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Wastewater characterization and monitoring and troubleshooting of an IASB reactor at a Fish-Canning Plant

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Summary

Fish-canning wastewaters have high lipid content. Lipids are ideal substrates for methane production, since theoretically their degradation produces more biogas with higher methane content, when compared with proteins or carbohydrates. Although, treatment of fish-canning wastewaters is particularly difficult due its high content of soluble organic matter and salts. In addition, they also contain a significant amount of oil and grease and have differing concentrations of them depending on the production process and on the type of raw material processed. This thesis reports on wastewater characterization and monitoring and troubleshooting of an *Inverted Anaerobic Sludge Blanket reactor* at *Poveira* fish-canning plant.

The anaerobic treatment system implemented at *Poveira* has three stages: equalization, anaerobic digestion in IASB reactor and biogas utilization.

It was verified that cleaning step has the biggest contribution (45%) of the total wastewater flowrate produced. The design flow of 40 m^3 for the IASB was exceeded for most of the monitoring time. Strong variations in flow might have adversely affected the stability and efficiency of the process. Therefore, a water reduction plan was implemented in order to reduce consumption and normalize flow to the bioreactor.

Homogenization of the influent was not being guaranteed in equalization step by the presence of a solidified layer of fat which occupied 50% of the volume of the equalization tank. Thus, three cleanup actions were taken in order to remove the layer of solidified fat.

The maximum amount of COD removal efficiency was 70% during monitoring period, however, the COD removal efficiency was below 50% for most of the time. The estimated production of biogas was determined to be 64 m^3 per day which could theoretically allow the biogas boiler to operate for approximately one hour per day. The stability of *Poveira´s* anaerobic wastewater treatment process was verified by the stability of pH inside IASB reactor, decreased organic loading rate, increased retention of biomass and subsequent decrease in effluent total suspended solids and influent homogenization composition. In order to achieve a better performance in the anaerobic process wastewater treatment, stable

conditions must be reached. These conditions can be achieved through implementation of the maintenance and analytical plans developed in this thesis.

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Ac – Acetate ion

AD – Anaerobic Digestion

BOD – Biological Oxygen Demand

CDM – Clean Development Mechanism

Cl^- – Chloride
CO_2 – Carbon dioxide

-CH2- – Methyl group

-COOH- – Carboxylic group

COD – Chemical Oxygen Demand

CH_4 – Methane

$Ca(OH)_2$ – Hydrated lime
CaO – Quicklime
COD_{CH_4} – Load of COD removed and converted into methane

ELVs – Emission Limit Values

F/M – Food to Microorganism ratio
FA – Fatty Acid
FOG – Fat, Oil & Grease
H_2 – Hydrogen ion

HAc – Nonionized acetic acid

HRT – Hydraulic Retention Time
HS^- – Hydrogen sulfide
IASB – Inverted Anaerobic Sludge Blanket

$K\ (t)$ – Correction factor for temperature

K – COD corresponding to one mole of CH_4

LCFA – Long Chain Fatty Acid

Na+ – Sodium ion
Na_2CO_3 – Sodium carbonate
$NAHCO_3$ – Sodium bicarbonate
NaOH – Sodium hydroxide
NH_4HCO_3 – Ammonia bicarbonate
NaCl – Sodim Chloride
NH_3 – Free ammonia
NH_4^+ – Ammonium ion
N – Nitrogen
OLR – Organic Loading Rate
$\bar{p}_{CO2}$ – Partial pressure of carbon dioxide
PMP – Potential Methane Production
P – Phosphorus
P – Atmospheric pressure
Q - Flowrate
R – Gas constant
S_{Balk} – Bicarbonate alkalinity
S_{Talk} – Total alkalinity
S_{VFA} – Concentration of VFA
SAO – Syntrophic Acetate Oxidation
SAOB – Syntrophic Acetate Oxidising Bacteria
SMA – Specific Methanogenic Activity
S^{2-} – Sulfide ion
SO_4^{2-} – Sulfate ion
SRT – Solids Retention Time
TSS – Total Suspended Solids
T – Temperature
UASB – Upflow Anaerobic Sludge Blanket
V - Volume
VFA – Volatile Fatty Acid
VSS – Volatile Suspended Solids

VHL – Volumetric Hydraulic Load

V_{CH_4} – Volume of methane produced

X_{VSS} – Concentration of Volatile Suspended Solids

$Y_{acidogenic}$ – Yield coefficient for acidogenic microrganisms

$Y_{methanogenic}$ – Yield coefficient for methanogenic microrganisms

1. Introduction

1.1. Fish canning wastewater valorization

The fishing industry is of particular importance to Portugal as a country of great maritime tradition. When the fishes are caught in numbers, greater than the amount of consumption, their preservation becomes a necessity for their future use. Industrial manipulation had to occur in a short period of time in order to preserve fish quality and freshness due to the perishable character of the fish (Mata, 2009). The common processes in fish processing plants are filleting, freezing, drying, fermenting, canning and smoking (Palenzuela-Rollon, et al., 2002). Canning fish is a method of preservation in which the fish is sealed hermetically in a container and then sterilized and cooled to ambient temperature for subsequent storage.

The canning sector is generally seen as the main segment of the fishing manufacturing industry at national level. Canning fish represents a source of relatively inexpensive, nutritious and healthy food which is stable at ambient temperatures for long periods (Bratt, 2010).

There was 20 plants in operation producing 58,500 tons of canned fish in the year 2010 and seven of them are located north of Douro River (Castro and Melo, 2010) (Boaventura, et al., 2012). The production of canned fish, by species and in percentage is 48% sardines, mackerel, 27%, 24% tuna and 1% for the another ones.

This sector generates large quantities of wastewater due the consume of water in operations such as cleaning, washing, cooling, thawing, ice removal, etc. (Gaiker, Technological Center, 2005).

The treatment of fish-canning wastewaters and satisfy the emission limit values (ELVs) for industrial wastewaters (Decree-Law No. 236/98) is particularly difficult due its high content of soluble organic matter and salts, the significant amount of oil and grease they present and differing concentrations of them depending on the production process and on the type of raw material processed (Aurrekoetxea, et al., 2002).

Poveira is one of the oldest canned fish company in Portugal, working since 1938, located in Póvoa do Varzim, a small city by the sea in the North of the country.

Since 1938 that canned fish from *A Poveira* is in the most demanding markets, and their brands are recognized for quality, excellence and traditional manufacturing.

Poveira owns one of the best industrial plant in canning sector in Portugal (Figure 1), a brand new industrial complex that started its activity in January 2013.

Figure 1: The new fish-canning industrial plant for *Poveira*.

Poveira produces canned tuna, mackerel, codfish, sardine and anchovy. According to the type of canned, wastewater is produced at different stages of the process, showed in Table 1:

Table 1: Sources of wastewater production according to the type of canned fish processed.

Type of canned fish	Sources of wastewater production
Tuna	Baking Washing Sterilization
Mackerel **Codfish**	Thawing Brine Soaking

Sardine	Baking Washing Sterilization
Anchovy	Washing

These effluents are often subjected to pre-treatment before discharge to the sewage system for further treatment at an urban wastewater treatment plant, such the pre-treatment at *Poveira*. The effluent generated in fish canning factory *Poveira* is further treated at municipal wastewater treatment plant of Laúndos

Lipids are glycerol bonded to long chain fatty acids (LCFA), alcohols or other hydrophobic molecules by an ester or ether linkage. Fats and oils are a subgroup of lipids that have the alcohol groups esterified with fatty acids, predominantly in the form of triglycerides (glycerol backbone with three LCFA) (Alves, et al., 2009). Palmitic acid (hexadecanoic acid) and oleic acid (cis-9-octadecenoic acid) are, respectively, the most abundant saturated and unsaturated LCFA present in wastewater. Figure 2 shows a chemical structure representation of palmitic acid (trivial name) also called hexadecanoic acid (systematic name). The black balls represent carbon, the white balls represent hydrogen, and the red balls at the end represent oxygen.

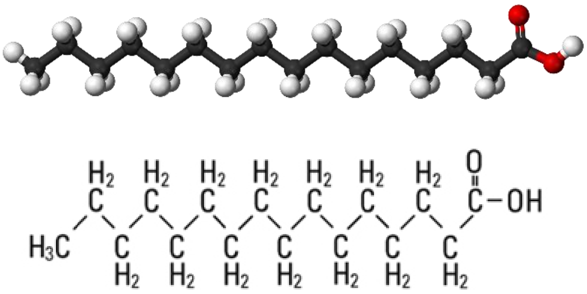

Figure 2: Palmitic acid chemical structure representation.

Lipids are amphipathic, that is, show properties of hydrophobicity and hydrophilicity (Madigan, 2003). LCFA are aliphitic chains of mainly methyl groups (-CH2-) with a carboxylic group (-COOH-) at one end of the chain. As such, they have a hydrophobic tail connected to a hydrophilic head. Sousa (2006) reported that lipid hydrolysis does not reduce the chemical oxygen demand (COD) but transfer over 90% of the COD to LCFA, for this reason lipids are ideal substrates for methane production, since theoretically their degradation produces more biogas with higher methane content, when compared with proteins or carbohydrates as shown in Table 2.

Table 2: Potential biogas production in different classes of substrates (Alves, et al., 2009).

Component	Methanogenic reaction	Biogas (L/g)	CH_4 (%)
Lipids	$C_{50}H_{90}O_6 + 24.5H_2O \rightarrow 34.75CH_4 + 15.25CO_2$	1.425	69.5
Carbohydrates	$C_6H_{10}O_5 + H_2O \rightarrow 3CH_4 + 3CO_2$	0.830	50.5
Proteins	$C_{16}H_{24}O_5N_4 + 14.5H_2O \rightarrow 8.25CH_4 + 3.75CO_2 + 4NH_4^+ + 4HCO_3^-$	0.921	68.8

Anaerobic digestion of lipids have a theoretical yield of 1.430 L biogas/g compared to 0.830 L biogas/g for proteins and 0.921 mL biogas/g for carbohydrates (Alves, et al., 2009). Theoretically lipids degradation produces more biogas with higher methane content, when compared with proteins or carbohydrates as shown in Table 2.

The net energy production increases significantly if a fraction of waste lipids is mixed in the feedstock due in part to the lower (more negative) mean oxidation state of carbon in lipids as compared to carbohydrates and proteins (Alves, et al., 2009) (Gujer, et al., 1983).

However, without a proper feeding strategy, addition of waste lipids to an AD plant is risky, if accumulation of long-chain fatty acids (LCFA) is not prevented biomass washout by flotation, granule instability and toxicity of microorganisms could occur (Alves, et al., 2009). Further delails of problems in anaerobic treatment of lipid/LCFA based wastewaters will be dealt in section 1.4.3.

1.2. IASB reactor technology

The IASB (Inverted Anaerobic Sludge Blanket) reactor is a high-rate anaerobic reactor able to treat directly industrial effluents with high lipid rate. The typical performance of high rate anaerobic processes is presented in Table 3.

Table 3: Typical high-rate anaerobic process performance *(Hall, 1992)*.

Parameter	Value
BOD_5 removal,	80 to 90%
COD removal, mass	1.5×BOD_5 removed
Biogas production	0.5 m^3/ kg COD removed
Methane production	0.35 m^3/ kg COD removed
Biomass production	0.05-0.10 g VSS/g COD removed

The IASB reactor incorporates two distinct separation mechanisms: floatation and settling.

Floatation is used to retain suspended solids with a lower density than water, while sedimentation is used to retain settling solids. Reactor influent is continuously mixed with the settled sludge recycle and then injected into reactor downers to induce circular mixing in the top section of the reactor. Further mixing is provided by the biogas produced: it enhances the mixing around the downers through gas lift action. In this way, there is no need for the application of a biogas compressor or blower to mix reactor contents. Fat adheres to the sludge, resulting in a floating layer of sludge, where fat is degraded and biogas is produced. Eventually sludge starts settling again and accumulates at the bottom of the reactor. Treated wastewater leaves the reactor through an inclined plate settler. Because LCFA is present, biogas production can result in foaming. To counteract foam formation,the reactor is equipped with treated wastewater sprays in the top of the reactor. Figure 3 gives a schematic representation of an IASB reactor.

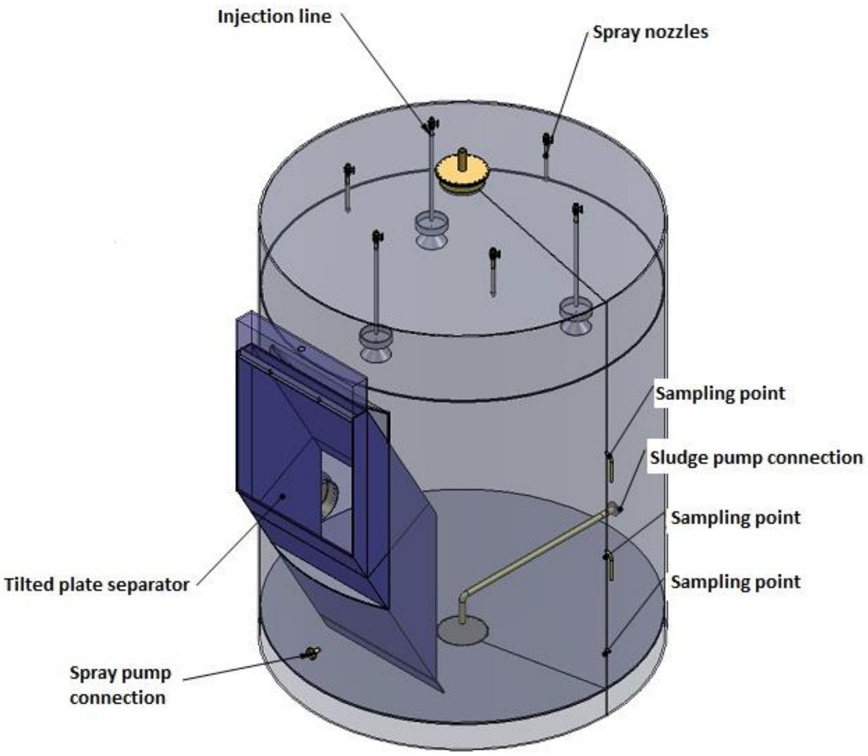

Figure 3: Schematic representation of an IASB reactor (Ambysis).

The IASB technology compared to conventional anaerobic treatment systems have the following advantages:

- Utilizes sludge floatation to maintain the sludge bed;
- no need for fat separation prior to anaerobic treatment, omitting associated investment costs;
- No fatty waste stream production and therefore no costs associated to disposal;
- Maximisation of renewable energy production as biogas due to direct conversion of fat into biogas and reduction of carbon dioxide emissions;
- No mechanical parts included in the reactor, reducing maintenance cost;
- Modular design for easy replication;
- The reduction of operational costs, the increase of market competitiveness of fish, dairy and meat processing industries and generating business

opportunities for companies (Sellers and Project Suppliers) and stimulating local economy are economic advantages of the present technology.

1.3. Anaerobic degradation

Anaerobic digestion (AD) of complex organic compounds is based on the biological process that occurs in absence of light or inorganic electron acceptors (oxygen, nitrate, sulfate, sulfur or oxidized metal ions such iron and manganese) which converts organic matter to the most oxidized and reduced forms of monoatomic carbon (carbon-dioxide and methane respectively) through four main steps, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis (Batstone, 2014). This stages are presented below in the Figure 4.

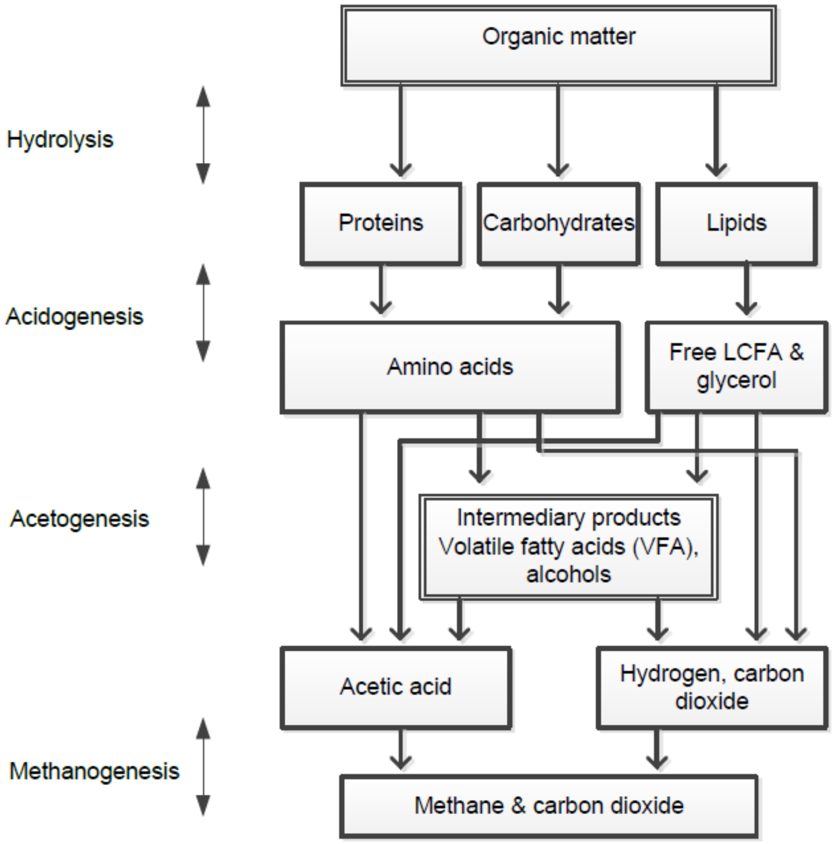

Figure 4: Schematic representation of anaerobic digestion process (Adapted from Caruana (2012))

The whole process is carried out at least by four physiologically different microbial groups: primary fermenting bacteria, secondary fermenting bacteria, hydrogenotrophic methanogens, and aceticlastic methanogens (Braguglia, 2009) (Hattori, 2008).

