FUNDAMENTALS OF ANAEROBIC DIGESTION, BIOGAS PURIFICATION, USE AND TREATMENT OF DIGESTATE

Airton Kunz
Ricardo Luis Radis Steinmetz
André Cestonaro do Amaral
Technical Editors

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Brazilian Society of Agricultural and Agroindustrial Production Waste Specialists – Sbera

Brazilian Agricultural Research Corporation
Embrapa Swine & Poultry
Ministry of Agriculture, Livestock and Food Supply

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Foreword

Environmental issues of different agro-industrial production chains have become increasingly important and challenging due to the transformation of production systems related to an increase in scale and concentration.

It makes us have to think about new strategies for the environmental sustainability of production systems. Agribusiness depends on it to continue to develop and mitigate environmental impacts.

In this context, the animal protein production chains, mainly those involving Confined Animal Production Systems such as swine and poultry, and, recently, cattle raising, require attention due to their potential impact.

Therefore, this book sought to gather and systematize the knowledge produced by national research for the management, treatment, and use of residues containing a high concentration of organic matter and nutrients. We hope that the content can serve as a qualified technical basis and assist in decision-making for the choice of technological routes adapted to different realities.

Enjoy the book!

Janice Reis Ciacci Zanella
Researcher at Embrapa Swine & Poultry

Preface

The high concentration of carbon and nutrients in animal protein production residues requires different strategies for their management.

The literature is rich in the discussion and design of effluent treatment systems for sanitary sewage. However, animal production chains still lack educational material considering the characteristics of their effluents. The topic is important and requires attention, as many production units may have an environmental impact equivalent to medium-sized cities.

This book brought together some specialists on this topic to contribute and support the discussion using a technical language, presenting the advances in knowledge in the area.

The subject that starts the discussion of the material gathered here is anchored in the anaerobic digestion process for carbon removal from biodegradable organic matter-rich substrates and its conversion into biogas.

The different biogas purification strategies are presented later, with a view to generating a quality fuel gas applicable to different situations.

The digestate produced in anaerobic digestion processes, a nutrient-rich material, is discussed from the perspective of agricultural use as fertilizers, considering the nutrient balance.

Nutrient removal from the digestate, for situations in which agricultural use is not possible, is presented in detail, covering nitrogen and phosphorus and the different strategies for their treatment.

It is important to emphasize that the management and treatment of residues do not present a single solution, but alternatives applicable to different realities. We hope that the material gathered here can technically contribute to this discussion.

Airton Kunz
Ricardo Luis Radis Steinmetz
André Cestonaro do Amaral
Technical editor

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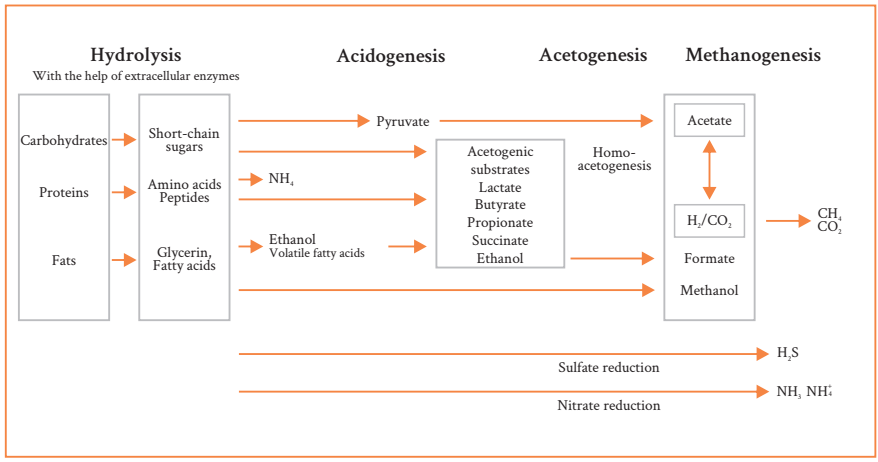
Chapter I

THE BIODIGESTION PROCESS

André Cestonaro do Amaral
Ricardo Luis Radis Steinmetz
Airton Kunz

Introduction

Anaerobic digestion is a complex metabolic process that requires anaerobic conditions (redox potential ≤ 200 mV) and depends on the joint activity of an association of microorganisms to transform organic material into carbon dioxide and methane. The process can be divided into four phases: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Each phase is performed by different groups of microorganisms, in syntrophy, and may require different environmental conditions (Figure 1).



Source: Adapted from Deublein and Steinhauser (2011).

Figure 1. Hydrolysis, acidogenesis, acetogenesis, and methanogenesis processes.

Hydrolysis

The hydrolysis phase degrades high molecular weight compounds, such as lipids, polysaccharides, and proteins into simpler (monomers) and soluble organic substances. This process occurs through the action of extracellular enzymes excreted by hydrolytic bacteria. The importance of the hydrolysis phase in the degradation rate depends on the characteristic of the involved substrate. Hydrolysis is of great importance in the global degradation rate when the organic matter present is complex and difficult to degrade, being considered a limiting step in the anaerobic digestion rate. The duration of the hydrolysis phase varies according to the substrate characteristics, that is, a few hours for carbohydrates and a few days for proteins and lipids. Lignocellulose and lignin are hydrolyzed more slowly, often incompletely.

Acidogenesis

Monomers formed in the hydrolytic phase are used as substrates by different anaerobic and facultative bacteria, being degraded in the acidogenic phase into short-chain organic acids, molecules with 1 to 5 carbons (e.g., butyric, propionic, and acetic acids), alcohols, nitrogen

oxides, hydrogen sulfide, hydrogen, and carbon dioxide. The hydrogen partial pressure during the process directly affects the oxidation state of the products. If it is too high, it will result in products with a higher amount of carbon.

Carbohydrates such as glucose are broken down to pyruvate in acidogenesis. This product is converted into lactic acid by *Lactobacillales* and ethanol by the action of yeasts. Fatty acids are degraded, for example, by *Acetobacter* by β-oxidation. Therefore, fatty acids must be linked to coenzyme A and oxidation occurs step by step through the sequential release of two carbon units in the form of acetate. Amino acids are degraded in pairs by *Clostridium botulinum* through the Stickland reaction (Figure 2), in which one amino acid serves as an electron donor and another as an acceptor. This reaction results in the formation of acetate, ammonia, carbon dioxide, and hydrogen sulfide.

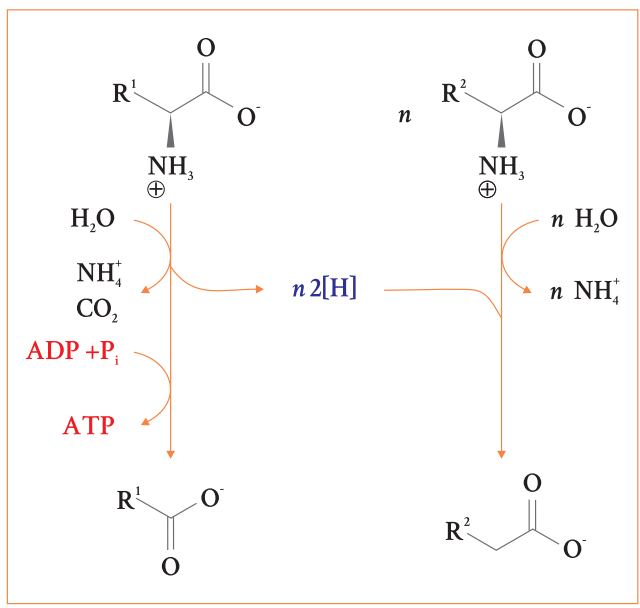


Figure 2. Example of a Stickland reaction, in which a pair of amino acids is degraded, forming acetate, ammonia, and carbon dioxide.

Acetogenesis

The third phase of anaerobic digestion is considered critical to the process, being conducted by a group of bacteria called acetogenic. The acetogenic reactions are endothermic (Table 1). For instance, the propionic acid degradation into acetate and carbon dioxide has $\Delta G = +74$ kJ.mol^{-1}.

Table 1. Acetogenic reactions. Decomposition to low molecular weight elements.

Name	Conjugate acid	Chemical reaction/chemical compound	ΔG (k J.mol^{-1})
Carbon dioxide/ hydrogen		$2CO_2 + 4H_2 \rightleftharpoons CH_3COOH + 2H_2O$	-104.6
Formate	Formic acid	HCOOH	
Acetate	Acetic acid	CH_3COOH	
Propionate	Propionic acid	$CH_3(CH_2)COOH + 2H_2O \rightleftharpoons CH_3COOH + CO_2 + 3H_2$	+76.1
Butyrate	Butyric acid	$CH_3(CH_2)_2COOH + 2H_2O \rightleftharpoons 2CH_3COOH + 2H_2$	+48.1
Lactate	Lactic acid	$CH_3CHOHCOOH + 2H_2O \rightleftharpoons CH_3COOH + HCO_3 + H^+ + 2H_2$	-4.2
	Ethanol	$CH_3(CH_2)OH + H_2O \rightleftharpoons CH_3COOH + 2H_2$	+9.6

Source: Adapted from Deublein and Steinhauser (2011); Chernicharro (2007).

Acetogenic bacteria establish a syntrophic relationship with methanogenic archaea and homoacetogenic bacteria. In this phase, long-chain acids are transformed into acids with only one or two carbon atoms (formic and acetic), with the concomitant hydrogen and carbon dioxide production. Homoacetogenic bacteria govern the balance of the direction of hydrogen and carbon dioxide consumption reaction for acetate production (Equation 1). A thermodynamically favorable formation of short-chain acids must occur associated with the consumption of gaseous hydrogen by methanogenic archaea. Syntrophy between organisms from different microbial groups allows both to grow, ensuring the feasibility of producing acetate from organic acids.

$$\boldsymbol{2CO_2 + 4H_2 \rightleftharpoons CH_3COOOH + 2H_2O} \qquad \textit{Equation 1}$$

Methanogenesis

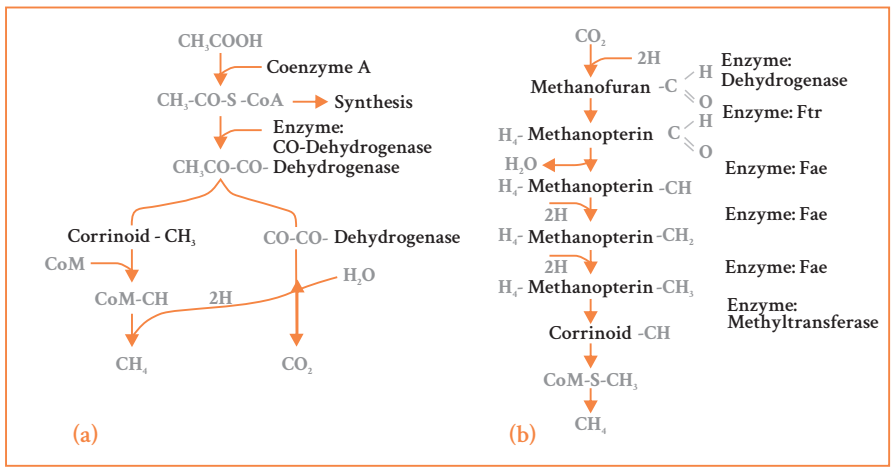
The final phase, methanogenesis, takes place under strictly anaerobic conditions. Thus, the carbon contained in the biomass is converted into carbon dioxide and methane through the action of methanogenic archaea. The archaeal domain is polymorphic, allowing it to be distinguished from other domains only by the 16S rRNA sequence. The reactions that occur in methanogenesis are exothermic (Table 2).

Table 2. Methanogenic reactions. Decomposition to low molecular weight elements.

Chemical reaction	ΔG (k J.mol^{-1})	Name
$4H_2 + HCO_3^- + H^+ \rightleftharpoons CH_4 + 3H_2O$	-135.4	Several species
$CO_2 + 4H_2 \rightleftharpoons CH_4 + 2H_2O$	-131.0	
$4HCOO^- + H_2O + H^+ \rightleftharpoons CH_4 + 3HCO_3^-$	-130.4	Several species
$CH3COO^- + H_2O \rightleftharpoons CH_4 + HCO_3^-$	-30.9	Some species
$4CH_3OH \rightleftharpoons 3CH_4 + HCO_3^- + H^+ + H_2O$	-314.3	
$CH_3OH + H_2 \rightleftharpoons CH_4 + H_2O$	-113.0	*Methanobacterium* *Methanospirillum*
$2CH_3CH_2OH + CO_2 \rightleftharpoons CH_4 + 2CH_3COOH$	-116.3	*Methanosarcina*

Source: Adapted from Deublein and Steinhauser (2011).

Methanogenic archaea are divided according to their metabolic pathways into acetoclastic and hydrogenotrophic. Acetoclastic methanogenic archaea (e.g., *Methanosarcina*) convert acetate into methane, while hydrogenotrophic methanogenic archaea (e.g., *Methanobacterium* and *Methanospirillum*) convert hydrogen and carbon dioxide into methane. Both reactions are exothermic. The pathways for methane formation via acetate or carbon dioxide are shown in Figure 3



Source: Adapted from Deublein and Steinhauser (2011).

Figure 3. (a) Formation of methane by acetate; (b) methane formation by carbon dioxide. CoA = coenzyme A; CoM = coenzyme M.

Many authors report a 70/30% collaboration in methane production between acetoclastic/hydrogenotrophic methanogenic archaea. However, recent studies have shown the dynamism of this relationship (Silva et al., 2014). Acetoclastic methanogenic archaea are more sensitive to changes in pH and high ammonia concentrations, which is a characteristic of substrates from agriculture (other than sanitary sewage). It may imply the predominance of methanogenic hydrogenotrophic archaea. The acetate produced during biodigestion in the absence of acetoclastic methanogenic archaea is oxidized by homoacetogenic bacteria, producing CO_2 and H_2 (Figure 1), which are used as a substrate by hydrogenotrophic methanogenic archaea, producing methane.

Process parameters

The metabolism of anaerobic mesophilic microorganisms depends on several factors (Table 3). Therefore, multiple parameters must be considered and controlled for an optimal fermentation process.

Table 3. Environmental requirements of anaerobic mesophilic microorganisms.

Parameter	Hydrolysis/Acidogenesis	Methanogenesis
Temperature	25 - 35	32 - 42
pH	5.2 - 6.3	6.7 - 7.5
C:N ratio	10-45	20-30
Dry matter concentration (%)	<40	<30
Redox potential (mV)	+ 400 to - 300	<-200
Required C:N:P:S ratio	500 : 15 : 5 : 3	600 : 15 : 5 : 3
Trace elements	-	Essential: Ni, Co, Mo, Se

Source: Adapted from Wellinger et al., (2013).

Hydrogen partial pressure

Hydrogen partial pressure plays a key role in methanogenesis. Therefore, a narrow symbiosis between H_2-producing and H_2-consuming microorganisms is necessary. Overall, a biochemical reaction needs to be exothermic for it to occur spontaneously, that is, the Gibbs free energy must be negative ($\Delta G < 0$).

The hydrogen concentration must be balanced, as methanogenic microorganisms need hydrogen to produce methane (hydrogenotrophic methanogenic archaea). On the other hand, the hydrogen partial pressure must be low enough (10^{-4} to 10^{-6} bar) so that acetogenic bacteria are not inhibited by excess hydrogen, paralyzing the production of short-chain acids.

The maximum hydrogen partial pressure depends on the involved microorganism species and also the substrate characteristics. The energy window is especially small for anaerobic conversion of propionate via acetic acid and carbon dioxide/hydrogen into methane. Low partial pressures can only be maintained if the formed hydrogen is quickly and effectively removed by hydrogen-consuming microorganisms.

Temperature

The temperature has important effects on the physicochemical properties of the components found in anaerobic substrates. It also influences the growth rate and metabolism of microorganisms and, therefore, the population dynamics in a biodigester. Microorganisms can be classified into three large groups, according to the temperature (Table 4).

Table 4. Classification of microorganisms according to the temperature.

	Optimal growth temperature (°C)
Thermophilic	60
Mesophilic	37
Psychrophilic	15

Acetoclastic methanogenic archaea are the group most sensitive to temperature increase. The temperature affects the hydrogen partial pressure in a biodigester, influencing the syntrophic metabolism kinetics. Thermodynamically, endothermic reactions under standard conditions, such as the breakdown of propionate into acetate, carbon dioxide, and hydrogen, become energetically more favorable at high temperatures, but exothermic reactions (e.g., hydrogenotrophic methanogenic) are less favored at high temperatures.

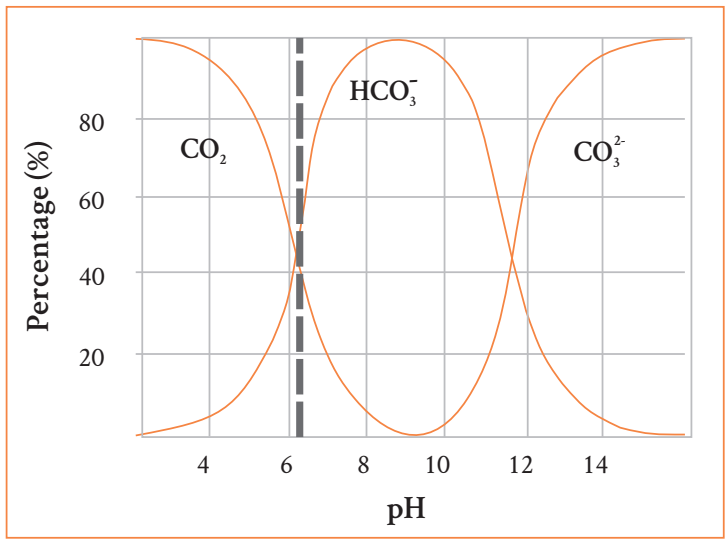
Increasing temperature has numerous benefits, including an increase in the solubility of organic compounds, improving the biochemical reaction rates. There is also an increase in the pathogen elimination rate. However, temperature influences parameters such as ammonia dissociation, which may have an inhibitory effect (Kunz; Saqib, 2016). The chemical balance is shifted from NH_4^+ to NH_3 (aqueous) as the temperature increases, which may lead to failure in the process. Free ammonia is toxic to methanogenic archaea, as it easily diffuses through the cell membrane of microorganisms, causing ionic imbalance and/or potassium (K^+) deficiency.

Biogas production in regions with a large thermal amplitude can be compromised due to high-temperature variations. The reactor temperature should not vary sharply more than 2 °C to avoid possible problems. Biomass temperature control is of paramount importance to ensure uniformity of biogas generation.

pH, alkalinity and volatily fatty acids

Each microorganism group has a different optimum pH value. Methanogenic archaea are extremely pH sensitive, with an optimum value between 6.7 and 7.5. Fermentative microorganisms are less sensitive and can adapt to greater pH variations between 4.0 and 8.5. The main products at low pH values are acetic and butyric acids, while the main products at pH close to 8.0 are acetic and propionic acids. Volatile acids produced during biodigestion tend to reduce the pH of the reaction medium. This reduction is usually countered by the activity of methanogenic archaea, which also produce alkalinity in the form of carbon dioxide, ammonia, and bicarbonate.

The system pH is controlled by the concentration of carbon dioxide in the gas phase and HCO_3-alkaline in the liquid phase (Figure 4). CO_2 is continuously released as a gas during biodigestion. A higher CO_2 amount will remain dissolved in the reaction medium if the system pH decreases excessively. On the other hand, dissolved CO_2 will form carbonic acid if the system pH increases, thus releasing hydrogen ions (Deublein; Steinhauser, 2011).



Source: Adapted from Deublein and Steinhauser (2011).

Figure 4. Chemical balance between carbon dioxide – bicarbonate ion – carbonate ion.

Almost all CO_2 will be in free molecule form at pH 4 and dissolved as carbonate in the substrate at pH 13. The chemical equilibrium point between gaseous and soluble forms in the system will occur at pH 6.52 (Figure 4). Therefore, the increase in pH will result in a lower CO_2 concentration in the gas phase. Bicarbonate has a strong buffering effect at concentrations of 2.5 $g.L^{-1}$ – 5 $g.L^{-1}$.

Most problems in anaerobic digestion can be attributed to the accumulation of volatile acids and, consequently, the decrease in pH. The main adverse effects of volatile fatty acis (VFAs) in the anaerobic digestion process are related to the fact that they are intermediate species. The decrease in pH below 6.6 implies the growth inhibition of methanogenic archaea. However, acidogenic bacteria continue their functions up to pH 4.5. The result is a rapid VFA accumulation.

A strategy for controlling the buffering system and indirectly monitoring the acids produced during the anaerobic digestion is the intermediate alkalinity/partial alkalinity (VFA/TA) ratio, the former providing values equivalent to the alkalinity by bicarbonate and the latter to

alkalinity from volatile acids. Table 5 shows the importance of monitoring the VFA/TA ratio and the reactor relationships and characteristics according to empirical experience.

Table 5. Evolution of the VFA/TA ratio and reactor characteristics.

VFA/TA ratio	Reactor characteristics
>0.4	Reactor under overload
0.3 – 0.4	Optimal range
< 0.3	Reactor under underload

Source: Adapted from Mézes et al., (2011).

The optimal value may vary depending on the reactor and substrate characteristics. It is recommended to monitor the VFA/TA ratio constantly, as observing sudden variations and taking corrective measures when necessary are the most important.

Nutrients

Cells of anaerobic microorganisms contain nitrogen, phosphorus, and sulfur at approximate dry matter proportions of 12%, 2% and 1%, respectively. The anaerobic process requires biological oxygen, N, and P demand ratios of 700:5:1. Sulfur, potassium, calcium, magnesium, chlorine, and sulfate ions are necessary for the proper functioning of anaerobic digestion. Trace elements such as iron, copper, zinc, magnesium, molybdenum, and vanadium are important for cell growth.

Sulfur compounds can cause problems for the anaerobic process, as they lead to the precipitation of essential nutrients at trace levels, such as iron, nickel, copper, and molybdenum, which are insoluble at low redox potentials (precipitation as a sulfide). Heavy metal ions such as Cu^{++} and Zn^{++}, alkali and alkaline earth metal ions, and NH_4^+ can also cause inhibitory effects. Toxicity is reversible in many cases and a high acclimatization potential is observed when sufficient time is given to anaerobic microorganisms.

Ammoniacal nitrogen and free ammonia

Ammonia is an essential nutrient for the growth of anaerobic microorganisms, but it can also be toxic at high concentrations. Fermentation of urea and protein-rich materials releases ammonia. A high generation of free ammonia may be reached as a function of the pH and temperature of the reaction medium (De Prá et al., 2013). The chemical equilibrium of the system for free ammonia (FA) formation can be calculated using Equation 2.

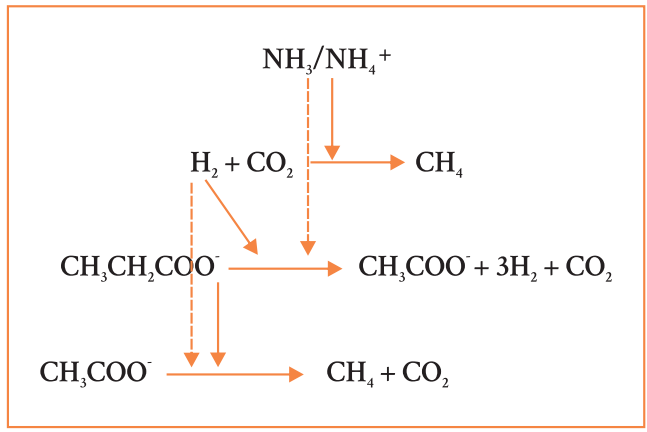
$$FA\ (NH_3, mg.L^{-1}) = \frac{17}{14}\ X\ \frac{[total\ ammonia\ as\ N] \cdot 10^{pH}}{e^{[6344/(273+T(°C))]} + 10^{pH}} \qquad \textit{Equation 2}$$

Table 6 shows the relationship between pH and temperature with free ammonia concentration in an effluent with a high ammoniacal nitrogen concentration, using Equation 2.

Table 6. Effect of pH and temperature on free ammonia (FA) concentration in an effluent with high ammoniacal nitrogen concentration.

NH_3-N ($mg.L^{-1}$)	Reactor pH	Temperature (°C)	FA ($mg.L^{-1}$)
3,000	5	20	0.14
3,000	7	20	14.34
3,000	9	20	1,031.68
3,000	5	37	0.47
3,000	7	37	46.58
3,000	9	37	2,055.77
3,000	5	55	0.15
3,000	7	55	139.51
3,000	9	55	2,911.66

Free ammonia is toxic to methanogenic archaea as it easily diffuses through the cell membrane of microorganisms. Figure 5 shows a scheme to exemplify the inhibitory action of free ammonia. Continuous arrows indicate reaction inhibition and dashed arrows indicate possible inhibitory actions.



Source: Wiegant and Zeeman (1986).

Figure 5. Scheme proposed to explain the inhibitory action of free ammonia. Horizontal arrows: inhibited reactions; vertical arrows: inhibitory action. Dotted arrows indicate possible inhibiting actions.

The literature shows anaerobic digestion inhibition at different free ammonia concentrations. Garcia and Angenent (2009) studied the digestion of swine manure and reported inhibition of methane production at concentrations from 200 $mg.L^{-1}$ at 35 °C with pH 7.6. Rodríguez et al., (2011) reported that levels of up to 375 $mg.L^{-1}$ of free ammonia did not affect the efficiency of the digestion process.

The acclimatization of microorganisms in the presence of free ammonia is a key factor for the process efficiency. It can occur due to the adaptation of methanogenic archaea species present in the reactor or through population selection, standing out species more adapted to the reactor conditions (Silva et al., 2014).

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Chapter II

IMPORTANT PARAMETERS FOR THE ANAEROBIC DIGESTION PROCESS

André Cestonaro do Amaral
Ricardo Luis Radis Steinmetz
Airton Kunz

Introduction

The anaerobic digestion process is biologically mediated and involves different types of microorganisms. Thus, specific conditions must be respected and followed for the correct functioning of biodigesters. In this chapter, we will discuss the main parameters and how to calculate them.

Alkalinity

Alkalinity can be understood as a measure of the buffering capacity of a system, that is, the ability to avoid sudden changes in pH. It is often attributed to the balance between CO_2 dissolution and carbonic acid (H_2CO_3) formation in anaerobic environments.

The total alkalinity is given by the sum of the concentration of ions hydroxyl (OH^-), carbonate (CO_3^{2-}), and bicarbonate (HCO_3^-), being expressed as the concentration of calcium carbonate ($CaCO_3$).

It can be determined by titrating the sample with a sulfuric acid solution (e.g., 0.1 mol.L^{-1}) up to pH 4.5 and applying the equation:

$$\boldsymbol{Alk = M \times E \times \frac{100,000}{D}}$$

Equation 1

Where:

Alk = Alkalinity (mg $CaCO_3.L^{-1}$)

M = Sulfuric acid solution concentration (mol.L)

D = Sample volume (mL)

E = Sulfuric acid titred volume (mL)

100,000 = Coefficient to adjust the unit of measure

Determination of the VFA/TA ratio

One of the monitored parameters is the relationship between the accumulation of volatile fatty acid and total alkalinity ratio, known as the VFA/TA ratio. The result is a simple value dependent on the relationship of these two parameters, relative to short-chain organic acids (VFA) and alkalinity (TA).

They can be calculated by titrating the sample with sulfuric acid, following the equation:

$$\boldsymbol{VFA/TA = \frac{\left((V_{pH4.4} - V_{pH5.0}) \cdot \frac{20}{V_{sample}} \cdot \frac{M_{acid}}{0.1} \cdot 1.66 - 0.5\right) \cdot 500 \cdot V_{sample}}{0.5 \cdot M_{acid} \cdot V_{pH5.0} \cdot M_{CaCO_3} \cdot 1,000}}$$

Equation 2

Where:

VFA/TA = Intermediate alkalinity to partial alkalinity ratio

$V_{pH4.4}$ = Titrated acid volume to pH = 4.40 (mL)

$V_{pH5.0}$ = Titrated acid volume to pH = 5.00 (mL)
V_{sample} = Centrifuged sample volume (mL)
M_{acid}= Acid molarity (molar concentration of the hydrogen ion (dissociated) in the acid) ($mol.L^{-1}$)
M_{CaCO3} = calcium carbonate molar mass in 100 $g.mol^{-1}$

Hydraulic retention time

Hydraulic retention time (HRT) is the mean time that the substrate remains inside the biodigester, that is, the ratio between the biodigester volume and the feeding flow rate, determined using Equation 3.

$$HRT = \frac{V}{Q} \qquad \textit{Equation 3}$$

Where:

HRT = Hydraulic retention time (d)
V = Biodigester volume (m^3)
Q = Feeding flow rate ($m^3.d^{-1}$)

Organic loading rate

Organic loading rate (OLR) represents the amount of substrate added to the biodigester in a given period of time. It is obtained using Equations 4 or 5.

$$VOL = \frac{(Q \times S_V)}{V} \qquad \textit{Equation 4}$$

$$VOL = \frac{S_V}{HRT} \qquad \textit{Equation 5}$$

Where:

OLR = Organic loading rate ($kg_{VS}.m^{-3}d^{-1}$)
Q = Flow rate ($m^3.d^{-1}$)

Sv = Concentration of volatile solids in the substrate ($kg.m^{-3}$)
V = Reator volume (m^3)
HRT = Hydraulic retention time (d)

OLR influences the entire dynamics of the anaerobic digestion process. An optimal organic loading rate provides adequate conditions for microorganism growth and, consequently, higher process stability. Low OLRs may represent a low food/microorganism ratio, which results in low biological activity. High OLRs may present a high food/microorganism ratio, which can lead to the accumulation of volatile fatty acis and process failure. The ideal OLR is related to the biodigester model, applied technology, and substrate type.

OLR x temperature relationship

Safley and Westerman (1990) presented Equation 6 using different versions of the van't Hoff-Arrhenius relationship to estimate the limit of the organic loading rate relative to changes in the biodigestion temperature:

$$\frac{OLR_2}{OLR_1} = e^{p(T_2 - T_1)}$$

Equation 6

Where:

OLR_1 and OLR_2 = Organic loading rate ($kg_{VS}.m^{-3}.d^{-1}$)
T_1 and **T_2** = Temperature (^oC)
p = Constante 0.1 ($^oC^{-1}$)

Example 1

There is an UASB biodigester treating swine manure. It is operated with a organic loading rate of 0.3 $kg_{VS}.m^{-3}.d^{-1}$ and a mean temperature of 18 °C. What OLR can this reactor be subjected to if a heating system is installed, increasing the temperature of the reaction medium to 35 °C?

A: The equation proposed by Safley and Westerman (1990) is applied.

Where:

OLR_1 = 0.3 $kg_{VS}.m^{-3}.d^{-1}$

OLR_2 = Unknown variable

T_1 = 18 °C

T_2 = 35 °C

$$\frac{OLR_2}{0,3} = e^{0,1(35-18)}$$

$$OLR_2 = 1.64\ kg_{VS}.m^{-3}.d^{-1}$$

Methods for evaluating anaerobic sludge and substrate quality

There are laboratory tests carried out on a small scale and under controlled or monitored conditions to evaluate the anaerobic digestion kinetics (microorganism activity and substrate degradability characteristics, among others). Characterizing the chemical and physical composition of residues is an essential step, but biokinetic tests are also extremely important to visualize the real interaction between microorganisms and substrates. These kinetic tests consist of anaerobic respirometric tests and usually involve the evaluation of biogas or methane production from the known mass of inoculum or substrate biomass (or organic matter defined as VS, COD, or TOC).

Several methods, standardized or not, are found in the literature for evaluating anaerobic kinetics. Some focus on the efficiency of microorganisms, others focus on substrate degradability under anaerobic conditions, and others aim to evaluate the biochemical potential of methane (or energy potential) of the substrate. Some evaluate the toxicity of inhibitory substances in the anaerobic process. However, all methods are based on the batch incubation of substrate(s) mixed with anaerobic inoculum under controlled conditions. Table 1 shows a summary of the usual and standard methods for studying anaerobic kinetics.

Table 1. Summary of anaerobic kinetic methods.

Method	Purpose and/or application
SMA	Measures the specific activity of methane production in anaerobic sludges. Used to make comparisons between inoculums or evaluate efficiencies in anaerobic reactors
ISO 13.641	Anaerobic toxicity test. It is based on the measure of inhibition of biogas production after three days of incubation. Used to evaluate the influence of different chemical compounds on aerobic digestion
ISO 11.734	Estimates the degradation of various organic substances by measuring biogas production
ASTM E2170-01	Estimates the degradation of chemical substances by measuring biogas production and by chemical analysis of the residual concentration. A recognized method in the USA
DIN 38.414-8	Measures the degradation of sludge and effluents by measuring biogas production
VDI 4.630	Method for measuring BBP and BMP. Applied to various types of substrates, including agricultural residues and crops. A recognized method in Germany and European countries

Specific methanogenic activity (SMA)

This test is primarily used to evaluate the performance of methanogenic microorganisms (or inoculum). According to Aquino et al., (2007), SMA can be used as a parameter for monitoring the "efficiency" of the methanogenic population in a biological reactor. The studies of Valcke and Verstraete (1983), Zeeuw (1984), and Dolfing and Bloemen (1985) were pioneers in the development and use of SMA tests as a tool to characterize and evaluate anaerobic reactors in sanitary effluents. All tests for determining SMA available in the literature are based on measuring the methane production rate as a function of inoculum concentration. However, there is no standardized methodology for this purpose, and, depending on the methodology, establishing a relationship between SMA results in the studies available in the literature is extremely difficult. Although SMA is a very important parameter, the available methods do not have standardization and can hardly be used to make comparisons between experiments.

Aquino et al., (2007) performed a literature review on the possible methodologies available on this subject. The methodological differences range from the use or not of the culture medium for inoculum conditioning to ways of measuring the gases produced in the digestion. The

first considerations about the test were made based on batch tests by Zeeuw (1984), who measured the methane production rate of sludges from a known organic load and VS concentration. The substrate applied in the study by Zeeuw (1984) varied from a mixture of volatile acids, usually acetic, propionic, and butyric acids, to the use of a single substrate, mainly acetate, besides adding metal solutions to the nutrient solution, ensuring that there were no limitations for methane production. This method measures gas production via liquid displacement, using a sodium hidroxide solution, in which carbon dioxide is dissolved in the medium, ensuring that the displaced liquid comes from the amount of methane released by the sludge.

Dolfing and Bloemen (1985) proposed a methodology based on the gas chromatography analysis of methane produced in the headspace of serum vials. In this method, the gas is sampled with a lock syringe to keep the gas at the same pressure as the vial. The mixture of acids or acids separately (e.g., only acetate or propionate) and an anaerobic buffer solution are added to the sludge. The use of volatile acids individually has some advantages, as the knowledge of their degradation allows estimating the maximum conversion rates for each substrate to obtain information about inhibition or limitation of the process by high or insufficient concentrations of some type of acid. Moreover, it can be useful in identifying the bacterial genera present in the sludge.

ISO 13641 standards

ISO 13641 standards – Water quality – Determination of inhibition of gas production: This procedure establishes protocols to determine the toxicity of possible substances in anaerobic systems. It is divided into two standards: part 1, which refers to the general test procedure; and part 2, which refers to adaptations of methodology for low concentrations of microbial biomass. Basically, the protocols define methodological guidelines for estimating the 50% inhibitory concentration (IC50) of biogas production. It is based on the incubation of an anaerobic inoculum together with a standard substrate and mixed to different concentrations of the inhibitory agent to be evaluated. The produced gas volume is measured after incubation for three days at 35 °C and compa-

red with the test gas production without inhibitor addition. This procedure is dedicated to the evaluation of acute toxicity to the anaerobic process. There are no standardized procedures for chronic toxicity testing (long-term testing).

ISO 11734:1995

ISO 11734:1995 - Evaluation of "ultimate" anaerobic biodegradability of organic compounds in digested sludge - Method by measurement of the biogas production: This standard presents the description of a standardized kinetic test to evaluate organic chemical compounds against anaerobic microorganisms. The test consists of exposing the anaerobic inoculum to the chemical compound of interest for a period more than 60 days. The evaluation of biogas production is carried out through manometric measurements.

