

Chapter 2 ~ Waste Stabilization Ponds and Nitrogen Chemistry

2.1 Introduction

A WSP system is a number of large shallow basins enclosed by earth embankments, where raw wastewater is treated by entirely natural processes, involving algae and bacteria (Mara, 2004). In order to construct WSP, earth must be moved to create the basin for the pond. Preliminary treatment, inlet and outlet structures for the ponds, embankment construction and pond lining material (which is required only if the soil in the excavated area is too permeable) are the only other essential civil engineering works required for pond fabrication (Mara, 2004).

The basic layout of a WSP system consists of a number of ponds connected in series, to complete wastewater treatment to a high quality standard. Typically, a whole system might include an anaerobic pond, followed by a facultative pond and the required number of maturation ponds. Anaerobic ponds receive all fractions of the raw wastewater entering the system which (usually in domestic wastewater) contains high levels of solid material, biochemical oxygen demand (BOD), and both organic and inorganic nitrogen fractions, as well as many other contaminants. Anaerobic ponds are usually 2–5 m deep and are devoid of dissolved oxygen due to the high organic loading they receive (Mara, 1976, 2004). Their primary function is the removal of BOD, suspended solids (SS) and helminth eggs; this is achieved through the action of sedimentation of the settleable solids fraction of the wastewater, where anaerobic digestion of these solids is carried out by strict anaerobic bacteria (Mara, 2004).

Anaerobic ponds feed facultative ponds, which are usually between 1–2 m in depth and are responsible for removing a large percentage of the influent BOD, SS, nutrient and pathogen contents of the wastewater. An ideal depth for these ponds is usually given as 1.5 m, as ponds over 2 m deep have been found to have very poor nutrient removal rates (Silva *et al.*, 1987). Both aerobic and anaerobic treatment of wastes occurs simultaneously within these ponds – thus their name, facultative – which describes the ability of the bacteria in them to live and

function in the presence, or absence, of dissolved oxygen. These ponds contain an appreciable amount of algae in the upper aerobic layer of the pond water.

Maturation ponds are fully aerobic and are 1–1.5 m in depth, and typically contain a larger number of algal species than those found in facultative ponds. They receive facultative pond effluent, and their function is to remove residual SS, BOD and nutrients from the wastewater. Alongside this, their most important function is to reduce the number of excreted pathogens – bacterial, viral, protozoan and helminthic – in the wastewater. This is partially achieved through solar disinfection, where solar rays penetrate the cell walls of pathogenic microorganisms and break the pyrimidine base dimers of the DNA base pair structure. This process deactivates the cell, and is further facilitated by oxygen-mediated exogenous photosensitization (Curtis, 1990). Pathogenic bacteria (with the notable exception of *Vibrio cholerae*) are also intolerant of high pH levels (which damage cells physically), and on sunny days pH levels of around 10 can be achieved in these ponds through the action of photosynthesis by algae which cause a shift in pond alkalinity, which results in rapid bacterial die-off.

The mechanisms and fundamentals of the design and operation of anaerobic and maturation ponds, and other pond types are not considered here; full descriptions can be found in Mara (1976, 2004).

2.2 Facultative ponds

Facultative ponds are extremely complex systems, and are perhaps the most biochemically complex of all WSP types. As previously mentioned, the name *facultative*, applied to these ponds, describes the occurrence of bacteria which can live in either aerobic or anoxic/anaerobic conditions. Facultative ponds are constructed directly after anaerobic ponds and treat anaerobic pond effluent. However, an anaerobic pond can be bypassed and a primary facultative pond used as the first pond in the treatment system after preliminary treatment (screening and grit removal). (Primary facultative ponds have served as the experimental systems used in this research). Although essentially little more than a shallow basin, their successful operation depends upon careful and correct design, assessing the prevailing climatic conditions ascertained for their location, and the

consistency of the characteristics of the wastewater they are designed to receive. The sum of these factors assures the successful integration and interaction of the biological, chemical and physical processes occurring within them.

2.2.1 Characteristics of facultative ponds and principles of their operation

Facultative ponds are distinguished by numerous unique and individual features, and can be sub-divided into layered zones where certain biological processes prevail. Experts have debated at length and in depth the merits of aerobic over anaerobic wastewater treatment systems, and vice versa (Gijzen, 2001). The beauty of facultative WSP is that both processes occur simultaneously, ensuring a more complete oxidation of all the waste components entering them.

The upper tier of the pond (extending from the pond surface to a depth of around 50 cm) is fully aerobic and characterised by a rich green colour attributable to the large quantities of algae in this stratum of pond water. Algae are diverse photoautotrophic microorganisms, which play an intrinsically important role in facultative pond biochemistry; without them facultative ponds would become anaerobic (Pearson *et al.*, 1987). The volume and the genera of algal species are often prolific within a well functioning WSP system. (The colonisation and role of algae within WSP are discussed in more detail in section 2.3.1).

Algae form a dense band at the pond surface, or just beneath it, where they congregate to move vertically up or down within this aerobic layer to optimise their photosynthetic capabilities within the photic zone (the zone within which effective solar radiation can penetrate) (Konig, 1984). The photic zone usually comprises the top 50 cm of the pond (Mara, 1976), but algae can form dense bands of around 10–20 cm thick around 10–50 cm below the surface of the pond (Mara, 2004). A healthy well operating facultative pond usually has algal concentrations of 500–2000 µg chlorophyll *a* per litre (Mara, 1997). In warm sunny climates, algal oxygen production can be so profuse that the upper tier of the WSP quickly becomes supersaturated with oxygen.

This layer of the pond is aerobic for these distinct reasons:

- Molecular oxygen diffuses into the pond water naturally from the atmospheric boundary layer.
- Wind mixing enables the rapid turnover of pond waters, especially at the pond surface. The physical turnover of waters by this action brings to the pond surface, waters from depths which would have been unaffected by natural diffusion of O₂ into the surface waters. The new flow of air brought by the wind over the pond also provides a continual supply of fresh O₂ with which to oxygenate the water.
- The turnover of thermal stratification within a pond can also aid the mixing of O₂ into the pond water. In warm climates thermal stratification plays a particularly important role within pond mixing. Surface layers of the pond are heated during the day, but in the cooler evening and night they lose heat to the atmosphere and cool rapidly, and thus the water becomes denser. This water is displaced downwards by the action of the less dense warmer subsurface water, which overturns it and rises to the top of the pond, enabling O₂ to diffuse into the warmer water. O₂ contained within the cooler water is carried downwards, and therefore its concentration is more uniformly distributed throughout the various strata of the pond.
- If a facultative WSP is operating correctly, by far the largest proportion of O₂ contributed to the system, as much as 80% (Mara and Horan, 2003; Oswald, 1988) comes from algal photosynthesis, which can reach very high levels on warm sunny days.

The main pathways of BOD removal within facultative ponds are summarised by Marais (1970) in Figure 2.1.

Moving longitudinally downward from the pond surface, the mid-layer of the pond beneath the aerobic layer is the facultative/anoxic layer. Some algal species are found within this layer, but it is inhabited mostly by an appreciable number of microorganisms which have the ability to function in the presence or absence of molecular oxygen. The facultative boundary occurs at the aerobic/anoxic interface which is largely where the photic zone ends and the thermocline occurs.

The thermocline is the point within the pond where a sudden drop in temperature occurs, as insolation is obscured by the murky upper pond layer and can no longer effectively penetrate and warm the pond waters beneath it. Some O_2 may exist within the upper portion of this layer although at very low concentrations. Anoxic conditions are achieved when all residual dissolved O_2 has been eliminated (Horan in Mara and Horan, 2003). Charpentier *et al.* (1987) favoured the use of oxidation-reduction potential (ORP) measurements to define, and more accurately determine anoxic conditions within a water body. Where ORP values of 1 to -300 mV occur, Charpentier *et al.* (1987) deemed a water to be anoxic. ORP values between these values often occur within facultative WSP at these pond depths.

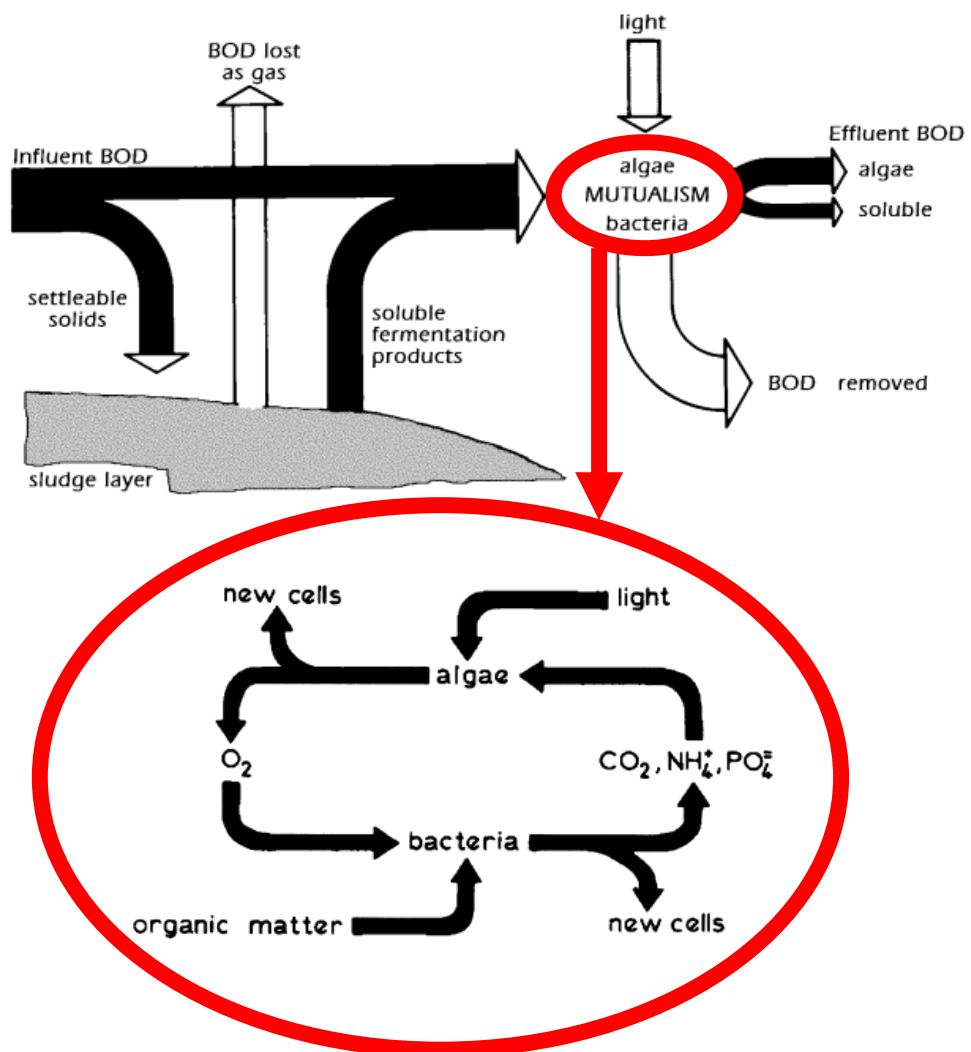


Figure 2.1: Diagram to show the major pathways of BOD removal within facultative WSP, and the mutualistic (or symbiotic) relationship between pond algae and bacteria (Marais, 1970; Mara, 1997).

A sizeable portion of BOD is removed within this facultative fraction of the pond. Oxygen produced by the algae from the aerobic layer above, coupled with oxygen-enriched water from wind and thermal mixing, is transferred via these mechanisms to the lower depths of the pond. There, facultative autotrophic and heterotrophic bacteria use oxygen as a terminal acceptor, and metabolise dissolved, colloidal and suspended organic matter in the oxidation of carbon and nitrogen compounds for cell growth, energy and synthesis (WHO, 1987). Facultative anaerobes colonise this portion of the pond, and as previously stated, they are active in the presence or absence of free molecular oxygen (Gerardi, 2003). If O_2 is present, it is used for enzymatic activity within the bacteria for the metabolism of wastes, but if it is absent, other molecules such as NO_3^- are used to degrade wastes such as methanol, where the chemically combined oxygen in the NO_3^- is used as an electron donor. The microbiological mechanisms in the aerobic and facultative layers of the pond also are responsible for a large amount of nutrient removal.

Beneath the facultative/anoxic zone at the bottom of the pond is the anaerobic zone, categorised by the complete absence of molecular oxygen and high negative ORP values of -300 mV or greater which deem it to be anaerobic (Brockett, 1976; Charpentier *et al.*, 1987). Here, a thick layer of sludge forms; this is built up over time by the continual sedimentation of settleable solids from the wastewater influent, fallout from the suspended solids fraction of the wastewater, from dead algal, bacterial and other microbial cells, in addition to other larger life forms in the pond. A major portion of the influent oxygen demand is almost instantaneously removed from a facultative pond via sedimentation mechanisms, and stabilized in this benthic layer (Bryant and Bauer, 1987). The sludge also incorporates a fraction of undegradable solids (Carré and Baron, 1987). This is a very complex section of the pond where a number of important activities take place. The bottom portion of the pond serves not only as an in-built sludge storer, but through anaerobic degradation processes the sludge is broken down and its various by-products recycled. These by-products include the gases CH_4 , CO_2 and N_2 as well as soluble products and other complex organic compounds (Marais, 1970).

The sludge is 'treated' by physical compaction over time, and digested by anaerobic microorganisms. The process of hydrolysis ensures that polymeric compounds, such as those that comprise carbohydrates, fats and proteins, are enzymatically broken down by anaerobic heterotrophs into their constituent monomers (Horan, 1990). Here, nutrients are released and can be recycled by bacteria at source or through the action of pond mixing by turnover of thermal stratification, are carried into upper layers of the pond where they are metabolised by other pond microorganisms. Natural solubilisation also plays a part in sludge degradation within a pond.

Volatile fatty acid (VFA) production is an important process which occurs here, and a considerable amount of gas production takes place within this anaerobic layer. VFA's are produced in anaerobic conditions as by-products of the metabolism of monomers from carbohydrates, fats and proteins through the action of anaerobic bacteria in the absence of oxygen. Methanogens colonise and dominate this portion of the pond as they reduce CO_2 and use VFA's for cell metabolism. Methane is given off as a by-product and rises to the pond surface and escapes into the atmosphere. Some methane is utilized by methane-oxidising bacteria, which use methane as their sole source of carbon and energy (Murrell and Radajewski, 2000). Denitrification also occurs here as this is a strictly anaerobic process. In high summer, frequent bubbling at the pond surface can be observed, as elevated temperatures are a driver for increased gas production. Small 'blobs' of sludge, and sometimes small blankets, rise to the surface as rapid gas production carries sludge from the base of the pond to surface waters as the bubbles of gas are generated.

