Chapter 5

Post-treatment of anaerobically digested sewage for nutrient removal

A. V. Haandel[1]* , S. L. Santos[2], T. Allegue[3] and J. M. Garrido[4]

[1]Department of Civil Engineering, Universidad Federal of Campina Grande, Campina Grande- PB, Brazil
[2]Department of Civil Engineering, Universidad Federal of Rio Grande do Norte, Natal – RN, Brazil
[3]Department of Chemical Engineering, Khalifa University, Abu Dhabi, United Arab Emirates
[4]Department of Chemical Engineering, Universidade de Santiago de Compostela, CRETUS, Santiago de Compostela, Galicia, Spain
*Correspondence: adrianusvh@gmail.com

ABSTRACT

Although modern anaerobic sewage treatment systems and in particular the upflow anaerobic sludge blanket reactor (UASB) have demonstrated remarkable performance in tropical and subtropical regions, the effluent quality is generally not compatible with discharge standards in many countries, so post-treatment becomes necessary. In practice, the most widely applied post-treatment options are polishing ponds (PPs) and other aerobic treatment bioreactors. PPs are treatment units treating anaerobically pre-treated wastewater, which distinguishes them from conventional waste treatment ponds, wherein normally raw wastewater is treated. Anaerobic pre-treatment has a very profound influence on the configuration and operation of PPs, as will be shown in this chapter. Other aerobic post-treatments can be realized with conventional activated sludge systems, or more complex technological solutions including membrane bioreactors (MBRs) or trickling filters. The anaerobic–aerobic treatment has important advantages compared to the purely aerobic treatment: the volume and sludge production is much smaller, there is a possibility that the anaerobic–aerobic system operates with energetic self-sufficiency and the concentration of excess sludge is much higher facilitating its processing. An important problem of anaerobic–aerobic treatment is the difficulty of producing a final effluent with low nutrient concentration. Anaerobic sewage treatment reduces the content of biodegradable organic matter in the effluent, limiting in practice the removal of phosphorus to the use of chemical precipitation systems. For the case of nitrogen, the alternatives only go through new processes, which complement the poor capacity of conventional denitrification due to the limited concentration of biodegradable organic matter. This limits in practice, the nitrogen elimination capacity of activated sludge systems or trickling filters treating effluents from UASB reactors. A notable breakthrough could occur if reliable partial nitrification anammox processes for the water line were developed, as these are still under development, or systems in which the dissolved methane present in the anaerobic effluents was used to denitrify, as it has already been observed in some MBRs.

Keywords: aerobic post-treatment, anaerobic sewage digestion model for pH change, nutrient removal, organic material removal, pathogen removal, per capita area in polishing ponds, polishing ponds, transfer ponds.

5.1 INTRODUCTION

Anaerobic sewage treatment was applied in the first stage, to give adequate treatment to urban wastewater, in warm regions of the planet, through a system with low energy demand and scarce sludge production. This system was able to provide a cost-effective response to the elimination of oxygen-consuming organic substances that were previously discharged into the aquatic environment with untreated sewage. On the other hand, there is a growing concern about developing energy self-sufficient processes, even in other colder regions of the world as the US and Europe, for treating wastewater (Delgado Vela et al., 2015; EEA, 2022), where energy recovery through the methanation of organic matter can be the way to increase sustainability of the WWTPs.

Although efficient, anaerobic sewage treatment does not produce an effluent quality that is compatible with most environmental standards, and post-treatment is required to reduce the concentrations of four components: organic material, nitrogen, phosphorus, and pathogens. Poor nutrient removal is one of the main drawbacks of the anaerobic sewage technology, so its application in nutrient-sensitive areas will require the development of post-treatment processes that effectively remove nitrogen and phosphorus compounds, avoiding those that might imply eliminating the energy benefits of anaerobic treatments. The elimination of a large fraction of the organic matter in anaerobic treatment system limits, in practice, the use of conventional biological nutrient elimination, using enhanced biological phosphorus removal (EBPR) or conventional nitrification–denitrification processes due to the low BOD_5/P and BOD_5/TN of anaerobic effluents.

The effluents from anaerobic upflow anaerobic sludge blanket (UASB) treating sewage contain COD values between 100 and 200 mg/L; BOD_5 60–120 mg/L; NH_4^+-N 30–50 mg/L, and PO_4^{3-}-P 10–17 mg/L (Foresti et al., 2006). Additionally, the presence of other compounds as dissolved methane and hydrogen sulphide, varies depending on temperature and sewage composition. In practice two post-treatment biological systems have been mostly applied for anaerobically treated sewage: (1) polishing ponds (PPs) and (2) other aerobic post-treatment systems.

Treatment through PP is an evolution of the waste stabilization ponds (WSP). WSP are the oldest communal sewage treatment units (Parker et al., 1950). The proposed Australian system had the limited objective of removing organic material. To achieve this objective, the system was composed of a series of three ponds: an anaerobic pond (AP), for the removal of organic material by anaerobic digestion; followed by a facultative pond (FP), with mixed anaerobic and aerobic conditions for additional organic removal; and, one or more maturation ponds (MP) for further organic material removal in a predominantly aerobic environment. For the FP, the main design criterion is that there must be an equilibrium between the production of dissolved oxygen (DO), due to the photosynthesis of algae present in the pond, and DO consumption, due to the oxidation of organic material by bacteria. This means that the FP must be at least partially in an aerobic environment. In MPs, DO production is normally larger than DO consumption, so that MPs are predominantly aerobic.

Marais and Shaw (1961) established that WSPs were also efficient in removing pathogens: helminth eggs by sedimentation, and thermotolerant coliforms (TTC) decay with time. Marais (1974) established that TTC decay was a first-order process and optimized the MPs, showing the advantage of subdivision. Thus, a system was developed by combining the Australian system for organic material removal and Marais' system for pathogen removal, but for both purposes, a long retention time was required. In the 1960s and 1970s, it became clear that nutrient removal was necessary to avoid eutrophication. Although nutrient removal was attempted in WSPs, Pano and Middlebrooks (1982), Bastos et al. (2018), and Zimmo et al. (2003) have shown that nitrogen removal is at most partial and phosphorus removal is poor (Gomez et al. 2000). Thus, even though much research has been conducted (Ho et al., 2017), much less progress has been made to improve the four main problems of WSPs: (1) large surface areas required, (2) the liberation of biogas from the AP releases odorous compounds and greenhouse gases into the atmosphere, (3) long outfall to combat odour problems for the contributing population and (4) poor effluent quality since efficient nutrient removal is impossible. Secondary problems due

to the use of AP are (5) that the sewage suspended solids accumulate, so that operation must be interrupted to remove them every few years, and (6) due to the odour problem the WSP system must be constructed far away from the urban region, which means that a long and costly outfall is necessary.

Most of the disadvantages of anaerobic ponds are eliminated when they are replaced by an efficient anaerobic treatment unit like UASB (Lettinga *et al.*, 1980). The biogas is captured and can be used productively or flared off so that methane is converted into less offensive carbon dioxide. Also, the sewage suspended solids accumulate in the anaerobic reactor and can be discharged and transformed into a semi-solid or a solid, with the possibility of being used as an organic fertilizer. In addition, since at least in principle there is no biogas emission to the atmosphere, the odour problem does not exist and construction near or even within the urban region to be served is feasible. Today, more than 900 units are operating in Brazil alone (Van Haandel & Van der Lubbe, 2019; Van Lier *et al.*, 2020).

As anaerobic pre-treatment has a profound impact on all aspects of pond design and operation, it is convenient to use the term WSP for raw sewage treatment (Mara, 1997), and PP for post-treatment of digested sewage (Cavalcanti, 2003). For instance, the need for an aerobic condition in the FP integrated in WSPs, leads to a large area, because the oxidation of the AP effluent requires much oxygen, and the photosynthesis rate is relatively low because of the low transparency (presence of colloids) of the AP effluent. By contrast, after efficient anaerobic treatment photosynthesis develops at a higher rate in the clear effluent, whereas the oxygen demand is relatively low, so the oxygen production rate will always be greater than the oxygen consumption rate. This in turn means that there is an equivalent consumption of carbon dioxide to oxygen production, and this will trigger a pH increase in the PP, a feature that is not normally observed in WSPs. The pH increase makes nutrient removal feasible: nitrogen removal by desorption of ammonium gas and phosphorus by precipitation of phosphate.

The pH increase in the PP has a strong influence on the processes that develop, especially concerning the nutrients. In Figure 5.1, the fractions of gaseous (NH_3), and saline ammonia (NH_4^+) have been plotted as a function of pH. The NH_3 fraction increases from a very small value at pH $= 7$ (the likely pH of anaerobic effluent) to a fraction of 50% of the total ammonium concentration at pH $= 9.3$. The mechanism of nitrogen removal in PP is the desorption of gaseous ammonia from the liquid phase. The desorption/absorption processes can be described in terms of Fick's law, which says that the rate of the processes is proportional to the inductive force of those processes. In the case of volatile components (CO_2 and NH_3), the inductive force is the difference between the saturation concentration and the actual concentration.

In Figure 5.2, the fractions of $H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-} are plotted as functions of pH. The removal mechanism of phosphate in PP is due to PO_4^{3-} precipitation with cations that may be present

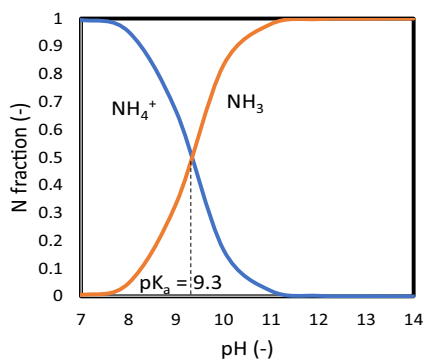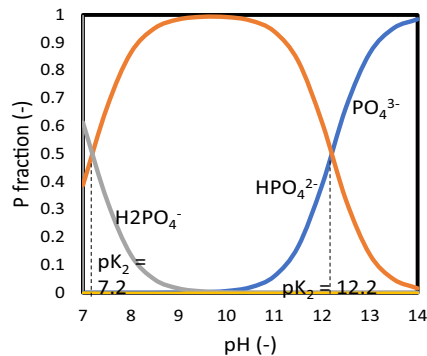**Figure 5.1** NH_3 and NH_4^+ fractions as a function of pH.

Figure 5.2 $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-} fractions as a function of pH (H_3PO_4 is not shown).

in the wastewater (like Ca^{2+}) or may be added during or after the treatment in the PP (like Al^{3+}). Figure 5.2 shows that the pH must be high before a considerable PO_4^{3-} fraction is formed and precipitation can take place. When Figures 5.1 and 5.2 are compared it is concluded that nitrogen will be removed before phosphate removal will occur.

In anaerobic–aerobic treatment systems, an aerobic post-treatment system like activated sludge or biofilm bioreactor is preceded by efficient anaerobic treatment for example in a UASB reactor. In principle, this anaerobic–aerobic set-up can produce a final effluent equivalent to purely aerobic systems except, as stated, for the possibility of removing nutrients efficiently: the removal of a large part of the biodegradable material in the anaerobic pre-treatment is an impediment for removal of nitrogen through nitrification and heterotrophic denitrification and of phosphorus through luxury uptake.

Important advantages of anaerobic pre-treatment are that all factors of investment and operational costs are strongly reduced: the anaerobic–aerobic system can be constructed with a much smaller volume than purely aerobic systems, and the costs for aeration energy and destination of sludge production are also significantly reduced. In many anaerobic–aerobic treatment systems, the UASB reactor is not only used for digestion of influent organic material but also for the stabilization of aerobic excess sludge. In this chapter, it will be shown that this procedure often leads to very serious operational problems and for that reason is not recommendable without a suitable adaptation which will be presented in the following sections.

Chemical precipitation of phosphorus is the only alternative available to reduce its concentration in wastewater with low organic matter content, such as anaerobic bioreactors effluents. For chemical precipitation, calcium, aluminium, or iron salts are used, which form an insoluble metal phosphate salt that can be removed by settling. Hydrated aluminium sulphate ($Al_2(SO_4)_3 \cdot 14H_2O$) is the most common aluminium salt used to remove phosphate. One mole, that is 594 g salt, reacts with 2 moles of phosphate containing 61.9 g of phosphorus to form 244 g of $AlPO_4$.

$$PO_4^{3-} + Al^{3+} \rightarrow AlPO_4 \downarrow$$
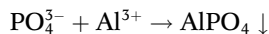
Iron salts are commonly used in the precipitation of phosphate from wastewater. Both ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions can be used in the form of chloride or sulphate. If ferrous salt is used, this should be oxidized to the ferric ion (Fe^{3+}), using oxygen, this should be taken in the oxygen balance. Stoichiometrically, 55.85 g of Fe ion will react with 30.95 g of P to form 150.8 g of $FePO_4$.

$$PO_4^{3-} + Fe^{3+} \rightarrow FePO_4 \downarrow$$
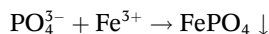
Unluckily, the metal ions should be present in excess relative to the phosphorus ion concentration to achieve a low level of dissolved phosphorus compounds, increasing the expected cost of chemical precipitation. The EPA Design Manual (1976) suggests that 20% excess of aluminium ions is necessary if an 80% reduction of phosphorus is desired, while 100% excess is recommended for 95% phosphorus removal. The exact dosage is determined by on-site testing and varies with the specific characteristics of the sewage.

Lime is used as an alkalinity and calcium source to promote chemical P precipitation. Although it is possible to calculate an approximate lime dose for phosphorus removal, this is generally not necessary. In contrast to iron and aluminium salts, the lime dose is largely determined by other reactions that take place when the pH of wastewater is raised. Only in waters of very low bicarbonate alkalinity would the phosphate precipitation reaction consume a large fraction of the added lime. Lime, which previously was one of the main chemicals used for phosphorus removal is nowadays less frequently used because of increased production of sludge as well as the operational and maintenance problems associated with its use. The presence of Ca and Mg in the treated sewage is the main cause of the phosphate removal described for PPs, as will be discussed much more thoroughly in this chapter, due to its precipitation determined by the pH increase generated by photosynthesis.

For the case of nitrogen, traditionally the denitrification process in biological systems is carried out by heterotrophic bacteria, which reduce nitrate to molecular nitrogen by oxidizing organic material. Mulder (1995) discovered anammox bacteria that are capable of autotrophic denitrification by oxidizing ammonia with nitrite anaerobically. If the concentration of organic material is low, such as in anaerobically pre-treated wastewater, heterotrophic denitrification may not be possible, because of the imbalance between the oxidant (nitrate) and the reductant (organic material in this process). In that case, autotrophic denitrification is very advantageous not only because no organic material is required, but also for the reduction of the oxygen demand. However, the removal of nitrogen in the water line through this process is still a challenge.

In this chapter are presented in more detail strategies to improve the removal of nutrients for anaerobically treated sewage with poor organic matter content, using PPs and aerobic biological systems.

5.2 POST-TREATMENT IN PPS

5.2.1 Main processes that develop in PPs

In a PP, the concentration of the many constituents of the digested sewage changes over time, because of biological, chemical, and physical processes taking place in the unit. The most important biological processes are: (1) photosynthesis, (2) oxidation of organic material by bacteria that use oxygen, and (3) fermentation of organic material by anaerobic digestion. These three processes directly affect the removal of organic material. Figure 5.3 shows a schematic representation of a pond and the interaction between the different biological processes. During photosynthesis the algae use solar energy to reduce carbon dioxide, transforming it into cellular organic matter. In a simplified way this process can be represented by Equation (5.1a) where the organic matter is expressed as chemical oxygen demand (COD):

$$CO_2 + H_2O \rightarrow COD + O_2 \tag{5.1a}$$

It is important to note that, necessarily, the generation of organic material (in terms of COD mass) accompanies the generation of oxygen, that is, the mass of oxygen generated by photosynthesis is exactly the stoichiometric amount required to oxidize the organic material that is also generated in the same process. However, this oxygen is generally not used for oxidation of cellular material of algae,

but rather to oxidize the faecal organic material present in the sewage. This bacterial oxidation can be represented by the following schematic equation:

$$COD + O_2 \rightarrow CO_2 + H_2O \tag{5.1b}$$

When analysing Figure 5.3, it is observed that the processes of photosynthesis and bacterial oxidation are complementary in the sense that the products of one process are the reactants of the other. This shows that, in principle, there can be no net removal of the organic material through oxidation if there is no source of oxygen other than photosynthesis, such as atmospheric oxygen uptake. However, the combined action of photosynthesis and oxidation may result in the removal of organic matter from the liquid phase by other mechanisms. The net result of the development of the two processes is the transformation of faecal organic matter into the mass of microalgae and bacteria. This mass of microorganisms can form flocs through spontaneous flocculation and these flocs can settle down and become part of the bottom sludge of the pond, where they can be digested or accumulated as non-biodegradable compounds (Figure 5.1).

The relative rates of the two biological processes, directly affect important parameters such as DO, CO_2, biochemical oxygen demand (BOD_5), COD, suspended solids (SS), and pH, while others are indirectly affected, such as alkalinity and nutrient concentrations. The relative rates of the two processes are determined mainly by the conditions of transparency, solar irradiation, temperature, depth of the pond available for photosynthesis, and concentration of the biodegradable organic material for bacterial oxidation.