ASTM E2170-01 (2008)

ASTM E2170-01 (2008) - Determining anaerobic biodegradation potential of organic chemicals under methanogenic conditions: Similar to the ISO standard, this standard, elaborated by the metrology agency of the United States, also presents the description of a kinetic test of degradation of organic chemical compounds under anaerobic conditions. The test consists of exposing the anaerobic inoculum to the chemical compound of interest for a period between 25 and 30 days. The evaluation of biogas production is carried out through manometric measurements. Both ISO and ASTM standards are mainly applied in the evaluation of substances used in the medical field.

DIN 38414-8

DIN 38414-8 - Determination of the amenability to anaerobic digestion: This German standard establishes basic conditions for the execution of kinetic tests in batches to evaluate the anaerobic degradation of organic substrates using volumetric tests of biogas production.

VDI 4630

VDI 4630 - Fermentation of organic materials – Characterisation of the substrate, sampling, collection of material data and fermentation tests: This German standard is recognized in the European Union and establishes conditions for carrying out batch, semi-continuous, and continuous kinetic tests to evaluate the anaerobic degradation of organic substrates. It is an improvement of the DIN 38414-8 standard and is widely used by the European community to evaluate the biochemical methane potential (BMP) of different substrates. It is also used as a reference for bench-scale process simulation to assist the operation of large-scale biogas production plants.

The VDI 4630 (2006) standard establishes rules and the need for equipment to carry out fermentation tests on organic materials. Batch tests can provide information on a) the possibility of biogas production and the anaerobic biological degradability of a given material or mixture of materials; b) the qualitative evaluation of the degradation rate of the material under study; and c) the evaluation of the inhibitory effect of the investigated material in a given time interval.

Batch tests do not generate information on a) the process stability with reactors continuously fed with the investigated material; b) the biogas production under practical conditions different from those under which the test was carried out due to possible positive or negative synergistic effects; c) the mono-fermentation of the substrate under process conditions; and d) the organic loading rate limits.

The results of fermentation tests depend primarily on the used sludge (anaerobic inoculum) activity. The inoculum is usually collected in a biogas plant to provide the highest diversity of anaerobic microorganisms possible. The inoculum must contain a concentration of dry organic matter (volatile solids) higher than 50% of the total solids.

Some restrictions must be considered to determine the amount of substrate and inoculum used in the test: a) the amount of substrate must not exceed the amount of inoculum ($VS_{substrate}/VS_{inoculum} \leq 0.5$) to predict inhibitions in the batch test; b) the biogas production from the substrate must be at least 80% higher than the inoculum contribution; and c) the

solids concentration in the batch test must not exceed 10%, ensuring adequate mass transfer during the test.

Materials with known biogas production capacity are used to ensure the activity of the anaerobic inoculum. Possible reference material is crystalline cellulose, which produces between 740 $L_N.kg_{VSadd}^{-1}$ to 750 $L_N.kg_{VSadd}^{-1}$. These values must be recovered by at least 80% in a control test. This recovery value ensures that the inoculum has satisfactory biological activity and is suitable for carrying out BMP tests.

Normalization of biogas volume

Biogas production must always be expressed in a normalized way to standard temperature and pressure conditions (273 K and 1,013 hPa). Equation 7 is used for normalization:

$$V_N = \frac{V \cdot (p - p_w) \cdot T_0}{p_0 \cdot T} \qquad \textit{Equation 7}$$

Where:

V_N = **Biogas volume normalized to normal temperature and pressure conditions** (mL, L or m^3)

V = Produced biogas volume (mL, L or m^3)

p = Biogas pressure at the reading time (hPa)

p_w = Water vapor pressure as a function of room temperature (hPa)

T_0 = Temperature under normalized conditions (273 K)

p_0 = Pressure under normalized conditions (1,013 hPa)

T = Biogas temperature (K)

Expression of results

Table 2 shows important parameters for monitoring bioreactors and their measurement units. The monitoring of these variables contributes to better process control and knowledge of the biodigester operating conditions.

Table 2. Definitions of important variables and measurement units for the biodigester control.

Parameter	Expression	Unit
Temperature	T	^{o}C, K
Substrate concentration	S_o	% (e.g., $g_{TS}.100\ g_{FM}^{-1}$) % (e.g., $g_{VS}.100\ g_{FM}^{-1}$) $g_{VS}.L^{-1}$ ou $kg_{VS}.m^{-3}$ $g_{VS}.kg_{FM}^{-1}$
Volatile fatty acis	VFA	$mg_{HAc}.L^{-1}$
Intermediate alkalinity to partial alkalinity ratio	VFA/TA	mg_{HAc}/mg_{CaCO3}
Particle size	ps	mm
Added organic loading rate	OLR_{add}	$kg_{VSadd}.m^{-3}_{reactor}.d^{-1}$
Removed organic loading rate	OLR_{rem}	$kg_{VSrem}.m^{-3}_{reactor}.d^{-1}$
Hydraulic retention time	HRT	h or d
Redox potential	E_H	mV
Biochemical methane potential	BMP	$L_{N\ CH4}.kg_{VSadd}^{-1}$, $L_{N\ CH4}.kg_{FMadd}^{-1}$
Biogas productivity	PB	$Nm^3_{biogas}.m^{-3}_{reactor}.d^{-1}$ $L_{N\ biogas}.L_{reactor}^{-1}.d^{-1}$
Methane productivity	PM	$Nm^3_{CH4}.m^{-3}_{reactor}.d^{-1}$ $L_{N\ CH4}.L_{reactor}^{-1}.d^{-1}$
Biogas production	PBd	$L_N.d^{-1}$
Biogas yield	BY	$L_{N\ biogas}.kg_{VSadd}^{-1}$, $L_{N\ biogas}.kg_{FMadd}^{-1}$
Methane yield	MY	$L_{N\ CH4}.kg_{VSadd}^{-1}$, $L_{N\ CH4}.kg_{FMadd}^{-1}$
Biogas composition (v v^{-1})	Bc	% CH_4($L_{N\ CH4}.100\ L_{N\ biogas}^{-1}$), % CO_2($L_{N\ CO2}.100\ L_{N\ biogas}^{-1}$), ppm$V_N$ H_2S ($mL_N.m^{-3}$)
Digestate composition	Dc	% (e.g., $g_{TS}.100\ g_{FM}^{-1}$), % (e.g., $g_{VS}.100\ g_{FM}^{-1}$), $g_{VS}.L^{-1}$, $kg_{VS}.m^{-3}$

HAc = acetic acid; MF = fresh matter; TS = total solids; VS = volatile solids; add = added; rem = removed; N = normal.

Source: Kunz et al., 2016.

Problems, causes, and solutions in the biodigester operation

Table 3 shows corrective actions for the most frequent operational problems that can occur in a biodigester.

Table 3. Possible problems found in the biodigester operation, causes, and measures to solve them.

Parameter	Expression	Unit
Redox potential close to zero	Anoxic or oxic condition	Measure DO Measure NO_X Check the inoculum activity
Excess scum and foam	System overload	Decrease the organic loading rate
Solid dragging	High flow rate	Decrease the system flow rate
Light gray colored sludge	Redox potential out of the anaerobic condition	Measure DO Measure the redox potential Check the inoculum activity
Biogas does not burn	Low methane concentration (less than 15%)	Check the inoculum activity Decrease the feeding flow rate
Accumulation of volatile fatty acis	Inhibition of methanogenesis	Decrease the feeding flow rate Check for changes in the substrate for the possible presence of inhibitors agents
Lack of alkalinity	Substrate quality	Supplement the alkalinity
Low pH	Accumulation of VFA	Decrease the flow rate Adjust the alkalinity
Temperature below the recommended operating condition	Heating system failure	Inspect the heating system
Sudden reduction in biogas production	System overload or underload Presence of inhibitory agents	Check the flow rate Check the S concentration in the substrate Evaluate whether there was a change in the substrate characteristic
High concentration of fixed solids in the sludge (> 50%)	Substrate characteristic	Controlled sludge disposal Substrate pre-treatment for FS removal

DO= Dissolved oxygen; $NO_x = NO_2^- + NO_3^-$; VFA = volatile fatty acis.

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Chapter III

BIODIGESTERS

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Introduction

The central point of an anaerobic treatment system is the biodigester model. Thus, projects adapted to the type of substrate to be treated, level of investment, and environmental conditions must be sought. Table 1 shows the key points for establishing the process.

Table 1. Important points for choosing the biodigestion system.

Technology	Key points	Options
Feeding system	Type of biodigester and raw material for feeding	Discontinuous feeding for batch biodigesters Continuous or semi-continuous feeding for plug-flow/CSTR biodigesters Solid or liquid feeding system, depending on the dry matter content of the substrate
Reactor temperature	Risk for pathogens*	Mesophilic temperatures when there is no risk of pathogens Thermophilic temperatures when there is a risk of pathogens (e.g., domestic organic waste)
Number of phases	Substrate composition, risk of acidification	One-phase systems when there is no risk of acidification Two-phase system for substrates with a high content of sugar, starch, and proteins, or substrates difficult to degrade
Stirring system	Dry raw material for feeding	Mechanical stirrers for high solids concentration in the biodigester Mechanical, hydraulic, or pneumatic stirrer systems for low solids concentration in the biodigester

*An alternative is the use of the heat treatment process (e.g., pasteurization).

Biodigester types

Biodigesters are characterized by the feeding regime (batch or continuous), feeding form (upward or laminar), solids concentration in the reactor (solid digestion >20%, semi-solid digestion from 10% to 15%, and wet digestion <10%), and stirring system (complete mixing, partial mixing, or no mixing). The most common models found in Brazil and the details will be discussed in this chapter.

Covered lagoon biodigester (CLB)

The covered lagoon biodigester is a tank dug into the ground, waterproofed, and covered with geosynthetic material (e.g., PVC and HDPE) characterized by low permeability to fluids and gases, and flexible enough to accumulate biogas. It has a rectangular base, with a trapezoidal section and variable slope inclination, according to the ground characteristics (Figure 1).

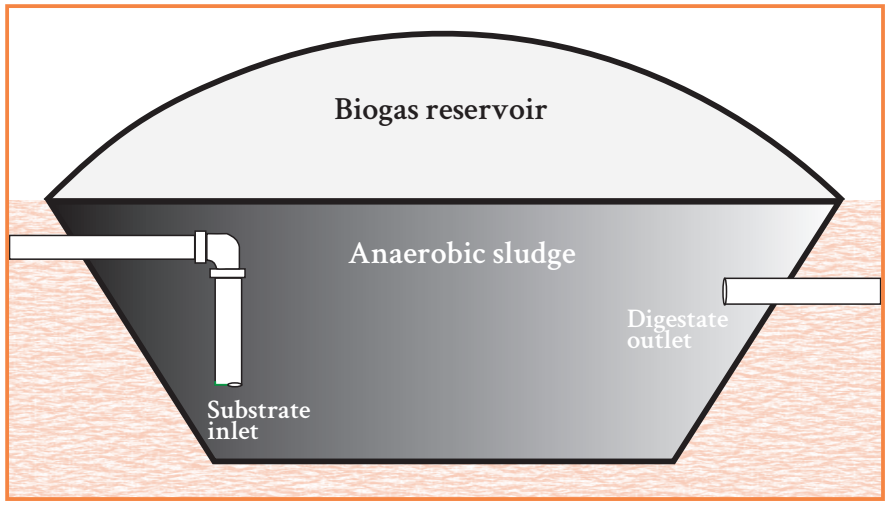


Illustration: Airton Kunz

Figure 1. Representation of the internal view of the covered lagoon reactor.

CLB has been widely used in rural areas to manage effluents from animal production. It is considered of low technological level, with ease of construction and operation. We usually find references to this model as "Canadian" or "canvas biodigester". In general, it does not have heating or stirring systems. Thus, in some cases, we also find reference to this model as "tubular", in which the constructive dimensions and the semi-continuous feeding regime end up generating flow configurations that vary between laminar and plugged (Figure 2). Another aspect of this model is the need for high hydraulic retention time (HRT), which increases the area required for installation. Example 1 presents the design of a CLB.

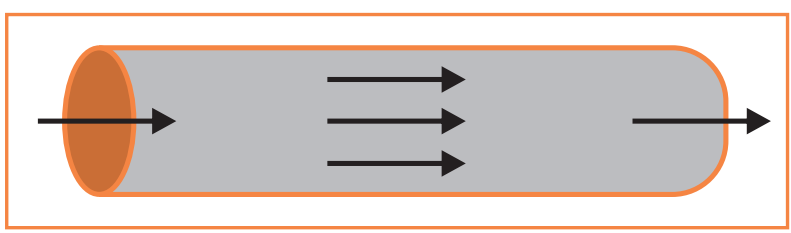


Illustration: Ricardo Steinmentz

Figure 2. Representation of a plug-flow reactor.

The absence of a heating system implies a variation in the biomass temperature of the CLB as a function of the ambient temperature, with direct implications for the capacity of biogas generation, being significantly affected in regions with a more severe winter (e.g., South of Brazil). There is a trend for sludge accumulation at the tank bottom (Example 2) due to the reactor hydraulic regime and the non-use of a solids removal system previously installed at the CLB, creating the need for disposal (Figure 3). It is often hampered by the biodigester dimensioning, preventing the efficient disposal of solids.

Photo: Pedro Colombari/Granja São Pedro

Figure 3. Sludge accumulation in a covered lagoon biodigester.

This biodigester model is generally used for treating effluents with low solids concentration, with up to about 3% (m v^{-1}) of total solids, and a low organic loading rate (OLR), between 0.3 $kg_{VS.}m^{-3}{}_{reactor}.d^{-1}$ to 0.5 $kg_{VS.}m^{-3}{}_{reactor}.d^{-1}$. Biogas productivity per reactor volume is between 0.03 $m^3.m^{-3}{}_{reactor}.d^{-1}$ and 0.15 $m^3.m^{-3}{}_{reactor}.d^{-1}$ (Catrell et al., 2008), varying according to the substrate type, OLR, operating temperature, and HRT.

Example 1

Dimensioning of a covered lagoon biodigester in a swine farm (weaning production unit) with 500 swine females (sows). The swine manure from this unit has a volatile solids concentration of 18 $kg_{VS}.m^{-3}$. The environmental agency of the State of Santa Catarina, where this farm is located, considers a waste production of 16.2 $L.sow^{-1}.d^{-1}$ (IN11 – IMA, SC).

$$\boldsymbol{Q = WPS \times NS} \qquad \textit{Equation 1}$$

Where:

Q = Waste produced daily ($m^3.d^{-1}$)

WPS = Waste production per sow ($m^3.sow^{-1}.d^{-1}$)

NS = Number of sows

Therefore:

$$Q = 0.0162 \times 500 = \mathbf{8.10\ m^3.d^{-1}}$$

The biodigester volume can be calculated considering a organic loading rate of 0.5 $kg_{VS}.m^{-3}.d^{-1}$, as follows:

$$\boldsymbol{V = \frac{Q \times S_o}{OLR}} \qquad \textit{Equation 2}$$

Where:

V = Biodigester volume (m^3)

Q = Substrate flow rate ($m^3.d^{-1}$)

$\mathbf{S_o}$ = Concentration of volatile solids in the substrate ($kg_{VS}.m^{-3}$)

OLR = Organic loading rate ($kg_{VS}.m^{-3}.d^{-1}$)

Therefore:

$$V = \frac{8.10 \times 18.0}{0.5} = \mathbf{291.60\ m^3}$$

HRT can be calculated by the equation below:

$$\boldsymbol{HRT = \frac{V}{Q}} \qquad \textit{Equation 3}$$

Where:

HRT = Hydraulic retention time (d)

V = Biodigester volume (m^3)

Q = Substrate flow rate ($m^3.d^{-1}$)

Therefore:

$$HRT = \frac{291.6}{8.10} = \mathbf{36\ d}$$

The CLB model has some particularities that must be respected in its construction: a) minimum length x width ratio of 2x1; b) depth of 3 m to 4.5 m; and c) slope inclination of about 45°, which may vary depending on the ground.

Besides organic matter, which is the substrate for biogas production, many effluents are also composed of inorganic materials, characterized as fixed solids (FS). These solids, as a rule, do not contribute to biogas production and can lead to the biodigester aggradation, decreasing HRT (Figure 3). Therefore, the sludge needs to be correctly managed in the biodigester.

Example 2

A CLB has 3,000 m3, a feeding flow rate of 100 $m^3.d^{-1}$, and an FS concentration in the substrate of 12 $kg.m^{-3}$. The FS concentration in the effluent (digestate) is 9 $kg.m^{-1}$. The estimate of FS accumulation in the CLB will be:

$$\boldsymbol{AcFS = \left(FS_{substrate} - FS_{digestate}\right) \times Q} \qquad \textit{Equation 4}$$

Where:

AcFS = Fixed solids accumulation ($kg.d^{-1}$)

$\mathbf{FS_{substrate}}$ = Fixed solids concentration in the substrate ($kg.m^{-3}$)

$\mathbf{FS_{digestate}}$ = Fixed solids concentration in the substrate ($kg.m^{-3}$)

Q = Flow rate ($m^3.d^{-1}$)

Therefore:

$$\text{AcFS} = (12 - 9) \times 100$$
$$\text{AcFS} = \mathbf{300\ kg_{SF}.d^{-1}}$$

There is an FS accumulation in the reactor of 300 $kg_{FS}.d^{-1}$. Thus, 108,000 kg of FS accumulates after one year of operation.

A sand density of 2,000 $kg.m^{-3}$ can be used to estimate the sludge volume to be discarded, as follows:

$$\boldsymbol{D = \frac{m}{v}} \qquad \textit{Equation 5}$$

$$2{,}000 = \frac{108{,}000}{\text{v}}$$
$$\text{v} = \mathbf{54\ m^3.year^{-1}}$$

A total of 54 m^3 of FS will accumulate in the biodigester after one year of operation, which means approximately 2% of the useful volume. It is worth noting that we are considering only FS, but other types of solids may accumulate in the biodigester (e.g., sludge generation by

biological processes). Thus, there is a recommendation for periodic disposal of this material. There is also the need to separate solids before the biodigester (e.g., sandbox) to avoid solids accumulation at the CLB bottom and a reduction in the useful volume of the tank. Reducing the useful volume of the biodigester will result in lower HRT and provide overload conditions.

UASB biodigester

The upflow anaerobic sludge blanket (UASB) biodigester is characterized by the upward flow of wastewater through a sludge blanket to the top of the reactor, where there is a three-phase separator (Figure 4).

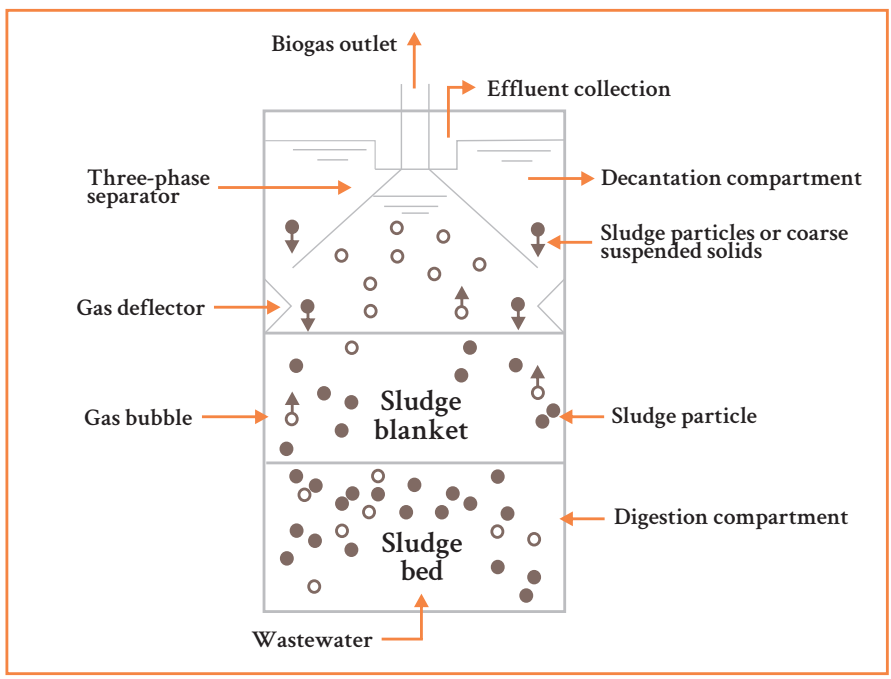


Illustration: Marcos Lins

Figure 4. Operating scheme of a UASB biodigester.

These reactors are characterized by their high biomass retention capacity, which allows them to work with a low hydraulic retention time (4 to 72 hours). In addition, UASB reactors are stable in situations of variations in the wastewater characteristics and support for high organic loading rates (0.5 $kg_{VS}.m^{-3}.d^{-1}$ to 8.0 $kg_{VS}.m^{-3}.d^{-1}$ or 2 $kg_{CODsoluble}.m^{-3}.d^{-1}$ to 32 $kg_{CODsoluble}.m^{-3}.d^{-1}$), especially under conditions in which the organic matter is solubilized.

The wastewater of the UASB reactor must have a low concentration of total solids (<2%) due to hydrodynamic reasons. It indicates that a pre-treatment of effluents from animal production is often necessary.

UASB reactor dimensioning

The organic loading rate, surface velocity, and effective treatment volume should be considered to determine the dimensions and required volume of a UASB biodigester. The effective treatment volume is the volume occupied by the sludge blanket (active biomass). There is an additional volume between the sludge blanket and the three-phase separator. The biodigester nominal volume is calculated based on the organic loading rate, as shown:

$$\boldsymbol{Vn} = \frac{(\boldsymbol{Q} \times \boldsymbol{S_o})}{\boldsymbol{OLR}} \qquad \textit{Equation 6}$$

Where:

Vn = Nominal volume (m^3)

Q = Wastewater flow rate ($m^3.d^{-1}$)

S_o = Wastewater concentration ($kg_{VS}.m^{-3}$)

OLR = Organic loading rate ($kg_{VS}.m^{-3}.d^{-1}$)

A correction factor is used to determine the total corrected liquid volume below the gas collection, indicating the fraction occupied by the sludge blanket. The total reactor volume can be calculated considering the correction factor, which can range from 0.8 to 0.9:

$$Vc = \frac{Vn}{E} \qquad \textit{Equation 7}$$

Where:

Vc = Corrected volume (m^3)

Vn = Nominal volume (m^3)

E = Correction factor (0,8 to 0,9)

The upward velocity is another important variable to avoid biomass carryover, being found by relating the wastewater flow rate with the cross-sectional area of the UASB biodigester:

$$v = \frac{Q}{A} \qquad \textit{Equation 8}$$

Where:

v = Upward velocity ($m.h^{-1}$)

A = UASB cross-sectional area (m^2)

Q = Wastewater flow rate ($m^3.h^{-1}$)

The upward velocity depends on the availability of organic matter present in the substrate. This relationship is shown in Table 2.

Table 2. Upward velocity and recommended height for UASB biodigesters treating different effluents.

Type of effluent	Upward velocity ($m.h^{-1}$)		Reactor height (m)	
	Range	Typical	Range	Typical
Totally soluble COD	1.0-3.0	1.5	6-10	8
Partially soluble COD	1.0-1.25	1.0	3-7	6
Domestic effluent	0.8-1.0	0.7	3-5	5

The biodigester liquid height can be determined using the following relationship:

$$H_L = \frac{V_C}{A} \qquad \textit{Equation 9}$$

Where:

H_L = Biodigester height based on the liquid volume (m)

V_C = Corrected volume (m^3)

A = UASB cross-sectional area (m^2)

The gas collector height is additional to the UASB biodigester height, approximately 25% more. Therefore, the total UASB biodigester height is defined as:

$$H_T = H_L + H_G \qquad \textit{Equation 10}$$

Where:

H_T = Total biodigester height (m)

H_L = Biodigester height based on the liquid volume (m)

H_G = Biodigester height based on the liquid volume (m)

Example 3

Many agro-industrial effluents have considerable concentrations of readily available organic matter, followed by a low concentration of volatile solids. It allows the use of UASB reactors for biogas recovery and waste stabilization. Dimension and determine the HRT for a UASB reactor treating agro-industrial effluent with the characteristics described in Table 3.

Table 3. Characteristics of an agro-industrial effluent.

Item	Unit	Value
Flow rate	$m^3.h^{-1}$	41.67
TS	$g.m^{-3}$	2,000
VS	$g.m^{-3}$	1,700
Alkalinity	$g.m^{-3}$ as $CaCO_3$	500
Temperature	°C	30

Answer:

Determine the UASB reactor volume based on a OLR of 8 $kg_{VS}.m^{-3}.d^{-1}$:

$$\boldsymbol{Vn = \frac{(Q \times S_o)}{OLR}} \qquad \textit{Equation 6}$$

In which:

$$\mathrm{Q} = 41.67 \times 24\ \mathrm{h} = \mathbf{1{,}000\ m^3.d^{-1}}$$

$$\mathrm{Vn} = \frac{1{,}000\ \mathrm{m^3.d^{-1}} \times 1.7\ \mathrm{kg.m^{-3}}}{8\ \mathrm{kg_{VS}.m^3.d^{-1}}}$$

$$\mathbf{Vn = 212.5\ m^3}$$

Determine the corrected reactor volume:

$$\boldsymbol{Vc = \frac{Vn}{E}} \qquad \textit{Equation 7}$$

$$\mathrm{Vc} = \frac{212.5\ \mathrm{m^3}}{0.85} = \mathbf{250\ m^3}$$

Determination of the UASB reactor dimensions:

First, the cross-sectional area is determined based on the surface velocity (Table 2). A value of 1.5 $m.h^{-1}$ was used because the VS/TS ratio in the effluent is 85%.

$$\boldsymbol{v = \frac{Q}{A}} \qquad \textit{Equation 8}$$

$$\mathrm{A} = \frac{1{,}000\ \mathrm{m^3.d^{-1}}}{(1.5\ \mathrm{m.h^{-1}}) \cdot (24\ \mathrm{h.d^{-1}})} = \mathbf{27.8\ m^2}$$

From this, the diameter is calculated:

$$\boldsymbol{A = \frac{\pi \cdot D^2}{4}}$$

$$27.8 = \frac{\pi \cdot D^2}{4} = \mathbf{6\ m}$$

The next step consists in determining the UASB biodigester liquid height:

$$\boldsymbol{H_L = \frac{V_C}{A}} \qquad \textit{Equation 9}$$

$$H_L = \frac{250\ m^3}{27.8\ m^2} = \mathbf{9\ m}$$

Finally, the total reactor height is determined, as follows:

$$\boldsymbol{H_T = H_L + H_G} \qquad \textit{Equation 10}$$

$$H_T = 9\ m + 2.25\ m = \mathbf{11.25\ m}$$

Note: H_G was calculated as 25% of the H_L height.

In short:

- Diameter: 6 m
- Height: 10 m
- Volume: 235 m^3

The HRT calculation considers the corrected reactor volume and the feeding flow rate:

$$\boldsymbol{HRT = \frac{V}{Q}} \qquad \textit{Equation 3}$$

$$\text{HRT} = \frac{250\ \text{m}^3 \times 24\ \text{h.d}^{-1}}{1{,}000\ \text{m}^3\text{.d}^{-1}} = \mathbf{6\ h}$$

CSTR biodigester

The continuous stirred-tank reactor (CSTR) is a biodigester model that supports high organic loading rates (1 $kg_{VS}.m^{-3}.d^{-1}$ to 4 $kg_{VS}.m^{-3}.d^{-1}$) and is characterized by its homogenized content due to the presence of a stirring system. It is the most used biodigester configuration in biogas plants, especially regarding the co-digestion (mixture of substrates) and a higher solid concentration (close to 10% $m.v^{-1}$). CSTR biodigesters represent approximately 90% of the reactors constructed in Europe.

The hydraulic retention time (HRT) and solids retention time (SRT) are the same for CSTR anaerobic reactors, as it is assumed that there is no sludge accumulation in the reactor. The minimum HRT in the reactor is usually between 15 and 20 days, which can vary greatly depending on the type of substrate to be digested. CSTR biodigesters without sludge recirculation are best suited for effluents with high solids concentrations.

The presence of a stirring system adds implementation and maintenance costs to a CSTR biodigester, but it assists in the heat transfer and keeps the solids in suspension, which improves the contact between organic matter and microorganisms. The temperature maintenance by heating systems ensures higher biogas production capacity, as it helps to stabilize the reactor and maintain the population of microorganisms.

Feeding system

The feeding system takes the substrate from the storage site to the biodigester. It can consist of simple transport structures, but it can also be complex systems coupled with methods of homogenization, crushing, and flow control. The level of technology applied is dependent on the project's need and budget.

Stirring system

The proper functioning of the stirring system is essential for the process stability in a CSTR biodigester. The use of a stirring system implies a 15% to 30% gain in biogas productivity (Karim et al., 2005). The importance of proper stirring applies is related to an increase in the distribution of substrates, nutrients, enzymes, and microorganisms in the biodigester. Stirring also contributes to the elimination/reduction of crusts and optimizes the release of biogas present in the sludge.

Two important aspects of stirring in a biodigester are intensity and timing. However, the information available in the literature on these aspects is still contradictory. Very intense stirring for long periods can lead to scum formation problems, affecting biogas release from the biodigester. Insufficient stirring leads to phase separation in the biodigester, interfering with inoculum/substrate contact, heat transfer, and biogas release. In summary, the influence of stirring on the biodigester efficiency depends on factors such as solids content, viscosity, fat content, and the presence of surface-active substances, which promote foam.

Stirring technologies are divided into mechanical, hydraulic, or pneumatic. Figure 5 shows schemes that exemplify the types of stirring. Hydraulic stirring (Figure 5a) occurs with recirculation inside the biodigester utilizing hydraulic pumps located inside or outside the CSTR reactor. Pneumatic stirring (Figure 5b) is established as a function of biogas recirculation, causing homogenization in the reaction medium by bubbling in the liquid or a process known as gas lift. Mechanical stirring (Figure 5c) is the most used in biogas plants and can have different intensities and stirrer models, as follows:

a) Submersible propeller motor pumps. It features high-speed ope ration (1,500 RPM) and good efficiency. It usually operates in discontinuous mode, that is, it turns on and off at programmed time intervals.

b) Long-shaft mixer (Figures 6a). It features an operation with speeds in the range of 10 RPM to 50 RPM. It usually operates in continuous mode, with the disadvantage of higher power consumption.

c) Horizontal paddle mixer (Figure 6b). It is characterized by low speed (2 RPM to 4 RPM). The operation of this stirrer is continuous, and its disadvantage is the difficulty in maintenance.

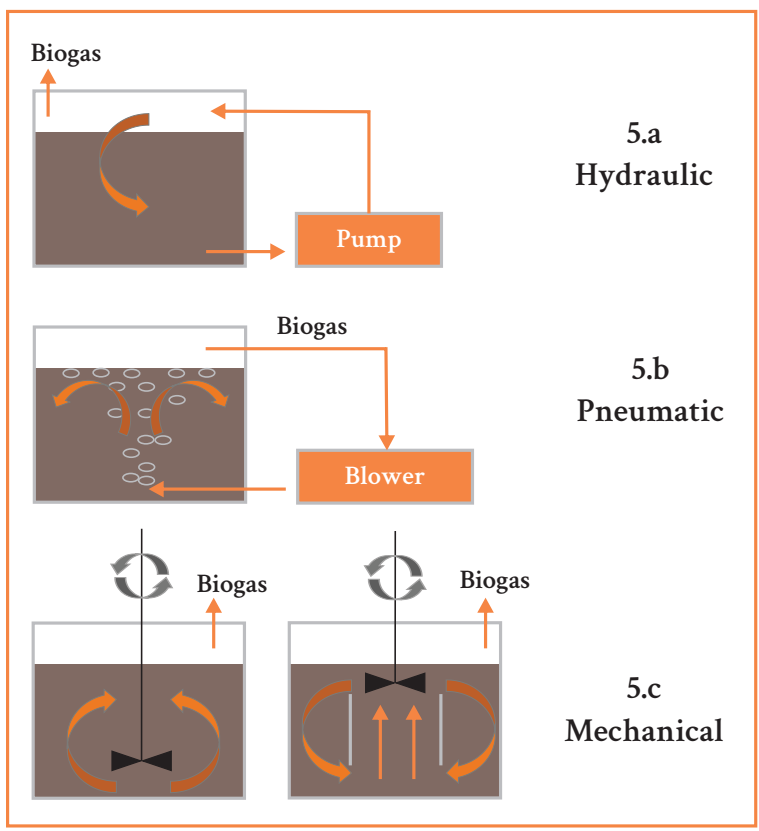


Figure 5. Examples of different stirring modes: a) hydraulic stirring, b) pneumatic stirring, and c) mechanical stirring.

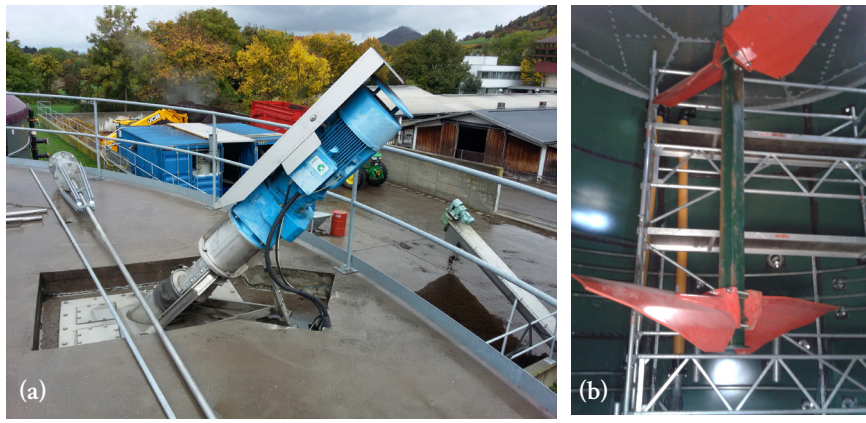


Photo: Ricardo Luis Radis Steinmetz

Figure 6. Examples of mechanical stirrers: (a) long-shaft mixer and (b) vertical paddle mixer.

Heating system

The biomass heating method is of paramount importance in continuous processes. The heat requirement is a function of the substrate flow, the specific heat capacity of the materials, the temperature difference between the substrate and the operating temperature in the biodigester, and the system heat loss.

There are several possibilities for heating biomass in a CSTR biodigester (Figure 7). Some systems opt for heating the substrate, others for direct biomass heating or even the circulation of heated water through coils inside the reactor.

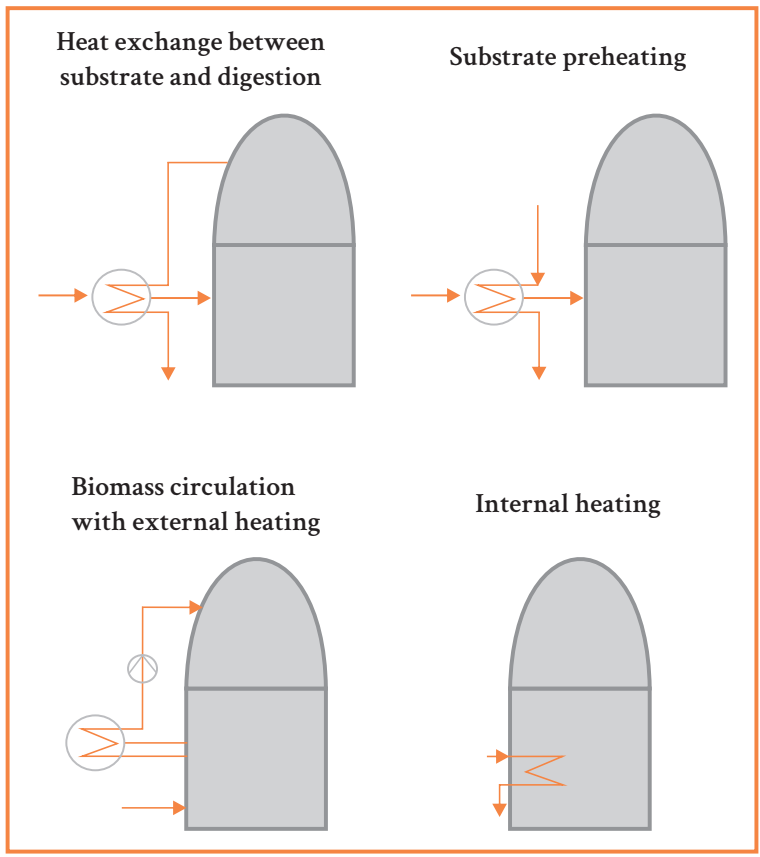


Illustration: Vivian Fracasso

Figure 7. Heating systems most used in anaerobic digestion.