2.2.2 Facultative pond design criteria

Facultative pond design is dependent on a variety of different criteria which must be considered carefully when design calculations are made in order to ensure that the pond will work properly. Primary and secondary facultative ponds are ideally designed and built rectangular in shape, with length-to-breadth ratios of 2–3 to 1, and a maximum depth of approximately 1.5 m (Mara, 2004). A facultative pond which follows an anaerobic pond (a secondary facultative pond), can have a larger length to breadth ratio of up to 10 to 1.

There are four principal parameters which need to be carefully considered during the design of a WSP:

1. Temperature: the mean air temperature of the coolest month of the year is used as the design temperature
2. Net evaporation: the value for mean monthly evaporation is also taken from data collected in the coolest month of the year. Net evaporation is simply calculated by evaporation minus rainfall.
3. Flow: the mean daily flow is used to calculate this
4. Weatherell *et al.* (2003) note that most WSP are designed on the basis of BOD loading: this can either be obtained through the collection and analysis of 24-hour flow-weighted wastewater composite samples, or through calculating the theoretical influent BOD from the equation:

$$L_i = 1000B/q \quad (2.1)$$

where L_i = the wastewater BOD measured in mg/l

B = the BOD contribution of each person in g/caput/day, and

q = the wastewater flow (l/caput/d) (Mara, 1997).

Many design equations have been advocated over the years for facultative pond design, and BOD removal kinetics operating under different hydraulic flow regimes. Distinguished researchers in the field include Herman and Gloyna (1958), Marais and Shaw (1961), Thirumurthi (1969), McGarry and Pescod (1970), Gloyna (1976), and Reed *et al.* (1988). All working design equations have been derived and tested empirically, through the analysis of performance data from WSP throughout the world.

The principle of facultative WSP design is based upon how much 'load', in terms of organic waste – i.e., BOD, they are capable of receiving from the strength of influent wastewaters and according to different design parameters. All waste contained within wastewater exerts an oxygen demand on that water body; oxygen is required by bacteria and other microbiological organisms in order to break down and oxidise this waste for their cell metabolism. The oxygen required by microorganisms for this is referred to as the biochemical oxygen demand (BOD) of a wastewater. A facultative pond is designed according to the BOD

load applied per unit area of pond surface area per day, (the units are commonly given as kg BOD/ha day), rather than the volumetric loading approach (given in g BOD/m³ day) of BOD per day, which is used to design anaerobic WSP. The surface loading approach is undertaken because the algae, which are the main providers of oxygen to the system, reside in the upper 50 cm of the pond; therefore their rate of oxygen production is a function of photosynthetic activity which in turn is a function sunlight incidence. It follows that oxygen production from algae is a function of area, thus the BOD load applied to the pond is also a function of area, and not of volume (Mara, 2004).

Mara (1987) modified the empirical equation derived by McGarry and Pescod (1970) to present a more robust global design equation for facultative WSP. McGarry and Pescod (1970) analysed performance data from 143 WSP systems which were operating under different conditions, and found good levels of BOD removal of ~70–90 %. The regression model they prepared, takes into account the relationship between ambient air temperature and the maximum BOD load which can be applied to a facultative pond before it turns anaerobic, and is given below in equation 2.2:

$$\lambda_s = 60(1.099)^T \quad (2.2)$$

where λ_s = surface BOD loading (kg/ha.d)

T = mean air temperature (°C)

Pond failure describes situations in which a pond becomes anaerobic throughout the day at all depths (McGarry and Pescod, 1970). Here algal activity is inhibited, thus oxygen is not transferred to the system, and subsequently the pond can become overloaded. Equation 2.2 served as an interim design equation, where the maximum BOD value, which could be applied to a pond before it failed, was generated – i.e., it was an envelope of pond failure. In 1987 Mara modified McGarry and Pescod equation to provide a more flexible design model, which factored in a safety margin to act as a buffer to prevent pond failure.

This is given in equation 2.3:

$$\lambda_s = 350(1.107 - 0.002T)^{T-25} \quad (2.3)$$

where λ_s = surface BOD loading (kg/ha d)

T = mean air temperature (°C)

The surface BOD loading applied to a pond is also related to the mean amount of influent BOD in the flow entering a system per day, and the area of the facultative pond. This is represented in equation 2.4:

$$\lambda_s = \frac{10L_iQ}{A_f} \quad (2.4)$$

where λ_s = surface BOD loading (kg/ha d)

L_i = the concentration of influent BOD (mg/l)

Q = mean flow (m³/d), and

A_f = facultative pond area (m²)

The area of a facultative pond is derived by rearranging equation 2.4 to give equation 2.5:

$$A_f = \frac{10L_iQ}{\lambda_s} \quad (2.5)$$

The retention time of a facultative pond can be simply determined by equation 2.6:

$$\theta_f = \frac{A_f D}{Q_i} \quad (2.6)$$

where θ_f = hydraulic retention time (d)

A_f = the surface area of the facultative pond (m²)

D = depth (m), and

Q_i = mean influent flow rate (m³/d)

Equation 2.6 is sufficient to a degree in calculating the theoretical hydraulic retention time, but it does not consider inevitable losses from the system, and so a more accurate equation for determining the mean hydraulic retention time is

presented in equation 2.7. This incorporates losses from the system attributable to net evaporation, as follows:

$$\theta_f = \frac{2A_f D}{(2Q_i - 0.001eA_f)} \quad (2.7)$$

where θ_f = facultative pond hydraulic retention time (d, days)

A_f = area of facultative pond (m²)

Q_i = mean influent flow (m³/d)

e = mean net evaporation (mm/d)

A model for determining the concentration of effluent BOD leaving a facultative pond was derived by Marais and Shaw (1961), shown by equation 2.8. This uses the first-order rate constant for BOD removal (derived by equation 2.9) and is based on facultative ponds operating under a completely mixed hydraulic flow regime:

$$L_e = \frac{L_i}{1 + k_1 \theta_f} \quad (2.8)$$

where L_e = unfiltered BOD of facultative pond effluent (mg/l)

L_i = BOD of facultative pond influent (mg/l)

k_1 = first-order rate constant for BOD removal (d⁻¹), and

θ_f = facultative pond hydraulic retention time (d)

The first-order rate constant (k_1) for BOD removal per day is given by equation 2.9:

$$k_{1(T)} = k_{1(20)}(1.05)^{T-20} \quad (2.9)$$

where $k_{1(20)}$ = the design value of 0.3 d⁻¹ for primary facultative ponds, and 0.1 d⁻¹ for secondary facultative ponds.

2.3 Facultative pond microbiology

Facultative pond microbiology is extremely complex, and as it is a natural system a huge variety of different organisms can coexist in a pond at any one time. The types of microorganisms shown in Table 2.1 are the main drivers of the principal biochemical reactions which take place within facultative WSP. In order to

understand pond process biology better, and the functions that each microbe fulfils, it is useful to distinguish microorganisms according to the ways in which they satisfy their requirements for carbon, energy, hydrogen and electrons (Mara and Horan, 2003). Table 2.1, adapted from Chan (2003), provides an excellent reference for classifying microorganisms according to their metabolic needs when describing the many complex biochemical reactions which occur within WSP. With respect to biomass yield in facultative WSP, it follows that microbial growth rate, enhanced by the mutualistic relationship shown in Figure 2.1 between bacteria and algae, is directly related to the specific substrate (i.e., the organic and inorganic oxidisable matter contained within the raw wastewater) removal rate from the WSP (Kayombo *et al.*, 2003).

2.3.1. Algae

Algae are unicellular eukaryotic photoautotrophs, which are green in colour due to their high content of the photosynthetic pigment chlorophyll *a*; they can exist in colonies of individual cells embedded in a polysaccharide matrix (Horan, 1990). They are vitally important in successful pond performance as they help to reduce odour emissions, are the oxygen providers for the efficient bacterial degradation of the organic load, help to contribute to enhanced conditions for pathogen reduction, and aid ammonia nitrogen removal (Weatherell *et al.*, 2003). The key parameters of organic loading, light penetration, sulphide and ammonia concentrations determine algal activity, biomass concentration and speciation within a pond (Pearson, 1988).

Time and season can also effect algal biomass: for example, episodes of predatory zooplankton grazing, and insufficient light penetration throughout the water column (particularly in winter months with limited day-light hours of low insolation intensity). Mara and Pearson (1986) proposed that the concentration of chlorophyll *a* in a thriving facultative WSP should be between 500 and 2000 $\mu\text{g/l}$, and that a value of $\leq 300 \mu\text{g/l}$ denotes an unstable system close to failure (Pearson, 1996), where 300 $\mu\text{g/l}$ is considered to be the minimum value required for a correctly operating pond (Mara and Pearson, 1986).

Table 2.1: Classification of microorganisms according to their nutritional type and energy requirements (adapted from Chan, 2003).

Nutrition type	Microorganism type	Energy source	Electron or hydrogen source	Carbon source
Photolithotrophic autotrophy	Photoautotrophs	Light	Inorganic compounds and H ₂ O	CO ₂
Photo-organotrophic heterotrophy	Photoheterotrophs	Light	Organic compounds	Organic compounds
Chemolithotrophic autotrophy	Chemoautotrophs	Inorganic compounds	Inorganic compounds	CO ₂
Chemo-organotrophic heterotrophy	Chemoheterotrophs	Organic compounds	Organic compounds	Organic compounds

Saqqar and Pescod (1996) further provide three criteria which can be used to distinguish between a healthily operating facultative WSP and one they deemed to be anoxic. In synopsis, they defined an anoxic pond to be a pond where oxygen consumption by microorganisms – mainly facultative bacteria – is close to, or slightly more than, the oxygen produced by the algae (Saqqar and Pescod, 1996). The three criteria presented which classify an anoxic pond are: 1) diurnal DO concentrations at depths of ≥ 10 cm are normally zero; 2) there will be an increase in SS in the pond effluent compared to the pond influent – but this does not apply to primary facultative ponds; and 3) the pH will be higher than found in an anaerobic pond (Saqqar and Pescod, 1996).

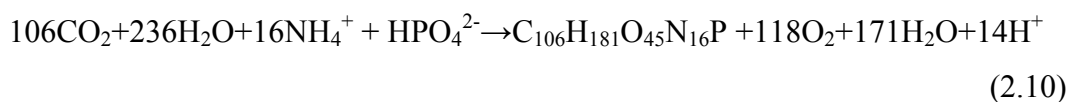
Many genera of algae exist within WSP, and a few of the predominant species are listed in Table 2.2. Apart from their taxonomic grouping, it is useful to classify algae into the two distinct groups of motile and non-motile genera, which is of real significance in terms of WSP oxygen production. Motile algae have flagella – a tail-like appendage which can induce movement of the alga, thus enabling them to migrate up and down the pond water column. Conversely, non-motile forms of algae are not flagellate, and they can move up and down a water column only if they are caught in the convective currents of thermally stratified pond water turning over, or by wind-induced mixing. If there is insufficient mixing,

these algae will remain at depths below the photic zone and respire, eventually dying, and so contributing to the BOD within the pond.

Table 2.2: Some of the most common algal genera found in facultative WSP (Mara and Horan, 2003; Mara, 2004).

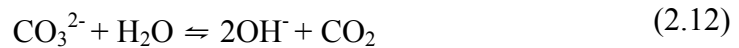
Motile algae	Non-motile algae
<i>Euglena</i>	<i>Eudorina</i>
<i>Phacus</i>	<i>Ankistrodesmus</i>
<i>Chlamydomonas</i>	<i>Chlorella</i>
<i>Chlorogonium</i>	<i>Micratinium</i>
<i>Pandorina</i>	<i>Scenedesmus</i>
<i>Pyrobotrys</i>	<i>Selenastrum</i>
<i>Carteria</i>	<i>Coelastrum</i>
<i>Volvox</i>	<i>Dictyosphaerium</i>
<i>Navicula</i>	<i>Oocystis</i>
<i>Rhodomonas</i>	<i>Cyclotella</i>
	<i>Oscillatoria</i>
	<i>Anabaena</i>

Flagellate algae tend to predominate in facultative ponds, and the algal species present can provide a good indication of how a pond is operating. It is recognised that algal speciation changes according to the pond surface organic load applied; typically at higher loadings, the diversity of algal species decreases (Marais, 1970; Konig, 1984; and Konig *et al.*, 1987; Pearson, 1988). Algae produce oxygen from the splitting of water molecules in order to use hydrogen. They use the carbon in CO₂ as their main carbon source, and use inorganic chemicals such as ammonia and phosphates to synthesise new cell material and cell energy. A complete mass balance equation for photosynthesis, shown in equation 2.10, was prepared by Oswald (1988):

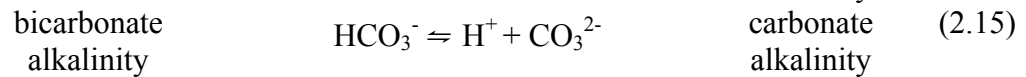
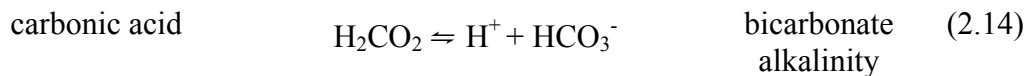
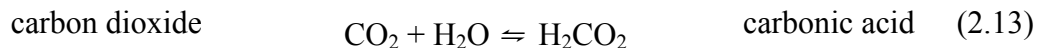


Under conditions of intense photosynthesis, the algae utilise CO₂ at a much faster rate than it can be replaced by the heterotrophic bacteria contained within the pond water. When free CO₂ concentrations drop in the pond water column below its equilibrium concentration with air, the algae have the ability to sequester CO₂ from carbonate and bicarbonate ions present in pond water. As CO₂ is removed from the water column, the alkalinity shifts from carbonate

alkalinity (CO_2^{3-}), to bicarbonate (HCO_3^-), to carbonic acid (H_2CO_3) and finally to CO_2 through the process of ionic dissociation. The product of this ionic dissociation is an increase in hydroxyl ions, which can elevate the pH to as high as 10 or more when photosynthesis is rapid. During these changes in pH, the equilibrium between each alkalinity contributing agent continually shifts, demonstrated accordingly in equations 2.11–2.15; the total alkalinity of the pond water remains the same (Sawyer, 1994). These changes in equilibrium can continue to occur as algae extract CO_2 from the pond water until an inhibitory pH is reached, usually in the range of 10 to 11 (Pipes, 1962; Azov and Shelef, 1987; Sawyer *et al.*, 1994). The equilibrium equations for alkalinity exist as follows:



A slightly different portrayal of the shift in the forms of alkalinity is shown by equations 2.13– 2.15:



The rate at which algal photosynthesis occurs within a WSP is directly proportional to the degree of light intensity and higher temperatures: as these latter two variables increase, so does the rate of algal photosynthesis. However, the amount of dissolved and readily available carbon dioxide in a wastewater can act as a limiting factor, which can partially abate photosynthetic activity if it is not present in sufficient quantities for algal utilisation. Algae preferentially use carbon dioxide as their carbon source. Should the quantity of carbon dioxide drop significantly within the photic zone, because the bacteria cannot provide carbon dioxide at the same rate as algae are depleting it, the available alkalinity, present in the form of carbonate and bicarbonate ions, dissociate, which provides more carbon dioxide (Mara, 1976). This results in an increase in alkaline hydroxyl ions which, in turn, increase the pH within the pond, so causing a shift in the ammonia-ammonium equilibrium (Mara, 1976). Figure 2.2 shows schematically the shifts in the forms of alkalinity with respect to pH.