The primary fermenting bacteria hydrolyse complex organic compounds to basic components such proteins to amino acids, fats to glycerol and long-chain fatty acids (LCFA), and polysaccharides to mono or disaccharides through the action of extracellular hydrolytic enzymes (Gray, 2004). This microbial group further ferments monomers to reduced compounds (alcohols, short-chain fatty acids, organic acids, and certain aromatics), hydrogen and CO_2 - acidogenesis process (Gray, 2004). The secondary fermenting bacteria or obligate proton reducers oxidize the reduced products to acetate, hydrogen and CO_2 - acetogenesis process. LCFA are converted to acetate and hydrogen through β-oxidation mechanism.

Interspecies hydrogen transfer between the secondary fermenting bacteria and the hydrogenotrophic methanogens is considered to be essential for the oxidation of the compounds produced in the acidogenesis phase because these reactions proceed if hydrogen partial pressures are kept low and this is achieved in the symbiotic relationship with hydrogenotrophic methanogens that utilizes hydrogen in the production of methane. In general, degradation of fatty acids to acetate and hydrogen or, in the case of propionate, to acetate, hydrogen, and CO_2 is far more endergonic under standard conditions than is the ethanol oxidation. Consequently, for fatty acid degradation, the hydrogen partial pressure has to be decreased to substantially lower values (<10 Pa) than with ethanol (<100 Pa) (Schink, 1997).

Methanogenesis is the final stage of the anaerobic digestion process where hydrogen and CO_2 are converted to methane by the hydrogenotrophic methanogens whereas acetate degradation can proceed through two different pathways: direct cleavage of acetate by aceticlastic methanogens or syntrophic acetate oxidation (SAO) (Koch, et al., 1984). SAO is a two-step reaction in which acetate is primarily oxidised to H_2 and CO_2 by syntrophic acetate oxidising bacteria (SAOB), followed by hydrogenotrophic methanogens that uses hydrogen as the electron donor and CO_2 as the electron acceptor to produce methane (Etienne, 2012).

Methane formation via syntrophic acetate oxidation pathways is thermodynamically favorable only at elevated temperature and/or high acetate concentrations (Koch, et al., 1984) (Petersen, et al., 1991).

The end-products of complete anaerobic digestion process are methane, carbon dioxide and anaerobic biomass.

The advantages of anaerobic treatment process in comparison to aerobic process are the less energy requirements, less biological sludge production, fewer nutrients requirements higher volumetric loadings (Srinivas, 2008), production of methane with high calorific value and the microorganisms can remain dormant for several months and become operational within a week of start-up (Jogdand, 2010).

However, the chemistry and the microbiology of the anaerobic processes are more complex than the aerobic ones (Braguglia, 2009). Anaerobic treatment process have disadvantages in comparison to aerobic process because anaerobiosis is relatively a slow process compared to aerobiosis (Srinivas, 2008), the anaerobes microorganisms are more sensitive to toxic compounds and operating parameters than aerobes (Braguglia, 2009). The products of biological decomposition are more odourous, corrosive and less stable (Srinivas, 2008), anaerobiosis operates in a narrow pH range and can not tolerate higher acidity and addition of alkalinity is needed (Srinivas, 2008).

1.4. Affecting parameters

1.4.1. pH, alkalinity and volatile acids

Based on the sequence of anaerobic reaction, microbial species involved can be roughly divided into the following three categories: (a) bacteria responsible for hydrolysis; (b) acidogenic bacteria; and (c) methanogens.

The acidogenic bacteria tolerate a low pH and have an optimal pH of 5.0 to 6.0; however, most methanogens can function optimally in specific optimal pH range of 6.8 to 7.4 (Bitton, 1999)(Ahn, et al., 2002). This explains why pH is more inhibitory to methane-producing bacteria than to acidogenic bacteria.

A decrease in pH increases the production of VFAs, particularly propionic and butyric acid, at the expense of acetic acid. If the increased VFA production rate exceeds the maximum capacity of the methanogens to use acetic acid and H_2, excess of VFAs will begin to accumulate, decreasing the pH and the enzime activity of methanogens is reduced to a low level that lead with the accumulation of VFAs and a further decrease in the pH. If this situation is left uncorrected, the result is a precipitous decrease in the pH, the accumulation of higher molecular weight VFAs, and a near cessation of methanogenic activity. Under normal operation conditions, the pH reduction caused by acidogenic bacteria can be buffered by bicarbonate produced by the methanogens. For an anaerobic process functioning within the acceptable pH range, the pH is controlled primarily by the bicarbonate buffering system. The concentration of bicarbonate alkalinity in solution is related to the carbon dioxide content of the gas space in the reactor and the reactor pH.

$$S_{Balk} = 6.3 \times 10^4 \times \left(\frac{\bar{p}_{CO2}}{10^{pH}}\right)$$

Where S_{Balk} is the bicarbonate alkalinity expressed as mg/L as $CaCO_3$ and $\bar{p}_{CO2}$ is the partial pressure of carbon dioxide in the gas space expressed in atmospheres. Figure 5 presents that relationship and illustrates that typical anaerobic processes operate with bicarbonate alkalinities in the range of 1,000 to 5,000 mg/L as $CaCO_3$ and carbon dioxide partial pressures of 25-45%.

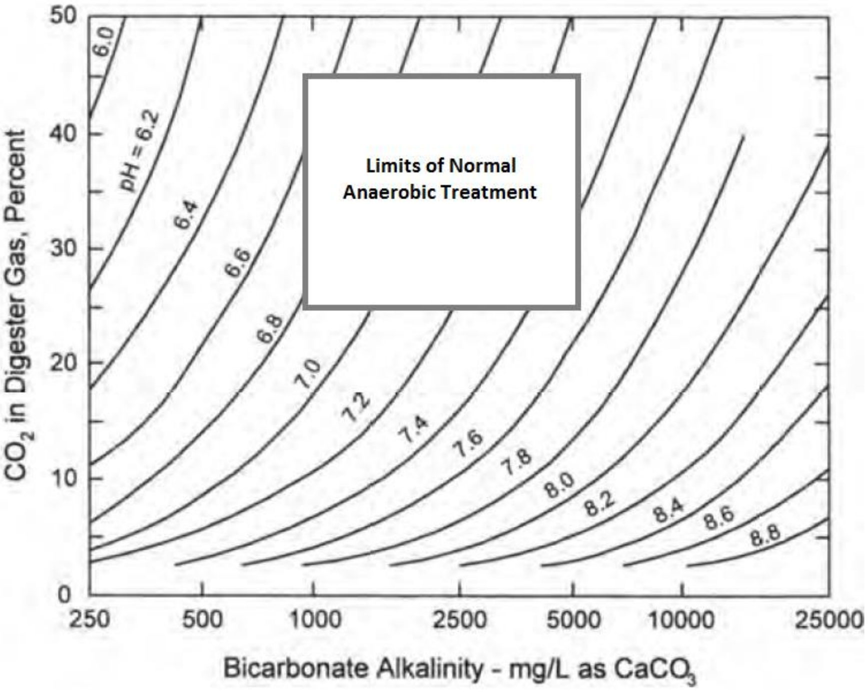

Figure 5: Effect of pH on the relationship between the bicarbonate alkalinity of the liquid phase and the carbon dioxide content of the gas phase in an anaerobic process *(Parkin, et al., 1986)*.

When VFAs begin to accumulate in an anaerobic process, they are neutralized by the bicarbonate alkalinity present. For example, acetic acid is released by acidogenic bacteria in nonionized form, but exists as acetate ion at neutral pH. The reaction of acetic acid with bicarbonate alkalinity to convert to acetate is:

$$HCO_3 + HAc \leftrightarrow H_2O + CO_2 + Ac$$

Where HAc represents nonionized acetic acid and Ac represents acetate ion. When a pH end point of 4.0 is used in the alkalinity analysis, acetate will be partially converted to acetic acid and will, therefore, register as alkalinity. Thus, if VFAs are present, the total alkalinity will represent the concentration of both bicarbonate ion and VFAs. If the concentration of VFAs is known and is expressed as acetic acid, the bicarbonate alkalinity can be calculated from the total alkalinity as:

$$S_{BAlk} = S_{Talk} - 0.71 \times (S_{VFA})$$

Where S_{Talk} is the total alkalinity expressed as $CaCO_3$ and S_{VFA} is the concentration of VFAs expressed as acetic acid.

Several chemical products can be used to control the pH of anaerobic processes, including hydrated lime ($Ca(OH)_2$), quicklime (CaO), sodium carbonate (Na_2CO_3), sodium bicarbonate ($NaHCO_3$), sodium hydroxide ($NaOH$) and ammonia bicarbonate (NH_4HCO_3).

Sodium bicarbonate is preferred for pH adjustment because its impact is longer lasting and its toxicity potential is low. It adjusts the pH by direct addition of bicarbonate ions which will result in a direct increase in the pH without affecting the carbon dioxide content of the gas space. However, the cost of the product is very high.

1.4.2. Temperature

Temperature has a significant effect on the performance of anaerobic processes. The control of a stable operating temperature is important, since the adaptation of microbes to temperature fluctuation is slow. Daily variations in temperature should not be allowed, with the change limited to ± 1 °C (Garber, 1982).

The relationship between ammonia and temperature was examined in anaerobic digestion process by Angelidaki and Ahring (1994) since ammonia is beneficial to the growth of anaerobic bacteria within the stimulatory range between 50 to 200 mg/L. More free ammonia is produced with increased temperature and aggravates the ammonia inhibition, thus this problem can be resolved by decreasing the temperature or limiting the amount of ammonia by decreasing the loading.

1.4.3. Long-Chain Fatty acids

LCFAs, the primary component of fat, oil & grease (FOG), are degraded anaerobically via the β-oxidation pathway to acetate and H_2, which are subsequently converted to methane. β-oxidation begins when the fatty acid is activated with coenzyme A and the resulting oxidation leads to the release of acetyl-CoA and the formation of a fatty acid chain, which is shortened by two carbons. Acetyl-CoA is oxidized by way of the citric acid cycle and the process of β-oxidation is repeated (Madigan, 2003).

Saturated fatty acids follow the traditional β-oxidation pathway, however, the exact pathway for degradation of unsaturated fatty acids is undetermined and two possible pathways have been suggested. Earlier studies have suggested that the degradation of unsaturated LCFA first required complete saturation followed by the typical β-oxidation pathway (Novak, et al., 1970). Another study suggested that β-oxidation of unsaturated fatty acids might occur before fatty acid saturation (Roy, et al., 1986).

The anaerobic treatment of lipid-containing wastewaters have different operational problems due to: sludge flotation leading to washout (Hwu, et al., 1998a) (Jeganathan, et al., 2006); transport limitation from bacteria being coated in a layer of LCFAs thereby hindering the cells access to substrates and its ability to release biogas (Pereira, et al., 2004); or a LCFA toxicity effect on acetoclastic and hydrogenotrophic methanogens (Koster, et al., 1987) (Hwu, et al., 1996) (Hwu, et al., 1997) (Alves, et al., 20012a,b).

Transport limitation has been attributed to LCFAs amphiphilic properties that allow them to be easily adsorbed onto the cell wall of the microorganisms (Jiang, 2012). The LCFA layer formed on the cell wall prevents the passage of essencial nutrients through the cell membrane,causing the death of microorganisms. The LCFAs have relatively low density and, when attached to the bacterial cell wall, can cause flotation of biomass and washout from the reactor (Hwu, et al., 1998). Sensitivity of microorganisms to LCFA seems to be related to their cell wall structure, with Gram-positive bacteria and methanogens being more easily inhibited than Gram-negative bacteria (Albagnac, et al., 1985).

Alves, et al. (2013) showed that unsaturated LCFA is more inhibitory to methanogens than saturated LCFA.

Hwu (1997) showed that although granular sludge was more resistant to LCFA toxicity than suspended and flocculent biomass, physical stability of granules is critical for lipid containing wastewaters. LCFA adsorb onto the biomass and induce granular sludge flotation, which occurs for concentrations far below the toxicity limit, independent of sludge origin and specific acetoclastic methanogenic activity and sludge adaptation to lipids.

1.4.4. Volatile fatty acids

Volatile fatty acids (VFAs) are weak acids that are largely dissociated at neutral pH. Normally acetate, butyrate, and propionate are the predominating VFAs found in the methane reactors (Jordening, et al., 1998) (Aguilar, et al., 1995). The presence of a relatively high concentration of short-chain (1–3 carbon units), nonionized volatile acids such as acetate, butyrate, and propionate causes a decrease in the concentration of alkalinity and a drop in pH (Gerardi, 2003). In order to avoid AD process failure, the specific VFA concentration has been monitored and used as a process indicator for a long time. Propionate is perhaps the most toxic of the volatile acids and may exert toxicity at concentrations less than 5 g/L (Barredo, et al., 1991) (Gerardi, 2003).

The ratio of propionate to acetate is suggested to show a stable process if its value is below 1.4. Dogan, et al. (2005) investigated the inhibition of volatile fatty acids (VFA), namely acetate, butyrate, and propionate, on the activity of acetoclastic methanogens within a full-scale upflow anaerobic sludge blanket (UASB) reactor using specific methanogenic activity (SMA) test and reported that maximum potential methane production (PMP) rates were obtained at 3 g/L acetate concentration, at butyrate concentration of 5 g/L, and at 1 g/L propionate concentration. Approximatelly. 50% and 100% inhibition occurred at acetate concentrations of 13 g/L and 25 g/L, butyrate concentrations of 15 g/L and 25 g/L, and propionate concentrations of 3.5 g/L and 5 g/L, respectively.

1.4.5. Nitrogen

Total nitrogen is comprised of organic nitrogen, ammonia, nitrite, and nitrate. The oxidized forms of nitrogen, such as nitrite or nitrate are not present in anaerobic processes, since they are immediately converted into nitrogen gas (N_2). Under anaerobic conditions, the degradation of organic nitrogen results in the production of ammonia. Thus, if there is an increase in the averaged concentration of ammonia in the anaerobic reactor, this can be considered an indicator of protein breakdown.

Ammonia nitrogen exists in aqueous solution as either the ammonium ion or ammonia, depending on the pH of the solution in accordance with the following equilibrium reaction:

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$$

Many researchers have investigated the effects of ammonia-nitrogen on anaerobic treatment. MacCarty (1964) reported that concentrations between 50 and 200 mg/L are beneficial, mainly because ammonia-nitrogen is a required nutrient and stimulates bacterial growth.

Both species are inhibitory, but at significantly different concentrations. Free ammonia (NH_3) is more inhibitory and can cause a toxic response at concentrations over 50 mg/L for unacclimated methane cultures (Kroeker, 1979) (Chin, et al., 1971) (McCarty, et al., 1961). Concentrations of ammonia above 50 mg/L can be tolerated by methane-forming bacteria if the bacteria have been acclimated. If methane-forming bacteria cannot be acclimated to free ammonia, digester pH can be decreased to 7 or digester feed sludge can be diluted to prevent ammonia toxicity (McCarty, et al., 1961). On the other hand, ammonium ion (NH_4^+) concentrations over 4 g NH_4^+-N/L are known to inhibit methanogenesis (Chen, et al., 2008).

At pH levels above 7, the equilibrium is displaced to the left, the free ammonia (NH_3) is predominant and results in increased toxicity because of the shift to a higher free ammonia to ionized NH_4^+ ammonia ratio at higher pH (Borja et al., 1996b). Process instability due to ammonia often results in volatile fatty acids (VFAs) accumulation, which again leads to a decrease in pH and thereby declining concentration of FA. The interaction between FA, VFAs and pH may lead to an inhibited steady state, a condition where the process is running stably but with a lower methane yield (Angelidaki, et al., 1993) (Angelidaki, et al., 1993). As illustrated in Figure 6, vastly different total ammonia concentrations can result in a toxic free ammonia concentration, above 50 mg/L, depending on the pH and temperature.