In the case of PP, the concentration of organic matter from the digested sewage is low and the ponds will have typical characteristics of maturing ponds in WSP, especially if they are shallow (larger area favourable to photosynthesis). Under these conditions, it is possible to achieve high oxygen concentration, and there may even be oxygen desorption from the supersaturated liquid phase into the atmosphere. The predominance of photosynthesis over oxidation has another consequence: CO_2 is consumed, which increases pH. In this respect, the PP is different from WSP where there is an equilibrium between oxygen production by algae and consumption by bacteria.
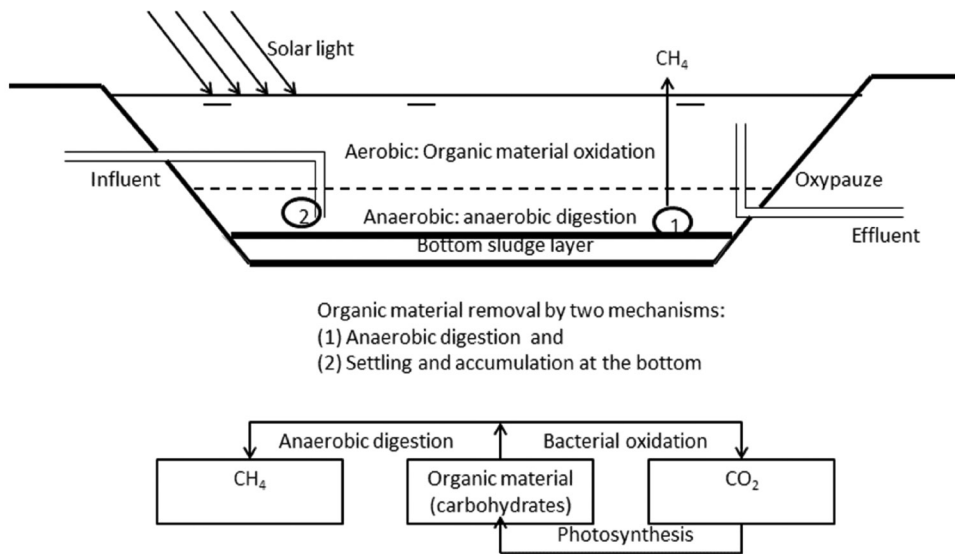
Figure 5.3 Schematic representation of a PP and of the biological processes that develop in it.

The third important biological process is anaerobic digestion, which develops at the bottom of the PP, where the oxygen from photosynthesis does not reach or, if it arrives, is immediately consumed. The process can be represented schematically as

$$COD \rightarrow CH_4 + CO_2 \tag{5.1c}$$

Strictly speaking, anaerobic digestion also does not destroy the organic material, merely turns it into a gas (methane) that is released, thus effecting the physical removal of the organic material from the liquid phase and therefore the effluent quality.

PPs are affected by five components of the anaerobic effluent: (1) the organic material concentration (BOD and COD), (2) pathogens, especially TTC, (3) pH, (4) nitrogen, and (5) phosphorus.

Concerning organic material removal in PP, Figure 5.3 shows that if photosynthesis has a higher rate than organic material oxidation, there is an increase of algae in the pond. However, on the contrary Aguiar *et al.* (2021) have shown that the BOD in PP is reduced to low values (order of 20–40 mg/L) after a short retention time of only 5 days. The final COD concentration after 5 days is much higher (150–250 mg/L). The explanation is that because the efficient pretreatment in the UASB reactor, it is much easier to remove the residual organic material in the PP. The BOD and COD tend to pass through a minimum at 5 days, whereafter their values tend to increase due to algae production.

Marais (1974), reporting on the pioneering work on kinetic modelling of decay (Marais & Shaw, 1961) has shown that this process can be described as a first-order reaction with the rate of reduction of TTC bacteria proportional to the existing number, that is, it occurs according to Chick's law (Chick, 1908):

$$r_d = (dN/dt) = K_b N_{ttc} \tag{5.2}$$

where r_d = decay (death) rate of the TTC; N_{ttc} = concentration of the TTC (UFC/100 mL); t = decay time (d); K_b = decay constant for TTC (per d).

In chemical engineering, particularly in reactor design calculations, first-order processes have been thoroughly studied, because they are the type that occurs very frequently in practice. It is possible to use the theoretical models developed for the calculation of reactors, to describe the process of decay of TTC in ponds. The most important aspect of pond design is the influence on bacterial decay is the required retention time for a particular TTC removal efficiency. The differential equation (5.2) can be solved for flow through ponds and sequential batch ponds. For a pond operating at constant flow and volume, the solutions are

(1) Sequential batch ponds: $N_e/N_i = \exp(-k_b R_h)$ (5.2a)

(2) Flow through ponds: $N_e/N_i = 1/(1 + k_b R_h)$ (5.2b)

(3) For a series of equal ponds (M), the expression becomes: $N_e/N_i = 1/(1 + k_b R_h/M)M$ (5.2c)

It can be noted that all expressions have the same dimensionless group $k_b R_h$.

where N_e = TTC concentration in the effluent; N_i = TTC concentration in the influent; R_h = retention time in a pond system; R_h/M = retention time in pond 'M' of a series; M = number of ponds in series.

Figure 5.4 shows a graphical representation of Equations (5.2a) to (5.2c): the removal efficiency of TTC (log scale) is plotted in function of the product $k_b R_h$, which is a dimensionless number. The value of the decay constant can be determined experimentally when the decay of TTC is observed as a function of time in batches of PPs at different temperatures and depths: Batista *et al.* (2021) proposed the following equation:

$$k_b = (1.6/H)1.07^{(t-25)}d^{-1} \tag{5.2d}$$

where: H = pond depth in m; T = temperature in °C.
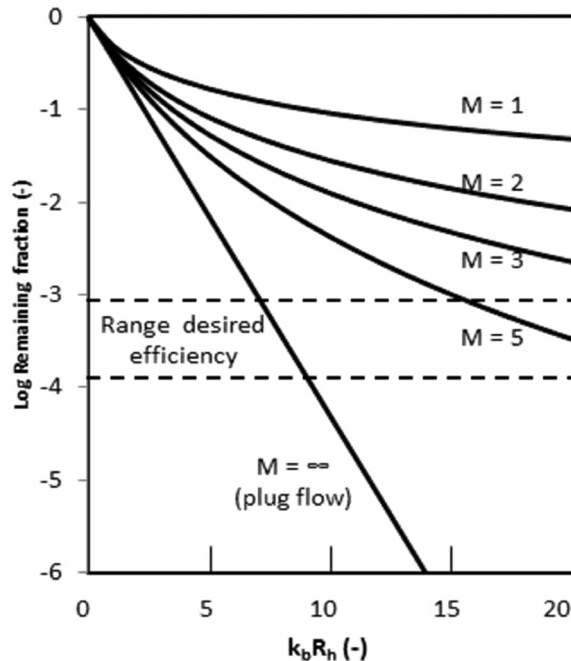
Figure 5.4 Relationship between the reminiscent fraction of TTC and the dimensionless product $k_b \times R_h$, for a number of lagoons, M, of 1, 2, 5, and infinite.

Digested sewage may have a concentration of 10^6–10^7 UFC $\times$ 100 per mL (i.e., 10% of the concentration in raw sewage), whereas the WHO standard specifies a maximum of 10^3 UFC $\times$ 100 per mL. Hence to comply with the recommendations of WHO for unrestricted irrigation, the reduction of the number of TTC must be of the order of 10^3–10^4 UFC $\times$ 100 per mL, that is, the desired removal efficiency must be of the order of 99.9 to 99.99%.

5.2.2 Hydrodynamic regime in PPs

The most common form of operation of PPs is a continuous flow regime (CFPP), due to greater operational simplicity. However, Albuquerque *et al*. (2021) showed that it is possible to operate them in sequential batch mode regime (SBPP) and that this alternative can have important advantages, such as a faster removal of nutrients and pathogens, resulting in a reduction of the required footprint. The main difference between CFPP and SBPP operation mode is the pH trend. In the CFPP pond, the pH has the maximum value during the entire retention time (which is also the discharge pH) and the high pH reduces the photosynthetic activity. On the contrary, in the SBPP, the pH is initially neutral (UASB effluent), increasing during the retention time, reaches its maximum value just before the effluent discharge. In Table 5.1, the differences between the various operating parameters in SBPP and CFPP are summarized.

While sequencing, batch PPs undoubtedly have significant advantages over continuous flow PP, a problem is that the sewage flow is continuous and the SBPP discontinuous. One solution for this problem is to introduce a transfer pond that continuously receives the UASB effluent, from where it can be distributed in batches to PPs as shown in Figure 5.5. The transfer pond would have at least the same volume as the PPs. The advantage of having a transfer pond is that besides transferring digested effluent to the PPs, it can realize several other functions, the most important being:

Table 5.1 Differences between SBPP and CFPP.

	SBPP	**CFPP**
Feed	Batch-wise	Continuous flow
DO concentration	Initially zero Final maximum	Constant, lower than maximum of SBPP
pH	Initially 7, Final maximum.	Constant lower than maximum of SBPP
BOD & COD removal	Slow COD and BOD increase due to algae flocculation	Rapid increase of COD and BOD
N removal	Minimum time for removal	Maximum time for removal
P removal	Minimum time for removal	Maximum time for removal
Thermotolerant bacteria removal	Minimum time for removal	Maximum time for removal
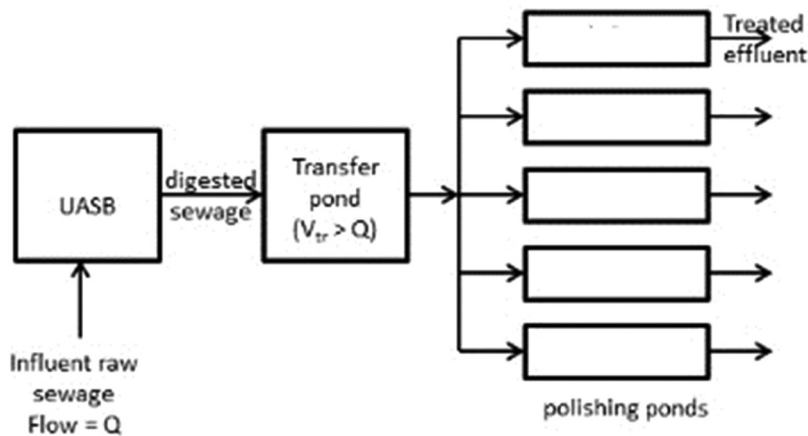
Figure 5.5 Schematic representation of the flow sheet and operation of PPs fed with sequential batches from the transfer pond.

- The transfer pond also functions as a settling tank for sludge solids eventually discharged from the UASB reactor. Part of the settled solids could then be returned to the UASB reactor, and the remainder be processed to produce a sludge cake or dry solids.
- If the transfer pond is a settler, there is no need to have a settler on top of the UASB reactor. If this structure is modified, the operation of UASB reactors becomes very easy. The principal operational problem of UASB reactors is the scum layer that forms at the surface of the interface under the separator elements. If the separator is not more necessary, the scum problem no longer exists.
- If the retention time of the transfer pond is high enough, the process of photosynthesis can begin and establish a population of algae in the discharge to the PPs, so photosynthesis can start immediately, and algae are transferred to the PPs at the beginning of their operation. If sufficient oxygen is produced by photosynthesis in the transfer pond it can oxidize sulphides that may have been formed in the UASB reactor, thus suppressing the bad odours that could otherwise arise.
- If the retention time in the transfer pond is sufficient, helminth eggs from the UASB reactor may be retained and not discharged into the PPs, thus ensuring that these pathogens will not be present in the final effluent. At any rate, a large fraction of the helminth eggs will be retained in the transfer pond and will end up in the excess sludge.

- The concentration of carbon dioxide dissolved in the UASB reactor effluent will be reduced by the desorption mechanism, reducing the acidity and facilitating the increase of pH by photosynthesis, thus enabling the removal of ammonia nitrogen from the liquid phase in the subsequent PP by desorption.

5.2.3 Experimental research

Even in efficient anaerobic treatment systems, the concentration of several constituents of the effluent is high and post-treatment is required. The most important constituents that normally need correction are (1) organic material, (2) nitrogen, (3) phosphorus, and (4) pathogens. An experimental investigation was carried out at a pilot scale to establish if the removal of these constituents is feasible in a sequencing batch PP and principally what are the required depths and retention times.

Having established that SBPPs are more efficient than FTPPs, experiments were carried out at a pilot scale to evaluate the feasibility of removing pathogens, residual BOD and nutrients in SBPP. Figure 5.6 is a flow sheet of the employed system and a photo of a series of four pilot scale ponds.
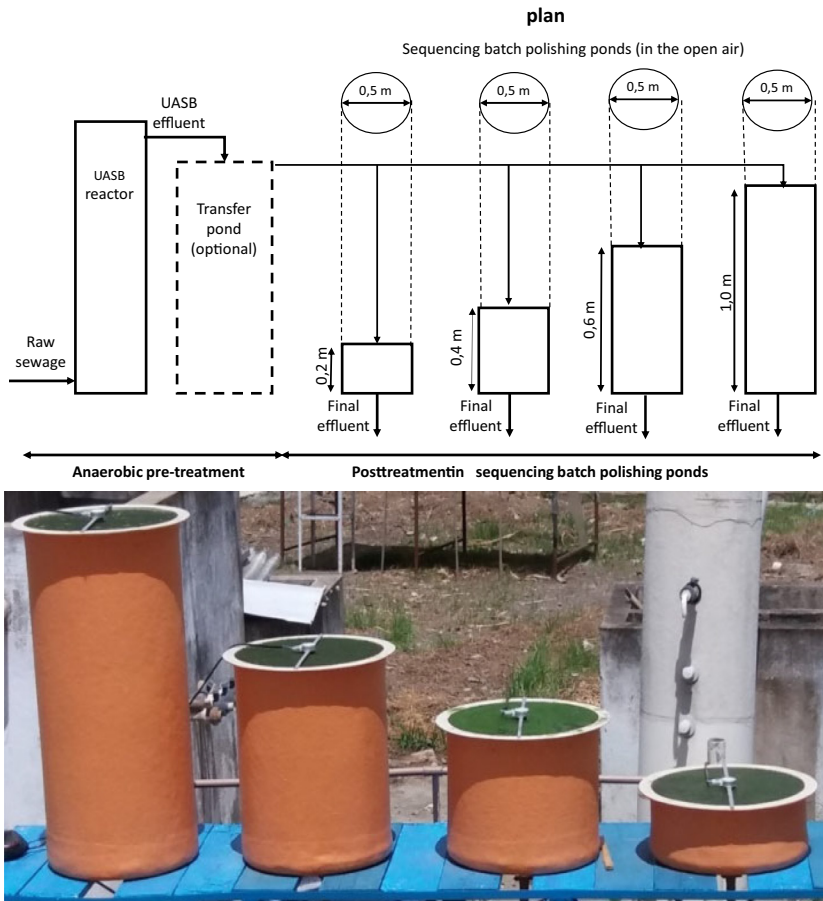

Figure 5.6 Flow sheet and photograph of a system of SBPPs with depths of 0.2, 0.4, 0.6, and 1.0 m with mild stirring of the pond contents.

The experimental investigation was carried out in Campina Grande-Brazil (7 degrees south), using municipal raw sewage as influent of a UASB reactor, operated at a retention time of 6 h. Shallow ponds were used, because it was shown that these operated at a high rate. These pond models were operated with very gentle superficial stirring with a shallow metal bar attached to a small motor (6 rpm) in order to re-suspend any algae, floated by bubbles of dissolved oxygen, evolving from the ponds when these were supersaturated with DO. At the same time, the agitation served to even out stratification in the liquid phase. Later experiments showed that this agitation had a negligible effect on the performance of the ponds.

The experiments were carried out on four glass fibre cylinders with a diameter of 0.5 m and depths of 0.2, 0.4, 0.6, and 1.0 m. The experiments were carried out in the open air over a period of nine months: during summer (6 months) and winter periods (3 months). In Campina Grande there is abundant sunshine and sewage temperature is always 25°C, but in 'winter' there is more rainfall.

Tests for DO, pH, alkalinity, COD, nitrogen and phosphorus were carried out, according to standard methods and procedures. Distilled water was used to compensate losses due to evaporation and sampling. SBPP operation was continued until pH reached a value of about 9.7 or when the maximum retention time of 30 days was exceeded. For measurements of DO, temperature, and pH, a multi-parameter instrument was used (Hanna, model HI 98196).

