batch reactors, the pH dropped to 5.0 from the initial 7.0. The completely anaerobic reactor did not recover to neutral pH in 52 days. The reactors supplied with limited oxygenations of 1 and 0.1 g/L.d returned to the initial pH after 34 and 28 days, respectively. Reduced VFA content and stripping of CO₂ and H₂ due to aeration were suggested as the reasons for this faster pH recovery. According to Zitomer [53], extra CO₂ generation due to aeration may demand higher alkalinity for neutralizing CO₂ acidity in anaerobic digesters, but this is not supported by experimental evidence.

Noticeable pH differences were not obtained in continuously fed anaerobic digesters operating under different oxygenation levels [28, 54]. This implies that limited aeration has no significant influence on pH under relatively stable operating conditions but it can prevent instabilities and augment recovery from pH instabilities.

2.9. Methanotrophic Effect

Presence of the specific microbial group called methanotrophes, which are capable of oxidizing CH₄ into CO₂ and water, might induce a negative effect on the methane potential of anaerobic digesters. These methanotrophes are mainly aerobic organisms and thus, in the presence of oxygen, they might play a significant role if sufficient amounts of organisms are available. It is estimated that some 17% of the natural methane generation in the world is degraded thanks to the action of methanotrophes in the open environment [1]. Some anaerobic methanotrophes have also been found [1].

The significance of methanotrophic processes in AD has not been widely reported. In several batch experimental series involving headspace aeration, Botheju and Bakke [47] detected a slight decrease in gas volume with time after the biogas generation had been halted due to substrate exhaustion. The methanotrophic methane consumption could have occurred throughout the experimental duration, but could only be detected when the gas generation stopped. Kato *et al.* [44] recognized methanotrophic bacterial groups in an anaerobic reactor exposed to prolonged aeration. Zitomer and Shrout [52] observed that exposing an anaerobic culture to 125% of oxygenation (as % of COD input) resulted in very little methane in the off-gas but the culture was showing a high methanogenic activity. They concluded that aerobic oxidation of methane could have been the reason for this. Zitomer [53] also suggested methanotrophic activity caused by aerobic or microaerophilic bacteria. Zitomer [53] further cited the possibility of methane-utilizing methanotrophes to carry out co-metabolic biodegradation of highly chlorinated substances like tri/tetra chloro-ethylene, using methane as a co-substrate. If this claim can further be substantiated, it would make anaerobic digestion under partially aerated conditions even more attractive as a treatment strategy for bioremediation of a multitude of halogenic substances.

2.10. Biogas Generation and Methane Yield

It has been observed that limited aeration can enhance the methane yield in anaerobic digestion [27, 46, 47]. Botheju *et al.* [27, 46] studied the effects of different air and oxygen headspace volumes in anaerobic batch bioreactors fed with starch. They observed positive response due to oxygenation for a range of oxygenation loads (0 – 16%) and also predicted the existence of an optimum oxygenation level corresponding to a maximum methane yield [46]. A few other studies also suggested that limited aeration can lead to enhanced methane generation [50, 74, 106]. Pirt and Lee [50] observed that traces of oxygen enhanced the anaerobic digestion of algal biomass in batch mode reactors. They reported that digesters fed with air produced biogas faster than strict anaerobic condition. Gerritse *et al.* [74] also reported that about a 20% increase in methane production was observed at low oxygen fluxes in a chemostat (dilution rate 0.06 h^{-1}) provided with headspace oxygen. Further increasing the oxygen flux resulted in the washout of methanogens and accumulation of substrate. Botheju *et al.* [106-108], described the use of limited aeration in AD for the treatment and bio-gasification of waste monoethanolamine (MEA) generated in post combustion CO_2 capture. Net methane generation by the degradation of waste MEA was positively influenced by the increasing oxygen loads (in the range of 0 - 0.39 ml O_2/ml reactor content). The effect was more pronounced at a higher initial equivalent MEA concentration of 500 mg/L compared to a 125 mg/L concentration.

Tang *et al.* [100] reported that a microaeration level of 7.5% (v/v) of the biogas generation rate did not make any difference in either biogas generation rate or methane concentration. However, their feed contained S compounds and they observed significant H_2S oxidation. The supplied oxygen could have been completely spent on H_2S oxidation. This observation is consistent with the study by Polanco *et*

al. [29] where an aeration load of 0.013 – 0.024 L $\text{O}_2/\text{L} \cdot \text{d}$ does not affect the biogas/methane generation but only the H_2S oxidation. It is clear that H_2S oxidation processes (both chemical and biochemical) are faster and can easily out-compete the other oxygen utilizing biochemical processes; even the aerobic respiration [86].