The most adopted practice is the use of coils as a heat exchanger, where heated water circulates through the biomass, maintaining the desired temperature. The substrate is heated to the desired temperature suitable for biogas production through heat transfer processes.

The need for heat to be generated by the heating system can be calculated by the equation:

$$Q_{sa} = m.c_{pa}(T_2 - T_1) \times \eta \qquad \textit{Equation 11}$$

Where:

Qsa = Need for heat (kJ)

m = Vheating fluid flow rate ($kg.s^{-1}$)

c_{pa} = Heating fluid specific heat ($kJ.kg^{-1}.^oC^{-1}$; for water: 1 $kJ.kg^{-1}.^oC^{-1}$).

T_1 = Substrate initial temperature (^oC)

T_2 = Reactor operating temperature (^oC)

η = Process efficiency (%)

The heat needed to heat the substrate to the desired temperature can be obtained by Equation 12:

$$Q = m_s\, c_e(T_2 - T_1) \qquad \textit{Equation 12}$$

Where:

Q = Energy required for wastewater heating (kJ)

c_e = Substrate-specific heat ($kJ.kg^{-1}.^oC^{-1}$)

m_s = Substrate mass (kg)

T_1 = Substrate initial temperature (^oC)

T_2 = Reactor operating temperature (^oC)

The following equation is used to estimate the substrate-specific heat, considering the total solids concentration:

$$C_e = 4.19 - 0.00275 \times S_{TS} \qquad \textit{Equation 13}$$

Where:

S_{TS} = Total solids concentration in the substrate ($g.L^{-1}$)

The heat needed to keep the temperature inside the biodigester constant is equal to the heat flux through the external surfaces and considers the construction material, which can be calculated by the equation:

$$Q_W = \frac{A(t_i - t_e)}{R} \qquad \textit{Equation 14}$$

$$R = \frac{e_x}{k_x} \qquad \textit{Equation 15}$$

Where:

Q_w = Heat flow through the contact surface ($W.m^{-2}$)

A = Surface area (m^2)

ti = Internal temperature (^oC)

te = External temperature (^oC)

R = Material thermal resistance ($m^2.^oC.W^{-1}$)

ex = Material thickness (m)

kx = Material thermal conductivity ($W.m^{-1}.^oC^{-1}$)

Example 4

A water heating system working with two heating elements of 1,800 W each was studied. These heating elements heat 55 L of water externally to a 10 m^3 biodigester, and the heated water is recirculated with a motor pump through coils in contact with the biomass in the biodigester.

The biomass temperature was indirectly controlled by the temperature of the water that recirculates in the coil. A set point could be determined for the coil water using the installed temperature controller.

The amount of energy used to heat the biomass was evaluated through the monitoring of the average time that the heating elements remained on.

The heating had the characteristic of turning on for 8.20 minutes, remaining turned off for an average of 16 minutes. It resulted in 7.50 hours connected per day.

The heating element power was calculated by multiplying current x voltage, resulting in 3.63 kW. Therefore, consumption reached 27.21 kWh d^{-1} or 816.44 kWh in a month.

This energy was enough to keep the water between 45 and 55 °C circulating in the biodigester coil, which allowed an average increase in the waste temperature of 5.6 °C, that is, from 24.70 °C to 30.30 °C in the biodigester.

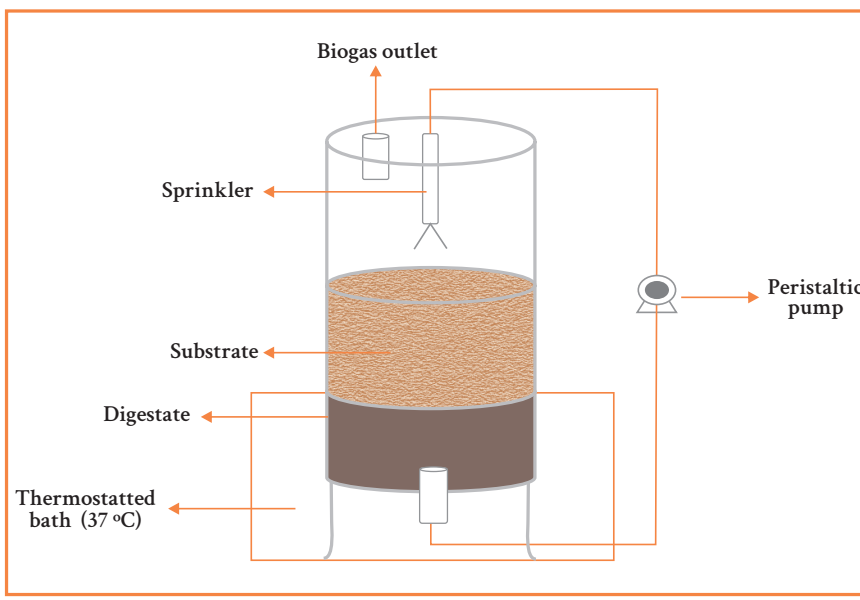
The biodigester operating conditions during this experiment were:

Flow rate = 560 $L.d^{-1}$

HRT = 18 d.

Solid-state biodigester (dry digestion)

Solid-state biodigesters are more common with a batch operation (Figure 8), being fed with waste containing between 20% and 40% solids. The substrate is added to the reactor together with inoculum (50% $m_{substrate}/m_{inoculum}$), with the percolated liquid recirculated over the solid fraction.



Source: Adapted from Marchioro et al. (2018).

Figura 8. Solid-state batch biodigester with inoculum recirculation.

The amount of solids in the biodigester affects its volume and the treatment process. Biodigesters with a smaller volume than the other technologies studied in this book are required due to the low water concentration in solid-state digestion systems. On the other hand, there is a need for pumps to recirculate the leachate.

The digestion time lasts from 2 to 4 weeks, depending on the type of substrate. The methane concentration in the biogas is relatively high, that is, approximately 80%. Solid-state digestion has some characteristics:

- Biogas productivity is 15% to 40% lower than wet digestion.
- Smaller biodigester volume.
- Supports substrates with higher solids concentration as well as larger particle size.
- No large substrate dilutions are required.
- The bioreactor needs to be opened to be filled in and/or emptied.
- The bioreactor feeding is discontinuous.

Safety in the biodigester operation and biogas handling

There is a wide range of hazards that exist in an anaerobic biodigester or biogas and/or biomethane plant. These hazards are related both to occupational and environmental risks and also the effectiveness of the biogas production process.

Biogas itself represents a hazard with chemical and physical risks due to its constituents. The gas mixture that composes the biogas has asphyxiating (suffocation) properties. Other aspects such as corrosivity and toxicity of hydrogen sulfide (H_2S), the toxicity of ammonia (NH_3), and inflammability of methane (CH_4) and hydrogen (H_2) must also be considered.

In the case of methane, the mixture with air in concentrations from 5% ($v.v^{-1}$) to 15% ($v.v^{-1}$) is sufficient for combustion to occur, and the vapors trigger an explosion if restricted in a confined space. Oxygen concentration in H_2S removal systems by injecting air or oxygen in situ in the anaerobic biodigester must never exceed 4.5% (Brasil, 2015a).

Therefore, periodic monitoring to evaluate whether there are gas leaks in the reactor, pipelines, and reservoirs is of paramount importance. A gas leak can be evaluated in several ways.

A 2% detergent solution can be used in low technological reactors (e.g., CLD in rural properties), being applied to connections, valves, gaskets, and canvas using a brush. The occurrence of bubbles would indicate gas leakage. Leaks in more advanced technological reactors (e.g., industrial-scale plants) can be monitored using special cameras, which generate images in the infrared region, allowing the identification of anomalies in the air.

The use of pressure gauges to monitor gas pressure is recommended to work around problems with excess pressure. Also, the need for valves and the possibility of flame arrester systems in risk areas must be verified. It is desirable to install at least one valve per anaerobic reactor with isolation from its respective gas chamber.

Gate and butterfly valves are the most used. Ball valves are often used in pipes with a nominal diameter of up to DN 50. Butterfly valves must be fitted with a stop. Valves made of nodular cast iron or higher quality steel should be used. Grey cast iron valves should not be used due to the possibility of a chemical attack by H_2S. Thus, valves must be made of materials resistant to the corrosion potential of biogas. Valves must be installed upstream and downstream of the flame arrester valves to allow maintenance activities to be carried out safely and prevent the entry of air into the biogas pipeline.

Alternatively, a very simple system called a water seal is used for pressure control in biodigesters operating at low pressure and small scale, as shown in Figure 9. It is a "U" tube filled with water to act as a hydraulic seal. The height is usually about 10 mm for covered lagoon biodigesters. The importance of internal pressure equalization is shown in Figure 10, in which the biodigester was displaced by excess stored gas, resulting in severe structural problems.

Photo: Ricardo Luis Radis Steinmetz

Figure 9. Gasometer pressure relief valves and simplified water seal system for pressure equalization inside a biodigester.

Photo: André Cestonaro do Amaral

Figure 10. Damaged biodigester in which there was an excessive biogas accumulation, and the water seal did not work properly.

Other risk factors involving electricity and heat should also be considered when designing a biodigester or biogas plant. Concern with the grounding of pipelines and equipment should be considered as a precaution against static electricity, avoiding sparks and electrical discharges.

Therefore, possible sources of ignition must be evaluated and avoided. The use of cell phones, smoking, or any other source of sparks or flames must not be allowed in the risk areas. In addition, the use of lightning rods must also be evaluated and considered.

The installation of burners for the disposal of excess gas is an important safety tool, but it also needs some care. The ABNT NBR 12.209 (1992) standard provided for a safe distance between burner and biodigester and/or gasometer of at least 30 m. Furthermore, the minimum distance to any other building should be 20 m.

These distances were disregarded in the updated version of this standard (2011), only indicating that the burners must be installed so that their flames, gases, and hot components do not pose a risk. In this case, the flame and the gas and smoke outlet must be at a minimum height of 3 m and the area within the 5 m radius of the burner must be free of vegetation (shrubs and trees). The enclosed burner must be installed at least 5 m away from buildings and traffic routes and open flame burners may require longer distances, which must be evaluated for each case. In all cases, the use of windshields and rain shields is recommended to improve the lighting and monitoring of the burner and pilot light, if any (Brasil, 2015a).

Other risks related to the handling of substrates or digestate must also be considered. In addition to the environmental risks, there is a biological occupational risk. Therefore, the requirements in the Regulatory Standards on Safety and Health at Work, especially standards 15 and 32 (Brasil, 2015b), should be considered.

The biological risk will depend on the type of substrate used in the biodigester and, therefore, safety precautions must be proportional. Use of personal protective equipment (e.g., gloves, closed-toe footwear, and safety glasses) should be prioritized when handling substrate and digestate samples.

Substrates with higher risk potential, such as the organic fraction of urban solid waste, domestic effluents, sewage sludge, and dead animals, may receive thermal processes such as pasteurization to reduce and control pathogens. The evaluation of technical and economic feasibility must be considered in all cases.

Inspections of hydraulic pumps and pipelines carrying substrate and digestate must be routinely carried out to avoid clogging and overpressure. Some liquids can form precipitates or deposits on the pipe walls (Figure 11), restricting the flow, causing an increase in pressure, work overload in pumps, leaks, and even a drop in biogas productivity.

In these cases, strategies for inspection of pipelines and leak containment systems must be evaluated. Alternatively, leak containment strategies should be considered. In these cases, the use of physical barriers, channels, and liquid storage tanks should be considered in all risk areas.

Photo: Lucas Scherer Cardoso

Figure 11. Struvite-encrusted digestate pipeline section.

Another basic precaution for accident prevention is the delimitation of areas where the biodigesters, reservoirs, and other facilities are located using fences and orientation notices (Figure 12).

Photo: Lucas Scherer Cardoso

Figure 12. The areas where biodigesters, reservoirs, and other facilities are located must be fenced and signposted.

Other issues such as the control of vegetation around the facilities and the control of rodents, which are largely responsible for damaging plastic membranes and electrical cables, can also avoid simple problems that impact the operation of a biodigester or biogas plant.

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Chapter IV

BIOGAS TREATMENT AND PURIFICATION

Marcio Luis Busi da Silva
Melissa Paola Mezzari

Introduction

The energy use of biogas in Brazil has been carried out for at least 40 years when it was initiated and integrated into the "green revolution" model in the 1970's. Biogas has been used in the last decade as a national energy source, mainly due to the stimulus of the carbon credit market, which seeks to reduce methane emissions – a biogas component that contributes to the greenhouse effect. Currently, Brazil has 123 operating biogas plants used to produce thermal, electrical, and mechanical energy and biomethane/CNG (Figure 1) (CIBiogás, 2016). The substrates used in the biodigestion come from different industrial and agricultural sources.

The degree of purity and concentration of methane are the main factors to be considered in terms of biogas calorific potential, which varies between 15 $MJ.Nm^{-3}$ and 30 $MJ.Nm^{-3}$ (Abatzoglou; Boivin, 2009). Hydrogen sulfide (H_2S), carbon dioxide (CO_2), and ammonia (NH_3) stand out among the main contaminants that affect the energy potential

of biogas. The raw biogas may present H_2S concentrations ranging from 100 ppm to 10,000 ppm ($mg.m^{-3}$) and, in extreme cases, up to 30,000 ppm, depending on the composition of the substrate used in the anaerobic digestion (Beil; Beyrich, 2013). H_2S is a gas that has a bad odor, in addition to being corrosive and toxic (Hendrickson et al., 2004; Ni et al., 2000). Regarding human health and toxicity, it is known that continuous exposure to low gaseous concentrations of H_2S (15 ppm – 50 ppm) results in mucosal irritation in the respiratory tract, which can cause headaches, dizziness, and nausea (MSDS, 1996). High concentrations between 200 ppm and 300 ppm result in respiratory arrest, and exposures to concentrations above 700 ppm for more than 30 minutes are fatal (MSDS, 1996). Despite being a flammable gas that contributes to the energy potential of biogas, the presence of H_2S causes corrosion in storage tanks, metallic pipes, and combustion engines, and results in the deterioration of the biogas production infrastructure (Garcia-Arriaga et al., 2010).

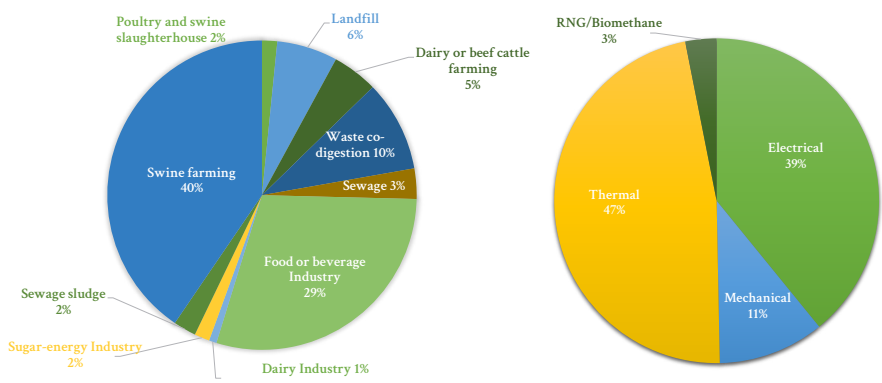


Figure 1. Distribution and variety of substrates used in anaerobic biodigestion (left) and energetic application of biogas use (right) in Brazilian operating plants.

Carbon dioxide (CO_2) is one of the main constituents of biogas, corresponding to a volume of 20% to 30% in substrates from agricultural residues (Wellinger et al., 2013). The presence of CO_2 directly interferes with the energy potential of biogas, as it is inert in terms of combustion and occupies a volume.

Ammonia (NH_3) is also another very common contaminant in biogas originating from agricultural residues, present at concentrations ranging from 50 $mg.m^{-3}$ to 100 $mg.m^{-3}$ (Wellinger et al., 2013). Ammonia has corrosive and toxic properties. The incomplete combustion processes in engines, for example, release nitrogen oxides (NO_x) into the atmosphere, which can contribute to the formation of acid rains and result in respiratory problems (Latha; Badarinath, 2004).

In addition to the mixture of gases, biogas also has water (moisture) in its composition at mean concentrations of 6% at 40 °C. The water can accumulate in pipelines via condensation processes when not removed from the biogas, resulting in corrosion problems and/or clogging in the event of freezing. Table 1 shows the main problems caused by contaminants present in the biogas and the maximum concentration allowed according to the application.

In this context, implementing biogas treatment technologies to generate a quality fuel that can be efficiently converted into thermal, electrical, and/or mechanical energy is necessary. Currently, biogas treatment and purification processes are based on: (1) calorific value adjustment and removal of contaminants that affect the biogas quality and the useful life of system components; and (2) biogas purification and biomethane concentration for its insertion in biogas distribution and transport lines (Figure 2).

This chapter provides information on the most commonly used biogas treatment techniques, including the advantages and disadvantages of each process, as well as the basics of dimensioning. It is important to point out that the choice of treatment technology directly depends on the flow rate of the produced biogas, the biogas composition, and, mainly, the biogas purification level to be achieved (Figure 2). More complex and costly treatment systems to implement and operate will allow achieving higher biogas purification levels, as they allow the removal of contaminants more effectively and efficiently. However, conditioning biomethane with a high degree of purification is not always necessary. In this case, simpler and cheaper treatment systems can be used, as long as they meet the minimum requirements for each type of application (Table 1).

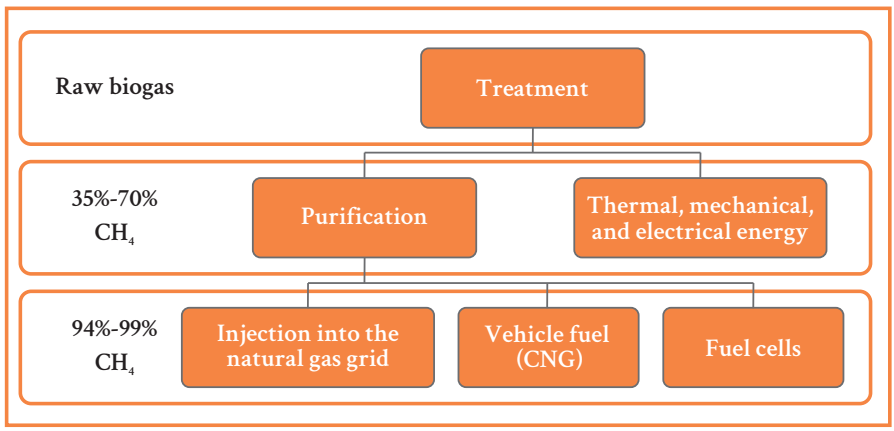


Figure 2. Biogas treatment and purification steps for biomethane concentration according to its final use.

The efficiency of removing contaminants in a treatment system is obtained through physicochemical analyses carried out to characterize the biogas at the entrance and exit of the treatment. In addition to specific analyses to determine contaminant concentrations, the Wobbe index, the relative density, and the calorific value of the biogas can also be calculated (Wellinger et al., 2013). The constituent concentrations must also be determined through specific analyses (e.g., BNT, ISO, and ASTM) in the case of conditioning or purifying the biogas for injection into the biogas network, in accordance with Resolution No. 16 of the National Agency of Petroleum, Natural Gas and Biofuels (ANP).

Table 1. Main biogas impurities, generated problems, and quality requirements for energy purposes.

Contaminant	Problems	Energy use requirements
Water	• Corrosion of compressors, fuel tanks, and engines due to the formation of acids with H_2S, NH_3 and CO_2 • Water accumulation in pipelines • Condensation or freezing due to pressure	Removal for performance in internal combustion engines, microturbines, molten carbonate fuel cells; conversion into biomethane
Particulate matter	Clogging by accumulation in compressors, fuel tanks and engines	
Oxygen	Danger of explosive mixtures due to the high O2 concentration in the biogas	
Ammonia	Corrosion by dissolution in water	
Hydrogen sulfide (H_2S)	• Corrosion of compressors, fuel tanks, and engines • Toxic concentrations in the biogas (>5 $cm^3\ m^{-3}$) • SO_x formation by combustion	• < 250 ppm for boiler heating • 545 ppm – 1,742 ppm for internal combustion engines • 2800 ppm for generators • 10,000 ppm for turbines and microturbines • 2 ppb – 15 ppb for injection into the biogas network • 5 ppb for vehicle fuel • 1 ppb – 5 ppb for fuel cells
Carbon dioxide (CO_2)	Low energy value	< 2% in the conversion into biomethane
Siloxanes	• Formation of SiO_2 and microcrystalline quartz by combustion • Deposition on spark plugs, valves, and cylinders	• 9 ppm – 44 ppm for internal combustion engines • 0.42 ppm for generators • 0.05 ppm – 0.08 ppm for turbines and microturbines • < 100 ppb for fuel cells
(Not generated by agricultural residues) Halogens	Corrosion in combustion engines	< 1 ppm for fuel cells

Source: Adapted from Ryckebosch et al. (2011) and Sun et al. (2015).

Water removal

The raw biogas obtained at the exit of the biodigester is saturated with moisture that must be effectively removed. The methods of water removal from the biogas generally also allow simultaneous removal of impurities such as particulate matter and foam (if any). Water removal is usually carried out at the first stage of biogas filtration to prevent corrosion of compressors and pipelines.

The physical separation of water by condensation or chemical drying are among the most used methods (Table 2). The main condensation techniques use cyclone separators, fine mesh screen demisters (porosity 0.5 ηm - 2 ηm), and pipes with a purge to separate and eliminate the condensed steam (Novak et al., 2016; Ryckebosch et al., 2011). Pipe cooling improves condensation and is generally more efficient in removing moisture from the biogas. However, the implementation and maintenance of this practice have a high cost, making it more complex with the installation of coolers and associated piping. Chemical drying includes the use of cylindrical reactors with absorbent materials in their internal volume, such as triethylene glycol or hygroscopic or adsorbent salts such as zeolites, silica gel, or aluminum oxide (Novak et al., 2016; Ryckebosch et al., 2011). Chemical drying is the predominant technique, but materials need to be changed and regenerated frequently to maintain the efficiency of moisture removal from the biogas (Table 2). Simultaneous removal of particles and hydrocarbons can also occur during the first step of the moisture removal treatment (Ryckebosch et al., 2011). Table 2 shows the advantages and disadvantages of conventional systems for removing the moisture from the biogas. Figure 3 shows a desiccator with absorbent material with the main function of removing water from the biogas.

Table 2. Advantages and disadvantages of techniques to remove water from the biogas.

Process	Technique	Advantages	Disadvantages
Adsorption	• Silica gel • Molecular sieve • Alumina	• Adsorbent materials can be regenerated • They can be destined for any use of biogas, with low operating costs	• Adsorption columns must be operated at a pressure of 6 bar – 10 bar • Particles and oil must be previously removed
Absorption	• Ethylene glycol • Selexol • Hygroscopic salts	• Materials can be regenerated • Simultaneous removal of particles and hydrocarbons • They are not toxic compounds • High removal efficiency	• Need for high temperatures and pressures for regeneration of the absorber solution • Need to treat high gas volumes (>500 $m^3.h^{-1}$) to be feasible economically
Cooling	• Cooling to 2 °C	• Most efficient technique for removing moisture from biogas	• High energy consumption to keep the system cool
Condensation	• Cyclone separator demisters • Pipe heat exchange systems • Piping with water vapor purge system	• They can be applied as a pre-treatment in all biogas generation systems • Reduction of water vapor by up to 0.15% of the initial volume • Elimination of hydrocarbon and oil particles, simple technology	• The pipes must be long and installed with a slope to allow purging of water vapor • The pipes must be resistant to freezing

Source: Adapted from Ryckebosch et al. (2011).

Source: AVP, Air & Vaccum, Inc. (http://airvacuumprocess.com).

Figure 3. Example of a desiccator for water removal from the biogas: reactor filled with adsorbent material (zeolites).

H_2S removal

The H_2S removal can be carried out by biological, physical, or chemical processes. The desulfurization process is divided into two phases: (1) primary phase, which reduces H_2S levels to <500 ppm, achieving an efficient removal of approximately 100 ppm; and (2) precision phase, which adjusts the H_2S concentrations to specifications and requirements for injection into the natural biogas network. In this case, the H_2S concentrations are reduced to less than 0.005 ppm. In addition to this classification, desulfurization methods can be divided into internal or external methods when occurring inside or outside the biodigester, respectively (Figure 4).

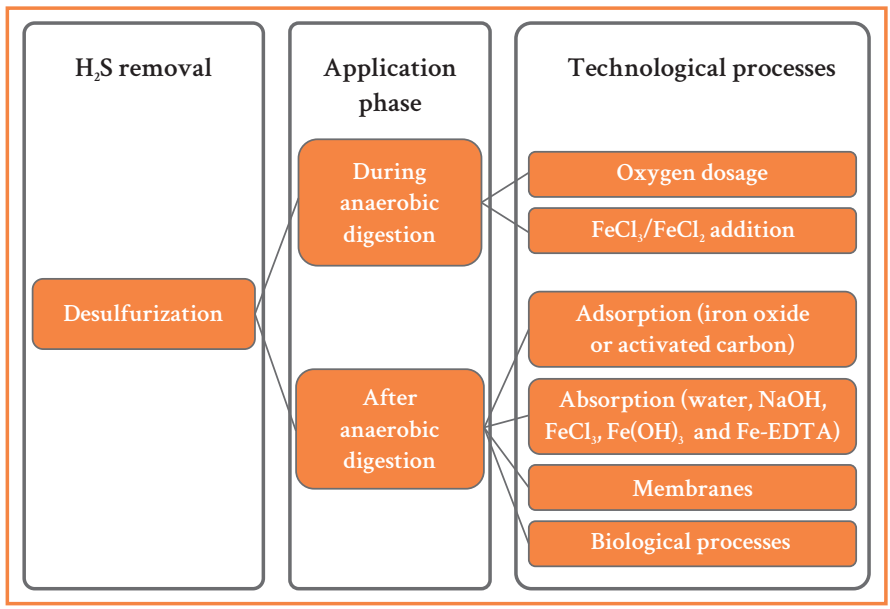


Figure 4. Most commonly observed technological processes for biogas desulfurization.

H_2S removal inside the biodigester

Oxygen injection

Desulfurization inside the biodigester occurs through micro-aeration or direct injection of air or pure oxygen through the use of special gas cylinders. The growth of bacteria that oxidize H_2S is stimulated in the presence of oxygen, occurring in the process of biological desulfurization of H_2S into elemental sulfur. Oxidation is carried out by the action of a specialized group of sulfide-oxidizing microorganisms, which are widely found in the anaerobic environment of biodigesters (e.g., *Thiobacillus* spp. and *Acidithiobacillus* spp.).

The oxygen supply needs to be carefully calculated and monitored to apply this technique. Sulfate (SO_4^{2-}) will be the main product to be formed if the molar ratio of oxygen consumption with sulfide (O_2/S_2^-) is higher than or equal to two (≥2). However, the products formed will be represented mostly by elemental sulfur if the ratio (O_2/S_2^-) presents values between 0.5 and 1.0.

Oxidation-reduction potential (ORP) meters are used to control the molar ratio between oxygen and sulfide (O_2/S_2^-)in the biodigester (Khanal; Huang, 2003). ORP values in the order of –100 mV to –400 mV indicate the formation of elemental sulfur as the main product, accounting for more than 80% of the removed H_2S (Janssen et al., 1998; Krishnakumar et al., 2005). A sensor for measuring the redox potential must be installed in the upper air part of the biodigester for the control of air injection provided by pumping (conventional injection pumps for aeration gases). An automatic controller triggers a solenoid valve to open or close the air injection system depending on the ORP setpoint.

However, there are disadvantages associated with the process despite the simple, efficient, and low-cost methodology for H_2S removal. Incorrect air dosing in the system can lead to potentially explosive mixtures (6%–12% $v.v^{-1}$ oxygen). The presence of oxygen in the biodigester can inhibit methane production, as methanogenic microorganisms are sensitive to the presence of oxygen. Furthermore, H_2S oxidation can generate elemental sulfur deposits inside the biodigester, increasing the sludge volume, which must be removed frequently. The presence of trace gases of oxygen and nitrogen resulting from the addition of air to the system may limit the biogas applicability.

Iron chloride addition

Iron chloride reacts with H_2S to form insoluble iron sulfide (FeS) through the precipitation reaction of the iron salt. Iron chloride addition (ferric chloride – $FeCl_3$, and ferrous chloride – $FeCl_2$) to the biodigester is carried out by direct dosing inside or externally, using a reactor installed in series in the biogas line. These chemical reactions are very efficient in reducing H_2S (Table 3), but the removal levels are not sufficient for limit concentrations established in fuel cells or injection in natural biogas pipelines. The concentrations achieved with this methodology are ≤ 100 ppm of H_2S (Ryckebosch et al., 2011), suggesting its application for biogas purification for use in boilers, engines, or turbines.

The sulfur precipitation reaction is pH-dependent despite being a low-cost and simple technique, with a lower efficiency under acidic conditions. However, methanogenic processes generally act to remove

the medium acidity (removal of H^+, CO_2, and organic acids used as biological substrates), which makes the medium naturally well buffered with pH ranging from 7 to 8. The lack of selectivity of reagents with sulfur enables parallel reactions to occur, resulting in reduced H2S removal efficiency (Devai; Delaune, 2002; Speece, 2008). In this case, a higher concentration of oxidizing agent (Table 3) is necessary to guarantee the stoichiometric efficiency of the reaction with sulfur, which implies higher amounts of reagents to be used, thus increasing the costs and complexity of the technique. The formation of precipitates inside the biodigester is a potential problem.

The sediment formed must be frequently removed from the biodigester to avoid loss of internal volume and alteration in the biogas production capacity due to changes in the hydraulic retention time. Biodigesters that do not have a complete mixture may present an inefficient H_2S removal due to the lack of homogeneity necessary to favor an efficient chemical reaction.

The stoichiometric demand in the chemical treatment processes allows calculating the theoretical amounts of reagents and products to be added for desulfurization. Table 3 shows the amount of reagent to be used based on these calculations.

Table 3. Stoichiometric calculations and demand in the physicochemical and biological treatment processes for H_2S removal from the biogas.

Tratamento	Reações estequiométricas	Demanda química
$FeCl_3/FeCl_2$	$2FeCl_3 + 3H_2S \rightarrow 2FeS + S + 6HCl$	0.31 g $FeCl_3$/g H_2S
Fe(OH)n	$2Fe(OH)_3 + H_2S \rightarrow 2Fe(OH)_2 + S + 2H_2O$ $2Fe(OH)_2 + H_2S \rightarrow FeS + 2H_2O$	0.16 g $Fe(OH)_3$/g H_2S 0.19 g $Fe(OH)_2$/g H_2S
Iron oxides	$FeO + H_2S \rightarrow FeS + H_2O$ $Fe_2O_3 + 3H_2S \rightarrow Fe_2S_3 + 3H_2O$	0.47 g FeO/H_2S 0.64 g $Fe2O_3$/g H_2S

Removal of H_2S downstream

Adsorption processes

Various adsorbent materials such as synthetic zeolites, activated carbon, silica gel, or alumina are used to remove H_2S, N_2, NH_3, and H_2O, among other compounds, from the biogas (Beil; Beyrich, 2013; Ryckebosch et al., 2011). Reactors with adsorbent materials are installed in series in the biogas line. These reactors in the form of columns operate at different stages, such as adsorption, depressurization, desorption, and pressurization. The adsorption of contaminants occurs under pressure (~800 kPa) and the desorption occurs by depressurizing the bed, allowing the removal of contaminants and regeneration of the adsorbent.

Although H_2S removal can be performed by this process, the recommendation is that it be previously removed through the other purification processes mentioned above, as the presence of H_2S can make the adsorption on the material irreversible (Ryckebosch et al., 2011). The presence of water in the biogas can also result in a rapid saturation of adsorbent materials and, therefore, water removal before the adsorption process is recommended. Gases such as O_2 and N_2 can be removed during adsorption, as long as the adsorbent material has selectivity to these elements and is applied under specific conditions of atmospheric pressure (Ryckebosch et al., 2011).

The most commonly used adsorbents consist of activated carbon and iron oxide (Abatzoglou and Boivin, 2009). Activated carbon can be impregnated with potassium permanganate ($KMnO_4$), potassium iodide (KI), potassium carbonate (K_2CO_3), or zinc oxide (ZnO), which act as catalysts, resulting in increased speed of the H_2S oxidation reaction (Petersson, 2013). Activated carbon impregnated with metal salts with catalyst effect has a higher capacity for H_2S adsorption due to the combination of microporosity and oxidative properties. Examples of metal oxides also include Fe_2O_3, Cu_2O, and MnO (Wiheeb et al., 2013).

H_2S readily reacts with iron oxide, iron hydroxide, and zinc oxide to form iron sulfide or zinc sulfide, respectively. The method is commonly known as an iron sponge because it uses a solid base, in this case,

steel wool covered with iron filings to form the reaction bed (Figure 5). An alternative to the use of steel wool is wood chips impregnated with iron oxide, which have been preferably used as a reaction bed.

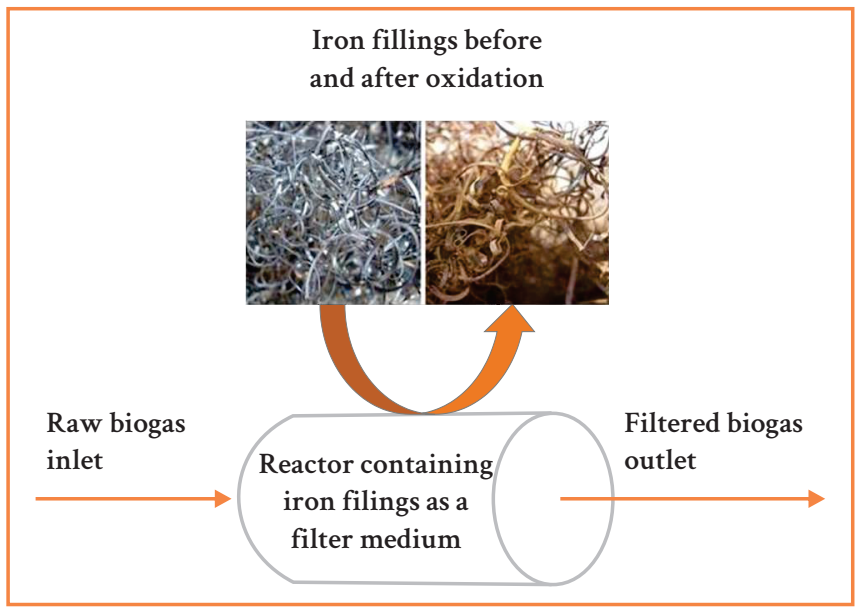


Illustration: Marcio Busi

Figure 5. Diagram of a filter installed in series in the biogas line containing iron filings as adsorbent material. Detail of iron filings oxidation.