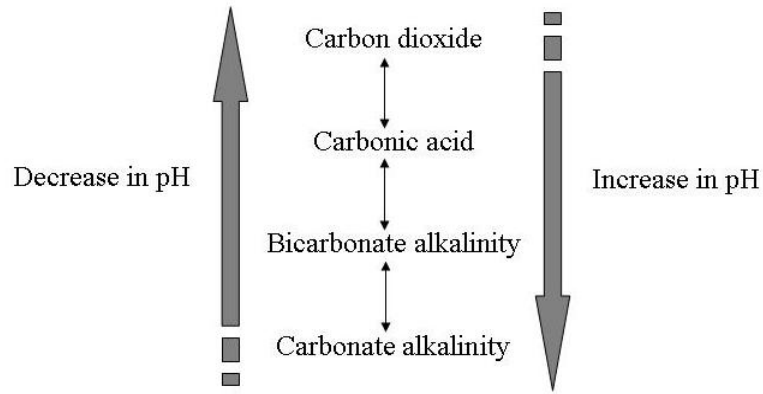


Figure 2.2: Diagram to show the shifts in the dominant forms of alkalinity with respect to pH.

So far, only photoautotrophic algae have been discussed. There are several algal species which can satisfy their nutrition through chemo-organotrophy and photo-organotrophy. These algae metabolise the substrates acetate, butyrate, and propionate which are part of the larger VFA family, produced by anaerobic digestion within the sludge layer of the facultative pond (Pearson, 1988; Gerardi, 2002). *Euglena* was found to have the ability to utilize butyrate. *Chlamydomonas*, *Chlorella* and *Euglena* were found to be able to grow in the dark using acetate, but only *Chlorella* was found to be able to use glucose, and under these conditions it was still only *Euglena* which could use butyrate (Pearson *et al.*, 1987a).

In order for a facultative pond to operate correctly, it is paramount that algal populations are properly maintained. Pond algae provide a valuable food source for predators and grazers such as *Daphnia*, *Paramecium* and various rotifers, and these can be a huge problem when they wipe out algal populations. Algal consumption by larger life-forms, can rapidly leave a pond with an expedient onset of anoxic conditions, particularly in facultative WSP; however, as with all natural cycles, once algal biomass is depleted below a level necessary for predatory sustenance, predatory populations start decline, allowing algal populations to start to regenerate, and thus the cycle starts all over again.

Algal activity in facultative WSP is arrested by ammonia and sulphide toxicity, where both the degree of inhibition is concentration and pH dependent (Pearson, 1988). Although ammonia is an essential requirement for algal cell synthesis,

concentrations above 28 mg NH₃/l are detrimental to algae. As ammonia is lipid soluble, it can traverse cell membranes easily and cause cellular damage (Konig *et al.*, 1987). Sulphide is much more toxic to algae than ammonia. Ammonia and sulphide are essential parts of a wastewater, but they are also generated through microbial activity within ponds (Pearson *et al.*, 1987a). Sulphate-reducing bacteria work to reduce sulphate to sulphide, which is also released through the breakdown of mercaptans. Sulphide toxicity is found to increase with decreasing pH, while conversely ammonia toxicity increases with increasing pH. The unionised sulphide (H₂S, dissolved hydrogen sulphide gas) inhibits algal functionality by diffusing through the algal cell membrane and adversely targeting the cells photosynthetic mechanism (Mara, 2004) by switching off photosystem II activity (Pearson, 1988). In general, soluble sulphide levels of above 8 mg/l, or 400 mg/l for sulphate, pose problems for algae (Pearson, 1988). A table summarising the findings of ammonia and sulphide toxicity on four algal species presented in Pearson *et al.* (1987b) is shown in Table 2.3.

However, the effects of ammonia and sulphide toxicity in algae are reversible if ameliorated in sufficient time. When the recovery of algae was investigated after exposure to sulphide toxic conditions (exposure to 250 µm total sulphide for >8 hours at pH 7.25), the photosynthetic capabilities of the algae were found to regenerate, although the longer the exposure time to sulphide, the slower the recovery of the algae (Pearson *et al.*, 1987b). The effects of ammonia toxicity and sulphide toxicity produced similar behaviour in the algae.

Table 2.3: Concentrations of ammonia and sulphide which cause toxic effects in algae at various pH (Pearson *et al.*, 1987b).

Alga	Ammonia causing 50% inhibition at pH 8.5 (mg/l NH₄-N)	Ammonia causing 50% inhibition at pH 8.5 (mM NH₄-N)	NH₃ causing 50% inhibition at any pH (mM)	Total sulphide causing 50% inhibition at pH 7.25 (µM H₂S)	H₂S causing 50% inhibition at any pH (µM H₂S)
Chlamydomonas	81	5.8	0.88	118.3	44.95
Chlorella	356	25.4	3.84	80.0	30.40
Euglena	87	6.2	0.94	27.5	10.45
Scenedesmus	150	10.7	1.62	58.3	22.15

In the same study conducted by Pearson *et al.* (1987b), the inhibition of algae to ammonia toxicity was monitored, and algae were found to fully recover after exposure to ammonia (exposure to 15 mM NH₃ at pH >8, for >4 hours). In both sulphide and ammonia toxic environments, *Euglena* fared badly and struggled to recover, especially after exposure to high sulphide. The sequence of relative tolerance to sulphide is presented in the following ascending order:

Euglena < *Scenedesmus* < *Chlorella* < *Chlamydomonas*

The ascending tolerance to ammonia toxicity is given as:

Euglena < *Chlamydomonas* < *Scenedesmus* < *Chlorella*

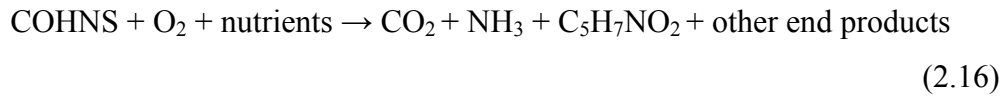
2.3.2. Bacteria

Bacteria play an intrinsically important role in wastewater treatment, and Horan (1990) stated that they comprise the largest microbial community component in any biological wastewater treatment processes. An empirical formula for a bacterial cell is given by Tebbutt (1998) as C₆₀H₈₇O₂₃N₁₂P. Some of the more common types of facultative WSP bacteria, cited most in congruent literature, are presented in Table 2.4, showing their nutrition type and activity within the pond system.

Bacteria have two metabolic strategies which mineralise organic matter: one is via aerobic mineralisation where oxygen is used as an electron acceptor to aid the degradation of organic matter, and the other occurs anaerobically, where inorganic electron acceptors such as nitrate (denitrification), sulphate (sulphate reduction) and carbon dioxide (methanogenesis) are used in the oxidation of organic matter (Gijzen, 2001). Both these processes occur simultaneously within facultative WSP.

In the aerobic tier of facultative ponds, the non-settleable fraction of BOD (i.e. the soluble and suspended organic fractions) is oxidised by heterotrophic and aerobic chemo-organotrophic bacteria such as *Pseudomonas*, *Flavobacterium*, *Archromobacterium*, and *Alcaligenes* spp for growth and energy production

(Soares, 1996). The stoichiometry of aerobic biological oxidation is presented in equation 2.16 (Metcalf and Eddy, Inc. 2003):



COHNS denoted in equation 2.16 is an expression for the organic matter contained within wastewater. More bacterial species grow on organic nitrogen than on ammonium salts and nitrate (Mara, 1974).

Anaerobic digestion or oxidation, plays a very important role in the stabilization of waste at lower regions of the pond. Essentially the oxidation of organic material in this region of the pond is incorporated into new cell material, and unstable organic acids such as alcohols, ketones and methane (Tebbutt, 1998). The four stages of anaerobic digestion are: hydrolysis, acidogenesis, acetogenesis and methanogenesis Each occurs in the following reactions shown in equations 2.17, 2.18, 2.19 and 2.20 respectively (Soares, 1996):

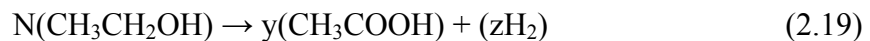
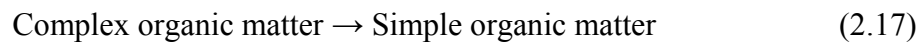


Table 2.4 is a compilation of just some of the many types of bacteria found within facultative WSP, and shows their role within a pond system. Sulphate-reducing bacteria are of notable importance, and are mentioned here as they provide a good visual indicator of pond operating conditions, where a change in a pond's surface colour from green to purple or brown (known as 'purple ponds') is a definite signal of the onset of anaerobism. This can be caused by pond overloading, where the toxic effects of sulphide inhibit algal function (as already described in section 2.3.1), so allowing anaerobic photosynthetic sulphur bacteria to predominate. These thrive in anaerobic conditions, and their nutritional type is photoautotrophic; thus photosynthetic sulphur bacteria require sulphide, light and anaerobic conditions to grow (Pearson, 1988).

Table 2.4: Bacterial types typically found within facultative WSP.

Bacterial type	Bacterial genera/species	Bacterial nutritional type	Bacterial description
Aerobic	<i>Beggiatoa alba</i> <i>Sphaerotilus natans</i> <i>Bacillus</i> <i>Zoogloea</i> <i>Pseudomonas</i> <i>Achromobacter</i> <i>Flavobacterium</i> <i>Alcaligenes</i>	Heterotrophic	Responsible for the release of carbonaceous and nitrogenous by-products as a result of the oxidation of organic material.
	<i>Nitrosomonas</i> <i>Nitrospira</i>	Autotrophic	Oxidisers of ammoniacal-N
Facultative	<i>Chromatiaceae</i> <i>Thiopedia</i>	Photoautotrophic	Photosynthetic purple sulphur bacteria, retain sulphur within cells
	<i>Chlorobiaceae</i>		Photosynthetic green sulphur bacteria, release sulphur
	<i>Rhodospirillaceae</i> <i>Rhodopseudomonas</i>		Photosynthetic non-sulphur purple bacteria
	<i>Chloroflexaceae</i>		Photosynthetic non-sulphur green bacteria
Anaerobic	<i>Achromobacter</i> <i>Alcaligenes</i> <i>Micrococcus</i> <i>Pseudomonas</i> <i>Thiobacillus</i>	Heterotrophic	Denitrifiers, reduce NO_3^- to N_2
	<i>Streptococcus</i> <i>Clostridium</i> <i>Flavobacteria</i> <i>Aerobacter</i> <i>Escherichia</i> <i>Staphylococcus</i>		These hydrolyse and solubilise complex compounds such as proteins and amino acids into simple monomers, NH_3 and CO_2 , H^+ & H_2S
	<i>Methanobacterium</i> <i>Methanospirillum</i> <i>Methanosarcina barkeri</i> <i>Methanosarcina hungatei</i>		Use acetate, carbon dioxide and hydrogen to produce CH_4
	<i>Desulfovibrio</i> <i>Desulfobacter</i>		Sulphate reducing bacteria

2.3.3 Other WSP dwelling organisms

A WSP can have a long hydraulic retention time, which can allow a vast number of organisms to colonise the pond, so much so that the accumulated flora and

fauna can often equal that of a natural ecosystem (Horan, 1990). Some organisms may play an important role in nutrient cycling within ponds, while some species are known to disturb pond microbiology, sometimes to the extent that desirable in-pond operating conditions and pond performance are adversely affected.

Organisms found within facultative WSP include viruses, fungi, crustaceans and insects. Viruses are parasitic microbes, and many in wastewater, including noroviruses and rotaviruses, are of human origin (Mara, 2004). Species of sewage fungi (which are multicellular chemoorganotrophs) may be present in WSP waters, but will seldom predominate and form large clusters. The groups of fungi which exist in wastewater streams play important roles in wastewater treatment, through their ability to mineralise organic carbon and metabolise organic matter (Soares, 1996).

Protozoa are unicellular eukaryotes, the majority being aerobic heterotrophs. In areas of the world where safe water supply is lacking, excreted pathogenic organisms in domestic wastewaters are responsible for a number of waterborne diseases (Mara, 2004), but many are non-pathogenic and have an important role in most aerobic biological wastewater treatment processes (Soares, 1996).

Protozoa can be classified into three groups (Mara, 2004): the amoebae (e.g. *Sarcodina*), the ciliates (*Ciliophora*), and the flagellates (*Mastigophora*). The ciliates have the greatest species diversity within wastewater treatment plants (but not necessarily the most prolific), and four distinct types of *Ciliophora* recognised: *Holotrichia*, *Spirotrichia*, *Peritrichia*, *Suctorina* (Horan, 1990). The *Mastigophora* (flagellate protozoa) are largely heterotrophic (Mara, 2004) and are subdivided into two classes. The first are the *Phytomastigophorea* which contain chloroplasts, and are capable of autotrophic as well as heterotrophic nutrition (Horan, 1990). The second are *Zoomastigophora*, more commonly associated with activated sludge and trickling filter processes in conventional wastewater treatment. Heterotrophic protozoa can contribute to overall BOD removal from a wastewater treatment system, as they uptake soluble organic material through diffusion or active transport, and flagellate protozoa actively compete with heterotrophic bacteria for free BOD (Horan, 1990; Soares, 1996;

Mara, 2004). Helminths (worms), also occupy WSP waters, are largely pathogenic; they are most prevalent in WSP in developing countries where infection, and thus excretion rates are much higher.

Rotifers and crustaceans are larger organisms, visible with the naked eye, and are commonly observed within surface layers of WSP. They are mostly aerobic heterotrophic multicellular organisms but their role in wastewater treatment is largely unknown.

Crustaceans dwell in WSP and are aerobic, feeding on bacteria and algae. By far the most common crustacean in WSP are *Daphnia* spp. These are particularly problematic as, in a few days, they can wipe a WSP almost entirely of its algal population. Pearson (2005) notes that although they help to decrease the content of BOD₅ and SS in the final effluent, disinfection processes within the pond are adversely affected as the algal mechanisms for increasing the pH to kill the bacteria have been removed.