Decreased amounts of methane generation under increased aeration conditions have also been widely reported [28, 54, 91, 92]. O'Keefe and Chynoweth [92] observed decreased amounts of methane generation coupled with oxidative loss of soluble organic matter while experimenting on aeration effects in simulated landfill cells. According to Mshandete *et al.* [91], 9 hours of aerobic pre-treatment enhanced the methane potential from mesophilic anaerobic digestion of sisal pulp waste, but noted that increasing further aeration only resulted in a significant loss of methane potential due to aerobic substrate consumption. Shen and Guiot [54] reported lower methane yields in UASB-like reactors under various oxygenation rates, allegedly caused by aerobic substrate utilisation. According to their experiment, different dissolved oxygen concentrations of 0, 0.6, 2.1, 4.3 and 8.1 mg/L were maintained in the liquid phase of the UASB-like reactor fed with a synthetic substrate composed of 75% sucrose and 25% acetate. These conditions resulted in the CH_4 yields (as % of input COD) of 64.1, 58.7, 55.1, 47.9 and 41.6 respectively and the CO_2 yields (L/g COD removed) of 0.23, 0.25, 0.33, 0.32 and 0.39 respectively. But, the specific anaerobic activity, related to the different substrates of glucose, propionate, hydrogen and acetate, of the granules were found to be not impaired by the aeration even after 3 months of operation. An exception to this was the 8.1 mg/L case in which the granular activity was reduced only after 3 months of operation. Interestingly, over a short period (1 month) even a higher activity was observed for acetate and glucose uptake, for the oxygenation level of 2.1 mg/L [54]. It is observed that reducing methane yields under increasing oxygen loads can approximately be described using near linear functions [25, 46]. The data reported by Shen and Guiot [54] support this idea.

Increased CO_2 generation can happen as a result of aerobic substrate consumption in partially aerated anaerobic digestion. Even in the cases where a mild aeration condition improved the methane yield, a still higher increase of CO_2 generation was observed, hence reducing the CH_4/CO_2 ratio upon increasing the aerobic load [27].

It is suggested that the response of oxygen in biogas generation can either be positive or negative based on several parameters, including the hydrolysis rate coefficient, biomass concentration, and in the case of continuous reactors, hydraulic retention time, organic loading rate, and the sludge age [46]. Increased hydrolysis rate coefficient (overall second order [25]) and increased biomass concentration will cause the optimum oxygenation point to shift towards zero. This means that under high "hydrolysisability" conditions, there would not be any positive response by oxygen addition on the methane yield and the sole effect would become negative [28, 46]. In continuous operation mode, it is likely that low HRT conditions which would give rise to lower biomass and a shorter time for hydrolysis may favor positive oxygen effects while higher HRT with sludge recycling might lead to negative effects.

Zeng and Deckwer [68] discussing the use of limited aeration in anaerobic production of 2,3 –butanediol (BD) pointed out that there is an optimal oxygen utilization rate which gives the maximum BD productivity for each hydraulic retention time (HRT). These authors also recognized the importance of biomass concentration in deciding the optimum aeration level.

3. OXYGEN EFFECTS ON MICROBIAL ECOLOGY

Tang *et al.* [100] concluded that limited aeration did not produce a significant phylogenetic diversity in their thermophilic (53 °C) anaerobic digester fed with synthetically made municipal solid waste, aerated continuously by air with a rate of 7.5% (v/v) of the biogas generation rate. This simply means that limited aeration may not lead to dramatic differences in the microbial community structure. Tang *et al.* [100] arrived at this conclusion by establishing and comparing the genetic diversity trees for the cultures grown in partially aerated and fully anaerobic reactors. Further they noticed that several other parameters including VSS digestion efficiency, TOC (total organic carbon) concentration, VFA concentration, biogas generation rate and also the concentration of methane were almost the same for two different digesters (partially aerated and fully anaerobic). However, the concentration of H₂S in the biogas was considerably lower for the partially aerated reactor. Interestingly, the authors also noticed that although H₂S concentration was low in the aerated reactor, according to the gene analysis there was no evidence for any reduced SRB activity in the partially aerated reactor. This may suggest that H₂S reduction in this partially aerated reactor was caused mainly by a chemical effect of oxidation of H₂S rather than a result of inhibition of SRB. The results of the study by Duangmanee *et al.* [98], which has been discussed earlier, can also be explained by this argument. Though limited aeration did not change the phylogenetic diversity, it did reduce the size of the population of a group of methanogens, *Methanosarcina*, by about 50%, and conversely the population size of another group of methanogens, *Methanoculleus*, was dramatically increased, by almost 100% [100]. This is evidently due to the difference in oxygen tolerability of different methanogenic organisms as discussed before; in the presence of oxygen, certain microbial species with higher oxygen tolerability can out-compete others having less oxygen tolerance.

Zee *et al.* [86] suggested that there is a possibility of growth of (poly) sulfide oxidizing bacteria in a partially aerated anaerobic digester fed with low sulfate vinasse. The sulfide removal rate was higher for the batch tests conducted with partially aerated culture compared to a complete anaerobic culture. Conversely, after sulfide reduction, sulfide reappearance was also faster in the partially aerated culture indicating the possibility of an increased population of polysulfide, sulfur and thiosulfate reducing bacteria [86]. Studying the sulfide removal by limited aeration in a UASB reactor fed evaporator condensate from a sulfite pulp mill, Zhou *et al.* [80] observed that aeration caused rod shaped methanogens to disappear from the sludge granules and afterwards the filamentous and cocci shaped methanogens predominated. Initially the granules consisted of mainly filamentous methanogens and some amounts of rod shaped methanogens. This observation suggests that certain shifts in methanogenic species population can occur due to aeration.