Adsorption processes are extremely efficient in reducing H_2S (< 5 ppm) and are widely applied when there is a need to achieve low H_2S levels in the biogas, such as fuel cells and biomethane (Beil; Beyrich, 2013). One of the great disadvantages of this filtering practice is the accumulation of ferric sulfide, which is formed from the oxidation reaction of H_2S with iron, reacting exothermically in the presence of air and causing instantaneous ignition and risk of explosion. This is known as a pyrophoric process. The chemical equation for the ferric sulfide formation is shown in Equation 1:

$$\boldsymbol{Fe_2O_3(iron\ filings) + 3H_2S \rightarrow 2FeS + 3H_2O + S}$$

Equation 1

Other disadvantages include the high cost of the system and difficulties in its operation and maintenance, which require regeneration or frequent exchange of adsorbent materials. There are also energy costs during the regeneration process, as high temperatures (450 °C) are required to achieve this goal. Neglecting rigorous maintenance results in a loss of contaminant removal efficiency, which compromises the quality and end-use of the biogas.

The adsorbent material is placed in a reactor installed in series in the biogas line to dimension the system. In general, these reactors are built using material inert to corrosion, such as PVC containers or even stainless steel, the latter presenting high costs. The quantity (or volume) of adsorbent material to be used is calculated according to the mass required to satisfy the reaction stoichiometry (Table 3). The use of activated carbon impregnated with oxidizing solution leads to an adsorption capacity of 150 milligrams of H_2S per gram of activated carbon. On the other hand, the adsorption capacity of non-impregnated activated carbon is reduced to 20 milligrams of H_2S per gram of activated carbon (Abatzoglou; Boivin, 2009). The activated carbon needs to be replaced after it reaches saturation and loss of adsorption efficiency and the consequent H_2S removal. The mass of activated carbon to be used per day (or months, according to the produced biogas flow rate and the adsorbent reactor size) can be estimated through the biogas flow rate (Q; $m^{3}.d^{-1}$) multiplied by the H_2S concentration found in the biogas (C; $kg.m^{-3}$). Based on the obtained result, the mass of activated carbon to be used is normalized by the maximum adsorption capacity mentioned above.

Absorption processes

Absorption processes encompass chemical methods using water, reagents, and/or organic solvents. Contaminants with a higher solubility are dissolved and removed along with water during biogas washing. The biogas washing to remove CO_2 can be carried out very effectively under high pressure (1,000 kPa–2,000 kPa). After decompression and desorption, CO_2 is released into the atmosphere, and water is regenerated. This simple, low-cost technology results in an efficient CO_2 removal (95%) (Beil; Beyrich, 2013; Ryckebosch et al., 2011). The final CH_4 concentrations range from 93% – 98%.

The presence of organic or chemical reagents has higher CO_2 absorption rates compared with water. Some examples of organic reagents include polyethylene glycol (Selexol®) and tetraethylene glycol dimethyl ether (Genosorb®) (Beil; Beyrich, 2013; Ryckebosch et al., 2011). Chemical solvents commonly used in the absorption process include aqueous amine solutions, alkaline salt solutions, or iron-containing solutions (Zhao et al., 2010). Chemical absorption uses low atmospheric pressure (50 mbar – 150 mbar), with the chemical solution being regenerated in the desorption column by heating processes (106 °C –160 °C) (Beil; Beyrich, 2013). The treated biogas must undergo a drying process to remove the water vapor formed due to the heating of the desorption column (Beil; Beyrich, 2013).

The CH_4 recovery rates are high, reaching values close to 99.9%. Although it is a very efficient technique for removing H_2S (>97%), the disadvantages include costs with chemical-oxidizing agents, energy demand for continuous pumping of the solution to wash the biogas, and, mainly, the generation of a final liquid effluent that needs to be regenerated or properly treated before its final disposal.

Biogas washing is usually carried out using reactors with corrosion-resistant material (PVC or stainless steel). The reactors are usually cylindrical, and their internal volume is filled with water containing or not chemical solutions. The internal circulation of the washing liquid medium occurs through pumping. The reactors are generally built with a longer length and smaller diameter and arranged in a vertical position to optimize the reaction time and contact between CO_2 and H_2S. The biogas enters the bottom of the reactor and flows counter-currently to the liquid flow to the top of the reactor, where it is collected.

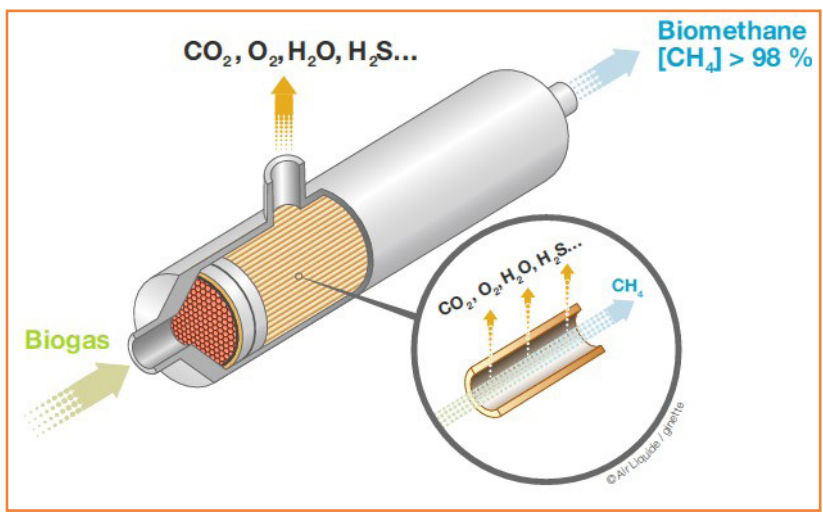
Selective membranes

The use of separation processes using membranes or gas permeation comprises the methane separation from other biogas contaminants by diffusion processes. The types of contaminants to be separated are associated with the type of membrane, structure, permeability, and lifetime. Polymeric or inorganic materials are the most used in the preparation of membranes for CO_2/CH_4 separation (Basu et al., 2010), and

polyimide and cellulose acetate are the most used (Budzianowski, 2016). Membranes composed of inorganic material have 5 to 10 times more selectivity than conventional polymeric membranes (Budzianowski, 2016).

Treatment using membranes (Figure 6) involves separation by high pressure (approximately 96% removal efficiency) or biogas-liquid absorption (98% efficiency) (Ryckebosch et al., 2011). The treatment process is performed in compact systems and generally does not require frequent maintenance (Zhao et al., 2010). Some examples of membranes available on the international market are PRISM®, SEPURAN®, Perm-Select®, and Valopur®.

One of the main disadvantages of the biogas treatment process using membranes is related to the high costs of the system. Therefore, this technique is only recommended when it is intended to obtain biomethane with a high degree of purity.



Source: http://www.apsleyfarms.co.uk/gas-to-grid

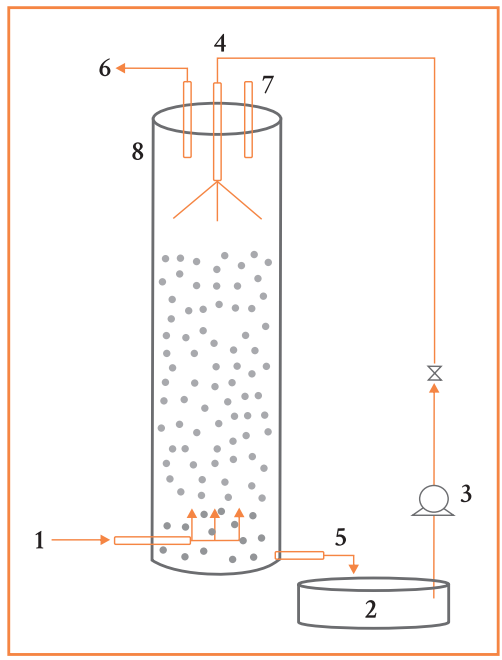
Figure 6. Representation of a biogas filtration system using membranes. The filter is installed in series in the biogas line.

Biological processes

The biological process is mediated by bacteria capable of oxidizing H_2S into sulfate (SO_4^{2-}) and/or elemental sulfur (S^0) in the presence of oxygen or nitrate as electron acceptors (Prescott et al., 2002). Examples of bacteria that oxidize H_2S are *Thiobacillus* sp., *Thermothrix* sp., and *Thiothrix* sp. (Stainier et al., 1986). These microorganisms are often found in various environments and are known as chemotrophs. The CO_2 present in the medium is used as a carbon source for cell multiplication. Therefore, the fortuitous CO_2 removal can also benefit from the use of a biofilter (Syed et al., 2006).

Biofilters are reactors usually made with corrosion-resistant materials with different configurations (Syed et al., 2006). In practice, these reactors are internally filled with porous materials (e.g., Bioballs®) (Pirolli et al., 2016), which have a high surface area for fixing microorganisms (biofilms), not restricting the biogas passage. A nutrient solution rich in nitrogen (preferably in the form of nitrates) and phosphorus is continuously pumped into the biofilter. This solution percolates on the internal porous material of the reactor to keep the population of bacteria metabolically active in the process of oxidizing H_2S. The sulfur removed by filtration, along with some nutrients still present in the liquid effluent, can be disposed of without the need for treatment and is also used as an excellent source of liquid fertilizer. Figure 7 shows a schematic illustration of the biofilter.

Biofilters are cost-effective in removing contaminants, being able to remove up to 100% of the H_2S present in the biogas. One of the drawbacks is the preparation of the nutrient solution, which needs to be changed regularly in the biofilter. However, the diluted effluent from the anaerobic biodigester can be used as a nutrient solution for the biofilter as a way to reduce labor and costs associated with nutrients (Pirolli et al., 2016). In this case, the digestate also serves as an excellent inoculum source for H_2S-oxidizing bacteria, which start to colonize the interior of the biofilter. Biofilters are generally limited by the low biological oxidation kinetics of H_2S. Thus, the process is mainly recommended for systems with low flow rates. The dimensioning of larger biofilters is possible to meet higher flow rates, but the costs become more significant.



Source: (Pirolli et al., 2016).

Figure 7. Representation of a biofilter. (1) Biogas inlet; (2) reservoir with a nutrient solution or diluted digestate; (3) circulation pump; (4) inlet of nutrient solution with an internal disperser to ensure uniform and homogeneous distribution across the entire surface of the internal porous material; (5) liquid nutrient solution outlet; (6) purified biogas outlet; (7) thermometer (not required); (8) PVC reactor with porous material for bacterial fixation (e.g., bio balls) inside.

The use of biofilters with microalgae has shown very promising results in the removal of CO_2 and H_2S from the biogas (Conde et al., 1993; Mann et al., 2009; Prandini et al., 2016). Usually, stabilization and treatment lagoons are used to treat the digestate produced by the biodigester. The digestate is an effluent rich in nitrogen and phosphorus, which can lead to eutrophication processes when dumped in these lagoons due to the high growth of microalgae (Figure 8).

Microalgae use CO_2 during the biogas filtration process as a carbon source for cell growth through photosynthesis. The oxygen generated by photosynthesis serves as an electron acceptor, which is used by H_2S-oxidizing bacteria. Thus, 100% of the CO_2 and H_2S are removed from the biogas. The high CO_2 concentration in the biogas stimulates

the growth of microalgae and hence increases the nutrient consumption rate (phycoremediation) and the digestate treatment efficiency. The advantage of the technique of CO_2 removal by microalgae is the development of an integrated platform between digestate treatment and biogas filtration. In practice, the biogas can be bubbled in closed photobioreactors containing diluted liquid effluent and microalgae (Figure 8). The biogas flow rate (Q, $m^3.day^{-1}$) to be used in the biofilter depends on the biological activity of removing contaminants by the microalgae. Kinetic tests are carried out in pilot systems to determine the biogas purification efficiency by microalgae.

Photo: Melissa Paola Mezzari

Figure 8. Eutrophication of treatment and stabilization lagoons due to the presence of microalga

Cryogenics

Biogas purification by the cryogenic process is a little-used technique and has been in continuous development (Ryckebosch et al., 2011). During the cryogenic treatment, the raw biogas is dried and compressed at high pressure (8,000 kPa) under controlled temperature conditions

(cooling), resulting in the separation of CH_4 (>97%) and CO_2 removal by condensation. Although the results obtained with cryogenic purification are very promising, the technology is unfeasible due to the high initial investment cost and high energy consumption (Budzianowski, 2016; Scholz et al., 2013).

Final considerations

Treatment or purification of biogas is a crucial process for its use as a source of biofuel. This chapter presents the processes most commonly used for the treatment and purification of biogas, including the removal efficiencies, as well as the advantages and disadvantages of each system. A correct dimensioning of the treatment and purification system requires the determination of the produced biogas volume, its physicochemical characteristics, and, mainly, its degree of purity to be achieved. H_2O, H_2S, CO_2, and NH_3 are among the main contaminants present in the biogas. Water is usually the first contaminant to be removed from the system, resulting in the minimization of corrosion of the entire infrastructure that composes a gas purification system, including compressors and pipelines. Water removal also reduces problems associated with the saturation of the filter material and adsorbents.

The removal of H_2S occurs inside or outside the biodigester through biological or chemical oxidative processes. The practice of injecting O_2 or air to remove H_2S must be considered carefully to avoid excess O_2 and the intrinsic risks of explosion. The amount of O_2 in the system must not exceed the maximum concentrations according to the rules and regulations in force for injecting biogas into the network. Biological processes are considered to have low implementation and maintenance costs and can significantly reduce H_2S and CO_2 sustainably. Furthermore, the integration of technologies in a modern biogas purification platform associated with the digestate treatment using microalgae has shown very promising results.

CO_2 can be removed during or after desulfurization processes by washing the gas with water and/or chemical reagents, using or not pressurized systems. The use of selective membranes for the removal of CO_2 and other contaminants such as H_2S and NH_3 can also be used.

Except for the cryogenic technique, all processes mentioned in this chapter have been used for biogas treatment and purification. The choice of the most appropriate technique to be used must consider the maximum levels of contaminants allowed in the biomethane composition, in addition to factors such as implementation and maintenance costs and the degree of complexity of the system and availability of parts and/or technical assistance.

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Chapter V

USE OF DIGESTATE AS FERTILIZER

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Introduction

Anaerobic digestion is still an emerging technology in Brazil (Kunz et al., 2009) despite the great potential for the energy use of agro-industrial residues available in the country (Abiogás, 2015). In Europe, this industry is already developed. A report by the European Biogas Association pointed out that there were already 17,240 biogas plants in operation in 2015 on that continent, notably in Germany, where around 60% of these plants were installed (EBA, 2015). Brazil had approximately 150 biogas plants operating in 2016, less than 1% of the installed capacity in Europe (Cibiogás, 2016).

One of the biggest challenges for the development of this industry is the need for the correct disposal of effluent from biodigesters (digestate). If, on the one hand, there are already technologies for the digestate

treatment (Chapters VI and VII), aiming at nutrient removal (nitrogen and phosphorus) and enabling the reuse of wastewater or its disposal into receiving water bodies, on the other hand, the use these technologies add costs that impact the economic viability of these projects (Miele et al., 2015). The recycling of digestate as a fertilizer in agriculture removes part of the added cost with the implementation and operation of digestate treatment systems, but aspects related to the supply of nutrients via digestate, the demand for nutrients in agricultural areas available for recycling, and the logistics of fertilizer distribution projects should be considered in these projects, as they also add costs and have technical limitations (Miele et al., 2015; Nicoloso, 2014).

The technical criteria necessary for the correct destination of digestate as a source of nutrients for agriculture will be discussed in this chapter. The concepts that will be exposed here are valid both for larger-scale projects (biogas plants) and for smaller-scale biodigesters for the treatment, for example, of animal manure and other residues from rural properties or decentralized energy generation condominiums (Olivi et al., 2015). The environmental impacts related to the use of digestate as a fertilizer and strategies for its mitigation will also be addressed.

Characterization of digestate as a fertilizer

The digestate quality and its potential for agronomic use depend on several factors, namely: (a) composition and variability of residues used as substrates for biodigestion (e.g., waste and carcasses of dead animals, agro-industry residues, residues, or plant biomass, among others); (b) type of biodigester and biodigestion technology; (c) segregation and loss of nutrients in the substrate and digestate storage structures; (d) efficiency of substrate pre-treatment systems (e.g., separation of phases before the biodigester) and/or digestate treatment; and (e) dilution of substrates and digestate with water. Table 1 shows the amount of nutrients (nitrogen, phosphorus, and potassium) associated with some residues of animal origin, plant biomass, and agro-industrial residues commonly used as substrates in biodigesters.

In addition to differences in the chemical composition and variability among substrates, the different proportions of substrate mixtures to be used in the feeding of the biodigester will also have a major impact on the nutrient composition of the digestate. Therefore, each project must have a specific analysis to determine the supply and nutrient content of the digestate available for recycling as fertilizer in agriculture. The values shown in Table 1 can be used for dimensioning the supply of nutrients via digestate that must be submitted to treatment or recycling in agricultural areas as fertilizer. However, the processes of loss and segregation of nutrients that can occur in the biodigester and effluent treatment or storage systems need to be considered. Vivan et al. (2010) found no significant variation in the concentration of TKN (total Kjeldahl Nitrogen), NH_3-**N** (N ammoniacal), and P (phosphorus) between the wastewater (liquid swine manure) and the digestate from a covered lagoon biodigester with a hydraulic retention time (HRT) of 45 days. However, reductions in the contents of these nutrients in the order of 50%, 30% and 77%, respectively, were observed after passing the digestate through an anaerobic lagoon with an HRT of 55 days. This reduction in N contents was attributed to ammonia volatilization losses, which can be increased by the mineralization of organic N during the biodigestion process. On the other hand, the reduction in P contents in the digestate was attributed to the physicochemical P precipitation, mostly in the form of calcium phosphate (Steinmetz, 2007). Therefore, P is not lost but segregated, as observed by the increase in the concentrations of this nutrient in the sludge deposited in the digestate storage lagoons (Zanotelli et al., 2005). In general, N losses of 50%–60% are expected for swine manure treated by biodigestion, also considering the digestate storage before its application to the soil (Fatma, 2014). The other nutrients have no considerable losses although the segregation of nutrients between the different types of effluents from biodigesters (e.g., sludge and liquid digestate) should be considered.

Table 1. Amount of nutrients associated with some animal residues commonly used as a substrate for anaerobic digestion.

Type	Source	Substrate production		Nutrient			
		Amount	Unit	N	P_2O_5	K_2O	Unit
Swine and cattle slurry[1]	Swine, finishing	1.64	m^3 piglet^{-1} year^{-1}	8.0	4.3	4.00	kg.piglet^{-1}.year^{-1}
	Swine, nursery	0.84	m^3 piglet^{-1} year^{-1}	0.40	0.25	0.35	kg.piglet^{-1}.year^{-1}
	Swine, PPU	8.32	m^3 sow^{-1} year^{-1}	25.7	18.0	19.4	kg.sow^{-1}.year^{-1}
	Swine, CC	17.2	m^3 sow^{-1} year^{-1}	85.7	49.6	46.9	kg.sow^{-1}.year^{-1}
	Cattle, milk	20.0	m^3 animal^{-1} year^{-1}	65.6	36.8	61.8	kg.animal^{-1}.year^{-1}
Litter[2]	Broiler chickens	2.36	kg animal^{-1} year^{-1}	67.0	71.0	62.0	g.animal^{-1}.year^{-1}
Animal carcasses[3]	Swine	75.0	kg (mean weight)	21.5	63.6	35.5	kg.ton^{-1}
	Broiler chickens	2.5	kg (mean weight)	30.5	57.1	24.5	kg.ton^{-1}
Plant biomass[4]	Corn silage	21.0	ton ha^{-1} (DM)	9.5	9.5	12.7	kg.ton^{-1} (DM)
	Sorghum silage	23.1	ton ha^{-1} (DM)	3.5	2.7	4.6	kg.ton^{-1} (DM)
	Sunflower silage	15.9	ton ha^{-1} (DM)	11.8	15.2	28.7	kg.ton^{-1} (DM)
Agro-industrial residues[5]	Sugarcane vinasse	13.0	L L^{-1} de ethanol	0.37	0.60	2.03	kg.m^{-3}
	Filter cake (sugarcane)	35.0	kg ton^{-1} sugarcane	1.40	1.94	0.39	% (DM)

[1]Calculated based on Nicoloso and Oliveira (2016) and Miele et al. (2015). Manure and nutrient production per housed animal: finishing units, considering 3.26 lots of finishing pigs per year; nursery, considering piglets up to 28 days old; the unit for piglet production units (PPU) and complete cycle (CC) is hosted sow, considering 2.35 farrows per year, 12 piglets per farrow, and 11.5 finished piglets per sow per farrow. [2]Calculated based on Nicoloso et al. (2016a) and considering 13 poultry housed per square meter, 0.10 m litter thickness, 600 kg litter density per cubic meter, and litter change every 15 lots of 42 days and 7 days apart. [3]TEC-DAM (2017). [4]Oliveira et al. (2010). [5]Soares et al. (2014); DM: dry matter.

A field survey carried out in a microbasin in the Santa Catarina State, Brazil, showed that the digestate from covered lagoon biodigesters treating the same type of substrate (e.g., swine manure) had high variability in terms of N, P_2O_5, and K_2O contents (Table 2). The biodigesters had similar characteristics despite the distinct origin of substrates (type of farm). In this case, the high variability of results was attributed to differences in the farm manure management (waste of water), biodigester operation (some of them had systems for separating coarse solids from the wastewater), occurrence of rainwater inlet in some facilities (poorly oriented drainage of the terrain), and, mainly, long digestate storage time in some of these units, which allowed P precipitation into the lagoon sludge, considerably reducing the P_2O_5 content of the liquid digestate (Olivi et al., 2015).

Table 2. Characterization of digestate from covered lagoon biodigesters treating liquid swine manure (Olivi et al., 2015).

Type of farm	Housed animals		Biofertilizer				
	Number	Category	TS	N	NH_3-N	P_2O_5	K_2O
				mg.L^{-1}	mg.L^{-1}	mg.L^{-1}	mg.L^{-1}
PPU	280	Sows	2.3	550	508	71	384
PPU	400	Sows	14.8	2.008	1,527	850	576
PPU	300	Sows	9.9	1,718	1,401	370	715
PPU	150	Sows	3.1	862	783	86	515
GFU	250	Swine	38.5	4,089	2,568	1,670	1,257
GFU	750	Swine	4.2	987	954	31	919
GFU	1,000	Swine	27.0	2,232	1,301	940	934
GFU	260	Swine	3.6	771	731	41	909
CC	150	Sows	1.7	125	94	29	447
NU	1,500	Piglets	19.4	2,376	1,843	352	1,438
Mean			13.1	1,644	1,232	435	866
Standard deviation			12.0	1,133	707	520	381

PPU: piglet producing unit; GFU: growing and finishing unit; CC: complete cycle; NU: nursery unit.

Furthermore, the use of different practices or processes for managing and treating the digestate (e.g., phase separation, composting, and drying) will also affect the availability of nutrients in the fertilizer.

A preliminary study for the construction of a biogas plant for treating different agricultural residues (swine manure, swine carcasses, poultry hatchery waste, sludge from a slaughterhouse treatment system, and poultry litter) using complete-mix biodigesters determined that two types of effluents would be produced in that plant: liquid digestate and organic compound obtained after a phase separation process of the effluent from the biodigester (Brasil, 2015; Nicoloso, 2014). The digestate and organic compound characteristics expected to be generated in the biogas plant are shown in Table 3.

Table 3. Characteristics of digestate, biodigester sludge, solid residue, and organic compound obtained under different arrangements of complete mix biodigesters in a biogas plant and upflow biodigester in a swine manure treatment plant.

Source	Fertilizer	DM (%)	N	P_2O_5	K_2O
			kg.m^{-3} or kg.ton^{-1} (wet basis)		
Biogas plant[1]	Liquid digestate	2.5	2.5	1.1	2.1
	Organic compound	25.0	93.0	121.0	47.0
SMTP[2]	Liquid digestate	1.0	2.0	0.6	1.1
	Biodigester sludge	6.5	5.1	7.2	1.1
	Solid residue	28.0	6.9	7.4	2.1
	Organic compound	54.5	8.5	12.1	3.3

[1]Complete mix biodigester treating a mixture of substrates (swine manure, swine carcasses, poultry hatchery waste, sludge from a slaughterhouse effluent treatment system, and poultry litter). Source: Brazil (2015) and Nicoloso (2014). [2]Swine manure treatment plant of Embrapa Swine and Poultry. Source: Nicoloso et al. (unpublished data).

The liquid digestate and organic compound expected to be generated in the biogas plant would present drastically different chemical composition and dry matter content. Similarly, high variability is observed regarding the composition of the different fertilizers obtained in a swine manure treatment plant (SMTP), where different treatment systems were installed, including rotary sieve brush, flotation-settling tank, upflow biodigester, and a composting system for the solid fraction of swine manure separated on the sieve (Table 3). Differences regarding the concentration and form in which the nutrients are available in fertilizers (organic or mineral) obtained from different treatment processes will considerably affect their agronomic efficiency, as discussed later (Nicoloso et al., 2016a). However, the logistics, cost, and feasibility of

transporting and distributing fertilizers are also affected (Miele et al., 2015; Nicoloso, 2014).

The results presented here show that the high variability of nutrient content in the digestate and other organic fertilizers makes laboratory analysis essential for fertilizer characterization (Nicoloso et al., 2016a). The analysis of fertilizer will allow its application at adequate doses in agricultural areas, supplying the crop demand for nutrients without excess in the soil and avoiding environmental impacts.

Criteria for the agronomic use of digestate

Fertilizer (mineral or organic) application to the soil aims to supply the nutrient demand of crops so that they express their productive potential. Plants explore the soil through their root system in search of water and nutrients, which can be originated in the soil or come from the applied fertilizer. Thus, more fertile soils require the application of lower doses of fertilizers than soils that have lower contents of available nutrients, as fertile soils can supply higher amounts of macronutrients (N, P, K, Ca, Mg, and S) and micronutrients (B, Cl, Cu, Fe, Mn, Mo, Co, Ni, and Zn) to the plants.

In general, fertilization recommendations aim to establish the most technically and economically efficient N, P, and K doses for different crops (Gatiboni et al., 2016). The focus on these three nutrients for fertilizer recommendation occurs because Ca and Mg are supplied through liming, S is recommended preventively for more demanding crops, and micronutrients are supplied in adequate amounts by the soil, without the need for their application via fertilizers, except under specific soil, climate, and crop conditions (Gatiboni et al., 2016). N recommendations are based on soil organic matter content and its decomposition rate, N cycling in the soil-plant system, losses of N applied via fertilizers (e.g., leaching, volatilization, and immobilization), and N demand by crops. Therefore, the construction of soil fertility in terms of N supply to plants is related to an increase in soil organic matter stocks in the long term and not directly to the application of nitrogen fertilizers. P and K fertilization recommendations are based on their availability in the soil, their losses when applied via fertilizers (e.g., adsorption and

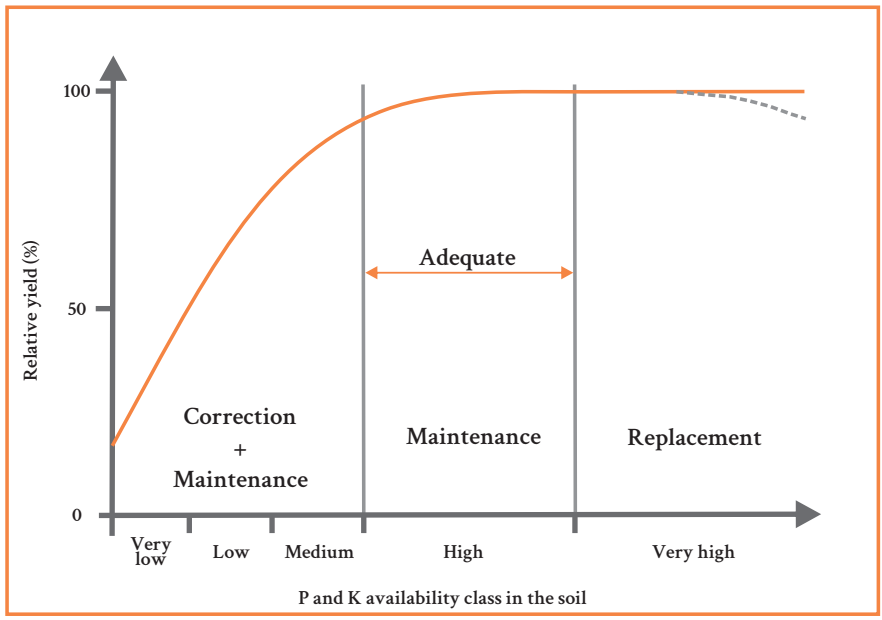
leaching), and their demand by crops. In this sense, three fertilization concepts are established for P and K recommendation, namely: corrective, maintenance, and replacement fertilization (Gatiboni et al., 2016).

Correction fertilization aims to raise P and K contents in the soil to the "critical content" of the crops (Figure 1). The critical content represents the concentration of P and K available in the soil necessary for a yield of approximately 90% of the maximum production of the crop to be fertilized. Crop yield below this critical content shows a high response to fertilization and an increase in soil P and K contents. Soils from Rio Grande do Sul and Santa Catarina present correction rates varying from 40 kg.P_2O_5.ha^{-1} to 160 kg.P_2O_5.ha^{-1} and 30 kg.K_2O.ha^{-1} to 120 kg.K_2O.ha^{-1}, according to their availability classes (very low, low, or medium) in the soil (De Bona, 2016). These doses recommended as correction fertilization have been determined only to increase soil nutrients contents, not considering that part of these nutrients is absorbed and exported by the plants. Thus, a maintenance dose must be added to this correction dose to meet the demand for P and K by crops. A significant increase in crop yield is not expected due to an increase in P and K contents in the soil above the critical level. Therefore, maintenance fertilization aims only to add the amounts of P and K removed by crops and exported through grains, forage, or biomass and also replace the losses of these nutrients in the soil, keeping the P and K contents stable in a range considered suitable for crop development ("high" nutrient availability class). On the other hand, replacement fertilization aims to add the amounts of P and K exported by crops and is recommended for soils with contents classified as "very high". Applying only the prescribed replacement doses can result in a reduction of P and K contents in the soil over time due to the nutrient losses that are likely to occur. Table 4 shows the amounts of N, P_2O_5, and K_2O suggested for maintenance and replacement fertilizations of the main grain crops grown in Rio Grande do Sul and Santa Catarina (De Bona, 2016).

Table 4. Maintenance fertilization and phosphorus and potassium replacement for the main grain crops grown in the Rio Grande do Sul and Santa Catarina States (adapted from De Bona, 2016).

Crop	Reference yield[1]	Maintenance fertilization[2]			Replacement fertilization[3]		
	ton.ha^{-1}	N (SOM 2,6%-5%)[4]	P_2O_5	K_2O	N	P_2O_5	K_2O
		kg.ha^{-1}			kg.ton^{-1}		
Canola	1,5	=40+20*(EY-RY)	=30+20*(EY-RY)	=25+15*(EY-RY)	20	15	12
Barley	3,0	=40+30*(EY-RY)	=45+15*(EY-RY)	=30+10*(EY-RY)	20	10	6
Sunflower	2,0	=40+20*(EY-RY)	=30+15*(EY-RY)	=30+15*(EY-RY)	25	14	6
Corn	6,0	=70+15*(EY-RY)	=90+15*(EY-RY)	=60+10*(EY-RY)	16	8	6
Soybean	3,0	=0	=45+15*(EY-RY)	=75+25*(EY-RY)	60	14	20
Sorghum	4,0	=55+15*(EY-RY)	=50+15*(EY-RY)	=35+10*(EY-RY)	15	8	4
Wheat	3,0	=60+30*(EY-RY)	=45+15*(EY-RY)	=30+10*(EY-RY)	22	10	8

[1]Reference yield is the minimum yield considered in this fertilization recommendation system. [2]The maintenance fertilization for phosphorus and potassium is calculated as a function of an additional maintenance dose to be applied according to the desired expected yield (EY) relative to the reference yield (RY). [3]Replacement fertilization as a function of nutrient export for each ton of grain produced. [4]Nitrogen fertilization for soils with organic matter (SOM) contents between 2.6% and 5%, medium-yielding predecessor crop.



Source: Adapted from Gatiboni et al. (2016).

Figure 1. Relative yield of crops as a function of P and K content in the soil and indications for correction, maintenance, and replacement fertilizations.

The data shown in Table 4 allow determining the available amounts of nutrients to be applied to the aforementioned crops considering the expected yield projected with fertilization. However, organic fertilizers may have reduced efficiency compared to mineral fertilizers because part of the nutrients is in forms unavailable to plants (Nicoloso et al., 2016a). In general, organic fertilizers with a higher proportion of nutrients in the organic form and high lignin and fiber contents have a lower decomposition rate in the soil and, therefore, a lower release and availability of nutrients for plants. For instance, poultry litter has an agronomic efficiency index for nitrogen of 0.5% or 50% (Table 5). It means that only 50% of the total N content present in the fertilizer will be available for the 1st cultivation after application to the soil (immediate effect). However, poultry litter still has a residual effect of 20% for N, which will be available for the subsequent crop (2nd cultivation). Table 5 lists the agronomic efficiency indices of some organic fertilizers often available in regions of intensive animal production.

Table 5. Mean values of nutrient efficiency of different organic fertilizers applied to the soil in two successive cultivations (Nicoloso et al., 2016a).

Fertilizer	Cultivation	Nutrient[1]		
			P	K
Poultry litter	1st cultivation (immediate effect)	0.5	0.8	1.0
	2nd cultivation (residual effect)	0.2	0.2	0.0
Swine slurry	1st cultivation	0.8	0.9	1.0
	2nd cultivation	0.0	0.1	0.0
Cattle slurry	1st cultivation	0.5	0.8	1.0
	2nd cultivation	0.2	0.2	0.0
Organic compost from swine manure[2]	1st cultivation	0.2	0.7	1.0
	2nd cultivation	0.0	0.3	0.0

[1]Total nutrients (mineral + organic). [2]Considering shavings and/or sawdust as substrate.

The organic fertilizer dose to be applied to the soil must consider the specific recommendations for the different classes of soil fertility, crop demand and expected yield, and content and agronomic efficiency index of the fertilizer to be used, being calculated according to the equations described below (Nicoloso et al., 2016a):

Solid fertilizers

$$A = \frac{QD}{\left(\left(\frac{B}{100}\right) \times \left(\frac{C}{100}\right) \times D\right)}$$

Equation 1

Liquid fertilizers

$$A = \frac{QD}{(C \times D)A}$$

Equation 2

Where:

A = Organic fertilizer dose to be applied to the soil (kg.ha^{-1} for solids and m^3.ha^{-1} for liquids)

B = Dry matter content of the solid organic fertilizer (%)

C = Concentration of N, P_2O_5, or K_2O in the organic fertilizer (% for solids and $kg.m^{-3}$ for liquids)
D = Fertilizer agronomic efficiency index. The term "B/100" can be eliminated from the equation for solid fertilizers in which the nutrient content is expressed on a wet basis.

Considering, for example, the mean data of nutrient concentration shown in Table 2 to calculate the amount of digestate (considering an efficiency index similar to swine manure, as shown in Table 5) to be applied for maintenance fertilization in the corn crop with a productivity expectation of 12 tons per hectare (Table 4), we can use Equation 2 as described below:

a) To meet the demand for N: A = 160/1.6 x 0.8 = 125 $m^3.ha^{-1}$.
b) To meet the demand for P_2O_5: A = 180/0.4 x 0.9 = 500 $m^3.ha^{-1}$.
c) To meet the demand for K_2O: A = 120/0.8 x 1.0 = 150 $m^3.ha^{-1}$.