2.4 Wastewater characteristics and composition

Wastewater is essentially the water supply of a community after it has been used in a variety of applications (Metcalf and Eddy, Inc., 2003). The domestic fraction of wastewater includes raw faecal material and urine and anything else which has been flushed away in a toilet, grey water or sullage which comprises bath and shower water, kitchen wastewater, laundry water and any other type of effluent discharged by a household. Included in this category is the water used by the industrial sector for manufacture and cooling, and many other purposes wide ranging in water use applications within industry, which contribute to a huge proportion of the municipal wastewater stream received by wastewater treatment works (WWTW's). Storm water, containing grit and high heavy metal concentrations, as well as other pollutants and items carried away by storm drains imbedded in roadsides, and wastewater from urban run-off and combined sewer overflows (CSO's) also contribute to the wastewater stream entering a municipal WWTW.

Wastewater contains a wide variety of microbial fauna both commensal and pathogenic in nature which are found in faeces and urine. A high content of solids and other colloidal material are always present, as well as fluctuating degrees of chemical compounds. Three principal properties characterise wastewater: these are a wastewaters physical, chemical and biological properties. According to Tebbutt (1998) a *typical* raw sewage contains around 1000 mg/l of solids in solution and suspension where 99.9% is water; therefore as little as 0.1% of sewage is comprised of solid waste fractions. Of this solid 0.1%, 70% is composed of organic fractions, which is composed of proteins (65%), carbohydrates (25%) and fats (10%). The remaining 30% of the solid fraction consists of inorganic materials such as grit, salts and metals.

As referred to at the beginning of this thesis, all wastewaters exert an oxygen demand within the receiving water body, as aerobic bacteria strip dissolved oxygen from the water to oxidise and stabilize waste through metabolic processes. The oxygen demand of a wastewater can be determined biologically through biochemical oxygen demand (BOD) tests, and chemically through the chemical oxygen demand (COD) test. The BOD test provides an indication of how much oxygen will be removed from wastewaters by microbial mechanisms, whereas the COD test incorporates the BOD fraction, plus the fraction of organic waste which cannot be oxidised microbially, but only chemically. Consequently the COD value of a water body is always higher than its BOD value.

The BOD test exists in two forms: that of BOD₅ and BOD_u. This procedure provides a measure of the capability of bacterial metabolism in breaking down the waste, as the bacteria rely on the dissolved oxygen content within the wastewater for these processes. The energy created by the biological oxidation of waster enables the bacteria to build new cells through the synthesis of complex molecules, such as proteins and polysaccharides (Mara, 1976). Therefore, it follows that the more oxygen removed from the water, the stronger the waste – or higher the organic content, as bacteria rely on more oxygen to bring about this process. The BOD₅ is the test commonly used in analysing wastewaters on a regular basis, as the ‘5’ denotes the five-day incubation period occupied by the test.

BOD_u is a measure of ultimate BOD, whereby organic matter undergoes complete oxidation, and both the carbonaceous and nitrogenous oxygen demands are satisfied. This process can be extremely lengthy, thus the BOD₅ tests is more commonly used, where, conveniently the largest proportion of available dissolved oxygen is used in metabolic processes within this five day period.

The COD test on the other hand relies on chemical reactions rather than microbiological reactions to oxidise the organic fraction of the wastewater. The oxygen supplied for the reaction is contained within a potassium dichromate salt (K₂Cr₂O₇), where the sample is added to this and combined with a strong acid and digested at 150°C for two hours. The oxidation of waste then occurs with the dichromate ion in equation 2.21 (Sawyer, 1994):



It must be noted that although the COD test is much quicker and more accessible than the BOD test, the oxidising capacity of the dichromate ion is so potent that non-biodegradable material (which would not normally be oxidised in the environment in absence of this ion) is oxidised, and this must be taken into account when analysing the results.

Mara (2004) has classified wastewater strength with respect to BOD₅ and COD concentrations for municipal wastewaters as shown in Table 2.5.

Table 2.5: Indicative guides of BOD₅ and COD with respect to the classified strength of a wastewater.

Strength	BOD₅ (mg/l)	COD (mg/l)
Weak	<200	<400
Medium	350	700
Strong	500	1000
Very Strong	>750	>1500

Wastewaters from industrial plants such as chemical works and leather production facilities produce COD, BOD, ammonia and other constituents of much higher concentrations than those presented here, but are not discussed because this research was conducted only using municipal wastewater for influent feed.

2.4.1 Nitrogen fractions within municipal wastewater

Nitrogen exists in four main forms in the water cycle (Tebbutt, 1998). In wastewater, three of these are inorganic, and the fourth is organic. A considerable amount of data which documents concentrations of various wastewater contaminants and constituents, has been collected globally. Table 2.6 provides results for only some of the many typical concentrations for nitrogen fractions found within raw municipal wastewaters, as reported by various researchers. Gloyna (1971) notes that although the basic content of human wastewaters within one country varies but little, the character and volume of wastes can differ widely from one country to another.

Gotaas (1956) reported that between 5–7% of human faecal material and 15–19% of human urine was comprised of nitrogen.

Table 2.6: Typical concentrations of various nitrogen fractions in raw wastewater.

Source	Ammonia-N	Nitrite-N	Nitrate-N	Organic N
Tebbutt (1998)	40 mg/l	–	< 1 mg/l	–
Schroeder (1977)	25 mg/l	0 mg/l	0 mg/l	15 mg/l
Shilton (2005)	24-53 mg/l	–	–	12-27 mg/l
Horan (1990)	22 mg/l	–	–	–
Metcalf and Eddy Inc., (2003)	12 mg/l ^a	0 mg/l ^a	0 mg/l ^a	8 mg/l ^a
	25 mg/l ^b	0 mg/l ^b	0 mg/l ^b	15 mg/l ^b
	45 mg/l ^c	0 mg/l ^c	0 mg/l ^c	25 mg/l ^c

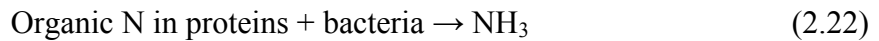
^aweak strength wastewater; ^bmedium strength wastewater; ^chigh strength wastewater.

2.4.1.1 Organic nitrogen sources

Organic nitrogen exists as part of more complex carbon containing compounds within the wastewater fraction. Organic nitrogen comes almost solely from waste by-products of human metabolism in the form of proteins, amino acids and urea (Tebbutt, 1998; Sawyer *et al.*, 1994). These originate from dead cellular material dispensed by the body, and also from unassimilated protein bound in faeces, and upon oxidation, the nitrogen fraction is usually liberated as ammonia in the process, ammonification (Tebbutt, 1998). Other organic sources of nitrogen may

come from other materials such as soil which can often enter a sewer through storm drainage.

Much of the nitrogen bound in inorganic substances seldom remains in this form, and rapidly undergoes transformation, being released as an inorganic source. One example of this is observed in the breakdown of urine in a wastewater stream. Urine contains nitrogen resulting from the metabolic breakdown of proteins; the nitrogen fraction exists principally as urea which is then rapidly hydrolyzed by the enzyme urease to the inorganic fraction of ammonium carbonate $(\text{NH}_4)_2\text{CO}_3$ (Sawyer *et al.*, 1994). Nitrogen bound in unassimilated protein is converted to ammonia by heterotrophic bacteria in aerobic or anaerobic conditions, by the following reaction given in equation 2.22 (Sawyer *et al.*, 1994):



The organic nitrogen fraction entering a wastewater treatment works is, in many cases, appreciably reduced in concentration by the time it reaches the works, as organic fractions readily undergo deamination and ammonification during this passage in the sewer (Walmsley and Shilton, 2005).

2.4.1.2 Inorganic nitrogen sources

Inorganic nitrogen is present in municipal wastewater containing the expulsion of human waste from a household. Further quantities of inorganic nitrogen can also be added directly from sources including artificial fertilisers used on farmland, farm animal waste, and a huge variety of manufacturing processes (Horan, 1990). The predominant form of inorganic nitrogen within raw wastewater is ammoniacal nitrogen, comprising the ionised form, NH_4^+ and free gaseous ammonia NH_3 , or that released directly from the breakdown of ammonium salts, e.g. ammonium chloride (NH_4Cl) and ammonium carbonate ($(\text{NH}_4)_2\text{CO}_3$). Ammonia is very water soluble: the shift between ammonium and ammonia is temperature and pH dependent.

As can be seen in Figure 2.3, neutral and acid pH favours the presence of ammonium (Soares, 1996). At a pH of above 6.6, the ammonium ions start to dissociate to yield ammonia (equations 2.24 and 2.25). Below a pH of 8, the

NH_4^+ ion is predominant (Nurdogan and Oswald, 1995), and at a pH of 9.2, NH_4^+ and NH_3 are equal in concentration. Konig *et al.* (1987) cited that at a pH of 9, around 40% of ammoniacal nitrogen exists as NH_3 , whereas at a pH of 10, the proportion of NH_3 will rise to almost 80%, and at pH of 12, all ammonium ions have dissociated to yield ammonia (Reed, 1985 in Abis, 2002).

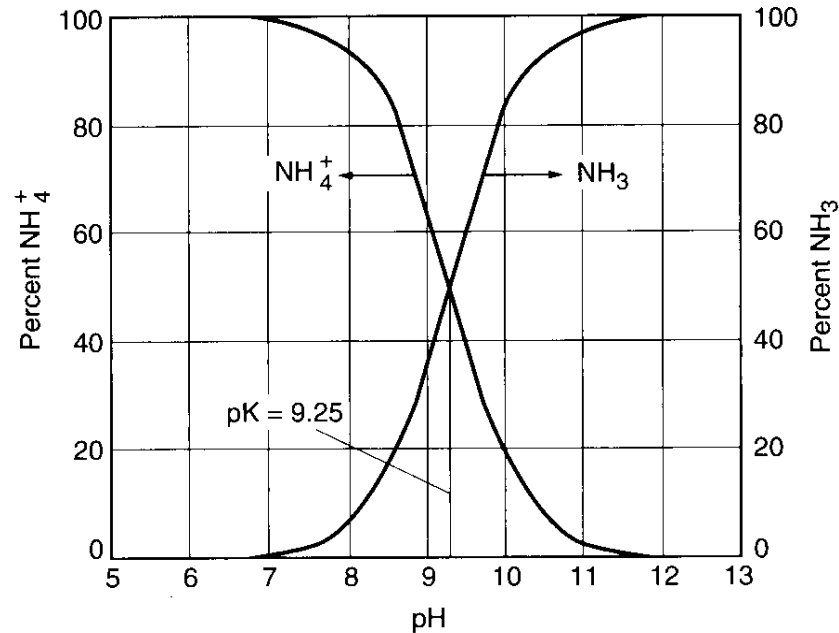


Figure 2.3: The relationship between ammonia and ammonium equilibrium with respect to pH (Metcalf and Eddy, Inc., 2003).

Nitrite is only ever present in very small amounts within any water body; it exists as an intermediate oxidation stage above the reduced nitrogen fraction ammonia, and below the fully oxidised nitrogen fraction of nitrate. It is not a very stable compound, and can either be very quickly reduced or oxidised and seldom exceeds 1 mg/l in wastewater (Metcalf and Eddy, Inc., 2003).

Nitrates originate in wastewater either from the chemical or biological oxidation of reduced nitrogen species (nitrification), or directly from the agricultural run off from fertilizers, or from the addition of industrial effluents to the wastewater stream.

2.5 The nitrogen cycle and its significance within wastewater treatment

The nitrogen cycle is one of the most important elemental cycling regimes on earth. Mammals depend on it for their nitrogen source, and it has immense socio-economic implications too – for example, crop production in western cultures relies heavily on nitrogen fertilization from synthetic nitrogen compounds produced through the Haber-Bosch process. Nitrogen is an essential element in the building blocks for proteins, peptides, nucleic acids and amino acids such as lysine, arginine and histidine, although it is only a minor constituent of living biomass when compared to the proportions of carbon, hydrogen and oxygen (Gijzen and Mulder, 2001). The nitrogen containing compounds Adenine and Guanine (both purines), and Thymine and Cytosine (both pyrimidines) comprise the four bases which form the genetic code in DNA (Cox, 1997). *Homo sapiens* and many other animals, with the exception of ruminants, do not have the capacity to utilize nitrogen from atmospheric or other inorganic sources to fix nitrogen directly, and are therefore dependent upon the consumption of plants, and animals who feed on plants, for their nitrogen requirement with which to satisfy protein production (Sawyer *et al.*, 1994). Many different nitrogen species exist in atmospheric, soil, and aquatic systems, but, as discussed in section 2.4.1, the species of principal importance within the wastewater nitrogen cycle are organic nitrogen, ammoniacal nitrogen, nitrite nitrogen and nitrate nitrogen. The nitrogen cycle in natural soil and aquatic environments is shown in Figure 2.4.

Equally, the success of a biological wastewater treatment relies heavily upon the nitrogen cycle as an intrinsically indispensable part essential for the overall wastewater treatment process. In the same way that many mammals depend on nitrogen, a vast range of wastewater microbiology needs nitrogen compounds for their cell synthesis and energy. The conceptual understanding of the nitrogen cycle is of fundamental importance to environmental and sanitary engineers, as the knowledge of how nitrogen is removed from wastewater and by what means can contribute to an efficiently designed and effective working system. The nitrogen cycle within wastewater is complex, as a whole variety of individual reactions contribute to it, and many different pathways and mechanisms of removal often operate simultaneously. The cycle has a further complexity in that

nitrogen abides in several oxidation states of -3 to $+5$ (Sawyer *et al.* 1994; Cox, 1997; Metcalf and Eddy, Inc., 2003); these are brought about by bacteria and other microbes and by the aerobic and/or anaerobic conditions in which they prevail, these determining whether the oxidation changes are positive or negative (Sawyer *et al.*, 1994; Metcalf and Eddy, Inc., 2003).

The nitrogen cycle for water portrayed in Figure 2.4 is not too different from the processes which occur naturally within a WSP – the sources of nitrogen addition to a wastewater (precipitation and dust-fall, wastewater nitrogen, overland runoff, atmospheric contributions, and input from soil systems) are essentially the same.

However, in recent years an alarming shift has occurred in the nitrogen cycle, due to anthropogenic activity disrupting the fragile balance of the natural cycle. The Harber-Bosch process, responsible for generating synthetic ammonia for agricultural fertilizer, and the industrial manufacture of nitric acid, have contributed huge quantities of reactive nitrogen to the atmosphere and biosphere (Cox, 1997; Aneja *et al.*, 2001). Gijzen and Mulder (2001) note that industrial N_2 fixation constitutes almost 30% of the total global nitrogen influx to the biosphere – a huge 9×10^9 kg/year, of which between only 10 and 15% is actually assimilated into food protein. The increase in reactive nitrogen levels is causing widely detrimental effects, which are becoming ever more apparent. Water quality of wetland ecosystems is also being adversely affected, (Fair and Heikoop, 2006) and causing mass eutrophication events globally, which is affecting aquatic microcosms through the increased oxygen demand exerted upon groundwater, river, lake and coastal water bodies, and overcrowding by aquatic plants (Gijzen and Mulder, 2001). Agricultural fertilizer may also be contributing to an atmospheric increase in nitrous oxide, while the production of various oxides of nitrogen from the transport industry, not only cause localised air pollution, but also acid rain (Cox, 1997). There has never been a more pressing need to thoroughly understand, and act upon, the nitrogen removal mechanisms and pathways within wastewater treatment, through which, the effects of the out of kilter global nitrogen cycle can be partially abated.