According to the study by Chen *et al.* [109], it was observed that anaerobic attached film expanded bed reactors (AAFEB) with pre-aerated biofilm carriers had an increased ability to withstand shock load conditions compared to the non pre-aerated ones and concluded that pre-aeration helped the growth of a more diversified and a viable culture. Pre-aeration further helped a faster biofilm formation, significantly shortening the start-up time. Faster growth of microbes under aerobic conditions can also generate higher amounts of exopolymers, which are the key binding agents for biofilm formation [110]. Additionally, aeration may have supported the growth of filamentous organisms which can capture free flowing organisms and accelerate biofilm growth [109]. It is conceivable that pre-aeration can be used as a technique to shorten the start-up time of regular UASB reactors by faster granule formation. Further studies are, however, needed to confirm this possibility.

Tango and Ghaly [89] and Ghaly and El-Taweel [88] demonstrated that the limited aeration greatly affects the cell growth. At a given substrate concentration, increasing aeration resulted in a higher specific growth rate, hence the culture became more tolerant against substrate inhibition. Furthermore, aeration shortens the lag period of the microorganism growth pattern. This indicates that partial aeration is a successful strategy for recalcitrant waste digestion. However, Conklin *et al.* [81] reported that micro-aeration lead to reduced digester reserve capacity (capacity to tolerate shock loads in AD), probably caused by the lesser activity of methanogens in the presence of oxygen. In contrast, Zitomer and Shrout [52] mentioned that the specific methanogenic activity was higher for some cultures which received oxygen than the cultures maintained under strict anaerobic conditions. Even though Shen and Guiot [54] have also reported such an observation over a short period (1 month) of operation of their UASB-like reactor, these observations must further be elucidated with more confirming experiments and theoretical explanations.

Under very low dissolved oxygen concentrations (<1µM), growth of a specific group of bacteria called “microaerophiles” can occur [66]. These non-fermentative bacteria do not belong to any of the conventional microorganisms groups of anaerobic, aerobic or facultative. This fourth kind of organism can only thrive within a niche of very low dissolved oxygen range which is left blank due to the low activity of anaerobic, aerobic and also facultative organisms at this specific low oxygen range [66]. Whether microaerophilic organisms can be present in significant numbers in anaerobic digesters maintained under limited aeration conditions or whether they would play any significant role in such cases is still unclear and should be addressed in future studies. But it can be predicted that their activity will mainly be restricted to aerobic respiration as this group of organisms are known to be non-fermentative.

4. OPERATION OF PARTIALLY AERATED ANAEROBIC BIOREACTORS

4.1. Co-Existing Anaerobic - Aerobic Cultures

Co-existence of anaerobic and aerobic respiring cultures is possible due to the several deterrence mechanisms explained before, namely, oxygen shielding effect by facultative organisms, diffusion barriers, and limited intrinsic

oxygen tolerance of some strict anaerobes. Simultaneous maintenance of both aerobic and anaerobic activities in (semi) continuous bioreactors has been demonstrated in a study conducted by Botheju *et al.* [27, 28]. Simultaneous oxygen consumption and methane generation was observed. In this study, methanogenic activity was sustained with no notable prolonged inhibition under the presence of oxygen (up to 10.1% of input COD) in a suspended biomass semi-continuously fed and partially aerated reactor system. The methane yield showed a negative impact by the aeration, but the lower COD content under partially aerated operation resulted in a superior effluent quality.

By carrying out a biofilm simulation on a membrane micro-aerated anaerobic digester (MMAD), Botheju *et al.* [79] indicated the possibility of having a fixed film of biomass which could perform aerobic metabolism even in an anaerobic bulk phase. A high amount of facultative organisms was seen growing at the bottom of the biofilm (on the membrane surface, where free oxygen is available), while anaerobic organisms dominate near to the membrane-bulk liquid interface. Zitomer and Shrout [52], also demonstrated the feasibility of maintaining aerobic respiration and methanogenesis using a common mixed culture in a single bioreactor. Zitomer [53] emphasized the necessity of investigating the co-existing anaerobic/aerobic activities in continuous feed reactors.