Figure 5.7 shows experimental results of the investigation. For comparison, only retention times of 30 days were investigated, as this is the normal retention time in WSP.
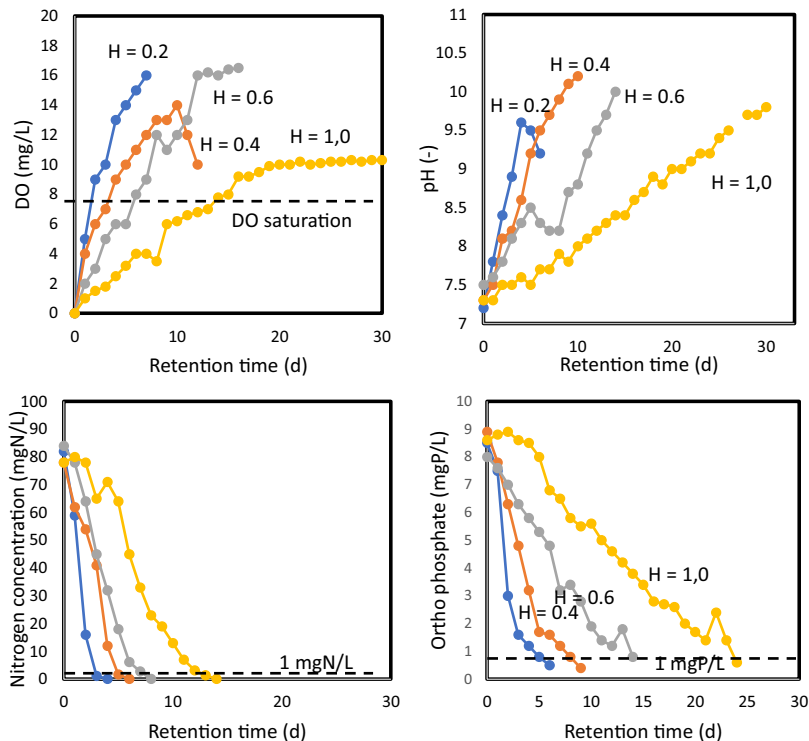
Figure 5.7 Average values of DO concentration, pH, ammonium nitrogen and phosphate concentrations as functions of time in SBPP with different depths (0.2–1.0 m).

Average values of the DO concentration, the pH value, ammonium nitrogen and phosphate were represented over time for the four investigated depths and led to the following observations:

The DO concentration increased in all SBPP from the first day onwards, but the rate of increase heavily depended on the depth of the pond. Very high DO concentration > 20 mg/L tended to become unstable, possibly due to a decreasing oxygen production at high pH values.

pH increased with time, but at very different rates, also depending on the pond depth. Very high pH values (pH >11) could not be sustained in the ponds, probably due to reduced photosynthetic CO_2 consumption.

Nitrogen removal was feasible in shallow ponds, but the required retention time was longer during rainy periods, when there was less sunshine.

The pond depth has a very strong influence on the time required for total nitrogen removal, varying from 4 to 14 days at an initial concentration of about 80 mg/L. A pH of 8.5 was sufficient to remove nitrogen.

Efficient phosphate removal was possible in all ponds, but the retention time varied strongly with depth (6–24 days) for depths of 0.2–1.0 m. A pH of 9.5–9.7 is required for efficient P removal.

It is important to note that the profiles of Figure 5.5 are all influenced by the climate so that under different circumstances the results will be different. Ideally for any project an experimental investigation should be carried out.

With the aid of the data in Figure 5.6, models were developed that can foresee the values of pH, nitrogen, and phosphorus vs time. The following sections show the development of the models for pH, nitrogen, and phosphorus removal.

5.2.4 Model for pH change and nitrogen removal in sequencing batch PPs

The pH value and its stability in sewage treatment plants are determined almost entirely by the carbonic system. If the state of the carbonic system is to be evaluated, the definitions of alkalinity and acidity are important. Loewenthal and Marais (1976) have proposed:

$$Alk = 2[CO_3^-] + [HCO_3^-] + [OH^-] - [H^+] \tag{5.3a}$$

$$Ac = 2[CO_2] + [HCO_3^-] + [H^+] - [OH^-] \tag{5.3b}$$

The pH variation in treatment systems is due to variations in alkalinity and acidity, which in turn are affected by the physical, chemical, and biological processes occurring in these systems. To analyse the pH variation, the Deffeyes diagram (Deffeyes, 1956), improved by Loewenthal and Marais (1976), is very useful. It shows that there is a linear relationship between alkalinity and acidity for any pH. By using the dissociation equations for the carbonic system, Equations (1a) and (1b) can be used to relate three variables: alkalinity, acidity, and pH:

$$Alk = [Ac + 10^{(pH-pK1)} - 10 - pH] * [1 + 2.10^{(pH-pK2)}] / [1 + 2.10^{(pK1-pH)}] \tag{5.4a}$$

where k_1 = first ionization constant of carbonic system (Harned & Scholes, 1943); k_2 = second ionization constant of carbonic system (Harned & Davis, 1943).

If the pH is in the neutral range (5 < pH < 9) the influence of the carbonic system is decisive and the influence of the ions H^+ e OH^- can be neglected, so Equation (2) is simplified to

$$Alk \approx Ac[1 + 2 \times 10^{(pH-pK2)}] / [1 + 2 \times 10^{(pK1-pH)}](5 < pH < 9) \tag{5.4b}$$

Figure 5.8 is a Deffeyes diagram, drawn from Equation (5.3). In the diagram, two values of the three variables: (i) alkalinity, (ii) acidity or (iii) pH define the ionic equilibrium point, which characterizes the condition of the liquid phase. Figure 5.8 shows how the ionic equilibrium point moves, when adding to or removing materials from the liquid phase. Thus, according to Equation (5.3a), the
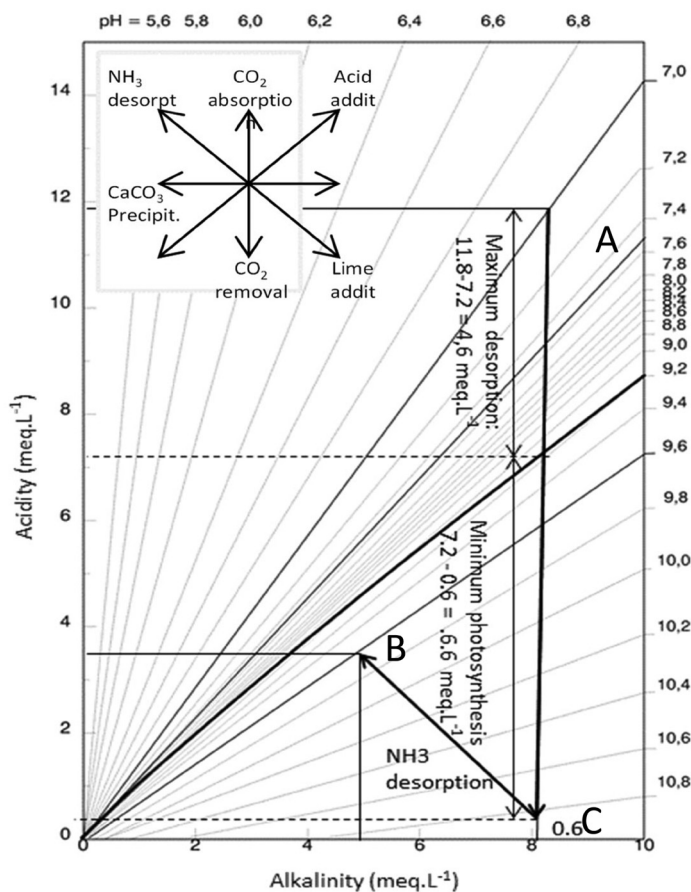
Figure 5.8 Deffeyes diagram (temperature of 25°C: $pK_1 = 6.33$, $pK_2 = 10.33$, $pK_w = 14$).

alkalinity does not change upon CO_2 removal, but the acidity decreases by 2 eq/mol. For example, if the effluent from a UASB reactor has a pH = 7 and an alkalinity of 8 meq/L, (point A), the acidity will be about 12 meq/L.

Once established, the processes occurring in the treatment system and knowing what are the effects of these processes on the alkalinity and acidity, it is possible to establish the variation of alkalinity and acidity and determine the pH in the treatment system and in the effluent. For example, when it is known that in the treatment system, the alkalinity decreases by 3 meq/L (with a final alkalinity of 5 meq/L) and if a final pH = 9.6 is desired, then B is the final ionic equilibrium point, with an acidity of 3.4 meq/L. For this to take place the acidity has to decrease by 0.6 from 12 to 11.4 meq/L. Such a decrease results from desorption or biological consumption of carbon dioxide.

In PPs, the main biological, chemical, and physical processes that can alter alkalinity and/or acidity and therefore the pH are: (1) biological or physical removal of CO_2 and (2) physical removal of NH_3 by desorption, which is equivalent to the addition of a strong acid. Although at high pH the liquid phase may be super saturated with calcium carbonate, precipitation of $CaCO_3$ does not take place in ponds. With Figure 5.7, it is possible to predict the effect of varying the concentration of carbon dioxide and ammonia on the value of alkalinity, acidity, and pH as shown in Table 5.2.

Table 5.2 Effect of CO_2 and NH_3 removal on alkalinity and acidity change and on pH variation in treatment systems. (OM = organic matter).

Process	Reaction	ΔAlk (eq/mol)	ΔAc (eq/mol)	ΔpH
Physical CO_2 removal	No	0	-2	Increase
Biological CO_2 removal	$CO_2 + H_2O \rightarrow OM + O_2$	0	-2	Increase
NH_3 desorption	$NH_4^+ \rightarrow NH_3 + H^+$	-1	1	Decrease

In PPs, it is important to know the time it takes for the pH to reach a certain value which depends on the kinetics of the relevant processes. To establish the retention time required to obtain a certain pH change in a SBPP it is necessary to formulate equations expressing the rates of the three pH affecting processes. The desorption rate of volatile components in water like CO_2 and NH_3 can be described by Fick's equation (Equation (5.4)), which states that the desorption rate of a volatile compound in a liquid is proportional to the degree of supersaturation existing between the current concentration of the compound and the saturation concentration:

$$r_d = k_d(C_l - C_s) \tag{5.5}$$

where: r_d = desorption of the volatile compound; k_d = desorption constant; C_s = saturation concentration of the volatile compound; C_l = concentration of the volatile compound in the liquid phase.

Thus, the rates of transfer for NH_3 and CO_2 can be expressed as

$$r_{dN} = k_{dN} \cdot [NH_3], \tag{5.5a}$$

$$r_{dC} = k_{dC} \cdot ([CO_2] - [CO_2]_s) \tag{5.5b}$$

When the differential equations (5.4a) and (5.4b) are integrated expressions found for CO_2 and NH_3 as functions of time so that the values of the constants can be determined experimentally:

$$Ln\{([CO_2] - [CO_2]_s) / ([CO_2]_o - [CO_2]_s)\} = -k_{dC}t \tag{5.6a}$$

$$Ln([NH_3] / [NH_3]_o) = -k_{dN}t \tag{5.6b}$$

Morais *et al.* (2023) determined experimentally the values of the transfer constants as functions of temperature (T) and pond depth (H):

$$k_{dC} = (0.56/H)1.056(T - 20) \tag{5.7}$$

$$k_{dN} = (0.20/H)1.095(T - 20) \tag{5.8}$$

For a specific depth and temperature (which define the values of k_{dC} and k_{dN}), the only process that is not yet defined in Table 5.2 is the rate of CO_2 consumption or oxygen production (OPR), but this can be measured as the rate of change in the DO concentration (Equation (5.9)). The rate of CO_2 consumption can be conveniently determined by the oxygen production rate in the pond by considering that if 1 mol of oxygen is produced 1 mol of CO_2 is consumed. The rate of oxygen production can be expressed as

$$OPR = -(dCO_2/dt)_c - (dDO/dt)_p \tag{5.9}$$

where:

$OPR = (dDO/dt)_p$ = DO production rate in the pond (mol DO/L/d)
    = photosynthesis rate – DO consumption rate
    = biological CO_2 consumption rate (mol CO_2/L/d)

Equation (5.9), together with Equations (5.5)–(5.8), are applied for the calculation of pH variation and ammonia removal in SBPP. Since these equations cannot be solved analytically, they must be solved numerically. The differential equations must be written in terms of finite time elements that will cause finite changes in the concentrations of CO_2 and NH_3, as in Equations (5.10) and (5.11):

$$\Delta[CO_2] / \Delta t = -k_{dC}([CO_2] - [CO_2]_s) - \text{OPR} \tag{5.10}$$

$$\Delta[NH_3] / \Delta t = [NH_3] - k_{dN}[NH_3]_o \tag{5.11}$$

These variations, in turn, result in alkalinity and acidity changes as well as ammonia concentration variations that can be expressed as

$$\Delta\text{Alk} = -k_{dN}[NH_3]\Delta t \tag{5.12}$$

$$\Delta\text{Ac} = -2\{k_{dC}([CO_2] - [CO_2]_s) - 2\text{OPR} + k_{dN}[NH_3]\}\Delta t \tag{5.13}$$

$$\Delta N_{tot} = N_{tot} - k_{dN}[NH_3]\Delta t \tag{5.14}$$

5.2.4.1 pH variation in SBPP

The pH variation can be calculated from the alkalinity and acidity changes. Considering that H^+ e OH^- concentrations are small and do not influence the alkalinity and acidity one has

$$\text{Alk} = \text{Ac}(1 + 2k_2 / [H^+]) / (1 + [H^+] / k_1) \tag{5.15}$$

This is an implicit quadratic expression and can be solved for $[H^+]$, resulting in

$$[H^+] = \{k_1(\text{Ac/Alk} - 1) / 2 + (k_1(\text{Ac/Alk} - 1)/2)2 - 4k_1k_2)0.5\}/2$$

Since $pH = -\log[H^+]$ one has

$$pH = -\log\{(0.5(k_1(\text{Ac1} / \text{Alk1} - 1) / 2 + ((k_1(\text{Ac1/Alk1/2})2 - 4k_1k_2)1/2)\} \tag{5.16}$$

5.2.4.2 Nitrogen removal in SBPP

To calculate nitrogen removal in SBPP, it is considered that the ammonia ion (NH_4^+) and gaseous ammonia (NH_3) are in equilibrium in the liquid phase:

$$NH_4^+ \leftrightarrow NH_3 + H^+ \tag{5.16a}$$

$$K_a = [H^+] \cdot [NH_3] / [NH_4^+] \tag{5.17}$$

Equation (5.17) shows that the removal of ammonium is equivalent to the addition of a strong acid, that is, per mmol of desorbed NH_3, there is a decrease of 1 meq of alkalinity and an increase of 1 meq of acidity.

According to Emerson *et al.* (1975), the ionization constant is given by Equation (5.19):

$$pK_a = 0.09018 + 2729.92 / T \ (T \text{ is in Kelvin}) \tag{5.19}$$

Thus, at a temperature of 298 K and a fixed solids concentration of 1 g/L (i.e., an activity coefficient of about 0.9), the dissociation constant is $pK_a = 9.1$. Therefore, for pH values greater than 9.1, the non-ionized form, NH_3, is predominant, according to Equation (9.13). In contrast, for neutral pH values the non-dissociated ammonia fraction is small (1% for pH = 7.1) and accordingly, the desorption rate NH_3, will be low.

Table 5.3 shows a summary of the equations to evaluate the effects of the above-mentioned processes on alkalinity and acidity and consequently on pH trends in PPs vs time. Now the basis for a model is developed, able to evaluate the variations in pH, alkalinity, acidity, and ammonium concentration as

Table 5.3 Equations to calculate the rates of processes and concentration trends of the key operating variables in SBPP.

DO production rate	$OPR = \Delta OD / \Delta t = -(\Delta CO_2 / \Delta t)_f$	(5.8)
NH_3 desorption rate	$r_{dN} = (\Delta[NH_3] / \Delta t)_d = k_{dN}[NH_3]$	(5.4a)
CO_2 desorption rate	$r_{dC} = (\Delta[CO_2] / \Delta t)_d = k_{dC}([CO_2] - [CO_2]_s)$	(5.4b)
Alkalinity change:	$\Delta Alc / \Delta t = -k_{dn}[NH_3]$	(5.11)
Acidity change:	$\Delta Ac / \Delta t = -2\{k_{dc}([CO_2] - [CO_2]_s) - 2OPR + k_{dn}[NH_3]\}$	(5.12)
pH change:	$pH = -\log\{(0.5(k_1(Ac / Alc - 1) / 2 + ((k_1(Ac / Alc / 2)^2 - 4k_1k_2)^{1/2})\}$	(5.15)
CO_2 conc. change	$\Delta[CO_2] / \Delta t = -k_{dc}([CO_2] - [CO_2]_s) - OPR$	(5.9)
NH_3 conc. change	$\Delta[NH_3] / \Delta t = -k_{dn}[NH_3]$	(5.13)
Change of total N	$\Delta N_{tot} / \Delta t = N_{tot} - k_{dn}[NH_3]$	(5.16)

Legend: K_{dN}, k_{dc} = desorption constant for NH_3 and CO_2, respectively; indices s and o stand for saturation and time = 0.

function of time in ponds operated in batch mode. The model is a set of differential equations defined by Equations (5.8)–(5.18).

The model illustrated in Table 5.3 can be used to calculate the values of the alkalinity, acidity, pH, carbon dioxide, and total nitrogen as a function of time in SBPPs. The simulated curves thus generated are compared to the experimental values in Figure 5.9a and b, where experimental values of pH and nitrogen (reported in Figure 5.6) and the simulated curves calculated from Table 5.3 are presented for the four investigated depths. There is a good correlation between the experimental and simulated values for all depths. The correlation for pH sometimes is not good for high pH values. This finding can probably be attributed to the fact that at very high pH values the photosynthesis rate decreases, so that the rate of pH increase is reduced and may even become negative, and this occurrence is not included in the model of Table 5.3.

The pH curves show that there are three phases of pH change: (1) an initial rapid change due to CO_2 desorption (2) a slower intermediate rate of pH change due to acidity production by ammonium desorption and (3) a quick final change due to completion of ammonia removal. Similarly, ammonia removal has also three phases: (1) a slow initial phase because the pH and ammonia desorption are still low, (2) a higher rate of ammonia removal when the NH_3 concentration is high, and (3) a low final rate of ammonia removal when the NH_3 concentration is low at the end of a batch, because the total N concentration is low even if the pH is high.