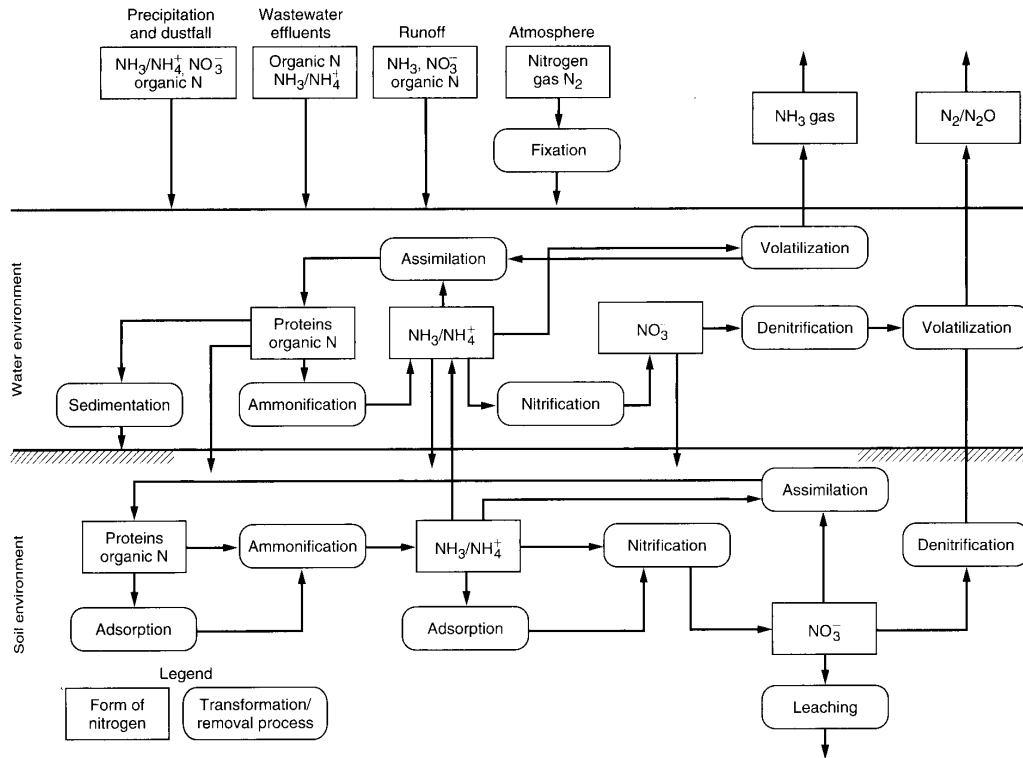


Figure 2.4: The nitrogen cycle in natural soil and aquatic environments (Metcalf and Eddy, Inc., 2003).

The transformation which various nitrogen species undergo in a wastewater treatment plant, occurs biologically in both solid and liquid phases – i.e., within the sludge layer of a reactor and in the mixed liquor overlying wastewater. The physical mechanisms of sedimentation and volatilization obviously play a part, but biologically speaking, the fate of nitrogen in a wastewater treatment plant occurs through autotrophic nitrification, heterotrophic denitrification, and through the autotrophic anaerobic oxidation of ammonia (Heit Wong *et al.*, 2003). These three pathways for nitrogen removal are discussed in more depth in sections 2.6.4, 2.6.5, and 2.6.6 respectively.

2.6 Nitrogen removal mechanisms and pathways in wastewater treatment and primary facultative waste stabilization ponds

To date, a wealth of literature has been written about nitrogen removal from WSP, within which are to be found some contradictory opinions and findings.

Middlebrooks *et al.* (1983) postulated that the four mechanisms and pathways below chiefly responsible for nitrogen removal within WSP:

- Ammonia volatilization
- Algal uptake through assimilation into the biomass
- Sedimentation and incorporation into the sludge layer, and
- Biological nitrification and denitrification.

These four mechanisms and pathways are still the most commonly cited, with some researchers emphasising a preference for one in particular. All of the above are perfectly valid, and, as the following literature review will reveal, some appear to be particularly suited to case-specific environments.

Figure 2.5 presents a more detailed summary of mechanisms and pathways for nitrogen removal within WSP, including the intermediary steps, which link one mechanism, or pathway, to another.

The scope of nitrogen removal within facultative WSP cannot, and should not, be confined to those occurring processes shown in Figure 2.5. The last few years has seen the adoption and application of existing technologies (such as DNA sequencing and other molecular microbiological analytical techniques) for use within the field of wastewater engineering. As a result, other exciting previously unknown pathways for nitrogen removal have come to light, through the discovery of anammox – the anaerobic ammonia oxidising bacterial community, and other ammonia oxidisers (discussed in section 2.6.7). The literature presented here is in no way conclusive, but it is a selection of some of the findings from nitrogen research conducted on facultative WSP.

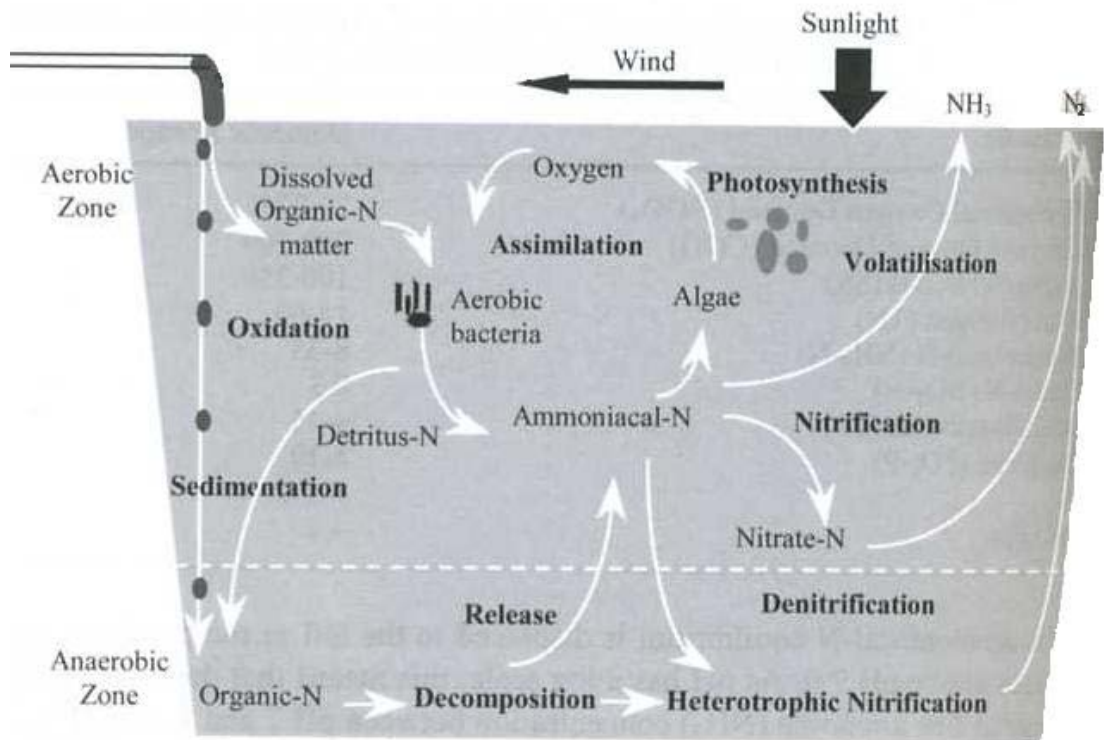


Figure 2.5: Some of the nitrogen removal processes operating within WSP.

2.6.1 Ammonia volatilization

The exact contribution which ammonia volatilization processes make to the overall nitrogen removal process from WSP's, remains a hotly contested and widely debated issue. Much research has been published over the last 30 years or so, in which researchers have advocated a variety of different theories and models in attempts to quantify how much nitrogen can be removed from a WSP via the ammonia volatilization route, in conjunction with other in-situ coexistent chemical parameters, and environmental variables which drive this phenomenon.

Despite findings presented, there is no universal agreement on the significance of this removal mechanism within WSP's or the tangible difference this mechanism makes to overall removal within a mass balanced-system. Some researchers are advocates of the mechanism (Soares *et al.* (1996), others hint that it may be a possible mechanism (Nurdogan and Oswald, 1995), while others have found that it made no significant difference to overall nitrogen removal. Indeed, it may be a more important mechanism for ponds situated in equatorial regions of the world, where temperatures, insolation and pH are much higher as they are the main drivers which enhance this mechanism.

Various gases are released from facultative WSP (CH₄, CO₂, NH₃, H₂S, N₂, and H₂ among others), especially when temperatures are higher and microbial reaction rates are greater. The gases released from WSP come from the anaerobic fermentative degradation processes of sludges and solids by microorganisms in the benthic layer at the base of the pond. Of the gases leaving through the pond surface, 70% is typically methane, 20% is nitrogen and 10% belong to other fractions (Oswald, 1988). Biogas production is clearly visible on hot days, where a rapid succession of bubbles can be observed bursting from a pond's surface. McGarth and Mason (2004) measured biogas production from a WSP treating dairy farm wastewater, and found that the amount of biogas captured by a 4 m² floating chamber was in the range 4–20 litres per 5 minute period. The predominant form of nitrogen leaving the pond surface as biogas is NH₃. Dinitrogen (N₂) is released through denitrification mechanisms, and NO_x species (oxides of nitrogen) and N₂O (nitrous oxide) through other in-pond mechanisms.

Ammonia volatilization, also known as ammonia stripping, describes the process whereby gaseous 'free' un-ionized ammonia (a volatile compound which is highly soluble in water) passes out of liquid embodiment via diffusion and desorption into the atmosphere in a liquid gas transfer process. Ammonia nitrogen exists in aqueous solution either as ammonia gas (NH₃) or as the ammonium ion (NH₄⁺) (Metcalf and Eddy, Inc., 2003). The ammonium ion and ammonia exist in the equilibrium reactions in water shown in equations 2.23 and 2.24. This equilibrium between the gas and liquid phase composition is strongly affected by both pH and temperature (Schroeder, 1977).



Metcalf and Eddy Inc. (2003) provide equation 2.25 when the law of mass action is applied to equation 2.24 and where the activity of water is 1:

$$\frac{[\text{NH}_3][\text{H}^+]}{[\text{NH}_4^+]} = K_a \quad (2.25)$$

where the equilibrium dissociation constant for acid ionisation (K_a) equals $10^{-9.25}$, or 5.62×10^{-10} .

Figure 2.3 shows the equilibrium of ammonia and ammonium nitrogen along which the concentration of the free ammonia gradient changes in water by the effect of rising pH. This relationship is also described mathematically in equation 2.26, where the percentage of ammonia in a water body is determined as:

$$NH_3, \% = \frac{[NH_3] \times 100}{[NH_3] + [NH_4^+]} = \frac{100}{1 + [NH_4^+]/[NH_3]} = \frac{100}{1 + [H^+]/K_a} \quad (2.26)$$

As temperature and pH rise to create more alkaline conditions, the equilibrium between NH_3 and NH_4^+ shifts further to the right of equations 2.23 and 2.24, and the ammonium ion dissociates to produce free ammonia and hydrogen ions (Sawyer *et al.*, 1994).

Figure 2.6 shows the liquid and gas phase NH_3 mole fractions as a function of temperature (Schroder, 1977). Green *et al.* (1996) note that since ammonia is more soluble in water at lower temperatures, the loss of ammonia to the atmosphere is more likely to occur during warmer periods.

Of the total amount of ammonia volatilized within a WSP, a proportion comes from the soluble fraction of NH_3 and the inter-conversion of the ionized fraction NH_4^+ to NH_3 within the upper aerobic tier of the pond (equations 2.23 and 2.24). The rest comes from the anaerobic degradation of organic material in the anaerobic sludge layer releasing ammonia as a by-product. The process of ammonia volatilization is complex, and includes many other factors which affect the ammonia flux between the liquid-gas interface at the atmospheric boundary. The mass transfer of ammonia via this mechanism, can be calculated with or without chemical reactions not only involving temperature and pH coefficients, but also encompassing molecular diffusion coefficients incorporating the Henry's law constant; this quantifies the partial pressure of a given gas at a specific temperature, and also the dissociation constants of ammonium and ammonia nitrogen (Aneja *et al.*, 2001).

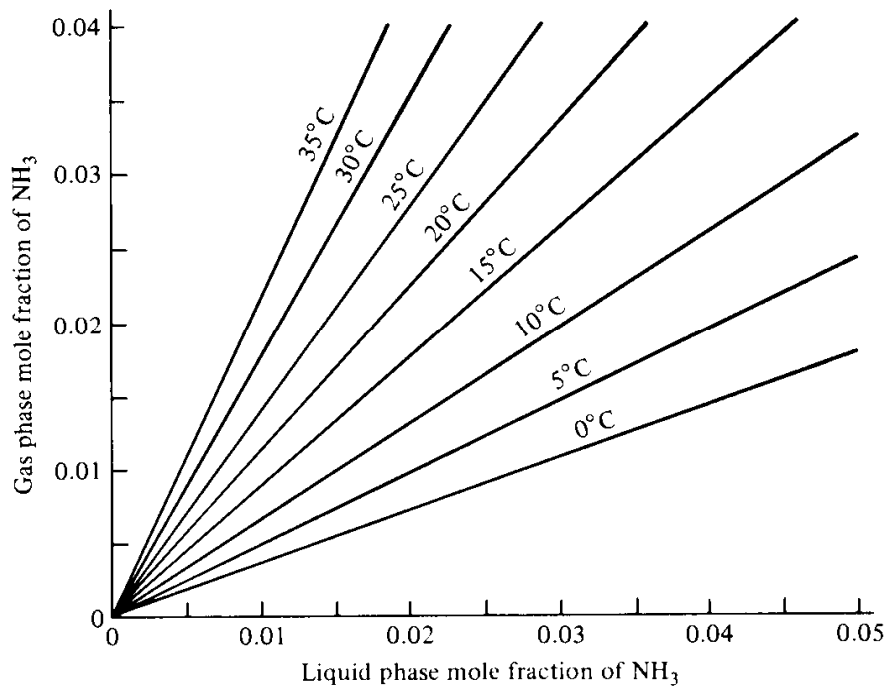


Figure 2.6: The liquid and gas phase fractions of NH₃ (mole) as functions of temperature (Schroder, 1977).

Horan (1990) notes that, although ammonia is a very soluble gas, it does not pass out of solution into the gaseous phase unless displaced by other gases; therefore, as ponds are usually quiescent in condition, it is unlikely that ammonia volatilization will be the predominant mechanism for nitrogen removal within these systems.