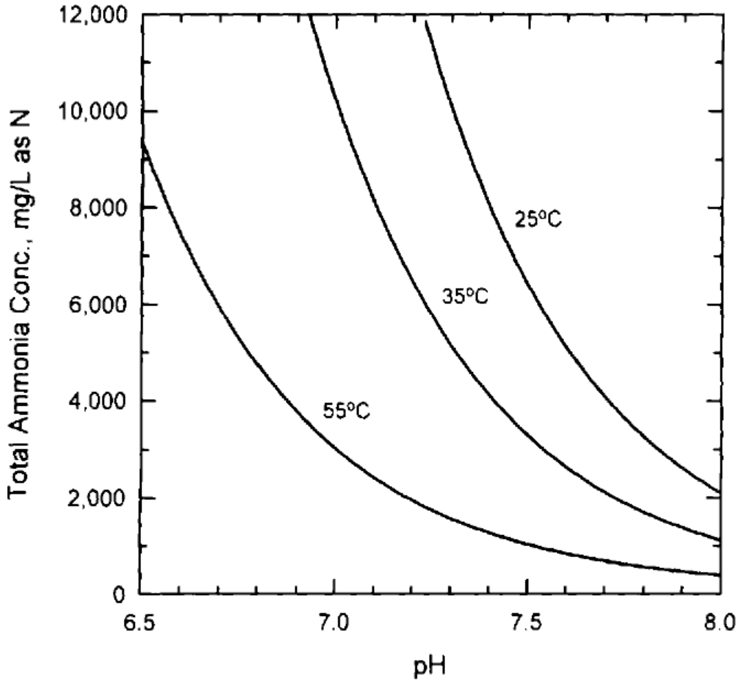

Figure 6: Effects of pH and temperature on the total ammonia-N concentration necessary to give a free ammonia concentration of 100 mg/L as N *(Grady, et al., 1999)*.

For mesophilic conditions (25°C and 35°C) the total ammnonia concentration can exceed 10,000 mg/L as N and the free ammonia concentration will still be below 100 mg/L as N at pH values of about 7.

The strategies for reducing amonia inhibition are the reduce of temperature, reduce of pH, or reduce the total ammonia concentration (Bhattacharya, et al., 1989).

1.4.6. Phosphorus

Phosphorus (P) is an essential nutrient to the growth of microrganisms. The usual forms of phosphorus found in aqueous solutions include the orthophosphate, polyphosphate, and organic phosphate. The orthophosphates, for example, PO_4^{-3}, HPO_4^{-2}, $H_2PO_4^-, H_3PO_4$, are available for biological metabolism without further breakdown. Polyphosphates and organic phosphate undergo hydrolysis in aqueous solutions and revert to the orthophosphate forms; however, this hydrolysis is usually quite slow (Lin, et al., 2007). The minimum recommended quantity of phosphorus should be 0.5 - 1% of COD (Gerardi, 2003).

1.4.7. Sulfate

Sulfate (SO_4^{2-}) itself is not inhibitory to anaerobic bacteria, but impacts anaerobic processes because under anaerobic conditions, the sulfate ion is reduced to sulfide ion, which establishes an equilibrium with hydrogen ion to form hydrogen sulfide, causing competition between sulfate reducing bacteria and methanogens.

Under anaerobic conditions, sulfate is reduced to hydrogen sulfide, which is a powerful inhibitor of methanogenesis. Hydrogen is needed to reduce sulfate to hydrogen sulfide. The need for hydrogen results in competition for hydrogen between two bacterial groups, sulfate-reducing bacteria and methanogens. Kinetic studies have shown that sulfate reducers generally have higher maximum growth rates and higher affinity for substrates (i.e., lower half-saturation constants Ks) than methanogens. K_s=9.5 mg/L and K_s=32.8 mg/L, respectively, for acetate substrate (Oremland, 1988) (Shonheit, et al., 1982) (Yoda, et al., 1987). This means that sulfate-reducing bacteria can out-compete methanogens under low acetate concentrations (Bitton, 2005).

Sulfate reducers and methanogens are very competitive at COD/SO_4 ratios of 1.7-2.7. An increase of this ratio is favorable to methanogens, whereas a decrease in the ratio is favorable for sulfate reducers (Bitton, 2005).

At pH values of 8 and above, most of the reduced sulfur exists in solution as HS^- and S^{2-} ions. At pH levels below 8, the equilibrium shifts rapidly toward the formation of unionized H_2S and is about 80 percent complete at pH 7.

Sulfide is the principle source of sulfur for metanogens. For sulfide to enter a bacterial cell, it must exist as nonionized hydrogen sulfide (H_2S). This form of sulfide occurs in a relatively high concentration within the pH range of 6.8 to 6.9, which is also near the pH of normal anaerobic digester operation.

Although sulfide is required in relatively high concentrations and is considered an obligate micronutrient, only soluble sulfides are inhibitory and concentrations greater than 200 mg/L at neutral pH, while concentrations up to 100 mg/L can be tolerated with little or no acclimation (Guerin, et al., 1964) (Amberg, et al., 1952) (Gerardi, 2003).

Sulfide reacts with heavy metals cations, including iron, forming highly insoluble precipitates like iron sulfide that gives anaerobic processes their characteristic black color (Grady, et al., 1999).

The sulfate may cause several problems in the actual digestion process, as well as in ancillary equipment (Rinzema, 1988). Part of the hydrogen sulfide is transferred to the biogas and may cause corrosion problems in boilers and internal combustion engines and the remaining part of the sulfide causes a high oxygen demand in the effluent, and potential malodour problems.

1.4.8. Salinity

In the food industry, saline effluents are mainly generated by the use of brine solutions and dry salt (NaCl) for obtaining the finished product. In the fish processing industry, the sources of pollution are initially related to the unloading of fish accompanied by seawater. The fisheries then generate wastewater rich in proteinic nitrogen, organic matter and salts.

Water containing a significant quantity of salt (mostly NaCl) is termed saline (Yang, et al., 2013). The presence of NaCl has a negative influence on the performance of anaerobic wastewater treatment (Lefebvre, et al., 2006), (Lettinga, et al., 1988), and the sodium ion is considered to be the strongest inhibitor on activity of bacteria among the cations, on the molar basis according to De Baere, et al. (1984) and Speece (2008) that reported that Na^+ concentrations exceeding 10 g/L showed a negative impact on the microbiological processes in anaerobic digestion with non-adapted sludge (Ikeda, et al., 2011), (Kargi, et al., 2004), (Krongthamchat, et al., 2007), (Delgenes, et al., 2007), resulting in lower methane production (Kugelman, et al., 1965), (Lettinga, et al., 1988). Meanwhile, Martini, et al. (2007) suggested that Cl^- concentrations higher than 35.5 g/L could inhibit aceticlastic methanogenesis. High Na^+ concentration in wastewater have and also a negative effect on the formation and stability of anaerobic granular sludge (Lefebvre, et al., 2006), which is a requirement for operating high rate anaerobic reactor systems.

Feijoo, et al. (1995) reported that when 11 g Na^+/L was abruptly added to a mesophilic UASB reactor supplied with the neutralized volatile fatty acids (VFAs) acetic, propionic, and n-butyric acid, the VFA-removal efficiencies decreased, as well as the methane production, by 90 %. In addition, Eikebrokk, et al. (2006) found that Na^+ concentrations of 10 g/L could have inhibited the anaerobic process, being responsible for the decrease in COD removal efficiencies from 55.2 to 36.7 %.

High salt concentrations significantly reduce the treatment efficiency of anaerobic processes under mesophilic conditions which can be due to cell plasmolysis and death of microorganisms because high sodium concentrations in wastewaters induce salt stress to microbial species, resulting in the inhibition of enzymes and a decrease in cell activity (Kargi, et al., 1997), (Uygur, 2006), (Kim, et al., 2008) that lead to biomass wash-out (Lema, et al., 1995) due to salt induced disintegration of flocs and granules.

Ismail (2013) reported that at sodium concentrations of 10 – 20 g Na^+/L calcium started leaching from anaerobic granules and these granules became more vulnerable to shear and wash-out of active methanogenic biomass in the effluent of the reactors was observed, likely as a result of the high Na^+ concentrations. Ismail (2013) suggest that calcium augmentation therefore may present a solution to sustain the strength of anaerobic granules when exposed to higher sodium concentrations.

1.4.9. Design, operation and performance parameters

There are two significant retention times in an anaerobic digester. These are hydraulic retention time (HRT) and solids retention time (SRT).

The volumetric hydraulic load is the volume of wastewater applied daily to the reactor, per unit of volume. The hydraulic retention time is the reciprocal of the volumetric hydraulic load,

$$VHL = \frac{Q}{V_R}$$

Where:

VHL= volumetric hydraulic load ($m^3/m^3.d$)

Q= flowrate (m^3/d)

V_R= total volume of the reactor (m^3)

$$HRT = \frac{1}{VHL}$$

where:

HRT= hydraulic retention time (d)

The hydraulic retention time (HRT) is if of fundamental importance, since it is directly related to the velocity of the anaerobic digestion process, and that, in turn, depends on the size of the reactor. Hydraulic detention times ranging from 8 to 10 hours, considereing the daily average flowrate, have been adopted for the treatment of domestic sewage at a temperature of approximately 20°C for UASB reactors.

The conversion of volatile solids to gaseous products in an anaerobic digester is controlled by the HRT. HRT values affect the rate and extent of methane production.

The SRT is the average time that bacteria (solids) are in the reactor. Solids retention time controls the types of microorganisms that can grow in the process and the extent to which various reactions will occur. One benefit of increased SRTs is increased hydrolysis and stabilization of particulate organic matter.

A proper design of SRT should be based on the rate-limiting step that is usually considered as the hydrolysis phase and leads to optimum system performances. A sufficient SRT can prevent bacteria washout, minimize the effect of temperature fluctuation, and reduce the risk of the occurrence of inefficient mixing. High SRT values are advantageous for anaerobic digesters because maximize removal capacity, reduce required digester volume, and provide buffering capacity for protection against the effects of shock loadings and toxic compounds in wastewaters and sludges. High SRT values also help to permit biological acclimation to toxic compounds. High SRT values may be achieved through two measures. First, the volume of the digester may be increased. Second, the concentration of the bacteria (solids) may be increased.

Organic loading rate (OLR) is defined as the amount (mass) of organic matter applied daily to the reactor, per volume unit. As such, this parameter integrates reactor characteristics, operational characteristics, and bacterial mass and activity into the volume of media.

The organic loading rate can be calculated by the following equation,

$$OLR = \frac{COD_{in}}{HRT}$$

$where\ OLR = Organic\ loading\ rate \frac{kg\ COD}{m^3 \times d}$,

$COD_{in} = Influent\ COD\ \left(\frac{kgCOD}{m^3}\right)$

An increase of OLR up to a certain limit will lead to operational problems like excessive foaming at the gas–liquid interface and accumulation of undigested ingredients. As a result, the treatment efficiency deteriorates (Sayed, 1987) (Blázquez, et al., 1997) (Kalyuzhnyi, et al., 1998)

Sludge loading rate (SLR) or food to microorganism ratio (F/M) refers to the amount (mass) of organic matter applied daily to the reactor, per unit of biomass present:

$$SLR = \frac{COD_{in} \times Q}{X_{VSS}}$$

SLR= Sludge Loading rate (kgCOD/kg VSS.d)

X_{VSS}= Concentration of volatile suspended solids present in the reactor (kg VSS/m^3)

1.5. Methane production

The methane production can be evaluated from the estimation of the COD degradation in the reactor, according to the following equation:

$$CH_4 + 2O_2 \leftrightarrow CO_2 + 2H_2O$$

The general expression that determines the theoretical production of methane per gram of COD removed from the wastewater is as follows:

$$V_{CH_4} = \frac{COD_{CH_4}}{K\ (t)}$$

where:

V_{CH_4}= Volume of methane produced (L)

COD_{CH_4}= Load of COD removed from the reactor and converted into methane (g COD)

$K\ (t)$= Correction factor for the operational temperature of the reactor (g COD/L)

$$K\ (t) = \frac{P \times K}{R \times (273 + T)}$$

where:

P= Atmospheric pressure (1 atm)

K=COD corresponding to onde mole of CH_4(64 g COD/mole)

R= gas constant (0.08206 atm.L/mole.ºK)

T= Operational temperature of the reactor (ºC)

Should be deducted the COD consumed for biomass production and the COD used for reduction of the sulfate. For the biomass production the following yield coefficients for acidogenic and methanogenic microrganisms: $Y_{acid} = 0.15$ and Y_{methan}=0.03 $gCOD_{cel}/gCOD_{remov}$.

COD load converted into acidogenic biomass:

$$COD_{acid} = Y_{acid} \times COD_{load\ removed\ in\ the\ system}$$

COD load converted into methanogenic biomass:

$$COD_{methan} = Y_{methan} \times (COD_{load\ removed\ in\ the\ system} - COD_{acid})$$

The COD used for reduction of the sulfate present in wastewater can be estimated by the following equation:

$$S^{2-} + 2O_2 \leftrightarrow SO_4^{2-}$$

1.6. IASB reactor technology – *Poveira*

Figure 7 shows the pre-treatment system installed at *Poveira*.

Figure 7:*Poveira* industrial wastewater treatment plant (Ambysis).

Figure 8 shows a simplified representation of the anaerobic treatment system implemented at *Poveira*. A more detailed diagram is shown in Appendix 1.

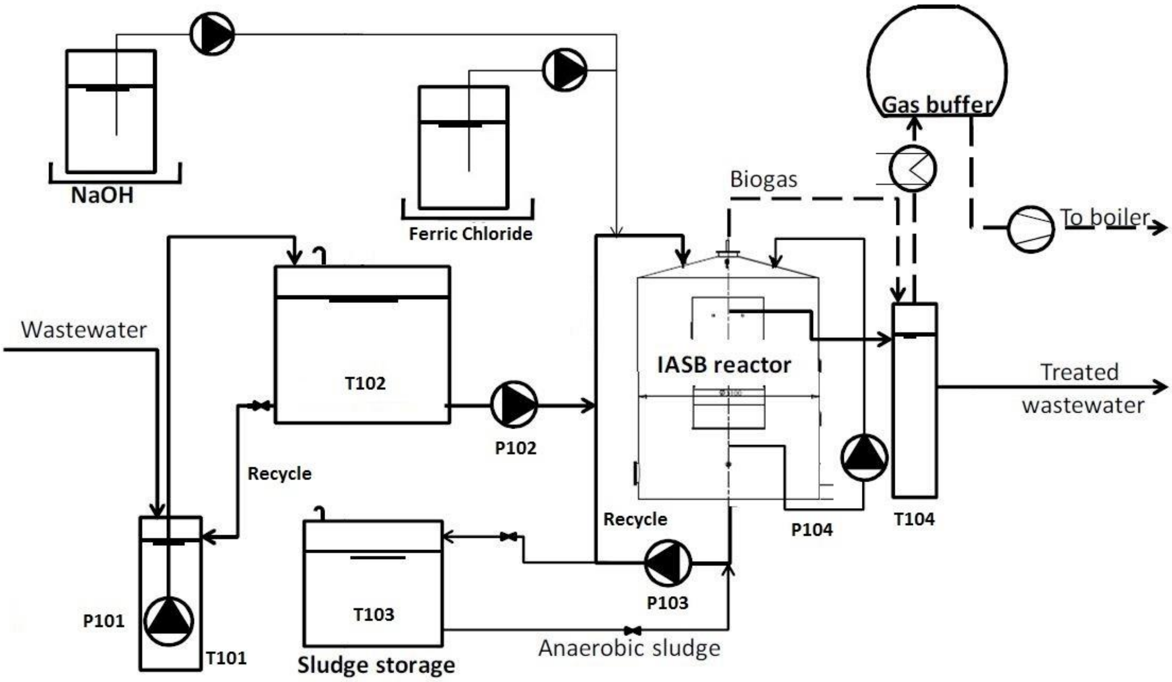

Figure 8 Simplified representation of the anaerobic treatment system with IASB reactor.