Gerritse *et al.* [74] observed that co-existing bacterial cultures of obligate aerobe *Pseudomonas testosterone* and anaerobe *Veillonella alcalescens* were sharing a common habitat and competing for a common substrate over a wide range of oxygen supply rates, confirming the possibility of the simultaneous maintenance of aerobic and anaerobic activities in a single bioreactor. Furthermore, Gerritse *et al.* [74] showed that the anaerobe *V. alcalescens* could not grow alone in an aerobic media and its growth was completely inhibited at a very low oxygen level which was even below the detection limits of the measuring instruments used. This highlights the fact that anaerobic organisms mostly depend on the aerobic activity of their counterparts to shield themselves from high oxygen tensions. Also, Gerritse *et al.* [74] were able to control the competition between these two organisms by controlling the oxygen supply rate. At increasing oxygen supply rates, CO₂ production increased due to the higher activity of *P. testosterone*. The acetate and propionate concentration decreased mainly due to the consumption by *P. testosterone*. Accordingly, it is deemed possible to manipulate the substrate competition between facultative organisms and strictly anaerobic methanogens by adjusting the oxygen input to the reactor, as also observed by Shen and Guioit [54]. This strategy enables bioreactors to perform simultaneous oxygen consumption, acid production and methane generation and also may improve the methane yield of the reactors. Kato *et al.* [44] discussed several such cases and suggested that enhanced methane generation could be realized due to the enhanced production of acids by facultative bacteria, hence stimulating the methanogenesis with more substrates (especially in the case of particulate feeds).

4.2. Degradation of Recalcitrant and Toxic Organics Using Aeration Assisted AD

Effluent streams generated by anaerobic digesters can contain an undigested COD fraction which is composed of

different refractory organic matter resisting anaerobic degradation [54, 111]. Various types of pre-treatment for AD has been suggested for enhanced waste treatment and stabilisation, such as thermal, ultrasonic, chemical (ozone, H₂O₂, etc.) and enzymatic pre-treatment [34, 97, 112-115]. The feasibility of many such treatment options are, however, not well proven either economically or technically. Partially aerated anaerobic digestion can be a viable and simple alternative for enhanced waste treatment.

Co-existing anaerobic - aerobic reactor systems may also open new opportunities for biodegradation and bioremediation of recalcitrant compounds and toxic xenobiotics [54]. There are a number of case studies showing that a combined activity of aerobic and anaerobic organisms is needed for degrading or mineralizing some chemical substances [44, 68]. Simultaneous aerobic/anaerobic reactor systems can be an attractive alternative because of significantly lower capital and operating cost.

According to a study on the use of partial aeration in AD treating monoethanolamine (MEA) wastes generated in post combustion CO₂ capture, Botheju *et al.* [106, 107] indicated the possibility of enhanced methane generation from MEA wastes using partial aeration assisted AD. This observation can be explained by the MEA degradation mechanisms suggested by Ohtaguchi *et al.* [116]. The presence of oxygen in the digester may have enabled it to degrade MEA to CH₃COOH with faster kinetics and hence the anaerobic culture did not have to cope with a high MEA concentration and resultant substrate inhibition. An associated BOD (biological oxygen demand) test also revealed that aerobic degradation of MEA is possible with faster kinetics and also with higher amine concentrations compared to anaerobic degradation [106]. The hypothesis of Tango and Ghaly [89] and Ghaly and El-Taweel [88] that enhanced cell yields by aeration lead to stronger cultures which are more tolerable for substrate inhibition, may also be relevant in explaining these observations.

Some compounds or micro-pollutants inducing a toxic effect on one group of organisms can be used as a substrate for other organisms. For example, in forest industry wastewater, the BOD caused by resin acids are toxic for anaerobic cultures but can be consumed by aerobic or facultative organisms [44]. Aeration assisted AD could therefore be suited for energy recovery from pulp/paper industry wastewater containing such resin acids.

The ability of the facultative microorganisms to break down azo-bonds in azo dyes used in textile industry, under low aeration conditions has been documented [117]. Azo compounds resist the oxidative degradation by aerobic bacteria [118], due to the chemical structure of the N=N azo-linkage. These compounds can however be reduced under either strict anaerobic or partially aerated conditions by the action of strict anaerobic or facultative anaerobic organisms [117]. This reduction process generates aromatic amines [119] which can be completely degraded by aerobic biodegradation. Hence, an anaerobic treatment design with limited aeration can achieve complete degradation of azo dyes containing textile wastewater. Use of an additional readily degradable co-substrate can sometimes be necessary [52, 106, 117]. Sandhya *et al.* [117] used starch as a co-substrate in their investigation. Botheju *et al.* [106] used this

co-metabolic approach with apple juice as the co-substrate for the successful anaerobic methanogenic degradation of waste MEA generated in post combustion CO₂ capture. This acidic co-substrate addition further helped to decrease the above-neutral pH conditions typical for amine wastes. Furthermore, a more suitable C/N ratio was realized by adding this C rich co-substrate. The usefulness of aerobic-anaerobic treatment schemes for recalcitrant, persistent and eco-toxic organic waste, has also been pointed out by Song and Logan [56]. A few case studies on the degradation of various chlorinated compounds and polycyclic aromatic compounds by Zitomer and Shrout [52] indicated that oxygen can promote different degradation reactions of various compounds by different mechanisms.