5.2.5 Model for phosphorus removal in sequential batch PPs

To model the P concentration in the PP is difficult because several compounds interact. In fact, there are different phosphorus compounds that may precipitate, and several cations in the liquid phase. Therefore, instead of trying to develop a model with a theoretical solution, an empiric approach has been applied.

In Figure 5.10, the experimental data of Figure 5.7 were used to plot experimental values of the logarithm of the total phosphate concentration, $\log(C_t)$, in the SBPP with depths varying from 0.2 to 1.0 m as a function of pH. The result of the experimental investigation is that for any pH and pond depth the data plot is, in good approximation, a straight line with an average slope of 0.43. The experimental results also indicate that the pH for a final P concentration of $C_t = 1$ mg/L or $\log(Ct) = 0$ is, in good approximation, 9.7 for all depths so that the average of the data leads to the following linear equation:

$$\text{Log}(C_t) = -0.43 \cdot pH + 4.29 \tag{5.20}$$
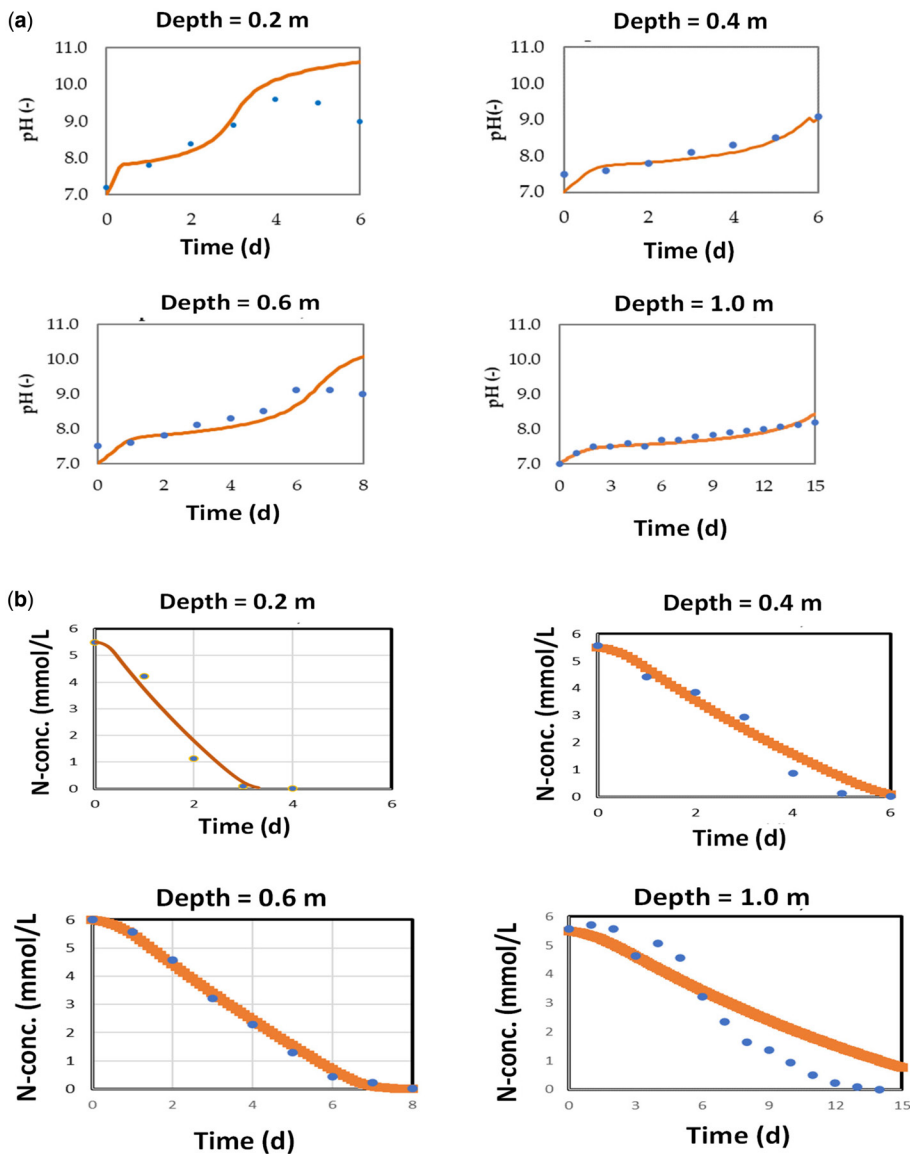
Figure 5.9 (a) Experimental data and simulated curves for pH vs time in SBPPs with depths varying from 0.2 to 1 m. (b) Experimental data and simulated curves for the nitrogen concentration and pH vs time in SBPPs with depths varying from 0.2 to 1.0 m.

Now the required pH for any final P concentration can be calculated. For example, the pH necessary for a final P concentration of 2 mg/L can be calculated as follows (See Fig 5.11):

For an effluent P concentration of 2 mg/L: $\log C_t = \log 2 = 0.32$;
The straight line with a slope of 0.43 and passing $C_t = 1$ or $\log C_t = 0$ has a pH value of 9.2 for $C_t = 2$ or $\log C_t = 0.32$.

(a)   **Depth = 0.2 m**

$y = -0.458x + 4.3709$
$R^2 = 0.9298$

log (Ct) vs pH (-)

**(b)**   **Depth = 0.4 m**

$y = -0.4363x + 4.2284$
$R^2 = 0.9481$

log(Ct) vs pH (-)

(c)   **Depth = 0.6 m**

$y = -0.3957x + 3.9035$
$R^2 = 0.8697$

log(Ct) vs pH (-)

(d)   **Depth = 1.0 m**

$y = -0.4617x + 4.3939$
$R^2 = 0.8968$

log(Ct) vs pH(-)
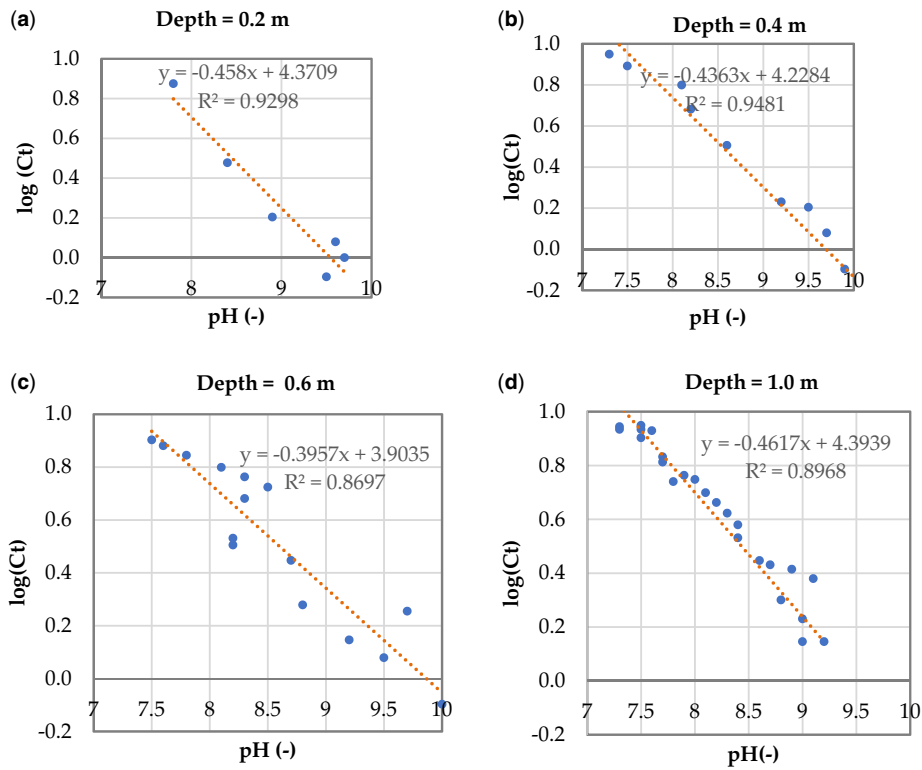
Figure 5.10 (a) Experimental profiles of the logarithm of the total phosphorus concentration as a function of pH for a pond depth of 0.2 m. (b) Experimental profiles of the logarithm of the total phosphorus concentration as a function of pH for a pond depth of 0.4 m. (c) Experimental profiles of the logarithm of the total phosphorus concentration as a function of pH for a pond depth of 0.6 m. (d) Experimental profiles of the logarithm of the total phosphorus concentration as a function of pH for a pond depth of 1.0 m.

Log (C$_t$) vs pH (-), C$_t$ (mg/L)

0.43
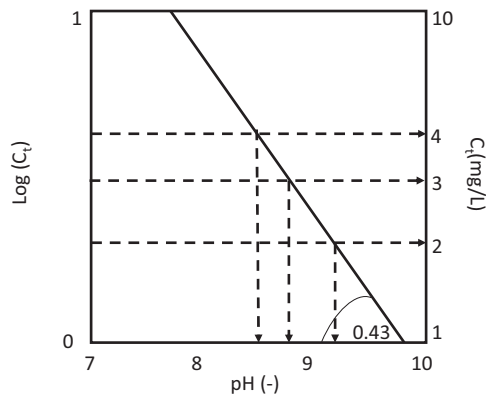
Figure 5.11 Logarithm of residual phosphorus concentration in SBPP as a function of the pH.

Figure 5.9 is a graphical representation of Equation (5.19), from which the residual phosphate concentration can be read off for any pH, or, conversely where the required pH can be read off for any desired residual phosphate concentration.

The retention time necessary to acquire the required pH for a particular residual phosphorus concentration can be determined by the pH-nitrogen model presented in Table 5.3.

It is important to note that Figures 5.9 and 5.10 are representatives only of the investigated wastewater and have no general applicability because in wastewater the concentrations of cations like Ca^{2+} are variable and this directly influences the solubility of phosphate for any particular pH.

5.2.6 Per capita area required for UASB–SBPP system

In Sections 5.4 and 5.5, the required retention times for the removal of the nutrients N and P are calculated. However, the variable of greatest interest for the design optimization is not the retention time, but the area per capita. This variable is related to depth. The area required for the construction of PPs can be calculated by considering that the retention time is the ratio between the volume of the pond and the applied daily flow so that the area per capita can be expressed as

$$A_{ie} = (R_h \times Q_{ie})/H \tag{5.21}$$

where: A_{ie} = area required for SBPP (m² per inhabitant equivalent (ie)); R_h = hydraulic retention time (days); Q_{ie} = daily contribution per inhabitant flow (m³/ie/d); H = pond depth in m.

Using the data in Figure 5.6, Table 5.4 shows the observed HRT for a final effluent with a concentration of <1 mgN/L and <1 mgP/L in ponds with different depths (columns 2 and 3). Columns 4 and 5 show the values of the area per capita for the residual concentrations of 1 mgN/L and 1 mgP/L, calculated with Equation (5.20) for a contribution per capita of 0.1 m³/d. In Figure 5.12 the values of the per capita areas have been plotted as a function of the pond depth. It can be observed that in shallow ponds it is feasible to obtain a very low nutrient concentration in SBPP, but also that the required area is much smaller than the area of conventional water sewage plant (WSP), where the area per capita is of the order of 3 m²/ie, but where the removal of nutrients is not possible. The figure can be easily adapted for other per capita contributions.

Data in Figure 5.12 show that

(1) In the range of 0.2–1.0 m there is little influence of the pond depth on the pond area. The optimal pond depth is about 0.5 m, which is much smaller than the depth of MP in WSP where the depth is usually in the range of 1.0–1.2 m. The shallow depth of the pond is an important factor in the reduction of construction costs.

(2) The area of shallow ponds is much smaller than 3 m² per inhabitant equivalent, normally used for the design of conventional WSP, where nutrient removal does not occur.

(3) The area required for P removal is about double of the value for N removal.

Table 5.4 Average values of the retention time and required area per ie for achieving concentrations <1 mg/L of nitrogen and phosphorus in the final effluent.

|	Retention Time (d)		Per Capita Area (m²/ie)	
Pond Depth	for 1 mgN/L	for 1 mgP/L	for 1 mgN/L	for 1 mgP/L
L_1 = 0.2 m	3	5	1.52	2.65
L_2 = 0.4 m	4.5	8.5	1.11	2.12
L_3 = 0.6 m	7	13	1.15	2.19
L_4 = 1.0 m	13	24	1.28	2.40
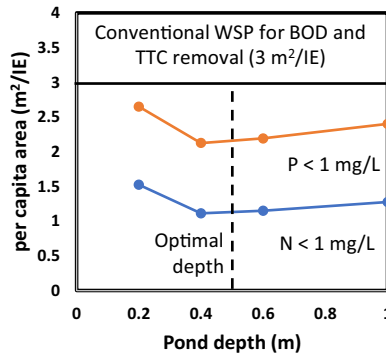
Figure 5.12 Per capita area for nutrient and pathogen removal in SBPP to produce effluent with less than 1 mgN/L and 1 mgP/L as a function of the pond depth.

5.2.7 Production of high-quality water

It is important to note that nutrient removal should be the goal of post-treatment only when the final effluent is not used in agriculture. For industrial reuse or discharge of final effluent into surface water, nutrient removal is required. When wastewater is treated for industrial reuse, it is also necessary to remove the algae growing during the post-treatment in SBPP. One possibility is clarification (coagulation–flocculation–sedimentation–filtration) of SBPP effluent. In this case, the use of a coagulant (usually poly aluminium chloride or aluminium sulphate) will also remove the phosphate, so, in that case, an additional removal unit is not required. Figure 5.13 shows the flowchart of a treatment system that can produce water for industry. The time required for each treatment step is also indicated. As in Figure 5.12, it was established that the retention time (and hence the pond area) for P removal is about double of the retention time for N removal. In cases where clarification is applied it is possible to reduce the retention time in the PP, however, a mechanism must be used to remove the algae.

The same figure also shows that it is possible to treat the effluent after clarification to remove dissolved salts by applying reverse osmosis, possibly preceded by micro or ultrafiltration. This demineralized water may be required in special industrial applications such as water for refrigeration as well as a source for public water supply. Clearly, the additional processes of clarification and reverse
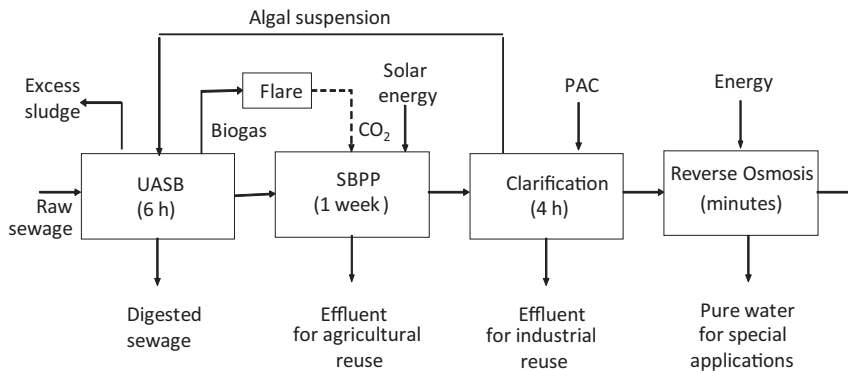
Figure 5.13 Flow sheet to produce water for industry for general use or to produce demi water for special applications.

osmosis will lead to an increase in the treatment costs and will normally be applied only if there is a specific demand for high-quality water. This is often the case in regions with scarce water resources.

5.3 POST-TREATMENT WITH ACTIVATED SLUDGE SYSTEMS AND MBRS

An important disadvantage of anaerobic pre-treatment is that conventional nutrient removal of nitrogen by nitrification and heterotrophic denitrification and phosphorus removal by luxury uptake is limited due to the low concentration of biodegradable organic material in the effluent of the anaerobic pre-treatment system. This low concentration affects both conventional denitrification after nitrification in the anoxic section and the feasibility of luxury P uptake (Van Haandel & Van der Lubbe, 2012). Two alternative biological processes may increase the removal of nitrogen in anaerobic–aerobic systems:

Autotrophic denitrification: the anammox system.
Denitrification coupled with methane oxidation.

This section presents the potential of various post-treatment technologies that can be used to improve the quality of anaerobically treated sewage, with particular emphasis on those that have promoted either the anammox or denitrification processes coupled with methane oxidation for improving nitrogen removal.

5.3.1 The anaerobic–aerobic treatment system

In this section, the feasibility of producing an effluent with activated sludge quality, after applying an anaerobic pre-treatment will be evaluated. Equation (5.24) shows that anaerobic treatment results in the division of influent organic material into three parts: (1) a part that is composed of soluble non-biodegradable as well as degradable material, but not digested in the reactor; (2) a part of suspended solids composed of biological sludge and non-biodegradable and particulate organic material from the influent; (3) a part transformed into methane. The objective of the anaerobic treatment is the maximization of the fraction transformed into methane and the minimization of the organic material in the effluent and the discharged sludge. In the aerobic treatment, the organic material is also divided into three fractions: (1) a fraction in the effluent, (2) a fraction in the produced sludge, and (3) an oxidized fraction of inorganic products. Marais and Ekama (1976) have presented a model for activated sludge behaviour in purely aerobic systems. Van Haandel and van der Lubbe (2012) have shown that the same basic theory also applies to the aerobic sections of anaerobic–aerobic systems.