The treatment process is divided through three different stages:

- Equalization
- Anaerobic treatment
- Biogas system

1.6.1. Equalization

Industrial wastewater is collected in influent tank T101 (10 m^3 of capacity) by level control. The level control is achieved by a pressure sensor installed at the bottom of T101. At a low level, the level control actuates a pneumatic valve to let fresh wastewater into the influent tank up to the wanted high level. The influent tank T101 was meant to homogenize the influent and prevent peak loads. In the influent tank T101 is installed a submersible pump P101 with a capacity of 40 m^3/h. The pump P101 is equipped with a cutting system to prevent large elements, including fish waste, remain in the influent and cause disturbances downstream. The influent is pumped from the influent tank T101 to an equalization tank T102 (capacity of 45 m^3). by the pump P101. Equalization tank is equipped with a temperature measurement and pH measurement for control of the assay of caustic soda. In the cooking step of tuna, sardine and mackerel at *A Poveira*, the effluent produced have high temperatures affecting anaerobic treatment. In order to equalize the temperature below 45ºC there is the possibility to recirculate the effluent of T102 to the influent tank T101 by opening valve vc101. Besides temperature uniformization, the recirculation also allows the equalization with respect to effluent quality by preventing organic load peaks.

Equalized effluent is pumped to the R101 reactor IASB (volume of 100 m^3) using a feed pump P102. The pump P102 is an excentric screw pump with frequency controller and capable of pumping a maximum flow rate of 7 m^3/h. The level measured in the equalization tank T102 determines the flow to be transferred to the IASB reactor.

1.6.2. Anaerobic treatment

The *Inverted Anaerobic Sludge Blanket* reactor R101 is the main element of the treatment system and have a total volume of 100 m^3. Basically, the reactor can be divided into five sections:

- Head space zone: The biogas is collected and the spray is powdered to prevent excessive foaming inside the reactor;

- Floating sludge zone at the top of the reactor: The anaerobic sludge is present here either as suspended sludge or as part of a floating fatty scum layer at the water surface to initial degradation;
- Mixing zone at the center of the reactor: Settleable sludges and floating sludges are separated;
- Settling zone at the bottom of the reactor: Settled sludge is removed using a vacuum manifold;
- Effluent discharge zone: Water is discharged through a tilted plate separator.

The effluent from equalization tank T102 is mixed with recirculated sludge from the bottom of the reactor R101 by an eccentric screw pump P103. Pump P103 has can change pumping rates and can achieve a maximum flowrate of 20 m^3/h. The mixture is fed to the IASB reactor through a distribution system consisting of three different points of injection installed at the top of the reactor. The injection lines have control valves to allow a sequential injection and an adequate control of the process.

Inside the IASB reactor R101 anaerobic sludge is separated into a floating fatty fraction and a settling fraction. A tilted plate separator with a separation area of 35 m^2 is included in the IASB reactor R101.The floating fatty fraction present at the top of the IASB reactor is anaerobic degraded and the produced sludge having a minimum settling velocity of 0.5 m/h is separated from treated wastewater and collected in the bottom of the reactor. The treated wastewater flowed by gravity from the reactor to effluent tank T104. Effluent tank T104 has a volumetric capacity of 10 m^3 and is equipped with an internal degassing drum. Between the reactor R101 and the effluent tank T104 is installed a pH measurement line for pH control.

The produced anaerobic sludge is storage in a sludge storage tank T103 with a capacity of 35 m^3 and equipped with a high and low level measurer. Stored sludge can be used for replacement and/or substitution of existing sludge inside the IASB reactor.

The biogas formation may induce foaming and to prevent this situation, a centrifugal pump P104 is available (capacity of 10 m^3/h). Three spray nozzles are installed in the headspace of the IASB reactor. The water spray is timed and injected sequentially. Caustic soda can be dosed in the spray line.

1.6.3. Biogas System

Biogas is collected from the top of the reactor R101 and from the effluent tank T104. The produced biogas is cooled using a heat exchanger H101 which allows the reduction of humidity (under 1%) and hydrogen sulfide (H_2S) for enhancing the use in an industrial boiler since hydrogen sulfide is easily converted into sulfuric acid, and it can cause corrosion. The biogas is then stored in a double membrane gasometer G101 with 100 m^3 of capacity and equipped with an ultrasonic level measurer that allows the compressor C101 control. The compressor C101 is used to transport the biogas from the gasometer to the industrial boiler with a constant flow of 50 m^3/h at a pressure of 50 mbar using a valve spring. The biogas is then consumed in the industrial boiler.

2. Materials and Methods

At *Poveira* a mini-lab was set-up including a spectrophotometer (Spectroquant® NOVA 30A, Merck Millipore, Darmstadt, Germany), a thermoreactor (Spectroquant® TR 320, Merck Millipore, Darmstadt, Germany), a precision balance (Pioneer™ PA214, OHAUS®, Parsippany, USA), a microcentrifuge (Micro Star 12, VWR®, Pennsylvania, USA). The following parameters were monitored:

2.1. Flow rate

The flow rate was measured by an electromagnetic flowmeter (ABB).

2.2. pH

The pH was measured with an electrode (VWR pH100) connected to a pH-meter (VWR pH110).

2.3. Conductivity

The conductivity at 20 ºC was determined using a conductivity probe and a conductivity meter (VWR CO310).

2.4. Sludge volume index

The sludge volume index (SVI) is the volume in milliliters occupied by 1 gram of a suspension after 30 minutes settling according to method 2540 F (APHA, 2005). Suspended solids concentration of a well-mixed sample of the suspension was determined and also the 30 minutes settled sludge volume. SVI is calculated by the following equation:

$$SVI = \frac{settled\ sludge\ volume\ (\frac{mL}{L}) \times 1000}{suspended\ solids\ (\frac{mg}{L})}$$

2.5. Oil & Grease

The determination of oil and grease was performed according to method 5520 D (APHA 2005). Reagents used were hydrochloric acid and n-hexane.

2.6. Dissolved organic carbon

Dissolved Organic Carbon (DOC) analyses were done in a TOC-500A apparatus. Briefly, after aspirating the sample, this equipment quantifies the total dissolved carbon (DC) and the inorganic carbon (IC) - the carbonate, bicarbonate and dissolved CO_2 - present in the sample, and determines the total organic carbon (DOC) by difference, which corresponds to all carbon atoms covalently bonded in organic molecules. Thus, calibration curves for total carbon and inorganic carbon were obtained. All TOC values reported are the average of at least two TC and IC measurements and only the values with a CV (coefficient of variation) less than 2 % were validated.

2.7. Total nitrogen

Organic and inorganic nitrogen compounds are transformed into nitrate according to Koroleff´s method by treatment with an oxidizind agent in a thermoreactor. In concentrated sulfuric acid, this nitrate reacts with a benzoic acid derivative form a red nitro compound that is determined photometrically. The digestion is analogous to DIN EN ISO 11905-1. An example of the procedure (including reagents) that came with the kits is located in the Appendix 3. Attempts were made to identify the reagents present within the test kits but were not successful.

2.8. Total phosphorus

In sulfuric solution orthophosphate ions react with molybdate ions to form molybdophosphoric acid. Ascorbic acid reduces this to phosphomolybdenum blue (PMB) and the total phosphorus was quantified by measuring the intensity of blue color photometrically. The method is analogous to EPA 365.2+3, APHA 4500-P E, and DIN EN ISO 6878. An example of the procedure (including reagents) that came with the kits is located in the Appendix 3. Attempts were made to identify the reagents present within the test kits but were not successful.

2.9. Chemical Oxygen Demand

The chemical oxygen demand (COD) test is used to measure the content of organic matter of both wastewater and natural waters. The COD expresses the amount of oxygen originating from potassium dichromate that reacts with the oxidizable substances contained in 1 L of water under the working conditions of the specified procedure.

The determination of COD at *Poveira* was performed with test kits where the water sample is oxidized with a hot sulfuric solution of potassium dichromate, with silver sulfate as the catalyst. Chloride was masked with mercury sulfate. The concentration of green Cr^{3+} ions was then determined photometrically. An example of the procedure (including reagents) that came with the kits is located in the Appendix 3. Attempts were made to identify the reagents present within the test kits but were not successful. The method corresponds to ISO 15705 and is analogous to EPA 410.4 and APHA 5220 D. The determination of COD in the composite sample was performed at *FEUP* according to method 5220 B (APHA, 2005), which quantifies the $K_2Cr_2O_7$ reduction by oxidizable organic and inorganic compounds in an open reflux digester (G.VITTADINI Record/6Test digester), at

150 °C for 2 hours. The remaining dichromate was determined by titration with ammonium iron sulfate.

2.10. CBO_5

The biochemical oxygen demand (BOD_5) quantifies the biodegradable organic matter. It was determined according to the procedure described in Method 5210 B (APHA, 2005). This method is based on the difference between the initial and final (after 5 days incubation at 20 °C - oven Memmert, Model 854 Schwarbatch) dissolved oxygen concentration (assessed with a Crison OXI 45 meter and the electrode WTW CellOx 325). The quantification of BOD_5 of wastewaters usually required a previous dilution of the sample.

2.11. Sulfate

The determination of sulfate at *Poveira* was performed in test kits in which sulfate ions react with barium ion to form slightly soluble barium sulfate. The resulting turbidity is measured in the photometer (turbidimetric method). The method is analogous to EPA 375.4 and APHA 4500-SO_4^{2-} E. An example of the procedure (including reagents) that came with the kits is located in the Appendix 3. Attempts were made to identify the reagents present within the test kits but were not successful.

2.12. Total Suspended Solids

Total suspended solids (TSS) was evaluated by gravimetry - Method 2540 B, using a glass microfibres filter with particle retention 1.2 µm.

2.13. Volatile Suspended Solids

Total suspended solids (TSS) were ignited to constant weight in a muffle furnace at a temperature of 550°C according to method 2540 E (APHA, 2005). The weight lost on ignition was the volatile suspended solids (VSS).

2.14. Wastewater characterization

Influent wastewater samples were collected manually at variable time (2 hours) with a constant volume (1 liter) during a week (5 days) which day of work with 8 hours. The samples were collected in a plastic container along the day and at the end of the week the samples were mixed (composite sample). The composite sample were kept in a refrigeration system set at 4ºC and preserved away from light. Composite samples provide a more representative sampling because the influent have heterogeneous characteristics that vary over the type of canned produced. Sampling of influent wastewater was collected in the influent tank (T101), which is a location before wastewater enters in the equalization tank.
Cations were determined by ion chromatography (Dionex DX-120) using Dionex Ionpac (column:CS12A 4x250 mm; suppressor: CSRS®300 4 mm). Anions were determined by ion chromatography (Dionex ICS-2100), usando Dionex Ionpac (column:AS 11-HC 4x250 mm; suppressor: ASRS®300 4 mm).

2.15. Jar test experiment

The sample for jar-test experiment was collected from inside IASB reactor on April 29 and the experiment was performed at FEUP on April 30. A standard jar test apparatus (Jar tester JLT6, VELP Scientifica) was employed for the coagulation tests. Three different organic coagulants RIPOL 070, RIFLOC 1815 and XTRAT XTL 200 and two inorganic coagulants ferric chloride and ferric sulfate were tested. In order to see if coagulant dosage influenced the wastewater treatment, several dosages were studied: 2-10 mL/L for RIPOL 070 and RIFLOC 1815; 1-3 mL/L for XTRAT XTL 200 and ferric sulfate; 0.5 - 3 mL/L for ferric chloride. Each jar was filled with 500 mL of sample from the IASB reactor and the coagulant dose for each jar was then added. These experiments were carried out at pH=7.0.Then, the experimental procedure consisted of a rapid mixing at 150 rpm for 3 minutes and, after that, in order to form flocs, the wastewater was moderately stirred at 20 rpm for 15 minutes. Finally, a sedimentation stage of 30 minutes allowed the flocs formed to settle.

3. Results and discussion

3.1. Wastewater characterization

Throughout the day, depending on the the type of fish being processed, streams that are being launched, the volume and characteristics of the final effluent changed significantly. The streams of wastewater were identified and its flowrates were quantified. This values serve as a basis for an estimation of the contribution for each step of the process that is shown in Figure 9:

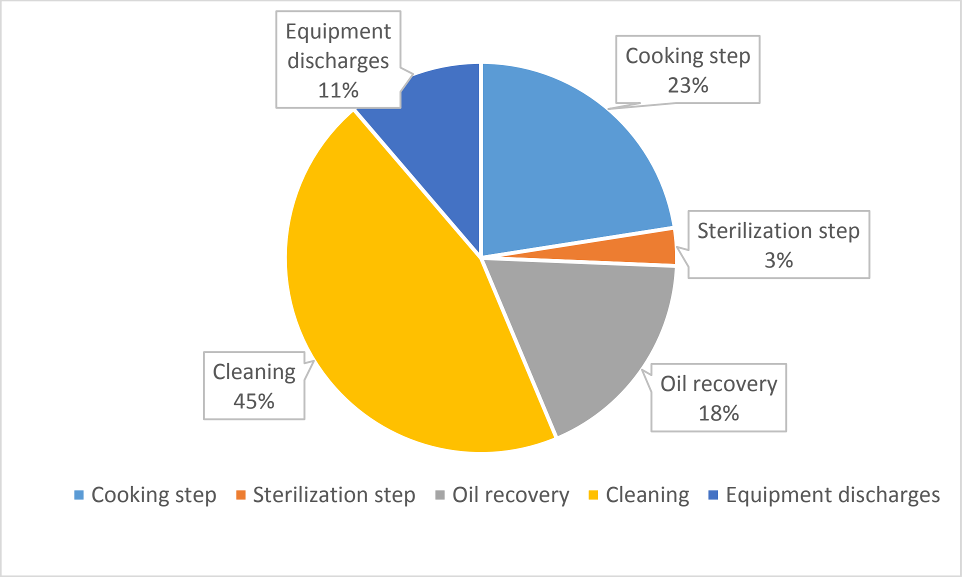

Figure 9: Estimation of the contribution of the main steps of fish-canning process for the total wastewater flowrate produced.

It was verified that cleaning has the major contribution at 45% of the total wastewater flowrate produced. This was followed by cooking (23%) and oil recovery (18%), then equipment discharges (11%) and finally sterilization (3%).

The wastewater composite sample was characterized for a variety of different parameters and the results are shown in Table 4.

Table 4: *Poveira* fish-canning wastewater composition.

pH	7.2
Conductivity (mS cm^{-1})	9.5
TSS (mg/L)	767 ± 61

VSS (mg/L)		732± 50
DOC (mg C/L)		559±16
COD (mg O_2/L)		4826 ± 82
BOD_5 (mg O_2/L)		3567± 310
P_{total} (mg P/L)		242 ±2
$N_{total\ soluble}$ (mg N/L)		228±1,3
$N_{ammoniacal}$ (mg NH_3/L)		197± 1
Oil and grease (mg/L)		4800
Anions	F^- (mg /L)	55.5
	Cl^- (mg/L)	3747.4
	NO^{2-} (mg/L)	23.8
	SO_4^{2-} (mg/L)	130.3± 12.3
	Br^- (mg/L)	<DL
	NO^{3-} (mg/L)	69.3
	PO_4^{3-} (mg/L)	157.9
Cations	Li^+ (mg/L)	<DL
	Na^+ (mg/L)	3149.2
	NH_4^+ (mg/L)	117.4
	K^+ (mg/L)	141.9
	Mg^{2+} (mg/L)	132.6
	Ca^{2+} (mg/L)	320.0

*DL – Detection limit

The possibility of sulfide inhibition should not be considered because the COD/SO_4 ratio is less than 7.5. Since every 96 mg of SO_4^{2-} present in the wastewater consume 64 mg of COD, the COD used for reduction of the sulfate is 87 mg/L.

Free ammonia (NH_3) can be inhibitory if it reaches concentrations of about 100 mg/L as N and the composite sample shows a higher value of free ammonia. Although, at pH 7.0 and at mesophilic conditions the total ammonia soluble concentration ($N_{total\ soluble}$) is lower than the concentration necessary needed to give a free ammonia concentration of 100 mg/L as N as shown in Figure 6. The COD concentration of the wastewater exceeds 4,000 mg/L which makes the anaerobic process competitive for fish-canning wastewater treatment. The concentration of magnesium (132.6 mg/L) is considered stimulatory for the microbial growth because is comprising in the concentration range 75 - 150 mg/L. Calcium concentration is considered stimulatory in the range 100 - 200 mg/L and moderately inhibitory in the range 2,500 - 4,500 mg/L, therefore the influent wastewater has a stimulatory effect in the microbial growth. The minimum concentration of potassium in the wastewater should be comprised in the range 200 - 400 mg/L to have a stimulatory efect, thus the concentration in the influent wastewater (142 mg/L) is under this range. The sodium concentration in the influent wastewater is considered moderately inhibitory because is near the range 3,500-5,500 mg/L.

3.2. Nitrogen and Phosphorus requirements

For anaerobic treatment process to be successful, nutrients necessary for the growth of microorganisms should be supplied in sufficient amounts. Table 5 resumes the calculation of nitrogen and phosphorus requirements and determination of COD:N:P ratio.

Table 5: Nitrogen and phosphorus requirements for *Poveira* fish-canning wastewater.