4.3. O₂ Effects in High Rate Aggregated Biomass Reactors

Quite different to the completely mixed suspended biomass (CSTR type) reactors, higher unintentional aeration can occur in high rate reactor configurations like up-flow anaerobic sludge blanket (UASB) and expanded granular sludge bed (EGSB), mainly due to the very high hydraulic loading conditions and extensive recycle streams [44]. Inflows of such reactors can carry a significant amount of oxygen load as dissolved oxygen and such reactors are often used for relatively low organic feed concentrations, leading to significant inflow O₂/COD ratios. Kato *et al.* [55] claimed that oxygen toxicity would never occur due to dissolved oxygen entering anaerobic digesters with feed influent streams. Granular/aggregated sludge provides diffusion protection from oxygen for strict anaerobes in such processes, preventing detrimental effects. Using microelectrodes and confocal scanning laser microscopy, de Beer *et al.* [120] showed that oxygen did only penetrate into the first 30 μm of a 90 μm thick biofilm cell cluster. However, oxygen was found in biofilm pores and channel-like structures. Methanogenic biofilms are known to be more porous and channeled than aerobic films, probably because methanogenic films generate large amounts of gas [120, 121]. Kato *et al.* [44] suggested that oxygen generally could not penetrate more than the first few hundred micrometers in thick biofilms. According to Chu *et al.* [51], methanogens in granular sludge are protected due to diffusion limitation of oxygen. Methanogenesis mainly occurs deep inside the granules while facultative and aerobic biomass dominate near the surface of the granules [54]. Kato *et al.* [55] observed that a granular sludge was capable of maintaining high methanogenic activity together with high oxygen tolerance. A considerable methanogenic activity was still present even at a dissolved oxygen concentration of 12.4 mg/L (using pure oxygen). Wu *et al.* [122] demonstrated the possibility of establishing anaerobic granular sludge in a UASB reactor seeded with floccular sludge from an aeration tank treating sewage.

Chu *et al.* [51], experimenting on a membrane bioreactor with granular sludge bed seeded with anaerobic granules, observed simultaneous removal of organic substrates and nitrogen from synthetic wastewater containing sugar, potato starch and peptone, under an oxygen limited operation. In this case, in addition to methane generation, nitrification may have occurred on and near the surface of the granules, with denitrification in deeper layers. Aeration was carried out by

15 min. on and 15 min. off cycles with a 3 - 4 g O₂/L.d load. It was observed that the granules adapted to the oxygen limited operation and became slightly lower in density (1030 kg/m³ for anaerobic and 1010 kg/m³ for partially aerated operation). The specific methanogenic activity decreased by ~ 25% (1.48 and 1.12 g CH₄-COD/g VSS. d for anaerobic and partially aerated, respectively). The experimental reactor used by Chu *et al.* [51] had two distinguishable reaction zones: A limited aeration zone within the granular sludge bed and a more aerobic zone above the granular sludge bed. Shen and Guiot [54], also observed that the sludge volume index (SVI) of a granular sludge increased due to exposure to increasing levels of oxygenation, resulting in reduced sludge settling and some sludge washout. The level of dissolved oxygen concentration was found to be correlated to the sludge washout rate [54]. This could have been caused by growth of a fluffy aerobic or facultative biomass on the granule surface.

Kato *et al.* [55] observed that the granule diameter did not correlate with the oxygen tolerance of anaerobic granular sludge. Also they did not detect any effect on the oxygen tolerance of granules even after they crushed the granules to reduce their size. This implies that granules generally have a much greater thickness (0.5 – 5 mm [123]) compared to what is needed to protect them from complete oxygen penetration. De Beer *et al.* [120] reported oxygen penetrated to only about 30 μm depth in a biofilm.

4.4. Limited Aeration as a Pre-Treatment in AD

Limited aeration can be used as a pre-treatment prior to anaerobic digestion to enhance the digestibility of different types of wastes. Limited aeration has been tested as a pretreatment prior to anaerobic digestion of municipal organic solid waste by Nguyen *et al.* [95] and Juanga *et al.* [124]. According to these two studies, waste bed reactors which were effectively partially aerated by sending air through the bed (flow rate 0.4 L/kg. hr.) in a “2 hr. run 4 hr. stop” mode for a 5 day period before inoculation and anaerobic digestion phase started, showed a considerably improved methanogenic stage performance compared to the reactors which were not aerated. Not only did the pre-aerated reactor produce a higher biogas volume but it also reached the active methane phase (50% methane in gas) quicker than the rest of the reactors. This is a clear indication of a positive effect that partially aerated pre-treatment can pose on anaerobic digester performance. The rapid increase in gas generation observed just after the lag phase may suggest that partial aeration could have also resulted in better acidification providing enough substrate for methanogens. Akashi *et al.* [125] reported a significant increase in biogas generation as a result of partially aerated pre-treatment. They observed an impressive 1.5 times higher biogas generation from biological sludge when pretreated by partial aeration than the anaerobic operation alone. Experimenting on the effect of thermophilic (65 °C) aerobic pre-treatment on biogasification, Hasegawa *et al.* [34] showed that a low aeration rate of 0.08 vvm. led to an increased biogas generation by a factor of 1.5, compared to an untreated sludge. The higher aeration rate of 0.4 vvm. had only a slight positive effect on biogas generation. Pre-aeration helped increase methane generation at the start-up of a biogas reactor treating organic

solid waste, by avoiding the digester souring, according to Simon [20].