Pano and Middlebrooks (1982) is without doubt the most cited and acknowledged work when discussing ammonia removal from WSP's. They advocate the volatilization of ammonia as the dominant mechanism of ammonia removal from facultative WSP. To date, their model formulated to describe this process mathematically, still retains its reputation as a valid working model describing ammonia removal from WSP. However, although the model has been proven to work when predicted values are compared with measured values, the advocacy by the researchers that the route mechanism of volatilization is responsible, remains questionable; other in pond processes which were not measured could account for the removal of ammonia.

The model presented for ammonia-nitrogen removal from facultative WSP was derived principally from a preconceived advocacy that ammonia volatilization was the main mechanism for ammonia removal from these systems. Their archetypal model (equations 2.27 and 2.28) was formulated using empirical data for temperature, pH, pond configuration, hydraulic loading rates and detention time as variables, and was tested utilizing data collected from three facultative WSP located in different parts of the USA (Pano and Middlebrooks, 1982). One of their main assertions was that, at low temperatures, ammonia stripping would be the main mechanism for nitrogen removal, where the model based on first order kinetic principles and a completely mixed hydraulic regime proposed temperatures below 20°C as conditions favourable in order for the model to work successfully. Two equations were used to define ammonia stripping from the three pond systems studied; one was derived for temperatures up to 20°C and one for a temperature range between 21 and 25°C. For temperatures $\leq 20^\circ\text{C}$, the following equation is given:

$$C_e = \frac{C_i}{\{1 + [(A/Q)(0.0038 + 0.000134T)\exp((1.041 + 0.044T)(pH - 6.6))]\}} \quad (2.27)$$

For temperatures of 21–25°C, the equation is modified to:

$$C_e = \frac{C_i}{\{1 + [5.035 \times 10^{-3} (A/Q)] [\exp(1.540 \times (pH - 6.6))]\}} \quad (2.28)$$

where C_e = Ammonia nitrogen effluent concentration (mg N/l)

C_i = Ammonia nitrogen influent concentration (mg N/l)

A = Pond surface area (m^2)

Q = Flow (m^3/d)

Many researchers have used the Pano and Middlebrooks model and found that actual results, when compared to theoretically generated values produced from the model, are satisfactory; for example, Soares *et al.* (1996) found excellent agreement of data gained in the field when tested with the Pano and Middlebrooks (1982) model for WSP in northeast Brazil. Conversely, many have found these theoretical values too low in actual comparison to the data gained from real-life

analysis of a pond system. The underlying assumption throughout this model, is that ammonia is removed solely from a WSP system by the ammonia stripping mechanism, and no allowance is factored in for algal assimilation, or in-pond transformations by microbial biochemical reactions. The constants presented in the equations were generated from empirical data generated from the three WSP studied.

Furthermore, the three study systems examined were very different from each other. On the one hand, it can be argued that this is not a cause of concern, as the model correspondingly fits with the systems studied, and that it is therefore more versatile in practice being adaptable and applicable to WSP systems whatever their characteristics, site and situation, climatic conditions, etc. Alternatively, the differences occurring between the three pond systems arguably raise questions over the formation and feasibility of the model. It is cited by the authors that the wastewater of the three plants at Eudora, Corinne and Peterborough were significantly different in character: the Peterborough ponds were anaerobic in the winter period, and even in the summer it had a low pH not exceeding 7.4, whereas the other ponds experienced higher in-pond pH values. This further suggests that any number of other biochemical processes could have been occurring within the ponds, which were simply overlooked at the time of study.

Although the three pond systems studied were subject to similar climatic conditions, each pond was fed with wastewater types which were significantly different, and each was very different in nature. The findings of this research must be analyzed with these facts in mind, and also that it is important to note that ammonia volatilization was not measured for in the field, so the basis of the model resides wholly on theoretical supposition.

In direct contrast to the above, research findings from Ferrara and Avci (1982) contradict the Pano and Middlebrooks (1982) assertion that the volatilization of ammonia explains ammonia removal from WSP (DiGiano, 1982). Ferrara and Avci (1982) analysed one of the same facultative ponds used in the Pano and Middlebrooks (1982) study but attributed biological uptake of ammonia, followed by the sedimentation of organic nitrogen, as the predominant removal mechanism.

DiGiano (1982) has presented a critique of the Pano and Middlebrooks (1982) model and comments that, because the field data support a first-order relationship, this is not sufficient proof that the removal mechanism can be attributed to ammonia volatilization. Indeed, as Pano and Middlebrooks (1982) found that ammonia removal correlated well with increased temperatures and pH, and worked on the assumption that volatilization was the removal mechanism, so Ferrara and Avci (1982) conclude that periods of higher temperatures and pH correspond with periods of high biological activity (Ferrara, 1982). Zimmo *et al.* (2003) rightly observes that both of these studies were based on theoretical assumptions and not on a complete nitrogen mass balance for the system in question.

Somiya and Fujii (1984) conducted research on a laboratory based pilot-scale oxidation pond, and analysed various chemical parameters within the pond water column, the settleable solids fraction of the wastewater, and the pond sediment. Ammonia volatilization was not actually measured, but Somiya and Fujii (1984) proffered that the ammonia volatilization rate was relatively low, and it was therefore implied that this mechanism for nitrogen removal was chosen for the completion of their mass balance analysis.

In 1995, Silva *et al.* purported that ammonia volatilization, ammonia assimilation into algal biomass, and biological nitrification coupled to denitrification were the three mechanisms for nitrogen removal within WSP. As in the Pano and Middlebrooks (1982) study in the USA, ammonia volatilization was not physically measured from this WSP system in northeast Brazil. Two equations were derived from the experimental data collected which modelled the theoretical (as it was not actually measured) volatilization of ammonia from the number of WSP which comprised the whole treatment system, using ammonia nitrogen concentrations in the pond column, and influent and other coefficients to describe the removal. The two alternative equations are shown in 2.29 and 2.30, where the actual measured values and those predicted by the models, compared favourably.

$$N_c = N_i[1+8.65 \times 10^{-3}(A/Q)\exp(1.727(\text{pH}-6.6))]^{-1} \quad (2.29)$$

$$N_c = N_i[1+38.8 \times 10^{-3}(A/Q)\exp(1.911(\text{pH}-7.5))]^{-1} \quad (2.30)$$

where N_c = pond water-column ammonia concentration (mg N/l)

N_i = pond influent ammonia concentration (mg N/l)

A = pond area (m²)

Q = flow (m³/d)

Soares *et al.* (1996), working on the same site in north-east Brazil as Silva *et al.*, (1995), found that the Pano and Middlebrooks (1982) model adequately described the results obtained from the ponds under study. First-order kinetic equations were also derived (similar to those presented in equations 2.29 and 2.30) for nitrogen removal within WSP, assuming that ammonia volatilization was the dominant mechanism (Soares *et al.*, 1996).

Pearson *et al.* (1996) also hypothesised that high ammonia removal rates in pond systems with pH values greater than 8.7 occurred via the ammonia volatilization mechanism. The high concentrations of ammonia, versus the low concentrations of TKN in the facultative ponds, supported the postulation that losses of ammonia via volatilization were masked by ammonification processes resulting from the release of ammonia-N from organic-N compounds (Pearson *et al.* 1996).

Shilton (1996) found that ammonia volatilization did indeed significantly contribute to overall nitrogen removal from a piggery pond system, operating under controlled conditions within a laboratory. It was observed that the mass transfer of ammonia volatilized from the piggery wastewater increased as the TKN and ammonia concentrations increased, during trials altering influent concentration within the simulated pond. This was because of the recorded elevated pH, which is indicative of higher concentrations of free ammonia (Shilton, 1996). Volatilization rates ranged from 355 to 1534 mg N/m² d over the study period. This experiment was not replicated on a full-scale pond in real time conditions, thus the actual quantification of ammonia volatilization from a full scale pond treating piggery waste, was not recorded. Shilton (1996) deemed that

to rely solely on the ammonia volatilization mechanism for nitrogen removal, in order to achieve high nitrogen removals from ponds, would be impractical.

Ammonia volatilization is readily recognised as the only significant mechanism for gaseous nitrogen removal within WSP's; other gaseous fractions such as N₂ produced from denitrification, and N₂O, have rarely (if ever) been measured from a WSP and might prove to be more significant in overall nitrogen removal than the ammonia volatilization route. Peu *et al.* (1999) found satisfactory removals of N₂O emissions from a floating capture chamber placed over farm scale treatment units for livestock wastes; as much as 91 mg N₂O-N/h m² was measurable in some places. It must be noted, that the waste type used in this study originated from slurry and other farm animal waste, significantly different in constitution from municipal wastewater; however the research still found low volatilization rates, and the emissions measured equated to less than 1% of the total nitrogen input to the system (Peu *et al.*, 1999).

Ammonia volatilization was also measured from pig wastes by Aneja *et al.* (2001) from a single WSP in North Carolina, USA, and comprehensively modelled to describe the mass transfer mechanism. Their results revealed that lagoon temperatures were one of the most sensitive factors affecting ammonia flux, as was wind speed during unstable conditions. The model predictions for ammonia volatilization indicated an exponential increase with increasing temperature and pH (Aneja, 2001). A linear increase was also established between ammonia volatilization, and in pond total ammoniacal nitrogen concentration (TAN), where ammonia volatilization rates were proportional to TAN increase within the pond.

As Shilton (1996) and Aneja *et al.* (2001) had found, Zimmo *et al.* (2003) also noted the linear relationship between in-pond ammonia concentrations, and the rate of ammonia volatilization.

Zimmo *et al.* (2003) conducted ammonia volatilization experiments on eight domestic WSP in Palestine; four ponds in series were covered with duckweed, and four ponds in series (but running in parallel to the duckweed-based ponds) were algal-based. Ammonia volatilization was captured in a transparent Plexiglas

chamber suspended over each pond in turn, and ammonia stripped from the pumped gas stream by a 2% boric acid solution, for a one and a half year period. Research findings confirmed that ammonia volatilization from WSP treating domestic wastewater only, accounted for a small fraction of total nitrogen removal from the system; in the duckweed-based ponds the range was 7.2–37.4 mg N/m² d, and in algal-based ponds 6.4–31.5 mg N/m² d. Ammonia volatilization approximated only 1.5% of total ammonium nitrogen removal. According to their data, the model presented in equation 2.31 was derived to define the amount of ammonia volatilization from WSP's:

$$Y = 3.30[\text{NH}_3] + 4.90 \quad (2.31)$$

where Y = ammonia volatilization (mg N/m² d)

NH₃ = calculated as a function of pH, temperature and ammonium concentration in pond water (mg N/l) (Zimmo *et al.*, 2003).

Caicedo Bejarano (2005) also studied ammonia volatilization in duckweed stabilization ponds, using a similar ammonia trap as that described in sections 3.4 and 4.6, and 2% boric acid as the ammonia stripping solution. In conclusion, ammonia volatilization was discounted as an important removal mechanism within duckweed stabilization ponds, where this mechanism achieved less than 1% of nitrogen removal from the whole system (Caicedo Bejarano, 2005).

Rockne and Brezonik (2006) studied the mass transfer of nitrogen, phosphorus and carbon within a facultative WSP in Minnesota, USA, which experienced extreme cold weather, including four months of ice cover. Research revealed the importance of volatilization as an ammonia removal mechanism, particularly in late spring, when the pH measured above 8, and ammonia levels were still high (Rockne and Brezonik, 2006). The mass transfer of ammonia across the air-water interface was calculated from variables recorded at the ponds, and used by the kinetic model produced by Jayaweera and Mikkelsen (1990a, b, & c). The variables used to compute this were: total ammoniacal nitrogen in the pond water column, pH, temperature, pond depth, and the wind speed profile above the pond surface (Rockne and Brezonik, 2006). The model developed by Jayaweera and Mikkelsen (1990a, b, & c) is presented in equation 2.32. As with other studies

documented within this section, the volatilization of ammonia was not actually physically measured, but, using a comprehensive calculation of nitrogen mass transfer within the pond system, which incorporated equation 2.32, it was predicted that 600 kg N was removed, for the study year via the volatilization of ammonia. The total N input over the 12-month period was 1,300 kg, where the remaining losses comprised 170 kg N/year via sedimentation, and 300 kg N/year lost through effluent washout, which produced an overall N removal efficiency of 80% (Rockne and Brezonik, 2006).

$$F_N = \frac{(C_{gN} - H_{nN}C_{lN})}{\left(k_{gN}^{-1} + \frac{H_{nN}}{k_{lN}}\right)} = \frac{\left(\frac{C_{gN}}{H_{nN}} - C_{lN}\right)}{[k_{lN}^{-1} + (H_{nN}k_{lN})^{-1}]} \quad (2.32)$$

where F_N = ammonia-N flux (mgN/m² day)

C_{gN} = bulk ammonia-N concentration gas phase mg/m³

C_{lN} = bulk ammonia-N concentration liquid phase mg/m³

H_{nN} = Henry's law constant

k_{gN} = ammonia-N gas exchange constant (m/d)

k_{lN} = ammonia-N liquid exchange constant (m/d)

To conclude this section, it has been difficult to determine the contribution of ammonia volatilization to overall nitrogen removal from WSP's, but the general consensus from the studies presented above, is that ammonia volatilization rates from WSP's are extremely low. However, this process is, in practice, one of the most difficult nitrogen removal mechanisms to measure. Further development of gas capturing chambers, and a standardised procedure, might lead to a highly useful compilation of ammonia volatilization data from WSP around the world.

2.6.2 Algal uptake through assimilation

The assimilation by algae of nitrogen into cell biomass from organic and inorganic forms has already been detailed in section 2.3.1 and expressed stoichiometrically by equation 2.10. With respect to photoautotrophic nutrition, it has long been established that, on the whole, algae preferentially uptake ammonium-N over nitrite and nitrate as their chosen source of nitrogen (Green *et*

al., 1996). It has been found, however, that while some types of algae show preferential uptake of ammonium-N, others uptake nitrate, and still others uptake either inorganic fraction equally well (Cohen and Fong, 2004). *Chlorella*, for example, has been found to grow just as well on nitrate as it does on ammonia (Konig *et al.*, 1987).

The process of nitrogen uptake and assimilation by algae involves the transport of nitrogen from the water column across the cell membrane and subsequent assimilation into organic compounds, followed by incorporation into macromolecules and proteins required for growth (Cohen and Fong, 2004). It is sensible to postulate that this pathway accounts for a sizeable proportion of nitrogen removal from WSP, in particular facultative ponds, as all microorganisms rely on nitrogen for cell growth, and biomass in well functioning ponds is prolific. Santos and Oliveira (1987) reported that large amounts of ammonia removal in a secondary facultative pond in Portugal was attributable to assimilation by algae and bacteria. Senzia *et al.* (2002) found that 17.4% of ammonia was taken up by microorganisms within a PFP in Tanzania, and that the nitrogen contained within microorganisms in the final effluent of the pond accounted for 50% of the total nitrogen removed from the ponds.