S_0	**g/L**	**4.826**
Y	**g VSS/ g COD**	**0.050**
N bac (N)	**g/ g VSS**	**0.065**
N bac (P)	**g/ g VSS**	**0.015**
TSS/VSS	**ratio**	**1.140**
N_r (N)	**g/L**	**0.018**
N_r (P)	**g/L**	**0.004**

The COD:N:P ratio is 1170:4:1. The influent wastewater has 4.826 g COD/L, the requirements for nitrogen and phosphorus is 0.018 g/L and 0.004 g/L, respectively. The requirements for these nutrients are satisfied for the concentration in the influent, 0.228 g N/L and 0.242 g P/L.

3.3. Process control

Monitoring and control are important strategies for achieving a better process stability and higher conversion efficiencies in anaerobic reactors. Monitoring is a requirement for process control. The monitoring period was from February 27 (day 0) to June 12 (day 110).

3.3.1. Flow rate

The *Poveira* industrial wastewater treatment plant was projected to treat a flow rate of 40 m^3 per day. The flowrate was daily measured and the values are shown in Figure 10.

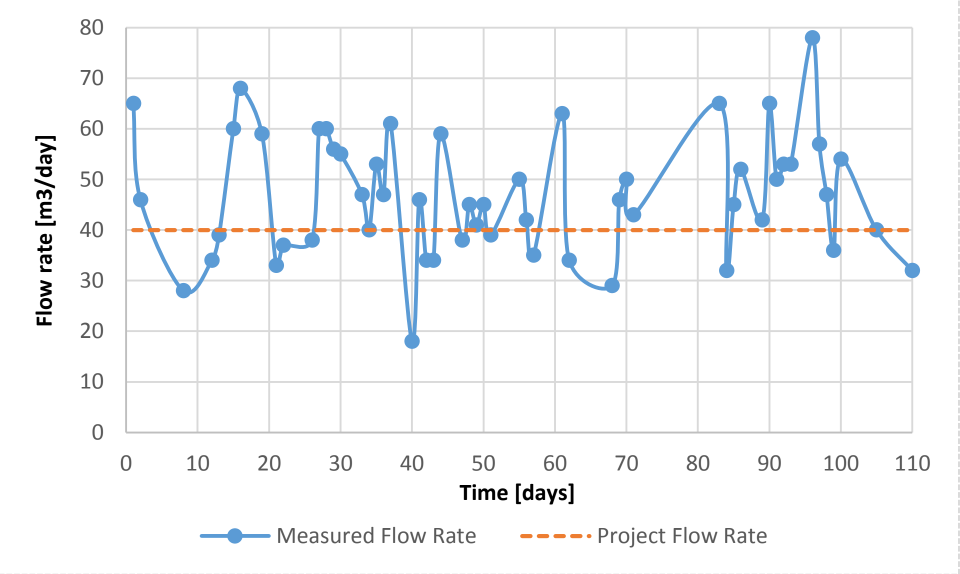

Figure 10: Control of the measure flowrate and comparison with the project flow rate.

Design flow of 40 m^3 was exceeded for most of the monitoring time. Strong variations in flow may adversely affect the efficiency of the IASB reactor and the accumulation of volatile fatty acids (VFA), a deterioration of the granules and consequently the biomass loss (washout) was observed during these periods of overloading. After every shock load, the reactor passed through a transient condition that lasted approximately 5 days before achieving the new ‘‘steady state’’. The spikes in flow were linked to the use of water from the cleaning and oil recovery steps. Therefore, a water reduction plan was implemented (flow reducers, etc.) in order to reduce consumption and normalize flow to the bioreactor.

3.3.2. Total suspended solids

Figure 11 shows the TSS concentrations in the effluent.

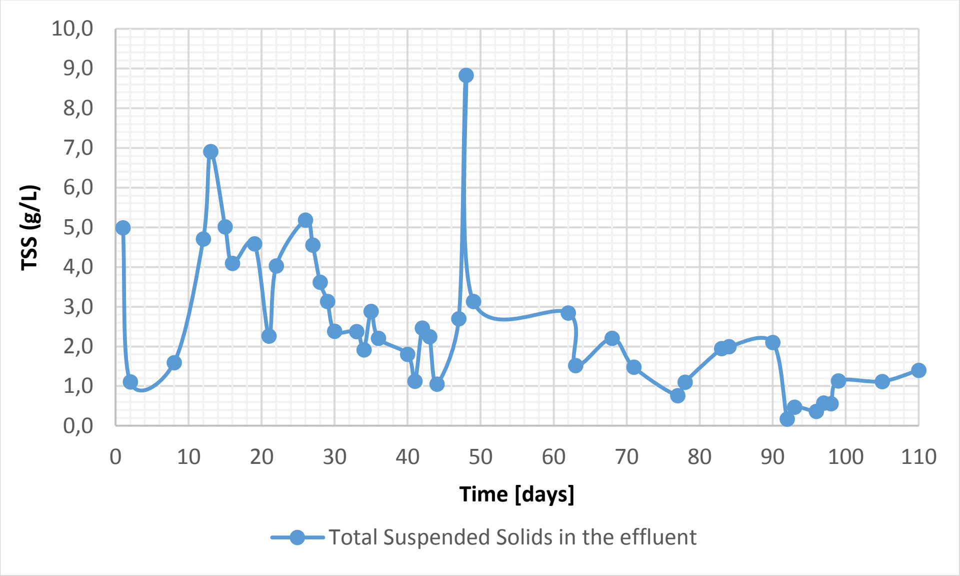

Figure 11: Total Suspended Solids (TSS) concentration control in the effluent.

As can be seen, the TSS oscillated between 1,000 and 7,000 mg/L between days 0 and 40. At day 43, a shock load of caustic soda occurred, which resulted in significant increase in TSS at day 49. Another shock load of caustic soda occurred at day 55 but no observable increase in TSS was seen. This was most likely because most of the biomass had already washed out during the first event (day 49), with little remaining biomass in the reactor. Afterwards, TSS concentrations were approximately 3,000 mg/L or less. Afterwards, biomass transfer was done during one week between the monitoring days 75 and 85. In the period between operation days 80 and 90, an increase in the TSS was observed most likely due to the washout of biomass after the new inoculation due to a higher flowrate than the projected (50 m^3 versus 40 m^3).

Effluent TSS concentrations were higher when the spray line was working due to the washout of the biomass. The spray probably affects the stability of granules. From the day 70, the spray line has been disconnected.

3.3.3. pH, conductivity, alkalinity

Figure 12 shows the range of influent and effluent wastewater pH .

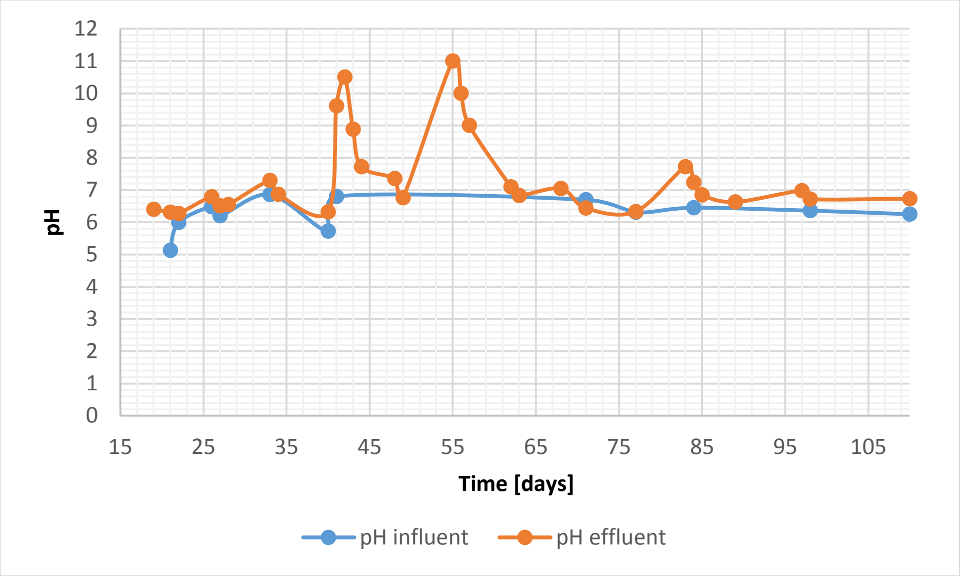

Figure 12: pH control in the influent and effluent wastewater.

The pH in the influent did not change significantly during monitoring time, ranging 6.0-7.0. The VFA production in the reactor leads to the decrease of effluent pH and the addition of caustic soda is needed to increase the pH. The two peaks of pH in the effluent occurring on days 45 and 55 of operation were associated with excessive dosing of caustic soda due to problems in the programming system. This value of pH led to the death or inactivation of activity of the microrganisms and consequent washout of the biomass that can verified in Figure 11.

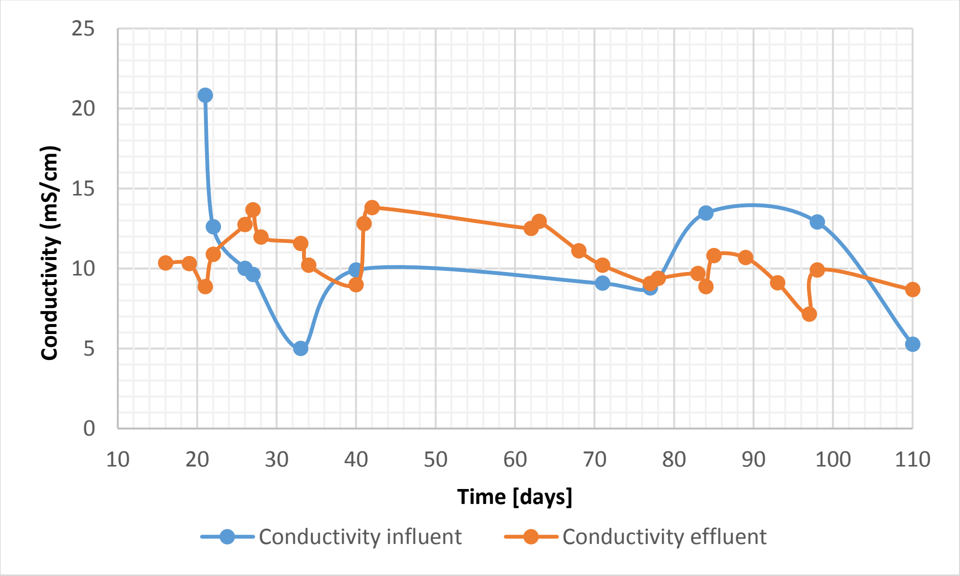

Figure 13: Conductivity control in the influent and effluent wastewater.

The increase of the effluent pH leads to the increase of the conductivity because the excessive dosing of caustic soda did increase the concentration of Na^+ in the effluent.

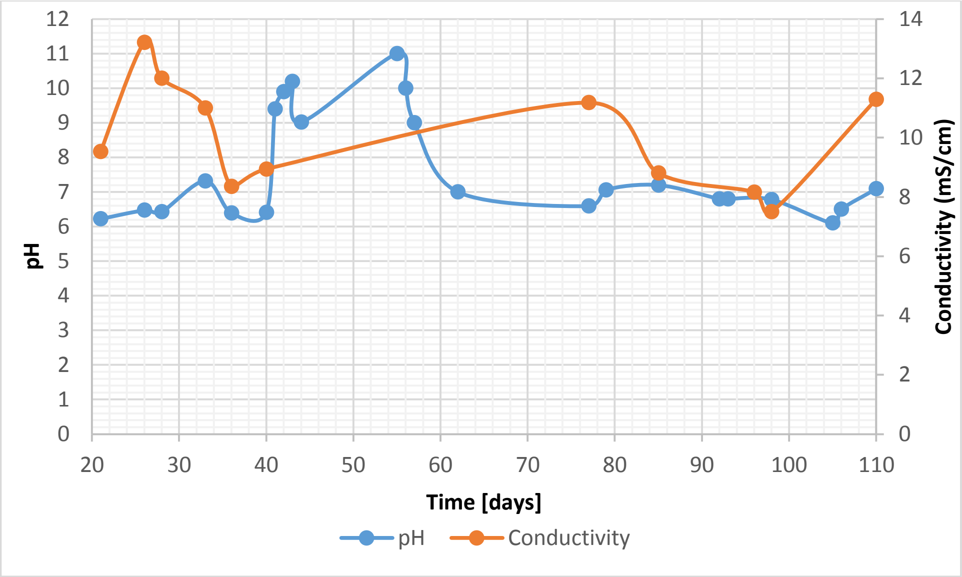

Figure 14: pH and conductivity control in the IASB reactor.

In the beggining of the operation, the pH inside the reactor was around 6. The two peak loads of pH were associated with the excessive dosing of caustic soda. From operation day 80, the pH stabilized near neutrality because after the events with excessive dosing of caustic soda, the pH inside the reactor became controlled manually instead of automatic. Alkalinity in the reactor at pH 6.8 was around 15 mg/L. At this concentration, the percentage of CO_2 in reactor gas is lower than 5%. Bicarbonate alkalinity is needed to stablize the process and produce better biogas content. The conductivity in the IASB reactor varies with the concentration of Na^+ and Cl^- of the influent which is mainly related to the number of discharges of cookers, washing baskets tank and tuna cooking tank.

3.3.4. Sulfate, total phosphate and total nitrogen

Influent and effluent sulfate, total phosphate and total nitrogen concentration were monitored and are shown in Figure 15 and 16, respectively.

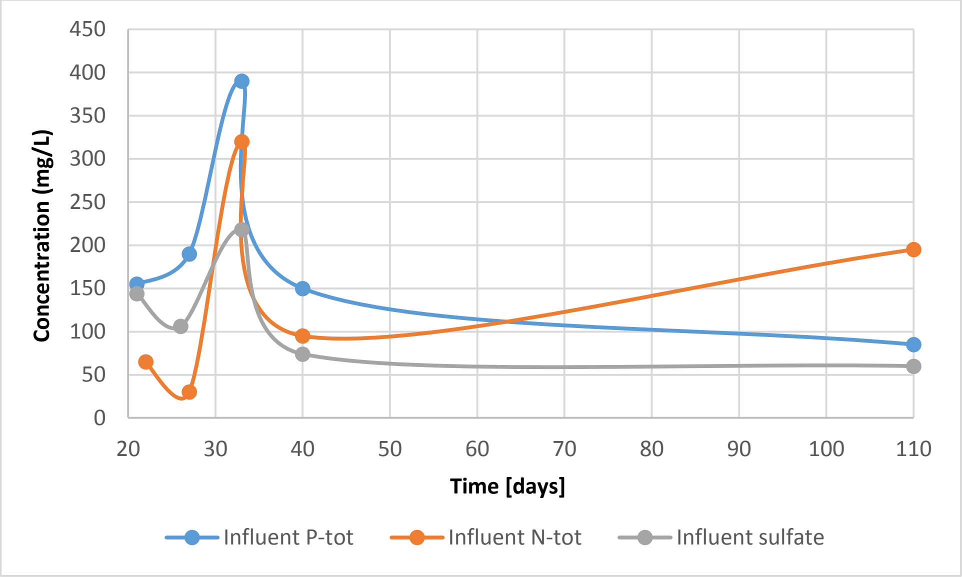

Figure 15: Influent phosphorus total, nitrogen total and sulfate concentration control.

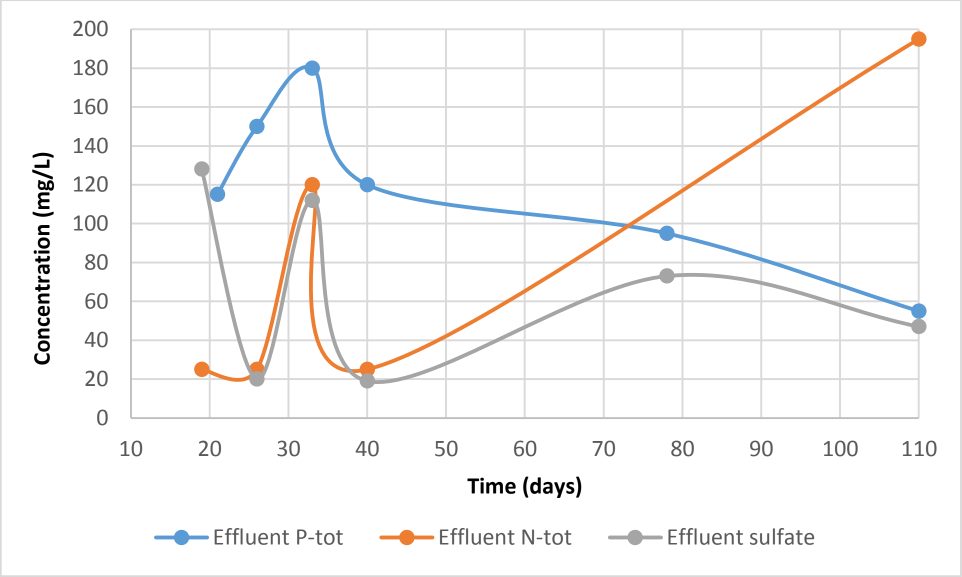

Figure 16: Effluent phosphorus total, nitrogen total and sulfate concentration control.