4.5. Post Anaerobic Aeration

Aerobic respiration activity can increase the sludge production [126, 127] of a digestion process. However, lower level of aeration may result in less production. Use of anaerobic digester sludge (digestate) as a valuable fertilizer or a soil amendment is promoted [13, 17-19, 128] as a sustainable nutrient recycling process. It has been observed that AD increases the plant available nutrient content of the organic wastes [13]. In that sense, partial aeration can result in a more stable and a safer product. This is due to the reduced contents of organics, malodorous products (H_2S , VFA) and pathogens [28, 29, 129]. Botheju *et al.* [19, 128] reported the possibility of using post-anaerobic nitrification to greatly reduce the content of toxic heavy metals content in an anaerobic digestate from a plant treating municipal organic wastes. This allows the digestate to be qualified as a high ranking organic fertiliser in addition to the achievement of N stability. Other authors have suggested post-anaerobic aeration as a polishing stage for anaerobic digestates in order to improve C and N removal [44, 130].

Nitrification by post aeration of anaerobic effluents as described by Botheju *et al.* [19, 128] can be a useful way to convert digestates rich in ammoniacal nitrogen into a valuable liquid N fertilizer product. The common practice today is to dewater the anaerobic effluents and to use only the solid fraction as a soil amendment or a fertilizer, while the liquid fraction is discarded as wastewater. This release of NH_4-N can account for up to 50% of the total ammonium load of a municipal wastewater treatment plant [131]. Post-anaerobic aeration of digestates is useful also as a secondary treatment to achieve better effluent quality and sometimes to comply with local regulations on land application of digestates [130]. Anaerobic digestion of typical municipal wastes often lead to effluent streams with 1000 - 3000 mg/L NH_4-N and COD loads higher than 1000 mg/L [19, 131, 132]. Nitrogen present in protein containing food residues ends up as NH_4-N in anaerobic effluents that cannot be further converted into nitrate (NO_3-N) without an aerobic treatment stage [133]. When the anaerobic effluent is used as N fertilizer, which is increasingly encouraged as a nutrient recycle strategy, nitrate (NO_3-N) is the preferred form of N, as ammoniacal nitrogen (NH_4-N) is more unstable and also can lead to root zone acidification in plants [134]. NO_3-N , on the other hand, is a highly mobile and easily available N source for crops [135]. Accordingly, the post anaerobic nitrification scheme proposed by Botheju *et al.* [19, 128] can be a reasonable way of recycling nutrients in AD treatment. It is shown that over 70% ammoniacal N conversions can be achieved and pH can be used as a simple and good enough monitoring parameter for the successful operation of the process [19, 131]. Interestingly, it has been reported that ammonia oxidizing (nitrifying) bacteria can survive unharmed in anaerobic digesters [130]. This suggests that co-digestion of waste activated sludge and municipal organic wastes can be useful in achieving high nitrifying biomass contents in post-anaerobic nitrification. Nitrifiers are slow growing microorganisms [133] and highly susceptible to washout and overload [19]. Furthermore, it may be worthwhile to study the possibility of simultaneous methanogenesis and

nitrification under oxygen limiting conditions. Some N loss through denitrification will, however, occur under reduced conditions. Even though, Lie and Welander [136] stated that denitrification can be inhibited at very low DO concentrations (< 0.1 mg/L), Bakke (R. Bakke - unpublished data) observed that denitrification occurred in partially aerated methanogenic reactors.

4.6. Self Heating Capacity

Aerobic respiration activity associated with an anaerobic process can lead to a significant biochemical heat generation [94]. This heat generation can be used to elevate the reactor temperature into the thermophilic range (~ 55 °C). Thermophilic anaerobic digestion generally delivers superior process efficiencies compared to mesophilic (~ 35 °C) digestion [114, 137], mainly due to the faster kinetics at elevated temperatures [22, 138]. Another aspect of great importance is that thermophilic anaerobic digestion can extensively reduce the pathogens content in the digestate [61, 129, 137]. Smith *et al.* [129] concluded that a mere 20 - 60 min. holding time was sufficient to achieve a complete pathogen destruction in thermophilic (at 55 °C) anaerobic digestion, while mesophilic digestion could not demonstrate adequate pathogen reduction. Anaerobic digester operators in some countries are legally directed to heat up their digestates in order to ensure pathogen reduction [139]. For that reason, some plants are designed to operate in sequential anaerobic-aerobic mode completing the pathogen destruction in the aerobic stage [139]. Aerobic-anaerobic sequential treatment can also be applied. In Norway, an upstream micro-aerobic hygenisation stage (temp > 70 °C and retention time > 2 hrs.) is used in digesters operating on municipal wastewater sludge [28]. This configuration was also applied to enhance the digester efficiency, probably caused by enhanced hydrolysis due to partially aerated pre-treatment and elevated temperature. By this means, a part of the energy lost owing to reduced methane yield due to aerobic respiration can be reclaimed as microbial heat. Further, a common observation is that thermophilic digesters are relatively unstable compared to the mesophilic operation [61, 138, 140]. However, partial aeration can successfully be used to enhance the stability of such digestion systems through reduced accumulation of VFAs [28].