Figure 5.14 shows the interaction of aerobic and anaerobic treatment and the possible advantages that can be obtained in comparison with conventional aerobic treatment. The advantages can be listed:

(a) Drastic reduction in investment cost by reducing the volume of the treatment system.
Research carried out in Campina Grande by Santos (2017) led to the development of empiric expressions for the three fractions or organic material in anaerobic treatment systems: (COD in the effluent, COD in the produced sludge and digested COD):

$$f_s = f_{ust} + f_{bst} = f_{us35}1.037^{(35-T)} + 0.20(1.011)^{(T-35)} \text{Exp}^{(-0.04(Rs-3.8\times1.06(35-T)))} \tag{5.22}$$

$$f_x = f_{up35}1.015^{(35-T)} + 0.16 \times 1.014^{(35-T)} \exp^{(-0.04(Rs-3.8\times1.06(35-T)))} \tag{5.23}$$

and

$$f_d = 1 - f_s - f_x \tag{5.24}$$

where: f_s = soluble COD fraction discharged in the effluent; f_x = COD fraction discharged as volatile solids in sludge; f_d = digested COD fraction; R_s = sludge age in the UASB reactor; T = temperature in °C; f_{us} = COD fraction in the influent that is non-biodegradable and soluble; f_{up} = COD fraction in the influent that is non-biodegradable and particulate.
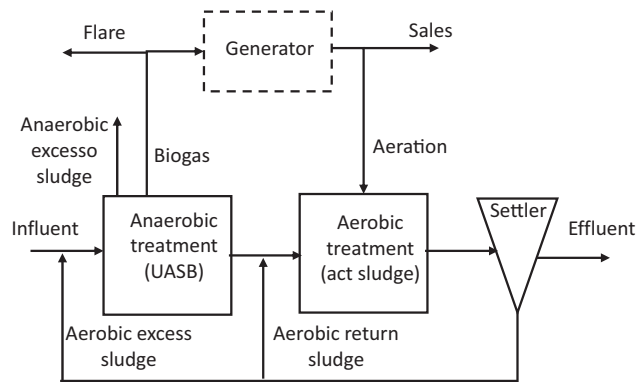
Figure 5.14 Basic scheme of the anaerobic–aerobic system composed of a UASB reactor and an activated sludge system.

The equations show that the division of the three fractions depends on the composition of the organic material in the influent (the non-biodegradable soluble and particulate COD fractions, f_{us} and f_{up}) and two operational variables: sludge age (R_s) and temperature (T). In anaerobic treatment, what matters is maximizing the digested fraction (f_d). Figure 5.15 shows the digested fraction as a function of sludge age at different temperatures. For all temperatures, the digested fraction of organic material is maximum when the sludge age is 100 days or more. Therefore, there is no merit in operating a UASB reactor with a sludge age of more than 100 days: only the volume of the UASB reactor increases, without this resulting in greater efficiency because the removal of the biodegradable organic material is already essentially complete.

It is not known *a priori* how long the liquid will remain in the UASB reactor to have a sludge age of 100 days. However, the Brazilian standard, NBR 12.209/2011, establishes the residence time for the maximum efficiency of the UASB reactor as a function of temperature. Figure 5.16 graphically shows the residence time for maximum efficiency in the UASB reactor as a function of sewage temperature.

As for the volume of the post-treatment system, it is necessary to optimize the volume of the aerobic reactor and the final settler. To optimize the post-treatment, it is sought to establish the smallest volume of the units of the aeration tank and final settler as a function of the sludge concentration. Van
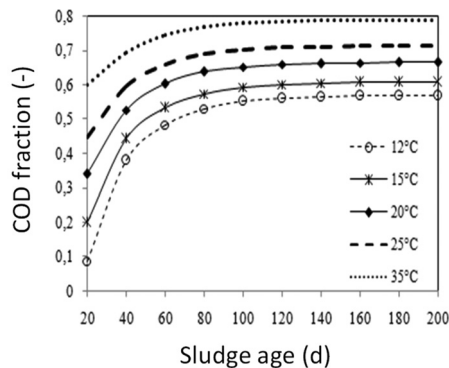
Figure 5.15 COD fraction converted into biogas as a function of the anaerobic sludge age for different temperatures (fus25 = 0.10 and fup25 = 0.08).
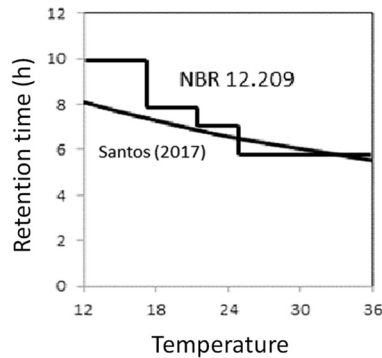
Figure 5.16 Retention time for maximum efficiency in a UASB reactor as a function of temperature conforming NBR 12.209/2011.

Haandel and van der Lubbe (2012) proposed the following two equations to calculate the volume of the reactor and the settler:

$$v_r = \left[(1 - f_{us} - f_{up})(1 + f_{bh}R_s)YR_s \,/\, (1 + b_h R_s) + f_{up}Rs/f_{cv}\right]Q_a S_{ta}\,/\,(S_{ta}f_v) \tag{5.25}$$

$$v_d = S_f \cdot H \,/\, v_o \cdot \exp^{(kXt)} \tag{5.26}$$

where: v_r = volume of the aerobic reactor per unit of applied sewage flow; v_d = volume of the final settler per unit of applied sewage flow; S_{ta} = total COD in the influent; f_{us} = non-biodegradable and soluble COD in the influent; f_{up} = non-biodegradable and particulate COD in the influent; f_v = volatile sludge fraction; k = sludge compressibility (Vesilind 1968; Leitão, 2004); v_o = limiting sludge settling velocity (Vesilind 1968; Leitão, 2004); R_s = sludge age; T = temperature; S_f = safety factor; H = reactor height; X_t = total sludge concentration.

Figure 5.17 shows the total retention time of anaerobic–aerobic treatment systems as a function of the anaerobic sludge age. The shortest retention time depends on several factors, among which the most important are: (a) concentration and composition of organic material in the influent; (b) sludge settleability; (c) sludge age; (d) temperature; (e) safety factor in the settler. In Figure 5.17, the retention
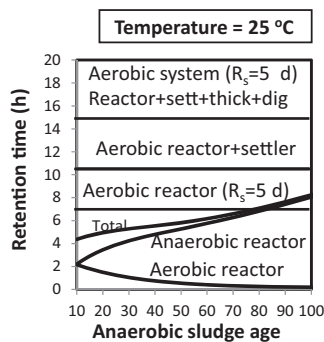
Figure 5.17 Hydraulic retention time in the anaerobic reactor and the complementary aerobic treatment, as well as the sum of the two, as a function of the age of the anaerobic sludge. The residence time in the aerobic system is indicated (Rsae = 5 days).

time of a purely aerobic system is also indicated for an aerobic sludge age of 5 days, which is the minimum for good effluent quality. It can be observed that the residence time of the anaerobic–aerobic system (total) is much shorter than for the complete aerobic system (reactor + settler + thickener + sludge digester). For an anaerobic sludge age of 100 days, the residence time of the anaerobic–aerobic system is around half the value for an aerobic system (aerobic sludge age of 5 days). Therefore, there is room for a very considerable investment cost reduction.

(b) Reduction of energy consumption for aeration: in principle, the system can be independent on the external energy by generating electricity from methane.

Energy consumption is affected by the two components of the anaerobic–aerobic system. In the pre-treatment, methane is generated that can be used directly to generate electricity. In post-treatment, the energy demand for aeration is reduced compared to the conventional aerobic system, because there is relatively little residual biodegradable material in the UASB effluent. The simplified model of the activated sludge system shows that energy consumption increases with aerobic sludge age because the demand for endogenous respiration increases. Depending on the age of the sludge, there may or may not be energy demand for nitrification.

Figure 5.18 shows the potential for energy production in the anaerobic system as well as the energy demand for aeration in the aerobic post-treatment (both in W/ie) for the case with and without nitrification at 25°C. To estimate the energy production potential, a biogas leakage of 30% and a methane-to-electric energy conversion efficiency of 40% were adopted, which means a production of a power of 0.2 kW per $kgCH_4$/d was applied to the generator. To estimate the energy demand, an oxygen transfer efficiency of the aerators of 2 kgO_2/kWh was adopted. It is observed that the potential for energy production is much greater than the consumption for aeration so that, in principle, there is a production potential that generates a surplus. In any case, the energy consumption in the anaerobic–aerobic system will be much lower than in the purely aerobic system, even in cases where the energy production potential is not used.

(c) Considerable reduction in sludge production, reducing the cost of its final disposal.

Equations (5.22) and (5.23) allow the comparison between sludge production in the anaerobic–aerobic system and the purely aerobic system. Figure 5.19 shows the sludge production in the two systems as a function of the anaerobic sludge age for a temperature of 25°C. The graphs show that there is indeed a large reduction in sludge production in the anaerobic–aerobic system, especially when the anaerobic sludge age is high, which are normally applied in practice.
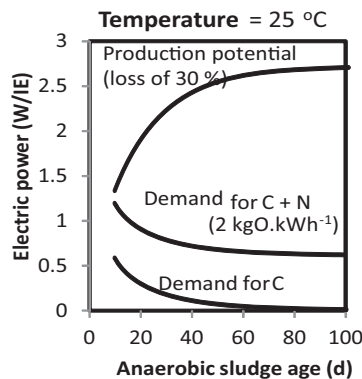
Figure 5.18 Per capita energy consumption for aeration and electric energy generation potential as a function of the anaerobic age of the sludge at 25°C. The aerobic sludge age applied is 5 days and cases with (C + N) and without (C) nitrification are considered.
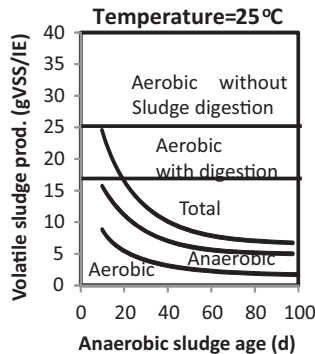
Figure 5.19 Anaerobic sludge production and excess aerobic sludge as a function of the anaerobic sludge age and excess aerobic sludge production at 25°C (aerobic sludge age = 5 days).

(d) The concentration of the digested sludge is high, facilitating its final disposal.The average sludge concentration in the UASB reactor is 25–30 gTSS(total solids concentration)/L, while in the aerobic system, it is normally not more than 5–10 g/L Therefore, the mixture of the two sludges from anaerobic–aerobic systems will have a concentration much higher than sludge generated in purely aerobic systems, which can greatly facilitate pumping and dehydration.

(e) The excess aerobic sludge can be stabilized in the anaerobic pre-treatment system.In anaerobic–aerobic systems, the possibility exists of using the anaerobic pre-treatment reactor not only for the anaerobic treatment of raw sewage but also for the stabilization of excess sludge generated in the aerobic post-treatment. The excess sludge generated in the anaerobic pre-treatment is already stabilized and does not require specific treatment before its dehydration. To assess the feasibility of using the anaerobic reactor for stabilization of post-treatment active sludge, it is necessary to verify that:

  (1) The introduction of excess aerobic sludge into the UASB reactor does not affect its performance as an anaerobic pre-treatment unit for the sewage to be treated.
  (2) Stabilization of the aerobic sludge in the anaerobic reactor is feasible.
  (3) The aerobic sludge digestion efficiency is equal to the value obtained in a conventional activated sludge digester.
  (4) The non-digestible part of the stabilized sludge mixes with the anaerobic sludge remains in the pre-treatment reactor and is discharged together with the excess anaerobic sludge.

de Silva Filho and van Haandel (2014) showed experimentally that the first three assumptions materialize, but the fourth condition does not: after aerobic sludge digestion, the non-digestible part of the aerobic excess sludge is expelled from the anaerobic reactor, because of its weak settleability. The consequential accumulation of these non-biodegradable solids in the aerobic reactor continues until the sludge concentration becomes so high, that the settler cannot separate the solids from the liquid phase and the system starts discharging sludge along with the effluent.

It is concluded that, although it is possible to digest the aerobic excess sludge in the anaerobic pre-treatment reactor, operational difficulties tend to arise that make this operation not feasible, as in practice has been experienced in many anaerobic–aerobic systems: non-biodegradable residual of aerobic sludge digestion will form an accumulate in the reactors and eventually will be released through the single outlet which is discharged in the final settler as indicated in Figure 5.20. Therefore, practice and theory show that of the five possible advantages of anaerobic–aerobic treatment four can
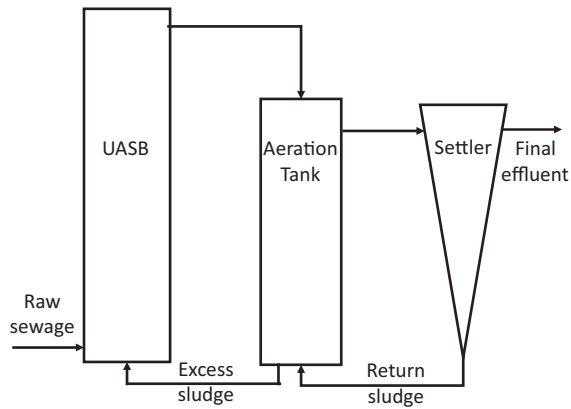
Figure 5.20 Layout of the activated sludge UASB reactor configuration without intermediate settler for stabilization of excess aerobic sludge in the UASB.

be materialized, but the fifth (the stabilization of aerobic excess sludge in the anaerobic reactor) leads to very serious operational problems.

A solution to this problem is to separate the non-biodegradable solids in the anaerobic effluent before discharge into the aerobic reactor, for example by introducing an intermediate settler as indicated in Figure 5.21. In that case, the accumulation of these solids in the treatment system and the associate operational problems can be avoided.

5.3.2 Application of the anammox process

As it was stated in the introduction of this chapter, phosphorus removal could be carried out by chemical precipitation of the orthophosphate ion with calcium, aluminium, or iron salts. On the other hand, conventional nitrogen removal is at most partial in anaerobic–aerobic treatment systems.

Autotrophic nitrogen removal, through the combined action of anammox and ammonium oxidizing microorganisms, is undoubtedly one of the most promising nitrogen removal processes for anaerobically treated effluents. In a first stage, the oxidation of around 50% of the ammonium to
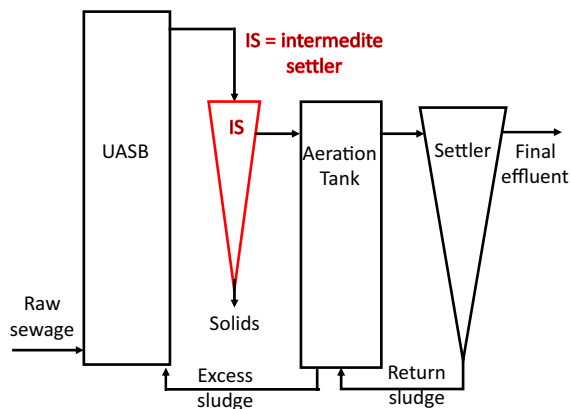
Figure 5.21 Layout of the activated sludge UASB reactor configuration with an intermediate settler for stabilization of excess aerobic sludge in the UASB.
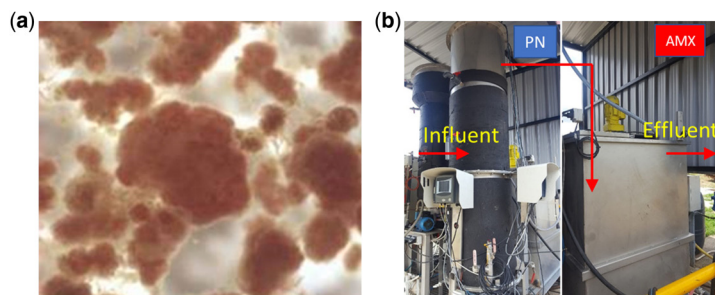
Figure 5.22 Left (A), anammox granules, anammox biomass is characterized by its intense reddish colour; right (B) picture of the two-stage PN/A prototype operated by Pedrouso *et al.* (2023).

nitrite is sought by means of nitrite-oxidizing autotrophic microorganisms (Equation (5.27)). In a second stage, the ammonium that has not reacted is used as an electron donor to denitrify nitrite, using anammox autotrophic microorganisms (Equation (5.28) and Figure 5.22a).

$$1.32\,NH_4^+ + 1.98\,O_2 \rightarrow 1.32\,NO_2^- + 1.4\,H_2O + 2.64\,H^+ \qquad \text{ammonia oxidation} \tag{5.27}$$
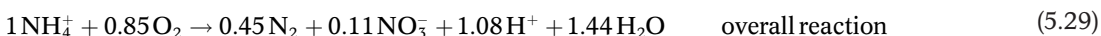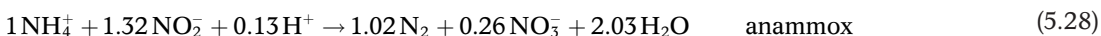
$$1\,NH_4^+ + 1.32\,NO_2^- + 0.13\,H^+ \rightarrow 1.02\,N_2 + 0.26\,NO_3^- + 2.03\,H_2O \qquad \text{anammox} \tag{5.28}$$

$$1\,NH_4^+ + 0.85\,O_2 \rightarrow 0.45\,N_2 + 0.11\,NO_3^- + 1.08\,H^+ + 1.44\,H_2O \qquad \text{overall reaction} \tag{5.29}$$

Because anammox sludge is autotrophic, the CO_2 used in sludge production must be partially reduced (oxidation number from 8 to 4), which is possible by partial nitrite to nitrate oxidation. According to Equation (5.29), the overall nitrogen efficiency is limited to a maximum of 89%, due to the production of nitrate. The real efficiency of the process could be affected by the presence of some residual ammonia concentration in the effluent.