The COD:N:P ratio is 1170:4:1. The influent concentration of COD varied from 2,000 – 9,000 mg/L and the minimum requirements for nitrogen and phosphorus should be in the range between 10 – 35 mg/L and 2 – 10 mg/L, respectively. The concentration required was satisfied during monitoring time.

A peak of influent and effluent phosphorus and total nitrogen and sulfate concentration occurred at day 33 possibly because the sample was a grab sample and the wastewater was not well mixed in the equalization tank due to the presence of the solidified fat layer.

3.3.5. Organic loading rate

Figure 17 shows the organic loading rate (OLR) applied to IASB reactor during monitoring period.

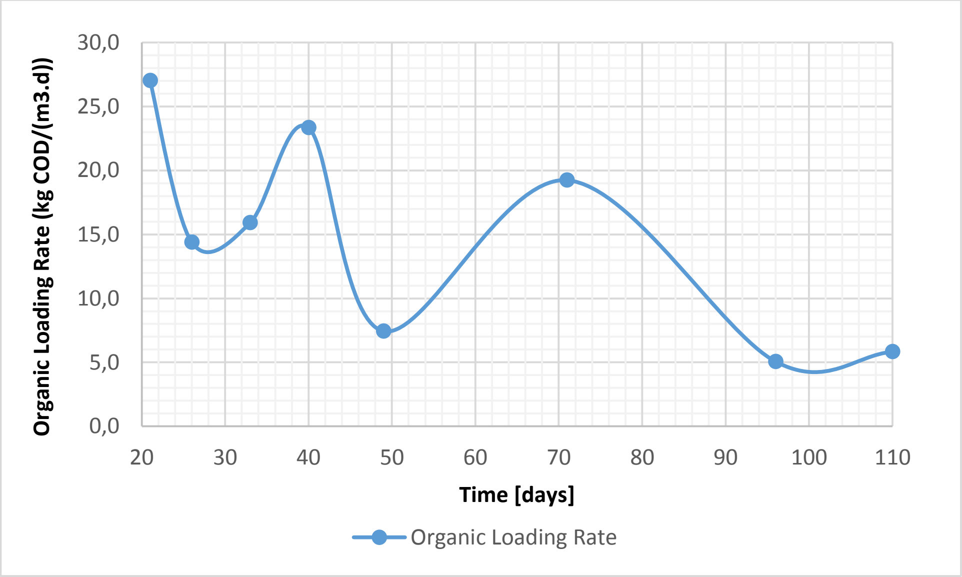

Figure 17: Control of Organic Loading Rate (OLR) applied to IASB reactor.

At the beginning of the operation the organic loading rate was greater than in the end, 25 kg COD/m^3.d and 6 kg COD/m^3, respectively. It was found that almost 50% of the equalization tank volume was occupied by solidified fat (see appendix 2) reducing equalization capacity. The decreased equalization capacity led to the existence of peaks of OLR because although the flowrate was approximately constant the COD applied to the reactor was very variable. In order to correct this situation, three cleaning actions of the equalization tank were schedule and approximately 38 tons of solified fat were removed and sent for treatment.

Punal and Lema (1999) have used a 380 m^3 UASB reactor for the treatment of fish-canning factory wastewater. The wastewater was a mixed effluent of tuna, sardine and mussel processing. The total alkalinity of more than 3 g $CaCO_3$/L was maintained to operate the system properly and to allow the biomass to resist load shocks. An HRT of 2 days was maintained and the OLR was varied from 1 kg COD/m^3 day to 8 kg COD/m^3 day and a 80-95% COD removal was achieved.

3.3.6. COD removal efficiency

Figure 18 shows the COD removal efficiency during monitoring time.

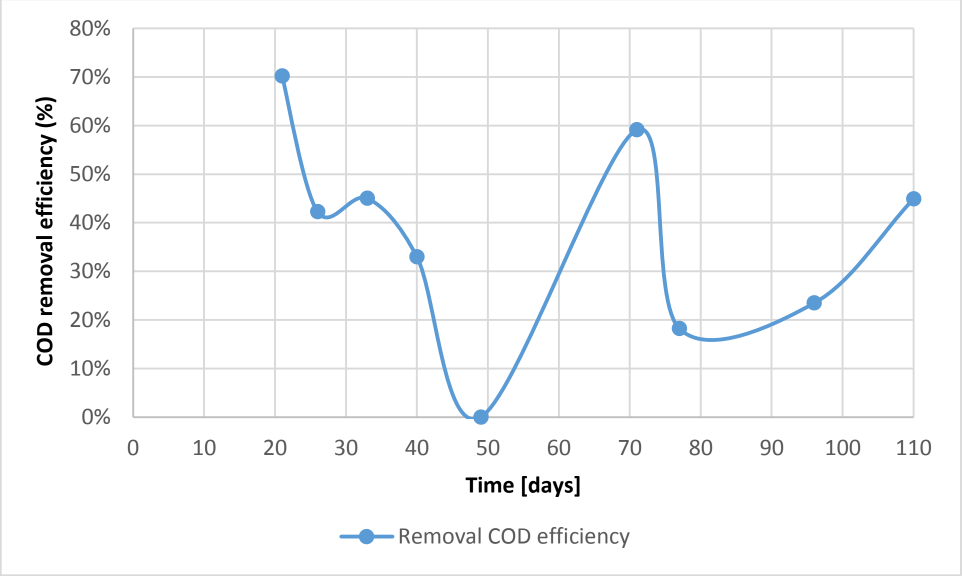

Figure 18: Control of removal COD efficiency by anaerobic IASB reactor.

The removal COD efficiency fluctuated between 0% - 70%. Sampling points for the determination of COD were situated before the reactor (R101) and after the equalization tank (T104). Null removal COD efficiency was verified after the excessive dosing of caustic soda and the washout of biomass occurred and could be seen with the increase of TSS concentration in the effluent in monitoring day 49. The biomass washout caused a lack of COD removal inside the reactor and is associated with low COD removal efficiency because the concentration of biomass inside the reactor was very low. Fluctuations in removal efficiency of COD may be related also to the lack of tank capacity in the equalization tank (T102) because homogenization of the influent was not guaranteed. The inhibition of the microorganisms could explain the low removal COD efficiency verified.

3.4. Estimation of the methane production

Knowing the chemical composition of the wastewater enables an estimation of the amount of methane that might be produced. According to (Chernicharo, 2007), the estimated production of biogas is 64 m^3 per day, of which 47 m^3 per day is methane, as shown in Table 6. The result of methane production includes a deduction for biomass growth and reduction of sulfate.

Table 6: Estimation of methane production in terms of COD removal for *Poveira* industrial wastewater treatment plant.

Q (m^3/d)	40
COD removal efficiency (%)	80
Y_{acid} (g COD cel/ g COD_{remov})	0.15
Y_{methan} (g COD cel/ g COD_{remov})	0.03
P (atm)	1
K (gCOD/mole)	64
R (atm.L/mol.°K)	0.0821
t (°C)	28
K (t) (gCOD/L)	2.59
COD (kg/d)	154
COD_{acid} (kg COD/d)	23
COD_{methan} (kg COD/d)	4
COD (SO_4^{2-}) (kg/d)	5
COD (CH_4) (kg COD/d)	122
V (CH_4) (m^3/d)	47
V (biogas)	64

The boiler installed at *Poveira* can use 60 m^3/h of biogas. According to the estimate for methane production in Table 6, the boiler will operate for approximately one hour per day. Care should be taken concerning the use of this estimate do to the fluctuations in the influent COD, COD removal, and biogas production that were observed during the course of this thesis.

3.5. Jar-test experiment

The results obtained during the experiment with XTRAT XTL 200, ferric sulfate and RIPOL 1815 did not show a formation of a clarified liquid layer and a bottom layer of solids and sedimentation was not observed. Regarding the results obtained with RIFLOC 1815 and ferric chloride, it was possible to observe sedimentation.

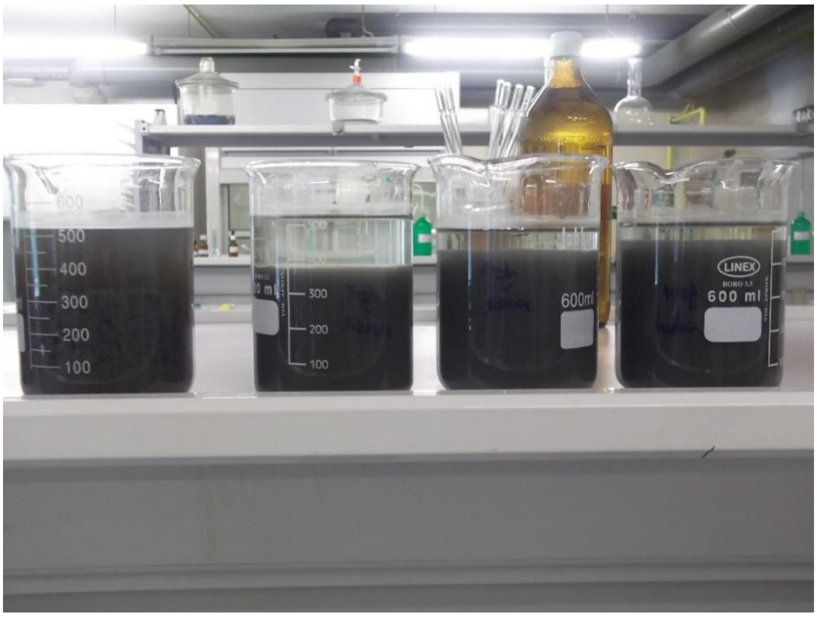

Figure 19: Jar results of decanted IASB reactor wastewater from the coagulation test using RIFLOC 1815 (from the left to right: blank, 4mL/L , 8mL/L and 10mL/L).

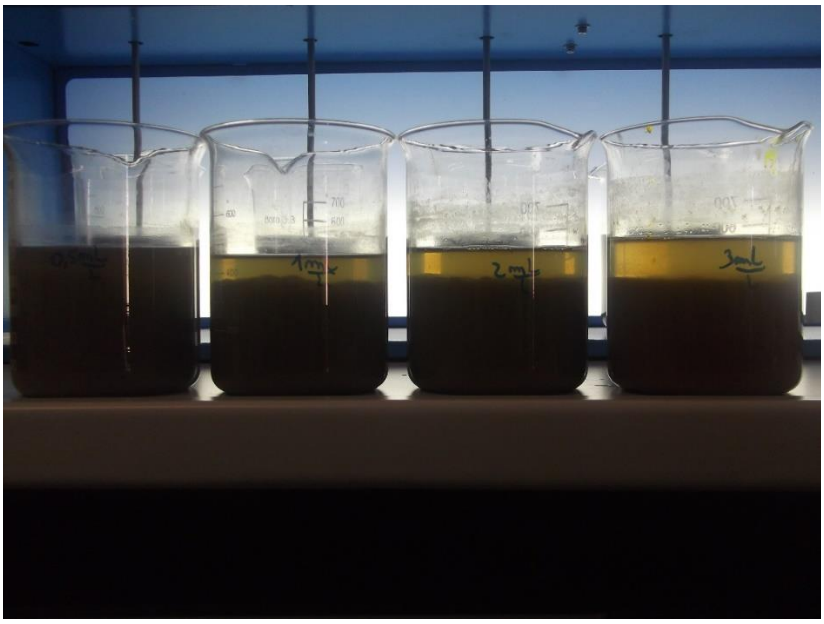

Figure 20: Jar results of decanted IASB reactor wastewater from the coagulation test using ferric chloride (from the left to right: 0,5 mL/L, 1 mL/L, 2 mL/L and 3mL/L).

3.6. Maintenance plan

The effective operational control of any wastewater treatment system will only be achieved from the implementation of an appropriate maintenance and monitoring programme, to enable both the verification of the operational parameters and the optimisation of the operational routine.

The maintenance plan should be broad enough to include all the aspects relevant to the operation of the treatment system, without disregarding the local reality and the availability of human resources and material. Therefore, not only do the development of physical-chemical and microbiological analyses become important, but also gathering of a series of information on the operation of the system. An operation and maintenance plan was developed and is shown in Table 8. This plan outlines the action to perform in each tank system and associated equipment.

Table 7: Operation and maintenance plan for *A Poveira* industrial wastewater treatment plant.

Local	Acção	Periodicidade	Operação n.º
Poço de bombagem (T101)	**Verificação da formação de camada flutuante de sólidos**	**D**	**1**
	Verificação do funcionamento das bóias de nível	**D**	**2**
	Verificação do funcionamento da recirculação entre T101 e T102	**D**	**3**
	Remoção de camada flutuante de sólidos	**D**	**4**
	Limpeza da linha de recirculação	**S**	**5**
Tanque de equalização (T102)	**Verificação do funcionamento do medidor ultrassónico**	**D**	**6**
	Controlo da alimentação do reactor IASB	**D**	**7**
	Remoção da camada de gordura existente	**De acordo com as necessidades**	**8**
	Limpeza da sonda ultrassónica	**S**	**9**
	Nível máximo no tanque de equalização	**De acordo com as necessidades**	**10**
	Funcionamento das válvulas controladas	**De acordo com as necessidades**	**11**
Reactor IASB (R101)	**Verificação da distribuição de lamas dentro do reactor**	**D**	**12**
	Verificação do funcionamento da linha de spray	**D**	**13**
	Redistribuição das lamas sedimentadas	**S**	**14**
	Adaptação da recirculação de lamas	**Decidido em conjunto com especialista**	**15**
	Adaptação da recirculação de spray	**Decidido em conjunto com especialista**	**16**

	Remoção de lamas acumuladas	A	17
Tanque de efluente (T104)	Verificação de fluxo a passar na linha de medição de pH	D	18
	Reintrodução de lamas sedimentadas no reator R101	S	19
	Limpeza da sonda de pH	S	20
	Reintrodução de fluxo pela linha de medição de pH	D	21
	Desentupimento do quebra-sifão	De acordo com as necessidades	22
Doseamento soda cáustica (P201)	Verificação do sistema de doseamento	D	23
	Ajuste do caudal de dimensionamento	Decidido em conjunto com especialista	24
Gasómetro (G101)	Inspeção visual do gasómetro	D	25
	Drenagem do permutador de calor	S	26
	Drenagem de condensados	S	27
Compressor de biogás (C101)	Verificação de pressão	D	28
	Verificação do funcionamento do compressor C101	D	29
	Ajuste de pressões de controlo e a montante e a jusante do compressor	Decidido em conjunto com especialista	30

A – Anualmente **D – Diariamente** **S – Semanalmente**

Table 8: Description of the operations included in the maintenance plan.