2.6.3 Deposition through sedimentation

Inevitably, primary facultative ponds, such as those at Esholt which are described in Chapter 4, will receive an appreciable amount of settleable material from the influent screened wastewater. The nitrogen bound up in this settleable fraction, is mostly in the form of organic nitrogen contained within proteinaceous faecal material, which settles out naturally to contribute to the sludge layer at the bottom of the pond.

A secondary source of nitrogen containing settleable material, is that deposited through dead algae and bacteria which settle out naturally after death. Green *et al.* (1996) cited that as much as 50–80% of dead algae from a high-rate pond which is paddle-wheel-mixed will be removed via sedimentation in a settling pond, whose hydraulic residence time is short, of no more than two days (values of 30–70% are reported for advanced integrated pond systems). Green *et al.* (1996) also stated

that the settled algae have low respiration rates, and if not removed, will concentrate on the pond floor for many months, or even years, without releasing significant amounts of nutrients. They may also possibly be consumed by *Chironomas* larvae which thrive in anoxic environments in rich organic sediments (Golueke *et al.*, 1957).

2.6.3.1 Nitrogen fluxes attributable to sludge feedback

Sediment nutrient release is a well studied phenomenon in limnology, and Nowlin *et al.* (2005) are just some of the many limnologists who have established that this process can influence water column concentrations and planktonic productivity. The WSP equivalent of nutrient cycling to the water column, via sludge breakdown, has been less well studied. WSP sludge acts not only as a sludge nitrogen sink, but also as a sludge nitrogen source. Seasonal variations also affect sludge feedback mechanisms as Lumbers and Andoh (1987) found, when modelling soluble COD mass balances, within planktonic and benthic regions of a facultative WSP situated in New Mexico, USA. Dead algal cells contribute nitrogen to the pond environment through their degradation: on an ash-free dry weight basis, around 50% of algal cells are composed of carbon, 8% of nitrogen, and 1% of phosphorus (Oswald, 1988). Many authors cite that benthos/sediment/sludge feedback mechanisms occur (Cohen and Fong, 2004), and that they provide useful substrates for algal and bacterial growth. However, not as many studies have been conducted on this nitrogen re-release flux. Indeed, Iwema *et al.* (1987) have said that it appears to be a practical impossibility to establish a short-term sedimentation balance *in situ*, on actively fermenting pond sludge.

Bronson (1963) using specially designed submergible gas collectors overlying the sludge layer, and just beneath the pond's surface in anaerobic ponds, concluded that large amounts of N₂ were being released from the benthic layer (between 25 and 35% N₂), and that the gas collected near the base of the pond was more N₂ enriched than the gas collected beneath the pond surface. This suggested that a large proportion of the N₂ produced had been absorbed into the water of the upper layers of the pond (Green *et al.*, 1996).

Brockett and Orchard (1975) measured ammonification from facultative pond sludges from the two different site depths of 135 cm, and 230 cm within the same facultative pond in New Zealand. Their research found that the degree of ammonification from pond sludge did not differ between these two sites, showing that ammonification is a facultative process which can occur independently of aerobic and anaerobic conditions.

Nitrogen feedback from the sludge benthos can occur in the three physical states of solid (from organic material particles from the sludge), liquid, or gaseous species. Iwema *et al.* (1987) studied digestion processes operating within facultative and maturation pond sludges, and found that 17% of the gas mix collected from a gas collector placed on the sludge layer was N₂, and 16% N₂ was retrieved from a collector suspended 20 cm above the sludge layer which, when calculated from the gas collection rate from each measuring device equated 527 ml/m² day and 544 ml/m² day respectively.

Bryant and Bauer (1987), measuring nutrient feedback from benthic sediments in an aerated stabilization pond, found that nitrogen feedback from sludge varied significantly with temperature, sludge type and deposit volume; overall, though, nitrogen feedback varied in a complex manner and was difficult to simulate within a model. Carré and Baron (1987) found, upon the analysis of vertical sludge profiles, that as the porosity of the sludge decreases with sludge age within sludge core samples, the concentration of ammonia dissolved in the interstitial phase increased with sludge depth, and thus sludge age. However, no quantification of the mass transfer of ammonia from the sludge to the water column above was determined.

Denitrification is one mechanism by which nitrogen is removed from WSP's; this is discussed in section 2.6.6. The second major mechanism for nitrogen removal (or certainly nitrogen transformation) within WSP is ammonification. This is the process whereby inorganic nitrogen principally in the form of ammonia, is returned to the nitrogen cycle within wastewater by autolysis, putrefaction and decay of proteins and other nitrogenous organic matter (Postgate, 1998). Anaerobic digestion in facultative ponds can frequently increase ammonia

concentrations through proteolysis (Pearson, 1988). This process is highly prevalent in anaerobic ponds, where nitrogen, as such, is not removed, but the organic fractions which comprise the majority of faecal and urinal matter undergo rapid transformation to ammonia – principally in the form of ammonium carbonate. This reaction has already been described in section 2.2.1, and the reaction shown in equation 2.3.

A highly comprehensive three year study, conducted on real-sized WSP's in Bertrix wastewater plant in Belgium, by Namèche *et al.* (1997) has shown that sludge sediment/water column interactions, exchanges and feedbacks are much more complicated than classic denitrification and ammonification mechanisms. Their findings revealed that the dry solids content of nitrogen, within the sludges for the ponds studied, averaged 2.5%, and that less than 3% of the nitrogen contained within the deposits was water soluble, and this probably occurred in exchanges within the sludge/water interface without any solubilization process (Namèche *et al.*, 1997). Nitrate concentrations measured in the sludge, were lower than those found immediately above in the water column. Namèche *et al.* (1997) found that denitrification was a key factor which explained the very low level of nitrate ($> 0.1 \text{ mg N-NO}_3^-/\text{l}$) in the upper 4 cm of pond sludge, attributable to this transformation mechanism encouraged by the redox conditions. Nitrate concentrations were, however, found to increase beneath 4 cm into the sludge layer. The ultimate conclusion presented was that the different fluxes of nitrates and ammonia at the sediment/water interface, are incredibly complex, and cannot easily be defined by one single mechanism. It was proposed that further investigation into other transformation mechanisms for returning nutrients to the water column, such as induced turbulence, gas convection, bioturbation, molecular diffusion, and especially biological mechanisms (aerobic and anaerobic bacterial activity are mentioned), including those induced by the benthic macrofauna, was necessary (Namèche *et al.*, 1997).

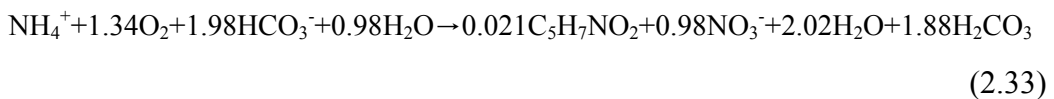
2.6.4 Nitrification

Nitrification is the biochemical process which occurs when ammonia, and other reduced nitrogenous products such as hydroxylamine (NH_2OH), undergo oxidation by autotrophic nitrifying bacteria in an aerobic environment. Complete

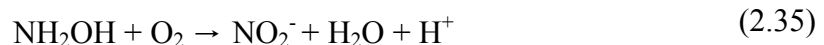
oxidation of ammonia to nitrate occurs in two stages; firstly ammonia is oxidised to nitrite, then nitrite is further oxidised to nitrate.

The bacteria which are responsible for the oxidation of ammonia within wastewater treatment, are from the chemolithoautotrophic groups of bacteria classified as what have become to be known as ammonia oxidising bacteria – AOB. A huge variety of AOB typically come from the genera *Nitrosomonas*, *Nitrosospira*, *Nitrosocystis*, *Nitrosoglea*, *Nitrosovibrio* and *Nitrosococcus* which are responsible for the conversion of ammonia to nitrite, and from *Nitrobacter*, *Nitrospira*, and *Nitrococcus* among other species, for the conversion of nitrite to nitrate (Randall *et al.*, 1992; Hiet Wong *et al.*, 2003). Through the complete conversion of ammonia to nitrate, these bacteria derive the energy necessary for their growth from the oxidation process (Sawyer *et al.*, 1994), where ammonia is the nitrifier substrate, and oxygen provides the terminal electron acceptor for their respiratory train (Hiet Wong *et al.*, 2003).

The overall equation given for the complete oxidation of ammonia to nitrate is given by Mara (2004), where it can be seen that the “nitrification oxygen demand” – i.e. the amount of oxygen needed to oxidise one mole of ammonium-N, is 3.1 g O₂ for every 1 g of nitrogen, and is shown in equation 2.33:



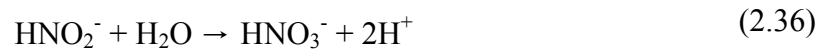
From this equation, it is possible to see that alkalinity is utilised in the nitrification process. The intermediate stages of complete ammonia oxidation of the conversion of ammonia to nitrite are presented in equations 2.34 and 2.35:



In the first stage of the nitrification process, ammonium is oxidised to nitrite with the intermediary stage being the production of hydroxylamine by the enzyme, ammonia monooxygenase (equation 2.34; Craggs, 2005). Hydroxylamine is then

converted into nitrite by the catalyst hydroxylamine oxidoreductase (equation 2.35) (Hiet Wong *et al.*, 2003).

The further stage of the oxidation of nitrite to nitrate can occur in two ways, where, the oxygen atom added to the nitrite ion is either donated from water (equation 2.36), or from molecular oxygen (equation 2.37). These reactions are carried out by nitrite oxidizers using the catalyst nitrite oxidoreductase (Hiet Wong *et al.*, 2003) or nitrite dehydrogenase (Craggs, 2005).



In essence, conventional wastewater treatment relies on the biological removal of ammonia through coupled nitrification and denitrification, catalysed by intense electrical and mechanical input. Ammonia is oxidised aerobically as presented by equations 2.33–2.37 to the end product, nitrate. Nitrate is subsequently reduced to dinitrogen by denitrification processes, where the wastewater is placed under anoxic conditions, and an organic carbon source such as methanol added, to catalyse the process (Sawyer *et al.*, 1994). In facultative WSP ponds however, these processes can often be quite hard to measure as they occur simultaneously and inclusively within a facultative pond – i.e., with nitrification occurring in the upper aerobic layer, and denitrification being confined to the lower anaerobic layer. Subsequently, it is much harder to isolate nitrification and denitrification mechanisms within a WSP environment, with many researchers claiming that nitrification mechanisms are masked by denitrification mechanisms, and vice versa.

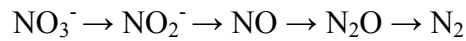
Nitrification mechanisms were found to be operating in the municipal WSP at the Western Treatment Plant, Melbourne, Australia. McLean *et al.* (2000) confirmed that active nitrification was confined to the upper sediments of the aerobic ponds, but occasionally, large nitrifier populations became established in the water column of lagoons; as a result much higher nitrification and ammonia removal rates were observed. This corroborated well with an earlier study conducted on the same WSP system, where Hodgson and Paspaliaris (1996) found that nitrogen

was being removed from pond system by ammonification, followed by simultaneous nitrification and denitrification. This research was followed by Lai and Lam (1997), who also found that nitrification coupled to denitrification was the major nitrogen removal pathway within the WSP system. Additional research by Hurse and Connor (1999) conducted on the same WSP as Hodgson and Paspaliaris (1996), confirmed the importance of coupled nitrification/denitrification as a nitrogen removal process, although values were not reported. Both Hodgson and Paspaliaris (1996) and Hurse and Connor (1999) found that nitrification rates within the Western Treatment Plant lagoons were higher in the summer than during the winter, reflecting a seasonal dependence of various nitrogen removal processes – in particular, nitrification and denitrification (Hurse and Connor, 1999).

Other WSP research describes nitrification processes to a greater or lesser degree. However, Hodgson and Paspaliaris (1996) deduced that two important variables feature significantly in relation to the rate at which this process takes place. The first is concerned with AOB numeration. Hodgson and Paspaliaris (1996) found that a nitrifier population of 10^8 AOB/ml are needed for good nitrification; the second, concerns the inhibition and eventual photodeactivation of AOB by UV radiation.

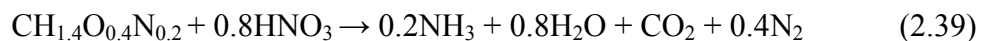
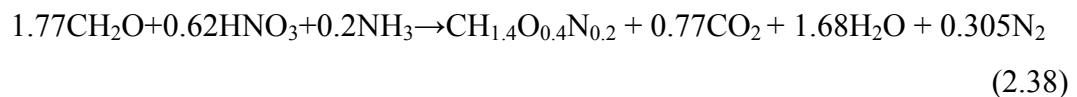
2.6.5 Denitrification

The opposite phenomenon to nitrification is denitrification, where nitrate is reduced along a respiratory electron chain through dissimilatory processes (Randall *et al.*, 1992). Various NO_x species, and ammonium, produced from the reduction of nitrate via biochemical assimilation reactions are also classified as a denitrification mechanism. In contrast to nitrification, many of the denitrifiers are strict anaerobes, where nitrate is reduced in the oxidation of organic matter via respiration (Sawyer *et al.*, 1994; Craggs, 2005). *Achromobacter*, *Alcaligenes*, *Bacillus*, *Micrococcus*, *Pseudomonas* and *Thiobacillus* are just some of the bacteria involved in denitrification processes (Mara, 2004; Craggs, 2005). The reduction of nitrate via denitrification occurs in the following stages (Randall *et al.*, 1992; Craggs, 2005):

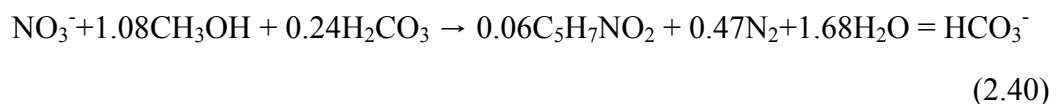


The reduction of NO_3^- to N_2 comprises the complete denitrification process, however, in practical terms, the end product, N_2 may not be reached within a pond environment, but nitrogen is still removed none the less. Bitton (1994, in Craggs, 2005) proposed the optimum conditions for denitrification to occur within ponds, is a pH of between 7.0 and 8.5, a temperature of $>10^\circ\text{C}$, and sufficient quantities of organic carbon to be present in the pond to act as an electron donor.

Denitrification occurs using organic substrates, or inorganic compounds. Organic denitrification is carried out by facultative heterotrophs who use nitrate and nitrite as terminal electron acceptors (and reduce these to dinitrogen), and oxidise organic matter to obtain the cellular carbon source, while producing carbon dioxide and water (Hiet Wong *et al.*, 2003). This is given in the reaction in equation 2.39. Equation 2.38 however shows the reaction where denitrifiers use organic carbon, ammonia as cell nitrogen, and produce acetate as the energy source (Hiet Wong *et al.*, 2003). Inorganic denitrification is discussed in the next section.