The process has multiple advantages, organic matter is not required to denitrify, oxygen consumption is significantly reduced, and a small amount of biological sludge is generated. This process has been successfully applied for the removal of nitrogen of anaerobic digestion centrates in the sludge line of urban STPs, with many installations built after the first full-scale system installed in the Rotterdam STP in 2002, and also for the treatment of certain industrial streams with high nitrogen concentration at temperatures of 30–35°C. At these high temperatures, the nitrite production rate is greater than the nitrate production rate, which is a key for the success of anammox.

Its application in the water line is especially interesting, as it would make feasible the energy sustainability of STPs treating organic matter and nitrogen. Two different reactor configurations could be applied for the autotrophic removal of nitrogen: a one-stage process, in which both partial ammonia oxidation to nitrite and anammox are carried out in the same bioreactor; and a two-stage process, in which partial ammonia oxidation into nitrite is carried out in a first reactor, and the anammox reaction in a second bioreactor coupled in series. From these two configurations, the use of a two-stage process is recommended for treating anaerobically treated sewage, since it gives better results in the water line at temperatures below 30°C (Pedrouso *et al.*, 2021; Wu *et al.*, 2020).

Nevertheless, most of the attempts carried out in the water line have generally been unsuccessful. The reasons are multiple:

(1) Anammox bacteria do not support high residual COD concentrations due to the competitive growth of heterotrophs. It is recommended that the COD/TN ratio of the wastewater be lower than 2–3 g/g, fixing a TN to be treated of 50 mgN/L. This means that the COD in the anaerobically treated sewage should be lower than 100–150 mg/L.

(2) At temperatures lower than 30°C, the anammox activity is reduced. Thus, the reactor volume and biomass concentration of the anammox process should be increased. Additionally, the temperature dependencies on the growth rate for ammonia oxidizers and anammox microorganisms are quite different, which can cause decompensation in the populations, and volumetric activities, of said microorganisms at lower temperatures (Pedrouso *et al.*, 2021; Wu *et al.*, 2020).

(3) Stable suppression of nitrite-oxidizing bacteria is a key aspect in bioreactors treating sewage, with low ammonia concentration, around 50 mgN/L and temperatures of 15–25°C, in comparison with the 500–1,500 mg/L observed in centrates at temperatures of 30–35°C. This complicates the required inhibition of nitrite-oxidizing bacteria by free ammonia (FA), which interferes negatively with the overall process by oxidizing nitrite to nitrate (Wang *et al.*, 2022). Nitrite oxidizers are inhibited at threshold FA concentrations of 0.08–0.82 $mgNH_3$-N/L. Additionally, nitrite oxidizers can also be inhibited by free nitrous acid (FNA) at concentrations higher than 0.02 $mgNO_2^-$-N/L (Pedrouso *et al.*, 2017, 2023; Zheng *et al.*, 2023). The use of a strategy to inhibit nitrite oxidizers by either FA or FNA, or even both, is conditioned by sewage alkalinity, pH, and TN in the wastewater stream. For hard waters, rich in ions such as magnesium and calcium, in which a pH higher than 7 can be easily maintained in the bioreactor, the alternative of free ammonia is more viable, since the presence of FA is favoured at higher pH. On the contrary, for soft waters, where the pH of the bioreactor can reach easily acidic values, decreasing FA concentration, the alternative of FNA can be the solution to inhibit nitrite oxidizers.

For all these reasons, nitrogen treatment, through the anammox process, is still a challenge for the treatment of the effluents from anaerobic systems in STPs. An exception would be hot climate zones with water temperatures above 25°C and the segregated treatment of black waters with much higher nitrogen content. For instance, autotrophic nitrogen removal was responsible for a large part of the nitrogen removal in the STP of Changi (Singapore), using a step-fed activated sludge secondary system, with water temperatures between 28 and 32°C (Wang *et al.*, 2022). Additionally, Zheng *et al.* (2023) treated urban sewage in a pilot system, using a high-rate activated sludge system and a two-stage hybrid pilot plant with both suspended biomass and biofilms, operating at temperatures between 20 and 32°C. The treated effluent contained around 10 mg/L of TN, mainly ammonia. Most of the nitrogen was removed autotrophically in the post-treatment system. It should be highlighted, that the observed COD removal in the first high-rate activated sludge system, around 60%, was quite similar to that observed in many anaerobic UASB reactors. Pedrouso *et al.* (2023) also treated sewage in a pilot system, composed of a first activated sludge reactor, and a two-stage partial nitrification–anammox system, observing a nitrogen removal efficiency of 80%, with temperatures between 23 and 26°C (Figure 5.22a). These studies clearly suggest that there is also an opportunity to treat nitrogen from anaerobic reactor effluents at such temperatures.

Another possibility is the segregated treatment of domestic black water and grey water. Black water contains more than 90% of the COD, and more than 80% of the TP, and TN daily load generated in a municipality. COD, TN, and TP concentrations usually are between 1,000–7,000, 100–1,000, and 25–200 mg/L, respectively (Pedrouso *et al.*, 2020). Thus, the segregated treatment of black water, using a first anaerobic treatment stage, makes it much easier for the application of an autotrophic TN removal post-treatment, due to the higher FA and FNA concentrations observed in the bioreactors. Pedrouso *et al.* (2020) were able to remove above 80% of the TN from anaerobically treated black waters, with around 120 mgN/L, using a one-stage autotrophic process.

5.3.3 Post-treatment with membrane reactors

MBRs are perhaps one of the technologies with the greatest potential for wastewater treatment, including the polishing of anaerobically treated sewage. Although there are various configurations

of biological membrane systems, this chapter refers to the use of aerobic MBRs using submerged membrane modules. MBR is one of the modifications of the activated sludge process, where the secondary clarifier has been replaced by microfiltration (MF) or ultrafiltration (UF) membranes, with a pore-size between 0.01 and 0.1 μm for UF membranes, and 0.1–0.4 μm for MF membranes. Through sieving, the membranes separate those components larger than the pore size, such as bacteria (>1 μm), helminth eggs (20–80 μm), protozoa (<50 μm), and for the case of UF even colloidal substances, making possible to obtain a high-quality effluent free of suspended solids and microbial indicators, facilitating the reuse of the reclaimed water. Thus, the use of MBRs may probably be the best technology available for water reuse. In any case, more information regarding MBRs and water reuse is provided in Chapters 3 and 8 of this book, respectively.

At present, anaerobic sewage treatment presents two main drawbacks: (i) negligible nitrogen removal, and (ii) the presence of dissolved methane in their effluents, which may be released into the atmosphere, notably increasing the greenhouse gas emissions of the process. Methane is a strong greenhouse gas with a global potential 28 times higher than carbon dioxide. Cakir and Stemstrom (2005) compared greenhouse emissions from both aerobic and anaerobic sewage treatment, concluding that aerobic technologies emit less greenhouse gases, especially for the treatment of diluted sewage, due to the dissolved methane emissions associated with anaerobic sewage treatment. There are various strategies to reduce the impact of dissolved methane emissions, including desorption-based techniques to recover part of the dissolved methane, and biological methane oxidation reactors, in which methane may be used to denitrify (Chapter 7 of this book; Stazi and Tomei, 2021).

An innovative MBR process developed at the University of Santiago de Compostela that aims to respond to the problems associated with anaerobic sewage treatment, the SIAM® treatment technology (US patent 9,725,345B2), obtains the removal of dissolved methane and total nitrogen, so killing two birds with one stone. This technology is based on the use of a hybrid membrane bioreactor (hybrid MBR) to treat the effluent of an UASB system fed with sewage.

The first development of the hybrid MBR of the SIAM® technology included two compartments: an anoxic compartment that accounted for 70% of the volume of the hybrid MBR; and an aerobic membrane filtration compartment, where ammonium was mostly nitrified to nitrate, and the remaining dissolved methane not eliminated in the anoxic stage was partly oxidized or desorbed to the atmosphere. A hollow-fibre ultrafiltration membrane was used to separate the permeate from the mixing liquor, preventing microorganisms from being washed out.
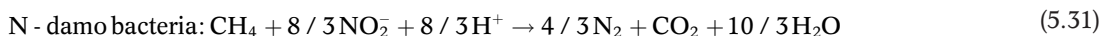
In the first development of the SIAM®, plastic carrier particles were placed in the anoxic compartment to promote the growth of a biofilm with methanotrophic or anammox microorganisms, which are maintained, unlike biomass in suspension, always in the anoxic conditions to favour growth.
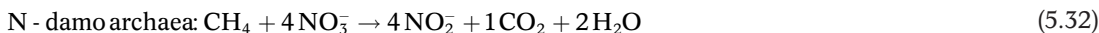
The elimination of nitrogen associated with the biological oxidation of methane can follow two pathways: one aerobic, known as aerobic methane oxidation coupled to denitrification (Amo-D); and another anaerobic, known as nitrite/nitrate-dependent anaerobic methane oxidation (N-damo).

In the Amo-D, aerobic methanotrophs are able to convert methane into methane oxidation products that are employed as a carbon source by conventional heterotrophic denitrifiers (Equation (5.30)) in a subsequent reaction (Thalasso *et al.*, 1997).

$$CH_4 + 1.1O_2 + 0.72NO_3^- + 0.72H^+ \rightarrow 0.36N_2 + CO_2 + 2.36H_2O \tag{5.30}$$

N-damo process is carried using either nitrite by N-damo bacteria (*Candidatus Methylomirabilis oxyfera*) or nitrate by N-damo archaea (*Candidatus Methanoperedens nitroreducens*) (Haroon *et al.*, 2013; Equations (5.31) and (5.32), respectively). Unfortunately, these newly discovered N-damo microorganisms are characterized by extremely slow growth rates of approximately 1–2 weeks (Ettwig *et al.*, 2009; Haroon *et al.*, 2013).
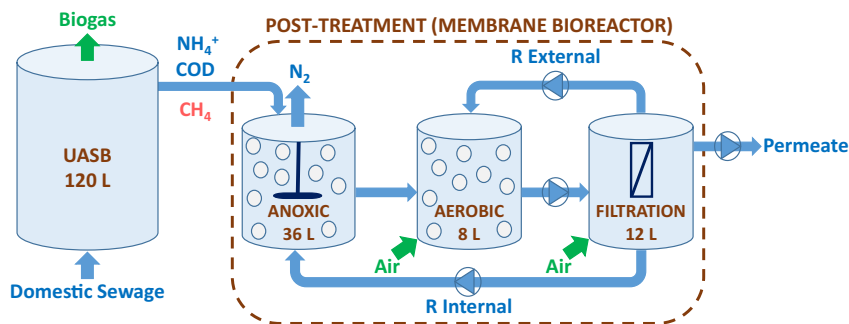
$$\text{N-damo bacteria:} \quad CH_4 + 8/3NO_2^- + 8/3H^+ \rightarrow 4/3N_2 + CO_2 + 10/3H_2O \tag{5.31}$$

$$\text{N - damo archaea: } CH_4 + 4NO_3^- \rightarrow 4NO_2^- + 1CO_2 + 2H_2O \qquad (5.32)$$

The methane removal rate in the first two-compartment pilot units reached up to 195 ± 17 mgCH$_4$/L/d, and the nitrogen removal rate, referred to the anoxic compartment, up to 144 ± 20 mgNO$_x$-N/L/d (Silva-Teira et al., 2017). The dissolved methane removal percentage reached 60–80%, while nitrogen removal was 15 mgN/L. From this concentration, and considering the difference between the COD fed to the MBR and that of the permeate, it was estimated that only 4 mgN/L was due to conventional heterotrophic elimination, estimating that aerobic and anaerobic methanotrophs were responsible for the remaining 11 mgN/L.

In a second development, a notable advance of the SIAM® process was the inclusion of an additional aerobic compartment, between the anoxic and membrane filtration compartments (Figure 5.23). This compartment also contained plastic supports for biofilm growth. This development boosted the nitrogen capacity of the hybrid MBR process. Nitrogen removal increased from 15 to 40 mg N/L, for sulphate depleted wastewater, to 60–70 mgN/L for sewage with around 50 mgSO$_4^{2-}$-S/L (Allegue et al., 2023). Those results were obtained in a small 176 L SIAM® pilot plant (120 L UASB + 56 L Hybrid MBR) treating synthetic sewage, maintaining HRT in the hybrid MBR around 0.41 days. Similar results were obtained in a SIAM® prototype with a 3.87 m³ UASB and a 2.06 m³ hybrid MBR, located in the STP of Cartagena (Murcia, Spain) in the frame of the European Union project Siamec (Domínguez et al., 2019; Figure 5.24) maintaining an HRT, referred to the MBR post-treatment system, of around 0.43 days. The TN removal efficiencies were around 75–80%, diminishing TN concentration from 75–90 to some 20 mg/L.

The presence of sulphate in raw sewage can alter the performance of the UASB system, as this ion is reduced in the anaerobic system to sulphur-reduced compounds including elemental sulphur and hydrogen sulphide. Sulphate content in raw sewage may have either an anthropic or natural origin or be caused by seawater intrusion in sewerage systems, especially in coastal towns. Sulphide may be utilized by autotrophic sulphur-oxidizing bacteria (SOB) as an electron donor for denitrification, which could enhance the nitrogen removal capabilities of UASB post-treatment systems. SOB can use reduced sulphur compounds, such as sulphide, as electron donor to transform nitrite/nitrate into N$_2$ (Equations (5.33) and (5.34)).

$$3HS^- + 8NO_2^- + 5H^+ \rightarrow 3SO_4^{2-} + 4N_2 + 4H_2O \qquad (5.33)$$

$$5HS^- + 8NO_3^- + 3H^+ \rightarrow 5SO_4^{2-} + 4N_2 + 4H_2O \qquad (5.34)$$

Figure 5.23 Schematic of the second development of the SIAM® process, with a first anaerobic UASB stage for removing COD; and a hybrid MBR post-treatment for polishing the effluents of the first anaerobic stage. Plastic carrier particles were introduced in both the anoxic and aerobic compartments of the hybrid-MBR system.

Figure 5.24 Image of the SIAM® prototype (using the second development) operated in the sewage treatment plant of the city of Cartagena (Spain), in the frame of the European Union Life Siamec project.

In principle, the presence of either dissolved methane or sulphur-reduced compounds in the anaerobically treated sewage increases the TN removal capacity of the post-treatment system, as was previously observed for the two SIAM® plants. However, hydrogen sulphide generated in the UASB stage may also inhibit the activity of microorganisms involved in the TN and dissolved methane removal, as was reported for nitrifying (Bejarano Ortiz *et al.*, 2013), anammox (Carvajal-Arroyo *et al.*, 2013) and methane-oxidizing microorganisms (Xu *et al.*, 2020). The inhibition of nitrification was observed in the SIAM® prototype, around day 480 (Figure 5.25) due to an electrical failure in the plant. This caused a rapid loss of nitrification, and no nitrate ions entered the anoxic compartment to oxidize the reduced sulphur products. This led to a vicious circle in which nitrification was not
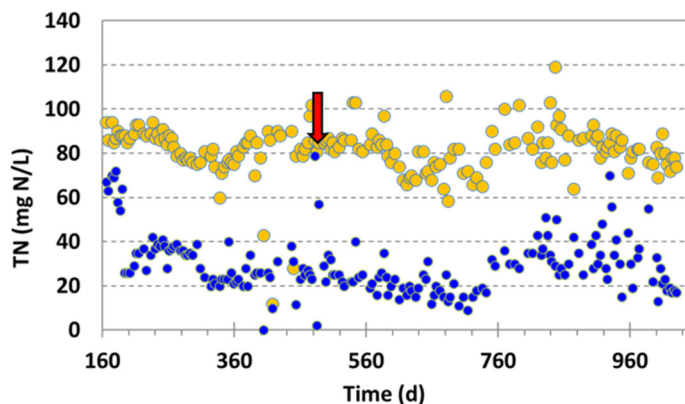
Figure 5.25 Evolution of the TN in the hybrid MBR stage of the SIAM® prototype operated in the STP of the city of Cartagena (Spain), in the frame of the EU Life Siamec project: (○) TN in the anaerobically treated sewage; (●) TN measured in the permeate of the MBR system. The red arrow indicates a nitrification inhibition event due to the accumulation of reduced sulphur compounds, which occurred around operating day 480.

achieved due to inhibition, and the inhibitor was not eliminated due to the absence of nitrate and nitrite in the anoxic compartment. In the prototype plant, this was solved by reducing the flow of residual water fed for a few days.

One of the advantages of using the SIAM® process, as occurred with some other MBR processes, is that it facilitates water reuse of the reclaimed water. Microbial (*E. coli*; *Legionella* spp.; Nematodes eggs) and chemical indicators (TSS, BOD_5, turbidity) in the permeate of the SIAM® prototype treating sewage were below the limits for water reuse in agriculture set by the EU regulation 2020/741 for reclaimed water quality class A. On the other hand, the observed overall sludge production is similar to that referred for anaerobic UASB systems, around 0.1 gTSS/gCOD, reducing the cost associated with sludge management.