The option for the highest dose (500 $m^3.ha^{-1}$) to meet the demand for P_2O_5 would result in an excessive application of 480 $kg.N.ha^{-1}$ and 280 $kg.K_2O.ha^{-1}$, which should be avoided to mitigate possible environmental impacts, especially related to nitrate and potassium leaching, ammonia volatilization, and nitrous oxide emission (Aita et al, 2014). In this case, the technically correct option would be to opt for the lowest dose (125 $m^3.ha^{-1}$) to meet the demand for N by the corn crop and complement the fertilization with P and K using another source of mineral fertilizer (Nicoloso et al., 2016a). Table 6 shows the results of an experiment of four growing seasons of corn fertilized with different sources of fertilizers (mineral, liquid swine manure, swine manure digestate, organic compound from swine manure, and control without fertilization) in a Nitisol (26% clay) under no-tillage and conventional tillage systems (Nicoloso et al., unpublished data). In this experiment, the total N dose applied to all treatments was 140 $kg.N.ha^{-1}$ only in the corn crop (spring/summer). P and K applications were carried out to meet the corn demand, according to De Bona (2016).

Tabela 6. Nitrogen absorption, biomass production, and grain yield of corn (accumulated from four growing seasons) fertilized with mineral fertilizer, liquid swine manure, swine manure digestate, and organic compound from swine manure under conventional and no-tillage systems (adapted from Nicoloso et al., unpublished data).

Parameter	Tillage system	Fertilization					Mean
		CTR	MIN	SS	SMD	COMP	
		---------- kg.ha^{-1} ----------					
Nitrogen	CT	599	759	751	741	647	700 ns
	NT	536	680	782	711	583	659
	Mean	567 b[1]	719 a	766 a	726 a	615 b	679
Biomass	NT	43.716	47.548	50.620	49.890	46.652	47.685 ns
	PD	39.808	46.794	51.004	49.901	43.393	46.180
	Mean	41.762 c	47.171 ab	50.812 a	49.895 ab	45.023 bc	46.932
Grains	NT	32.108	35.158	37.198	36.292	33.756	34.902 A
	PD	28.477	33.754	36.952	36.538	30.092	33.163 B
	Mean	30.293 d	34.456 bc	37.075 a	36.415 ab	31.924 cd	34.032

CTR: control without fertilization; MIN: mineral fertilization; SS: liquid swine slurry swine manure; SMD: swine manure digestate; COMP: organic compound from swine manure; CT: conventional tillage; PD: no-tillage; ns: not significant. [1]Means followed by the same lowercase letter in the row and uppercase letter in the column do not differ from each other by t-test ($p<0.05$).

No differences were observed between tillage systems for N accumulation and biomass production in the corn crop. However, corn grain yield was higher in conventional tillage areas due to a higher mineralization rate of soil organic matter induced by soil tillage. N accumulation and biomass production in corn were similar between treatments that received mineral fertilizer (urea), liquid swine manure (SS), and swine manure digestate (SMD). Grain yield was higher in the treatment that received SS than in the treatment with mineral fertilizer. The SMD treatment had intermediate productivity, not differing from each other. The treatment that received organic compost (COMP) had lower N accumulation, biomass production, and corn grain yield than the other treatments, indicating the lower N availability of this fertilizer (Nicoloso et al., 2016a) (Table 5). These results show that digestate and other organic fertilizers can efficiently and safely replace mineral fertilizers when the technical criteria set out here are observed, reducing the production costs in agriculture (Miele et al., 2015).

Requeriments of agricultural areas for digestate recycling

The dimensioning of the agricultural area necessary for the disposal of effluents from a biodigester combines the concepts discussed earlier in this chapter, namely: nutrient supply by the digestate and nutrient demand in the agricultural area. These same principles allow performing the reverse calculation to dimension the substrate offer and the biodigester size as a function of the agricultural area available for digestate recycling. This analysis is valid for small biodigesters operating on rural properties and large-scale biogas plants. However, this dimensioning must be carried out considering both factors (nutrient demand and supply) in the long term.

As previously discussed (Figure 1), correction fertilization aims to increase soil nutrient contents (P and K) to adequately supply the crop demand, reducing fertilizer consumption. However, only maintenance fertilization is used when the critical nutrient content in the soil is reached, keeping the crop productivity close to the productive potential, and replacing the loss of nutrients in the soil. In this sense, the recommen-

dation for maintenance fertilization is the dose to be used for dimensioning the demand for nutrients to keep the soil nutrient contents stable and the enterprise sustainable in the long term (Nicoloso and Oliveira, 2016). The option for dimensioning considering the recommendations for soil fertility correction would cause the gradual and excessive accumulation of nutrients in the soil, with negative effects on the environment over time. Similarly, the digestate supply dimensioning according to the replacement recommendations would promote a reduction in soil fertility and the need for the additional input of mineral fertilizers, as these recommendations do not predict soil nutrient loss. Thus, the dimensioning of nutrient supply and demand can be determined from the following equation (adapted from Nicoloso and Oliveira, 2016):

$$\boldsymbol{\Sigma\left[NS \times \frac{(100 - L)}{(100 \times AE)}\right] = \Sigma ND - \Sigma NSM} \qquad \textit{Equation 3}$$

Where:

NS = Mean annual nutrient supply (N, P_2O_5, or K_2O) in the substrates that will feed the biodigester, plant, or enterprise under analysis ($kg.year^{-1}$).

L = Nutrient losses (N, P_2O_5, or K_2O) that occur during the biodigestion process, treatment, and storage of substrates and effluents (%).

AE = Agronomic efficiency index of nutrients (N, P_2O_5, or K_2O) of each effluent.

ND = Mean annual nutrient demand (maintenance recommendation for N, P_2O_5, or K_2O) in agricultural areas available for recycling effluents from the biodigester, plant, or enterprise under analysis ($kg.year^{-1}$).

NSM = Mean annual nutrient supply from mineral sources or other organic sources used in the fertilization of agricultural areas available for recycling effluents from the biodigester, plant, or enterprise under analysis ($kg.year^{-1}$).

The determination of the average annual demand for nutrients in agricultural areas receiving digestate and other liquid effluents and solid waste generated by the biodigester, plant, or enterprise under analysis considers the used crop system, which normally varies over the years. Thus, the ideal is to carry out long-term planning (>4 years) for fertilizer use (Fatma, 2014). Another important factor is to determine which

nutrient (N, P_2O_5, or K_2O) will be used as a limiting factor for the dimensioning. Usually, P or N is used as a limiting nutrient, as K has little relevance from an environmental point of view for most residues. The sugarcane vinasse is an exception due to the high K concentration compared to other nutrients in this residue (Soares et al., 2014). P is used as a limiting nutrient for residues of animal origin (e.g., swine manure), as its supply in this type of residue meets the demand for this nutrient in most crops, without promoting an excessive supply of N or K in the soil (Fatma, 2014; Nicoloso and Oliveira, 2016).

Environmental limits for digestate application

Excessive fertilizer application may cause significant environmental impacts regardless of their mineral or organic origins, especially due to increased nutrient loss in the soil and the transfer to the environment (Aita et al., 2014; Escosteguy et al., 2016; Soares et al., 2014). Thus, numerous research initiatives have been seeking to establish indicators and critical environmental limits (CELs) of nutrient availability in the soil to reduce environmental pollution risks. CELs can be considered as indicator values of soil quality that impose limits on fertilizer application to the soil. In this sense, CELs can be used by regulatory and supervisory agencies to establish maximum acceptable doses or even prohibit the application of any source of nutrients to the soil, including digestate, agro-industrial residues, or mineral fertilizers. However, CELs cannot be confused with soil nutrient availability classes determined for fertilization purposes (Gatiboni et al., 2016), as not always soil nutrient contents classified as "very high" from an agronomic point of view (Figure 1) indicate a potentially deleterious effect on the environment (Escosteguy et al., 2016).

Although N is one of the most studied nutrients due to its high potential for environmental impact derived from its rapid transformations and losses in the soil, there are currently no CEL indicators in Brazil relating the concentrations of this nutrient in the soil to the environmental pollution risk. Moreover, we need to consider that more than 90% of the N in the soil is associated with SOM and, therefore, the total N contents are not good indicators of environmental risk. Initiatives to

establish CEL for N are, therefore, based on the most abundant reactive forms of this nutrient, such as N in the form of nitrate. The Water Protection Act (2008) in Canada (province of Manitoba) establishes that nitrogen fertilization should be planned so that the residual amount of NO_3 (nitrate) in the 0 cm–60 cm soil layer at the end of the crop cycle is not higher than 33 kg ha^{-1} to 157 kg.ha^{-1}, according to land use classes. In Europe, the Nitrates Directive 91/676/EEC does not set limits on nitrate in the soil, but it prohibits the waste or manure application during the winter and limits the doses of these residues to up to 170 kg to 250 kg of N.ha^{-1}, according to the country, in areas identified as vulnerable to groundwater contamination by this nutrient. The purpose of this legislation is to ensure that the nitrate content in groundwater and surface water in these regions does not reach the critical limit of 50 mg.L^{-1} (van Grinsven et al., 2012). In Brazil, Conama Resolution 420/2009, based on Ordinance 518/2004 of the Ministry of Health, establishes the limit level of nitrate in groundwater at 10 mg.L^{-1} (Brasil, 2009). This value should not be confused as a limit for nitrate concentration in the soil solution. Moreover, member countries of the European Union have also established national programs to control air pollution to reduce ammonia and nitrous oxide emissions from agricultural sources (Loyon et al., 2016). These programs are based on the adoption of good management practices and nitrogen fertilizer application, such as acidification and injection of liquid waste into the soil, incorporation of manure and solid mineral fertilizers, use of urease and nitrification inhibitors, split application, irrigation control, and verification of climate and soil conditions at the time of application (Unece, 2014).

Gatiboni et al. (2015) performed a first approximation to establish critical environmental limits for P (CEL-P) in soils that receive frequent organic residue applications. The developed method allows calculating the maximum available P content that can exist in the soil without high risks of its transference to the environment, considering the soil clay content. The calculation equation is described below:

$$\boldsymbol{CEL - P = 40 + \%Clay} \qquad \textit{Equation 4}$$

Where:

CEL-P = Maximum available P content determined by the Mehlich-1 method (mg. dm^{-3}) that soil can present without a high risk of pollution

%Clay = Soil clay content expressed as a percentage. This indicator has been adjusted and is only valid for the 0 cm–10 cm soil layer

According to the proposed method, sandy soils are more sensitive, whereas clayey soils can support higher amounts of P without making them available in large amounts to the environment. Briefly, the soil is considered a safe reservoir of P when its contents are below the CEL-P, even if these contents are classified as "very high" relative to P availability for crops (Gatiboni et al., 2016). However, soil can become a P source for the environment when its contents exceed this limit value, promoting the eutrophication of surface water reservoirs when lost from agricultural areas, mainly by runoff. This methodology is currently used by the Environmental Foundation of the State of Santa Catarina (Fatma, 2014) to classify the environmental risk of soils with the application of swine manure. However, the authors emphasize that the method is an incipient proposal and lacks a more intense field calibration and the inclusion in the model of factors other than soil texture, such as terrain slope and soil conservation practices, which can also affect soil P losses.

Although K is not considered a nutrient with high potential for environmental impact in most situations, the application of high doses of sugarcane vinasse or other effluents containing high K concentrations may promote excessive K accumulation, affecting soil and water quality. The excessive K accumulation in the soil in areas where vinasse is recycled as fertilizer can impair Ca absorption, promoting its deficiency in the plant (Vitti; Mazza, 2002) and soil salinization in extreme situations through the concomitant supply of Na and Cl by this effluent (Soares et al., 2014). The increase in soil K contents also causes its higher mobility in the soil profile and higher contamination risks of the water table. The consumption of water with high K contents can promote metabolic diseases in individuals with renal dysfunction (Rocha, 2009). The Environmental Company of São Paulo State established limits for vinasse application based on the K saturation in the soil cation exchange capacity (CEC) and the capacity to extract and export this nutrient by crops

(Cetesb, 2006). According to "Technical Standard P4.231 – Vinasse: criteria and procedures for application to agricultural soil", a maximum of 5% of the CEC can be occupied by K, considering the 0 cm–80 cm layer of soil depth.

Other elements, especially micronutrients and heavy metals, do not present a large number of regionalized studies in Brazil establishing CELs. However, Conama resolution 420/2009 establishes soil quality guiding values regarding the presence of some trace elements (Cd, Pb, Co, Cu, Cr, Hg, Ni, Zn, and V) for the entire Brazilian territory (Brasil, 2009). Despite this, these values need to be validated regionally both for the definition of quality reference values (QRV), indicating the natural abundance of a certain element in the soil without anthropogenic influence, and for CEL establishment. A survey carried out to define QRVs in soils of the plateau region of the state of Rio Grande do Sul found higher values for Co, Cu, Cr, and Ni than the prevention (PRV) and investigation reference values (IRV) indicated by Conama resolution (Fepam, 2014). These data reinforce the need for the development of regionalized CELs, especially for micronutrients or trace elements, which present high variability according to the type of material that originated the soil.

Environmental indicators of soil quality, such as CEL-P and others, aims to establish limits and guide the rational use of fertilizers in a technically correct and environmentally safe manner. The indiscriminate disposal of digestate or other agro-industrial residues directly on the soil, although accepted in the past (Decree-Law 303/1967; Brasil, 1967) is currently an inadmissible practice due to immediate and cumulative environmental impacts. The modernization of the environmental legislation in Brazil and other countries has advanced in this direction, requiring environmental licensing of areas where agro-industrial residues are applied according to the size of the enterprise (Cetesb, 2006; Fatma, 2014). The environmental licensing process includes the preparation of an environmental impact study and report, planning for residue recycling in available agricultural areas, and soil quality monitoring based on CELs and specific quality standards for each type of agro-industrial activity.

Mitigation of greenhouse gases due to the agronomic use of digestate

In the agricultural sector, greenhouse gas (GHG) mitigation strategies can be summarized as: (a) reduction of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) emissions; (b) replacement of GHG emissions from fossil fuels by renewable energy sources; and (c) atmospheric CO_2 sequestration by photosynthesis and its storage in stable or slow cycling compartments in the global C cycle (Smith et al., 2007). It is noteworthy that CH_4 and N_2O have a global warming potential (GWP) 28 and 265 times higher than CO_2, respectively (Myhre et al., 2014). Ammonia is not considered a GHG, but it can also indirectly affect N_2O emission during and after its nitrification when it returns to the soil (Singh et al., 2008).

Worldwide, the agricultural sector has the potential to offset approximately 10% of anthropogenic GHG emissions at their current levels, while in Brazil it can reach from 20% to 30% of the country's GHG emissions (Bayer, 2007). It is estimated that 89% of the technical potential for GHG mitigation in this sector is related to soil C sequestration, 9% is associated with the reduction of CH_4 emissions (flooded rice, ruminant management, treatment of waste and agro-industrial residues), and 2% is dependent on the reduction of soil N_2O emissions through the management of nitrogen fertilization (Smith et al., 2007).

Biodigesters and composting are currently the most widespread technologies to treat swine manure in Brazil (Kunz et al., 2009). Biodigesters have good potential for GHG mitigation, as CH_4 produced by the anaerobic decomposition of manure and other organic residues can be converted into CO_2 by controlled biogas burning (Kunz et al., 2009). In this sense, the ABC Plan (Low Carbon Emission Agriculture) of the Brazilian Government provides for the treatment of 4.4 million tons of manure through biodigestion or composting by 2020 (Barros et al., 2015) as one of the strategies for Brazil to meet the GHG emissions mitigation commitments (Intended Nationally Determined Contributions – iNDC) submitted to the Paris Agreement (Brasil, 2016).

However, GHG mitigation strategies employed in the agricultural sector can affect more than one GHG by more than one mechanism in processes that can even be opposed. Thus, the net benefit of adopting these strategies must be assessed by the combined effect on all GHGs (Robertson and Grace, 2004; Schils et al., 2005; Koga et al., 2006). Furthermore, the effect of a mitigation strategy can vary in time differently between GHGs: some can be mitigated indefinitely, while others are temporarily affected (Six et al., 2004; Marland et al., 2003). Thus, the GHG emissions that occur after the biodigestion or composting process, when the organic compound, digestate, sludge, and other effluents from biodigesters are applied to the soil as fertilizers, need to be considered regarding the treatment of manure and other agro-industrial residues.

The application of animal manure and other organic residues to the soil, especially those rich in ammoniacal nitrogen, is expected to accelerate the decomposition (and CO_2 emission into the atmosphere) of crop residues (N-poor grass straw). However, Aita et al. (2006) did not observe this effect when adding liquid swine manure to black oat crop residues (C/N = 44/1). In this case, the oat straw did not show a sufficiently high C/N ratio and, therefore, the microbial population did not need external mineral N for the decomposition of crop residues. Moreover, the authors reported that the occurrence of rain after manure distribution on crop residues may have transported the ammoniacal N applied to the soil with the manure beyond the residue decomposition zone. However, Grave et al. (2015a) observed an increase in the CO_2 emissions from soil fertilized with liquid swine manure only in the first 30 days after its application. On the other hand, the soil fertilized with swine manure treated by biodigestion did not show the same increase. Therefore, this effect was attributed to the decomposition of C applied to the soil by manure and not to the decomposition of crop residues (wheat straw) present in the soil. Field experiments have shown, in some situations, only an initial peak in CH_4 emission in the first hours after manure application, which has been attributed to CH_4 that is dissolved in the effluent (Sherlock et al., 2002). Thus, the application of organic fertilizers, especially those treated by biodigestion, has a limited effect on the increase in soil CO_2 and CH_4 emissions. However, these fertilizers can significantly contribute to the sequestration of atmospheric CO_2 and its stabilization as soil organic matter.

The impact of organic fertilizers on soil C sequestration rates depends on the quantity and quality of the residue to be applied. Mafra et al. (2014) observed a linear increase in soil C sequestration rates (–0.21 Mg C $ha^{-1}.yr^{-1}$ to 1.69 Mg C $ha^{-1}.yr^{-1}$) due to an increase in liquid swine manure application rates (0 $m^3.ha^{-1}.yr^{-1}$ to 200 $m^3.ha^{-1}.yr^{-1}$) on an Oxisol cultivated with corn and black oat. Although a large proportion of this increase in C sequestration rates is related to nutrient input to the soil and higher biomass production by corn and oat, another fraction can be directly attributed to C input by swine manure. However, residues characterized by a higher proportion of recalcitrant C and slowly decomposing in the soil, such as residues that undergo composting (Grave et al., 2015a), may have a higher impact on soil C accumulation. Nicoloso et al. (2016b) observed that C sequestration rates in a Chernozem cultivated with corn and fertilized with liquid cattle manure increased significantly when the fertilizer source was replaced by organic compound generated from cafeteria waste, considering the same N input to the soil from both sources. Conversely, the treatment of agro-industrial waste and residues by biodigestion can reduce C content in the digestate and limit soil C sequestration rates. Grave et al. (2015a) observed that the treatment of liquid swine manure by biodigestion reduced C input to the soil by approximately 50% compared to untreated manure. After three years of application of different sources of organic fertilizers for corn (140 kg.N.ha^{-1}), these authors did not observe significant differences between C stocks in soil fertilized with digestate and mineral fertilizers (unpublished data). Thus, the possible increase in C stocks in soil fertilized with digestate and other effluents containing low C contents can be attributed mainly to the input of nutrients and improvement in soil fertility rather than to a direct C input by the organic fertilizer.

Several biotic and abiotic processes are involved in the N_2O production and emission in agricultural soils. Heterotrophic and autotrophic nitrification, nitrification coupled with denitrification (different microorganisms), denitrifying nitrification (same microorganism), and denitrification are the main biological processes that control N_2O emissions in aerated soils (although under partial O_2 availability) (Butterbach-Bahl et al., 2013). These processes are mainly controlled by pH, temperature, moisture, oxygen diffusion, and soil C and N availability (Giles

et al., 2012). Therefore, soil management and fertilizer application play a major role in regulating the substrate availability for these processes and, consequently, soil N_2O emissions. The increased soil moisture promotes a reduction in oxygen diffusion (e.g., 65%–70% of the porosity filled by water) and an increase in soil nitrate (NO_3-) concentrations prevents its complete denitrification into N_2, contributing to N_2O accumulation as an intermediate metabolite (Panek et al., 2000; Giles et al., 2012).

In this sense, animal manure, especially liquid and with high availability of ammoniacal N and labile C, may favor soil N_2O emissions compared to mineral fertilizers, as observed in different soil and climate situations (Rochette et al., 2004; Perälä et al., 2006; Chantigny et al., 2010; Damasceno, 2010; Schirmann, 2012). This effect of manure on the increase in N2O emissions is attributed to several causes, especially the following:

a) Manure adds labile C to the soil, which is used for biomass and energy production by denitrifying bacteria and other heterotrophic soil microorganisms, reducing O_2 availability through its respiratory activity.

b) The liquid fraction applied to the soil with manure, composed of a mixture of water and urine, also contributes to reducing O_2 availability, an essential condition for N_2O emission through nitrification and denitrification.

c) Ammoniacal N from manure is rapidly nitrified in the soil, which, associated with the reduced O_2 availability, can result in N_2O emission during nitrification and denitrification when the produced NO_3 can be used as an alternative to O_2 in the respiratory chain of denitrifying bacteria.

In addition to these effects attributed to manure on favoring N_2O emissions, other additional factors inherent to no-tillage can contribute to increasing these emissions. The reduction in macroporosity, the soil compaction due to the movement of machines, and moisture preservation are characteristics of no-tillage, which, alone or together, can reduce soil O_2 availability, favoring denitrification. Moreover, soil organic matter (SOM) accumulation and the presence of crop residues in the no-till system increase C availability to heterotrophic bacteria, responsible

for denitrification. Thus, animal manure treatment using biodigestion has been an efficient technology to reduce N_2O emissions from soil managed under the no-tillage system (Table 7).

Table 7. Accumulated N_2O emissions (64 days) from a Nitisol fertilized with organic fertilizers under no-tillage and conventional tillage system (Grave et al., 2015b).

Fertilization	Tillage system		t-test (p-value)
	Conventional	No-tillage	
	---------- kg.N_2O.ha^{-1} ----------		
CTR	1,42 ± 0,18	1,85 ± 0,73 c[(1)]	0,948
MIN	1,87 ± 0,72	3,52 ± 0,65 ab	0,120
SS	2.55 ± 0.51 B	5.60 ± 1.38 A a	0.050
SMD	2.10 ± 0.40	2.94 ± 1.18 bc	0.606
COMP	1.56 ± 0.13 B	4.67 ± 1.70 A ab	0.017
Teste t (valor p)	0.443	0.004	-

CTR: control without fertilization; MIN: mineral fertilization (urea); SS: swine slurry; SMD: swine manure digestate; COMP: compound from swine manure. 1Means ± standard error (n=4) followed by the same lowercase letter in the column or uppercase letter in the row do not differ from each other by the t-test ($p<0.05$).

The accumulated N_2O emission at 64 days after the application of different sources of fertilizers was higher in the soil managed under the no-tillage system than in the soil submitted to conventional tillage, especially in areas fertilized with liquid swine manure (LSM) without treatment or submitted to composting (COMP) (Grave et al., 2015b). The authors attributed these results to the higher soil moisture content under no-tillage, as N availability and soil labile C contents did not vary between tillage systems. SS application to the soil under the no-tillage system increased N_2O emissions by 59% compared to the soil fertilized with urea (MIN) due to the input of labile C to the soil, which favored the proliferation of denitrifying microorganisms under high moisture and NO_3 availability conditions. These factors prevented the complete denitrification of NO_3 into N_2, resulting in N_2O accumulation as an intermediate metabolite and its emission into the atmosphere. As expected, the treatment of manure by biodigestion (SMD) or composting (COMP) limited the input of labile C and mineral N to the soil, reducing N_2O emissions into the atmosphere by 47% and 17% compared to the soil under no-tillage system and fertilized with SS.

These results are especially relevant for Brazilian agriculture, as Brazil has one of the largest cultivated areas under the no-tillage system in the world (Febrapdp, 2016). In this sense, the treatment of swine manure by biodigestion or composting and its recycling as sources of nutrients for agriculture contribute to the potential of GHG mitigation in the Brazilian agricultural sector by increasing C sequestration rates and mitigating soil N_2O emissions. However, for the potential of these technologies to be fully evaluated, it is essential that the GHG mitigation verified during the treatment of manure and other organic residues, which is currently accounted for in the ABC Plan, is also added to those observed in agricultural areas used for recycling organic fertilizers from different treatment systems

Final remarks

Technologies for the management of agricultural and agro-industrial residues have evolved significantly in recent decades. This evolution was followed by an increase in size and scale of production in rural properties and agribusinesses, providing alternatives for an environmentally adequate destination of residues generated by these activities in response to increasingly restrictive environmental legislation. In this sense, recycling organic residues as a source of nutrients for agriculture has proven to be a technically and economically viable alternative. However, this practice must follow the fundamental principles of fertilizer management and soil fertility already established and constantly refined by research. Failure to comply with these principles and the inadvertent disposal of these residues directly on the soil is a waste of nutrients from both an agronomic and economic point of view and can promote severe environmental impacts. Therefore, the establishment of environmental limits and their adoption by regulatory agencies as references for licensing processes and environmental monitoring is complementary to agronomic recommendations for fertilizer applications to ensure soil and environment quality conservation. Organic residues when properly managed constitute a safe source of nutrients for agriculture that can efficiently replace mineral fertilizers, with positive impacts on the environment and contributing to the economic viability of agricultural and agro-industrial enterprises.

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Chapter VI

DIGESTATE TREATMENT
Nitrogen Removal

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Introduction

Digestate is often poor in biodegradable organic matter, but it is an effluent rich in nitrogen and phosphorus, as most of the biodegradable organic matter has already been consumed in the anaerobic digestion process. Thus, this nutrient-rich effluent has great potential to negatively impact the environment when its agricultural use is limited or inadequate.

Problems associated with excess nutrients in the aquatic environment are worrying. High concentrations of ammoniacal nitrogen may lead to serious ecological implications, for instance, strongly influencing dissolved oxygen dynamics in the medium, as 4.6 mg of O_2 are required to oxidize 1 mg of NH_3.

Moreover, nitrogen and phosphorus in aquatic environments can cause eutrophication both in lentic and lotic environments, as well as

nutrient accumulation in the soil, entering a vicious circle of difficult environmental recovery if not stagnant at release (Hauck et al., 2016).

Nitrogen compounds in the different oxidation states can pose serious risks to human health from a public health point of view. Nitrate can cause methemoglobinemia (blue baby syndrome), the result of the reduction of NO_3- to NO_2- by bacteria in the intestinal tract and consequent oxidation of Fe^{2+} to Fe^{3+} of hemoglobin, forming methemoglobin, which is unable to bind to O_2, thus preventing the gas exchange in the human organism (Knobeloch et al., 2000).

Nitrite can also be combined with secondary amines from the diet, forming nitrosamines, which have known carcinogenic and mutagenic potential (Hu et al., 2012; Sadeq et al., 2008). Table 1 summarizes the major impacts that nutrients from digestate can cause when inappropriately released into the environment.

Table 1. Impacts caused by the most common forms of nitrogen in liquid effluents.

Oxidation state	Effect on the environment	Consequence
Reduced forms such as ammonia and ammonium	Increased oxygen requirement	The oxidation of ammonia that is released into the aquatic environment reduces O_2 concentration in the liquid medium
	Aquatic toxicity	Ammonia in the non-ionic form is toxic to many aquatic organisms
Oxidized forms such as nitrite and nitrate	Effects on human health	Nitrite can cause methemoglobinemia, known as blue baby syndrome
	Eutrophication	Nutrients cause excessive algae growth, which reduces O_2 overnight and produce organic compounds that cause odor and taste to water

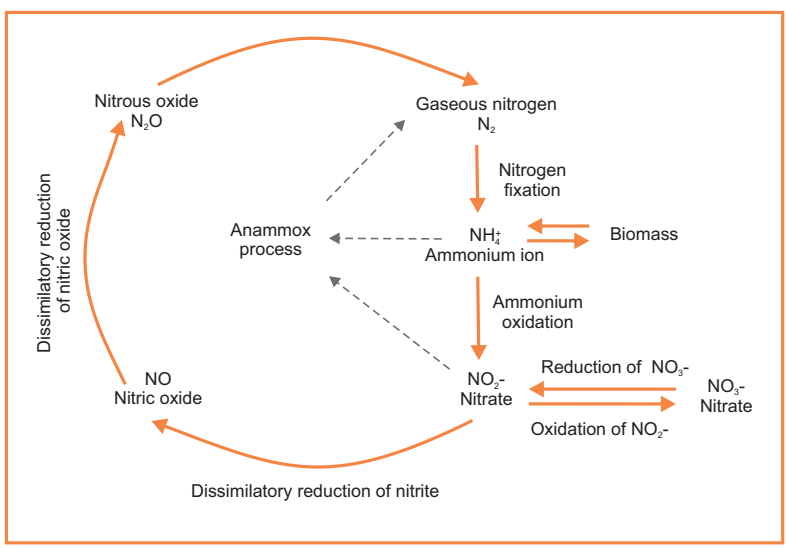
Given this scenario and facing environmental risks, digestates need to meet strict nitrogen and phosphorus concentration standards to be discarded at the end of treatment. Currently, few treatment systems contemplate nutrient removal, being associated with activated sludge systems in which, in the best scenario, nitrogen is only converted into nitrate without worrying about the environmental impact that it can cause.

The requirements regarding the management criteria for effluents from digesters have been increasing, making them significantly more restrictive and entailing the need for evolution in the effluent treatment processes that lead to a satisfactory reduction in nutrient concentration (Brasil, 2011; Fatma, 2014).

Nitrogen in the digestate and its main chemical transformations

Nitrogen is a nutrient present in the digestate in two main forms and oxidation states, being dissolved and particulate organic nitrogen and ammoniacal nitrogen (NH_3/NH_4^+).

The nitrogen cycle is carried out by a complex combination of various microorganisms and chemical reactions. Figure 1 shows the transformations of nitrogen compounds in the nitrogen cycle, resulting from microbial metabolism in the processes of fixation, nitrification, dissimilatory reduction of nitrite, denitrification, and anammox (anaerobic ammonia oxidation bacteria).



Source: Adapted from Ye; Thomas (2001).

Figure 1. Representation of reactions involved in the nitrogen cycle.

Figure 1 shows that nitrogen goes through several transformations, changing its oxidation state from the most reduced form to the most oxidized form. Table 2 shows the different chemical species of nitrogen that appear in the digestate and other effluents.

Table 2. Impacts caused by the most common forms of nitrogen in liquid effluents.

Chemical species	Description	Number of nitrogen oxidation (Nox)	Observation
$NH_3 + NH_4^+$	Total ammoniacal nitrogen (TAN)	-3	Independent of the medium pH
NH_3	Ammonia or free ammonia	-3	Varies depending on the medium pH
NH_4^+	Ammonium ion	-3	Varies depending on the medium pH
$NH_3 + NH_4^+ + N_{organic}$	Kjeldahl total nitrogen (KTN)	Indefinite	Total ammoniacal nitrogen added to the nitrogen present in organic matter
NO_2^-	Nitrite	+3	Generated through TAN oxidation
NO_3^-	Nitrate	+5	Generated through NO_2^- oxidation

The main source of ammoniacal nitrogen comes from metabolic reactions of bacteria that degrade organic substances, mainly urea, generating NH_3/NH_4^+. In contrast, gaseous nitrogen (N_2) can be converted into another form, mainly NH_3, by nitrogen-fixing bacteria (Hocking, 1985).

The formed ammonia can be anaerobically oxidized (together with nitrite) by bacteria with anammox activity or oxidized to nitrite by aerobic processes, which occurs with some frequency in effluents in the presence of oxygen. Nitrite can also be oxidized to nitrate or directly converted to gaseous nitrogen via nitric and nitrous oxide. Nitrate is the most oxidized form of nitrogen in nature and is often found in rivers and lakes, with the incorporation of oxygen from water movement (Galloway et al., 2008; Ye; Thomas, 2001).

Nitrogen in the ammoniacal form can still be assimilated by bacteria or oxidized to nitrite, which occurs with some frequency in effluents in the presence of oxygen. Other reactions of the microbiological nitrogen cycle, which is shown in Figure 1, commonly occurring in effluents with high ammoniacal nitrogen concentrations consist of the oxidation of nitrite to nitrate and dissimilatory reduction of nitrate to nitrite (Bailey et al., 2002; Gerardi, 2003; Ye; Thomas, 2001).

The pH and temperature influence the form that nitrogen is found in the digestate. The relationship between the concentrations of the two forms of ammoniacal nitrogen, ammonia and ammonium, and the relationship between nitrite and nitrous acid concentrations vary with the medium pH and temperature. The dissociation equilibria between these forms are described in Equations 1 and 2.

$$NH_4^+ \rightleftharpoons NH_3 + H^+$$ *Equation 1*

$$NO_2^- + H^+ \rightleftharpoons HNO_2$$ *Equation 2*

This equilibrium between the concentrations of ammoniacal nitrogen forms in an aqueous medium at 25 °C occurs at a pH of 9.25, in which 50% of both forms are observed. There is a predominance of ammonium ions at pH below the equilibrium point. However, the equilibrium is shifted to the formation of ammonia at pH values above 9.25, as shown in Figure 2.

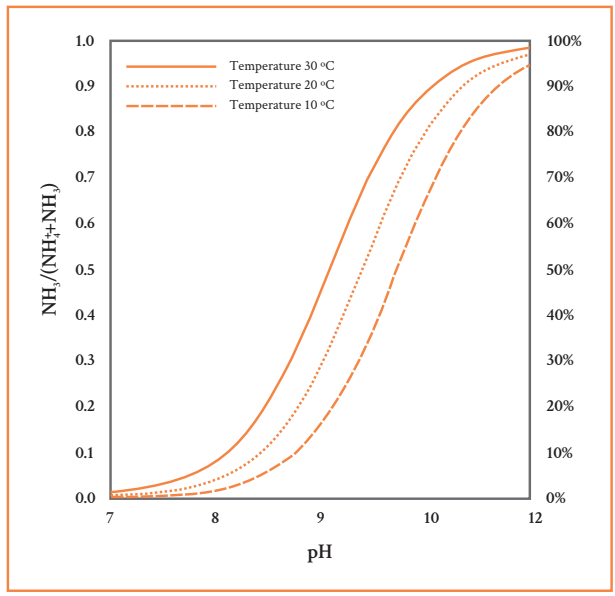


Figure 2. Influence of temperature and pH value on the equilibrium of NH_3 and NH_4^+ species.

Equation 3 is used to calculate ammonia and ammonium concentrations at any pH and temperature.

$$[NH_3\text{-}N] = \frac{[TAN]}{(1+K_{d,\,NH_3} \cdot 10^{pH})} \qquad \textit{Equation 3}$$

In which:

$$K_{d,\,NH_3} = e^{\frac{6344}{(273+T)}} \qquad \textit{Equation 4}$$

Where:

T = Medium temperature (°C)

$[NH_3\text{-}N]$ = Ammonia concentration

[TAN] = Total ammoniacal nitrogen

pH = Potential of hydrogen of the medium

Similarly, nitrous acid formation is a function of the temperature and pH of the medium. Figure 3 shows NO_2- and HNO_2 concentrations as a function of pH and temperature.

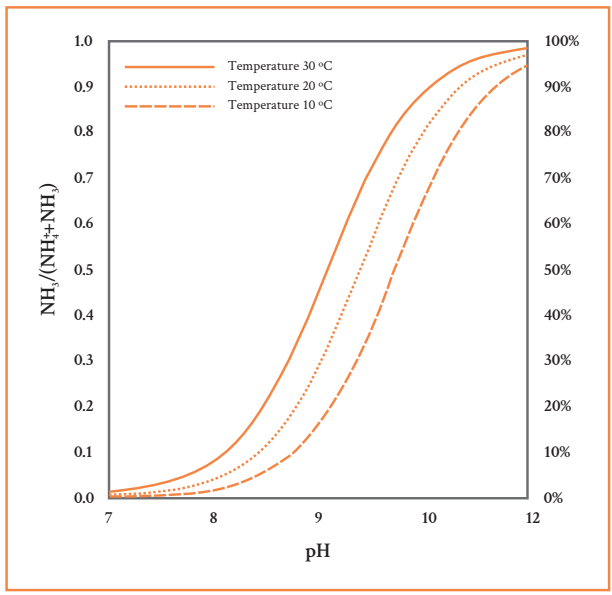


Figure 3. Influence of temperature and pH value on the equilibrium of NO_2- and HNO_2 species.