A complete equation given by Horan (1990) for denitrification is presented in equation 2.40 where:



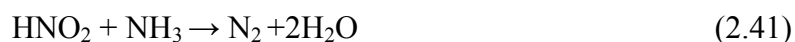
Pond water alkalinity is regenerated from denitrification which Mara (2004) notes is nearly half of the alkalinity used in nitrification processes; and Craggs (2005) gives that 3.6 g of CaCO_3 is produced for every gram of NO_3^- reduced.

2.6.6 Anammox

In recent years the novel discovery that ammonia oxidation can be undertaken by a group of anaerobic microorganisms belonging to the order Planctomycetales (Schmidt *et al.*, 2001), has offered renewed interest in nitrogen removal pathways within wastewater treatment reactors. In the process of anammox (ANAerobic AMMonium OXidation) (Bartlett, 2005), dinitrogen gas is formed via the oxidation of ammonia and nitrite in the absence of molecular oxygen (Shivaraman and Shivaraman, 2003) by using, instead, using oxides of nitrogen (including nitric oxide, nitrogen dioxide and nitrogen tetroxide but preferentially nitrite) as the terminal electron acceptor (Schmidt *et al.*, 2001). Methanotrophs are also capable of using ammonia to produce hydroxylamine (a vital intermediary stage in the anammox process) via methane monooxygenase (Ye and Thomas, 2001). Schmidt and Bock (1997) presented data which showed that ammonia could also be oxidised anaerobically by *Nitrosomonas* spp.

These processes have begun to be extensively studied within wastewater engineering, with the application that system design can be improved to encourage anammox bacterial conditions for growth. Furthermore, the application of molecular microbiological analytical tools, such as DGGE (denaturing gradient gel electrophoresis) and FISH (fluorescent in-situ hybridisation), are being extensively used within the field of environmental engineering, enabling new bacterial communities to be discovered, and studied (Milner *et al.*, 2008). As Schmidt *et al.* (2002) note, the discovery of these organisms have challenged the established notion that the oxidation of ammonia can only occur aerobically, and revealed that this concept is no longer solely true. The identification of the group was first established by PCR (polymerase chain reaction) amplification, and final identification through FISH when it was observed from a denitrifying pilot plant that ammonia and nitrate were disappearing from the plant with a concomitant increase of dinitrogen production (Schmidt *et al.*, 2002) by Arnold Mulder in the early 1990's (Mulder *et al.*, 1995; Kuenen and Jetten, 2001).

The stoichiometry of the anammox process is as follows:



All anammox research undertaken so far has taken place in conventional wastewater treatment systems, in particular in activated sludge processes. To date, there has been no published work which has used molecular microbiological analytical tools to investigate whether anammox bacteria exist in WSP.

2.7 Stable isotope tracer studies in wastewater treatment

The use of stable isotope tracking techniques in experimental systems have been well documented and represents an excellent and highly powerful tool enhancing the study of a huge range of soil, and aquatic environments (Wyffels *et al.*, 2003; Ashkenas *et al.*, 2004; Herrmann *et al.*, 2005), especially those geochemical cycles concerning the concentrations of nitrates in groundwater (Bottcher *et al.*, 1990; Wassenaar, 1995; Feast *et al.*, 1998). In the analysis of other aquatic environments, Nielsen (1992) describes using ^{15}N -enriched NO_3^- as an accurate and easy method for the measurement of denitrification in sediments within streams. The use of the stable isotope of nitrogen provides invaluable opportunity to study nitrogen transformations and mass transfer mechanisms within wastewater treatment processes (Reddy, 1983; Van de Graaf *et al.*, 1997; Lund *et al.*, 2000; Wyffels *et al.*, 2003), and also to assess the implications that the release of treated wastewater effluent has on the receiving environment (Fair and Heikoop, 2006).

Two stable isotopes of nitrogen exist: by far the most dominant fraction is ^{14}N which has an atmospheric relative abundance of 99.64% (Faure and Mensing, 2005). The stable isotope – ^{15}N , comprises the other fraction and has a natural abundance of 0.3663% (Hoefs, 1987; Wyffels *et al.*, 2003; Faure and Mensing, 2005); thus the $^{15}\text{N}/^{14}\text{N}$ ratio in the atmosphere is 0.0036765 (Junk and Svec, 1958, in Faure and Mensing, 2005). Isotopic effects occur because of the differences in chemical and physical properties of an isotope arising from the differences in the isotopes atomic mass (that cause quantum mechanical effects), which can cause small perturbations in chemical behaviour (Hoefs, 1987).

Isotopic fractionation describes the change in $^{15}\text{N}:^{14}\text{N}$ ratios through various nitrogen transformation mechanisms and pathways in the classic nitrogen cycle routes of the utilization of atmospheric N_2 for nitrogen fixation, nitrification, and denitrification (Faure and Mensing, 2005). This process describes the partitioning of isotopes between two substances with different isotopic ratios, and the two main phenomena producing fractionations are isotope exchange reactions and kinetic processes which depend upon the differences in the reaction rates of isotopic molecules (Hoefs, 1987). Faure and Mensing (2005) note that the extent of kinetic isotopic fractionation during nitrification processes is dependent upon the rate limiting steps between the conversion of ammonia into nitrite, and the conversion of nitrite to nitrate, thus by the time the complete oxidation of nitrogen has occurred, the nitrate is depleted in ^{15}N (by as much as 20-35‰ (Mariotti *et al.*, 1981 in Faure and Mensing, 2005). Conversely in denitrification processes, the reduction of NO_3^- to N_2 causes extensive depletion of ^{15}N within N_2 , thus residual nitrate concentrations are complementarily ^{15}N enriched (Faure and Mensing, 2005).

With the addition of $^{15}\text{NH}_4\text{NO}_3$ and $\text{NH}_4^{15}\text{NO}_3$ to estuarine waters in the analysis of algal behaviour, Cohen and Fong (2004) found the uptake rate of $^{15}\text{NH}_4^+$ in *Enteromorpha intestinalis* to be higher than that of $^{15}\text{NO}_3^-$. The study revealed that isotopic fractionation occurred at minimal levels as the isotopic composition of nitrogen in the surrounding medium did not affect the nitrogen species preference of ^{14}N or ^{15}N uptake (Cohen and Fong, 2004). Naldi and Wheeler (2002) also studied the incorporation of ^{15}N assimilated into the cellular tissue of the macroalgae *Ulva fenestrata* and *Gracilaria pacifica*. Numerous nitrogen rich incubation studies revealed that on average 96% of $^{15}\text{NH}_4^+$, and 99% $^{15}\text{NO}_3^-$ was recovered from the growth medium (the external nitrogen pool) and the algal tissue (the internal nitrogen pool) (Naldi and Wheeler, 2002). Isotope dilution effects were deemed negligible, which suggested that there was no discrimination between the uptake of ^{14}N and ^{15}N . This research also revealed that the two types of algae studies both preferentially took up ammonia over nitrate as their designated nitrogen source.

Reddy (1983) is apparently the only researcher prior to this study to use $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ tracking techniques to determine the fate of labelled nitrogen specifically within pilot-scale WSP. His focussed mainly of the nitrogen removal pathways provided by vascular aquatic macrophytes located on the pond surface, and emitted a complete mass balance of the system as not all nitrogen removal mechanisms and pathways were measured. In one of the systems (with no aquatic macrophytes) algae were found to assimilate only 4.6% of the added $^{15}\text{NH}_4^+$, and a small proportion recovered from the sediment layer. In the same system, algae also assimilated 4.3% of the added $^{15}\text{NO}_3^-$, and it was also found that significant quantities of labelled ammonium were converted into labelled nitrate which suggested active nitrification (Reddy, 1983). In the macrophytes system it was found that 34–40% of the total labelled nitrogen was removed ($^{15}\text{NH}_4^+ + ^{15}\text{NO}_3^-$) was removed through plant uptake, and 45–52% remained unaccounted for. In the system without macrophytes, 41% of the added ^{15}N was no accounted for; in both cases, nitrification coupled with denitrification and ammonia volatilization mechanisms were presented as feasible routes to explain the unaccounted labelled nitrogen from both systems (Reddy, 1983).

2.8 Hydraulic flow patterns and models in WSP

The use of models provide powerful tools in WSP design and they are very important not only for determining removal rates and decay constants from contaminants within the wastewater, but also enable the hydraulic performance and characterisation of a system to be found. Their use can pinpoint systems operating with poor hydraulic performance, which will inevitably affect their treatment capacity (Persson and Wittgren, 2003).

Chemical reaction engineering uses the characterisation of different flow patterns to describe the state of flow within chemical or biological reactors. Three prominent types of flow exist; they are: a) a completely-mixed flow regime, b) a dispersed, intermediate or arbitrary flow, and c) plug flow. Completely mixed and plug flow patterns, which both represent 'ideal flow' regimes, are polarised and represent the two extreme conditions for hydraulic characterisation. A completely-mixed regime occurs when the concentration of a substance within a vessel is completely homogenised – i.e., uniform throughout, and the

concentration of that substance has the same concentration at the exit stream, as it does within the vessel (Tomlinson and Chambers, 1979). Thirumurthi (1969) succinctly describes plug flow as a flow pattern within a vessel where there is no lateral diffusion or mixing and where a given mass of liquid passes through the vessel without lateral disturbance. In practice, it is near impossible to obtain plug-flow hydraulic characteristics within a reactor. Flow patterns in real reactors can approximate one or the other ideal flow patterns, but for the majority of reactors the flow patterns lie in the middle of the two. Dispersed, intermediate, or arbitrary flow is the term used to describe these flow types, which are also known as 'non-ideal flow'.

Over the years much distinguished research has integrated first-order kinetic reactions for BOD removal with various flow patterns (Banda, 2007). Wehner and Wilhelm (1956) were some of the first researchers to model BOD removal from a dispersed flow reactor (Mara, 2004). Following this, in 1961, Marais and Shaw produced a model which modelled the first-order removal of BOD in a waste stabilisation pond. Thirumurthi (1969) used the Wehner and Wilhelm (1956) equation to produce a graphical display for the equation (Mara, 2004) which incorporates reaction rates within WSP, the percentage of BOD remaining in the pond effluent, and the corresponding dispersion numbers. Reed (1988) used a plug flow model to describe BOD removal in PFP's, which incorporated a plug-flow first-order reaction rate, and hydraulic retention time as parts of the equation.

Polprasert and Bhattarai (1985), Agunwamba *et al.* (1992), Yanez (1993) and von Sperling (2002) are just some researchers who have sought to produce working models to obtain dispersion numbers from PFP's and maturation ponds. However, where time and resources allow, there is no substitute for undertaking a real hydraulic tracer study to determine the average hydraulic retention time of a pond.

Tracer studies are undertaken to map, monitor and model a flow pattern through real reactors (Levenspiel, 1999). The principle is to add a known amount of a slug in the form of a fluorescent dye, or an inorganic salt, as a single pulse with

the influent flow. The passing out of the slug from the reactor is measured at the exit point by a fluorimeter. From this data, a number of highly useful characteristics about the vessel can be obtained, such as the actual mean hydraulic retention time of the system, indices of dead spaces and hydraulic short-circuiting, including the calculation of the dispersion number. Tomlinson and Chambers (1979) and Levenspiel (1999) present a number of sequential calculations to produce a dispersion number from a hydraulic tracer study, where the dispersion number is defined as follows:

$$\sigma^2_{\theta} = 2\frac{D}{uL} - 2\left(\frac{D}{uL}\right)^2\left(1 - e^{-\frac{uL}{D}}\right) \quad (2.42)$$

where σ^2_{θ} = the dimensionless variance of the tracer curve

$\frac{D}{uL}$ = the dimensionless dispersion number

u = velocity of flow, and

L = the length of the liquid travelled

The dispersion number (D/uL) is presented by Levenspiel (1999) as a dimensionless group characterising the spread in the whole vessel, where D = the axial dispersion coefficient for a flowing fluid, u is the mean velocity of the flowing fluid, and L is the flow length (Muttamara and Puetpaiboon, 1996). Firstly, before equation 2.42 can be solved, the mean hydraulic retention time (\bar{t}) of the tracer within the vessel, and the variance (the measure of the spread of the curve produced) must be obtained. The mean hydraulic retention time is expressed in equation 2.43 as:

$$\bar{t} = \frac{\int_0^{\infty} tCdt}{\int_0^{\infty} Cdt} \quad (2.43)$$

where \bar{t} = the mean hydraulic retention time of the tracer within the pond
(days)

t = time

C = the concentration of the tracer, and

dt = the time intervals in between samples

For ease of calculation, equation 2.43 is written in discrete form as:

$$\bar{t} = \frac{\sum t_i C_i \Delta t_i}{\sum C_i \Delta t_i} \quad (2.44)$$

The variance is calculated by equation 2.45, and represents the square of the spread of the distribution as it passes out through the exit of the vessel (Levenspiel, 1999):

$$\sigma^2 = \frac{\int_0^{\infty} (t - \bar{t})^2 C dt}{\int_0^{\infty} C dt} \quad (2.45)$$

where σ^2 = variance squared

Again, for the practicalities of data calculation, the discrete form of equation 2.45 is written as:

$$\sigma^2 = \frac{\sum t_i^2 C_i \Delta t_i}{\sum C_i \Delta t_i} - \bar{t}^2 \quad (2.46)$$

The final step needed to be taken before the dispersion number can be calculated, is to obtain the dimensionless variance of the tracer curve which is given by:

$$\sigma_{\theta}^2 = \frac{\sigma_t^2}{\bar{t}^2} \quad (2.47)$$

In many ways, the dispersion number gained from a pond tracer study may have been influenced by any number of physical variables. Shilton and Harrison (2003) note that the volume of sludge within a WSP, a (more than likely) constantly changing flow rate, hydraulic short circuiting, the number of dead spaces, and environmental physical influences such as wind, and temperature

(which can induce thermal stratification and density differences in water within a WSP), could all combine so that a WSP may function either above or below its theoretical hydraulic retention time. It therefore follows that the dispersion number will be a function of these same variables, which will undoubtedly influence the behaviour of the tracer within a pond system and thus have some effect on the dispersion number. Normalising the data gained from a tracer study is a useful exercise, which makes the units of concentration and time dimensionless. This produces a residence time distribution (RTD) curve (describing the residence time of the tracer within the reactor), and allows for the direct comparison of different tracer curves from different studies undertaken at different times of year, and for ponds operating with different parameters.

A dispersion number of $\frac{D}{uL} = 0$, represents negligible dispersion – thus the plug flow conditions are reached within a vessel. Where $\frac{D}{uL} = \infty$ (or certainly larger than 2), large dispersion and mixed flow are achieved (Levenspiel, 1999). Certainly, for WSP, dispersion numbers obtained are highly likely to lie within the arbitrary flow region and, as the dispersion number increases, approximate a completely mixed flow regime.