Operação n.º	Descrição da operação
1	A presença de uma camada espessa de sólidos flutuantes pode impedir o funcionamento correto das bóias de nível e provocar uma sobrecarga de sólidos no reator IASB. De forma a evitar esta situação, deve-se, diariamente, remover a camada superficial de sólidos flutuantes existentes no T101. No caso da camada ser bastante fina ou, excepcionalmente, não houver possibilidade de remoção, poder-se-á aumentar temporariamente a recirculação entre T101 e T102 ou remexer a camada com água.
2	O funcionamento das boias de nível poderá ser influenciado pela formação de uma camada espessa de sólidos flutuantes e impedir o seu funcionamento normal. O funcionamento poderá ser verificado recorrendo ao autómato. No gráfico do autómato, as 4 bóias de nível são representadas por 4 círculos dentro do poço de bombagem. Quando a bóia não foi acionada, o círculo é cinzento. Quando acionadas por causa do aumento do nível dentro de T101, os círculos ficam verde ou vermelho. Os níveis aos quais isso acontece são fixas e se as boias não são acionadas a esses

	níveis, será necessário proceder para remoção de sólidos flutuantes para normalizar o funcionamento.
3	Se a concentração de sólidos for elevada, isso poderá resultar no entupimento da linha de descarga entre T102 e T101, assim impedindo a agitação do poço e do tanque de equalização. Poder-se-á verificar o entupimento esperando até a recirculação ser acionada no autómato, momento em que a válvula VC01 apresentar um círculo verde. Se não houver descarga dentro de T101, dever-se-á proceder para limpeza da linha de descarga.
4	Os sólidos deverão ser removidos, uma vez por dia, com um balde com fundo furado, para a água escorrer, e uma vara.
5	A linha de recirculação está equipada com uma picagem para colocação de uma sonda de pH. Esta picagem pode ser utilizada para limpar a linha, colocando uma mangueira para injeção de água. Os sólidos acumulados dentro da linha serão descarregados no poço de bombagem T101. • Abrir a picagem de colocação da sonda de pH; • Inserir pistola da mangueira e abrir a água; • Continuar o fluxo de água até só água sair; Fechar a água, tirar a pistola e fichar a picagem.
6	O funcionamento poderá ser verificado recorrendo ao autómato. O volume contido em T102 é indicado como uma percentagem da capacidade máxima do tanque T102 (50 m^3). Se esta percentagem mostrar grandes flutuações, será necessário prosseguir para limpeza da sonda. Poderá acontecer que a percentagem indicada no autómato é estável, mas não haver água no tanque de equalização. Neste caso, a bomba de alimentação P102 estará a bombear ar e isso consegue-se ouvir na linha de compressão da bomba: ouve-se ar a borbulhar. Será necessário desligar imediatamente a bomba.
	Anomalias na bomba P102 poderão resultar na produção de barulhos estranhos. Se houver um entupimento na linha de aspiração, parece que se está a bater com força na linha de bombagem com um pau de metal. Se isso acontecer, será necessário desligar a bomba, desmontá-la para limpeza e verificação de danos, e limpar a linha a

7	montante. Pode acontecer que a bomba esteja a aspirar ar. Se isso acontecer, será necessário desligar a bomba imediatamente para evitar danos a mesma. Se houver um entupimento na linha de pressão, as tubagens começarão a vibrar com força. A bomba P102 deverá ser desligada imediatamente no autómato. Se a abertura de uma das válvulas na linha de alimentação não for detetada pelo autómato, a bomba P102 será automaticamente desligada. Deverá ser verificado o funcionamento das válvulas.
8	Flutuações de temperatura no interior do tanque T102 leva, por vezes, ao espessamento da gordura e consequente acumulação. A gordura acumulada não é bombeada, ficando a ocupar volume no tanque, reduzindo o seu volume útil disponível. A redução do volume útil do tanque afeta a capacidade de equalização e consequentemente a eficácia de tratamento. Aconselha-se a verificação mensal da espessura da camada de gordura. No caso desta ser reduzida, é possível bombear água quente para o interior tanque de equalização de forma a dissolver a gordura. No entanto, esta operação deverá ser acompanhada por um técnico especializado, uma vez que requer o conhecimento do sistema de forma a não provocar danos. No caso da espessura da camada ser elevada, deve-se recorrer a uma empresa especializada para recolha e encaminhamento deste resíduo.
9	Para limpar a sonda é necessário subir o tanque de equalização. • Desenroscar o medidor de nível; • Limpar com um pano seco para remover condensados acumulados no sensor; • Recolocar a sonda de nível.
10	Poderá acontecer a situação em que o poço de bombagem T101, bem como o tanque de equalização T102 estão ao nível máximo. No caso de T102, isso acontece aos 95%. Em caso de emergência, T102 pode receber mais do que isso simulando um nível mais baixo dentro do tanque. • Desenroscar a sonda de nível e apontar para a parede da fábrica, assim simulando um tanque mais vazio; • Eventualmente, a bomba do poço de bombagem P101 começará a bombear.

	Deixar bombear até o tanque encher; • Quando não se consegue ouvir água a cair, o tanque está cheio e dever-se-á recolocar a sonda de nível na posição normal no tanque de equalização.
11	Em caso de incorreto funcionamento das válvulas deverão ser seguidos os seguintes passos. • Deverá ser verificada a pressão de ar para o controlo pneumático das válvulas. Se não houver pressão suficiente, as válvulas não abrirão em condições. Normalmente isso é devido ao compressor de ar comprimido estar desligado. Será uma questão de ligar o compressor. • Se houver pressão suficiente e as válvulas abrem e fecham manualmente, o problema poderá ser um ou mais fusíveis queimados dentro do autómato. Se houver um fusível queimado associado ao funcionamento das válvulas, o autómato poderá não detetar a abertura das válvulas. Dever-se-á prosseguir para substituição dos fusíveis e verificar o bom funcionamento. Depois de resolução do problema, basta carregar no botão OK no ecrã do controlo da bomba P102 e P103.
12	Com a variação do afluente, a distribuição de lamas dentro do reator varia. Para evitar concentrações excessivas de lamas na saída do reator, é necessário obter uma distribuição uniforme entre lamas flutuantes e lamas que sedimentam. Deverão ser tiradas amostras de cada ponto de amostragem para um teste de sedimentação/flutuação utilizando um cone Imhoff. De acordo com os resultados, poderá ser necessário adaptar o caudal de recirculação de lamas.
13	Os sprays poderão entupir na presença de resíduos de maiores dimensões nomeadamente, plásticos proveniente de vassouras e luvas. Quando isso acontecer, ao longo do tempo a bomba de spray P104 irá ficar cada vez mais quente. Para verificar o aquecimento, basta colocar a mão no corpo de bombagem. Poderá acontecer que só um spray esteja entupido. Será necessário verificar se as linhas de spray ficam pressurizadas depois da atuação da bomba. Se facilmente se conseguir desenroscar os sprays em cima do reator, as linhas não estão entupidas.
14	Se houver uma grande acumulação de lamas sedimentáveis no fundo do reator IASB, poderá ser necessário aumentar o caudal de recirculação de lamas. Este aumento deverá ser gradual e poderá ser feito no autómato, adaptando a velocidade da bomba

	P104.
15	A bomba de recirculação de lamas aspira do fundo do reator IASB em 2 pontos separados e portanto, poderá haver acumulação de lamas nas áreas mais afastadas destes pontos de aspiração. Há a possibilidade de redistribuir as lamas no fundo utilizando a bomba de spray P104. Basta abrir a válvula manual em cima do reator IASB durante algum tempo para injetar água no fundo do reator, assim possibilitando a redistribuição das lamas.
16	A recirculação de spray serve 2 objetivos: quebra de espuma e humidificação de lamas flutuantes. No entanto, a passagem de água pelos sprays cria muita fricção, que poderá resultar em desativação parcial da biomassa que passa pelos mesmos. É portanto importante limitar o caudal diário de spray e minimizar o mesmo.
17	Ao longo do tempo, verifica-se o aumento excessivo da quantidade de biomassa no interior do reator, sendo necessária a sua remoção. Para tal, há possibilidade de transferência de biomassa para o tanque T103. No caso do tanque T103 não apresentar volume disponível, a biomassa pode também ser transferida para o tanque que é da propriedade da Ambysis, no caso de aprovação por parte da empresa proprietária e caso haja volume disponível. Se não existirem as alternativas anteriormente apresentadas, então dever-se-á contactar uma empresa especializada para remoção e encaminhamento deste resíduo. É possível verificar que o reator está cheio através do autómato, que emite um aviso de nível máximo no reator ou então devido à saída de efluente pelo respiro do tanque T104.
18	Sem afluência de água residual pode acontecer, depois de retoma da afluência de água residual, de não haver fluxo a passar pela linha de medição de pH. Deve-se abrir a válvula manual que permite a passagem de água para o exterior, para verificar se existe fluxo a passar pela linha de medição de pH. No caso de não existir fluxo, seguir a descrição da operação n.º21.
	Durante a operação haverá alguma acumulação de lamas no tanque T104. Recorrendo a bomba P104, estas poderão ser transferidas para o reator. • Desligar a bomba P104 no autómato; • Desligar a bomba P102 da alimentação do reator no autómato; • Abrir a válvula manual na linha de spray em cima do reator IASB. Isso é de grande importância para evitar entupimento dos sprays com material inerte e

19	grosseiro acumulado em T104; • Fechar a válvula manual na linha de aspiração do reator R101 para bomba P104; • Abrir a válvula manual na linha de aspiração do tanque T104 para P104; • No autómato, abrir em manual a válvula de controlo na linha de spray da qual foi aberta a válvula manual. As outas 2 válvulas de controlo devem ser fechadas manualmente no autómato; • Por a bomba de spray P104 em automático e deixar funcionar durante o tempo pretendido; • Depois deste tempo, desligar a bomba P104; • Fechar a válvula manual na linha de aspiração do tanque T104 e abrir a válvula manual na linha de aspiração do reator R101; • Ligar outra vez a bomba P104 e deixar passar água do reator pelo menos 1 vez antes de fechar a válvula manual em cima do reator. Isto é para remover material grosseiro da linha e evitar entupimento dos sprays; • Por todas as válvulas de controlo do sistema de spray em automático.
20	A sonda de pH está diretamente exposta a águas residuais contendo gorduras e, ao longo do tempo, acumulará sujidade. Para efetuar a limpeza, deve-se: • Fechar as 2 válvulas manuais na linha de medição de pH; • Desenroscar a sonda e tirar da linha de medição de pH; • Passar a sonda por água; • Inserir a sonda em acetona para remover gordura acumulada; • Passar outra vez por água; • Recolocar a sonda de pH na linha de medição de pH; • Abrir as válvulas manuais na linha de medição de pH.
21	• Abrir um pouco a válvula de drenagem da linha de medição de pH para deixar escorrer água num balde; • Enquanto água está a escorrer, fechar a válvula de membrana a jusante da sonda de pH; • Abrir por completo a válvula de drenagem e deixar escorrer enquanto se abre outra vez a válvula de membrana; • Fechar a válvula de drenagem.

22	No caso de grande formação de espuma, o quebra-sifões poderá ficar entupido, resultando na perda de biogás pela descarga de água residual. O quebra-sifões pode ser desentupido com um jato de água.
23	Diariamente deverá ser verificado se o sistema está a funcionar em condições. Isso passa basicamente por uma inspeção visual para: • Fugas de soda cáustica, que pode indicar vedação inadequada da cabeça de bombagem; • Nível de óleo dentro da câmara de bombagem; • Nível de soda cáustica dentro do unitanque. Se o nível estiver baixo (menos de 250 L) dever-se-á prosseguir para encomenda de mais NaOH; • Posição da linha de aspiração de soda cáustica. Pode acontecer que a válvula de antirretorno e o peso se soltem da linha, resultando na flutuação da linha e potencial aspiração de ar. A aspiração deve ser evitada, uma vez que poderá resultar na secagem e quebra dos vedantes da cabeça de bombagem. Se aspiração de ar é verificada, a bomba de doseamento dever-se-á desligar imediatamente.
24	O caudal de doseamento pode ser ajustado na bomba. No entanto, o ajuste só pode ser feito enquanto a bomba estiver em funcionamento. Com o manípulo que se encontra na bomba, o caudal pode ser variado entre 10% e 100% do caudal máximo (50 L/h).
25	O gasómetro deverá ser inspecionado por anomalias todos os dias, como por exemplo: • Fugas de biogás; • Funcionamento de medidor ultrassónico de nível.
26	O permutador de calor está equipado com uma válvula para drenar condensados acumulados no mesmo. Basta abrir a válvula para deixar sair a água acumulada.
27	A armadilha de condensados também está equipada com uma válvula para drenagem. Basta abrir para descarregar a água acumulada.
28	Dois manómetros com leitura local estão instalados na linha de biogás. Diariamente deverá ser feita a leitura das pressões para registar potenciais alterações. Uma diminuição da pressão a montante do compressor pode por exemplo ser causada por acumulação de condensados.

29	Verificação passa pela inspeção visual e verificação por barulhos estranhos, que poderão ser indicação de anomalias.
30	Ações de limpeza e resolução só deverão ser feitas com um especialista presente.

3.7. Analytical monitoring plan

The control of the anaerobic process is accompanied by an analytical monitoring plan for the liquid fraction (Table 10) and solid fraction (Table 11).

Table 9: Analytical monitoring plan for the liquid fraction.

	pH	Conductivity	COD	VFA	Alkalinity	N-tot	P-tot	Sulfate
T102	D	D	W	M	D	W	W	W
R101								
H1	D	W		M	D			
H2	D	W		M	D			
H3	D	W	W	M	D	W	W	W
H4	D	W		M	D			
T104	D	D	W	M	D	W	W	W

D – Daily **M – Monthly** **W – Weekly**

Table 10: Analytical monitoring plan for the solid fraction.

	TSS/VSS	TS/VS	Imhoff test	LCFA	Methanogenic activity
T102	W	W	D	M	
R101					
H1	W	W	D	M	M
H2	W	W	D	M	M
H3	W	W	D	M	M
H4	W	W	D	M	M
T104	M	M	D		M

D – Daily **M – Monthly** **W – Weekly**

4. Conclusions

The stability of the anaerobic wastewater treatment process installed at *Poveira* began from May 16 (monitoring day 80) until June 16 (monitoring day 110) by stabilizing the pH inside IASB reactor, decreasing organic loading rate applied to

IASB reactor (identical to those recorded in the literature for fish canning anaerobic wastewater treatment), increased retention of biomass inside the reactor and subsequent decrease in total suspended solids in the effluent and homogeneization of the influent composition.

The maximum achieved COD removal efficiency was 70% but during the monitoring period, the COD removal efficiency was below 50% for most of the time. Different strategies should be tested to increase COD removal efficiency such as increasing the hydraulic retention time in the equalization tank and in the IASB reactor, keeping constant the organic loading rate (OLR) applied to the reactor, guarantee a biomass concentration sufficient to degrade the influent organic matter and increasing the alkalinity of the reactor to the range 1,500 - 3,000 mg/L.

The boiler should operate for approximately one hour per day because it consumes 60 m^3 of biogas per hour and the methane production estimated is 64 m^3 per day.

The ferric chloride dosing was suspended because it was observed that its addition was contributing to the adhesion of the biomass to the fat and induced flotation of the biomass rather than sedimentation. The Jar-test results demonstrated that the blank test had the highest volume of settled sludge.

Measures for the reduction of wastewater production, were implemented, such as awareness of the workers concerning the use of hose nozzles for cleaning procedures, installation of a flow control system in the hoses and in the recovery oil equipment.

Three cleaning procedures were done in the equalization tank to remove a solidified fat layer that was affecting the influent composition homogeneization and the available volume of the tank.

The spray line was deactivated since its use induced an increased turbulence inside the reactor and interfered with the stability of granular sludge.

In short, in order to achieve a better performance in the anaerobic process wastewater treatment (and any treatment process in general), stable conditions much be reached. These conditions can be achieved through an analytical control plan of the system and problem identification, interpretation and troubleshooting.

5. Recomendations

5.1. Implementation of Maintenance and Analytical Plans

The maintenance and analytical plans developed in this thesis (sections 3.6 and 3.7) should be implemented as quickly as possible, in order to ensure the proper operation of the bioreactor. Not only will this lead to better bioreactor performance but also cost savings to *Poveira*, amongst other things.

5.2. Jar-test experiment

The characteristics of produced wastewater may vary due to changes in canning production process. Therefore, jar-test experiments should be repeated in order to verify if a coagulant or flocculant may improve sludge settling.

5.3. Specific Methanogenic Activity (SMA) tests

The success of high-rate anaerobic processes depends fundamentally on the maintenance (inside the reactor) of an adapted biomass with high microbiological activity that is resistant to shock loads. In this respect, it is recommended to perform specific methanogenic activity (SMA) tests. In practice, the SMA tests consist of the evaluation of the capacity of methanogenic bacteria (specifically acetoclastic methanogens) to convert organic substrate into methane and carbon dioxide. It is recommended to use SMA tests in the following applications:

- to evaluate the behavior of biomass under the effect of an optimal dosage of coagulant used to improve the settleability of the biomass;
- to evaluate methane production;
- to determine the relative toxicity of chemical compounds such sodium, chloride and VFA that are present inside the reactor;

- to determine the maximum organic load that can be applied to the biomass
- to evaluate kinetic parameters.

For the development of the test, it is necessary to use anaerobic sludge from inside the reactor, organic substrate (usually sodium acetate is used), buffer and a nutrient solution and batch reactor flasks with rubber stoppers for sample removal and analysis.

5.4. Biogas prodution monitoring

The real biogas production at *Poveira* can only be monitored volumetrically by measuring the increase of the gasometer volume. The quality of the biogas must be occasionally verified by gas chromatography.

5.5. VFA monitoring

The monitoring of VFA concentration in the reactor was not possible at *Poveira* because there is no equipment for this analysis. Total VFA concentration can be measured online by titration (Feitkenhauer, et al., 2002), or indirectly where light spectroscopy is correlated to total VFA concentrations, by using near infrared spectroscopy (NIR) (Holm-Nielsen, et al., 2008) (Jacobi, et al., 2009). However, to measure individual VFA, online monitoring is more complex. The online monitoring of individual VFA has been based on sample filtration followed by analysis in a gas chromatograph (Pind, et al., 2003), or using headspace extraction followed by analysis in a gas chromatograph (Boe, et al., 2007).