Equations 5 and 6 can be used to calculate NO_2- and HNO_2 concentrations at any pH and temperature.

$$[HNO_2\text{-}N] = \frac{[N - NO_2]}{(1 + K_{d,\,HNO_2} \cdot 10^{pH})} \qquad \textit{Equation 5}$$

In which:

$$K_{d,\,HNO_2} = e^{\frac{-2300}{(273+T)}} \qquad \textit{Equation 6}$$

Where:

T = Medium temperature (°C)

[HNO_2-N] = Nitrogen concentration in the form of nitrous acid

[NO_{2-} -N] = Nitrogen concentration in the form of nitrite

pH = Potential of hydrogen of the medium

The actual NH_3 and HNO_2 concentrations are of paramount importance for the control of biological processes. Firstly, both ammonia and nitric acid are believed to be the actual electron donors, that is, they are effectively the substrates involved in the nitrogen oxidation processes by microorganisms in the aqueous medium, requiring less energy to be transported into the cell compared to ionized forms (Wiesmann et al., 2007).

In addition to the substrate, the importance of knowing NH_3 and HNO_2 concentrations is related to the toxic potential of these two nitrogen species to ammonia-and nitrite-oxidizing microorganisms (De Prá et al., 2016).

The data have shown that there may be inhibition of microorganisms by the presence of excess ammonia or nitrous acid depending on the concentration of ammoniacal nitrogen and nitrite in the medium, even at a pH close to neutrality (Anthonisen et al., 1976).

Case study 1 – Fractions of ammoniacal nitrogen in effluents

A digester operating under continuous flow rate is fed with 250 $m^3.day^{-1}$ of swine manure. The digestate of this digester has a TAN concentration of 1,450 $mg.L^{-1}$ and is at a temperature of 26 °C and pH 8.4. Considering the equilibrium between ammonia and ammonium, determine the distribution of ammoniacal nitrogen fractions, according to the chemical equilibrium between the species.

- [TAN] = 1,450 $mg.L^{-1}$
- T = 26 °C
- pH = 8.4

The nitrogen concentration in the form of ammonia present in the sample can be calculated by Equation 7, which was obtained by substituting Equation 4 in Equation 3.

$$[NH_3\text{-}N] = \frac{[\text{TAN}]}{\left(1 + e^{\frac{6344}{(273+T)}} \cdot 10^{-pH}\right)} \qquad \textit{Equation 7}$$

$$[NH_3\text{-}N] = \frac{1450}{\left(1 + e^{\frac{6344}{(273+26)}} \cdot 10^{-8.4}\right)}$$

$$[NH_3\text{-}N] = \frac{1450}{(1 + 16.39 \cdot 10^{8} \cdot 10^{-8.4})}$$

$$[NH_3\text{-}N] = \frac{1450}{(1 + 6.525)}$$

$$[NH_3\text{-}N] = \frac{1450}{7.525}$$

$$[NH_3\text{-}N] = 192.7 \text{ mg } L^{-1}$$

$$[NH_3\text{-}N] = \frac{192.7}{1450} \cdot 100 = 13.29\%$$

$$[NH_4^+\text{-}N] = \frac{1450 - 192.7}{1450} \cdot 100 = 86.71\%$$

Case study 2 – Free nitrous acid concentration

An activated sludge reactor treating digestate from a digester is at a temperature of 33.4 °C and pH 6.42. A sample collected from the liquid medium of the reactor had a concentration of 572.3 mg.L^{-1} of -NNO_2-N. Based on these data, calculate the HNO_2 concentration present in the medium at the time of collection.

- $[NO_2\text{-}] = 572.3 \text{ mg.}L^{-1}$
- T = 33.4 °C
- pH = 6.42

Equations 5 and 6 allow calculating the HNO_2 concentration in the collected sample. Equation 8 is obtained by substituting Equation 6 in Equation 5.

$$[HNO_2\text{-}N] = \frac{[N\text{-}NO_2]}{\left(1+e^{\frac{-2300}{(273+T)}} \cdot 10^{pH}\right)} \quad \textit{Equation 8}$$

$$[HNO_2\text{-}N] = \frac{[N\text{-}NO_2]}{\left(1+e^{\frac{-2300}{(273+T)}} \cdot 10^{pH}\right)}$$

$$[HNO_2\text{-}N] = \frac{572.3}{\left(1+e^{\frac{-2300}{(273+33.4)}} \cdot 10^{6.42}\right)}$$

$$[HNO_2\text{-}N] = \frac{572.3}{(1+5.495 \cdot 10^{-4} \cdot 10^{6.42})}$$

$$[HNO_2\text{-}N] = \frac{572.3}{(1+1445)}$$

$$[HNO_2\text{-}N] = \frac{572.3}{1446}$$

$$[HNO_2\text{-}N] = 0.3958 \text{ mg.L}^{-1}$$

Consolidated technologies for biological nitrogen removal

Currently, there are numerous alternatives (biological and physicochemical) for removing nitrogen compounds. Biological processes are usually low cost and require less labor than others, which is why they have been widely used for treating digestates.

Among the main biotechnological processes for nitrogen removal are: nitrification-denitrification, known as the conventional process, of which the first studies date back to 1890 (Khin; Annachhatre, 2004);

partial nitrification process, one of the most recently proposed alternatives to nitrification (Hellinga et al., 1998); anammox process, anaerobic oxidation of the ammonium ion (Mulder et al., 1995); and combined deammonification processes, which aim to combine the last two processes in a single reactor (Third et al., 2001).

Nitrification process

Conventional nitrification is a microbiological reaction of nitrogen oxidation with oxygen as the final electron acceptor. This reaction occurs in two steps. The first step is where ammonia-oxidizing bacteria (AOB) oxidize NH_3-N (-III) to NO_2-N (III), with hydroxylamine as an intermediate product (Equation 9). The genus Nitrosomonas is often referred to in the literature as the most common AOB genus found in the environment. In the second step, nitrite-oxidizing bacteria (NOB) oxidize NO_{2^-}-N (III) to NO_{3^-}-N (V) (Equation 10). At this step, Nitrobacter is the genus of NOB most commonly found in the environment (Grady et al., 2011).

$$NH_4^+ + \frac{3}{2}O_2 \xrightarrow{\text{ammonia-oxidizing bacteria}} NO_2^- + H_2O + 2H^+ + \Delta G$$

Equation 9

$$NO_2^- + \frac{1}{2}O_2 \xrightarrow{\text{nitrite-oxidizing bacteria}} NO_3^- + \Delta G = -76\ kJmol^{-1}$$

Equation 10

The growth of AOB is more favored than NOB. It proves that cell growth is proportional to the energy released in the reaction. The ammonia oxidation reaction can be approximately 3.8 times more thermodynamically favored than the nitrite oxidation (Wiesmann et al., 2007).

According to Henze (2010), the equation that determines the oxidation reaction of ammonia to nitrate, as a single step, is shown in Equation 11.

$$NH_4^+ + 1.86O_2 + 1.98HCO_3^- \rightarrow 0.02C_5H_7NO_2 + 0.98NO_3^- + 1.88H_2CO_3 + 1.04H_2O$$

Equation 11

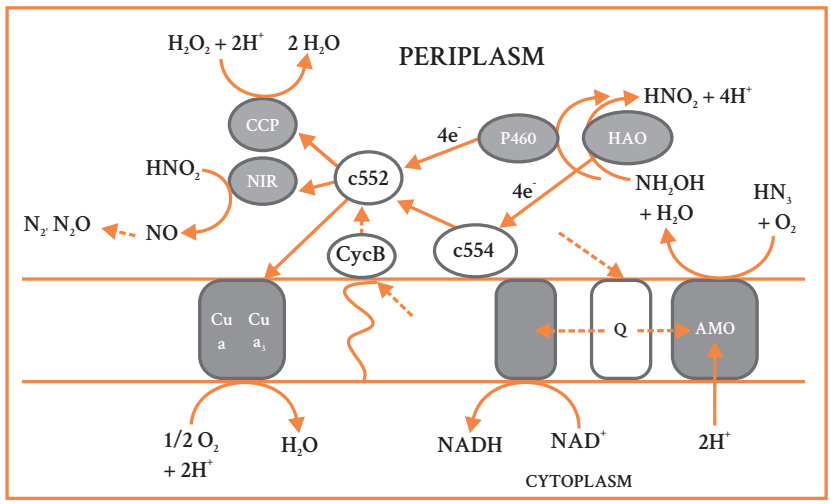
Biochemistry of nitrification

The reactions in the different steps of the NH_3-N oxidation (Nox -3) to NO_{3-} -N (Nox +5) are catalyzed by specific enzymes. The most complex reactions occur in the first step, that is, the nitritation or NH_3 -N oxidation to NO_{3-} -N, in which intermediates such as NH_2OH (hydroxylamine) appear. Two enzymes that participate in these reactions are the most important: ammonia monooxygenase, which acts to convert NH_3 to NH_2OH, and hydroxylamine oxidoreductase, which acts to convert NH_2OH to HNO_2.

Other enzymes catalyze reactions in the cell wall region of ammonia-oxidizing bacteria (Figure 4), such as nitrite reductase, which reduces HNO_2 to NO, nitric oxide reductase, which catalyzes the reduction of NO to N_2O, and nitrous oxide reductase, which catalyzes the reduction reaction of N_2O to N_2 (Hooper et al., 1997; Klotz; Stein, 2008; Bock; Wagner, 2013).

The oxidation of nitrite-N (III) to nitrate-N (V) occurs in a second step (Figure 5), that is, nitratation. Bacteria of the genus Nitrobacter participate in this step. The reaction is catalyzed by the enzyme nitrite oxidoreductase (NXR). This enzyme is found inside the cell wall and acts both in the oxidation of nitrite to nitrate and the reduction of nitrate to nitrite. Thus, the reaction is reversible.

Cytochrome a3 HCO (heme-copper oxidase) is another enzyme that plays an important role in this reaction. It is a group of proteins with a copper atom in the heme group that are part of the electron transport system of mitochondria and act as intermediate coenzymes in the cellular respiratory chain (Hooper et al., 1997; Klotz; Stein, 2007; García--Horsman et al., 1994).



Source: Adapted from Hooper et al., 1997(HOOPER et al., 1997).

Figure 4. Components of the nitrogen oxidation and electron transport system in Nitrosomonas. AMO – ammonia monooxygenase; HAO – hydroxylamine oxidoreductase; P460 – cytochrome P460; Q – ubiquinone-8; CycB – tetra-heme cytochrome c of the membrane; c552 – cytochrome c552; cp – di-heme c553 peroxidase; NiR – nitrite reductase; NOR – nitric oxide reductase; N_2OR – nitrous oxide reductase. Solid lines represent known mechanisms and dotted lines represent mechanisms not completely known, therefore, hypothetical.

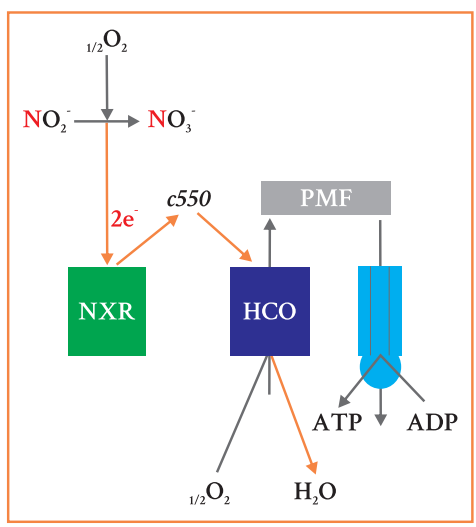


Figure 5. Components of the nitratation reaction system and their corresponding enzymes. NXR – nitrite oxidoreductase; c550 – cytochrome c550; HCO – heme-copper oxidase; PMF – proton motive force; ATP – adenosine triphosphate; ADP – adenosine diphosphate.

Nitrifying bacteria are autotrophic and, therefore, cannot incorporate exogenous organic compounds, obtaining energy from the oxidation of inorganic compounds. Many of the equations that define the growth kinetics of nitrifying bacteria do not consider that carbon dioxide is the only required carbon source. Moreover, the maximum growth rate of nitrifying bacteria is much lower than the growth rate of heterotrophic bacteria (Grady et al., 2011).

Although the nitrification process is autotrophic, it can also occur through the action of heterotrophic bacteria, which use organic carbon and oxidize ammonia to nitrate, such as *Arthrobacter* and *Thiosfera pantotropha* (Bitton, 2005).

Dissolved oxygen, pH, and growth of nitrifying biomass

The growth rate of nitrifying biomass is low and depends on growing conditions. Several parameters influence the nitrification performance of populations of nitrifying bacteria, such as dissolved oxygen (DO), pH, temperature (T), hydraulic retention time (HRT), and cell retention time (CRT). Among them, DO and pH are the most important (Wiesmann et al., 2007).

DO must be monitored in a reactor where complete nitrification is aimed, mainly because it can present a form of selection of different populations. It happens naturally, regardless of the goal.

Populations of nitrite-oxidizing bacteria are easily inhibited by the limitation of dissolved oxygen. This event is evidenced in Table 3, which shows that the ratio between the cell concentration of populations of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria (X_{NS}/X_{NB}) increases considerably when the DO of the medium is restricted (Canziani et al., 2006).

Table 3. Calculated and measured parameters of populations of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria. μNS – specific population growth rate of ammonia-oxidizing bacteria; μNB – specific population growth rate of nitrite-oxidizing bacteria; DO – dissolved oxygen in the medium.

μ_{NS} (d^{-1})	μ_{NB} (d^{-1})	X_{NS}/X_{NB}	Observations
0.625	0.555	2.96	DO > 2.0 $mg.L^{-1}$
0.450	0.129	16.54	DO between 0 $mg.L^{-1}$ and 0.5 $mg.L^{-1}$
0.468	0.192	25.02	
0.474	0.256	42.43	
0.632	0.395	31.66	DO between 0.5 $mg.L^{-1}$ and 1.5 $mg.L^{-1}$
0.582	0.275	18.97	

Source: Adapted from Canziani et al. (2006).

Regarding the oxygen requirement in the aerobic oxidation process of ammonia to NO_3, Equation 11 shows the need for 1.86 moles of oxygen for the complete oxidation of 1 mole of ammonium into nitrate. Converting the values in moles into mass in grams, the complete nitrification requires 4.25 g of oxygen per gram of NH_4^+-N.

The calculation of oxygen requirement in aerobic reactor designs aiming at nitrification to nitrate requires the concentration and load of nitrogen in the form of ammonium at the inlet of the aerobic reactor.

Case study 3 - Daily requirement of oxygen (Rd_{O2})

A digester generates 178 $m^3.d^{-1}$ of digestate, which is sent to an aerobic nitrifying reactor. The total ammoniacal nitrogen concentration is 1,385 $mg.L^{-1}$ and BOD (biochemical oxygen demand) is 3,630 $mg.L^{-1}$. Calculate the daily oxygen requirement needed in the reactor for the oxidation of all nitrogen in the form of ammonium to nitrate.

Initially, we need to know the nitrogen load at the inlet of the aerobic reactor. Therefore:

$$\boldsymbol{C = [TAN].Q} \qquad \textit{Equation 12}$$

Where:

c = Nitrogen load ($kg.d^{-1}$)

[TAN] = Total ammoniacal nitrogen ($kg.m^{-3}$)

Q = Flow rate ($m^3.d^{-1}$)

The daily nitrogen load at the inlet of the aerobic reactor can be calculated using Equation 12.

$$C = [TAN].Q$$

$$C = 1385\ \frac{mg}{L}.\ \frac{g}{1000\ mg}\ .\ 178\ \frac{m^3}{d}$$

Considering:

$$\frac{g}{L} = \frac{kg}{m^3}$$

$$C = 246.5\ kg\ d^{-1}$$

The nitrogen load allows calculating the daily oxygen requirement (Rd_{O2}) in the aerobic reactor as a function of Equation 12.

$$Rd_{O_2 \rightarrow NAT} = 246.5\ \frac{kgNAT}{d}\ .\ 4{,}25\ \frac{O_2}{NAT}$$

$$Rd_{O_2 \rightarrow NAT} = 1047.63\ \frac{kgO_2}{d}$$

The calculation of the oxygen requirement for the oxidation of organic matter can be carried out analogously. The calculation of oxygen requirement is equal to the daily BOD load.

$$C = 3630\ \frac{mg}{L}.\ \frac{g}{1000\ mg}\ .\ 178\ \frac{m^3}{d}$$

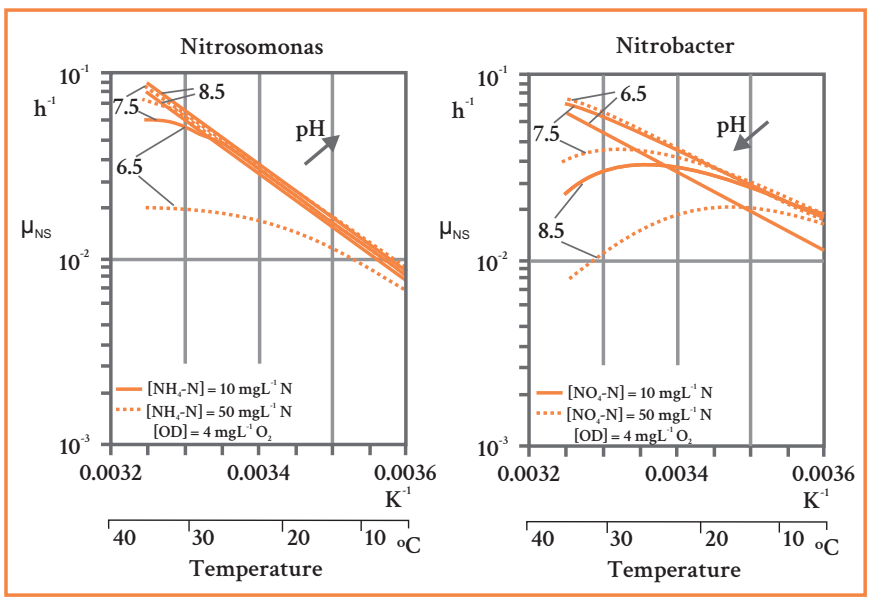
$$l = 646.1\ \frac{kg}{d}$$

$$Rd_{O_2 \rightarrow BOD} = 646.1\ \frac{kgO_2}{d}$$

In addition to the oxygen requirement necessary for the complete nitrogen oxidation in the nitrification process, pH has significant importance in terms of cell growth and stability of the nitrification process, as shown in Figure 6.

In the operation of nitrifying reactors, the pH contributes to the availability of the actual substrates of the process in addition to governing the equilibrium of the forms of ammonia (NH_3) and ammonium (NH_4), as well as nitrite (NO_2) and nitrous acid (HNO_2), as previously seen.

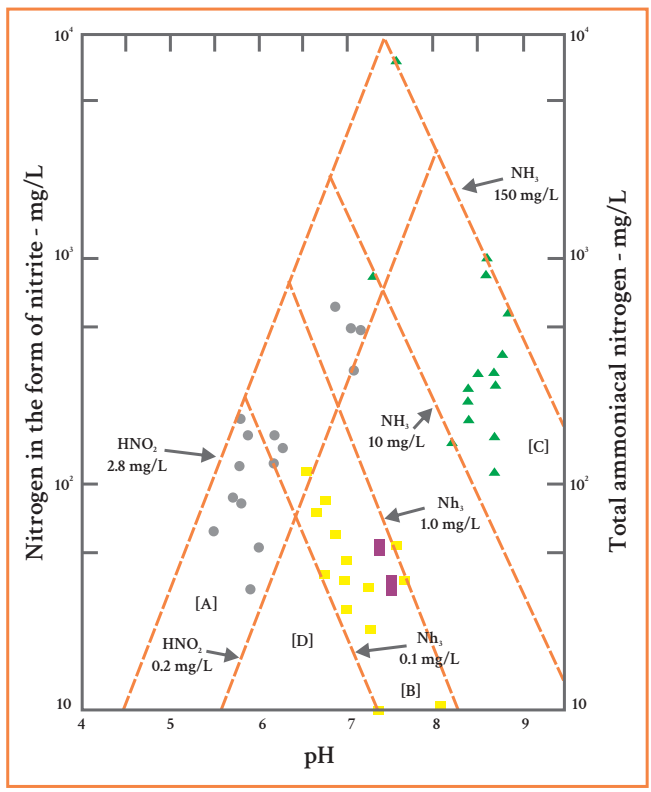
Only ammonia and nitric acid are effective substrates, as the cell uses less energy to transport these forms through the cell wall than the ionized forms ammonium and nitrite (Wiesmann et al., 2007).



Source: Adapted from Wiesmann et al., (2007).

Figure 6. The specific growth rate of bacteria of the genera Nitrosomonas (μ_{NS}) and Nitrobacter (μ_{NB}) as a function of pH, temperature, and concentration of the feeding medium.

The inhibitory effect of pH can be observed even at values close to neutrality, depending on the concentration of total ammonia and nitrite in the medium. The inhibitory effect of ammonia and nitrous acid on populations of *Nitrosomonas* (main population ammonia-oxidizing bacteria) and *Nitrobacter* (main population of nitrite-oxidizing bacteria) as a function of pH variation is shown in Figure 7.



Source: Adapted from Anthonisen et al., (1976).

Figure 7. Behavior of bacteria of the genera *Nitrosomonas* and *Nitrobacter* at different concentration ranges of free ammonia and nitrous acid as a function of pH.

The area identified as [A] in Figure 7, between 0.2 $mg.L^{-1}$ and 2.8 $mg.L^{-1}$, indicates the beginning of inhibition by HNO_2 for ammonia and nitrite-oxidizing bacteria, with a complete inhibition above 2.8 $mg.L^{-1}$ of HNO_2. The area [B] (0.1 $mg.L^{-1}$ to 1.0 $mg.L^{-1}$ of NH_3) identifies the beginning of inhibition of nitrite-oxidizing bacteria, while area [C]

(10 mg.L^{-1} to 150 mg.L^{-1} of NH_3) identifies the beginning of inhibition of ammonia-oxidizing bacteria (Anthonisen et al., 1976).

The pH control is essential for the maintenance of the nitrification process. The pH fluctuations can be minimized through a minimum amount of alkalinity, which acts by increasing the buffering power of the medium.

The alkalinity requirement can be determined by the stoichiometry of the TAN oxidation reaction. The stoichiometric requirement of bicarbonate (HCO_3-) is 2 mols for oxidation and 1 mol of NH_4^+, which corresponds to 4.36 grams of HCO_3 per gram of TAN (Galí et al., 2007).

The analytical methodology for total alkalinity in water and effluents determines the alkalinity in calcium carbonate ($CaCO_3$) (Rice et al., 2012) and, therefore, the alkalinity value in HCO_3- needs to be converted into Ca_2CO_3. Thus, the alkalinity requirement for complete oxidation of one gram of TAN is 7.14 grams of $CaCO_3$.

Case study 4 - Alkalinity requirement calculation

A nitrifying reactor is fed with digestate containing 2,190 mg.L^{-1} of N-NH_4+ at a flow rate of 135 $m^3.d^{-1}$. A concentration of 6,450 mg. $CaCO^3.L^{-1}$ was found after quantifying the total alkalinity in the digestate. Based on the data, calculate the daily alkalinity supplementation requirement in $CaCO_3$ for complete oxidation of NH_4^+ to NO_3-.

Initially, the daily load (L) of nitrogen and alkalinity present in the digestate that feeds the nitrifying reactor needs to be calculated before determining the daily requirement, using Equation 12.

$$C\left(\frac{kg}{d}\right) = [TAN] \,.\, Q$$

$$C = 2190 \frac{mg}{L} . \frac{g}{1000\ mg} . 135\ \frac{m^3}{d}$$

$$C = 2.19 \frac{g}{L} . 135\ \frac{m^3}{d}$$

$$C = 295.65 \frac{kg\,TAN}{d}$$

Analogous to calculating the nitrogen load, we can calculate the alkalinity load in $CaCO_3$ that feeds the nitrifying reactor daily.

$$C\left(\frac{kg}{d}\right) = [CaCO_3] \,.\, Q$$

$$C = 6450\frac{mg}{L} . \frac{g}{1000\ mg} . 135\ \frac{m^3}{d}$$

$$C = 6.45\frac{g}{L} . 135\ \frac{m^3}{d}$$

$$C = 870.75\frac{kgCaCO_3}{d}$$

Considering that 7.14 grams of $CaCO_3$ per gram of NH_4^+-N are needed, we can calculate the required daily alkalinity load through the product of the nitrogen load and alkalinity requirement.

$$\text{Alkalinity requirement} = 295.65\frac{kg\,TAN}{d} . 7.14\frac{kgCaCO_3}{kgTAN}$$

$$\text{Alkalinity requirement} = 2110.9\frac{kgCaCO_3}{d}$$

The daily alkalinity deficit for nitrification is obtained by subtracting the daily alkalinity load present in the digestate from the alkalinity requirement based on the TAN load that feeds the nitrifying reactor.

$$\text{Alkalinity deficit} = \text{Daily alkalinity load} - \text{Daily alkalinity requirement}$$

$$\text{Alkalinity deficit} = 870.75\frac{kgCaCO_3}{d} - 2110.9\frac{kgCaCO_3}{d}$$

$$\text{Alkalinity deficit} = -1240.15\frac{kgCaCO_3}{d}$$

Therefore, the alkalinity present in the digestate is not sufficient for the nitrification of this effluent, making it necessary to complement the alkalinity so that the pH of the system does not decrease to inhibitory levels.

Part of the alkalinity in processes with denitrification returns to the system and, when the processes are coupled, the alkalinity generated in the denitrification offsets part of the nitrification requirement, as discussed below.

Denitrification process

Denitrification is part of the nitrogen cycle. It consists of the transformation of NO_3 to N_2 under O_2 absence conditions. It is a reductive process and, therefore, a type of respiration. It occurs in four stages, according to Equation 13. Microorganisms oxidize an organic substrate as an energy source, producing numerous reduction equivalents.

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad \textit{Equation 13}$$

Each step in this reaction is catalyzed by specific enzymes. The nitrogen reduction steps have been widely studied. The structures of these enzymes have been visualized in high resolution, except for nitric oxide reductase. Furthermore, it is already known that there may be more than one type of reductase per step.

In general, the enzymes required for denitrification are only produced under or close to anaerobic conditions, but they are readily inhibited if anaerobic growing cells are exposed to O_2. Furthermore, the reactions are catabolic, that is, governed by heterotrophic microorganisms, requiring organic matter as a source of carbon for their cellular synthesis (Richardson et al., 2007; Mendonça, 2002; Tchobanoglous et al., 2013).

The microorganisms most frequently found in nature capable of reducing oxidized nitrogen consist of the genera *Pseudomonas* and *Alcaligenes*. However, other microorganisms have been described in the literature, such as *Achromobacter*, *Acinetobacter*, *Agrobacterium*, *Arthrobacter, Bacillus, Brevibacterium, Chromobacterium, Corynebacterium, Flavobacterium, Hyphomicrobium, Moraxella, Neisseria, Paracoccus, Propionibacterium, Rhizobium, Rhodopseudomonas, Spirillum,* and *Vibrio* (Tchobanoglous et al., 2013).

Biochemistry of denitrification

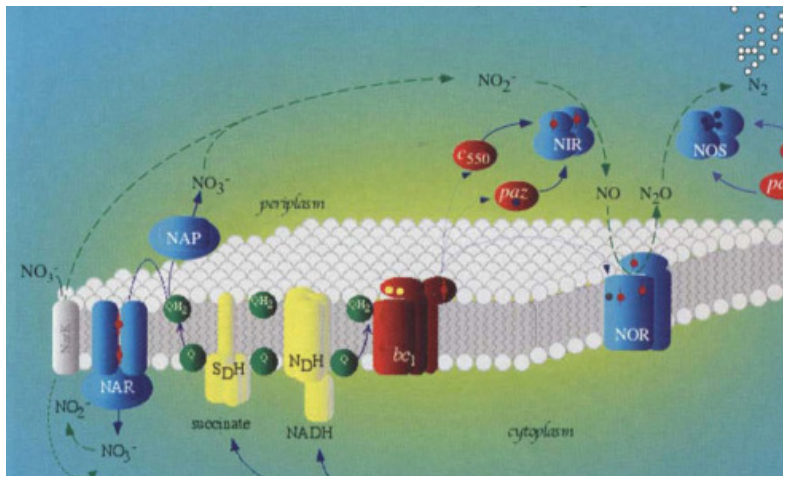
The enzymes responsible for denitrification in most bacteria receive electrons from the currents of the respiratory systems in the cytoplasmic membrane. In other words, denitrification is a form of respiration and a part of respiration with the electron transport system.

Denitrification occurs with the participation of specific components, such as ubiquinol/ubiquinone. The reduction reaction of ubiquinone to ubiquinol occurs using electrons from reductants such as NADH, volatile organic acids, and succinate. In denitrification, ubiquinol is directly oxidized in the cytoplasmic wall by nitrate reductase. There is a corresponding crystalline structure for this enzyme, commonly known as Nar, thus allowing knowing in detail how the enzyme works.

In summary, ubiquinol is oxidized towards the periplasmic membrane surface, with the release of H^+ into the periplasm, but electron transfer occurs across the membrane to the active site, which is located in a globular domain projected into the cytoplasm. The key point to note here is that electron transfer by Nar, together with the release of H^+ and absorption on both membrane sides, generates a driving force of protons across the membrane.

The location of the NO_3- reduction site on the cytoplasmic side of the membrane requires a NO_3- transport system, as shown in Figure 8. This task is believed to be the function of the NarK protein, which is a transporter both from the outside to the inside of the cell and the inverse. Normally, the NarK protein is the fusion of two proteins. Evidence suggests that one of these proteins catalyzes the entry of NO_3- into the cell with one or more H^+. It would allow NO_3- to enter the cell to initiate respiration.

In the steady-state, the import of NO_3- would be in exchange for the export of NO_2- to the periplasm, a process that would be the neutral exchange of electrons, thus neither affecting nor dissipating the proton motive force. The export of NO_2- to the periplasm is necessary because it is where nitrite reductase (NIR in Figure 8) is located in denitrifying systems (Moir; Wood, 2001; Spanning et al., 2007).



Source: Adapted from Spanning et al., (2007).

Figure 8. Scheme of the complete denitrification process in *Paracoccus denitrificans*. Dashed lines: transport of nitrogen oxides; solid lines: transport of electrons. SDH, succinate dehydrogenase; NDH, NADH dehydrogenase; Q, quinone; bc_1, cytochrome bc_1 complex; c_{550}, cytochrome *c*; paz, pseudoazurin; NAR, nitrate reductase of the membrane; NIR, cd_1-type nitrite reductase; NOR, *BC*-type nitric oxide reductase; NOS, nitrous oxide reductase; NarK, NO_3-/NO_2- transporter.

Electrons are delivered to cytochrome cd_1 by a mono-heme cytochrome c, cytochrome c_{550}, or the cupredoxin protein known as pseudoazurin. These two periplasmic, water-soluble proteins are reduced by the integral membrane complex, called the cytochrome *bc*1 complex, which in turn is reduced to ubiquinol. This complex is not specific for denitrification, occurring in several respiratory systems in all bacteria and mitochondrial electron transfer.

Nitric oxide is generated by nitrite reductase, but at low concentrations due to its toxicity. However, it is still an intermediary free from denitrification. Nitric oxide reductase is an enzyme present in the cell membrane, participating in the reduction of nitric oxide to nitrous oxide. It is believed, but not yet proven in the laboratory, it is provided by pseudoazurin or cytochrome c_{550} in common with nitrite reductase. The final step of denitrification is catalyzed by nitrous oxide reductase, another periplasmic enzyme, and acts to reduce nitrous oxide to gaseous nitrogen.

According to Wrage et al. (2001), the microorganisms responsible for denitrification are facultative anaerobes. That is, they are capable of using both oxygen and NO_3- and NO_2-. Therefore, the denitrification process is inhibited even at low dissolved oxygen concentrations.

Regarding the intermediates in the denitrification process, NO and N_2O are gaseous and accumulate in the medium when their enzymes are mainly inhibited at acidic pH.

Organic carbon and alkalinity in the denitrification

Denitrification is a heterotrophic process and needs a source of organic carbon to be carried out. There are two main forms in which denitrifying microorganisms obtain the organic carbon needed for reactions. It is called endogenous when the source comes from the cellular material. The other form is through an exogenous source, that is, an organic substrate, organic effluent, acetate, methanol, among others.

The organic carbon found in the composition of natural effluents comes basically from proteins, carbohydrates, and fats (Gerardi, 2002). In general, effluents after anaerobic treatment show the prevalence of short-chain carboxylic acids, such as acetic, propionic, and butyric acid (Miller; Varel, 2003; Ziemer et al., 2009).

An extra carbon source is required when there is not enough organic carbon present in the effluent. Methanol is commonly used in this role in industrial effluent treatment processes (Tchobanoglous et al., 2013). The denitrification reactions from acetate (Equation 14) and methanol (Equation 15) are shown below.

$$5CH_3COOH + 8NO_3 \rightarrow 4N_2 + 10CO_2 + 6H_2O + 8OH^-$$

Equation 14

$$5CH_3OH + 6NO_3 \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^-$$

Equation 15

Organic carbon availability in a given effluent is measured by the ratio between the available organic carbon mass and the nitrogen mass to be reduced in the denitrification. This ratio is usually called the C/N (carbon/nitrogen) ratio.

The C/N ratio has a great influence on the competition between the dissimilar reduction of nitrate to gas products (denitrification) and ammonification (Yoon et al., 2015). In laboratory tests with adapted biomass, the denitrification efficiency is not compromised when the C/N ratio is above 1 using methanol and above 2 using acetic acid as a carbon source (Her; Huang, 1995).

The magnitude of organic carbon is often expressed in the literature as the chemical oxygen demand (COD), which indirectly expresses the amount of organic matter present in the sample, accounting for the need for oxygen to oxidize the present organic carbon. Also, the relationship between the magnitudes TOC and COD is a function of factors such as the composition of organic matter and the presence of inorganic compounds, which consume oxygen in the oxidation of organic matter by the COD method, which may not occur in TOC determination methods.

The differences observed regarding the denitrification efficiency when comparing different substrates are due to the carbon bioavailability in each substrate. Studies have indicated the preference of denitrifying microorganisms for the use of short-chain carboxylic acids as a carbon source in denitrification (Elefsiniotis; Wareham, 2007; Adouani et al., 2010; Ahn et al., 2010).

Besides the preference for short-chain carboxylic acids and the high affinity of denitrifying microorganisms mainly for acetic acid, the denitrification rate using this acid as the main carbon source is more than twice the denitrification rate using propionic acid (Elefsiniotis; Wareham, 2007). It suggests that the use of longer-chain carboxylic acids by denitrifying microorganisms is complex and difficult.

Therefore, the C/N ratio must be observed considering only the soluble organic carbon, discarding the particulate material, when using the MLE (modified Ludzak and Ettinger) process aiming at the removal of nitrogen, regardless of the form in which the organic carbon is found in the digestate.

The organic carbon requirement is approximately 1.1 g per gram of nitrogen in the form of nitrate-based, considering the stoichiometry of the denitrification reaction (Equation 13). However, organic carbon is also consumed for cell synthesis and endogenous respiration in the denitrification process (Henze, 2010). In this sense, the denitrification reaction will occur without limitations if the digestate shows a C/N ratio above 2 or a COD/N ratio above 5 (Velho et al., 2017; Kishida et al., 2004; Chung et al., 2004).

Case study 5 - C/N ratio calculation

The concentration of TOC of 3,350 $mg.L^{-1}$ and total ammoniacal nitrogen (TAN) of 1,200 $mg.L^{-1}$ was observed in a digestate from a digester. Suppose the digester feeding flow rate is 10 $m^3.h^{-1}$ and 8 $m^3.d^{-1}$ of digester sludge is discarded. Calculate the C/N ratio and say if the denitrification process can be applied to this case.