Concerning the microbial population developed, the biofilms of the anoxic and aerobic compartments were enriched in anammox microorganisms. N-damo microorganisms (detected by q-PCR, Illumina, and FISH) were only observed in aerobic and anoxic biofilms of the prototype treating urban sewage in Cartagena, around 0.02% by Illumina. The presence of aerobic methanotrophs was always observed in the hybrid MBR, both in suspension and the biofilms, with values between 1.5 and 8%.

Despite the low N-damo content, denitrification associated with anaerobic methane oxidation was always detected in batch activity tests (Silva-Teira *et al.*, 2017). Xie *et al.* (2023) also detected some kind of N-damo activity without any known N-damo, in a moving-bed biofilm reactor (MBBR) under hypoxic conditions, with only 0.02 mg/L of dissolved oxygen (DO). The presence of aerobic methane oxidizers was remarkable, despite the low DO. They suggested the possibility that some unknown strains may harbour uncharacterized N-damo capacity that contributed to nitrogen removal.

The use of a hybrid MBR process with several anoxic and aerobic chambers, as proposed in the SIAM® system, basically is in line with what has been observed for anammox systems in the water line, using hybrid bioreactors (Zheng *et al.*, 2023). The use of carrier particles promotes the growth of sensitive microorganisms, avoiding the strong dissolved oxygen variations to which the biomass in suspension is subjected.

To interpret the obtained results in a more analytical way, a mathematical model to describe the biological processes in the hybrid MBR was developed taking account of the kinetic and stoichiometric parameters of existing microbial populations: heterotrophs, sulphate reducing bacteria, sulphide oxidizing bacteria, aerobic and anaerobic methanotrophs (including N-damo bacteria and archaea), ammonium and nitrite oxidizing bacteria and anammox. The model considered only the existence of suspended biomass, neglecting the presence of biofilms, and the mass balances in the hybrid MBR considered three tanks connected in series, representing the anoxic, aerobic and membrane filtration compartments of this system. The mathematical model developed may be considered a way of describing the system under study, the hybrid MBR, through mathematical equations, allowing the simulation, once it has been correctly adjusted, to verify the behaviour of the system.

The model developed is similar to some of the existing activated sludge models (ASM1 and ASM2) and contained 22 components, including sulphur compounds, dissolved methane and oxygen. The microbial reactions were modelled by 27 kinetic processes (Liñares-Lamas, 2018; Mauricio-Iglesias *et al.*, 2016) and contained 97 kinetic and stoichiometric parameters. Some of the parameters of the model were calibrated using the experimental results obtained during 248 days of operation, and others fixed considering the typical values referred to the ASM models. The model was verified considering that the experimental results obtained in a later period fit correctly with the predictions obtained.

The use of the model allows the study of the impact of various operating conditions different from those tested, allowing the identification of which variables can positively or negatively affect the biological elimination of total nitrogen or dissolved methane in the hybrid MBR.

Heat maps were used to represent the different operating conditions on the removal of total nitrogen and dissolved methane. Figure 5.26 shows the total nitrogen and dissolved methane removal predicted by the model, as a function of TSS in the aerobic compartment and HRT of the hybrid MBR.
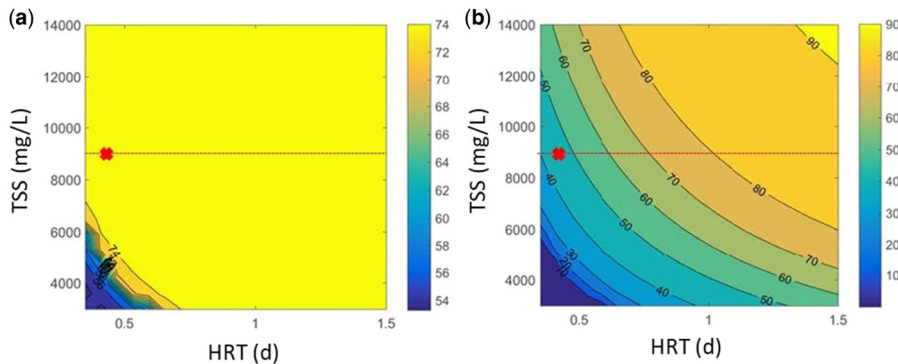
Figure 5.26 (a) Percentage of total nitrogen removal and, (b) percentage of dissolved methane removal predicted in the hybrid MBR process as a function of the HRT and TSS concentration in the aerobic compartment (Liñares-Lamas, 2018). The red dot indicated the experimental results obtained in the hybrid MBR of a SIAM® prototype.

The red dot indicates the observed experimental result observed maintaining HRT, DO and TSS at 0.4 day, 1 mg/L and 9 gTSS/L, respectively.

At short HRT the model predicts that nitrogen removal depends notably on the TSS concentration of the post-treatment bioreactor (Figure 5.26a), especially at low HRT. On the other hand, it might be necessary to operate with high HRT, greater than 0.5 days to obtain high nitrogen efficiency, when the post-treatment bioreactor operates below 4000 mgTSS/L, a concentration typically observed in activated sludge bioreactors.

Figure 5.26b shows the percentage of biological methane removal in the system carried out by aerobic methanotrophic organisms as a function of solids concentration and HRT. Since these microorganisms have a slow growth, below a certain value of solids they were not able to compete with the rest of the microorganisms and end up disappearing from the system. Therefore, methane removal increases with increasing solids concentration and with increasing HRT. Therefore, the use of an MBR system that allows maintaining high sludge concentrations may be a very good option for the removal of dissolved methane and nitrogen in anaerobically treated sewage.

Figure 5.26 shows the dissolved methane and nitrogen removals based on dissolved oxygen concentrations observed in the aerobic compartment of the hybrid MBR and the HRT applied. This parameter, DO, is one of the operating variables that is usually controlled, in fact, in aerobic bioreactors. The removal of nitrogen is practically constant as a function of the DO of the aerobic compartment (Figure 5.27a) since the volume of the membrane chamber is sufficient to carry out nitrification, in fact the removal range depicted in the heat map varies only between 74.2 and 75.4%. However, for the removal of methane (Figure 5.27b) an optimum value of oxygen is observed. Below this, oxygen limits the activity of aerobic methane oxidizers, while with a higher value, methane desorption due to the stripping to the atmosphere, increases. It is observed that the DO optimum values are between 0.4 and 1 mg O_2/L, and that methane removal would be strongly encouraged if the volume of the MBR was increased. The model predicts that methane removal could be increased from 45%, obtained in the experimental system, to more than 80%, if the HRT were increased from 0.4 to 1 day, maintaining the same concentration of dissolved oxygen. This would obviously increase the volume of the post-treatment system, increasing the capital expenses of the sewage treatment plant.

The model indicates that the elimination of dissolved methane depends strongly on the concentration of organic matter in the anaerobically treated sewage fed to the MBR, with a COD of around 300 mg/L, improving the removal significantly if the COD efficiency of the UASB increased. In fact, the removal of dissolved methane determined in a hybrid MBR, fed with an
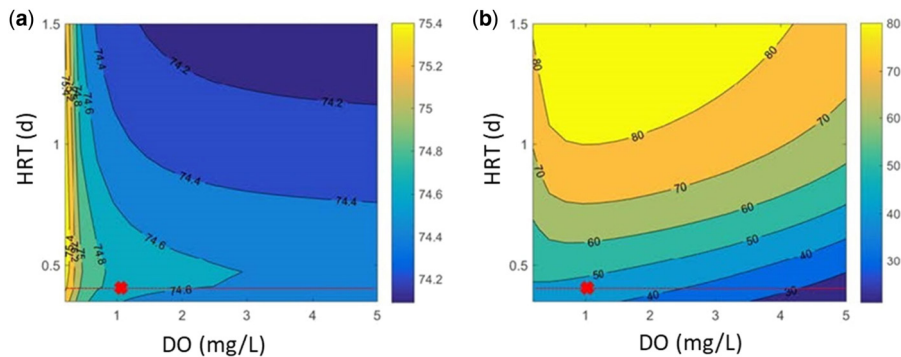
Figure 5.27 (a) Percentage of total nitrogen removal, and (b) percentage of dissolved methane removal predicted in the hybrid MBR process as a function of the HRT and DO concentration in the aerobic compartment (Liñares-Lamas, 2018). The red dot indicated the experimental results obtained in the hybrid MBR of a SIAM® prototype.

anaerobically treated wastewater containing less than 100 mgCOD/L, were higher, around 70–80% (Alvarino *et al.*, 2019; Silva-Teira *et al.*, 2017). This is due to the lower presence of conventional heterotrophs in the sludge when COD fed diminishes. On the other hand, the model indicates that the development of N-damo microorganisms is problematic under the operating conditions used. This agrees with the experimental results, where these microorganisms were present in a very low proportion in the biofilms, although some anaerobic methane oxidation activity was detected (Silva-Teira *et al.*, 2017).

The model indicates that most of the methane removal takes place by aerobic methanotrophic bacteria, which were detected in the sludge samples. In any case, the role of biofilms or the development of unknown anaerobic methanotrophic communities as referred in the literature (Xie *et al.*, 2023) could open new ways to improve the simultaneous removal of dissolved methane and total nitrogen from anaerobically treated sewage.

5.4 POST-TREATMENT WITH BIOFILM REACTORS

5.4.1 Trickling filters

Trickling filters are a type of non-submerged biofilm three-phase bioreactors, in which a plastic or rock packing material contained in a tower, is used to promote biofilm growth. Sewage is sprayed over the top of the trickling filter, and trickles to the bottom, wetting the biofilm. The treated sewage is collected at the bottom of the tower. Part of the biodegradable organic matter and ammonium contained in the sewage are oxidized by the biofilm, thanks to the oxygen transferred through natural or forced draft aeration.

Rocks with a nominal size of around 5–20 cm, random plastic packing media, and plastic bundles are the typical packing materials used in TFs. The height of the trickling filter is conditioned, among others, by the weight of the packing material, limiting the height of the trickling filters to 1–3 m or 4–12 m if rock or plastic packing materials are used, respectively.

Although trickling filters with rock are still in use, there is a tendency to replace them with plastic packing materials, due to the higher specific surface area, better air circulation, and the absence of ponding, which is caused by solids accumulation in the rock material (Bressani-Ribeiro *et al.*, 2017). This not only improves the efficiency of the filter but also reduces the nuisance problems or the proliferation of insects associated with the use of rock in TFs.

It is recommended to pretreat the sewage fed by primary sedimentation in conventional TF facilities. Trickling filters used as the post-treatment stage of UASB effluents are widely used in countries such

as Brazil. Several studies have demonstrated that the combined UASB + TF systems used for sewage treatment have a similar overall BOD removal efficiency than the conventional primary settling + TF sewage treatment (Bressani-Ribeiro et al., 2017).

The size of the TF required can be estimated in terms of the hydraulic loading rate (HLR; m³/m²/d) and the organic loading rate (OLR; kgBOD/m³/d) selected. TF are classified in five different categories depending on the HLR, OLR and the nature of the packing material used (Metcalf & Eddy 2014): low rate with rocks (1–4 m³/m²/d and 0.07–0.22 kgBOD/m³/d); intermediate rate with rocks (4–10 m³/m²/d and 0.24–0.48 kg BOD/m³/d); high rate with rocks (10–40 m³/m²/d and 0.4–2.4 kgBOD/m³/d); high rate with plastic (10–75 m³/m²/d and 0.6–3.2 kgBOD/m³/d); and roughing TFs with plastic packing (40–200 m³/m²/d and >1.5 kgBOD/m³/d). According to Bressani-Ribeiro et al. (2017), most of the UASB + TF full-scale plants in Brazil were designed as high-rate TFs with rocks.

Nitrogen or phosphorus removal with the above indicated TFs is negligible, and only certain ammonia removal could be expected, depending on the operational conditions, especially the applied loading rate and temperature.

5.4.2 Down-flow hanging sponge

Down-flow hanging sponge (DHS) technology can be considered an improvement of the trickling filter process, where the packing material is made up of polystyrene sponges. This technology, developed in Japan, has been successfully applied to the post-treatment of effluents from UASB systems, in urban STPs. This effluent trickles down through the sponges by gravity. In DHS systems the growth of a biofilm, covering the polystyrene particles used, eliminates in contact with air a large part of the organic matter and TKN fed. The sponge used as carrier in DHS is characterized by its large porosity (around 98.5%), which favours the retention of water, increasing the contact time between the residual water and the attached microorganisms, regarding other packing materials used in trickling filters, enhancing the pollutants removal.

Okubo et al. (2015) operated a full-scale UASB + DHS with polyurethane foam sponge as DHS support, for 5 years, without any significant sponge damage that could endanger the process.

DHS operated at short HRT, around 1.5 h referred to the sponge volume in full-scale facilities (Okubo et al., 2015; Mazhar et al., 2021). DHS towers contained around 24.7–33% carriers. Sludge retention time is around 90–120 days (Tandukar et al., 2007; Tawfik et al., 2006). DHS has low energy requirements regarding aerobic activated sludge systems, caused natural draft aeration. Additionally, excess of biomass growing onto the carrier is easily detached by the water that is percolating in the system, avoiding the clogging of the filter media. Excess biomass could easily be removed by secondary sedimentation, and this stage could even be dispensed in those locations where the TSS discharge limits are not rigorous (Bressani-Ribeiro et al., 2017). Biomass yield observed in DHS is very low, around 0.04 gTSS/gCOD as stated by Okubo et al. (2015).

In DHS, most of the COD is removed in the upper part of the filter. Organic matter removal efficiency of UASB + DHS systems are comparable with those of conventional activated sludge reactors (Tandukar et al., 2007), with removal BOD and COD efficiencies of around 90% for pilot plants (Tandukar et al. 2007; Tawfik et al. 2006). For two full-scale facilities located in India, Agra (Aamir Mazhar et al., 2021) and Karnaoba (Okubo et al., 2015), the COD efficiencies were 83 and 89%, respectively. The lower COD efficiency of the Agra facility might be caused by the absence of secondary sedimentation of the DHS effluent. TSS of the final effluent was higher, 42.7 ± 10 versus the 19 ± 8 mgTSS/L observed in the Karnaoba facility.

COD removal is hardly affected by temperature in UASB + DHS systems, with a slight improvement in any case at temperatures above 20°C (Tandukar et al., 2007). A large fraction of the ammonium fed is oxidized to nitrate in the lower part by aerobic nitrifiers. Around 50–70% of the fed ammonia is oxidized to nitrate in DHS (Okubo et al., 2015). The existence of anaerobic and anoxic environments inside the sponges of the lower part facilitates the elimination of part of the fed TKN due to denitrification processes. DHS systems may eliminate a fraction of the fed TN, around 6–12 mg/L,

mainly due to biological nitrification–denitrification processes, as the contribution of assimilation to nitrogen removal is almost negligible (Okubo *et al.* 2015; Onodera *et al.* 2014; Tawfik *et al.* 2006).

5.5 CONCLUSIONS

5.5.1 Combination of anaerobic pre-treatment and PPs

Anaerobic pre-treatment in combination with PP is highly advantageous when compared with conventional WSP:

- A large reduction of the pond can be achieved.
- Nutrient removal becomes feasible in PP, but not in WSP.
- There is a possibility of productive use of biogas and sludge production.
- Decreasing of effluent quality and quantity by evaporation is reduced.

The preferable hydrodynamic regime for PPs is sequential batch (SBPP) rather that continuous flow (CFPP).

- SBPP can remove nutrient more rapidly than CFPP, but it requires a transfer pond to feed the SBPP.
- Transfer ponds can have important secondary advantages: settling of anaerobic solids and odour reduction.

The increase of pH in PPs is due to CO_2 removal by photosynthesis and desorption. Ammonium desorption tends to reduce pH.

The principal mechanism for ammonium removal is desorption, although some nitrogen may be removed by incorporation in the produced biosolids.

The principal mechanism for phosphorus removal in PPs is precipitation with naturally present or added cations, although some phosphorus may be removed by incorporation in the produced biosolids. High-quality effluent may be produced by clarification of PP effluent and can further be improved to pure water by advanced treatment like inverse osmosis.

The construction costs for SBPP are much smaller than for WSP for three reasons:

- A per capita reduction of the pond area of 2/3 (67%) can be achieved if only nitrogen is to be removed from sewage. If nitrogen and phosphate are to be removed the reduction is 1/2 (50%).
- The depth for SBPP (0.5 m) is much smaller than for WSP (1.2 m), so that much less excavation is required, and the slopes are much smaller. The SBPP do not need to have all the same bottom level, again reducing excavation costs.
- The reduction of odour from SBPP plants allows construction near the contributing population reducing the costs for the outfall.

5.5.2 Anaerobic pre-treatment and other aerobic activated sludge post-treatment systems

Aerobic post-treatment, using activated sludge systems (after anaerobic pre-treatment) removes organic material and suspended solids with the same efficiency as purely aerobic sewage treatment, but has great advantages:

- Strong reduction in the volume of the treatment system, lowering the investment costs.
- Reduction of energy demand with the possibility of being self-sufficient through using the energy from the methane generated in the pre-treatment reactor.
- Reduction of sludge production, thus reducing operating cost.
- Increase in excess sludge concentration, facilitating dehydration and final disposal.
- Nitrogen removal is difficult and is limited by the low COD contained in the anaerobically treated effluent.