5.6. Hydrodynamic characterization

Since the flow rate and the influent COD concentration are stabilized, a hydrodynamic characterization should be developed in order to understand the hydraulic mechanisms and, therefore, to know exactly the actual HRT, and subsequently the behavior of the particles. Through the hydrodynamic characterization, dead zones and preferential channels of flow inside the reactor, and the average residence time of the particles can be determined.

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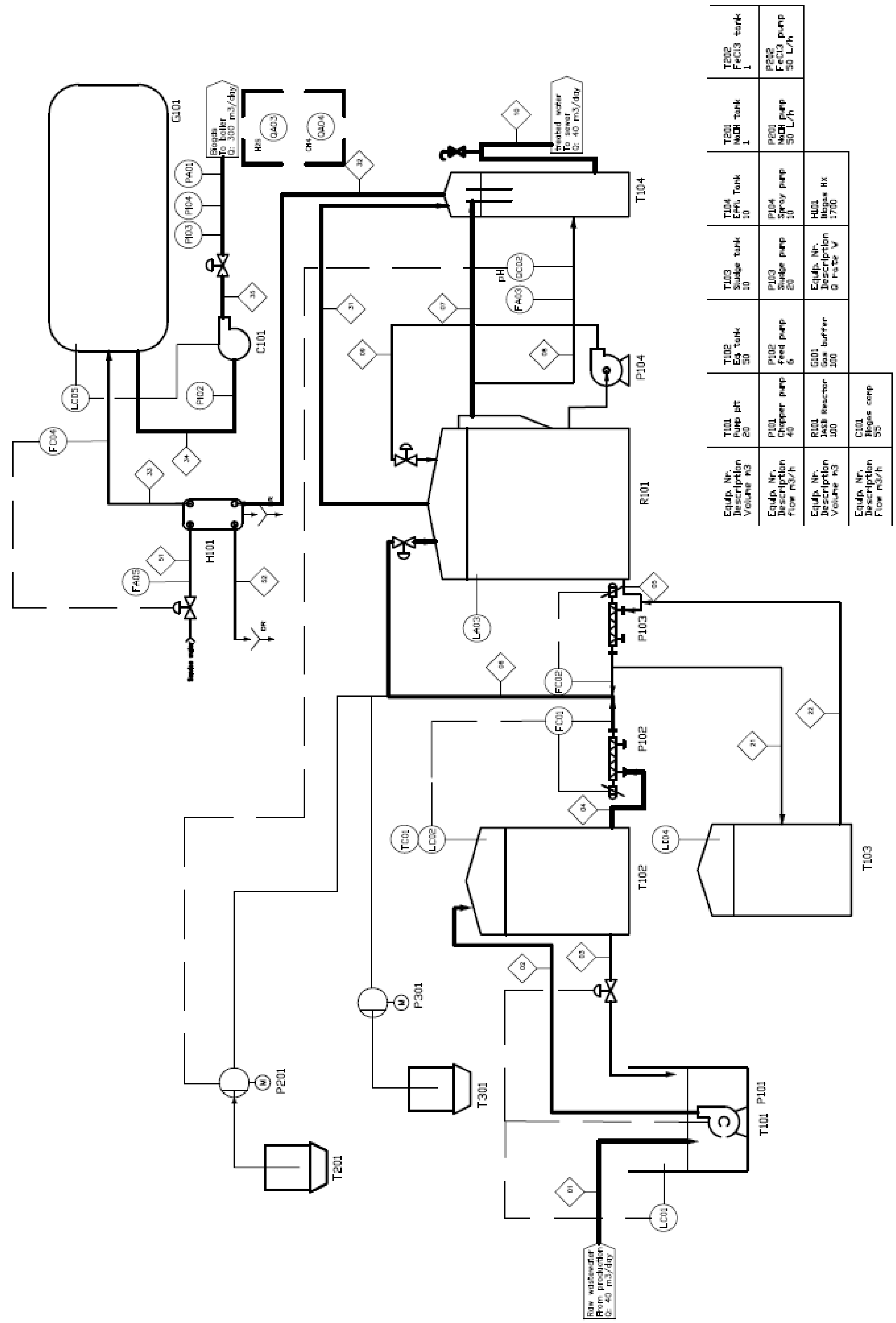
7. Appendices

Appendix 1:Process Diagram of the wastewater treatment plant from *Poveira*

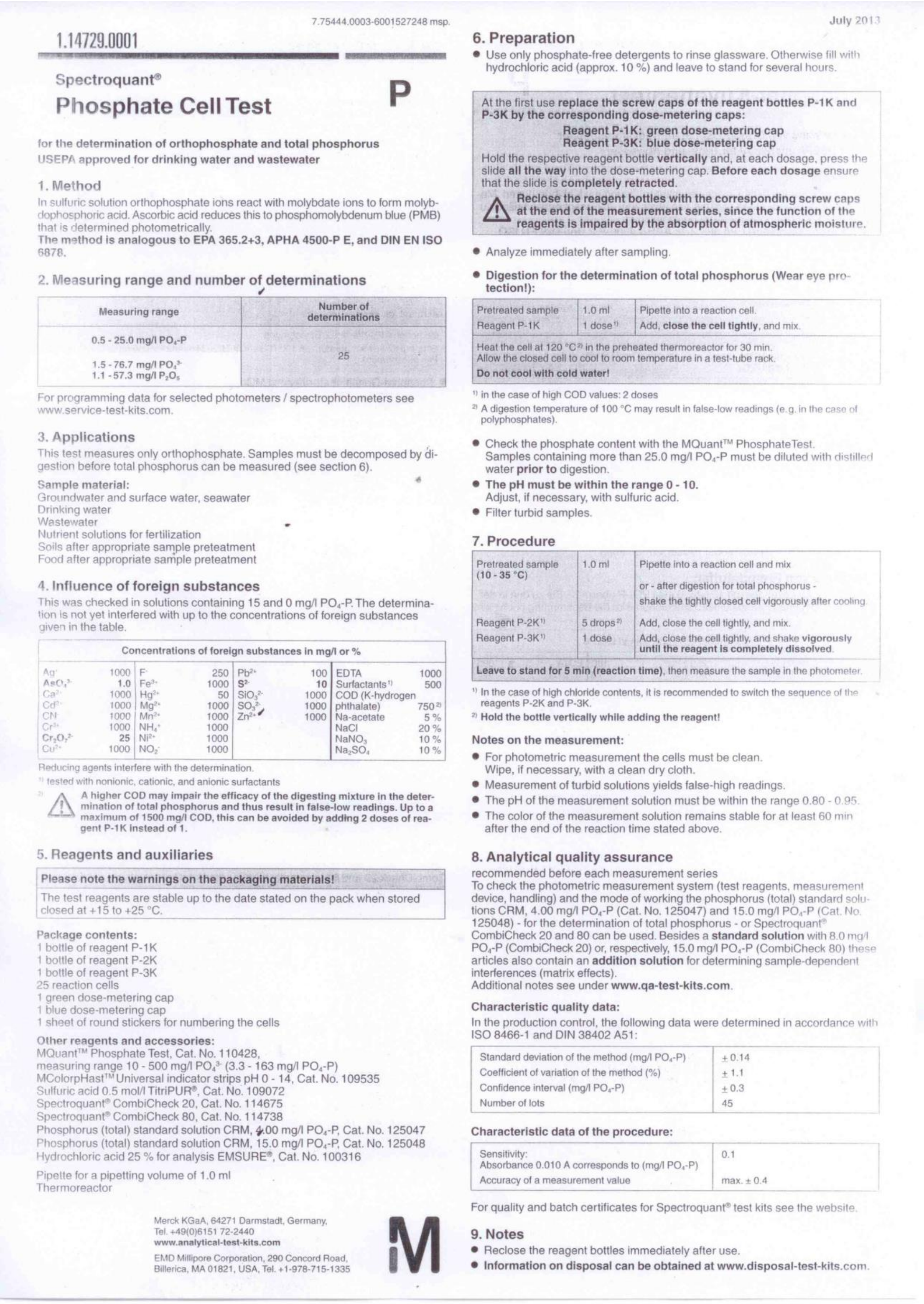


Equip. Nr. Description Volume m3	T101 Pump pit 20	T102 Eq. tank 50	T103 Sludge tank 10	T104 Effl. Tank 10	T201 NaOH tank 1	T202 FeCl3 tank 1
Equip. Nr. Description flow m3/h	P101 Chopper pump 40	P102 feed pump 6	P103 Sludge pump 20	P104 Spray pump 10	P201 NaOH pump 50 L/h	P202 FeCl3 pump 50 L/h
Equip. Nr. Description Volume m3	R101 UASB Reactor 100	G101 Gas buffer 100	Equip. Nr. Description Q rate W	H101 Biogas HX 1700		
Equip. Nr. Description Flow m3/h	C101 Biogas comp 55					

Appendix 2: Equalization tank (T102) and the solified fat layer

Figure 21: Equalization Tank (T102) and the solified fat layer.

Appendix 3: Test-kit procedure –An example for determination of phosphate total.



7.75444.0003-6001527248 msp.

1.14729.0001

Spectroquant®

Phosphate Cell Test

P

for the determination of orthophosphate and total phosphorus
USEPA approved for drinking water and wastewater

1. Method

In sulfuric solution orthophosphate ions react with molybdate ions to form molybdophosphoric acid. Ascorbic acid reduces this to phosphomolybdenum blue (PMB) that is determined photometrically.
The method is analogous to EPA 365.2+3, APHA 4500-P E, and DIN EN ISO 6878.

2. Measuring range and number of determinations

Measuring range	Number of determinations
0.5 - 25.0 mg/l PO_4-P 1.5 - 76.7 mg/l PO_4^{3-} 1.1 - 57.3 mg/l P_2O_5	25

For programming data for selected photometers / spectrophotometers see www.service-test-kits.com.

3. Applications

This test measures only orthophosphate. Samples must be decomposed by digestion before total phosphorus can be measured (see section 6).

Sample material:
Groundwater and surface water, seawater
Drinking water
Wastewater
Nutrient solutions for fertilization
Soils after appropriate sample preteatment
Food after appropriate sample preteatment

4. Influence of foreign substances

This was checked in solutions containing 15 and 0 mg/l PO_4-P. The determination is not yet interfered with up to the concentrations of foreign substances given in the table.

Concentrations of foreign substances in mg/l or %							
Ag^+	1000	F^-	250	Pb^{2+}	100	EDTA	1000
AsO_4^{3-}	**1.0**	Fe^{3+}	1000	**S^{2-}**	**10**	Surfactants[1]	500
Ca^{2+}	1000	Hg^{2+}	50	SiO_3^{2-}	1000	COD (K-hydrogen phthalate)	750[2]
Cd^{2+}	1000	Mg^{2+}	1000	SO_3^{2-}	1000		
CN^-	1000	Mn^{2+}	1000	Zn^{2+}	1000	Na-acetate	5 %
Cr^{3+}	1000	NH_4^+	1000			NaCl	20 %
$Cr_2O_7^{2-}$	**25**	Ni^{2+}	1000			$NaNO_3$	10 %
Cu^{2+}	1000	NO_2^-	1000			Na_2SO_4	10 %

Reducing agents interfere with the determination.

[1] tested with nonionic, cationic, and anionic surfactants

[2] ⚠ **A higher COD may impair the efficacy of the digesting mixture in the determination of total phosphorus and thus result in false-low readings. Up to a maximum of 1500 mg/l COD, this can be avoided by adding 2 doses of reagent P-1K instead of 1.**

5. Reagents and auxiliaries

Please note the warnings on the packaging materials!

The test reagents are stable up to the date stated on the pack when stored closed at +15 to +25 °C.

Package contents:
1 bottle of reagent P-1K
1 bottle of reagent P-2K
1 bottle of reagent P-3K
25 reaction cells
1 green dose-metering cap
1 blue dose-metering cap
1 sheet of round stickers for numbering the cells

Other reagents and accessories:
MQuant™ Phosphate Test, Cat. No. 110428,
measuring range 10 - 500 mg/l PO_4^{3-} (3.3 - 163 mg/l PO_4-P)
MColorpHast™ Universal indicator strips pH 0 - 14, Cat. No. 109535
Sulfuric acid 0.5 mol/l TitriPUR®, Cat. No. 109072
Spectroquant® CombiCheck 20, Cat. No. 114675
Spectroquant® CombiCheck 80, Cat. No. 114738
Phosphorus (total) standard solution CRM, 4.00 mg/l PO_4-P, Cat. No. 125047
Phosphorus (total) standard solution CRM, 15.0 mg/l PO_4-P, Cat. No. 125048
Hydrochloric acid 25 % for analysis EMSURE®, Cat. No. 100316

Pipette for a pipetting volume of 1.0 ml
Thermoreactor

Merck KGaA, 64271 Darmstadt, Germany,
Tel. +49(0)6151 72-2440
www.analytical-test-kits.com

EMD Millipore Corporation, 290 Concord Road,
Billerica, MA 01821, USA, Tel. +1-978-715-1335

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6. Preparation

- Use only phosphate-free detergents to rinse glassware. Otherwise fill with hydrochloric acid (approx. 10 %) and leave to stand for several hours.

At the first use **replace the screw caps of the reagent bottles P-1K and P-3K by the corresponding dose-metering caps:**

Reagent P-1K: green dose-metering cap
Reagent P-3K: blue dose-metering cap

Hold the respective reagent bottle **vertically** and, at each dosage, press the slide **all the way** into the dose-metering cap. **Before each dosage** ensure that the slide is **completely retracted.**

⚠ **Reclose the reagent bottles with the corresponding screw caps at the end of the measurement series, since the function of the reagents is impaired by the absorption of atmospheric moisture.**

- Analyze immediately after sampling.
- **Digestion for the determination of total phosphorus (Wear eye protection!):**

Pretreated sample	1.0 ml	Pipette into a reaction cell.
Reagent P-1K	1 dose[1]	Add, **close the cell tightly**, and mix.
Heat the cell at 120 °C[2] in the preheated thermoreactor for 30 min. Allow the closed cell to cool to room temperature in a test-tube rack. **Do not cool with cold water!**		

[1] in the case of high COD values: 2 doses

[2] A digestion temperature of 100 °C may result in false-low readings (e.g. in the case of polyphosphates).

- Check the phosphate content with the MQuant™ PhosphateTest.
  Samples containing more than 25.0 mg/l PO_4-P must be diluted with distilled water **prior to** digestion.
- **The pH must be within the range 0 - 10.**
  Adjust, if necessary, with sulfuric acid.
- Filter turbid samples.

7. Procedure

Pretreated sample **(10 - 35 °C)**	1.0 ml	Pipette into a reaction cell and mix or - after digestion for total phosphorus - shake the tightly closed cell vigorously after cooling.
Reagent P-2K[1]	5 drops[2]	Add, close the cell tightly, and mix.
Reagent P-3K[1]	1 dose	Add, close the cell tightly, and shake **vigorously until the reagent is completely dissolved.**
Leave to stand for 5 min (reaction time), then measure the sample in the photometer.		

[1] In the case of high chloride contents, it is recommended to switch the sequence of the reagents P-2K and P-3K.

[2] **Hold the bottle vertically while adding the reagent!**

Notes on the measurement:

- For photometric measurement the cells must be clean.
  Wipe, if necessary, with a clean dry cloth.
- Measurement of turbid solutions yields false-high readings.
- The pH of the measurement solution must be within the range 0.80 - 0.95.
- The color of the measurement solution remains stable for at least 60 min after the end of the reaction time stated above.

8. Analytical quality assurance

recommended before each measurement series
To check the photometric measurement system (test reagents, measurement device, handling) and the mode of working the phosphorus (total) standard solutions CRM, 4.00 mg/l PO_4-P (Cat. No. 125047) and 15.0 mg/l PO_4-P (Cat. No. 125048) - for the determination of total phosphorus - or Spectroquant® CombiCheck 20 and 80 can be used. Besides a **standard solution** with 8.0 mg/l PO_4-P (CombiCheck 20) or, respectively, 15.0 mg/l PO_4-P (CombiCheck 80) these articles also contain an **addition solution** for determining sample-dependent interferences (matrix effects).
Additional notes see under **www.qa-test-kits.com**.

Characteristic quality data:

In the production control, the following data were determined in accordance with ISO 8466-1 and DIN 38402 A51:

Standard deviation of the method (mg/l PO_4-P)	± 0.14
Coefficient of variation of the method (%)	± 1.1
Confidence interval (mg/l PO_4-P)	± 0.3
Number of lots	45

Characteristic data of the procedure:

Sensitivity: Absorbance 0.010 A corresponds to (mg/l PO_4-P)	0.1
Accuracy of a measurement value	max. ± 0.4

For quality and batch certificates for Spectroquant® test kits see the website.

9. Notes

- Reclose the reagent bottles immediately after use.
- **Information on disposal can be obtained at www.disposal-test-kits.com.**