Ideally, the carbon and nitrogen load are calculated and, subsequently, the relationship between the loads is verified to reduce the possibility of errors when calculating the C/N ratio.

Therefore:

$$C_{TOC}\left(\frac{kg}{d}\right) = Q\frac{m^3}{d} \,.\, [TOC]\frac{kg}{m^3}$$

In this case, specifically, the digester feeding flow rate is not the same as the outlet flow rate, as there is sludge disposal. Therefore, the outlet flow rate is the feeding flow rate minus the sludge disposal flow rate.

$$C_{TOC} = \left[Q_{feeding}\frac{m^3}{d} - Q_{sludge\ disposal}\frac{m^3}{d}\right] \,.\, [TOC]\frac{mg}{L}$$

$$C_{TOC} = \left[10\frac{m^3}{h} . \frac{24\ h}{d} - 8\frac{m^3}{d}\right] \,.\, 3350\frac{mg}{L}\frac{g}{1000\ mg}$$

$$C_{TOC} = \left[240\frac{m^3}{d} - 8\frac{m^3}{d}\right] \,.\, 3.35\frac{kg}{m^3}$$

$$C_{TOC} = 232\frac{m^3}{d} \,.\, 3.35\frac{kg}{m^3}$$

$$C_{TOC} = 777.2\frac{kg}{d}$$

The daily nitrogen load is calculated analogously.

$$C_{TAN} = \left[Q_{feeding}\frac{m^3}{d} - Q_{sludge\ disposal}\frac{m^3}{d}\right] \,.\, [TAN]\frac{kg}{m^3}$$

$$C_{TAN} = \left[Q_{feeding}\frac{m^3}{d} - Q_{sludge\ disposal}\frac{m^3}{d}\right] \,.\, [TAN]\frac{mg}{L}$$

$$C_{TAN} = \left[10\frac{m^3}{h}.\frac{24\ h}{d} - 8\frac{m^3}{d}\right] \,.\, 1200\frac{mg}{L}\frac{g}{1000\ mg}$$

$$C_{TAN} = 232\frac{m^3}{d} \,.\, 1.2\frac{kg}{m^3}$$

$$C_{TAN} = 278.4\frac{kg}{d}$$

The daily nitrogen load is calculated analogously.

$$\frac{C}{N}ratio = \frac{C_{TOC}}{C_{TAN}}$$

$$\frac{C}{N}ratio = \frac{777.2\frac{kg}{d}}{278.4\frac{kg}{d}}$$

$$\frac{C}{N}ratio = 2.79$$

The C/N ratio is 2.79, and it is expected that the denitrification process will not have its efficiency impaired due to the need for carbon because the amount of nitrogen is adequate.

The alkalinity equilibrium in the nitrogen removal process is a sensitive step. The generation of alkalinity equivalents can be observed in the complete denitrification cycle (Equations 14 and 15). Eight hydroxyl ions are generated for every 5 moles of acetate, that is, 3.57 g of alkalinity (as $CaCO_3$) is generated by reducing 1 g of NO_3-N(Tchobanoglous et al., 2013; Van Rijn et al., 2006). However, the values are lower than those from the stoichiometry on a real scale, that is, from 2.95 (Jeris; Owens, 1975) to 2.89 mg (Horstkotte et al., 1974) of $CaCO_3$ per mg of reduced nitrogen.

Thus, each gram of reduced nitrogen generates 3 grams of alkalinity in the form of $CaCO_3$, considering the stoichiometric value and the values observed in real-scale reactors for denitrifying reactor designs (Scheible et al., 1993).

A significant advantage of this excess alkalinity generated in denitrification is observed when thinking about the global nitrogen removal process via nitrification and denitrification. Given that alkalinity is consumed in the nitrification and alkalinity is generated in the denitrification, there is a compensation for the total alkalinity requirement when the processes are coupled.

Combined nitrification and denitrification process

The first configuration aiming at nitrogen removal consisted of the process proposed by Ludzak and Ettinger (1962), which was later modified and called modified Ludzak and Ettinger (MLE), shown in Figure 9. It is one of the most used processes for nitrogen removal in effluent treatment. The process consists of an anoxic tank before the aerobic tank, where the nitrification occurs. The nitrate produced in the aerobic tank returns to the anoxic tank.

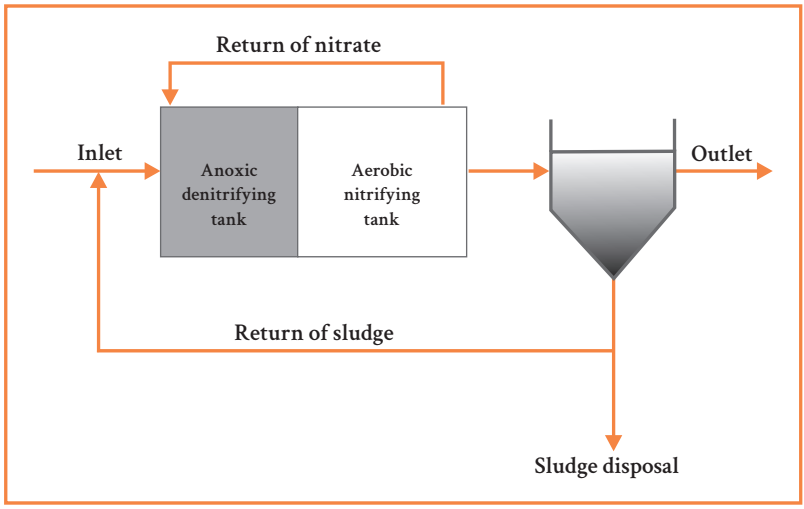


Figure 9. Representation of a complete mix reactor system using the modified Ludzak--Edinger process.

Heterotrophic microorganisms and the largest amount of organic carbon that will serve as electron donors in the nitrate reduction are in the anoxic tank. The process is also known as anoxic pre-denitrification because the anoxic tank precedes the aeration tank (Tchobanoglous et al., 2013; Wiesmann et al., 2007).

The recirculation rate of the liquid medium from the nitrifying reactor to the denitrifying reactor must be controlled. The higher the recirculation rate is, the higher the nitrogen removal. The overall system efficiency can be calculated by Equation 16.

$$E = 1 - \frac{[N_T]_s}{[N_T]_e} \qquad \textit{Equação 16}$$

Where:

$[N_T]_e$ = Total nitrogen concentration at the MLE system inlet ($mg.L^{-1}$)

$[N_T]_s$ = Total nitrogen concentration at the MLE system outlet ($mg.L^{-1}$)

Both reactors in the MLE system are full-mix and continuous flow rate, and the nitrogen removal efficiency is dependent on the ratio of the total recirculation flow rate from the nitrifying reactor to the denitrifying reactor and the system feeding flow rate (Equation 17). The total recirculation flow rate is the sum of the recirculation flow rate between the nitrifying and denitrifying reactor and the recirculation flow rate of the sludge from the sludge settler to the denitrifying agent (Equation 18).

$$R_T = \frac{Q_{RT}}{Q_{feeding}} \qquad \textit{Equation 17}$$

Where:

R_T = Total recirculation ratio

Q_{RT} = Recirculation flow rate ($m^3.d^{-1}$)

$Q_{feeding}$ = Feed flow rate of the MLE system ($m^3.d^{-1}$)

$$Q_{RT} = Q_{R\text{-}ND} + Q_{R\text{-}L} \qquad \textit{Equation 18}$$

Where:

$Q_{R\text{-}ND}$ = Recirculation flow rate between the nitrifying and denitrifying reactor ($m.d^{-1}$)

$Q_{R\text{-}L}$ = Sludge recirculation flow rate ($m^3.d^{-1}$)

Therefore, the maximum theoretical nitrogen removal efficiency for the MLE reactor system configuration is directly dependent on the total recirculation ratio (QRT) between reactors, as shown in Equation 19.

$$\frac{[N_T]_s}{[N_T]_e} = \frac{1}{1 + R_T} \qquad \textit{Equation 19}$$

The total recirculation ratio influences the system efficiency, as it is the basis of the nitrogen removal process. It can be used as a process control parameter. However, the ideal Q_{RT} value ranges between 3 and 6 times the feeding flow rate (Tchobanoglous et al., 2013; Chung et al., 2004), usually being set at 4.5 times the feeding flow rate ($Q_{feeding}$). The

sludge recirculation ratio is fixed at 1, as the sludge recirculation flow rate ($Q_{R\text{-}S}$) has the sole purpose of preventing excessive loss of biomass from the system.

The MLE process is very versatile, and results have demonstrated an efficiency above 90% of nitrogen removal from swine effluents. However, these effluents have a high concentration of total suspended solids, which can cause disturbances in the reactor operation, evidencing the attention that must be paid regarding this factor to avoid the fixed solids accumulation in the reaction tanks.

It is based on the nitrogen removal process via nitrification and denitrification and, therefore, there is a need for equilibrium between the amount of organic carbon and nitrogen, as previously mentioned, with the C/N ratio being essential in this process.

Most animal manure has enough carbon for denitrification because there is a large amount of available organic carbon, that is, a high C/N ratio. However, the amount of organic carbon available in the digestate is lower than that found in raw manure. The C/N ratio may decrease 20 times after the effluents and/or animal manure go through an anaerobic digestion process (Rico et al., 2011), which may remove nitrogen from the digestate by the MLE process unfeasible. A situation in which there is a lack of organic carbon available for denitrification requires the carbon supplementation or bypass from the digester directly to the denitrifying reactor.

Regarding dimensioning, the MLE system needs attention relative to two points, in addition to those already discussed: the volumetric nitrogen load (L_{vN}) and the hydraulic retention time (HRT).

The volumetric organic nitrogen load expresses the mass of nitrogen that feeds the MLE system per day as a function of the nitrifying reactor volume, according to Equation 20. The volumetric nitrogen load influences the nitrogen removal efficiency from the system. The MLE system operates with a nitrogen removal efficiency above 95% and L_{vN} ranging from 0.26 $kg.m^{-3}.d^{-1}$ and 0.41 $kg.m^{-3}.d^{-1}$. Therefore, an L_{vN} of 0.35 $kg.m^{-3}.d^{-1}$ is recommended to be used for the dimensioning of MLE systems (Bortoli, 2014; Vanotti et al., 2009; Park et al., 2004; Chung et al., 2004).

$$L_{vN} = \frac{[TAN]\frac{kg}{m^3} \cdot Q\frac{m^3}{d}}{V_{nitrifying\ reactor\ (m^3)}} \qquad \textit{Equation 20}$$

The same volume of the nitrifying reactor is usually used for the dimensioning of the denitrifying reactor, which can be dimensioned with a volume up to 20% smaller than that of the nitrifying reactor, with no efficiency loss (Park et al., 2004; Chung et al., 2004).

Finally, the calculation of the sludge settler (Equation 21) is performed using the hydraulic loading rate (HRR), which is based on the flow rate applied by the settler area. Conventionally, values between 1.5 $m^3.m^{-2}.h^{-1}$ and 4.33 $m^3.m^{-2}.h^{-1}$ are adopted. The sludge decanter volume is recommended to be between 5% and 10% of the nitrifying reactor volume as a safety parameter.

Considering that the system sludge is largely biomass and that its sedimentation is rapid, the sludge settler is established to not exceed three hours of HRT, when possible, to avoid sludge flotation and biomass loss (Wiesmann et al., 2007).

$$S_{settler} = \frac{Q_{feeding}}{SRR} \qquad \textit{Equation 21}$$

Case study 6 - Dimensioning of nitrifying/ denitrifying reactors

A piglet production unit with 4,800 sows has a digester for biogas generation and uses biofertilizer in arable areas belonging to partners close to the property. The need to treat the digestate for discharge into the receiving water body was highlighted in the new stage of environmental licensing. Considering that each sow produces an average of 32 L of manure per day and based on the digestate characteristics data presented below, determine the nitrifying reactor volume, the denitrifying reactor volume, and the dimensions of the sludge settler. Moreover, express the flow rates of feeding, recirculation from the nitrifying reactor to the denitrifying reactor, and sludge recirculation from the settler to the nitrifying reactor. Finally, determine the HRT of each reactor and sludge settler.

Digestate characteristics

Total ammoniacal nitrogen (TAN) ($mg.L^{-1}$) 2,200

Total organic carbon (TOC) $mg.L^{-1}$ 6,000

The calculation of volumes requires the establishment of some assumptions based on the literature.

1. The total recirculation ratio (RT) will be 5.0.
2. The nitrogen load should not exceed 0.35 $kg.m^{-3}.d^{-1}$.
3. The denitrifying reactor must be 20% smaller than the nitrifying reactor.
4. Surface runoff rate (TES) of 4 $m^3.m^{-2}.d^{-1}$, with a maximum HRT of 1 hour.

First, the manure flow rate is calculated.

$$Q = \text{No. of animals} \cdot \text{Manure production per animal}$$

$$Q = 4800 \cdot 32 \frac{L}{d}$$

$$Q = 153{,}600 \frac{L}{d} \frac{m^3}{1000L}$$

$$Q = 153.6 \frac{m^3}{d}$$

The C/N ratio in the digestate needs to be verified to assess the feasibility of using the nitrification and denitrification process.

$$\frac{C}{N}\text{ratio} \geq 2$$

$$\frac{C}{N}\ ratio = \frac{C_{TOC}}{C_{TAN}}$$

$$\frac{C}{N}\text{ratio} = \frac{Q.[TOC]}{Q.[TAN]}$$

$$\frac{C}{N}\ \text{ratio} = \frac{[TOC]}{[TAN]}$$

$$\frac{C}{N}\ \text{ratio} = \frac{6000\ \text{mg/L}}{2200\ \text{mg/L}}$$

$$\frac{C}{N}\text{ratio} = 2.72$$

There is the potential to use the coupled nitrification/denitrification process (MLE) because the C/N ratio is higher than 2.

Subsequently, Equation 20 is used to calculate the nitrifying reactor volume.

$$C_{vN} = \frac{[TAN]\frac{kg}{m^3}\ .\ Q\frac{m^3}{d}}{V_{\text{nitrifying reactor}}\ (m^3)}$$

Rearranging Equation 20, we have:

$$V_{\text{nitrifying reactor}} = \frac{[TAN]\frac{kg}{m^3}\ .\ Q\frac{m^3}{d}}{L_{vN}}$$

$$V_{\text{nitrifying reactor}} = \frac{2200\frac{kg}{m^3}\ .\ 153.6\frac{m^3}{d}}{0.35\ kg/m^3.d}$$

$$V_{\text{nitrifying reactor}} = \frac{2200\frac{kg}{m^3}\ .\ \frac{kg}{1000\ g}.\ 153.6\frac{m^3}{d}}{0.35\ kg/m^3.d}$$

$$V_{\text{nitrifying reactor}} = 965.5\ m^3$$

From the calculation of the nitrifying reactor volume and considering the established assumption, the denitrifying reactor volume will be 20% smaller.

Therefore:

$$V_{\text{denitrifying reactor}} = V_{\text{nitrifying reactor}} - (V_{\text{denitrifying reactor}} \,.\, 0.20)$$

$$V_{\text{denitrifying reactor}} = 965{,}5\ \text{m}^3 - (965.5\ \text{m}^3 . 0.20)$$

$$V_{\text{denitrifying reactor}} = 965.5\ \text{m}^3 - 193.1\ \text{m}^3$$

$$V_{\text{denitrifying reactor}} = 772.4\ \text{m}^3$$

Finally, the sludge settler volume of the system is calculated using the surface area value, according to Equation 21.

$$S_{\text{settler}} = \frac{Q_{\text{feeding}}}{\text{SRR}}$$

$$S_{\text{settler}} = \frac{153.5\ \text{m}^3/\text{d}}{4\ \text{m}^3/\text{m}^2.\text{d}}$$

$$S_{\text{settler}} = 38.4\ \text{m}^2$$

Setting the volume through the maximum HRT of three hours, the maximum volume will be:

$$V_{\text{settler}} = Q_{\text{feeding}} \,.\, \text{HRT}$$

$$V_{\text{settler}} = 153.5\ \frac{\text{m}^3}{\text{d}} . 3\ \text{h}$$

$$V_{\text{settler}} = 153.5\ \frac{\text{m}^3}{\text{d}} . 3\ \text{h} \,.\, \frac{d}{24\ \text{h}}$$

$$V_{\text{settler}} = 19.18\ \text{m}^3$$

Because the calculated settler volume value is lower than 5% of the nitrifying reactor volume, the highest value is adopted for safety reasons. Therefore, the sludge settler will have a volume of:

$$V_{dec} = V_{\text{nitrifying reactor}} . 5\%$$

$$V_{dec} = 965.5\ m^3.5\%$$

$$V_{dec} = 48.3\ m^3$$

Dividing the volume by the surface area, we have the height of the settling bed.

$$h = \frac{V_{dec}}{S_{dec}}$$

$$h = \frac{48.3\ m^3}{38.4\ m^2}$$

$$h = 1.26\ m \quad \rightarrow \quad h = 1.3\ m$$

Finally, the settler diameter is calculated from the surface area, considering a circular settler.

$$S_{dec} = \pi . r^2$$

$$r = \sqrt{\frac{S_{dec}}{\pi}}$$

$$r = \sqrt{\frac{38.4}{3.14}}$$

$$r = 3.5 \ \rightarrow \ D = 7.0\ m$$

Thus, from the volumes, the dimensioning of the finished system is shown in Table 4.

Table 4. Dimensioning of nitrifying/denitrifying reactors and sludge settler.

	Volume (m^3)	HRT (d)
Nitrifying reactor	965.5	6.3
Denitrifying reactor	772.4	5
Sludge settler	48.3	0.3

Nitrification and denitrification via nitrite

Unlike the conventional nitrification/denitrification process via nitrate, the nitrification/denitrification process via nitrite is mediated by the presence of NO_2-. This process is based on the fact that nitrite is the intermediate product of both the nitrification and denitrification process, thus being produced during nitrification and, subsequently, reduced to N_2 during the subsequent denitrification (Ciudad et al., 2005; Ruiz et al., 2006).

Figure 10 shows the paths of nitrification/denitrification via nitrate and denitrification via nitrite. The denitrification via nitrite reduces the ammonia oxidation pathway, making the oxidation of NO_2- to NO_3- unnecessary. An advantage is the 25% reduction in oxygen consumption in the aerobic phase, which implies a 60% energy saving in the entire process.

Moreover, the demand for electron donors, that is, organic carbon for denitrification, is 40% lower, and the denitrification rate via nitrite is 1.5 to 2 times higher than the denitrification rate via nitrate, which is technically feasible and economically favorable, especially when it comes to effluents with a high ammonia concentration or a low C/N ratio (Yang; Yang, 2011). Therefore, the ideal C/N ratio changes from 2 for the conventional denitrification to a C/N ratio of 1.2 for the denitrification via nitrite.

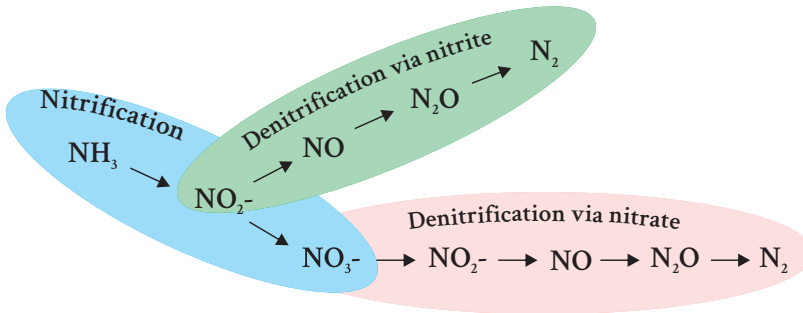


Illustration: Marcelo Bortoli

Figure 10. Representation of the of nitrification, denitrification via nitrite, and denitrification via nitrate paths.

Case study 7 - Calculation of the daily requirement of nitritation oxygen ($Rd_{O_2\text{-}NO_2^-}$) and C/N ratio for nitritation/denitritation

A biodigester generates 26 $m^3.h^{-1}$ of digestate, operating for eight hours a day. The digestate is sent to an aerobic reactor with the nitritation and denitrification process via nitrite. The total ammoniacal nitrogen concentration is 1,640 $mg.L^{-1}$ and the total organic carbon concentration (TOC) is 2,000 $mg.L^{-1}$. Calculate the daily oxygen requirement needed in the reactor for the oxidation of all nitrogen in the form of ammonium to nitrite to occur and calculate the C/N ratio, highlighting whether it would meet the denitrification process via nitrite.

Initially, the nitrogen daily load at the aerobic reactor inlet needs to be found. Therefore:

$$C = [TAN] \cdot Q$$

Where:

$$C = \text{nitrogen daily load } (\frac{kg}{d})$$

$$[TAN] = \text{total ammonia nitrogen concentration } (\frac{kg}{m^3})$$

$$Q = \text{flow rate } (\frac{m^3}{d})$$

The daily nitrogen load at the aerobic reactor inlet can be calculated using Equation 12.

$$C = [TAN] \cdot Q$$

$$C = 1640 \frac{mg}{L} \cdot \frac{g}{1000\ mg} \cdot 26\ \frac{m^3}{h} \cdot 8\ \frac{h}{d}$$

$$C = 1.64\ \frac{g}{L} \cdot 208\ \frac{m^3}{d}$$

Considering:

$$\frac{g}{L} = \frac{kg}{m^3}$$

$$C = 341.1\ \frac{KgN\text{-}NH_4^+}{d}$$

The nitrogen load value allows the calculation of the daily oxygen requirement ($Rd_{O_2\text{-}NO_2^-}$) in the aerobic reactor, using Equation 12.

$$Rd_{O_2\text{-}NO_2^-} = 341.2\ \frac{kgTAN}{d} \cdot \left[3.43\ \frac{gO_2}{gTAN}\right]$$

$$Rd_{O_2\text{-}NO_2^-} = 1170.3\ \frac{kgO_2}{d}$$

The C/N ratio can be calculated as follows:

$$\frac{C}{N}ratio = \frac{C_{TOC}}{C_{TAN}}$$

$$\frac{C}{N}ratio = \frac{Q[TOC]}{Q[TAN]}$$

$$\frac{C}{N}ratio = \frac{26\frac{m^3}{h} \cdot 8\frac{h}{d} \cdot 2000\frac{mg}{L}}{26\frac{m^3}{h} \cdot 8\frac{h}{d} \cdot 1640\ \frac{mg}{L}}$$

$$\frac{C}{N}ratio = 1.22$$

Therefore, the denitrification process via nitrite will not have its efficiency impaired since the C/N ratio is 1.22.

Recent technologies for biological nitrogen removal

Fundamentals and mechanisms

Digestates from efficient anaerobic digestion systems have been increasingly showing low carbon concentrations, resulting in an effluent with a low carbon/nitrogen (C/N) ratio. The trend in the absence of carbon makes it difficult to remove soluble nitrogen by the conventional nitrification/denitrification process detailed in the previous sections. It occurs because effluents that have a low C/N ratio may not have enough (and necessary) bioavailable organic carbon to carry out denitrification. In these cases, the addition of an external source of organic carbon is often necessary, which implies an increase in the operating cost of the conventional nitrification/denitrification process.

Effluents highly concentrated in nitrogen and little concentrated in carbon lead to difficulties in the dimensioning and operation of conventional systems, as seen in the previous sections. For this reason, new proposals have emerged to carry out this task. Recent studies on nitrogen removal aim to improve efficiency and reduce costs by optimizing available treatment strategies or implementing new processes and possibly new microorganisms capable of converting ammoniacal nitrogen into gaseous nitrogen, its inert form.

All these new processes seek to carry out the nitrogen elimination using nitrite as an electron acceptor and not nitrate, as there is a clear saving of oxygen for ammonium oxidation. The volumetric oxygen transfer coefficient (kLa) for different values of hydraulic retention time is approximately 25% lower for oxidation to nitrite than for nitrate, which results in energy savings in this process (De Prá et al., 2013).

Nitritation (or partial nitrification) is necessary to ensure nitrite availability, preventing the subsequent oxidation of nitrite to nitrate. According to Wiesmann et al. (2007), the ammonia oxidation reaction

is 3 to 3.8 times more thermodynamically favorable (240 KJ.mol^{-1} to 350 KJ.mol^{-1}) than nitrite oxidation (65 KJ.mol^{-1} to 90 KJ.mol^{-1}). Considering that the cell growth of the bacteria involved in this process is proportional to the energy released in their reaction, we can say that the growth of ammonia-oxidizing bacteria (AOB) is more favored than that of nitrite-oxidizing bacteria (NOB), which is advantageous when the objective is to accumulate nitrite in the reactor.

Some difficulties regarding the establishment of these processes are found due to the required control, especially when dealing with long periods of operation. Also, the steady-state phase is often difficult to achieve. Thus, some attention must be paid to most of these processes regarding the possible elimination of remaining nitrite into the environment due to its considerable toxicity.

The new proposals for processes for nitrogen removal via nitrite present in the literature will be mentioned with details below.

Partial nitritation process

The partial nitritation process is a technology based on the selection and favoring of ammonia-oxidizing bacteria (AOB), working as a pre-treatment capable of producing an effluent with ideal characteristics for feeding reactors with Anammox activity (as presented in the next section).

The strategy for the effectiveness of this process is to stop the oxidation of ammonia to nitrite (preventing oxidation to nitrate) and, at the same time, control the proportion of oxidized ammonia so that a portion of residual ammonia is maintained. In microbiological terms, it means disfavoring NOB activity, standing out bacteria of the genus *Nitrobacter*, allowing only AOB activity, standing out bacteria of the genus *Nitrosomonas* (De Prá et al., 2013; Yamamoto et al., 2006).

In summary, partial nitritation must limit the amount of ammonia oxidized by AOB activity, in addition to preventing the conversion of NO_2- to NO_3- by inhibiting NOB. Only 50% of the ammoniacal nitrogen should be oxidized to nitrite to make the stoichiometry according to the Anammox reaction, as described in Equation 22.

$$NH_4^+ + 0.75\ O_2 \Rightarrow 0.5\ NO_2^- + 0.5\ NH_4^+ + 2\ H^+ + H_2O$$

Equation 22

In this context, the effluent of this reactor, containing NH_4^+ and NO_2- without the complete oxidation of NH_{4+} to NO_2-, estimating a conversion of only 50%, would be suitable for feeding a subsequent reactor with Anammox activity to complete the intended degradation (Yamamoto et al., 2011).

However, some difficulties regarding the establishment of this process are found due to selectivity, especially related to long periods of operation. The physiological differences between AOB and NOB become extremely important in the process stability due to this condition. In this context, some operational strategies can be used to influence nitrite generation by favoring AOB due to the higher AOB sensitivity to certain environmental conditions (De Prá et al., 2013; Volcke et al., 2005).

The main alternatives to favor nitrite accumulation in biological systems are based on the proper regulation of control parameters such as dissolved oxygen (DO), aeration time, temperature, hydraulic retention time (HRT), solids retention time (SRT), pH, free ammonia (FA), free nitrous acid (FNA), and chemical inhibitors (Cui, 2012; De Prá, 2013). However, the economic feasibility of the process, in addition to its advantages and limitations, needs to be evaluated when using these strategies.

In this sense, the strategies for AOB selectivity cannot be generalized regarding nitrogen conversion using digestates, as the physicochemical characteristics of the effluent vary according to the production process and are dependent on its origin. Therefore, the process can be more or less efficient depending on the type of production.

Anammox process

Based on methodologies for identifying microorganisms and the type of metabolism developed by specific populations, the existence of a new segment of the nitrogen cycle was discovered in the 1990s, known

as anaerobic ammonium oxidation (Anammox) (Mulder et al. al., 1995). The anammox process evolved over the next few years from a largely unexplored part of the nitrogen biological cycle to become a key part of the overall nitrogen cycle. It is currently seen as a revolutionary technology for wastewater treatment (Scheeren et al., 2011).

The process involves an alternative route that consists of the anaerobic ammonium oxidation via specific microorganisms directly to N_2, using nitrite as an electron acceptor, with a small nitrate production. The free energy for this reaction is in the same order of magnitude as the free energy of the aerobic nitrification process, demonstrating that the anaerobic ammonium oxidation process is as favorable as the aerobic nitrification process. The stoichiometry of the anaerobic ammonium oxidation is presented in Equation 23 (Jetten et al., 2009).

$$NH_4^+ + 1.31\,NO_2^- + 0.066\,HCO_3^- + 0.13\,H^+ \Rightarrow$$
$$1.02\,N_2 + 0.26\,NO_3^- + 0.066\,CH_2O_{0.5}N_{0.15} + 2.03\,H_2O$$

Equation 23

Anaerobic chemolithoautotrophic microorganisms are responsible for these reactions, easily adhering to any solid surface, not existing uniformly within bioreactors (Isaka et al., 2006). Also, the culture of anammox microorganisms has excellent granulation properties, which allows the use of upflow reactor technologies to work with intense cell recycling, providing shorter reactor startup times (Kartal et al., 2011; Lotti et al., 2015).

Strous et al. (1998) combined the maximum bacterial activity and the substrate-to-biomass conversion factor to estimate the doubling time of bacteria with anammox activity between 9 and 11 days. The process produces a small volume of sludge due to this low growth rate, in addition to preserving approximately 60% of the oxygen used in the process, reducing treatment costs compared to the conventional nitrification/denitrification method (Ali et al., 2015; Jetten et al., 2001; Wang et al., 2016).

Several processes using bacteria with anammox activity have been implemented to optimize autotrophic nitrogen removal in wastewater since the discovery of anaerobic ammonia oxidation. Casagrande et al. (2013) observed high nitrogen removal loads (up to 20 $kgN.m^{-3}.d^{-1}$) when working with reactors with anammox activity, reaching values 66 times higher than the conventional process. These results demonstrate the potential efficiency that these processes can achieve and justify the worldwide trend of using these microorganisms in the treatment of effluents concentrated in nitrogen.

Like any biological process, bacteria with anammox activity can be inhibited under certain operating conditions or the presence/absence of any specific compound. In addition to oxygen, the process can be affected by pH, temperature, shear stress, and concentration of substrates and products. Therefore, the control and optimization of the process when applied is extremely important for the overall efficiency of the nutrient removal system.

Combined of deammonification process

The discussions presented in the previous sections allow for reflection on the combination of partial nitritation and anammox processes in terms of a new technology proposal for nitrogen removal. Deammonification is any technology that operates simultaneously with both partial nitrification and anammox processes (De Prá et al., 2012; Dosta et al., 2015; Gilbert et al., 2015; Magrí et al., 2012).

This technology appears as a promising alternative for eliminating high nitrogen loads in digestates and can be carried out using two or even a single reactor. As mentioned before, the bacteria responsible for the partial nitritation process are aerobic and, therefore, need oxygen during their metabolic activity.

On the contrary, anaerobic bacteria are responsible for the anammox process, with their activity stagnant when subjected to certain dissolved oxygen concentrations. These two processes are usually operated separately due to this condition, aiming at greater operational control and efficiency in nitrogen removal.

However, with the evolution and development of new technologies, recent studies have proposed that both bacteria can coexist in a single reactor as long as the system is maintained under limited dissolved oxygen conditions (Wett et al., 2007).

A representation of combined processes of deammonification is reproduced in Figure 11.

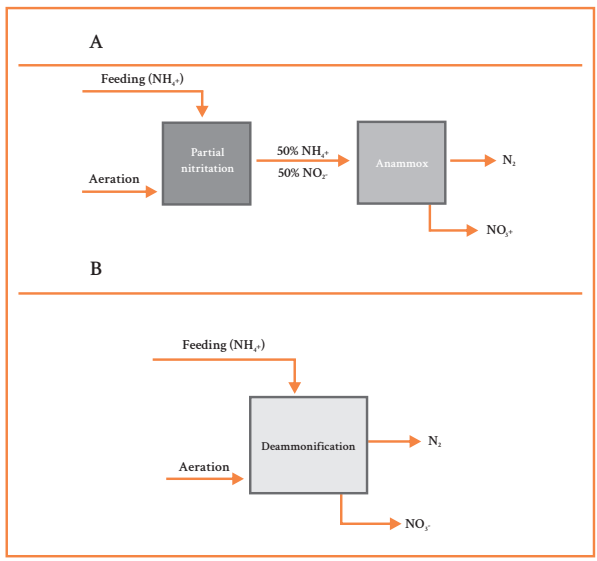


Figure 11. Representation of the partial nitritation + Anammox process using two (A) and a single (B) reactor for the operation of the deammonification technology.

In fact, it is easy to imagine a situation as shown in Figure 11A, where there is a first reactor operating under aerobic conditions, with only AOB activity, and a second reactor operating under anaerobiosis, with only anammox bacteria activity. However, this ammonia oxidation ratio in the first partial nitritation reactor can be difficult to maintain, leading to further problems in the anammox reactor (Cho et al., 2011). Thus, the overall nitrogen elimination efficiency in these systems is limited by the nitritation process of the first reactor. It occurs because there is a high requirement for operational oxygen control, in addition to ammonia oxidation, so that the partial nitritation process consumes all the dissolved oxygen from the liquid before entering the subsequent anammox reactor.

The overall nitrogen removal efficiency will be compromised if any imbalance occurs, and a higher oxygen concentration enters the anammox reactor. It demonstrates the importance of the operational process control to maintain the activity stability of ammonia-oxidizing bacteria not to reduce the nitrogen removal efficiency in the anammox process.

Although contradictory, the demand for operational control and technical demand decreases when we operate in a single reactor instead of two, as shown in Figure 11B. In this technology, aerobic ammonia-oxidizing bacteria are in symbiosis with the anammox anaerobic bacteria to form a single consortium for nitrogen elimination.

In terms of reaction, this process consists of the partial oxidation of ammonia to nitrite (by AOB activity) under limited oxygen conditions and, subsequently, the conversion of the nitrite produced together with part of the remaining ammonium to gaseous nitrogen (by the activity of anammox bacteria), forming a small amount of nitrate. The reaction combination of both processes results in the overall nitrogen removal reaction described in Equation 24.

$$NH_{4^+} + 0.85\ O_2$$

$$0.44\ N_2 + 0.11\ NO_{3^-} + 1.43\ H_2O + 1.14\ H^+$$

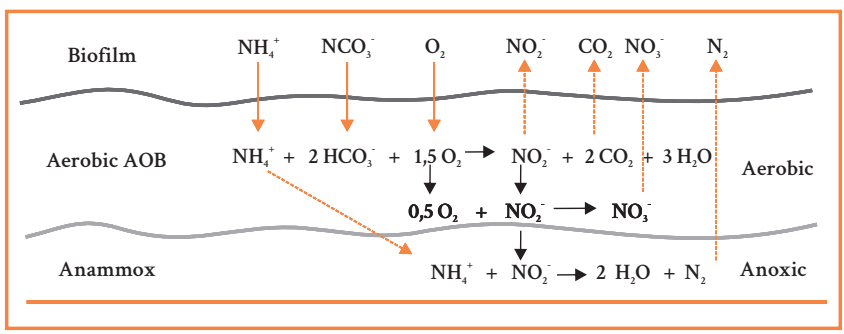
Equation 24

The Canon (completely autotrophic nitrogen removal over nitrite) process is a well-known single-step deammonification process initially proposed to operate sequencing batch reactors (SBR) at 35 °C (Figueroa et al., 2012; Third et al., 2001). However, new configurations have been proposed in recent years to perform nitrogen removal at lower operating temperatures (Chang et al., 2013; González-Martínez; Gonzalez-Lopez, 2016; Laureni et al., 2016; Veys et al., 2010), all aimed at reducing production costs and ease of operation to increase the process scale.

Reactor configuration

The deammonification process was originally proposed in sequencing batch reactors (SBR), but current technologies have evolved, and the proposal is also valid for continuous systems, with biofilm and airlift reactors (Egli et al., 2003; Leix et al., 2017; Reino et al., 2016).