5.5.3 Post-treatment with membrane and biofilm reactors

The use of MBRs, as post-treatment systems, allows obtaining a high-quality effluent, facilitating the reuse of water. Additionally, the higher microbial concentrations and the use of a membrane that prevents the washing of slow-growing microorganisms, facilitate the use of dissolved methane as an additional carbon source for denitrification.

The integrated SIAM® process, which consists of a UASB system coupled in series to a hybrid MBR, is effective in removing total nitrogen and dissolved methane. The development of second-generation SIAM® systems, in which the hybrid MBR consists of three chambers, allows the removal of total nitrogen to be significantly improved, going from a TN removal of 15–20 mg/L previously obtained, to more than 40 mg/L, and maintaining the removal of dissolved methane.

The use of trickling filters, using DHS systems, significantly improves the results previously observed for traditional trickling filters with stones or conventional plastic fillers. TN removal is limited to some 10 mg/L and cannot be easily controlled.

REFERENCES

Aguiar M. R. L., Santos S. L. and van Haandel A. (2021). Polishing ponds operating in batch mode for post-treatment of UASB reactors: organic material removal. Revista DAE: Edição especial, São Paulo, n. 229, março 2021.

Albuquerque M. S., Santos S. L. and van Haandel A. (2021). Influence of the hydrodynamic regime on the performance of polishing ponds. Revista DAE: Edição especial, São Paulo, n. 229, março 2021.

Allegue T., Arias A., Liñares A., Omil F. and Garrido J. M. (2023). Impact of dissolved sulfide on a hybrid membrane bioreactor treating the effluent of a mainstream up-flow anaerobic sludge blanket. *Environmental Science: Water Research and Technology*, **9**, 2733–2744, https://doi.org/10.1039/D3EW00404J

Alvarino T., Allegue T., Fernandez-Gonzalez N., Suarez S., Lema J. M., Garrido J. M. and Omil F. (2019). Minimization of dissolved methane, nitrogen and organic micropollutants emissions of effluents from a methanogenic reactor by using a preanoxic MBR post-treatment system. *Science of the Total Environment*, **671**, 165–174, https://doi.org/10.1016/j.scitotenv.2019.03.169

Bastos R. K. X., Rios E. N. and Sánchez I. A. (2018). Further contributions to the understanding of nitrogen removal in waste stabilization ponds. *Water Science and Technology*, **77**(11), 2635–2641, https://doi.org/10.2166/wst.2018.218

Batista M. M., Lambais G. R., Santos S. L. and van Haandel A. (2021). Bacterial decay in polishing ponds treating UASB reactor effluents. Revista DAE: Edição especial, São Paulo, n. 229, março 2021.

Bejarano Ortiz D. I., Thalasso F., de Cuervo López F. M. and Texier A. C. (2013). Inhibitory effect of sulfide on the nitrifying respiratory process. *Journal of Chemical Technology and Biotechnology*, **88**(7), 1344–1349, https://doi.org/10.1002/jctb.3982

Bressani-Ribeiro T., Brandt E. M. F., Gutierrez K. G., Díaz C. A., Garcia G. B. and Chernicharo C. A. L. (2017). Potential of resource recovery in UASB/trickling filter systems treating domestic sewage in developing countries. *Water Science and Technology*, **75**(7), 1659–1666, https://doi.org/10.2166/wst.2017.038

Cakir F. Y. and Stenstrom M. K. (2005). Greenhouse gas production: A comparison between aerobic and anaerobic wastewater treatment technology. *Water Research*, **39**, 4197–4203, https://doi.org/10.1016/j.watres.2005.07.042

Carvajal-Arroyo J. M., Sun W., Sierra-Alvarez R. and Field J. A. (2013). Inhibition of anaerobic ammonium oxidizing (anammox) enrichment cultures by substrates, metabolites, and common wastewater constituents. *Chemosphere*, **91**(1), 22–27, https://doi.org/10.1016/j.chemosphere.2012.11.025

Cavalcanti P. F. F. (2003). Integrated Application of the UASB Reactor and Ponds for Domestic Sewage Treatment in Tropical Regions. Doctoral thesis, University of Wageningen, The Netherlands.

Chick H. (1908). An investigation of the laws of disinfection. *Journal of Hygiene*, **8**(1), 92–158, https://doi.org/10.1017/S0022172400006987

Deffeyes K. S. (1956). Carbonate equilibria: a graphic and algebraic approach. *Limnology and Oceanography*, **10**, 412–426, https://doi.org/10.4319/lo.1965.10.3.0412

Delgado Vela J., Stadler L. B., Martin K. J., Raskin L., Bott C. B. and Love N. G. (2015). Prospects for biological nitrogen removal from anaerobic effluents during mainstream wastewater treatment. *Environmental Science and Technology Letters*, **2**(9), 234–244, https://doi.org/10.1021/acs.estlett.5b00191

de Silva Filho J. A. and van Haandel A. C. (2014). Simultaneous sludge stabilization of aerobic post treatment and sewage digestion in an anaerobic pre-treatment reactor. *Revista Dae*, **194**, 86–102.

Domínguez J., Rodríguez L., Silva-Teira A., Serna T., Sánchez E., Simón P., Moya N., Arias A., Lema J. M. and Garrido J. M. (2019). Removal of dissolved methane and nitrogen from anaerobically treated sewage using the SIAM process. (proceedings) Anaerobic Digestion Conference (AD16), Delft, The Netherlands.

Emerson K., Russo R. C., Lund R. E. and Thurston T. T. (1975). Aqueous ammonium calculations: effect of pH and temperature. *Journal of the Fisheries Research Board of Canada*, **23**, 2379–2383, https://doi.org/10.1139/f75-274

Environmental European Agency (2022). Beyond water quality. Report 05/2022. Publications Office of the European Union, Luxembourg, https://www.eea.europa.eu/publications/beyond-water-quality-sewage-treatment

Ettwig K. F., Van Alen T., Van De Pas-Schoonen K. T., Jetten M. S. M. and Strous M. (2009). Enrichment and molecular detection of denitrifying methanotrophic bacteria of the NC10 phylum. *Applied and Environmental Microbiology*, **75**, 3656–3662, https://doi.org/10.1128/AEM.00067-09

Foresti E., Zaiat M. and Vallero M. (2006). Anaerobic processes as the core technology for sustainable domestic wastewater treatment: Consolidated applications, new trends, perspectives, and challenges. *Reviews in Environmental Science and Bio/Technology*, **5**(1), 3–19, 2744, https://doi.org/10.1007/s11157-005-4630-9

Gomez E., Paing J., Casellas C. and Picot B. (2000). Characterisation of phosphorus in sediments from waste stabilization ponds. *Water Science and Technology*, **42**, 257–264, https://doi.org/10.2166/wst.2000.0656

Harned H. S. and Davis R. (1943). The ionization constant of carbonic acid in water from 0 to 50°. *Journal of the American Chemical, Society*, **65**(10), 2030–2037, https://doi.org/10.1021/ja01250a059

Harned H. S. and Scholes S. R. (1943). The ionization constant of HCO3-from 0 to 50 deg C. *Journal of the American Chemical, Society*, 1706.

Haroon M. F., Hu S., Shi Y., Imelfort M., Keller J., Hugenholtz P., Yuan Z. and Tyson G. W. (2013). Anaerobic oxidation of methane coupled to nitrate reduction in a novel archaeal lineage. *Nature*, **500**, 567–570, https://doi.org/10.1038/nature12375

Ho L. T., Van Echelpoel W. and Goedhals P. (2017). Design of waste stabilization pond systems: a review. *Water Research*, **123**, 236–248, https://doi.org/10.1016/j.watres.2017.06.071

Leitão R. C. (2004). Robustness of UASB reactors treating sewage under tropical conditions. PhD thesis, Wageningen University, Netherlands.

Lettinga G., van Velsen A. F. M., Hobma S., de Zeeuw W. and Klapwijk A. (1980). Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. *Biotechnology and Bioengineering*, **22**(4), 699–734, https://doi.org/10.1002/bit.260220402

Liñares-Lamas A. (2018). Modeling of the sulfur cycle in post-treatment of anaerobic reactor effluents. Master thesis, Universidade de Santiago de Compostela, Spain (in Galician).

Loewenthal R. E. and Marais G. V. R. (1976). Carbonate Chemistry of Aquatic Systems: Theory and Application. Ann Arbor Sci. Publishers, Michigan, USA.

Mara D. D. 1997. Design Manual for Waste Stabilization Ponds in India. Lagoon Technology International, Leeds, 125 p.

Marais G. V. R. (1974). Fecal bacteria kinetics in stabilization ponds. *Journal of the Environmental Engineering Division ASCE*, **100**(1), 119–139, https://doi.org/10.1061/JEEGAV.000013

Marais G. V. R. and Ekama G. A. (1976). The activated sludge process part I: Steady state behaviour, Water SA, 163–200.

Marais G. V. R. and Shaw V. A. (1961). A rational theory for design of waste stabilization ponds in South Africa. *Transactions of the South African Institute of Civil Engineers*, **3**, 205–227.

Mauricio-Iglesias M., Garrido J. M. and Lema J. M. (2016). Operation of an innovative WWTP with environmental objectives. A model-based analysis. *IFAC-PapersOnLine*, **49**(7), 539–543, https://doi.org/10.1016/j.ifacol.2016.07.398

Mazhar M. A., Khan N. A., Khan A. H., Ahmed S., Siddiqui A. A., Husain A., Rahisuddin Tirth V., Islam S., Shukla N. K., Changani F., Yousefi M., Hassaballa A.E. and Radwan N. (2021). Upgrading combined anaerobic-aerobic UASB-FPU to UASB-DHS system: Cost comparison and performance perspective for developing countries. *Journal of Cleaner Production*, **284**, 124723, https://doi.org/10.1016/j.jclepro.2020.124723

Metcalf E. and Eddy M. (2014). Wastewater Engineering: Treatment, Disposal and Reuse. 5th edn. McGraw-Hill, New York, USA.

Morais C. A., dos Santos S. L. and van Haandel A. C. (2023). A predictive model for ammonium removal in polishing ponds operated in sequential batch mode. *Water Science and Technology*, **87**(1), 13–26, https://doi.org/10.2166/wst.2022.418

Mulder A., van de Graaf A. A., Robertson L. A. and Kuenen J. G. (1995). Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor. *FEMS Microbiology Ecology*, **16**(3), 177–184, https://doi.org/10.1111/j.1574-6941.1995.tb00281.x

Okubo T., Onodera T., Uemura S., Yamaguchi T., Ohashi A. and Harada H. (2015). On-site evaluation of the performance of a full-scale down-flow hanging sponge reactor as a post-treatment process of an up-flow anaerobic sludge blanket reactor for treating sewage in India. *Bioresource Technology*, **194**, 156–164, https://doi.org/10.1016/j.biortech.2015.07.015

Onodera T., Tandukar M., Sugiyana D., Uemura S., Ohashi A. and Harada H. (2014). Development of a sixth-generation down-flow hanging sponge (DHS) reactor using rigid sponge media for post-treatment of UASB treating municipal sewage. *Bioresource Technology*, **152**, 93–100, https://doi.org/10.1016/j.biortech.2013.10.106

Parker C. D., Jones H. L. and Taylor W. S. (1950). Purification of sewage in lagoons. *Sewage and Industrial Waste*, **22**(6), 760–775, https://doi.org/10.2307/25031317

Pano A. E. and Middlebrooks J. (1982). Ammonia Nitrogen Removal in Facultative Wastewater Stabilization Ponds. *Journal Water Pollution Control Federation*, **54**(4), https://doi.org/10.2307/25041312

Pedrouso A., Morales N., Rodelas B., Correa-Galeote D., Val del Rio A., Campos J. L., Vazquez-Padin J. and Mosquera-Corral, A. (2023). Rapid start-up and stable maintenance of the mainstream nitritation process based on the accumulation of free nitrous acid in a pilot-scale two-stage nitritation-anammox system. *Separation and Purification Technology*, **317**, 123851, https://doi.org/10.1016/j.seppur.2023.123851

Pedrouso A., Val del Río Á, Morales N., Vázquez-Padín J. R., Campos J. L., Méndez R. and Mosquera-Corral A. (2017). Nitrite oxidizing bacteria suppression based on in-situ free nitrous acid production at mainstream conditions. *Separation and Purification Technology*, **186**, 55–62, https://doi.org/10.1016/j.seppur.2017.05.043

Pedrouso A., Tocco G., Val del Río A., Carucci A., Morales N., Campos J. L., Milia S. and Mosquera-Corral A. (2020). Digested blackwater treatment in a partial nitritation-anammox reactor under repeated starvation and reactivation periods. *Journal of Cleaner Production*, **244**, 1–9, https://doi.org/10.1016/j.jclepro.2019.118733

Pedrouso A., Val del Rio A., Morales N., Vazquez-Padin J. R., Campos J. L. and Mosquera-Corral A. (2021). Mainstream anammox reactor performance treating municipal wastewater and batch study of temperature, pH, and organic matter concentration cross-effects. *Process Safety and Environmental Protection*, **145**, 195–202, https://doi.org/10.1016/j.psep.2020.07.052

Santos S. L., Simões J. P. O, Paiva F. V. and Van Haandel A. (2017). Design optimization of a simple single family anaerobic sewage treatment system. *Water Practice and Technology*, **12**, 55–71, https://doi.org/10.2166/wpt.2017.010

Silva-Teira A., Sánchez A., Buntner D., Rodríguez-Hernández L. and Garrido J. M. (2017). Removal of dissolved methane and nitrogen from anaerobically treated effluents at low temperature by MBR post-treatment. *Chemical Engineering Journal*, **326**, 970–979, https://doi.org/10.1016/j.cej.2017.06.047

Stazi V. and Tomei M. C. (2021). Dissolved methane in anaerobic effluents: A review on sustainable strategies for optimization of energy recovery or internal process reuse. *Journal of Cleaner Production*, **317**, 128359, https://doi.org/10.1016/j.jclepro.2021.128359

Tandukar M., Ohashi A. and Harada H. (2007). Performance comparison of a pilot-scale UASB and DHS system and activated sludge process for the treatment of municipal wastewater. *Water Research*, **41**(12), 2697–2705, https://doi.org/10.1016/j.watres.2007.02.027

Tawfik A., Ohashi A. and Harada H. (2006). Sewage treatment in a combined up-flow anaerobic sludge blanket (UASB)-down-flow hanging sponge (DHS) system. *Biochemical Engineering Journal*, **29**(3), 210–219, https://doi.org/10.1016/j.bej.2005.11.018

Thalasso F., Vallecillo A., García-Encina P. and Fdz-Polanco F. (1997). The use of methane as a sole carbon source for wastewater denitrification. *Water Research*, **31**, 55–60, https://doi.org/10.1016/S0043-1354(96)00228-X

Van Haandel A. C. and van der Lubbe J. (2012). Handbook Biological Wastewater Treatment: Design and Optimization of Activate Sludge Systems. IWA Publishing, London, UK.

Van Haandel A. C. and van der Lubbe J. (2019). Anaerobic Sewage Digestion: Theory and Applications. IWA Publishing, London, UK.

Van Lier J. B., Mahmoud N. and Zeeman G. (2020). Anaerobic Wastewater Treatment. IWA Publishing, London, UK, https://doi.org/10.2166/9781789060362_0701

Vesilind P. A. (1968). Theoretical considerations: design of prototype thickeners from batch settling tests. *Water and Sewage Works*, **115**, 302–307.

Wang Z., Zheng M., Duan H., Yuan Z. and Hu S. (2022). A 20-year journey of partial nitritation and anammox (PN/A): from sidestream toward mainstream. *Environmental Science & Technology*, **56**(12), 7522–7531, https://doi.org/10.1021/acs.est.1c06107

Wu P., Zhang X., Wang X., Wang C., Faustin F. and Liu W. (2020). Characterization of the start-up of single and two-stage anammox processes with real low-strength wastewater treatment. *Chemosphere*, **245**, 125572, https://doi.org/10.1016/j.chemosphere.2019.125572

Xie T., Liu X., Xu Y., Bryson S., Zhao L., Huang K., Huang S., Li X., Yang Q., Dong H. and Winkler M. H. (2023). Coupling methanotrophic denitrification to anammox in a moving bed biofilm reactor for nitrogen removal under hypoxic conditions. *Science of the Total Environment*, **856**, 158795, https://doi.org/10.1016/j.scitotenv.2022.158795

Xu M., Zhou H., Yang X., Angelidaki I. and Zhang Y. (2020). Sulfide restrains the growth of *Methylocapsa acidiphila* converting renewable biogas to single cell protein. *Water Research*, **184**, 116138, https://doi.org/10.1016/j.watres.2020.116138

Zheng M., Li H., Duan H., Liu T., Wang Z., Zhao J., Hu Z., Watts S., Meng J., Liu P., Rattier M., Larsen E., Guo L., Dwyer J., Van Den Akker B., Lloyd J., Hu S. and Yuan Z. (2023). One-year stable pilot-scale operation demonstrates high flexibility of mainstream anammox application. *Water Research X*, **19**, 100166, https://doi.org/10.1016/j.wroa.2023.100166

Zimmo O. R., van der Steen N. P. and Gijzen H. J. (2003). Comparison of ammonia volatilization rates in algae and duckweed-based waste stabilization ponds treating domestic wastewater. *Water Research*, **37**(19), 4587–4594, https://doi.org/10.1016/j.watres.2003.08.013