Joss *et al.* [93] stated that partial aeration in anaerobic digestion can supply a heat yield of 14.5 MJ/kg O_2 .

4.7. Explosion Safety

Methane and oxygen can form dangerous and explosive gas mixtures [31, 141] within the explosive range of 5 - 15% of CH_4 [142]. There is a likelihood of creating such gas mixtures in a partially aerated anaerobic reactor. Hence, any experimental or large scale bio-gasification process involving oxygen/aeration must consider this fact and consider necessary safety designs. If such a possibility is identified, actions must be taken to adjust the aeration rate so that the explosion range will be avoided. It is, nevertheless, observed that the presence of various inert gases like CO_2 , water vapour and N_2 can greatly reduce the explosion characteristics of methane air mixtures [143]. When adequate and standard safety measures such as venting and spacing, flame traps at gas inlets/outlets, and inline CH_4 sensors with alarm systems are incorporated into the plant

design, any significant threat from an explosion hazard can be minimized.

4.8. Different Means of Partial Aeration in AD

Supply of air directly into the liquid media of the reactor is the commonly used way of oxygen induction, adopted in many studies [28, 49, 53, 59, 100]. A part of the oxygen supplied can however escape into the headspace. This escaped amount of the supplied oxygen can diffuse back into the liquid medium if the aeration is semi-continuous; otherwise a part can be lost with the biogas stream.

Supply of air in headspace in equilibrium with the liquid phase has been used in some studies [25, 27, 47, 74, 106]. This is a suitable way of aeration when only a minute amount of oxygen is intended to be given, especially for research purposes. Use of pure oxygen instead of air is an alternative [47, 105]. According to Guiot *et al.* [105], air addition did not cause a significant change in the methane production rate in a UASB reactor while pure oxygen addition led to reduced CH₄ generation. Pure oxygen could have diffused deeper in the sludge granules and caused inhibition of strict anaerobes, while air aeration could have only had a no or milder inhibition effect due to less oxygen concentration. In air aeration, dilution of biogas by N₂ is a disadvantage.

Rathnasiri [30] introduced a novel approach of using a dense polydimethyl siloxane (PDMS) membrane through which oxygen saturated water was circulated to diffuse traces of oxygen into an anaerobic reactor. This technique allows the application of traces of oxygen in a more controlled manner.

We suggest *electrolytic aeration* as a new concept where an electric current can be used to introduce an aeration effect through water electrolysis. This concept has not yet been tested in any study, but can be a useful method to introduce limited aeration in AD. Electrolytically generated hydrogen, together with oxygen, can also become a substrate for AD. An electrochemical combustion process can also happen concurrently, improving the waste digestion. Another plausible advantage of this method is to very precisely control the level of aeration based on reactor performance data. Feed back control of aeration using a rapid responding control parameter such as hydrogen concentration in biogas stream [144] can be established. This technique must be investigated further to have a greater insight into its applicability. Electrically enhanced hydrolysis is another plausible scenario.

5. MODELING OF OXYGEN EFFECTS IN AD

Different models have been proposed to describe the complex and interrelated biochemical processes taking place in anaerobic digestion. Among them, the generic model of ADM 1 [23] presented by the IWA mathematical modeling task group has been a subject of great interest during the last decade. There are various other models also describing the anaerobic digestion process to a level of significant accuracy. A good example is the model suggested by Angelidaki *et al.* [145]. Prediction accuracies of different models is a subject of controversy, naturally, as for all the models. Fuentes *et al.* [146] reported, for example, that the model by Angelidaki *et al.* [145] was accurate in predicting the behavior of their

mesophilic lab scale fluidized bed reactor. There are also numerous case specific models developed to describe specific reactors with defined type of substrates. Sometimes they may predict those specific cases more accurately than the generic models, like ADM 1.

There was no comprehensive model available to explain the behavior of anaerobic digesters under minute free oxygen load conditions until recent times. Botheju *et al.* [25] developed a model for this purpose based on ADM 1, to accommodate some of the known oxygen effects in anaerobic digestion. This model, named ADM1-Ox, predicted quite accurately the observed behavior of lab scale anaerobic digesters under different oxygenation conditions and substrates [25, 27, 28, 46, 106]. ADM1-Ox includes some of the plausible oxygen effects in anaerobic digestion by including seven aerobic uptake processes for the direct hydrolysis products of monosaccharides (simple sugars), amino acids, long chain fatty acids, and the four volatile fatty acids of acetic, propionic, butyric and valeric. The model also consists of modified hydrolysis rate equations to include the acidogenic biomass concentrations, as well as oxygen inhibition factors in the anaerobic processes. All aerobic uptake processes are catalyzed by the facultative acidogenic biomasses. No new biomass type is introduced in the ADM1-Ox, in addition to the seven types of trophic groups originally presented with ADM 1. This model predicts the existence of an optimum oxygenation point corresponding to a maximum methane yield. Furthermore, it can be used to identify different operating conditions giving rise to positive or negative impacts of oxygenation on biogas/methane generation. This model, in addition to the conventional CSTR arrangement, was also implemented in a biofilm configuration using the Aquasim Biofilm Reactor Compartment [85], following the methodology described previously [147]. ADM 1-Ox was also capable of predicting the oxygen effects observed in an experimental study where the degradation of MEA/waste MEA was investigated [106, 107]. For that purpose, two additional processes of aerobic and anaerobic MEA uptake had to be included in the model [107].