Reactors in systems with biofilm eliminate nitrogen by forming a film, concentrating AOB in its external part and bacteria with anammox activity in its internal part. Thus, theoretically, the partial conversion of ammoniacal nitrogen to nitrite will occur on the biofilm surface and, subsequently, in its anoxic zone, with nitrite and the remaining residual ammonia being converted to N_2 by the anammox activity, as shown in Figure 12.



Source: Adapted from Zhu et al. 2008

Figure 12. Representation of the biochemical transformations of nitrogen in the deammonification process in biofilms.

In the same context as systems with biofilm, reactors that work with suspended biomass also have a concentration gradient of the substrate and DO, that is, the outside of the granule remains under aerobic conditions to perform the partial nitritation whereas the inside maintains anaerobic condition for anammox activity to occur (Figure 13).

This reactor configuration has been gaining prominence and preference for use due to its ability to reach higher N removal loads, related to the larger surface area for mass transfer. This condition opens the possibility for the deammonification application at low temperatures, without significantly losing efficiency in nitrogen removal compared to systems with biofilm at 30 °C -35 °C.

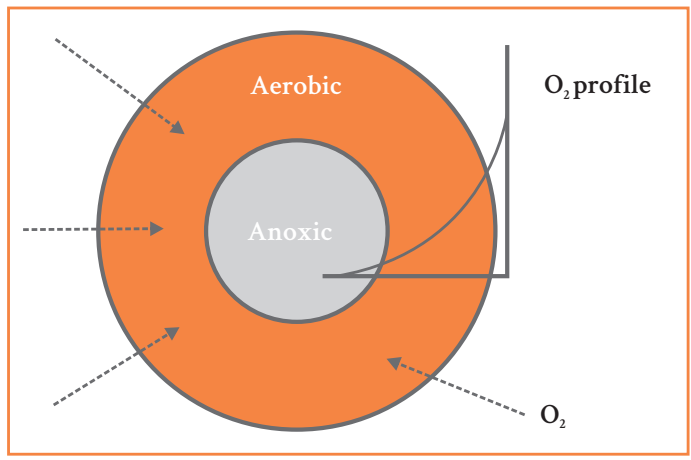


Figure 13. Representation of the oxygen concentration profile in a microbial flake.

Reactors that work with suspended biomass can be limited by resistance to mass transfer. Oxygen is the main factor for controlling the overall rate as the nitrite produced in the outer granule layer is consumed by bacteria found in the inner part, which can be attributed to diffusion in the granule or transfer of gas-liquid in the medium.

Control parameters

The control of the deammonification process follows almost entirely the parameters referring to the partial nitritation and anammox processes. The difference consists of the choice of the parameter used for selective inhibition of NOB and the effects it can cause to anammox bacteria. However, the deammonification process efficiency is usually directly related to three main factors: dissolved oxygen concentration, ammonia concentration, and control of the AOB population.

Dissolved oxygen is the electron acceptor in the partial nitritation process, being the main factor for controlling the global stoichiometry of the process, besides being directly related to the mass transfer and conversion of ammonia to nitrite. High dissolved oxygen concentrations can lead to the inhibition of the deammonification process for anammox bacteria (which are anaerobic) and AOB suppression, with excessive production of nitrite, which, in turn, is toxic for anammox activity depending on its concentration (De Prá et al., 2016).

Ammonia concentration is directly related to oxygen availability, but it can be critical for the process, as it serves as a substrate for both AOB and anammox. Thus, the process will substantially reduce efficiency if there is ammonia accumulation in the reactor or all ammonia is oxidized to nitrite due to the system imbalance. Many studies on the deammonification process have been conducted on a laboratory scale, but the applied volumetric load is lower than that applied to anammox due to operating conditions. However, significant savings have been observed as only one reactor is required, which can be advantageous depending on the effluent to be treated.

Regarding microbial populations, the interaction between aerobic and anaerobic bacteria present in the system plays an essential role in deammonification development. AOB require ammonia and oxygen as a source of substrate and electrons, while anammox bacteria require ammonia and nitrite. NOB require nitrite and oxygen and can interrupt the deammonification process if present in the medium due to competition for oxygen with AOB and nitrite with anammox bacteria. Therefore, maintaining the selectivity and interrelationship of microbial populations is essential in the deammonification process.

Usually, studies have been conducted mainly at high temperatures because temperatures above 25 °C favor anammox activity and act by expanding the differences between the growth rate of AOB compared to NOB. According to Veys et al. (2010), the ideal temperature for operating the deammonification process is 30 °C-35 °C, but recent studies have shown better advantages in operating reactors with lower applied loads but at room temperature (20 °C-25 °C) due to difficulties and energy costs (Chang et al., 2013; Cui, 2012; Wett et al., 2015).

This process has been a revolutionary technology for removing nitrogen. However, further investigations and research can contribute to this process to gain wide dissemination and become fully consolidated.

Trends and other processes in development

As already mentioned, the deammonification process has been one of the most innovative alternatives for the biological treatment of wastewater in recent years. An entirely new way of removing nitrogen was made available with its discovery in the 2000s. Many technologies have been developed and studied over the past few years for applicability in real effluents and several of them have already managed to transfer this technology to the full scale of operation.

Since the first full-scale anammox reactor for wastewater treatment in Dokhaven, Rotterdam, The Netherlands, set up and stabilized in 2002, there are 114 deammonification units reported around the world, including ten under construction and eight in the design phase (Lackner et al., 2014), and this number is increasing rapidly. Most plants (88 out of 114) were built in Europe, followed by China and North America, according to data from 2014.

The deammonification reactor capacity has been increasing rapidly despite the first anammox reactor was only 70 m^3 in volume (Lackner et al., 2014; Van Der Star et al., 2007). Full-scale plants with more than 142,000 m^3 of volume capacity are currently in operation, with the capacity to treat 134 tons of nitrogen load per day. Most of these full-scale treatment plants have been set up to treat municipal wastewater. However, they have not yet been applied on a full scale for the treatment of agro-industrial or agricultural effluents to date.

Initially, the plants have utilized the deammonification process at two stages and two reactors aiming at better operational control, using already consolidated partial nitritation systems. However, the focus has shifted mainly to single-stage deammonification with the setup experience and, since then, the trend towards single reactor setup has been increasing year after year. According to Lackner et al. (2014), approximately 88% of the full-scale plants are currently operated in a single-step deammonification configuration.

Several plants have implemented their own deammonification strategies, with differences mainly in the feeding cycle (intermittent vs. continuous), biomass disposal (suspended vs. fixed), and aeration con-

trol (intermittent vs. continuous). Another fact to be highlighted is that traditional technologies have also been modified and used efficiently for the application of the single-step deammonification process.

The deammonification process is likely to be implemented on a larger scale in the next few years due to cost savings, facility stability, and ease of control, associated with more stringent nitrogen removal requirements. In addition to application in municipal wastewater, industrial effluents have the potential for use.

Deammonification technologies will certainly be suitable for digestate within a technological package and aim to comply with current environmental legislation. Its application and demand for unit operations are directly related to the type of effluent and are paths to be scientifically explored to transform this technology into a reality on a large scale of operation in Brazil shortly.

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Chapter VII

DIGESTATE TREATMENT
Phosphorus removal

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Introduction

Phosphorus is one of the main constituents of fertilizers used in agriculture despite the environmental problems arising from its presence at high concentrations. Most of the used phosphorus comes from natural deposits found in phosphate rock mines. However, these deposits are located only in some regions of the planet. The demand for phosphate fertilizers in Brazil has increased significantly and currently, around 60% of the used inorganic P is imported due to the large increase in agricultural areas in recent decades (Associação Nacional para Difusão de Adubos, 2018).

Direct digestate application to the soil can be performed to take advantage of the fertilizer potential of this fraction, which is rich in nitrogen, phosphorus, and potassium. Bachmann et al. (2016) compared the use of digestate with untreated effluent and commercial fertilizer and found a higher P absorption by plants (corn, amaranth, and sorghum) from the digestate than from other fertilizers. However, the cost

involved with transporting the digestate to the farm makes its direct use difficult. Thus, the conversion of this nutrient into a solid form followed by separation from the digestate can be advantageous because it significantly reduces the volume to be transported. In this sense, phosphorus removal from the digestate for later use as a fertilizer, called second-generation fertilizer, is a sustainable solution (Withers et al., 2018).

The phosphorus removal processes can be classified into chemical, physical, and biological. The removed phosphorus is converted into a solid fraction in almost all processes, and the purity in P content depends on the process and the treated effluent composition.

The main processes used to remove phosphorus from digestate are summarized in Table 1, which compares their main characteristics.

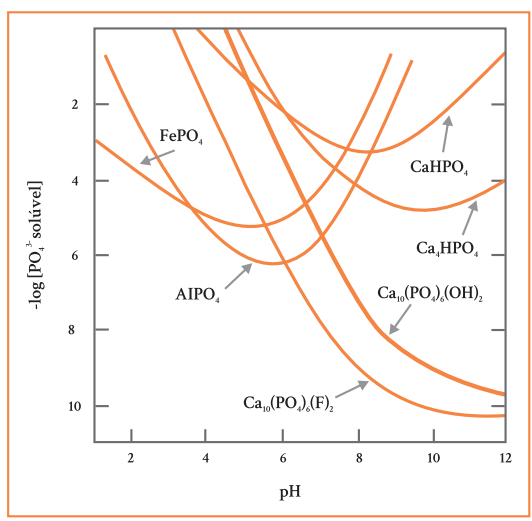
Table 1. Comparison of the main processes used to remove phosphorus from digestate.

Treatment	Optimal pH	Advantages	Disadvantages
Crystallization			
Quicklime	> 9.0	• Relatively cheap • Allows P recovery for use as fertilizer or in industry • Allows inactivation of pathogens (Viancelli et al., 2015)	• Requires high pH • Additional neutralization step may be required • Relatively high generated sludge volume
Struvite	7.5 – 9.0	• Simultaneous removal of N and P	• Mg addition is usually required
Fe	> 7.0	• Relatively cheap • Effective in P precipitation	• Precipitate unsuitable for use as a fertilizer
Al	> 6.3	• Effective in P precipitation • Biological process can be associated with Al precipitation at pH 6	• Expensive process • Precipitate unsuitable for use as a fertilizer
Physical processes			
		• Good P removal efficiency	• Use as little studied fertilizer • Expensive process
EBPR			
Enhanced Biological Phosphorus Removal		• Used associated with crystallization processes (struvite and quicklime) • Sustainable process • Requires little or no addition of chemicals	• Requires higher control of operating conditions compared to other processes • Complexity of facilities • Requires more physical space

Chemical processes are most suitable for phosphorus removal and recovery from the digestate due to their low cost, ease of setup and operation, and high efficiency (Peng et al., 2018). The most used consist of precipitation processes in the form of calcium phosphate and struvite. Some precipitation processes use iron and aluminum salts (Raptopoulou et al., 2016). Biological processes, also known as EBPR (Enhanced Biological Phosphorus Removal), are the most suitable for phosphorus recovery from the point of view of process sustainability, although the higher complexity and operational difficulties make their use difficult (Enhanced..., 2005). In general, physical processes present a good phosphorus removal efficiency although process costs are usually high.

Phosphorus removal by chemical processes

Most of the phosphorus present in the digestate is in the chemical form of soluble orthophosphate. Orthophosphates form insoluble compounds with metals such as calcium, magnesium, iron, and aluminum, allowing crystallization reactions to occur in water. These crystallization reactions are pH-dependent, as shown in Figure 1 (Stumm and Morgan, 1996). Thus, the pH adjustment of the process is essential to obtain higher P removal efficiencies.



Source: Stumm and Morgan (1996).

Figura 1. Diagram of the solubility of Fe, Al, and Ca phosphates as a function of pH.

The main crystallization processes used to remove phosphorus from digestate consist of the reaction with calcium at alkaline pH and formation of struvite, according to the following reactions:

$$5Ca^{2+} + 3HPO_4^{2-}OH^- \rightarrow Ca_5(PO_4)_3OH \quad \textit{Equation 1}$$

$$Ca^{2+} + HPO_4^{2-} \rightarrow CAHPO_4 \quad \textit{Equation 2}$$

$$3Ca^{2+} + 2PO_4^{3-} \rightarrow Ca_3(PO_4)_2 \quad \textit{Equation 3}$$

$$Mg^{2+} + H_nPO_4^{n-3} + NH_4^+ + 6H_2O \rightarrow MgNH_4PO_4.6H_2O + nH^+$$

Equation 4

Where:

n = 1, 2, 3, etc. corresponds to the solution pH

Phosphorus removal through precipitation with Ca

Phosphorus removal with calcium compounds is a relatively low-cost process that can be implemented without much difficulty using slaked lime as a calcium supply. In addition, the sludge generated has potential use as a fertilizer (Melia et al., 2017). In addition to removing P, the use of slaked lime also works to clarify the effluent if it has particulate material capable of coagulation/flocculation.

The treatment with slaked lime consists of adding a volume of $Ca(OH)_2$ solution necessary for P (orthophosphates) precipitation. According to Fernandes et al. (2012), the phosphorus removal efficiency is higher than 90% with the addition of $Ca(OH)_2$ solution up to pH 8.5 and higher than 96% at pH 9. In this study, the treatment with slaked lime was applied to the effluent that underwent an anaerobic digestion process in a UASB reactor, followed by treatment in a nitrification reactor. The treatment with the addition of slaked lime is advantageous, as it allows satisfactory phosphorus removal, and the pH of the final effluent does not need to be adjusted. This effluent has characteristics that enable its use for cleaning of facilities and irrigation (reuse water), for example.

Another advantage of using precipitation with slaked lime for phosphorus removal is the inactivation of pathogens, which occurs due to an increase in pH. This effect was demonstrated by Viancelli et al (2015), who observed total inactivation of *E. coli*, *Salmonella*, and P. *circovirus* type 2 at pH 10.

Factors that interfere with the phosphorus removal process with slaked lime

Considering that P is in the form of orthophosphate and according to the reaction below:

$$\mathbf{3Ca(OH)_2 + 2PO_4^{3-} \rightarrow Ca_3(PO_4)_{2(s)} + 6(OH)^-} \quad \textit{Equation 5}$$

Three moles of Ca^{2+} are required for two moles of PO_4^{3-}, that is, a Ca/P ratio of 1.5. The reaction of Ca with PO_4^{3-} can lead to the formation of several compounds, including hydroxyapatite [$Ca10(PO_4)_6(OH)_2$], tricalcium phosphate [$Ca_3(PO_4)_2$], octacalcium phosphate [$Ca_8(HPO_4)_2(PO_4)_4.5H_2O$], dicalcium phosphate [$CaHPO_4$], and calcium hydrogen phosphate dihydrate [$CaHPO_4.2H_2O$], in increasing order of solubility. In addition to the formation of these compounds with a crystalline structure, an amorphous compound known as amorphous calcium phosphate, with an approximate formula of $Ca_3(PO_4)_2.xH_2O$, similar to tricalcium phosphate, can be formed. The Ca/P molar ratio ranges from 1.3 to 2 for all these compounds. However, the digestate usually has high alkalinity mainly due to carbonate and bicarbonate ions. These ions also react with Ca^{2+}, as shown in the following reactions, causing a higher consumption of slaked lime than calculated by the Ca/P molar ratio:

$$\mathbf{CO_3^{2-} + Ca(OH)_2 \rightarrow CaCO_3 + 2(OH)^-} \quad \textit{Equation 6}$$

$$\mathbf{CaHCO_3^- + Ca(OH)_2 \rightarrow 2CaCO_3 + 2H_2O} \quad \textit{Equation 7}$$

The solubility product constant (k_{sp}) of the $CaCO_3$ precipitation reaction (k_{sp} = 3.36 x $10^{-9)}$ is higher than the ksp of the $Ca_3(PO_4)_2$ precipitation reaction (k_{sp} = 2.07 x 10^{-33}), that is, under this aspect, it could be assumed that the carbonate ion precipitation would only occur after the total precipitation of PO_4^{3-}. However, it is known that the reaction between $Ca(OH)_2$ and bicarbonate is complete above pH 9.5 while the reaction between $Ca(OH)_2$ and PO_4^{3-} starts above pH 7 and is very slow below pH 9. Therefore, the precipitation of carbonate and bicarbonate ions occurs concomitantly since the addition of quicklime up to a pH higher than 9 is necessary for treatments that use slaked lime to remove P.

The presence of ammonia, which also occurs in the digestate, is another factor that causes an increase in $Ca(OH)_2$ consumption. NH^{4+} ions react with $Ca(OH)_2$ according to the reaction:

$$\boldsymbol{Ca(OH)_2 + 2NH_4^+ \rightarrow 2NH_3 + Ca^{2+} + 2H_2O} \qquad \textit{Equation 8}$$

Thus, a larger amount of quicklime is needed to raise the pH until the complete precipitation reaction of orthophosphates (Szogi; Vanotti, 2009).

Furthermore, a high carbon concentration in the digestate causes co-precipitation of part of the organic matter, reducing the purity of sludge and, consequently, its added value for reuse as fertilizer or other products.

Considering the interferences of alkalinity, ammonia, and organic matter, the process of removing phosphorus from the digestate using precipitation with slaked lime must be implemented after an ammoniacal nitrogen removal process, especially if the alkalinity consumption also occurs in this process (Vanotti et al., 2003).

Phosphorus removal through struvite formation

Phosphorus precipitation in the form of struvite occurs when concentrations of the chemical species Mg^{2+}, NH^{4+}, and PO_4^{3-} exist in a 1:1:1 molar ratio and exceed the solubility product constant, respectively (Peng et al., 2018). Struvite crystallization has a low impurity con-

tent, which is important for phosphorus recovery and reuse (Zhou et al., 2015). This process occurs when the PO_4^{3-} concentration is between 100 $mg.L^{-1}$ and 200 $mg.L^{-1}$ and pH above 7.5. As can be observed, simultaneous phosphorus and ammonia removal occur in this case, which may be advantageous for some types of effluents.

$$Mg^{2+} + NH_4^+ + PO_4^{3-} + 6H_2O \rightarrow MgNH_4PO_4.H_2O_{(s)}$$

Equation 9

The Mg^{2+} concentration in most effluents is lower than necessary for struvite crystallization and, therefore, its addition is necessary, which is normally carried out by adding magnesium chloride.

Struvite can also precipitate undesirably in the lines that transport the digestate, which can lead to their obstruction, as shown in Figure 12 of Chapter III. To prevent this from happening, it is not recommended that the effluent remain stationary in the transport line. Washing it in an acidic medium (e.g., muriatic acid) will contribute to its solubilization and subsequent unblocking.

Phosphorus removal through crystallization with Fe and Al ions

Fe^{3+} and Al^{3+} ions react with phosphate to form $FePO_4$ and $AlPO_4$, as shown in the following equation:

$$X^{3+} + H_nPO_4^{3-} \rightarrow XPO_{4(s)} + nH^+$$

Equation 10

Where:

X = Al^{3+} or Fe^{3+}

Iron and aluminum compounds are widely used in wastewater treatment due to their properties as flocculants for particulate material removal by coagulation/flocculation. Regarding phosphates, the phase separation after the precipitation reaction is performed after floccula-

tion and sedimentation. The phosphorus removal efficiency obtained with the use of Fe or Al is considered high. Usually, iron or aluminum sulfate or chloride are used. Another advantage of using Fe and Al in phosphorus removal is the wide application range since precipitation occurs at any phosphorus concentration range, different from what occurs in the struvite formation process.

Fe^{3+} has been used due to its lower cost. The ideal pH for the use of Fe^{3+} ions is above 7. The colloidal characteristic of $FePO_4$ requires an excess of Fe^{3+} for the colloidal precipitate formation which, in turn, will aggregate other $FePO_4$ colloids and adsorb other chemical P species (Loehr et al., 1973).

Satisfactory results have been reported up to pH 7.5 when using Al^{3+} although the ideal pH for the $AlPO_4$ formation reaction is around 6.3, which in many cases avoids the need to adjust the pH of the digestate before the treatment.

The main disadvantage of phosphorus precipitation with Fe or Al is related to the possibility of phosphorus reuse. The formed compounds are not suitable for use as a fertilizer, as they prevent the solubilization of phosphorus in the soil, making it unavailable to plants. The use of tannin, a natural polymer, as a coagulation aid, can increase the sedimentation rate of precipitates and also enable the reuse of the generated sludge as fertilizer (Zhou et al., 2008).

Types of reactors used in chemical phosphorus removal processes

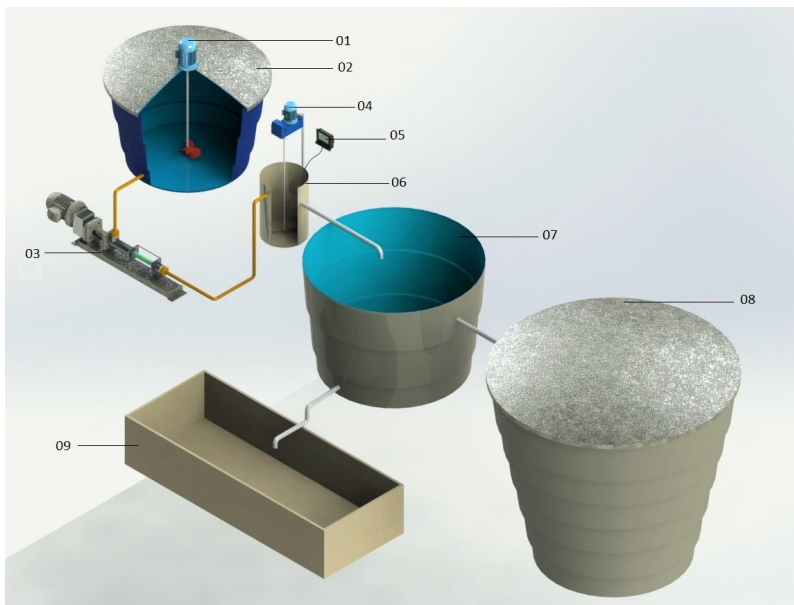
The crystallization reactions of orthophosphate with metal ions occur quickly and hence fast-mix, continuous, or batch reactors, equipped with a stirring system, are normally used.

The mixing time is greatly reduced depending on the reaction speed, but a complete homogenization needs to be ensured. Therefore, a hydraulic retention time of one minute is sufficient for the mixture between the effluent to be treated and the $Ca(OH)_2$ solution to be carried out.

The added reagent volume for crystallization with slaked lime can be determined by controlling the pH (Fernandes et al., 2012; Vanotti et al., 2009), that is, crystallization will occur keeping the pH above 9 and, consequently, precipitation.

Although pH is the main control parameter, Suzin (2016) demonstrated that the use of 7.3 $mL.L^{-1}$ of a 10% (m/v) $Ca(OH)_2$ solution in effluent from a nitrifying reactor with alkalinity below 300 $mg.L^{-1}$ of $CaCO_2$ allowed maintaining phosphorus removal above 90%. Therefore, the injection of 8 mL of solution per liter of effluent to be treated can be safely indicated for effluents with low alkalinity and ammonia.

The separation of phases (sludge and supernatant) can be conducted in a slow mixing unit or even a settler, which can be installed after the fast mixing unit, as shown in Figure 2. Studies have shown that three hours of hydraulic retention time at this stage are enough for phosphorus precipitation and elimination of several pathogens that may be present in the effluent (Viancelli et al., 2015; Suzin, 2016).



Source: Adapted from Suzin (2016).

Figure 2. Representation of a phosphorus removal system. 1 and 4: mechanical stirrer; 2: tank for preparation of $Ca(OH)_2$ solution; 3: $Ca(OH)_2$ metering pump; 5: pH controller; 6: quick mixing unit; 7: settler; 8: reuse water reservoir; 9: sludge drying bed.

Case study: dimensioning of a phosphorus removal system by chemical precipitation

A covered lagoon digester (CLD) is fed with a flow of 220 $m^3.d^{-1}$ and 8 m^3.sludge.d^{-1} is discarded. The digestate is treated by the nitrification/denitrification process, from which 6 m^3.sludge.d^{-1} is discarded. The effluent from the nitrogen removal process should be sent to a phosphorus removal system by chemical precipitation through the calcium hydroxide ($Ca(OH)_2$ addition. Considering that the CLD has a total phosphorus removal efficiency of 86% and nitrification/denitrification has a phosphorus removal efficiency of 75%, dimension a phosphorus removal system as shown in Figure 3. Assume that the total phosphorus concentration in the CLD feed is 1.33 $g.L^{-1}$ and the alkalinity and ammoniacal nitrogen concentrations at the outlet of the nitrification/denitrification system are negligible. Calculate the demand for $Ca(OH)_2$ (assuming 90% purity) in $kg.d^{-1}$ so that the phosphorus removal efficiency is 90% in the phosphorus removal module.

Initially, the concentration of total phosphorus (P) at the outlet of the nitrification/denitrification system needs to be determined. Therefore, the phosphorus concentration at the digester outlet is estimated considering that 86% of the P is naturally transferred to the CLD sludge.

$$[P]_{outlet\text{-}dig} = [P]_{inlet\text{-}dig} - ([P]_{inlet\text{-}dig} \cdot Ef_{P\ removal\text{-}dig})$$

$$[\mathrm{P}]_{\text{outlet-dig}} = 1.33\ \frac{\mathrm{g}}{\mathrm{L}} - \left(1.33\ \frac{\mathrm{g}}{\mathrm{L}}\ .\ 0.86\right)$$

$$[\mathrm{P}]_{\text{outlet-dig}} = 0.186\ \frac{\mathrm{g}}{\mathrm{L}} = 0.186\ \frac{\mathrm{kg}}{\mathrm{m}^3}$$

Equation 11

Considering that the total phosphorus concentration at the digester outlet is equal to the concentration on the inlet of the nitrification/denitrification system.

$$[P]_{outlet\text{-}dig} = [P]_{inlet\text{-}nit\text{-}denit} \qquad \textit{Equation 12}$$

$$[\mathrm{P}]_{\text{inlet-nit-denit}} = 0.186\ \frac{\mathrm{kg}}{\mathrm{m}^3}$$

The concentration at the outlet of the nitrification/denitrification system is calculated considering a total phosphorus removal efficiency of 75% in the nitrification/denitrification system.

$$[P]_{outlet\text{-}nit\text{-}denit} = [P]_{inlet\text{-}nit\text{-}denit} - ([P]_{inlet\text{-}nit\text{-}denit} \cdot Ef_{P\ removal\text{-}nit\text{-}denit})$$

Equation 13

$$[\mathrm{P}]_{\text{outlet-nit-denit}} = 0.186\ \frac{\mathrm{kg}}{\mathrm{m}^3} - \left(0.186\ \frac{\mathrm{kg}}{\mathrm{m}^3}.0.75\right)$$

$$[\mathrm{P}]_{\text{outlet-nit-denit}} = 0.0466\ \frac{\mathrm{kg}}{\mathrm{m}^3}.1000$$

$$[\mathrm{P}]_{\text{outlet-nit-denit}} = 46.6\ \frac{\mathrm{mg}}{\mathrm{L}}$$

The final concentration of total phosphorus can be estimated from the total phosphorus concentration at the outlet of the nitrification/denitrification system, based on the phosphorus removal efficiency of the removal system of 90%.

$$[\mathrm{P}]_{\text{final}} = 46.6\frac{\mathrm{mg}}{\mathrm{L}} - \left(46.6\ \frac{\mathrm{mg}}{\mathrm{L}}.0.90\right)$$

$$[\mathrm{P}]_{\text{final}} = 4.66\ \frac{\mathrm{mg}}{\mathrm{L}}$$

The calculation of the quick mixing unit and settler volumes is carried out by the outflow of the nitrification/denitrification system and HRT of one minute for the quick mixing unit and six hours for the settler. For this, the outflow of the nitrification/denitrification system needs to be known.

$$Q_{outlet\text{-}dig} = Q_{inlet\text{-}dig} - Q_{sludge\ disposal\text{-}dig}$$ *Equation 14*

$$Q_{outlet\text{-}dig} = 220\frac{m^3}{d} - 8\frac{m^3}{d}$$

$$Q_{outlet\text{-}dig} = 212\frac{m^3}{d}$$

$$Q_{inlet\text{-}nit\text{-}denit} = Q_{outlet\text{-}dig}$$

$$Q_{outlet\text{-}nit\text{-}denit} = Q_{inlet\text{-}nit\text{-}denit} - Q_{sludge\ disposal\ nit\text{-}denit}$$

$$Q_{outlet\text{-}nit\text{-}denit} = 212\frac{m^3}{d} - 6\frac{m^3}{d}$$

$$Q_{outlet\text{-}nit\text{-}denit} = 206\frac{m^3}{d}$$

$$Q_{outlet\text{-}nit\text{-}denit} = Q_{inlet\text{-}P\ removal}$$

$$Quick\ mixing\ unit\ volume\ (m^3) = Q_{inlet\text{-}P\ removal} \cdot HRT_{quick\ mixing}$$

Equation 15

$$\text{Quick mixing unit volume } (m^3) = 206\frac{m^3}{d} \cdot 1\ \text{min}$$

$$\text{Quick mixing unit volume } (m^3) = 206\frac{m^3}{d} \cdot \text{min} \cdot \frac{1\ d}{1440\ \text{min}} \cdot \frac{1000\ L}{m^3}$$

$$\text{Quick mixing unit volume } (m^3) = 143\ L$$

$$Settler\ volume\ (m^3) = Q_{inlet\text{-}P\ removal} \cdot HRT_{settler}$$

Equation 16

$$\text{Settler volume } (m^3) = 206\frac{m^3}{d} \cdot 6\ \text{h} \cdot \frac{1\ d}{24\ \text{h}}$$

$$\text{Settler volume } (m^3) = 51.5\ m^3$$

The dosage of slaked lime is a function of the stoichiometry of the reaction between Ca^{2+} and PO_4^{3-} ions to form $Ca_3(PO_4)_2$. Thus, 3 moles of Ca^{2+} are required to precipitate 2 moles of PO_4^{3-}. Thus, 3 moles of $Ca(OH)_2$ = 222 g and 2 moles of P = 62 g, considering the atomic masses of these chemical species and that we are working with P concentration and not with the orthophosphate ion. The $Ca(OH)_2$ dosage at 10% (m/v) (equivalent to 100 $g.L^{-1}$) can be calculated:

$$\boldsymbol{Ca(OH)_2\ dosage}\left(\frac{\boldsymbol{L}}{\boldsymbol{m^3}}\right) = \left(\frac{\dfrac{\boldsymbol{mass\ 3\ moles_{Ca(OH)_2}}\cdot\ [\boldsymbol{P_{effluent}}]}{\boldsymbol{mass\ 2\ moles_P}}}{\boldsymbol{100\ \frac{g}{L}}}\right).\boldsymbol{1000\ L}$$

Equation 17

$$\text{Ca(OH)}_2\text{ dosage}\left(\frac{\text{L}}{\text{m}^3}\right) = \left(\frac{\dfrac{222\text{ g}.\ 0.0466\text{ g}}{62\text{ g}}}{100\ \frac{\text{g}}{\text{L}}}\right).1000\text{ L}$$

$$\text{Ca(OH)}_2\text{ dosage}\left(\frac{\text{L}}{\text{m}^3}\right) = 1.7\ \frac{\text{L}}{\text{m}^3}$$

The quicklime dosage allows calculating the daily requirement for $Ca(OH)_2$, considering the $Ca(OH)_2$ purity of 90%.

$$\boldsymbol{Daily\ Ca(OH)_2\ requirement}\left(\frac{\boldsymbol{kg}}{\boldsymbol{d}}\right) = \frac{\boldsymbol{Q_{inlet\text{-}P\ removal}\cdot\ [solution]\ .\ dosage}}{\boldsymbol{purity}}$$

Equation 18

$$\text{Daily Ca(OH)}_2\text{ requirement}\left(\frac{\text{kg}}{\text{d}}\right) = \frac{206\frac{\text{m}^3}{\text{d}}\ .\ 10\%\ .\ 1.7\ \frac{\text{L}}{\text{m}^3}}{0.9}$$

$$\text{Daily Ca(OH)}_2\text{ requirement}\left(\frac{\text{kg}}{\text{d}}\right) = \frac{206\frac{\text{m}^3}{\text{d}}\ .\ 100\ \frac{\text{kg}}{\text{m}^3}\ .\ 1.7\frac{\text{L}}{\text{m}^3}\cdot\frac{\text{m}^3}{1000\text{ L}}}{0.9}$$

$$\text{Daily Ca(OH)}_2\text{ requirement}\left(\frac{\text{kg}}{\text{d}}\right) = 38.91\frac{\text{kg}}{\text{d}}$$

Phosphorus removal by physical processes

Some physical processes have been studied for phosphorus removal. The main ones consist of the use of membranes (Bolzonella et al., 2018), electrodialysis, and adsorption processes (Kunaschk et al., 2015). However, their high costs still limit the full-scale application despite the good efficiency in phosphorus removal.

Phosphorus capture and recovery with biochar have also been studied (Shepherd et al., 2017). In this case, phosphorus adsorption occurs through the interaction with Fe or Mg. There is the possibility of reusing the adsorbed material, but no information on the phosphorus bioavailability can be found in the literature.

Bolzonella et al. (2018) compared different nutrient recovery systems (P and N) used on a commercial scale in northern Italy. These systems combine physical and chemical processes: drying followed by acid recovery, stripping followed by acidic recovery, and membrane separation. The authors observed that the use of membranes allowed obtaining a high purity liquid effluent (reuse water) although it is the treatment with the highest cost among those studied. The digestate in the treatment with membranes and stripping initially undergoes a centrifugation process and most of the phosphorus remains in the solid fraction at this stage. The solid residue undergoes an acid treatment to recover nitrogen in the form of ammonium sulfate in the drying and stripping processes. Moreover, the digestate is subjected to a water evaporation process at drying.

Phosphorus removal by biological processes

Biological phosphorus removal is performed by microorganisms or some types of aquatic plants. The process known as EBP has been used to recover phosphorus, mainly in sanitary sewage effluents. This process consists of the intracellular bioaccumulation of polyphosphates by phosphate-accumulating organisms (assimilation and disassimilation processes) under aerobic and anaerobic conditions. In the anaerobic phase, bacteria assimilate volatile fatty acids, which are stored as polyhydroxyalkanoates and then metabolized in the aerobic phase to provide

the energy needed for the phosphate accumulation process. In addition to bacteria, microalgae have also been investigated for their phosphate assimilation capacity (Melia et al., 2017). The EBPR process has also been used to concentrate phosphorus in the sludge for further chemical treatment for struvite crystallization, in the case of effluents with lower phosphorus concentrations. P concentration must be between 100 and 200 $mg.L^{-1}$ for struvite crystallization.

Phosphate-accumulating microorganisms are heterotrophic and, therefore, a sufficient amount of bioavailable carbon is required for the operational success of a treatment system that uses the EBPR process. The availability of an electron acceptor, which can be nitrate or oxygen, is another determining factor for the process. Furthermore, the presence of cations, such as magnesium and potassium, is also important to facilitate phosphate assimilation and disassimilation by microorganisms. Phosphorus is released together with cations of magnesium and potassium in the anaerobic phase, while the assimilation of these chemical species occurs in the aerobic phase

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Embrapa

Swine & Poultry

The management and treatment of liquid effluents with high concentrations of organic matter and nutrients require a different approach due to their peculiarities, which are often not revealed in other effluents. The great technological challenge that is imposed is the combination of processes to mitigate environmental impacts and strategies to add value to these effluents. In this sense, this book is an intertwined approach to processes and practices involving anaerobic digestion, biogas purification, and agricultural use of digestate. Nitrogen and phosphorus removal is presented and discussed in detail considering the specificities of its treatment for situations in which the agronomic valuation of the digestate is not possible. This book brings together the knowledge accumulated over more than a decade by Embrapa and its network of partners in the development of practices and processes adaptable to tropical conditions.

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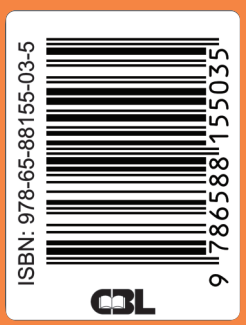

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