Another model which might have the potential ability to simulate simultaneous aerobic and anaerobic effects is the one proposed by de Gracia *et al.* [148]. This is a completely new model developed to describe the wastewater sludge (primary and secondary) digestion under aerobic or anaerobic conditions. The basic aim of this model was to facilitate connecting aerobic and anaerobic stages of biological sludge digestion; however, the model is also found to be suitable for simulating the aerobic/anaerobic digestion under oxygen limiting conditions. The model is capable of predicting the formation of VFA when the oxygen supply is limited in aerobic sludge digestion. Inclusion of an energy balance in de Gracia *et al.*'s [148] model allows the prediction of reactor temperature variation due to aerobic biological heat generation which can be a significant effect in highly aerated conditions. It also includes *thermal hydrolysis* as a physiochemical transformation process. This allows the model to reproduce digester behavior in *auto-thermal aerobic digestion*, which is basically an aerobic sludge digestion process using biochemical heat to elevate the reactor temperature without an external heat source. Generation of VFA in auto-thermal aerobic digestion is well

documented [101-104]. The applicability of this model for different substrates other than biological sludge has not been tested. It is also not clear whether, or to what extent, this model could be used to analyze the effects of oxygenation on bio-gasification. The process rate equations are not obvious in the publication [148]. It is however observed that two different formulations have been used to describe the hydrolysis under aerobic and anaerobic conditions, separately.

It may also be worthwhile to study the plausibility of using simple mathematical or empirical models (such as the Gompertz equation, e.g. Donoso-Bravo *et al.* [149]) to predict the oxygen effects in methane generation potential. Such equations can be based on simple mass balance and kinetic analyses. Those kinds of rudimentary formulations are sometimes convenient and cost effective to be used as a "rules of thumb" for basic estimation purposes compared to complicated model simulations requiring more time, effort and expertise.

6. CONCLUSIONS

Oxygen can have multifaceted effects in anaerobic bio-gasification. The overall impact of aeration on the methane yield of a digestion system thus depends on the resultant of these diverse biochemical and physiochemical reactions involving oxygen. The influence of the different reactions eventually depends on the operating conditions of a specific digestion system, such as the nature and concentrations of substrates (soluble, particulate or other specific ingredients like S), hydraulic and operational configuration of the reaction system (CSTR, UASB, batch or continuous) and the characteristics of the inoculum used (biomass concentration, oxygen adaptation, aggregated biomass, etc.).

In spite of the negative impact of oxygen on anaerobic organisms, digesters have a significant capacity to maintain stable performance under a broad range of oxygen input. Anaerobic and aerobic cultures can co-exist in a single reactor environment. Oxygen shielding effect by facultative biomass, diffusion barriers due to aggregated biomass and some intrinsic oxygen tolerance of anaerobic organisms are the main factors contributing to this.

Several experimental studies have been conducted on the oxygen effects in anaerobic digestion. Mechanistic models have been proposed to describe the oxygen effects in AD.

Biogas/methane generation and gas quality can be negatively influenced by aeration, but it can also be affected positively under certain operating conditions.

Oxygen can enhance the hydrolysis and even the acidogenesis stage of AD. Aeration does lead to oxidation of some available substrate by aerobic respiration, removing some of the methane potential. This can be more than compensated for by enhanced hydrolysis in some hydrolysis limited AD cases. An optimum oxygenation level corresponding to a maximum methane yield exists in such cases.

It is suggested that in phase-separated digestion systems, partial aeration can most suitably be incorporated with the initial hydrolysis stage. This arrangement would facilitate enhanced hydrolysis while minimizing the negative impacts of aeration.

Limited aeration can be a useful strategy to limit VFA accumulation during start-up and transition periods of digesters, avoiding instabilities due to pH drops and inhibition caused by VFA. This can be used for enhanced starting-up performance in AD and stable digester operation.

Enhanced effluent quality can be achieved in AD by limited aeration. Partial aeration assisted anaerobic digestion can be an especially useful means of treating a variety of recalcitrant, persistent and eco-toxic organic wastes which are difficult to treat either by aerobic or anaerobic treatment alone. Partial aeration can also help to avoid the formation of toxic H₂S in the digestion of S rich substrates, leading to better effluent water and biogas quality.

Some, but not dramatic, changes in the microbial community structure can be expected due to limited aeration in AD.

It is suggested that partial aeration assisted anaerobic digestion can become a useful waste treatment and a renewable energy generation scheme with multiple benefits. Further research on the topic, especially related to full scale applications, must be performed in order to establish this as a standard treatment option.

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